Climate Change Resilience: Sustainable Bioconservation Strategies for Built Heritage

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Abstract

Climate change poses a significant threat to the cultural and historical significance of built heritage. Rising sea levels, extreme weather events, and shifting environmental conditions endanger not only the physical structures themselves but also the cultural knowledge and traditional practices embodied within them. Ignoring this threat would represent a profound loss, severing the intergenerational transmission of invaluable knowledge and skills while eroding the very context and meaning of these landmarks.

This research proposes innovative bio-driven solutions to enhance existing heritage conservation practices in the face of climate change. Recognizing the inherent sustainability embedded within traditional building techniques, the study explores the potential of harnessing new bioprotective technologies, such as bioenhanced limewash with microorganism-driven functionalities, to create long-lasting, protective coatings for built heritage. By utilising microbial properties like biomineralisation and the synthesis of secondary metabolite antimicrobials, these bio-driven approaches offer promising avenues for shielding heritage structures from the forces of climate change, ultimately contributing to a more resilient and sustainable built environment.

The research focuses on two key bioprotective initiatives. First, bioenhancing traditional materials through experimental and in-situ practice. The primary objective is to improve the structural integrity of surface coatings, minimise building maintenance requirements, lower carbon emissions, and align with circular economic principles. Second, it utilises lichen-derived secondary metabolites as a sustainable bio-based protection against mould and bacterial growth. By exploring synergistic combinations and targeted delivery systems, this approach paves the way for reducing reliance on synthetic chemicals and fostering a more environmentally responsible future for heritage conservation.

However, careful evaluation and mitigation strategies are crucial to address potential ecological disruptions and unintended consequences associated with bioenhancement interventions. Further research is required to bridge knowledge gaps in our understanding of how climate change specifically impacts different heritage materials and microbial communities. Long-term research on the combined effects of climate and biodegradation is critical for developing accurate predictions and effective adaptation strategies.

Building trust and collaboration with local communities is essential for bioenhancement to become a viable and successful approach to heritage conservation. By positioning heritage as a focal point for climate education and action, we can raise awareness, inspire collective efforts, and unlock vital resources for adaptation and resilience strategies. Repurposing and retrofitting existing buildings for energy efficiency, while safeguarding their historical significance, offers a powerful solution to reduce reliance on new construction and protect vulnerable green spaces.

Public perception and ethical considerations add another layer of complexity to this multi-faceted challenge. Addressing concerns from both the public and heritage professionals regarding the use of biotechnologies in heritage conservation is crucial. Open and transparent communication, alongside rigorous scientific research and ethical evaluation will be essential for ensuring that bioenhancement becomes a responsible and effective tool for safeguarding our built heritage in the face of climate change.

In summary, a novel biomineralisation technique using cyanobacteria and biopolymers has been demonstrated to effectively mitigate weather-induced erosion in a manner that offers sustainable and environmental benefits. This method can be further enhanced by incorporating antimicrobial

secondary metabolites extracted from lichens to prevent unwanted surface colonisation. However, further research is necessary to optimise the approach and ensure its efficacy and compatibility with existing biomineralisation processes. This study paves the way for the development of sustainable bioprotective technologies for cultural heritage conservation but necessitates a critical and balanced approach that considers potential risks and ethical implications.

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Declaration

I, Peter Anthony Booth, declare that I carried out the work in this thesis in the Department of Creative Arts, University of Hertfordshire. It is my own work and to the best of my knowledge the work has not been conducted previously. All results other than my own are cited clearly in the references.

Date: 8 February 2024

Signed:

Introduction

Context

In March 2023, the Intergovernmental Panel on Climate Change (IPCC), a prominent United Nations organization responsible for the evaluation of climate change-related scientific knowledge, released The AR6 Synthesis Report. This comprehensive document serves as a culmination of insights derived from the preceding three volumes, encompassing studies on physical science, mitigation, and adaptation, developed over several years.

The primary focal point of this report underscores the imperative need for immediate action to mitigate the burgeoning threat to society, arising from escalating greenhouse gas emissions. Failure to curb these emissions is anticipated to result in global warming exceeding 1.5 °C, which would have far-reaching and adverse consequences. The report conveys a profound sense of urgency, emphasizing the monumental scale of measures required to address this crisis. Importantly, it underscores that achieving a reduction in emissions to half of 1990 levels by 2030, to meet the 1.5 °C target is attainable with only a 50% probability.

Established in 1988, the IPCC has periodically issued a series of synthesis reports, encompassing five previous iterations in 1990, 1995, 2001, 2007, and 2014. These reports have systematically encapsulated the collective assessments stemming from various working groups and specialised reports, delving into facets such as mitigation, impacts, adaptation, as well as the effects on terrestrial, oceanic, and cryospheric environments

Since the inaugural report in 1990, the IPCC has acknowledged the progress made in the rate and extent of governmental efforts to combat climate change. It is worth noting that the current plans in place are deemed inadequate to address the impending environmental climate changes. In 2023 there was a decreased level of contention and resistance regarding the factual underpinnings of climate impact when compared to the era of the first report. Nevertheless, despite this growing consensus, the definitive and impactful actions that stem from the prevailing levels of acceptance remain insufficient to persuade less vulnerable populations and governments to undertake resolute measures. This situation is compounded by the disconcerting estimate that global net anthropogenic greenhouse gas (GHG) emissions have surged by 54% compared to the levels observed in 1990, with a significant portion of this increase attributed to carbon dioxide (CO_2) emissions, followed by a notable upswing in methane production. It is important to underscore that, as of 2019, a staggering 79% of global GHG emissions emanated from the sectors of energy, industry, transport, and buildings, (IPCC, 2023).

Elevated GHG levels induce climate pressure, impacting natural and built infrastructure. How each environment responds to the physical, chemical, and biological implications is not fully understood and is subject to intensive research, *Figure 1*. Climate change is leading to catastrophic consequences and irreversible losses, as can be observed by the rise in sea level, flash flooding, fires from extreme heat, intense storms, and landslides. The change extends globally such that it is estimated the earth has lost 28 trillion tons of ice since the mid-1990s, a trend that appears to be accelerating, (Harvey, 2021).

Under increasing pressure, governmental bodies are now engaging with the growing list of climate-related threats and developing climate risk profiles and mitigation strategies. Strategies to leverage the carbon benefits of historic properties remain loosely defined. Gaps in strategy provide an opportunity to develop technologies redefining the contribution repurposed historic buildings offer and encourage novel ways to increase carbon storage and reduce GHG emissions.

Background to the research - climate change and heritage

The Climate Change Act of 2008 stipulates a regular, five-year requirement for the UK Government to release a Climate Change Risk Assessment (CCRA). This assessment serves to elucidate both the climate-related risks and opportunities. Each CCRA is underpinned by rigorous scientific evidence and plays a pivotal role in shaping governmental strategies and policies throughout the United Kingdom. The findings of these assessments are collated in an Independent Assessment of UK Climate Risk, overseen by the Climate Change Committee.

The UK Climate Change Risk Assessment of 2022, in a comprehensive evaluation, scrutinised sixty-one climate risks that span the entire UK, impacting various facets of the national economy. Within the evaluation, eight risk domains were singled out for concentrated attention, a strategic focus extending up to the year 2024. The highlighted risk domains exhibit significant diversity in their scope and are primarily centred around the potential ramifications for the UK's economic landscape. The adaptation plans related to these climate risks exhibit variance across the devolved governments within the UK. Notably, the Welsh Government has taken the lead in the preservation and safeguarding of heritage, as evidenced by their adaptation plan, which is oriented towards the historic environment sector.

The UK's forward-planning financial risk register, covering the period from 2050 to 2080, operates on the premise of an anticipated increase in temperature ranging from 2°C to 4°C, though the current iteration of this register remains unable to quantify the financial risks attributed to the threat posed by climate change on the built heritage sector.

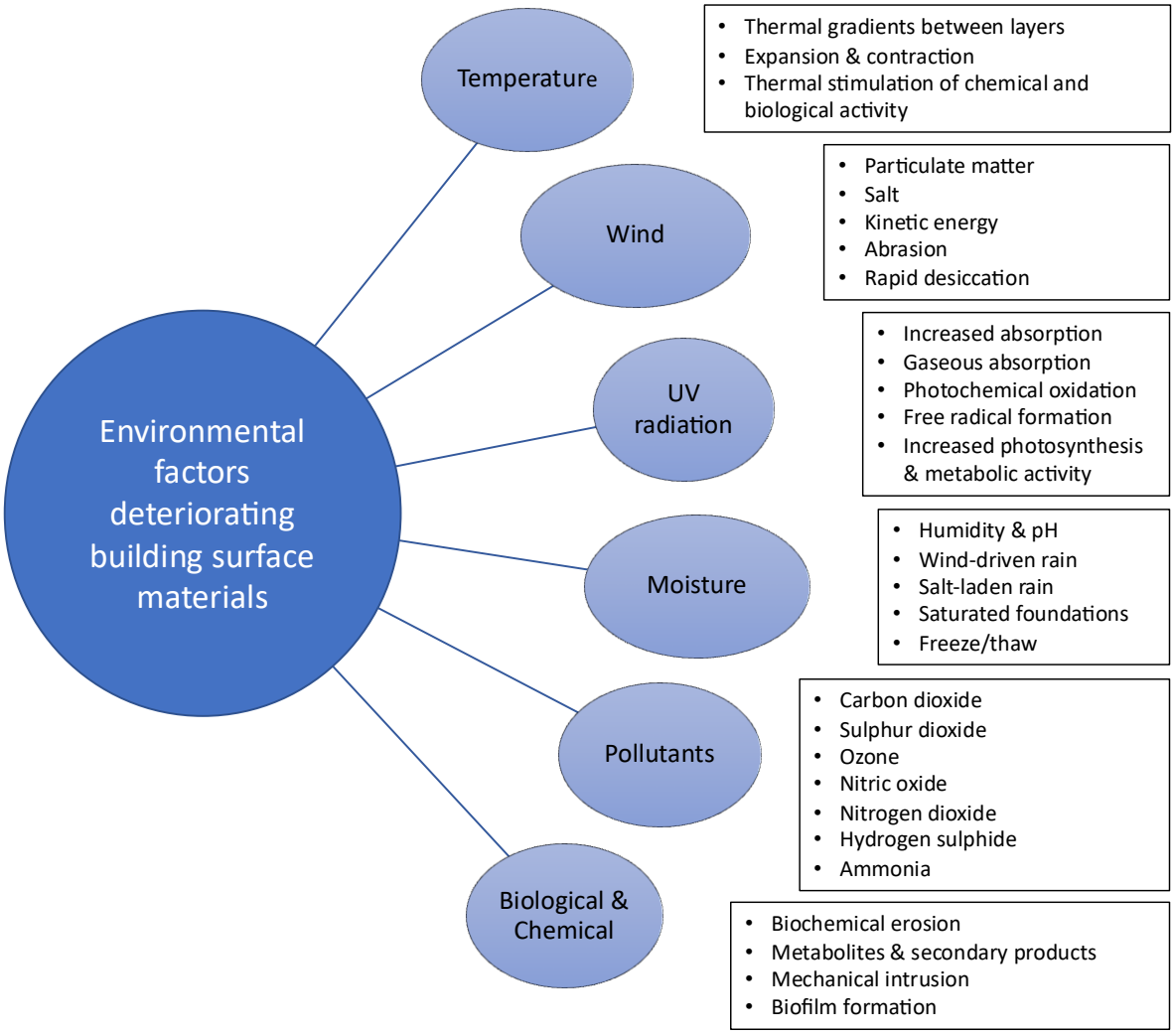


Figure 1: Changes to multiple environmental factors arise as a consequence of anthropogenic GHG emissions and the consequential effect on the climate. These parameters, whether in isolation or conjunction, exert a pronounced impact on the speed of deterioration experienced by building materials. If these factors are disregarded, they will ultimately culminate in costly restorative interventions or, ineluctably, the failure of traditional material performance.

In England, around 20% of homes built predating 1919 are of traditional construction methods and materials. Based on the English housing stock report 2014/2015, this equates to approximately 4.68 million buildings (IHBC, 2020). For Historic England (HE) to meet the 2040 vision for the conservation of heritage, HE has developed a three-strand strategy underpinning climate mitigation, managing risk, and adaptation. Each aspect of the strategy aspires to support government-mandated net zero emissions by 2040, though there are areas which remain unclear as to components within the strategy. A salient concern in this preservation endeavour is the ownership structure of heritage assets, most being in private hands. It is conspicuous that the three-strand strategy proposed lacks a comprehensive plan for enrolling private owners in this conservation initiative. Furthermore, the fiscal responsibilities and substantial compliance costs that will inevitably arise remain inadequately addressed by the strategy.

Historic Environment Scotland (HES) has taken an important step by declaring a climate emergency and documenting the HES Climate Action Plan for the historic environment for 2020-2025. Similarly,

the Historic Environment and Climate Change in Wales Sector Action Plan has developed a seven-point plan ushering in three core objectives to increase and augment knowledge, capacity, and resilience for the historic environment. With each of these plans, numerous multifaceted challenges frustrate the aims being achieved, encompassing issues related to financial constraints and the availability of skilled resources which may impede the realisation of the outlined aims and goals.

Issues being addressed

In 2021 the latest iteration of the National Planning Policy Framework (NPPF) delineated the planning policies of the government for England and guided their application. The framework primarily focuses on the concept of sustainable development, with an emphasis on an environmental imperative to protect and enhance the historic environment. Notably, aspects of the framework are subjective, introducing terminology such as 'substantial harm' in the context of designated heritage assets. This confers a degree of latitude in the interpretation and scope to local authorities when administering and applying the consent process. The potential for subjective standards when applied by local authorities may present problems to initiatives seeking approval for new technologies to enhance traditional conservation processes, (Ministry of Housing, 2021).

The preservation of endangered heritage assets hinges on the availability of a proficient workforce capable of executing the requisite work. In April 2019, a report by the Centre for Economics and Business Research (Cebr) on behalf of Higher Education (HE) conducted an analysis of skills deficits and scarcities within the heritage sector. The report documented a depletion of 7.4% in the overall heritage workforce, attributable to the diminished presence of European Union (EU) nationals following the repercussions of Brexit. This situation was further exacerbated by a substantial reduction in EU funding. Notably, the shortfall revealed that 89% of individuals engaged in construction activities about historical structures were employed by general construction firms, with a considerable 75% of such workers lacking specialised training pertinent to buildings constructed before 1919.

The potential loss of built heritage represents a critical threat to the perpetuation of intergenerational equity as a strategic resource for community development. It is important to acknowledge that the promotion of heritage and community resource development should not be solely reliant on governmental entities, as an over-dependence on public funding can impede entrepreneurial local development (Gustafsson, 2008). The establishment of cultural districts may offer a mitigating approach to some of these limitations. However, the degree to which this benefits the conservation of privately owned historical assets remains a matter of uncertainty, (Borin et al., 2012). To fully realize the cultural and economic advantages offered by the built environment, heritage conservation must also confront the mounting challenges posed by climate change. Encouragingly, there is ongoing cautious progress in adapting traditional conservation principles, influenced by the expanding body of literature pertaining to sustainable practices, renewable energy utilisation, and energy conservation within historic edifices (Forster et al., 2011).

Importance of the research

The escalating challenges of coastal erosion, propelled by rising sea levels, elevated tides, and heightened storm frequency, pose a substantial and escalating threat to the preservation of architectural heritage situated in exposed locations, (Bertolin, 2019). The confluence of oceanic alterations and the intensification of severe meteorological events synergistically accelerates the degradation of both structural and material components, ultimately culminating in the potential

failure of critical architectural elements. An illustrative case is the increasing stress experienced by building facades, driven by multidirectional wind-borne rain, leading to saturation and the infiltration of dampness (Orr et al., 2018).

The prolonged saturation of the foundation, induced by persistent heavy rainfall, results in the upward migration of moisture through the capillary action of the building materials, compromising the structural integrity and potentially leading to the development of deleterious microclimates within the interior envelope. This phenomenon is exacerbated by the overflow of inadequately designed gutters and the augmented runoff generated by oblique, wind-driven rain (Brimblecombe, 2014). The introduction of sea salt, carried by gale-force winds, coupled with the heightened acidity of rainwater due to increased levels of dissolved atmospheric CO_2, exacts a particularly corrosive toll on metal and stonework (Doehne, 2002).

The loss of cultural heritage buildings due to environmental threats poses a significant risk to the social and economic well-being of communities. Cultural heritage buildings play a vital role in fostering community cohesion and generating tourism revenue, which is particularly important for remote regions with limited income prospects, (Nasser, 2003). One indicator of this interconnectedness within a community is the pivotal role that heritage buildings play in generating tourism revenue, (Carr, 2008). The erosion of cultural heritage due to climate change can have far-reaching consequences, impacting both the social fabric and economic livelihood of communities, (Mckercher & Du Cros, 2012).

Mindful of the substantial contribution of cultural heritage to local economies, communities are inclined to harness the potential of cultural heritage tourism, though the endeavour to attract larger numbers of tourists is not universally met with enthusiasm (Del Chiappa et al., 2018). The loss of locally built heritage assets due to climate-induced erosion encompassing physical, chemical, and biological damage is therefore likely to result in the irreversible erosion of community cohesion, leading to local economies enduring economic challenges, (Blennow et al., 2019).

Pre-1919 properties play a pivotal role in mitigating the acute housing crisis currently afflicting the United Kingdom. The Centre for Cities (CFC) charity has identified a significant housing deficit of 4.3 million homes in the national housing market. Based on the previous commitments to construct 300,000 homes annually, the CFC estimates that rectifying this issue will likely exceed five decades (Watling & Breach, 2023). To alleviate the pressure on the housing shortage and preserve pre-1919 structures mandates a departure from conventional methodologies and an embrace of innovative conservation technologies adept at addressing environmental challenges.

In 2019, Historic England (HE), increasingly attuned to the challenges posed by climate change, commissioned Carrig Conservation International to investigate and quantify carbon dynamics in historical environments. Findings revealed that the construction of new buildings contributes to 31.3% higher carbon emissions compared to a mere 2.1% in the case of refurbishing a Victorian terrace. Furthermore, heritage properties demonstrate superior long-term performance in terms of comparative lifecycle carbon emissions. Even studies that account for the carbon footprint associated with manufacturing, transporting, and erecting materials for heritage retrofits consistently affirm that retrofitting exhibits more favourable carbon emissions when juxtaposed with the alternative of demolition and reconstruction, (Wise et al., 2019).

The research purpose

In light of the increasing climate-related threats to architectural heritage, the research purpose is to formulate and implement novel technological solutions for biological self-repair mechanisms and antimicrobial shielding. These innovations were designed to augment conventional conservation methodologies, with each technique aiming to fortify the resilience of traditional construction materials against the adverse impacts of climate-induced degradation. Consequently, this research introduces biotechnological interventions as a means of enriching conservation practices, thereby upholding the fundamental ethos and principles underpinning heritage preservation.

Aim of the research

The research aim is to address environmental weathering through the development and integration of sustainable, renewable, and durable organic materials within the framework of heritage conservation, thereby enhancing the sustainability and resilience of conservation practices associated with built heritage.

Thesis position

While traditional repair methods struggle to keep pace with the intensifying threats of climate change, bioinspired and physicochemical organic compounds offer a revolutionary path towards sustainable, self-healing, and adaptable materials for built heritage preservation. This thesis posits that the strategic design and fabrication of these compounds, drawing inspiration from the resilience shown by biological systems, can demonstrably enhance the durability and lifespan of conventional materials, enabling built heritage to not just survive, but thrive, in the face of climatic extremes.

Research questions

The research poses two sets of questions.

The first is framed around the mechanisms of biodegradation. How do climate change factors such as temperature, humidity, and precipitation, impact the interactions between biochemical, physical and material properties, and ecological elements that drive the biodegradation of traditional construction materials? Extending on from this is how do these key biodegradation pathways involved in biomineralisation and bioerosion threaten specific heritage materials under different climate scenarios?

The second is whether, through bioremediation and the biosynthesis of sustainable materials, innovative solutions can counter the erosive threat. To what extent can targeted microbial biosynthesis strategies be used to design and fabricate novel bio-based materials for bioremediation and protection of built heritage? Can these bio-inspired materials be tailored to exhibit desired properties such as water resistance, antimicrobial activity and self-healing while ensuring compatibility with specific historical materials and conservation ethics?

Methodology

The interdisciplinarity of the DHeritage professional doctorate is advantageous to this study. It facilitates the alignment of diverse methodologies, enabling a robust approach to research questions

within the complex context of heritage. This convergence of perspectives furthers discussion into the nuanced understanding of heritage as a multifaceted phenomenon embedded within social, cultural, and environmental systems. Consequently, the research can delve deeper into the meteorological, environmental, physicochemical and biological science dynamics at play potentially leading to richer and more comprehensive research outcomes. This framework ultimately serves as a foundation for knowledge production and dissemination within the field.

The initial phase of this research endeavour involved an extensive literature review to identify key concepts to investigate, bolster the thesis position, discern knowledge gaps, and facilitate the development of research methodologies. Within the literature review, salient issues emerged, particularly in the realm of environmental challenges and the knowledge gaps that exist between traditional conservation practices and the biodeterioration of built heritage. These knowledge gaps directed the research focus towards the refinement of experimental methodologies.

This comprehensive analysis of existing literature revealed critical knowledge gaps that has informed the conceptual framework and research direction of this thesis. These gaps were identified across five primary domains: the impact of climate change on cultural heritage, the biodeterioration of traditional materials, the role of biomineralisation in natural material synthesis, the design of bio-delivery systems, and the development of sustainable biocides.

Preservation and adaptation strategies for cultural heritage in the face of climate change are increasingly adopting a multidisciplinary approach to assess the scientific complexities involved (Bertolin, 2019; Sesana et al., 2018, 2021). While the imperative for adaptive measures in response to identified climate risks has been acknowledged, the majority of research has centred on environmental, planetary, and material sciences. This study posits a significant role for biological sciences in addressing decay processes affecting heritage materials and climate modelling, as well as in developing practical solutions and tools for the climate change adaptation of traditional materials.

The accelerated degradation of stone monuments and buildings has prompted investigations into the potential correlation between climate fluctuations and the equilibrium between bioprotection and biodeterioration, (Liu et al., 2022). Current research has focused on defining metrics for the relative bioprotective ratio and examining the influence of environmental factors on this balance. However, a standardised methodology for biodeterioration measurement is lacking, which may hinder the accurate assessment of competing biomineralisation processes.

Research on biomineralisation has primarily concentrated on reinforcing microfractures in concrete and exploring the potential for bioconcrete repair, (Castro-Alonso et al., 2019; Seifan et al., 2016, 2018). While both passive and active approaches have been explored, the aggressive nature of concrete has necessitated the development of robust frameworks, such as ceramics and graphite tubes, for bacterial attachment. An opportunity exists to develop biodegradable delivery vehicles capable of controlled release to target specific locations within construction materials.

The engineering of biodegradable and sustainably sourced microbial delivery systems is well established in the pharmaceutical and food industries but remains relatively unexplored in the context of construction materials. This disparity can be attributed to the harsh chemical environment of building materials and limited understanding of the interactions between biopolymers and construction compounds, (da Silva et al., 2014; Gunzburg et al., 2020; Wang et al., 2012).

The methodological approach adopted in this study is characterised as exploratory and positivist, commensurate with the conventions of experimental research within the natural sciences. This

approach leveraged observable and quantifiable methods. The interpretation of quantitative data derived from the experimental work played a pivotal role in informing the empirical assumptions underpinning the thesis, (Creswell, 2014). The investigative procedures employed were tailored to the specific requirements of the experimental hypotheses, yielding precise and replicable quantitative data that could be subjected to statistical validation.

This research employs a two-pronged practice-based methodology, detailed in Chapters 3 and 4, to gather novel, scientifically verifiable data. Each component directly informs the research argument, strengthening the thesis and generating new knowledge relevant to bio-based strategies in built heritage conservation. The rationale for this dual approach, unfolding across the chapters, fosters a cohesive synthesis of research elements and contributes meaningfully to the advancement of bio-based conservation strategies.

This methodological orientation may allude to a similarity with the generation of academic knowledge as traditionally associated with the conventional PhD, as argued by (Lee et al., 2000). Upon reflection, it becomes evident that the underlying philosophical framework of this thesis not only nurtures the cultivation of new knowledge within a disciplinary context governed by natural laws, (Hamilton, 2005), but also fosters the development of experimental methodologies with practical applicability. This approach has yielded a comprehensive spectrum of theoretical and practical perspectives aimed at addressing real-world, practice-based challenges. These contributions are well-suited for integration into the knowledge economy, aligning with arguments put forward in the literature, (Nowotny et al., 2003; Usher, 2002).

Research structure

This research adopts an interdisciplinary framework, incorporating the disciplines of climate science, biosciences, and biomaterial design. Its primary objective is to enhance and expand the preservation of built heritage and harness the latent capabilities of heritage science as a catalyst for future conservation efforts.

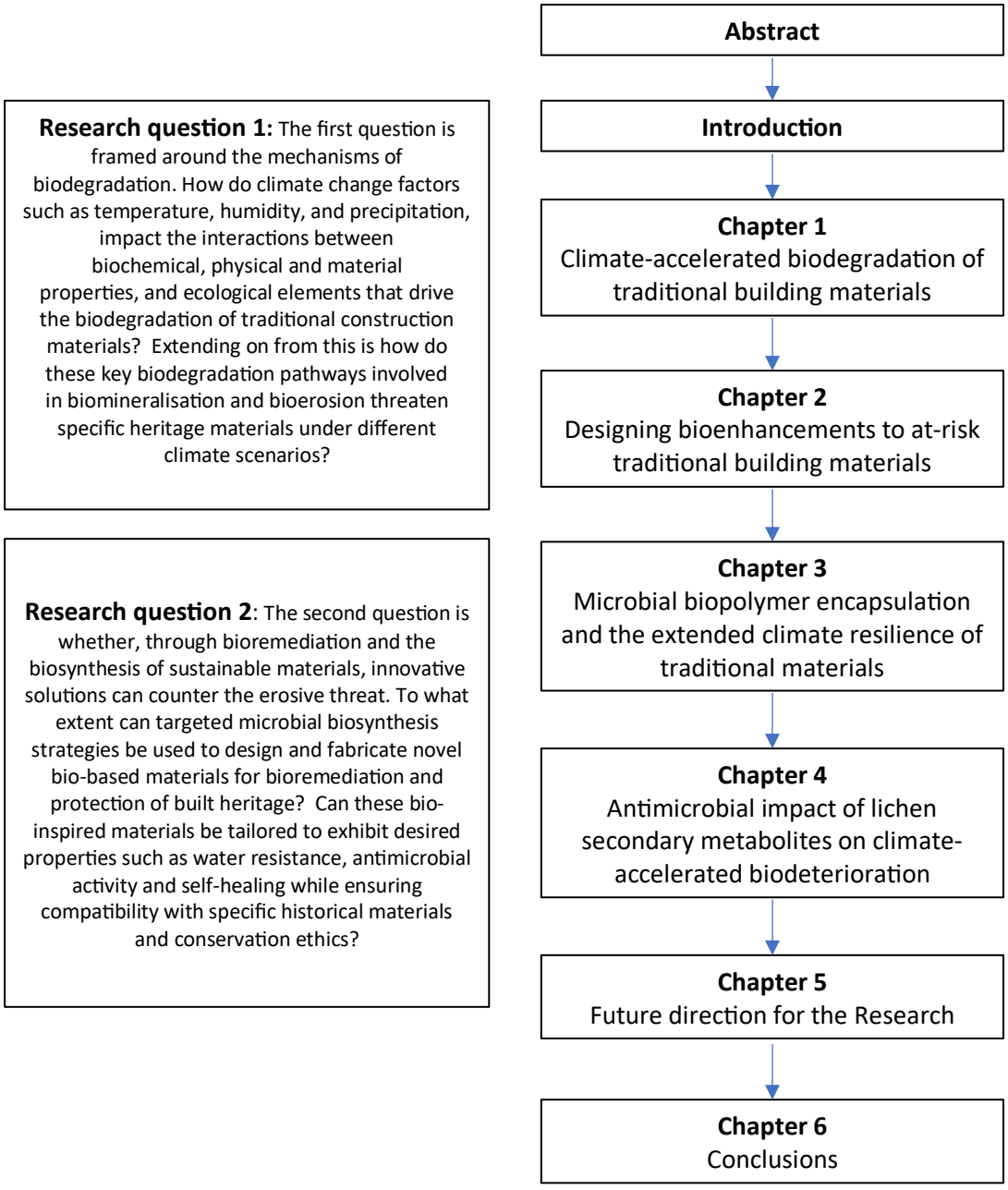


Figure 2: Overview of the research questions and the chapter structure

Chapter overview – the synopsis of the research design

This investigation delves into the multifaceted ramifications of climate-induced deterioration on the preservation of built heritage. Extreme weather events trigger the degradation of building materials, acting as potent catalysts for microbial proliferation, which may, in severe cases, compromise structural integrity. To effectively address this challenge, robust and adaptive approaches for the conservation of materials embedded within heritage sites are urgently required.

The research methodology adopted embraces a multifaceted approach, encompassing both observational and experimental components. Each subsequent chapter contributes to the advancement of traditional material conservation through a synergistic interplay of literature review, systematic observation, and in chapters three and four, empirical experimentation, *Figure 2*.

Chapter One undertakes a critical review of existing knowledge about the accelerated biodeterioration of traditional materials under the influence of a changing climate. This establishes a critical foundation for subsequent investigations, informed by the literature review.

Chapter Two leverages systematic observation to gain deeper insights into specific aspects of biodeterioration and potential mitigation strategies. The chapter investigates the biosynthesis of calcium carbonate and its potential application in enhancing the protective qualities of limewash applied to receptive renders and sets the context for the experimental work in subsequent chapters.

Chapter Three proposes and elaborates upon an experimental procedure for the encapsulation of autotrophic and heterotrophic microbial cells within biopolymer coatings derived from sustainable materials. This chapter explores the potential of biopolymer encapsulation to enhance biomineralisation processes within limewash, promoting climate resilience in traditional materials.

Chapter Four focuses on the identification and extraction of lichen-derived secondary metabolites with antimicrobial properties. This chapter evaluates the potential of these metabolites as environmentally sustainable alternatives to conventional chemical treatments for combating microbial decay in heritage buildings.

Chapter Five delves into the complexities and issues where climate change and the bioconservation of built heritage intertwine. It critically examines opportunities and potential challenges unravelling a path forward for the practical implementation of bioenhancement strategies. The chapter illuminates avenues for further exploration both within this specific bioenhancement research and beyond.

This research injects crucial new perspectives into heritage conservation by examining the impact of climate-driven biodeterioration on traditional materials. It pioneers the exploration of environmentally-sustainable strategies for microbial control and material protection, culminating based on the outcome of Chapter Four, in the proposal for industry-specific databases of secondary metabolites. This framework unlocks the potential of these metabolites as tools for the building sector and redefines the boundaries of heritage conservation by venturing beyond established practices.

The potential to extend the lifespan of heritage buildings, thereby alleviating the financial burden on property owners, materialises through this research as a tangible outcome. Furthermore, carbon

sequestration enhanced by biomineralisation processes, offers a potent strategy in the fight against climate change, potentially contributing to a net-zero approach. The second research initiative extends beyond the mitigation of biodeterioration; it presents a breakthrough innovation, offering ecological alternatives to traditional chemical treatments for microbial control in built heritage contexts.

Ultimately, this research paves the way for a fundamental shift in our understanding of climate-induced biodeterioration and its mitigation. By elucidating the complexities of this challenge and crafting robust, sustainable solutions, it lays the groundwork for the preservation of irreplaceable built heritage for future generations.

Value and contribution to knowledge

The following chapter proposes a framework for integrating innovative biotechnologies into the management of climate-induced degradation in traditional building materials. The research design seeks to achieve the systematic development of two primary outcomes to address this degradation, the bio-enhancement of lime-based material surface coatings and the bio-sourcing of secondary metabolites as antimicrobial agents to control surface moulds and harmful bacterial contamination.

In the first experimental design, biopolymer-encapsulated bacteria are incorporated into limewash, utilising either photosynthesis and atmospheric CO_2 or redox reactions and carbonate in solution to increase calcium carbonate density. Biomineralisation strengthens the sacrificial carbonate layer, extending its lifespan against extreme weather events. Reduced material erosion translates to lower maintenance demands, minimised repair costs, and decreased CO_2 emissions associated with labour-intensive repairs. The development of a sustainable, locally sourced conservation product presents opportunities for wider commercialisation and manufacturing, with an initial focus on protecting lime-rendered historic buildings. Furthermore, the application may hold potential for concrete structure maintenance, widely expanding its commercial appeal.

The second research initiative addresses the increasing prevalence of mould and bacterial growth on building surfaces due to warmer and more humid environments by mobilising the extraction and isolation of antimicrobial agents from lichens. These agents effectively inhibit mould and bacterial cell growth and biofilm formation, offering a non-toxic and environmentally sustainable alternative to harsh chemical treatments or damaging mechanical removal methods. The production of biosynthesised, environmentally friendly antimicrobial products for treating biological infestations opens doors for new commercial and manufacturing ventures, promoting both building and occupant health. Furthermore, these antimicrobial conservation products possess broader commercial applicability as mould and bacterial treatments across diverse traditional and contemporary structures.

By systematically developing evidence-based methodologies focused on biosynthesis and application of organic materials, Chapters Three and Four provide a solid foundation for addressing the climate-driven deterioration of traditional materials and extending the lifespan of these valuable cultural assets. The outcomes not only contribute to the development of renewable and sustainable advancements in material science but also comprise a strategy for mitigating carbon emissions associated with the maintenance of heritage buildings.

Expected outcomes

This research delves into the potential of biopolymer-encapsulated microbes and microbial secondary metabolites to contribute new knowledge to the field of heritage science. The outcome is anticipated to encompass a two-pronged approach:

First, the aim is to enhance the resilience of traditional lime render against harsh climatic conditions. This will be achieved through the development of an innovative limewash incorporating biopolymer-encapsulated microbes. Microbes will enable the formation of a denser sacrificial carbonate layer, utilising environmentally available carbon thereby strengthening the natural limewash shield against environmental degradation.

Second, to explore the efficacy of biologically synthesised antimicrobial agents in combating microbial deterioration, a major threat to traditional materials and potentially occupant health. The versatility and eco-friendliness of these antimicrobial metabolites hold promise for preserving heritage materials and buildings, potentially extending their lifespan for future generations.

The outcome of the research strives to extend the boundaries of conventional heritage science, forging a novel nexus between physicochemical and biological principles. By unlocking the potential of bio-based interventions, it paves the way for innovative and sustainable approaches within the cultural heritage sector.

Research next steps

Environmental fluctuations and their multifaceted impact on the microbiome inhabiting traditional building materials pose a significant challenge for predicting biodegradation and bioprotection processes. This complexity stems, in part, from the difficulty in anticipating climate shifts and deciphering their potential consequences for microbial populations, particularly regarding their environmental adaptations at the omic level.

The research outcome contributes to bridging the knowledge gap by laying the groundwork for two crucial research avenues that can improve our understanding and predictive capabilities in this domain.

The first research opportunity delves into the development of 3D and 4D biopolymer encapsulation mechanisms for controlled delivery and triggered release of microbial and chemical payloads within the context of construction and repair. Biopolymer capsules, designed for self-repair functionality and comprising of intelligent biopolymer materials, would deliver microbial or chemical agents to bolster material resilience against extreme weather events.

The second avenue focuses on designing a framework leading to establishing a comprehensive database for identifying lichen secondary metabolites with potential applications in the construction industry, particularly heritage preservation. This initiative hinges on an analysis of lichen metabolites, many of which remain largely unexplored in terms of their structural and functional properties. These metabolites hold promise as natural deterrents against the physicochemical and biodegradation processes that threaten traditional building materials. A dedicated research database would serve as a building materials-centric knowledge repository, facilitating the development of lead compounds, synthetic derivatives, and enhanced functionalities.

CHAPTER ONE: Climate-accelerated biodegradation of traditional materials

Chapter Abstract

Maintaining and retaining our built heritage is not merely an act of historical conservation; it is a commitment to intergenerational equity and a testament to human ingenuity. This chapter explores the multifaceted challenge posed by climate change to our architectural heritage. Climate change impacts heritage preservation directly through extreme meteorological events and indirectly by altering ecosystems and environmental conditions in which biodegradation takes place.

Directly, severe storms, torrential downpours, and flooding pose immediate threats to historic structures, causing irreparable damage. Indirectly, climate change disrupts the ecosystems and natural cycles leading to increased invasive growth and species proliferation which may encourage the introduction of larger organisms that undermine structural integrity.

This subtle interplay between climatic conditions, bio-infestation, and the physical properties of building materials fuels biodeterioration. Even slight changes in climate create favourable conditions for the attachment and growth of microorganisms, further accelerating the overall deterioration. The biological deterioration of traditional materials is multifaceted, an interaction between complex mechanisms through which climate change, accelerates the deterioration of our heritage and repercussions on occupant health. Understanding these mechanisms and developing environmentally sustainable, low-carbon strategies to safeguard these invaluable links to our past is essential for the benefit of local communities and future generations.

Emerging from this understanding is the promising yet uncharted territory of microorganism-supported bioprotection. Harnessing this innate ability of microbes will unlock a sustainable and low-carbon weapon in the fight against decay. This chapter concludes by exploring the potential and challenges of this novel approach, outlining the considerations for implementing diverse bioprotection strategies. In so doing, it illuminates the sustainable future of heritage conservation, paving the way for the next chapter, exploring bio-enhanced building materials and an opportunity to commit to the preservation of intergenerational equity in the face of a changing climate.

1.1 Introduction

The architectural heritage of a nation bridges the chasm between contemporary society and the echoes of history. This tangible testament to past endeavours not only illuminates the trajectory of bygone eras but also subtly influences our present and future. Stewardship of this cultural heritage reflects a fundamental commitment to intergenerational equity, preserving historical narratives that nourish local communities.

However, a threat looms across this canvas, the direct and indirect impact of climate change. It manifests in dual forms – the indirect acceleration of biodegradation in traditional building materials as a result of a faster breakdown of organic matter releasing CO_2 into the surroundings, thereby fostering a niche for microorganisms, and the direct erosion wrought by extreme weather events. Severe, high-energy storms, heavy rainfall, and rampaging floods impact historic edifices, when reinforced by biodegradation, potentially cause irreversible harm.

Climate monitoring initiatives, such as Copernicus, a cornerstone of the European Union's Space Programme, are essential for informing environmental management and climate change mitigation strategies. Collaborations with entities like the European Organisation for the Exploitation of Meteorological Satellites have established a robust global observation platform supporting critical research.

The escalating impacts of climate change, as underscored by the Intergovernmental Panel on Climate Change's (IPCC) Sixth Assessment Report (AR6), demand urgent action. The report's stark warnings of a potential 4°C temperature rise by century's end, (Calvin et al., 2023) and the inexorable rise in sea levels, (Met Office, 2024) highlight the imperative for immediate emissions reductions. The credibility and comprehensive nature of climate monitoring programs, exemplified by Copernicus, the IPCC, and the Met Office, provide a solid empirical foundation to counter economic and political opposition to climate mitigation measures

At the regional level, climate monitoring data underpins local authority efforts to develop and implement climate action plans. Historic Environment Scotland (HES) has prioritized energy efficiency and carbon management in alignment with Scottish Government targets. A 2018 climate change risk assessment, involving local authorities, identified vulnerabilities within the historic estate. This assessment has informed maintenance planning and resource allocation. However, the severity of climate-induced threats, particularly coastal erosion, has necessitated difficult decisions, including the designation of certain sites as unrecoverable. The Scottish Government's acknowledgment of rising temperatures, increased rainfall, and accelerating sea-level rise since the 1960s, (Hyslop et al., 2017), underscores the challenges faced by heritage conservation. The financial and resource implications of managing climate erosion are substantial, necessitating innovative approaches to protect historic structures from increasingly extreme weather events.

These environmental shifts have cascading consequences. Disrupted ecosystems and altered natural cycles lead to invasive species encroaching upon historical structures, their anchorage weaving into the material substrate, compromising structural integrity. Intertwined with these biological incursions are geomorphic processes, comprising a potent duo known as biogeomorphology, further weakening the building. As temperatures and moisture levels rise, the decay of materials fuels a cycle, supplying nutrients, thereby attracting microbial colonisers which contribute to accelerated deterioration.

Nutrients from organic decay and anthropogenic pollutants further expedite the deterioration of building materials. Physical stressors like vehicular vibrations and ground tremors inflict micro-

fractures, their cumulative effect causing cracks and material displacement. Rising temperatures and altered precipitation patterns elevate humidity, degrading stone, wood, and wall renders. Chemical agents, such as acid rain, atmospheric pollution, and industrial emissions, will corrode construction materials, particularly metals and compounds with high levels of mineral salts. This leads to corrosive chemical processes within substances like stone, causing alterations in composition, structural weakening, as well as discolouration, surface pitting, microfracture formation, and eventual building collapse.

Against this physicochemical backdrop, biodeterioration emerges as a complex interplay between substrate conditions, the nature of the bio-infestation, and the prevailing climate. Even minor climatic shifts create favourable conditions for the attachment and growth of new fungi and bacteria, as depicted in *Figure 3*.

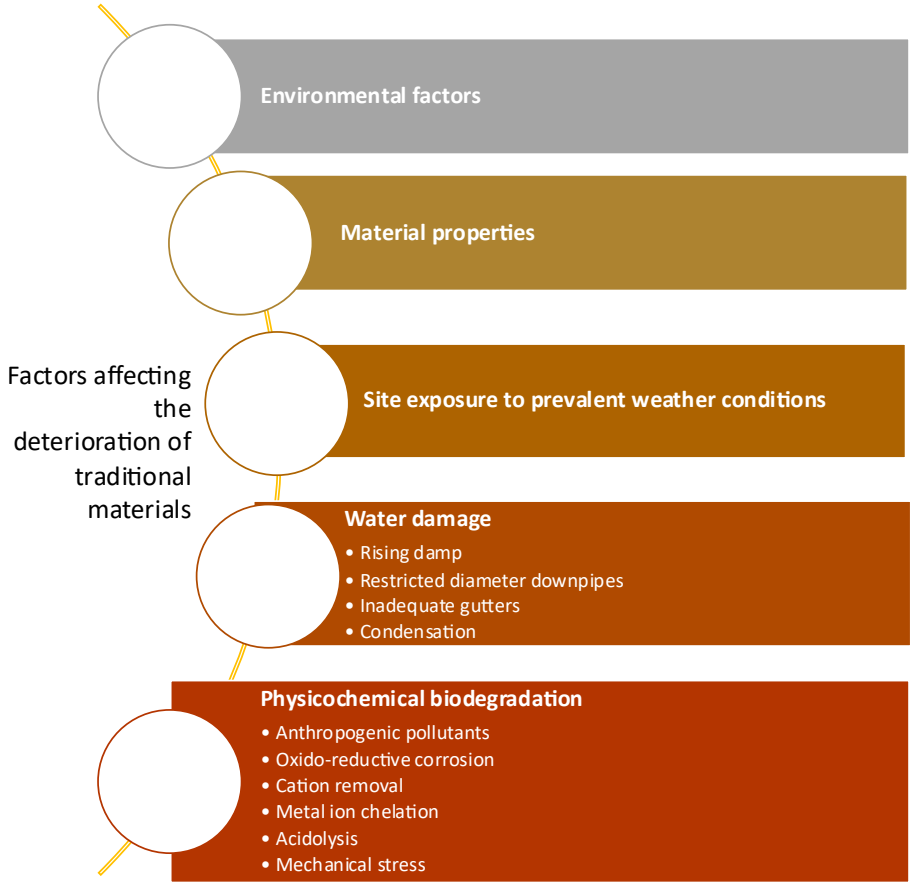


Figure 3: Factors affecting the deterioration of traditional materials including environmental, erosive weather, porosity of materials, saturation and permeability, antimicrobial resistance, site exposure and building orientation. The primary cause of deterioration is the entry of water and saturation of building materials which in turn encourages corrosion, bio-corrosion, organic acids, biofilm formation and the adhesion of anthropogenic pollutants which provide nutrients for microbial growth (Steiger et al., 1993)

The synergistic action of biological and non-biological degradation processes initiates the formation of microcracks within the building substrate. These microcracks act as pathways for fungal hyphae,

establishing favourable surfaces for the proliferation of bacterial biofilms. Within these microfractures, microorganisms secrete extracellular polymeric substances (EPS). Exposure to moisture triggers the transformation of EPS into a colloidal hydrogel, inducing significant alterations in the thermal and moisture properties of the material. The expansion and contraction cycles of the EPS exert pressure on the microcrack walls, further propagating fractures deeper into the substrate, thereby exacerbating the degradation process.

1.2 Mechanisms involved in the microbial biodegradation of traditional building materials

While the definitions of biodegradation and biodeterioration often overlap in the literature, suggesting a shared underlying biological mechanism, this study proposes a nuanced distinction based on perspective. "Biodegradation" is employed here as a broader term encompassing all microbial breakdown processes and their vital role in natural environmental cycling. "Biodeterioration," conversely, focuses on the same microbial activity, but from a perspective specifically highlighting economically undesirable degradation leading to structural failure in built environments, (Eggins & Oxley, 2001). This distinction, crucial for understanding the specific implications of microbial activity, is further elaborated upon in section 1.2.6.

1.2.1 Biodeterioration

Biodeterioration is a multifaceted phenomenon, culminating in adverse mechanical, chemical, and aesthetic detriments inflicted upon building materials. The deterioration arises from the physicochemical interplay between microorganisms and the underlying supportive substrate.

The microbial degradation of conventional construction materials encompasses the gradual erosion of these materials by microorganisms, primarily bacteria and fungi. This phenomenon exhibits variability contingent upon the intrinsic characteristics of the material, the prevailing environmental conditions, and the specific taxonomic classification of the microbial species involved, as depicted in *Figure 4*. Several of the terminologies which follow have been developed to elucidate the functioning of the wide range of biodegradation mechanisms that may be found in the construction industry, (Daval & Xu, 2023).

1.2.2.Biodissolution

Biodissolution is a fundamental biological process wherein solid materials undergo breakdown into subunits due to the catalytic actions of biological agents, primarily enzymes produced by microorganisms, (Potysz & Bartz, 2022). This decay involves the gradual degradation and disassembly of the material under the influence of metabolic by-products. The primary objective of biodissolution is to interact with the substance, resulting in its fragmentation into smaller constituents that eventually dissolve into a liquid medium (Wild et al., 2022; Wu et al., 2022). Microorganisms have evolved such biochemical mechanisms to harness dissolved components as sources of nutrients or energy.

This degradation of organic matter by microorganisms assumes a central role in natural recycling processes. Secreted enzymes cleave complex molecules into simpler building blocks, subsequently

absorbed into the microbial cells and integrated into various metabolic pathways. Numerous microorganisms actively partake in the biodissolution of mineral ores, contributing to bioremediation processes and the natural cycling of solubilised elements. Organisms such as lichens and fungi exemplify both mechanical and physical degradation of substrates. Fungi, for instance, extend hyphae and microtubules into microcracks, anchoring themselves to surfaces. Leveraging the hydrophilic nature of their cytoplasm, they can exploit the expansion and contraction of the fractures, facilitating water ingress and ensuing erosion. Regions featuring densely clustered microcracks on substrates are promptly colonised, potentially resulting in weakened areas leading to removal of surface layers. This interplay of biological and environmental factors underscores the multifaceted significance of biodissolution in ecological and geological contexts.

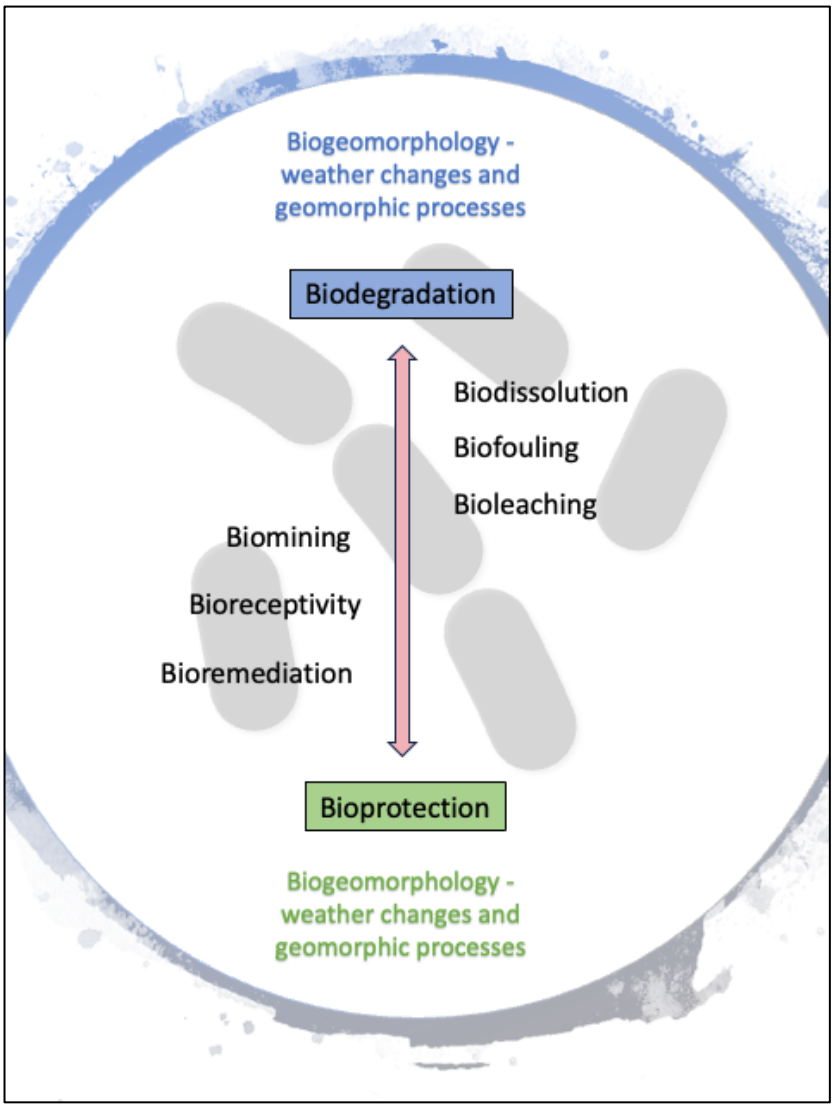


Figure 4: Environmental factors, including fluctuations in temperature, humidity, and pH, impact the colonisation patterns of microbial communities. Fungi exhibit distinct responses to varying weather conditions. Under consistent environmental conditions, fungi gradually penetrate the microcracks in substrates by extending their hyphae, generating corrosive acids and progressively delaminating the underlying stone. Conversely, during extended periods of severe weather conditions, the presence of lichens can function as a protective barrier against the erosive effects of climate shifting the delicate equilibrium between biodeterioration and bioprotection. The lichen effectively forms a bioprotective, self-repairing outer layer, offering a robust physical defence mechanism against erosion induced by extreme weather. Consequently, the formation of a protective layer can potentially outweigh the slower and less severe biodegradation brought about by the lichen's inherent biological processes in the context of extreme weather-induced erosion

1.2.3 Bioleaching

Microorganisms employ bioleaching as a mechanism for the extraction of metal ions from mineral ores and rocks. Acidophilic bacteria, most notably *Acidithiobacillus ferroxidans* and *Acidithiobacillus thiooxidans*, assume central roles in the bioleaching process. These microorganisms catalyse chemical reactions that culminate in the dissolution of metallic constituents from solid matrices. Within the bioleaching framework, microorganisms undertake the oxidation of metal sulphides inherent to the geological substrates, yielding sulphuric acid as a consequential by-product. Sulphuric acid actively participates in the dissolution of metal ions, a procedure of industrial significance for the recovery and reclamation of various valuable metals, including but not limited to copper, gold, silver, uranium, and nickel, (Adetunji et al., 2023; Saldaña et al., 2023).

The perpetual leaching of metals from geological substrata, encompassing even conventional stone formations, has a consequential effect. The leaching process weakens the structural integrity of the materials, leading to a concomitant reduction in compressive strength. Simultaneously, protracted leaching introduces toxic elements into the adjacent ecosystems, prompting concerns about the environmental consequences.

1.2.4 Biomining

Biomining comprises a dual-stage procedure. The initial stage, bioleaching, is characterised by the dissolution of metals from ores and discarded materials. The second stage, bioremediation, extracts minerals from solutions with low-grade concentrations, an action that stabilises the metals within the cellular matrix. These stages facilitate the detoxification of waste and introduce a low-energy extraction method, ultimately minimising the ecological footprint, (Johnson, 2014).

A salient environmental benefit of bioleaching, when contrasted with traditional metal extraction methods, lies in its capacity to bypass conventional mining and smelting processes. This circumvention serves to mitigate the generation of excessive waste and substantially reduces the dependency on energy-intensive procedures.

The economic viability of bioleaching, in comparison to standard mining techniques, is advantageous for its application on ores with low-grade concentrations that would otherwise be uneconomical to extract using conventional means, (Saldaña et al., 2023).

In synergy with the broader field of biomining, bioleaching offers a promising avenue for mitigating the environmental impact of industrial waste and facilitating the recycling of metals for use in manufacturing, (Adetunji et al., 2023).

To address the limitations associated with the use of microorganisms for metal recovery from polluted effluents, ongoing advancements are being made through genetic engineering. An example is the engineering of iron-sulphur microbes designed to overcome challenges such as extended processing periods and limited metal selectivity, (Chen et al., 2022).

1.2.5 Biofouling

Microbial biofouling represents an unconventional approach to the degradation of traditional materials, emerging as a consequence of the undesirable accumulation of organic compounds. Microorganisms adhere to material surfaces, forming biofilms that engender challenges for historic structures. These challenges encompass the obstruction of drainage systems and the deterioration of aging sewage conduits, stemming from the effects of biofilm-induced corrosion. Another repercussion of biofouling is the emergence of pigmented metabolic by-products, which leads to unwelcome deposits and the development of staining patinas on the external façades of buildings, (Gaylarde & Morton, 1999). Efforts to eradicate deleterious biofilms from building exteriors using mechanical and chemical interventions, including biocides, may inadvertently inflict more enduring harm upon the building materials and the surrounding ecosystem than the original damage caused by biofilms.

Biofilms materialise through the extracellular secretion of polymeric substances by microorganisms, culminating in the formation of protective microenvironments that foster the colonisation of a diverse and heterogeneous array of microbial species, embedded within an augmented surface-bound film. The collective metabolic influence of the biofilm matrix on the substrate surface contributes to the dissolution of solid compounds, such as calcium carbonate. The process involves the conversion of insoluble carbonate into soluble salts and the chelation of available ions for absorption, thereby further weakening the substrate. The impact of biofilms extends to the realm of bioweathering, as the biopolymeric matrix expands and contracts with fluctuations in water availability during weather cycles. Biofilm mechanisms that confer bioprotection to building surfaces include both passive and active ion pumps, which counteract the movement of chloride, magnesium, and hydroxide ions in materials such as ordinary portland cement. This phenomenon also extends to the mitigation of dissolved atmospheric acids and other corrosive chemicals, (Ariño et al., 1995; Carter & Viles, 2003; Garcia-Vallés et al., 2003; Shuying & Xiaoning, 2018).

When biological mechanisms like biofouling can be effectively managed it will open new research avenues to develop advantageous long-term biocolonisation strategies. Promoting biocolonisation as a methodology for safeguarding bioprotective layers could foster synergistic, sustainable habitats and diverse communities. These communities could provide protective biocolonising layers, in contrast to relying on mechanical or biocidal cleansing methods in attempts to preserve architectural surfaces (Bone et al., 2022; Harilal et al., 2020). Taking an assertive stance to encourage biocolonisation might encompass strategies such as adjusting the nutrient composition of external porous materials, modifying surface humidity and temperature, and pH buffering. Further research is necessary to comprehend the repercussions of these alterations on the overall performance and durability of surfaces on historic buildings functioning as an ecological haven.

1.2.6 Physicochemical biodegradation mechanisms employed by microorganisms

Biodeterioration is primarily instigated by the active biochemical processes orchestrated by microorganisms dwelling within epilithic biofilms. These microorganisms collaborate in the metabolic breakdown of anthropogenic contaminants and the assimilation of mineral nutrients acquired from the dissolution of the surface of the substrate. The distinction between biodegradation and biodeterioration carries distinct ecological implications. Biodegradation denotes the natural decomposition of complex organic compounds into simpler constituents, orchestrated by microorganisms employing metabolic by-products and enzymes. This natural process fosters the recycling of organic matter and the reintroduction of nutrients into ecosystems. In contrast, biodeterioration encompasses analogous processes that result in the undesirable degradation of both organic and inorganic materials, resulting in detrimental consequences for traditional material integrity, aesthetic appeal, and economic value.

Fungal and bacterial substrate anchoring mechanisms are identified as major drivers of biodeterioration in traditional materials. Hyphae and biofilms attach to porous or fissured materials when specific environmental conditions and nutrient availability are met. The structures extend within the material, securing the organism and extracting nutrients from the substrate matrix, (Money, 2004). The pressure exerted by hyphae varies within a range of 0.3 N mm-2 to 2.5 N mm-2, contingent upon the fungal species and substrate composition, (Lew et al., 2004). The composition of materials such as lime, hydraulic lime, and concrete, in conjunction with localised environmental conditions, plays important roles in influencing the degree and pace of microbial colonisation and ensuing substrate surface degradation. Substrates that result in rough surfaces or lower pH levels due to the dissolution of CO_2 in rainwater render surfaces more susceptible to colonisation (Shirakawa et al., 2003).

The observation that certain stones readily support microbial colonisation led to the introduction of the concept of bioreceptivity. This term embodies the inherent susceptibility of stone or similar material to microbiome establishment, a factor contributing to both biological and non-biological deterioration processes, (Guillitte, 1985; Miller et al., 2012). Several fields, including environmental science and architectural design, accord substantial attention to material bioreceptivity in the pursuit of sustainable design practices. Urban planners capitalise on bioreceptive surfaces and incorporate supporting structures to enhance biodiversity and ecological resilience, with the aim of creating sustainable and aesthetically pleasing environments. The interplay between biological growth and geomorphological factors establishes a framework for examining the processes by which rocks and stone undergo degradation and recycling in addition to the triggers that induce bio-protective responses on cultural heritage, (Favero-Longo & Viles, 2020; Gadd & Dyer, 2017). Various environmental conditions significantly influence whether identical microbial species or the introduction of new ones alter their contributions to biodeterioration and material dissolution or foster the formation of bio-protective shields guarding the underlying substrates. Several mechanisms contribute to biofilm degradation on building surfaces, as illustrated in *Figure 5*.

Sulphur-oxidising bacteria, when confined within anaerobic environments, produce organic acids that reduce the pH and corrode the compounds present in cement or lime walls. This phenomenon has been observed in the anaerobic conditions prevalent in Victorian sewer systems. Moreover, an increase in temperature and relative humidity contributes to historical sewer system failures, (Okabe et al., 2007). Consequently, the microbial-induced deterioration of sewage systems beneath historic structures can indirectly result in structural damage. This process also leads to the emission of sulphur compounds, causing aesthetic degradation on the exterior of the structure, as well as subsidence in the surrounding areas.

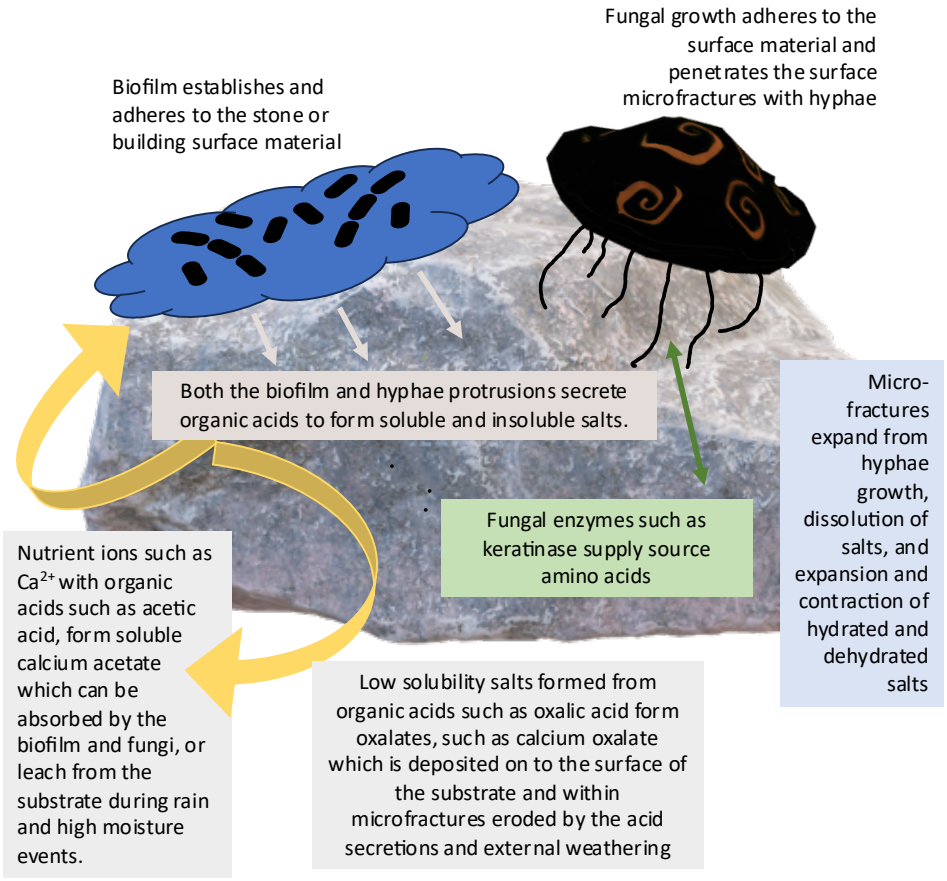


Figure 5: An illustration elucidating the shared characteristics and disparities inherent in the bacterial biofilm and fungal biodeterioration processes responsible for the degradation of stone substrates. The activity of bacterial biofilms, is similar, characterised by the infiltration of stone surfaces by hyphal structures. In conjunction with successive delamination cycles catalysed by the organic acids generated by biofilms, initiate and perpetuate cumulative damage to the stone matrix, *(De Windt & Devillers, 2010; Sterflinger, 2000)*

Bacteria and fungi both employ a wide spectrum of physiological mechanisms, *[Appendices: Table 21]* affording them a remarkable capacity for adaptability when colonising surfaces such as concrete and stone, *Figure 5*. Fungi demonstrate resilience in the face of extreme environmental conditions including sites with wide variations in pH. Fungal survival strategies encompass a multifaceted repertoire of adaptations, including the generation of spores which enables endurance of extended periods of nutrient and water scarcity, and the ability to conserve essential nutrients through the process of autolysis. Fungi demonstrate the ability to regulate nutrient distribution over significant spatial scales by harnessing the intricate connectivity of their mycelial network.

The mycelial network takes advantage of the readily available supply of calcium in lime and concrete materials which derives from the secretion of organic acids and enzymes like keratinase. These compounds lead to the dissolution of calcium carbonate compromising the stability of the substrate. In older buildings, lime mortar and render contain hard-to-degrade keratin, which comes from the inclusion of horsehair or other animal hair, an artisanal practice intended to enhance substrate curing and strength. Keratinase, a proteolytic enzyme abundant in fungi and certain bacteria, degrades tough keratin proteins, such as those found in hair, liberating essential amino acids that promote the proliferation of keratinase-producing organisms (Kuddus, 2017).

The dissolution of animal keratin in the lime aided by the action of organic acids on exposed calcium carbonate culminates in mechanical deterioration and the formation of insoluble salts within the microfractures resulting from the intrusion of fungal hyphae. The hyphae continue the mechanical pressure by expanding and contracting in response to climate-induced rain entering the widening fractures (Jiang et al., 2022).

Devising efficacious strategies to mitigate biodeterioration necessitates a comprehensive understanding of these processes and their responsiveness to gradual and extreme environmental fluctuations. Comparative analysis between the erosion induced by extreme weather conditions and alterations in biodeterioration processes is imperative for assessing the potential of bioprotective coatings against deleterious climate influences while also considering the pace of weather erosion concerning microbial biodeterioration effects.

Intrinsic determinants play a pivotal role in determining the susceptibility of different substrates to microbial colonisation and growth. These determinants encompass factors such as pore availability, surface texture, water presence, chemical composition and accessibility.

Substrates exhibiting a high degree of porosity, characterised by open pores constituting more than 14% of the volume and with a mean radius of up to 10 µm, facilitate moisture retention and a texture conducive to microbial colonisation. Stone with large pores retains water for shorter durations, making long-term colonisation unsustainable. Rough-textured building surfaces attract pollutants, dust, organic residues, and external nutrients, thereby augmenting the ease of colonisation.

The mineral composition of the stone confers advantages beyond its mineral nutritional value. Surfaces containing more than 3% $CaCO_3$ w/v, such as lime renders, mortars, sandstone, and concrete, can create a buffering environment. This buffering mechanism counters the acidic by-products produced by microbes and maintains a pH favouring bacterial growth, (Castanier, Le Métayer-Levrel, et al., 1999). In the case of historical lime render and mortar, the introduction of organic additives or pozzolans during mixing, such as straw, hair, melted and ground bone, cartilage, and blood can further augment microbial growth. Consequently, the combined effects of ongoing deterioration of the underlying stone, the presence of anthropogenic pollutants, and the incorporation or accumulation of organic materials often render historical substrates more susceptible to biological colonisation compared to newly constructed surfaces.

Controlling environmental and precursor elements can mitigate the deleterious metabolic processes of microorganisms and the persistent factors contributing to building substrate deterioration. These elements encompass natural and artificial light, as well as humidity – all of which are crucial for the growth of phototrophic microorganisms. Similarly, the availability of inorganic compounds from both natural and anthropogenic sources is vital for chemolithotrophic microorganism proliferation.

An evaluation of the site and the presence of these factors serves as the initial step in determining critical measures to minimise the facilitation of growth. Climatic conditions also play a pivotal role. For instance, in hot and sunny climates, strategies like removing adjacent trees might aid in the rapid evaporation of rainwater, whereas the same approach could exacerbate the issue in colder, damper climates (Liu et al., 2018).

Introducing novel materials within the design process presents a promising avenue for restricting the availability of moisture accessible for microbial growth. The application of chemical coatings to susceptible substrates, like lime render, has the potential to impede the infiltration of water into the

larger and deeper pores of the material. While employing silica nanoparticles and silicone polymers might achieve this objective, they could adversely impact the porosity and moisture-handling capabilities of the lime.

The central challenge resides in the identification of coatings that not only align with principles of environmental conservation and sustainability but also simultaneously mitigate water ingress while preserving the porosity and moisture-handling capability of the material. This challenge serves as the cornerstone of biodesign enhancements aimed at safeguarding traditional building materials at risk of deterioration. The topic is reviewed in Chapter Two and further elaborated upon in Chapter Three.

1.3 Influence of climate on microbial degradation of traditional building materials

The response of microbial communities on building surfaces is shaped by a variety of factors associated with the building itself. These factors encompass the intrinsic characteristics of the structure, the surrounding external environment, the effects of climate conditions, and any modifications made to the building systems and structure. Many of these adaptations by microbial communities are prompted by shifts in climate conditions.

For instance, alterations to building systems can wield a substantial influence on microbial colonisation. Traditional passive ventilation systems may be substituted with more advanced heating, ventilation, and air conditioning (HVAC) installations. Furthermore, adjustments to plumbing, drainage, sewage, and water storage systems could necessitate alterations in the original building design to adapt to local environmental factors. Additionally, the utilisation of chemical products, even those with short-term risk assessments, can potentially disrupt the natural rhythms of microbial communities on building surfaces over prolonged usage, impacting their ecological dynamics. Such modifications to microbial patterns on surfaces can lead to changes in community composition and the metabolic products they generate (National Academies of Sciences, 2017).

Building materials, susceptibility to water infiltration, erosion, wind-related damage, acid rain, anthropogenic pollutants, and alternating humidity and temperature cycles are all directly influenced by shifts in climate patterns. Indirectly, climate change also affects buildings through the actions inhabitants take to mitigate these direct impacts. Both the direct and indirect consequences stemming from climate changes exert influence on the colonisation and behaviour of microorganisms on material surfaces. The interplay of these multifaceted factors ultimately shapes the functional attributes of the building, its environmental impact, and the well-being of occupants – all of which are tightly interconnected to the microbiome residing within the structure.

Alterations in the behaviour and life-cycle patterns of insects, birds, and plants due to climate fluctuations can introduce new microbes to heritage materials. Consequently, this microbial introduction can disrupt the established growth and production of chemical metabolites in these materials. The introduction of novel bacteria to existing microbial communities often leads to horizontal gene transfer, where genes associated with survival are exchanged and retained. This process reinforces the prevalence of more resilient microbial profiles within these communities.

Water infiltration, whether due to excessive saturation or insufficient upkeep and repair, stands as the primary catalyst for microbial proliferation within structures. Severe climatic conditions such as intense downpours that exceed the carrying capacity of gutters, lead to overflow in downspouts and drainage systems. This, in turn, results in undue water saturation of neighbouring walls and foundations. Additionally, significant shifts in temperature can trigger condensation, luring the

growth of mould in areas like roof and wall spaces, where ventilation struggles to modulate the increased humidity levels.

The addition of insulation into traditionally constructed buildings can lead to the development of poorly ventilated cold areas. These cold zones, where condensation accumulates, provide a breeding ground for fungi and bacteria when proper ventilation is hindered by the newly installed insulation. This microbial growth produces volatile organic compounds, which are responsible for the distinct damp odours (Mendell et al., 2018; Mendell & Kumagai, 2017).

Heavy rainfall swiftly saturates compacted soil, causing groundwater to infiltrate the foundations of the building. Historic houses built before 1875 typically lack a physical damp-proof course, resulting in hydrostatic pressure that forces water through the wall materials. Incorporating contemporary materials into heritage properties results in water absorption, prompting the proliferation of mould spores that may have been introduced during the manufacturing process or accumulated during transportation and installation, (Andersen et al., 2017).

In consideration of the materials themselves, lime, gypsum, and cement are building products with porous structures that allow for water absorption and storage. Gypsum possesses an interior surface area of approximately 0.2 m^2g^{-1}, while cement has a much larger surface area of around 20 $m^2 g^{-1}$. The process of water absorption in these materials occurs in two stages: first, water molecules are attracted to the internal surfaces through adsorption, and then they enter the pores via capillary suction or absorption. This leads to a substantial increase in the water content of the material, potentially up to four times the original amount.

Distinguishing between the physicochemical degradation processes of lime and cement mortars is paramount due to their distinct chemical compositions. Concrete undergoes chemical degradation from the sulphates and chlorides present in salt-laden rain. This process rapidly undermines the surface integrity, forming microcracks. These microcracks, in turn, facilitate the ingress of water. Together with the salts present in the concrete, this intrusion causes expansion and contraction, resulting in the enlargement of cracks and more severe structural harm. Although calcium carbonate in lime is susceptible to chemical assault from soluble acids in rainwater, its surface is less prone to cracking. Nonetheless, it erodes at a faster rate compared to concrete surfaces.

Various materials like lime, brick, cement, wood, and gypsum respond with different equilibriums concerning relative humidity. To cope with fluctuations in moisture availability, microorganisms have developed efficient water conservation strategies. These strategies include forming biofilms or possessing water-retentive properties, as observed in lichen thalli. Such adaptations support microorganisms allowing them to thrive in varying environmental conditions, further underscoring the complexity of microbial interactions with building materials.

The interplay between relative humidity equilibriums of diverse materials and the moisture resistance mechanisms of microbial communities presents a complicated challenge in the assessment and quantification of moisture levels and microbial proliferation. Even in the evaluation of surface moisture on materials, several variables come into consideration. These factors encompass adaptations exhibited by microbial species, temperature fluctuations, nutrient availability, and the duration of moisture and arid periods. Consequently, delineating the critical moisture threshold at which microbial growth becomes inhibited remains a complex endeavour, (Dedesko & Siegel, 2015).

Fungal colonisation initiates on surfaces when the critical variables of moisture and relative humidity stabilise at approximately 80%, (Jain et al., 2009). Fungi can be categorised into three distinct categories based on their thermal preferences: psychrophiles, thriving in temperatures ranging from 0°C and 5°C; mesophiles, flourishing between 20°C and 45°C; and thermophiles, with growth occurring at or above 55°C. Notably, fungal growth demonstrates an accelerated pace when subject to consistent temperatures of 15°C or higher.

Contemporary adaptations made to accommodate heritage structures and changes in occupant behaviour due to evolving climatic patterns impact indoor humidity levels within these edifices. Instances of contemporary contributors to heightened water vapour emission include the utilisation of wood or wood-like flooring necessitating wet mopping and the installation of synthetic carpets that, unlike wool, cannot absorb and release water vapour. Furthermore, facilities such as hot tubs, whirlpool jets, and inadequately ventilated bathrooms may potentially facilitate the aerosolization of pathogenic bacteria like *Mycobacteria sp.* and *Legionella sp.*

Integrating concrete into the construction of traditional buildings has gained prominence over time. Commencing from the mid-nineteenth century, the utilisation of ordinary portland cement has witnessed an upsurge, particularly in the context of refurbishing traditional edifices originally constructed using softer lime mortar and render. Over time, it has become evident that the use of cement-based materials has adverse effects on the overall state of these structures, resulting in both functional and aesthetic deterioration, *Figure 6*.

Figure 6: Photograph illustrating the insertion of lime and concrete into stonework in a weather-exposed wall section in the Northern Isles, Scotland. Original protective limewash coatings have eroded leaving underlying lime render exposed. Weathering and trapped moisture has resulted in render loss and the formation of large cracks as a result of the introduction of concrete. The imbalance between the lime and concrete traps moisture resulting in an uneven erosion of lime and delamination of the stone surface. (Image source: author)

One consequence of introducing cement-based materials is the impact of moisture on the condition of the building materials. In cases of heavy rainfall, moisture is absorbed by the softer lime mortar joints and as the rain subsides, subsequently evaporates, leaving the stone components unaffected. Conversely, cement mortar possesses significantly lower porosity than stone, directing moisture through the stone itself, where it subsequently evaporates. This process results in the accumulation of salts at the peripheries of the mortar joints and contributes to the degradation of the stone surface, a phenomenon known as spalling.

Moreover, buildings constructed with cement mortar tend to retain dampness, remaining persistently moist. This dampness hinders the ability of the structure to achieve the superior thermal performance exhibited by buildings constructed with lime mortar and render.

In summary, the growing prevalence of concrete, particularly ordinary Portland cement, in traditional architectural construction has introduced substantial challenges. The contrast between the impacts of lime mortar and cement mortar on moisture absorption, salt deposition, and thermal performance underscores the disadvantages associated with cement-based materials in preserving the integrity and longevity of historical structures.

A comprehensive five-year-long investigation conducted at the archaeological site of Pompeii, Italy delved into the role of wind-driven rain in fostering microbial colonisation (Traversetti et al., 2018). This phenomenon became particularly conspicuous in areas characterised by compromised ventilation, cooler temperatures on northern-facing sections, and portions of structures with limited exposure to sunlight and wind. These conditions led to increased moisture accumulation in the stone, a pivotal factor for the growth of microorganisms (Abuku et al., 2009; Adamson et al., 2013).

Another key role in promoting the rapid colonisation and growth of microorganisms is the accumulation of both organic and inorganic substances on building exteriors. Beyond biotic deposits like bird faeces, deceased insects, and microbial layers on stone, anthropogenic residues deposit elements such as nitrogen, sulphur, chlorides, and petrochemical organic compounds, which serve as crucial nutrients. If left untreated, the expanding layers of microbial material on the stone create an environment conducive to the attachment and proliferation of more aggressive plants with robust root systems. This, in turn, generates a moist, nutrient-rich habitat that may encourage insect infestations. Alterations in the surface ecosystem can disrupt the balance of species dominance, allowing invasive species to take root, potentially displacing, or weakening the native microbial inhabitants. Such alterations in the species profile may reduce the resilience of the organic surface against mechanical weathering and expedite the instability of the material (Coombes et al., 2017; Jurgens & Gaylord, 2018; Pappalardo et al., 2018)

Microorganisms generate a diverse array of organic acids with varying levels of corrosive properties as shown in *[Appendices: Table 22].* The impact of these acids on structures can diverge based on factors like concentration, duration of exposure, and the specific materials in question. These acids expedite the process of corrosion, deterioration, and disintegration in various building elements, encompassing metals, concrete, masonry, and wood. Consequently, this can lead to structural impairment, reduced longevity, and aesthetic concerns within affected structures. By comprehensively addressing the fundamental activities of these microorganisms, it becomes feasible to avert or mitigate these detrimental consequences.

However, the regulation of these biodegradable organic acids and the production of secondary metabolites by microorganisms is subject to climatic influences. Within stable ecological cycles, microbially synthesised organic acids play a pivotal role in various ecological functions, including

decomposition, fermentation, and nutrient cycling. Elevated temperatures, prolonged warmer seasons, and increased humidity levels serve to stimulate metabolic activities, increasing the production of organic acids, thus expediting the decomposition process. A summary of the ramifications of shifting weather patterns on bacteria and fungi is presented in *[Appendices: Table 23]*.

Aside from increased organic acid production, climate variations impact the metabolic synthesis of secondary compounds, potentially contributing to the adaptability of microorganisms to abiotic stressors. One hypothesis posits that discrete biosynthetic gene clusters govern the production of secondary compounds and may manifest structural disparities in response to divergent climatic conditions (Singh et al., 2021). Examination of samples derived from *Umbilicaria pustulata* inhabiting both warm and cold-temperate environments uncovered three gene clusters exhibiting noteworthy differentiation. Within these clusters, one manifested exclusive occurrence in cold-temperate climes, a second exhibited compromised functionality in cold temperatures, while a third remained invariant across all cold-temperate populations. Analysis of allele frequencies along temperature gradients indicated that the observed variations in gene clusters likely arose as a consequence of positive selection and gene hitchhiking, rather than stochastic genetic drift. The precise secondary compounds generated by these gene clusters in the investigation were not explicitly identified; nevertheless, this research provides a foundational framework for prospective inquiries into the implications and prospects of climate-induced alterations in natural product synthesis.

Upcoming advancements in sequencing technologies hold the promise of expediting and enhancing the resource-efficient examination of microbial biochemical processes implicated in the degradation of building materials. This endeavour necessitates a heightened focus on quantifiable parameters, including the expression of organic and inorganic acids by fungi and bacteria that establish colonies in diverse environmental conditions and consideration of factors such as acid concentration, degradation kinetics, and the responses of the materials under review. These parameters can be systematically juxtaposed with evolving cellular 'omic' profiles. Of interest is the influence of climate-related factors on the upregulation of genes, leading to the augmented production of quantifiable acid levels. This phenomenon, when observed concurrently with identified gene clusters, can provide valuable insights into the development of potential bioprotective strategies aimed at mitigating the climate-induced deterioration of conventional construction materials

As climate patterns evolve and change, variations in meteorological conditions furnish valuable insights into the interplay between environmental variables and the biological processes affecting traditional materials. It can be argued that the interpretation and contrasting of the ramifications of biodeterioration requires a comprehensive consideration of factors which extend beyond climatic influences. Factors such as the nature of the underlying substrate material can play a role and merit examination. This is exemplified by the divergent physicochemical characteristics inherent to materials such as sandstone, lime, granite, and concrete, which as discussed, confer distinctive inorganic nutrient compositions and water retention capacities, exerting a discernible impact on colonisation dynamics.

Furthermore, cyclic fluctuations, such as alternating exposure to light and darkness, as well as temperature fluctuations, impose abiotic stressors that exert effects on genomic expression and biochemical outcomes. Consequently, further analysis of the multifaceted interplay between weather patterns, substrate materials, and abiotic stressors is essential to develop a comprehensive profile of the processes associated with the biodeterioration of heritage structures.

1.4 Potential threats to occupant health from climate-enhanced microbial growth in heritage buildings

The association between inhabiting moisture-damaged and damp edifices and a decline in health, notably manifesting as respiratory afflictions and allergic reactions to microbial toxins and fungal spores, has been firmly established (Mendell et al., 2011). A comprehensive understanding of the mechanisms underlying the growth and alterations in microbial attributes within deteriorating, moisture-laden buildings and their impact on occupants' health remains notably constrained. This knowledge, arguably, assumes a central role in guiding strategies to safeguard occupant health while preserving traditional building construction.

Several studies have catalogued a diverse array of bacterial and fungal species inhabiting interior building surfaces, including *Candida albicans, E. coli*, and *Streptococcus sp.*, alongside *Pseudomonas aeruginosa, Klebsiella pneumonia*, and *Staphylococcus sp.*, (Haleem et al., 2013). Microbial pathogens such as *Staphylococcus, Propionibacteria, Corynebacteria,* and enteric bacteria, in conjunction with several fungal spores, are also readily transported by human activity from the external environment into indoor living spaces, (Täubel et al., 2009).

Within the interior of buildings, microbial agents responsible for eliciting physiological responses and health deterioration primarily emanate from chemical and biological sources. Noted issues result from the generation of bacterial and fungal spores and the emission of chemical compounds. Such consequences arise from alterations in the microbial environment induced by recurrent wet-dry cycles, fostering spore production and the release of metabolites and mycotoxins from microbial cells.

Moisture infiltration, stemming from the degradation of renders, mortars, compromised brickwork, and damaged stonework, as well as ingress through deteriorated or improperly fitting structural elements, serves as the chief catalyst for the proliferation of microorganisms within and upon buildings, (Andersen et al., 2011; Hyvarinen et al., 2002). A promising direction for further exploration lies in identifying the key microbial constituents intertwined with moisture ingress in historical structures. Such identification will reveal a more comprehensive understanding of the health issues arising from occupant exposure while simultaneously advancing knowledge of the underlying mechanisms, microbial agents, and their interactions with structural damage resulting from moisture infiltration.

This correlation between elevated indoor moisture levels and the emergence of microbial colonies, contributing to heightened health risks for occupants, is not fully understood. To progress this, the establishment of a comprehensive framework that interconnects moisture assessment tools, a classification system for assessing the extent and nature of moisture penetration, the collection of microbial taxonomic data, and genetic profiling via DNA sequencing during fluctuations in moisture levels undeniably holds the potential to enhance understanding of the relationship binding moisture, microbial proliferation, and virulence.

Conducting DNA sequencing commonly employs quantitative PCR analysis of isolated DNA and sequencing of bacterial 16S rRNA. In one study, PCR analysis identified an escalation in *Staphylococcus* infection within the human skin microbiota, concomitant with a decline in the fungal presence of *Phoma, Botrytis,* and *Monographella* during the restoration of moisture damage. The findings derived from this examination of the impacts of moisture damage on microbiota indicate discernible shifts in the abundance of bacterial taxa, albeit not within the overall community structure, and yielding inconclusive results for fungi. While the correlation between an increase in dampness in architectural structures and the biodegradation process was limited in scope, this

investigation underscored the utility of contrasting pre- and post-moisture-damaged buildings as a methodological approach for elucidating shifts in microbial populations resultant from heightened moisture conditions, (Jayaprakash et al., 2017).

Numerous studies have provided insightful, albeit inconclusive, evaluations of the influence of compromised residential dwellings on bacterial community configurations and the potential implications for the well-being of occupants. Findings derived from the examination of the consequences of moisture-induced damage on microbiota dynamics have revealed shifts in the abundance of bacterial taxa within a relatively stable overall community structure. The same study found variable outcomes for fungal communities. Although the association between elevated moisture levels in buildings and the biodeterioration process is widely acknowledged, the study underscores the significance of comparing pre- and post-water damage states of buildings as a method to elucidate alterations in microbial populations resulting from increased moisture presence.

This investigation into characterising the diverse spectrum of fungal species inhabiting construction materials within environments afflicted by mould infestations has undergone scrutiny. The primary objective of the investigation was to elucidate potential associations between the identified fungal taxa and material degradation. The method employed for this characterisation involved the application of V8 contact plate sampling, which necessitates the utilisation of specialized Petri dishes containing appropriate growth agar media. The culture media for fungal sampling employed the use of malt agar. In this methodology, the agar surface extends beyond the rim of the Petri dish, enabling the transfer of microorganisms from the sampled surface onto the agar medium. The inoculated agar plates were incubated at temperatures within the range of 30 to 35 degrees Celsius, fostering the proliferation of microorganisms, enabling the counting of Colony-Forming Units (CFU) per sample, facilitating taxonomic identification and robust statistical analysis. The research findings identified *Penicillium chrysogenum* and *Aspergillus versicolor* as the predominant fungal species, frequently co-occurring with *Stachybotrys sp.* in the surveyed structures afflicted by water-related damage (Andersen et al., 2011). The presence of *Stachybotrys chartarum* in indoor environments has been correlated with various health concerns in humans, including the development of severe pulmonary hemosiderosis in infants, (Haugland et al., 1999, 2004).

Epidemiological investigations and meta-analyses such as these have substantiated a link between indoor damp conditions with the presence of mould and an elevated risk of developing asthma. This connection extends to encompass a spectrum of respiratory diseases, including dyspnoea, wheezing, cough, respiratory infections, bronchitis, allergic rhinitis, eczema, and upper respiratory tract maladies. Furthermore, these associations bear relevance to individuals of both allergic and non-allergic dispositions.

Indoor dampness and the presence of mould allergens serve as a catalyst for the exacerbation of asthma, with a pronounced emphasis on its impact on paediatric populations. A positive correlation is evident between the visible presence of dampness or mould and the occurrence of various allergic and respiratory afflictions. It must also be noted that the associations between specific microbial species identified in dust samples and the aforementioned health symptoms are ambiguous, exhibiting both affirmative and negative correlations.

The overall evidence supports proactive measures aimed at preventing and mitigating indoor dampness and mould infestation, which should improve the attendant health hazards. To date, quantitative data establishing conclusive evidence between dampness, microbial species, and the ensuing health symptoms remains incomplete, (Mendell et al., 2011).

The relationship between microbial exposure and various respiratory symptoms observed remains poorly understood. The aetiology of sick building syndrome is a subject of uncertainty, commonly postulated as a cumulative effect of toxins, allergens, and unidentified physiologically active agents. A study conducted to investigate respiratory symptoms among individuals working in both moisture-damaged and control buildings examined the release of inflammatory mediators, such as interleukin (IL)-1, IL-4, IL-6, tumour necrosis factor-α (TNF-α), and cell counts in nasal lavage fluid and induced sputum samples. The findings unveiled a substantial upsurge in IL-1, TNF-α, and IL-6 levels in nasal lavage fluid, as well as heightened IL-6 levels in induced sputum samples, particularly among occupants of moisture-damaged buildings. Simultaneously, individuals exposed to the moisture-damaged environment reported various symptoms, including sore throat, phlegm production, eye irritation, rhinitis, nasal obstruction, and cough, which were in concordance with the outcomes observed for the inflammatory agents. These results suggest a plausible connection between microbial exposure in moisture-damaged buildings and manifested symptoms, as well as changes in pro-inflammatory agents detected in both the upper and lower airways, (Purokovi et al., 2001).

Historical residences, particularly those subjected to renovation, may incorporate crawl spaces located beneath the ground level. These areas, situated beneath the lowest floor, serve the purpose of elevating the joists above the ground or soil, typically providing only limited vertical space, sufficient for crawling but not for standing. While relatively less prevalent in homes in the United Kingdom, crawl spaces are frequently encountered in the southern regions of the United States.

Crawl spaces are most beneficial in arid climates, as they tend to instigate drainage problems when located in humid environments. In such settings, they can foster the growth of mould, resulting in the development of damp and musty odours within the ground-floor living spaces. These spaces are usually equipped with passive ventilation systems through built-in grids within the foundations of the building. If, over time, these ventilation openings become obstructed or, during the renovation of historical structures, they are covered, it will hinder the crucial circulation of air. Elevated levels of rainfall and potential flooding can encourage the accumulation of moist air and water beneath the structure, ultimately leading to condensation in the cooler regions beneath the floor. A persistently damp environment becomes conducive to mould growth, which ultimately leads to the prolonged deterioration of wooden floor joists and supporting structures.

The proliferation of mould within these crawl spaces can extend across substantial areas, presenting health risks to occupants in multiple living spaces. Investigations conducted in regions with harsh climatic conditions, such as Sweden, where homes predominantly rest on two types of foundations—concrete slabs and crawl spaces—have revealed that crawl spaces can experience relative humidity levels ranging from 80% to 100% for consecutive months. In these studies, *Penicillium corylophilum* emerged as the predominant microbial growth on building materials.

Another study, which examined various isolates of *P. corylophilum* across Canada, identified several secondary metabolites in filtrate samples which may act as virulent agents toward occupant immune systems. These included sesquiterpene phomenone, meroterpenoids citreohybridonol, andrastin A, koninginin A, E and G, as well as three new alpha pyrones and four new isochromans. Additionally, various mycotoxins were identified in this study, (Bok, Hallenberg and Åberg, 2009).

Infections resulting from *Penicillium* sp. are commonly grouped under "penicilliosis." This category encompasses a variety of conditions, including keratitis, endophthalmitis, otomycosis, pneumonia, endocarditis, and urinary tract infections, (Egbuta et al., 2017).

Extensive research has been conducted on *Staphylococcus aureus* bacteria in various settings, such as hospitals, care homes, animal breeding centres, wastewater treatment plants, schools, and

residential buildings (Agostino et al., 2017; Denis, 2017). The frequency of epidemiological risk reports and reports of antibiotic resistance in *S. aureus* has shown an upward trend. It is estimated that 11-53% of bacteraemia-related complications in *S. aureus* treatment are attributed to the source, (Keynan & Rubinstein, 2013). *S. aureus* deploys diverse infection mechanisms, characterised by its high invasiveness into the human body, resulting in a spectrum of symptoms driven by its toxic attributes. This can culminate in sepsis and potentially fatal outcomes, (Agostino et al., 2017).

Of equal concern is the propensity of *S. aureus* to acquire antibiotic resistance, frequently through horizontal gene transfer from other resistant bacterial strains. Additionally, the airborne and direct transmission of *S. aureus* via hands or oral routes complicates its control. The pathogenic strain of *S. aureus* can endure on dry surfaces, regaining its contagious potential when exposed to moisture. The risk of exposure is particularly noteworthy in historical residential and public buildings, as transmission can occur through infected domestic animals originating from wastewater sites, animal husbandry locations, and carriers in the community, (Boopathy, 2017; Boyce, 2007; Conceição et al., 2013; DeLeo et al., 2010; Wardyn et al., 2015).

The heightened occurrence of extreme weather events amplifies the risk of flooding and the dissemination of inadequately treated wastewater from treatment facilities. This results in an increased likelihood of domestic animals coming into contact with contaminated water, potentially leading to the contamination of natural water reservoirs and soil. The wind-borne dissemination of bacterial spores, including *S. aureus*, is a further concern. Consequently, the exploration of strategies to protect stone heritage buildings from bacterial assaults has gained significance. This includes the application of antimicrobial oxide nanoparticles like Ag, ZnO, and TiO_2 for surface protection and preservation, with the potential to reduce infection risks for occupants of such buildings, (Fruth et al., 2021).

Staphylococcus has been detected in air samples from a range of public buildings in China (Li et al., 2015), residential flats in South Korea (Moon et al., 2014), as well as various public and private locations in Italy and Poland (Messi et al., 2015; Pastuszka et al., 2000). In the Italian study, two *S. aureus* isolates were found to be resistant to antimicrobials erythromycin and tetracycline, while in Poland, *S. aureus* strains resistant to erythromycin and clindamycin, another antimicrobial, were isolated (Lenart-Boroń et al., 2017).

The potential rise in antimicrobial-resistant *S. aureus* strains on historical property surfaces due to climate change-induced increases in temperature and humidity is raising concerns about occupant health. Despite efforts with antibiotics and similar-acting drugs targeting genomic or proteomic regulation, they have fallen short in effectively controlling *S. aureus* over the long term. While multidrug treatments may offer temporary success, the unchecked use of antibacterial agents renders this strategy insufficient to prevent eventual drug resistance (Kozajda et al., 2019).

Exploring natural products within shared heritage ecosystems that serve as defensive agents against competitive organisms like *S. aureus*, and mould colonisation presents a promising avenue for bio-toxic tools that can be employed in conservation practices. These natural products also referred to as secondary metabolites, provide environmentally sustainable solutions that effectively inhibit the growth and propagation of biodegrading microorganisms utilising a range of inhibitory mechanisms. Furthermore, these compounds exhibit potential as innovative treatments to counteract the spread of drug-resistant pathogenic microorganisms such as *S. aureus*.

Several interventions to reduce the risk of microbial growth in heritage buildings are proposed. Periodic ventilation of the building eliminates moisture and prevents the growth of mould and bacteria. This can be achieved economically by preserving passive ventilation systems inherent in

the original house structure such as ventilation grids, open fireplaces, and air vents on chimney pots. Avoiding attempts to impede air movement between walls and floors is essential to maintaining an effective ventilation system. Regular Inspection of the building for signs of moisture damage is critical. Swift repairs of any issues threatening the integrity of the building to the weather are essential to prevent microbial colonisation. Buildings exposed to high-impact and prolonged periods of wind-driven rain are at an elevated level of risk of supersaturated mortar and wall renders. Ensuring unobstructed moisture movement between building elements including walls, roofs and floors helps reduce moisture build-up, microbial colonisation, and long-term structural damage.

The consideration of energy-related factors influencing the shift in manufacturing materials for constructing structural components is important. Climate change has a notable impact on the degradation caused by microbial colonisation and should be factored into the decisions to make changes to material components. Materials derived from wood and cellulose, despite their relatively lower energy-intensive production processes, become vulnerable to microbial expansion when exposed to moisture. Within a few months following construction, damp structural elements become conducive to the proliferation of various moulds, such as *Aspergillus, Penicillium*, and *Stachybotrys.* These moulds can manifest on both external and internal material surfaces, as well as within the enclosed gaps of walls and ceilings, (Godish, 2005).

1.5 Impact of biocides on mitigating bacterial and fungal colonisation

Conservationists undertake multifaceted approaches to mitigate the colonisation of harmful microorganisms, safeguarding buildings, and cultural artifacts. In addition to formulating chemicals with biocidal properties, conservation specialists explore both direct and indirect strategies in this endeavour.

Indirect methods encompass interventions aimed at altering environmental conditions to create an inhospitable habitat for microbial growth. The complexity of this approach arises from the challenges associated with regulating anthropogenic pollutants, which contribute to the formation of nutrient-rich layers. Furthermore, the varying factors of humidity and temperature, particularly for structures exposed to ever-changing weather conditions, make environmental manipulation challenging, (Charola et al., 2011).

Conversely, direct methods offer a more straightforward approach involving mechanical, inorganic, organic, and biochemical techniques to eliminate undesirable microbial colonies from building surfaces. Mechanical techniques, while effective, may inadvertently damage the underlying structure or expose concealed layers to accelerated erosion. Similarly, the use of inorganic and organic chemicals can have adverse environmental consequences, generating toxic waste, including oxidising compounds such as hydrogen peroxide and chlorine. These substances not only harm substrates and nearby vegetation but also release ammonia, which poses environmental toxicity risks. Early synthetic biocides, while effective in killing undesired microorganisms, exhibited indiscriminate toxicity, leading to the demise of beneficial microorganisms and higher plant life in the treated areas, (Fidanza & Caneva, 2019).

A more ecologically responsible and economically viable approach involves the utilisation of allelopathic methods. This approach involves the development of natural biocides derived from plant products and microorganisms. The potential for advancing natural biocidal agents in the realm of built heritage, a relatively overlooked domain, is substantial, particularly as local authorities grapple with the need to ensure the habitability of existing housing and the reuse of commercial and industrial structures amidst limited land and building resources, (Jeong et al., 2018).

The selection of a natural biocide product hinges upon avoiding ecotoxic effects on local flora and fauna. A comprehensive understanding of the microbiocenosis of the specific habitat under examination is essential to prevent unforeseen toxicity to other species. The development of databases containing information about natural biocides obtained from plant extracts and microbial secondary metabolites holds promise for designing and producing biofungicides and biopesticides without detrimental effects on the surrounding environment, (Fidanza & Caneva, 2019; Isola et al., 2022; Mateus et al., 2023). Early research in this field has yielded encouraging outcomes, such as the antifungal properties of biosurfactant lipopeptides produced by bacterial strains like *Bacillus subtilis* and *Bacillus amyloliquefaciens*, effectively inhibiting microbial growth on mural paintings, (Marin et al., 2016; Silva et al., 2015).

Notwithstanding, modifications to conventional materials necessitate extensive research, particularly to understand the long-term effects of interventions. The incorporation of polymeric materials, additives, and agents to enhance performance or resistance to erosion may introduce compounds susceptible to fungal and bacterial deterioration due to favourable alterations in physical and chemical conditions for microbial growth. In-situ testing, involving real-world simulations, plays a pivotal role in evaluating environmental impact and material effectiveness.

Given the evolving environmental dynamics resulting from climate change, efforts to mitigate biodeterioration of heritage structures must emphasise sustainable and economically viable strategies. Environmental fluctuations are also altering the delicate balance between biodeterioration and bioprotection on traditional materials. When the erosive effects of the environment on external structures surpass the gradual decay from microbial colonisation, the possibility of leveraging colonisation as a protective or biocidal layer becomes a worthwhile consideration. Even minor fluctuations in temperature, precipitation, and sunlight can determine whether lichen or microbial coverage results in surface damage or serve as a shield against wind-driven rain and solar radiation, slowing the erosion of the upper substrate layer.

Furthermore, beyond the synthesis of chemical agents with inherent toxicity towards biological organisms, conservationists must embark on a comprehensive exploration of both indirect and direct approaches. These endeavours should aim at mitigating bacterial and fungal colonisation, consequently diminishing the adverse impact on architectural structures and heritage artefacts. Such measures play a pivotal role in safeguarding the preservation of our cultural heritage

1.6 Leveraging the changing balance between biodegradation and bioprotection processes resulting from the effects of climate change on cultural heritage

Promoting microbial colonisation on building materials to induce bioprotection constitutes an intriguing concept situated within the domain of bio-inspired or biophilic design. Traditionally, efforts have predominantly focused on averting the growth of microbes on building surfaces to preclude structural decay. In light of the mounting conservation challenges presented by climate change and extreme meteorological events, some heritage experts are intrigued by the potential benefits of deliberately encouraging microbial colonisation under controlled conditions. A variety of considerations and prospective methodologies are available to stimulate the proliferation of microbial life on building exteriors.

To enable colonisation, the selection of materials must be conducive to microbial proliferation. Surfaces characterised by porosity and texture facilitate the adhesion of lichens, fungi, and bacterial biofilms, providing a substrate for biofilm adherence and a conduit for hyphae to establish

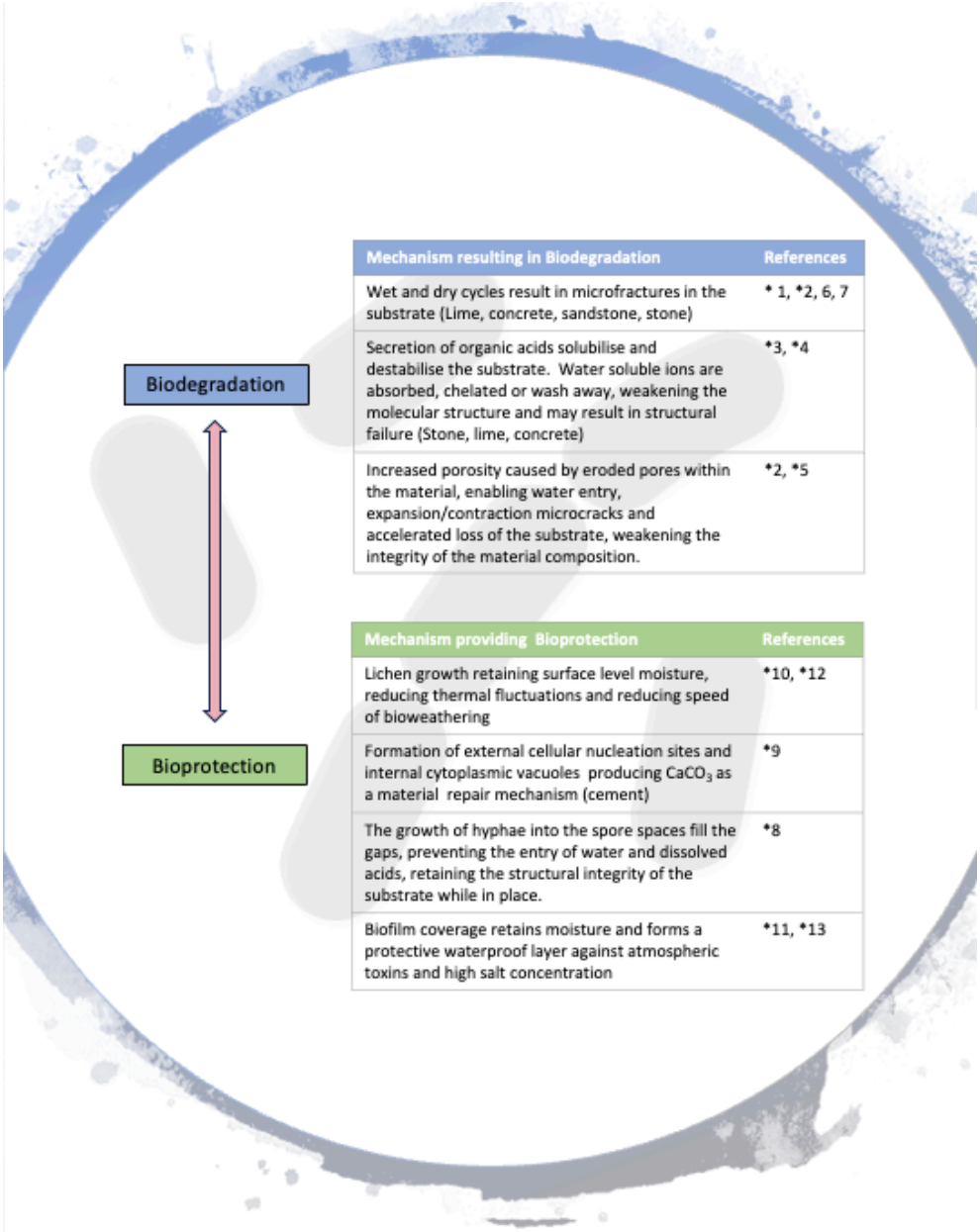
themselves on and within the material. In cases where contemporary materials exhibit reduced porosity or texture, the application of a specially designed and targeted microbial-friendly nutrient coating may prove advantageous.

Devising a strategy to foster uniform microbial surfaces with bioprotective attributes presents a challenge. Crustose lichens, while robust and resistant to detachment, present complications due to their sluggish growth rate, which limits their suitability for establishing protective colonisation patterns on stone surfaces. Therefore, evaluating the bioprotective efficacy of microbial colonies should ideally transpire in their natural environment, enabling the examination of external variables such as temperature and humidity, as well as the intricate ecosystem interactions absent in laboratory conditions.

The implementation of regular in-situ quantitative methodologies, involving measurements encompassing substrate erosion, chemical runoff, compression strength, and material degradation, introduces additional complexities to long-term monitoring. Nevertheless, these assessments are indispensable for establishing the microbial equilibrium that concurrently safeguards against deterioration and induces biodeterioration under fluctuating environmental conditions.

Conventional surfaces of historical structures are unlikely to naturally foster the desired bioprotective microorganisms. The introduction of selected microbial communities to heritage building surfaces, whether during construction, post-construction, or as part of a restoration process, is optimally achieved, through a counter-intuitive process, that is, within a supportive nutrient solution, thereby promoting the development of the intended microbial profile.

The determination of whether a specific species or microbial community exerts a net bioprotective or biodeteriorating influence in the context of extreme weather conditions hinges on the interplay of numerous interconnected and unpredictable factors, *Figure 7*. A study from Falsarna, Crete demonstrated a reduction in microbial deterioration of limestone when algae began to proliferate on the same substrate, (Naylor & Viles, 2002). Similarly, research comparing rates of weathering on carbonate rocks in the Algarve indicates that the brown canopy algae *Fucus* sp. mitigated temperature and humidity fluctuations, thereby reducing the extent of weather-induced degradation, (Baxter et al., 2022; Moura et al., 2012). Comparable reductions attributable to the presence of secondary microorganisms were noted in the context of saltwater weathering, (Goudie & Viles, 2010), as well as in mitigating erosion caused by direct solar radiation and thermal stress, (Gowell et al., 2015).



Mechanism resulting in Biodegradation	References
Wet and dry cycles result in microfractures in the substrate (Lime, concrete, sandstone, stone)	* 1, *2, 6, 7
Secretion of organic acids solubilise and destabilise the substrate. Water soluble ions are absorbed, chelated or wash away, weakening the molecular structure and may result in structural failure (Stone, lime, concrete)	*3, *4
Increased porosity caused by eroded pores within the material, enabling water entry, expansion/contraction microcracks and accelerated loss of the substrate, weakening the integrity of the material composition.	*2, *5

Mechanism providing Bioprotection	References
Lichen growth retaining surface level moisture, reducing thermal fluctuations and reducing speed of bioweathering	*10, *12
Formation of external cellular nucleation sites and internal cytoplasmic vacuoles producing $CaCO_3$ as a material repair mechanism (cement)	*9
The growth of hyphae into the spore spaces fill the gaps, preventing the entry of water and dissolved acids, retaining the structural integrity of the substrate while in place.	*8
Biofilm coverage retains moisture and forms a protective waterproof layer against atmospheric toxins and high salt concentration	*11, *13

*Figure 7: The evaluation of microbial colonisation as a factor in the biodegradation or bioprotection of building materials is impacted by the influence of climate change on the durability of these materials. Assessing the potential harm caused by darkened zones resulting from biofilms or calcium oxalate deposits poses a challenge to conventional conservation practices, as it questions whether these phenomena are detrimental to the heritage experience or represent a natural adaptation in the form of a protective patina against climate change. Source: [*1] - (Gómez-Pujol et al., 2007), [*2] - (Coombes et al., 2011), [*3] - (Flemming, 1993), [*4] - (Scheerer et al., 2009), *5 - (Cwalina, 2008), [*6] - (Morrison et al., 2009), [*7] - (Jayakumar & Saravanane, 2009), [*8] - (Garcia-Vallés et al., 2003), [*9] - (Gadd & Dyer, 2017), [*10] - (Carter & Viles, 2003), [*11] - (Shuying & Xiaoning, 2018), [*12] - (Kennedy et al., 2019), [*13] - (Trenhaile, 2017)*

Establishing a specific microbial colony necessitates the meticulous provision of essential nutrients. The formulation of a nutrient base is a critical step in minimizing the potential for invasive damage or the extraction of material surface nutrients (bioleaching) by microorganism colonisation.

The management of moisture levels presents a more formidable challenge, particularly in light of increasingly frequent cycles of drought and heavy rainfall resulting from shifting climate patterns. Adequate moisture is imperative for ensuring the adhesion and long-term viability of microbial colonies. Nevertheless, prolonged periods of excess moisture can inadvertently promote the growth of more well-adapted moulds and other undesirable microorganisms during the surface inoculation phase. Thus, meticulous planning and investment in protective measures are imperative to maintain appropriate moisture levels on large structures until the microbial community becomes firmly established.

Several other environmental factors are paramount in creating favourable conditions for microbial growth. These factors include maintaining temperature ranges, humidity levels, and light exposure that align with the specific requirements of the microbial community. Consequently, an understanding of the environmental preferences of these microorganisms is indispensable, and adjustments to the environment or the timing of interventions should be made accordingly.

While the notion of promoting microbial colonisation on building materials is relatively recent, the potential long-term advantages offer the promise of bolstering the resilience of traditional materials against the mounting impacts of extreme weather events. However, a prudent approach to this endeavour over the long term is indispensable, as it is essential to address potential challenges associated with controlled microbial colonisation. This is crucial to ensure that it does not inadvertently facilitate the proliferation of detrimental microorganisms, which could compromise the structural integrity of the building and pose health risks to occupants

1.7 Chapter Summary

Traditionally, conservation science has viewed microorganisms as adversaries, attributing cultural heritage deterioration to their intricate metabolic processes and synergistic action with anthropogenic pollutants. This perspective has spurred diverse interventions, spanning controlled environments to invasive treatments like mechanical removal, biocides, and UV irradiation. It is important to underscore that any antimicrobial strategy must prioritise environmental sustainability, (Pinna, 2021).

This chapter proposes a paradigm shift, advocating for microorganisms as allies in sustainable cultural heritage preservation. Microbes hold potential to protect architectural structures from harsh weather while not significantly altering the physicochemical properties of traditional building materials, as Pinna (2017) points out. Unveiling the complex interactions between diverse microbial communities and these materials remains a largely unexplored frontier.

With growing concerns regarding the environmental footprint of modern building materials, low-energy alternatives are gaining traction. Fabrication of steel, aluminium, and concrete involves significant fossil fuel consumption and carbon emissions, contrasting with the energy-efficient production of cellulose and wood fibre-based materials, (Pinna, 2017).

Nevertheless, cellulose-based products, despite their sustainability advantage, are vulnerable to biodeterioration in damp conditions. Responding to the first research question, this chapter has delved into the mechanisms of microbial degradation and how climate change influences them. Deterioration can begin within a month, often featuring prolific mould growth of, for example, *Aspergillus* and *Penicillium* species. Unchecked mould and bacteria thrive in damp, warm environments, posing serious health risks to building occupants. Integrating cellulose-based materials as substitutes necessitates concurrent solutions to biodeterioration, necessitating a shift in

conservation mindsets to embrace materials not originally part of the historical structure. The potential biodeterioration of these organic materials can be mitigated using natural antimicrobial secondary metabolites as biocides against unwanted microorganisms.

Microbial proliferation on other organic elements like wallpaper further challenges historical interior preservation. Dutch researchers have developed a material susceptibility classification system to inform biodeterioration mitigation strategies. The paramount objective is to fortify materials against biodeterioration without resorting to synthetic fungicides, which offer only reprieve and limited efficacy.

The investigative approach of this research starts with encapsulating heterotrophic and autotrophic non-pathogenic cyanobacteria within biopolymeric matrices. This innovative technique promises extended longevity for sacrificial limewash coatings by increasing calcium carbonate density and enabling self-repair mechanisms. Furthermore, extracts derived from secondary metabolites of lichens, adapted to similar environmental extremes, may exhibit antimicrobial properties against climate-induced microorganisms responsible for the biodeterioration of traditional building materials. Chapter Two explores the impact of these biodeterioration and bioconservation effects.

Subsequent chapters (Chapters Three and Four) present the practical development of biologically-inspired alternatives to combat climate-induced material degradation. These offer a plethora of benefits: efficacy, minimal ecological footprint, enhanced durability, environmental sustainability, and reduced risks for personnel involved in historic building conservation and restoration. The ramifications of this inquiry extend beyond cultural heritage, impacting cementitious materials and traditional building components like wood and stone.

CHAPTER TWO: Designing bioenhancements to at-risk traditional building materials

Chapter Abstract

Traditional building materials are woven into the fabric of cultural heritage and architectural history. The changes in meteorological conditions, an interplay of climate change, increased precipitation, and microbial activity expose material vulnerabilities, accelerating decay and presenting an existential threat to heritage structures. This chapter delves into the pioneering concept of designing bioenhancements, proposing a novel approach to safeguard at-risk traditional building materials against environmental and microbial degradation while preserving their historical integrity.

Advances in self-healing cementitious materials harnessing biotechnologies present three intriguing avenues: incorporating immobilised biomineralising bacteria, direct engineering materials with biopolymers, and prolonging the bacterial self-healing lifespans. While biopolymers are favoured for cell immobilisation, substantial optimisation is necessary to balance material properties, environmental impact, and long-term efficacy. This discussion analyses the principles, methods, and potential advantages of bioenhancements for traditional materials, highlighting opportunities for environmental sustainability and economic benefits for the custodians of historic buildings.

Nevertheless, crucial research gaps remain in optimising these promising avenues and translating them into practical applications. This chapter concludes by highlighting the need for further investigations in areas such as enhanced encapsulation techniques for bacterial longevity and activity, biopolymer modification to optimise mechanical properties, the scalability and cost-effectiveness of bio-based production methods and long-term performance evaluation.

2.1 Introduction

Extreme climatic events give rise to global phenomena, including droughts, floods, wind-induced precipitation, alterations in pH levels, and biological assaults, as summarized in *Table 1*. Environmental challenges pose a direct threat to the enduring legacy of heritage structures. The ever-evolving landscape of climate change compounds these issues, necessitating a re-evaluation of established conservation policies for designated heritage structures. This re-evaluation introduces potential clashes with ambitious zero-carbon initiatives and retrofits involving insulation, (Brimblecombe et al., 2011).

This chapter further develops on the literature review presented in the preceding chapter. It narrows its focus to delve into the intricacies of traditional materials, particularly those based on lime, which faces heightened susceptibility to biodeterioration. Within the purview of this chapter, attention shifts towards the innovative domain of microbial biosynthesis, biopolymer printing and antimicrobial secondary metabolites. These approaches are introduced and explored as novel strategies to fortify and protract the life expectancy of traditional building materials that are susceptible to growing environmental threats.

Table 1: Environmental factors influenced by climate change and the impact on lime-based traditional building materials

Environmental factors influenced by climate change	**Challenge to lime-based traditional building materials**	**References**
Temperature	In 2021, the maximum temperature reached was 32.2°C compared to the average hottest day during the period 1961-1990 of 31.4°C. In 2022, the maximum temperature was 40.3°C recorded at Coningsby, Lincolnshire, and a new provisional temperature for Scotland was set at 38.7°C. Compared to extensive studies on the impact of higher temperatures on cement-based products, there is limited research on the impact on heritage materials such as lime, other than the effect of fire. Temperatures below 5°C and higher than 18°C restrict natural carbonation taking place.	(Doleželová et al., 2018; Pachta et al., 2018; RMetS, 2022)
Sea level	From the early 1900s sea levels have risen around the United Kingdom by 16.5 cm and continues to rise. In addition to coastal erosion, the frequent storm surges result in the generation of wind-driven salt-saturated rain. The Roman architect Vitruvius in his work De Architectura developed a salt-resistant lime mix to withstand the eroding effect of sea water by adding finely ground natural mineral marble powder in a ratio of one part lime to three parts pozzolan, in this instance volcanic ash. The growth of salt crystals within the lime reduces the tensile strength of the render. Pozzolans may effectively lower salt erosion but similarly reduce the tensile strength of the lime.	(Morgan, 1960)
Changes in pH	The increased absorption of CO_2 acidifies the ocean and ocean spray while acid rain forms due to the sulphur dioxide and nitrogen oxide released from power stations and volcanoes. An increase in volcanic activity results from climate change as ice-loss reduces the pressure on the land mantle enabling volcanic venting.	(Tuffen, 2007)
Biological attack	An increase and extension of wet/dry weather cycles encourage fractures in the lime allowing moisture penetration. Microorganisms and the biofilms produced, retain moisture resulting in an enlargement of microfractures and cracks permitting access to further erosion and microbial colonisation.	(Viles, 2002)

Research into bio-augmented self-restoration, the utilisation of ecologically procured bio-polymers, and the development of antimicrobial interventions present a compelling avenue for enhanced conservation approaches for the revitalisation of historic buildings. These initiatives hold potential to ameliorate structural integrity, curtail maintenance requirements and costs, support zero carbon and lower energy initiatives and protract the longevity of cultural heritage.

2.2 Biopolymer encapsulated enhancement of limewash surfaces

The degradation of limewash sacrificial layers is exacerbated by wind-driven rain, alternating between intense dry periods and increased moisture levels and temperature. In coastal areas exposed to the elements, the interval for reapplying the sacrificial limewash layer has diminished from five years to three years or less. One potential solution involves substituting the limewash layer with a more durable synthetic coating. Synthetic sealants degrade at a slower rate by sealing the pores of the underlying substrate, thus preventing water and moisture ingress. However, diminishing the porosity of lime leads to significant moisture-related issues, including vapour accumulation, dampness, and condensation problems.

In warm and humid conditions, organic materials and anthropogenic pollutants adhere to the sealants, providing a nutrient base for microorganisms that deteriorate the sealant surface. Therefore, enhancing the durability of the limewash layer necessitates maintaining its chemical and biological protective function. An effective approach to improve the limewash durability should focus on enhancing the overall density of the lime without excessively reducing its porosity. A key aspect discussed in Chapter Three is the incorporation of calcium ion nucleating microorganisms capable of generating biotic extracellular $CaCO_3$ into the limewash. This forms the basis for the experimental design aimed at developing microbial encapsulation and biomineralisation for inclusion during the limewash application without impairing the natural limewash functionality.

The sequestration of carbon through encapsulated bacteria integrated into the limewash requires a comprehensive methodology that encompasses biopolymer synthesis, encapsulation technologies, biomineralisation, and the creation of microbially sustainable environments. The development of techniques for harvesting biopolymers derived from natural sources including biofilms, provides an innovative vehicle for bacterial encapsulation and nutrient delivery, presenting an opportunity to create biocompatible environments conducive to the precipitation of $CaCO_3$ by microorganisms.

Chapter Three addresses the second research objective, proposing an organic approach to create a bioactive, carbonating limewash. The approach aims to extend the sacrificial lifespan of the limewash by counteracting the erosive impact of weather conditions on the external rendering of structures, reduce the carbon footprint of building maintenance and restrain the rising costs of built heritage upkeep.

2.3 Literature review

Carbonates, primarily found in various forms of limestone, constitute approximately 42% of the total carbon reservoir on Earth, with a substantial portion originating from biogenic sources, (Zhu & Dittrich, 2016). Microbial carbon sequestration through CO_2 fixation has emerged as a promising and acknowledged technology, (Rossi et al., 2015). Photosynthetic microorganisms, primarily cyanobacteria and microalgae, play a pivotal role in capturing carbon dioxide from the atmosphere, (Kumar et al., 2011). Beyond the evident environmental advantages, the commercial prospects associated with harnessing environmentally beneficial microbial products are compelling. As of

2015, microbial biologics were valued at an impressive US$ 277 billion and are projected to reach US$ 400 billion by 2025, (Grand View Research, 2017). Microbes contribute toward generated lime-concrete CO_2 micro-encapsulating pastes (Wang, Soens, et al., 2014), biopolymers (Moradali & Rehm, 2020), biocides and biosurfactants (Fidanza & Caneva, 2019; Płaza & Achal, 2020), biofilm generated bioelectricity (Nealson, 2017), biofuels (Kumar et al., 2018) and brownfield site bioremediation (Megharaj & Naidu, 2017). The economic value of the carbon-fixing global market in the future is likely to be significantly greater than past estimates.

2.3.1 Lime render and weather erosion

Lime production is initiated through the high-temperature calcination of calcium-based rocks, typically at 900°C, culminating in the formation of calcium oxide (CaO) as depicted in *Figure 8*. Subsequently, calcium oxide may undergo "slaking" or hydration upon contact with water, yielding lime putty or, when combined with dampened aggregate, leading to the creation of "hot lime." Notably, the addition of water to calcium oxide is characterised by a vigorously exothermic reaction, generating a thixotropic wet hydrate, a choice conventionally favoured by skilled artisans. Hydrated lime subsequently undergoes an induration process, which is influenced by atmospheric carbon dioxide, commencing at temperatures above 5°C and contingent on residual moisture levels. Carbonation occurs as atmospheric CO_2 is absorbed, progressing at a rate of 5 mm per month, commencing from the external surface and progressing inwards (Young, 2008).

Climate change exerts several types of erosive force upon the exterior surface of the building, as illustrated in *Table 1*. Accelerated erosion occurs after prolonged cycles of drought and flooding, heightened rainfall acidity characterised by the presence of H_2SO_3 and HNO_3, consequential pH alterations, escalating temperatures, biological degradation, and the formidable impact of storm-force winds, (Sabbioni et al., 2008). Wind-driven rain and the salination of rainwater in coastal regions introduce salts that accumulate within the microcracks of weathered structures. During periods of drought, the crystallization of salts exerts substantial pressure within the structural interstices of lime-based materials, ultimately leading to material failure.

By the 19th century, the use of lime mortar diminished as the widespread adoption of Portland cement gained momentum. Portland cement is characterised by its ease of use, rapid curing properties, and high compressive strength. The strength of concrete is accompanied by two inherent limitations: its susceptibility to fracture and brittleness, and its significant role as a major contributor to atmospheric CO_2, (Blankendaal et al., 2014). Consequently, researchers are actively exploring strategies to mitigate the environmental impact of the extensive utilisation of concrete in construction practices, although the economic feasibility of such endeavours remains a formidable obstacle (Scrivener et al., 2018).

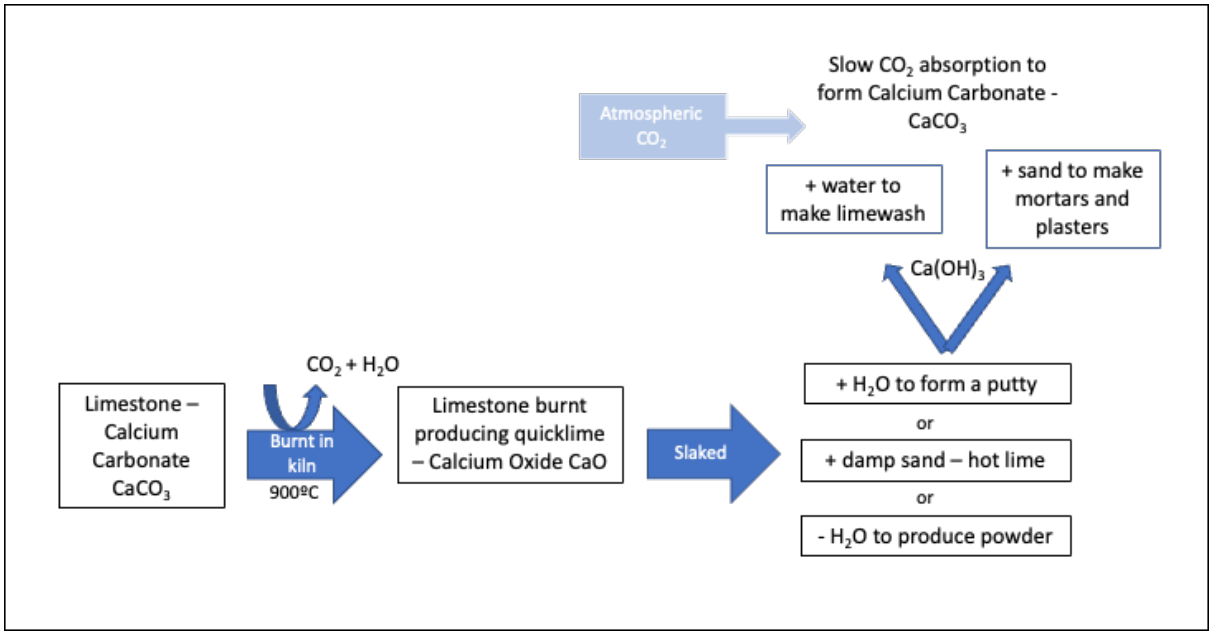


Figure 8: The lime cycle

In comparison, the utilisation of lime as a construction material is characterised by a protracted application process, contingent upon various factors including accommodating weather conditions and the availability of proficient craftsmanship. Unwise preservation methods, such as the use of epoxy resins and barium hydroxide ($Ba(OH)_2$) solution, not only contribute to environmental toxicity but also impede moisture permeability, leading to irreversible microstructure impairment of the lime (Rodriguez-Navarro et al., 2003). To bolster the adhesive, waterproofing, and antiseptic qualities of lime, additives like sealants and pozzolans have been introduced. Past building records reveal diverse additives, ranging from marine salt in 1811 to unusual substances like skimmed milk (1881), warm slaughterhouse blood mixed with stale beer (1883), flour (1887), sugar (1890), and molasses (1913), (Taliaferro, 2015). Artisans posited that the inclusion of blood could enhance binding strength, weather resistance, and carbonation, which may be due to the hydrolysis of blood proteins within the alkaline environment though there are limited experimental studies on this topic, (Fang et al., 2015).

Despite the convenience of application and high compressive strength, concrete is susceptible to cracking, leading to increased brittleness that permits the ingress of water and pollutants, ultimately compromising its long-term structural integrity. The thermal expansion mismatches among its constituent materials contribute to reduced material strength and ensuing environmental contamination, (de Muynck et al., 2008).

Early endeavours to fortify concrete through microbial repair mechanisms involved the incorporation of *Bacillus* spores and calcium lactate ($C_6H_{10}CaO_2$) as a nutrient, and both embedded in clay pellets. In this innovative approach, as cracks develop, water triggers the revival of dormant bacteria within the pellets. These reactivated bacteria then generate calcium carbonate ($CaCO_3$) deposits, effectively sealing the cracks, (Jonkers, 2007; Wiktor & Jonkers, 2011). The microbial repair process led to a discernible enhancement in the compressive strength of concrete, (Bang et al., 2010). Nevertheless, anaerobic ureolytic bacteria, during the biomineralisation process, yield environmentally harmful by-products, such as ammonia, while precipitating calcium carbonate. This contributes to toxic runoff. In contrast, autophototrophic bacteria engage in the fixation of

atmospheric carbon, forming robust calcite layers within cracks without generating toxic by-products (Zhu & Dittrich, 2016).

2.3.2 Overview of mechanisms of bacterial precipitation of calcium carbonate

Microbial precipitation arises from metabolic processes that can be categorised as either heterotrophic, exemplified by urea hydrolysis, or autotrophic. The outer cell membrane, characterised by a negative charge, can bind divalent cations, such as Ca^{2+}. This binding event leads to the creation of a nucleation site either within or external to the organism, where crystal formation commences. During the process of urea hydrolysis, which yields CO_2 and ammonia, there is a concurrent increase in the pH of the surrounding environment, coupled with an elevation in carbonate concentration, (Chahal, Rajor and Sidique, 2011). The active microbial precipitation of $CaCO_3$ is observed to occur at a considerably higher rate in comparison to passive chemical precipitation, (Stocks-Fischer, Galinat and Bang, 1999). This phenomenon has been substantiated through empirical evidence, demonstrating enhancements in CO_2 sequestration in organisms like *Chlorella* sp. and *Spirulina platensis*, with sequestration rates reaching up to 46%, (Ramanan et al., 2010).

Three autotrophic metabolic pathways are involved in bacterial calcium carbonate formation (Castanier et al., 1999), non-methylotrophic methanogenesis, anoxygenic photosynthesis and oxygenic photosynthesis, *Figure 9*. All three pathways use CO_2 as a carbon source and in the presence of calcium ions, produce a precipitation of calcium carbonate.

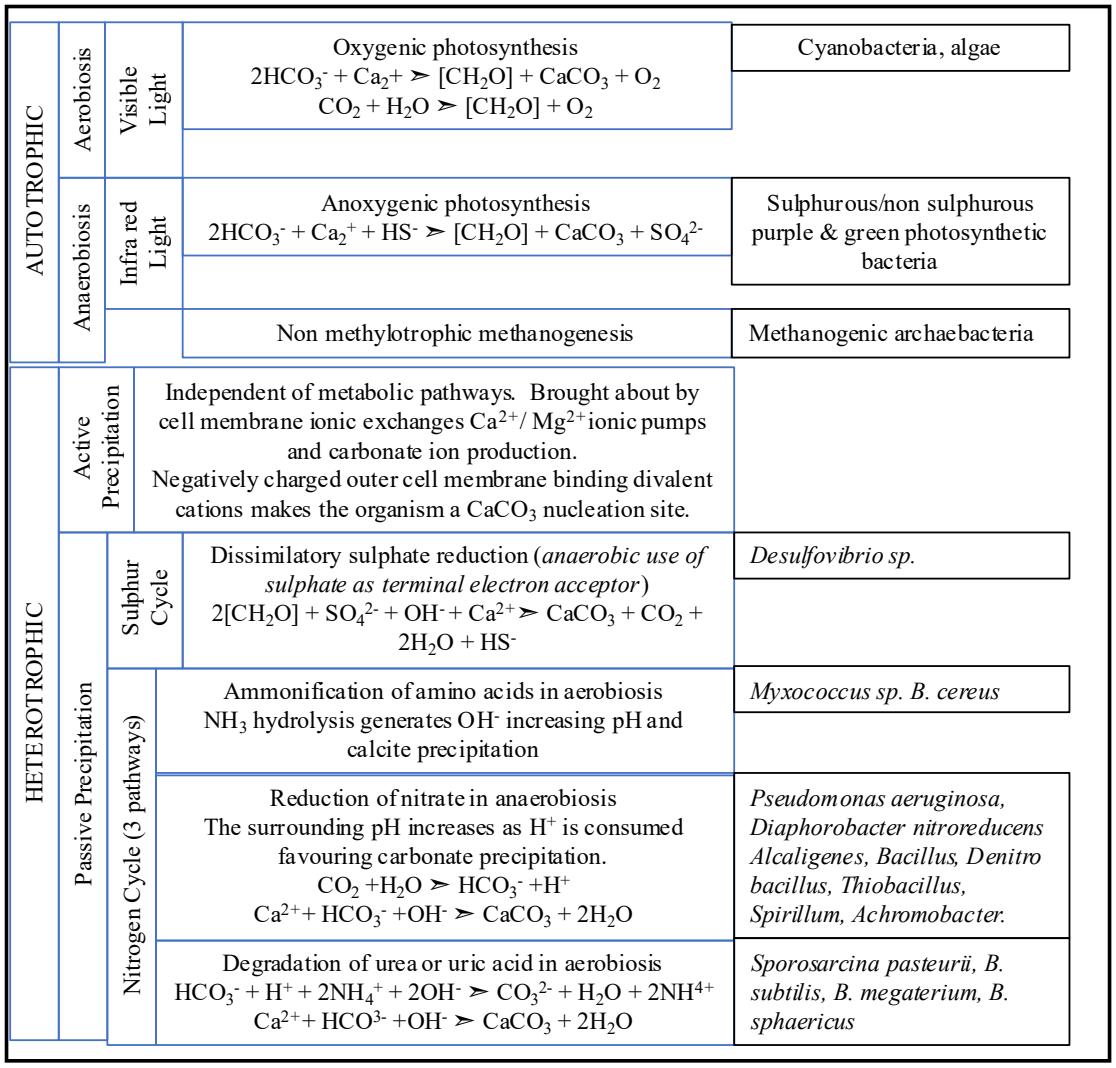


Figure 9: Comparison between autotrophic and heterotrophic bacterial production of CaCO3. A redox-generated high environmental pH is common across metabolic pathways. Autotrophic bacteria: Aerobiosis ((Dupraz & Visscher, 2005), Anaerobiosis (Baumgartner et al., 2006), Heterotrophic bacteria: Active Precipitation (Stocks-Fischer et al., 1999), Passive precipitation (sulphur cycle) (Baumgartner et al., 2006; Braissant et al., 2007), (nitrogen cycle pathways) (Achal & Mukherjee, 2015; Erşan et al., 2015; González-Muñoz et al., 2010; Jroundi et al., 2010; Kavazanjian & Karatas, 2008; Lee, 2003; Rodriguez-Navarro et al., 2003; Wei et al., 2015).

Photosynthesis is the principal contributor to the production of carbonate rocks (Altermann et al., 2006) such as cyanobacteria formation of stromatolitic carbonate speleothems in the photic zone of carbonate caves (Léveillé, et al., 2007). Photosynthesis leads to calcite precipitation by conducting an HCO_3^-/OH^- exchange across the cell membrane increasing the pH around the cells. By diffusion or via a symporter, CO_2 enters the cell wall (Espie and Kandasamy, 1992), the CO_2 is then synthesised into organic matter while bicarbonate is converted to CO_2 and OH^-, the latter released out of the cell increasing the pH of the external environment. Cyanobacteria are the only organisms that utilise H_2O as an electron donor during photosynthesis and the degree of light intensity is critical for this photosynthetic pathway (Kumar et al., 2011). Low-intensity light limits biomass productivity whereas high intensity can cause photo-inhibition. (Rubio Camacho et al., 2003).

During the formation of microbial carbonate, the carbonate adheres to the original material while retaining moisture permeability (Rodriguez-Navarro et al., 2003). Importantly, the deposition of microbial $CaCO_3$ aligns with conservation standards and does not introduce alterations to the physical appearance of the stone (Jroundi et al., 2010).

2.3.3 Methods for bacterial inclusion into a cementitious matrix

Bacterial inclusion into a cement or lime material follows three widely used methods, direct application, immobilisation, and encapsulation, *Table 2,* (Griño, Daly and Ongpeng, 2020). The simplest method directly applies live bacterial cells or spores with or without supporting nutrients into the concrete mix. Any micro spaces existing in the concrete are filled with calcite precipitate which improves the overall compression strength (Ghosh et al., 2005). Due to the highly alkaline environment, researchers have used alkaliphilic or alkali-tolerant strains that are capable of spore formation such as *Bacillus sphaericus*, a ureolytic, alkali-tolerant spore-forming microbe. It is crucial to acknowledge that unprotected bacterial cells may struggle to withstand the harsh environmental conditions associated with concrete and may consequently fail to persist for a sufficient duration to facilitate effective calcite-mediated repair (Wang and Soens, 2014; Li et al., 2019).

Table 2: Comparison of three methods employed to inoculate cement paste with bacterial cells or spores

	Direct application	**Immobilisation**	**Encapsulation**
Advantages	The most cost-effective approach entails the on-site introduction of bacteria or spores, with subsequent direct application of the material containing these microorganisms to the surface. (Khaliq & Ehsan, 2016)	Enables the cells to tolerate the alkaline conditions of the cement paste and survive the mixing process. Immobilisation in sepiolite a hydrous magnesium silicate increases the viability of bacterial cells and extends the calcite precipitation fracture healing process (Bang et al., 2001; Sandalci et al., 2021; Seifan et al., 2018)	Encapsulation protects the bacterial cells or spores against the harsh alkaline environment and reduces damage from mixing and application. Encapsulation enables the introduction of nutrients into the capsule to extend bacterial performance (Oyen, 2014; Wang, Soens, et al., 2014)
Disadvantages	The harsh alkaline environment and limited availability of nutrients result in a high cell mortality and extensive physical damage to live cells (Jadhav et al., 2018)	Incurs additional costs and requires off-site preparation of bacteria and immobilisation material. Antimicrobial qualities of immobilisation materials may reduce bacterial performance (Shaheen et al., 2018)	Discarded capsules may reduce the integrity of the concrete matrix and undermine the benefits of calcite precipitation. Thick capsule walls may impede cell resuscitation preventing the cells from entering the microfractures

To improve live cell viability and precipitation, bacterial cells can be immobilised within a protective material. Bacteria immobilised within graphite nano-platelets and lightweight aggregates can extend calcite precipitation for up to 28 days (Khaliq & Ehsan, 2016). Other protective materials include limestone powder (Shaheen et al., 2018), iron oxide nanoparticles (Seifan et al., 2018), polyurethane (Bang et al., 2001) and sepiolite (Sandalci et al., 2021) which, subject to availability of nutrients, can extend viability for up to a year. A third method for inclusion into a cement or lime paste is to

encapsulate the bacteria or spores within a biodegradable capsule providing a mechanical buffer during application and enclosing essential nutrients to extend cell viability.

Table 3: Applications utilising bacterial inclusion and encapsulation techniques employed

Encapsulation application	Encapsulation technique	References
Food bio-products (Such as protection from oxidation, adverse chemical reactions, evaporation)	Spray drying, spray cooling, extrusion, co-crystallisation, coacervation	(Abd El Kader & Abu Hashish, 2020; da Silva et al., 2014; Poornima & Sinthya, 2017)
Phase change materials (PCMs)—organic, inorganic, eutectic (Protection from flammability, PCM agent separation, thermal instability)	Emulsions, electroplating, solvent evaporation, precipitation	(Gao et al., 2022; Milián et al., 2017)
In-situ biodegradation and bioremediation (Cell immobilisation for use in contamination sites)	Spray-drying, extrusion, freeze-drying, electrospinning, coacervation, liposomes, ionic gelation, molecular inclusion	(Bamidele & Emmambux, 2020; Gao et al., 2020; Khotimchenko, 2020; San Keskin et al., 2018; Sarma et al., 2011; Valdivia-Rivera et al., 2021)
Drug delivery (Colon-targeted antitumour drugs—acid tolerant pectin polymers to release active drugs at site)	Hydrogels, pellets, microspheres, microsponges	(Khotimchenko, 2020) (Asua, 2002; Blaiszik et al., 2009; Feng et al., 2008; Souradeep & Kua, 2016; J. Y. Wang, Soens, et al., 2014; Wiktor & Jonkers, 2011; Zhang et al., 2021)
Enhanced construction materials (Concrete- strengthening enhancements)	Polymeric microcapsules incorporating chemical healing agents prepared by an oil-in-water dispersion mechanism based on an emulsion polymerisation technique. Sonification using a hydrophobic solution to generate microcapsules. Polymer encapsulation of bacterial spores *Bacillus sphaericus* using a melamine-based microcapsule system. Spores embedded in nutrient-enriched hydrogels are mixed directly into the mortar. Porous expanded recycled glass granules hold the spores and nutrients and trigger as the crack forms, promoting substrate repair	

2.3.4 Introduction to bacterial encapsulation

Encapsulation serves to mitigate the potential damage of physical or chemical harm to cells or spores before release into the cementitious matrix. The design of the encapsulating material must not impede carbonate precipitation or obstruct access to water. It must also not affect the chemical composition of the lime or cement matrix or diminish its compression strength. The efficacy of cell encapsulation hinges on several key factors, surface texture, shell thickness, and capsule diameter, (Joseph et al., 2010). Alterations in the wet/dry curing environments can also exert a notable impact on the self-healing response of the encapsulated cells, (Wang et al., 2012). When juxtaposed with

other methods of microbial immobilisation, encapsulation emerges as a more resilient approach as compared to solid or fluid microbe inclusion in terms of robustness. For encapsulation to be deemed economically viable, it is essential to identify criteria demonstrating improvements in robustness, attesting to microbial survival, long-term viability during transportation, and ease of application at the intended site.

2.3.5 Hydrogels and encapsulation polymers

The food, medical and environmental sectors practice encapsulation for the introduction of targeted microbial cells employed to extend the viability and efficacy of the beneficial metabolic processes specific to each application, *Table 3*.

(Bashan et al., 2002) inoculated soil utilising microbeads generated through a low-pressure spray of bacterial culture suspended in a highly nutrient-liquid base blended into an alginate solution. The resultant suspension manifested as small diameter droplets, which when sprayed through a calcium chloride solution solidified to form 100–200 µm microbeads containing colony-forming units (CFUs). The microbeads exhibited viability and exhibited resilience when subjected to a conventional freeze-drying procedure. Within a moist environment, the microbeads effectively biodegrade within a span of 15 days.

This example illustrates one method used to immobilise and encapsulate at the micro level. Other techniques include flocculation, adsorption to surfaces, covalent bonding to a carrier, intercellular cross-linking, polymer-gel encapsulation, and matrix entrapment (Cassidy et al., 1996). The selection of an appropriate polymer is pivotal for each of these technologies, tailored to the specific requirements of the application. The available spectrum of polymers spans both synthetic and natural variants. In pursuit of environmentally sustainable objectives, natural biopolymers often exhibit greater compatibility compared to their synthetic counterparts, and they are better equipped to provide a supportive niche for microbial growth when employed as the encapsulating medium.

Polymers refer to a wide range of natural or synthetic compounds composed of many multiples of simple chemical monomer units. The linked monomer units form a polymer backbone consisting of repeat units of carbon atoms which during the polymerisation process have released chemical groups to allow the polymeric linkage between the units. Synthetic polymers, particularly those derived from petroleum products, have achieved widespread usage, though their structural robustness has introduced various environmental challenges. In contrast, biopolymers are found throughout nature categorised into three principal groups, polysaccharides, polypeptides, and polynucleotides. Certain biopolymers such as rubber and cotton were familiar and in everyday use prior to the advent of chemically synthesised plastics. Biopolymers originating from living organisms represent an environmentally sustainable alternative to synthetic counterparts finding applications in the pharmacological, medical, and agricultural sectors.

2.3.6 Algal polysaccharides

Alginate and κ-carrageenan are two naturally occurring polymers demonstrating distinctive properties when exposed to Ca^{2+} ions due to the presence of guluronic acid in alginates. Alginates are linear polymers of β (1,4)—D-mannuronic acid and a (1,4)- L-guluronic acid monomers found in nature in varying configurations exhibiting a wide range of properties, (da Silva et al., 2014;

Dhamecha et al., 2019). The resultant cross-linked matrix creates enclosed spaces capable of entrapping, protecting, and immobilising cells. Calcium alginate manifests a dense, large pore alginate matrix ideal for bacterial occupation, (Voo et al., 2016). Bacteria encapsulated within calcium alginate beads demonstrate enhanced calcite precipitation compared to control groups, (Soysal et al., 2020). As calcium alginate beads biodegrade, they supply a sustained supply of nutrients and calcium ions for continuous microbial carbonate precipitation. Microorganisms are encapsulated into the alginate using the syringe method to produce the alginate beads which form in the range of 0.5–3.5 mm in diameter, (Lancy & Tuovinen, 1984). Advanced techniques have further enabled the reduction of bead size down to 120 µm, (Musgrave et al., 1983).

Algal polysaccharide beads increase the surface for cell attachment for encapsulated microorganisms facilitating a substantial increase in cellular metabolism. Alginate-encapsulated *Saccharomyces cerevisiae* cells produced 80% more ethanol when compared to planktonic cells (Galazzo & Bailey, 1990).

Carrageenan derived from red algae such as *Chondrus crispus* offers a varied structural diversity composed of linear chains of β(1,3)-D-galactose and α(1–4)-D-galactose units which form a robust encapsulation gel, (Perrechil et al., 2020). The encapsulation gel is generated by extruding carrageenan and cell suspensions at a temperature of 42°C into a cold solution of potassium chloride. The risk of denaturing several of the temperature-sensitive proteins within the cells can be mitigated through the addition of lotus bean or carob bean gum. This technique has found large-scale application for the encapsulation of microorganisms for the treatment of contaminated soil sites, (Hulst et al., 1985). Various manufacturing techniques, such as resonance nozzles, rotating disk atomizers, low-pressure ultrasonic nozzles, and parallel plate electrostatic droplet generators, have been employed to achieve industrial-scale production of encapsulated alginate at rates exceeding 24 lhr^{-1}, (Ogbonna et al., 1989; Stormo & Crawford, 1992). Alginate and carrageenan compounds enhance encapsulation by providing both chemical and mechanical stability, ensuring more effective release of capsule contents, and affording protection to encapsulated cells when exposed to freeze/thaw cycles, (Huguet et al., 1994; Malhotra & Basir, 2020; Sarıyer et al., 2020).

In similar comparison to alginate, pectin is an anionic polysaccharide derived from plant cell walls and is composed of long sequenced partially methyl-esterified (1–4)-linked a D-galactosyl uronic acid forming a natural hydrogel with the addition of Ca^{2+} divalent ions, (Yang et al., 2018). A simple hydrogel encapsulation technique incorporates the cell suspension with $CaCO_3$ and sodium alginate, which can be either extruded or applied as an emulsion, (Liu et al., 2020). The thick stable wall of the pectin capsule facilitates a controlled release and alleviates the stress on the encapsulated contents.

Initial encapsulation methods employed a porous aggregate to encase the bacterial spores and nutrients, (Jonkers, 2007). Hydrogels, a broader class of compounds encompassing alginate and pectin, are hydrophilic gels of cross-linked polymer chains that house bacteria or spores and from which water stored in the matrix is dispersed. Several hydrogels including calcium alginate represent a non-toxic, renewable natural source offering a well-structured matrix and large pores, rendering them ideal for encapsulating bacterial cells, (Voo et al., 2016). Hydrogel encapsulation effectively simulates an intracellular environment by maintaining a water content exceeding 90% water, (Oyen, 2014). The gradual release of water held within the hydrogel matrix extends protection to the cells from physical and chemical damage and facilitates metabolic $CaCO_3$ precipitation, (Wang, Snoeck, et al., 2014).

2.3.7 Biofilms – bacterially generated biopolymers

A challenge confronting biopolymer immobilisation technology is the relatively low mechanical strength of the capsule casing. Instability in the protective capsule will result in the untimely release of the contents and premature cell death. This can be addressed by reducing the size of the capsule to nano or micro dimensions. Refining the capsule size has been demonstrated to enhance cell survival rates and cell longevity, (Jampílek & Králová, 2017; Prasad et al., 2017).

A key advantage provided by bacterial biopolymers is a three-dimensional spatial matrix capable of accommodating microorganisms. Bacteria can synthesise four primary biopolymer classes, polysaccharides, polyesters, polyamides and inorganic polyanhydrides each expressing a diverse spectrum of properties. Microbial extracellular polymeric substances (EPS) or exopolymers, produced as a survival mechanism by bacterial cells, adhere to both hydrophobic and hydrophilic surfaces facilitating the formation of three-dimensional architectures constructing a biofilm, (Decho & Gutierrez, 2017). The production of biofilms provides several advantages to the cells contained within it including conferring a highly effective barrier to toxic molecules. The barrier raises the minimum inhibitory concentration of cytotoxins compared to that needed to destroy planktonic cells. Biofilm biopolymers are more resistant to mechanical stress, utilising electrostatic and hydrophobic forces and offering structural protection to resist deforming forces to the cell wall (Billings et al., 2015).

2.3.8 Biopolymer additives

Enhancing the performance, characteristics, and resilience of biopolymers can be accomplished through the incorporation of natural fillers and antioxidant stabilising compounds during their processing. The introduction of organic or inorganic additives into biopolymers during the encapsulation procedure serves to further augment the physical attributes pertinent to polymer encapsulation. Additives are specific and contingent on biopolymer application. In instances where there is an incompatibility between the additive and the polymer, it may hinder the encapsulation process, (Viveganandan & Jauhri, 2000). The incorporation of the additive can enhance the bio-carbonation process and is an avenue for future research to advance encapsulation technologies. An example is the addition of lectins which are carbohydrate-binding proteins to biofilm-derived which demonstrates an improvement in biopolymer linkages between the bacterial cells and the exopolysaccharides in the capsule wall thereby improving the encapsulation success rate, *Table 4*.

Table 4: Introduction of additives to biopolymers to deliver capsule performance improvement

Additive	Encapsulation advantages	References
Clay minerals	• Improved capsule wall thickness, • Extended bacterial survival rate • Reduced UV damage • Controlled cell release	(Liffourrena & Lucchesi, 2018; Zohar-Perez et al., 2003)
Skimmed Milk	• Increases cell count • Faster release of cells from the capsule	(Bashan et al., 2002; Power et al., 2011; Yu et al., 2001)
Starch (alginate)	• Improves capsule matrix strength reducing physical stress • Reduces exposure to UV radiation	(Dunkle & Shasha, 1989; Jankowski et al., 1997; Kim et al., 2005; Qi & Tester, 2019)

Chitin and chitosan	• Bioactive oligosaccharides improve resistance to pathogens and overall antimicrobial properties of the capsule	(Berger et al., 2014; Estevinho et al., 2013; Muxika et al., 2017; Nah & Jeong, 2021)
Humic acid	• Improved cell survival	(Young et al., 2006)
Sugars	• Protection from osmotic pressures • Improved resistance to desiccation	(Morgan et al., 2006; San Keskin et al., 2018; Schoebitz et al., 2013)
Proteins (hydrolysates, gelatine, albumin, elastin, casein, biofilm lectins)	• Enhance nutrient uptake by encapsulated cells • Bio-stimulants • Improved encapsulation rates • Improved linkage between microorganisms and exopolysaccharides	(Casadesús et al., 2019; Colla et al., 2017; Elzoghby et al., 2015; Nesterenko et al., 2013; Valdivia-Rivera et al., 2021; Vejan et al., 2019)

The incorporation of microfibres in conjunction with encapsulated bacteria into a lime medium represents an advancement of the bio-carbonation process. The synergy arises from the interaction between the microfibres loaded with encapsulated bacteria within the three-dimensional fibre matrix combined with the addition of calcium lactate as a precursor resulting in improved efficiency and an extended bio-carbonation period (Luo et al., 2015; Su et al., 2021). The use of environmentally compatible fibres such as cellulose appears to stimulate bacterial cells to produce EPS through mechanisms that may involve genomic or proteomic alterations within the cells (Gupta et al., 2017; Singh & Gupta, 2020).

2.3.9 Factors to consider in designing a limewash encapsulation technology

The optimisation of bio-carbonation efficiency hinges on the encapsulation of the most appropriate non-pathogenic bacteria. Within a recent comprehensive review of the performance of several *Bacillus* sp. incorporated into concrete mixes, *Bacillus halodurans* demonstrated the highest efficiency in spore formation, viability, and calcium carbonate formation, (Sri Durga et al., 2021).

(Souradeep & Kua, 2016) devised an eight-factor checklist to evaluate the effectiveness of a self-healing system within concrete substrates. Six of these criteria can be adapted to gauge the selection of a system for employment in limewash encapsulation.

1. The capsule wall must be sufficiently robust to safeguard the capsule contents during the mixing process yet thin enough to ensure a timely release of the microbial healing agent.
2. A homogenous distribution of the capsules throughout the limewash is important to guarantee a uniform release of cells into the application area.
3. The timing of the release of the healing agent must exhibit a responsiveness ensuring its availability precisely when and where the bio-carbonation is required.
4. As capsules fracture and discharge the microbial contents, the capsule fragments must not compromise the structural integrity of the limewash.
5. The release of the capsule contents should achieve a dual purpose, maintaining the viscosity of the limewash for uniform capsule distribution and adequately viscous to retain microbial cells at the point of application.
6. The survival rate of the bacteria is linked to the stability and release mechanism of the capsule polymer. Spores are more resilient prolonging microbial cell viability for up to 6

months whereas live bacterial cells though immediately responsive upon release have a limited lifespan, (Wiktor & Jonkers, 2011).

The merits of developing a bacterial-enabled limewash for use on lime render and lime composites are promising as a more resilient sacrificial limewash layer to extreme weather events has environmental and economic benefits.

This analysis identifies four areas that contribute to the formulation of limewash encapsulation and extend the natural carbonation process through bio-carbonation.

- Firstly, the selection of encapsulation technology at micro or nanoencapsulation dimensions as small as 100–200 µm to protect the capsule contents from physical stress.
- Incorporating the future development of a biopolymer or EPS-derived biopolymer possessing the capability to sustain living cells and control the timely release of the cells during the limewash application procedure.
- Assessing optimal additives and bio-enhancers such as microfibres and calcium carbonate nucleating microbial species is crucial to determine the optimal combination to maximise the bio-carbonation process as limewash carbonates during successive applications to stone or render surfaces.
- Lastly, it is essential to design a formulation with the appropriate density for the bacterial capsules. The formulation should permit a uniform cell release maintaining the integrity and performance of the limewash.

These four areas provide a structured framework for the design of limewash encapsulation, with a focus on extending the natural carbonation process through bio-carbonation.

2.4 Chapter Summary

Local authorities are constrained by statutory obligations inherent in heritage conservation policy, which may curtail their latitude in considering innovative alternatives during the consent process. These conservation principles also confront challenges in accommodating a primary goal outlined by the United Kingdom Government within the Government Construction Strategy, namely, the systematic reduction of carbon emissions. The concomitant surge in environmental legislation presents an auspicious opportunity for the introduction of novel technologies and alternative conservation materials to augment the traditional portfolio of skills and techniques necessary for mitigating the impact of extreme weather events. The integration of any new conservation material necessitates a comprehensive evaluation, resulting in the formulation of technical and safety data sheets, as well as environmental product declarations, reinforced by guidelines for building applications.

Research regarding the advancement of cementitious materials has focused on enhancing the strength of concrete, with a lesser focus on improving lime render performance. This emphasis on concrete is partly economic and stems from its susceptibility to fracturing over time and the inherent difficulty of repair due to accessibility limitations.

One avenue for self-healing explored in the literature involves the use of immobilised biomineralising bacteria. These bacteria can repair microfractures in render materials by producing calcium carbonate through ureolysis. This approach relies on anaerobic ureolytic bacteria, which generate environmentally undesirable by-products like ammonia.

Another strategy incorporates biopolymers directly into the cementitious mixture. This leverages the biopolymer's flexibility to complement the compressive strength of concrete. However, finding an optimal mixing ratio that maintains sufficient strength in the hybrid material remains a challenge.

Various attempts have been made to address these limitations. One approach involves introducing additives into the biopolymer, such as blood proteins for enhanced chemical bonding or physical scaffolding materials like horsehair to reinforce the polymer structure. Additives may have unanticipated consequences, negatively impacting the physicochemical properties of the material.

Other efforts have focused on extending the biomineralising self-healing lifespan of the bacteria. This includes inducing sporulation before incorporation into the cementitious material or utilising immobilisation frameworks like graphite nanoplates or melamine microcapsules. While these methods have achieved limited success, they fail to address the issue of environmentally harmful by-products. Additionally, robust immobilisation frameworks, while protecting the cells, can hinder their release and functionality.

Despite these challenges, biopolymers remain a preferred method for immobilisation and encapsulation of cells within a 3D polymer matrix. The use of naturally sourced and biodegradable biopolymers like alginate, cellulose, carrageenan, or harvested biofilm polymers offers promising potential for creating supportive structures to deliver and sustain organic payloads in a sustainable manner.

Overall, while substantial progress has been made in utilising biopolymers for self-healing cementitious materials, significant challenges remain regarding material optimisation, environmental impact, and long-term functionality.

Biodesigned materials tailored for utilisation within the construction sector can transcend the enhancements typically attributed to conventional heritage materials, such as lime. This research advocates the merits of microbial-encapsulated limewash and recommends continuous investment in microbial self-repair technologies. The incorporation of microbial materials in building construction is poised to become a mainstream technological advance, (Heveran et al., 2020).

Equally important are the prospects arising from this evaluation, which foster the development of alternative construction materials, exemplified by hemp-lime bio-composites fortified with carbon sequestration layers. By expanding the efficacy of microbe-encapsulated limewash beyond its applicability to listed buildings and cultivating innovative biodesigned active coatings to combat environmental pollutants and greenhouse gases, the outcome of this research has the potential to effectuate a global impact, both environmentally and economically. The availability of genome databases and further exploration into metabolic pathways will leverage transformative microbial advancements, yielding tangible environmental improvements for heritage structures and the broader construction industry.

CHAPTER THREE: Extending the climate resilience of traditional materials utilising microbial biopolymer encapsulation

Chapter Abstract

This chapter delves into the potential of microbial encapsulation of biopolymers as a novel strategy to bolster the climate resilience of historical buildings constructed with lime, stone, and brick. Expanding upon the concept of self-healing limewash introduced in the preceding chapter, this approach harnesses the biomineralisation abilities of microorganisms to fortify and safeguard the structural integrity of heritage structures. By encasing microbes within biopolymeric matrices, their metabolic activities can be harnessed to induce the formation of mineral deposits within the building materials, increasing the mineral density, thereby enhancing their resistance to environmental degradation and promoting self-healing functionalities. This bio-inspired approach shifts the focus from intrinsic material properties to harnessing the metabolic processes of microbes to induce the formation of protective bio-deposits within the building fabric, potentially offering a novel and biocompatible approach to heritage conservation in the face of increasingly erratic environmental conditions.

The cornerstone of this bioremediation strategy hinges upon the efficacious integration and enduring resilience of live cells or spores within the harsh chemical properties of limewash. This necessitates the advancement of encapsulation or immobilisation technologies, essentially constructing a protective biopolymer shell to safeguard the microorganisms and enable their active biomineralisation within the limewash matrix.

To achieve this, the chapter reviews pertinent technologies and principles. Key areas of focus include biopolymer formulation, 3D extrusion and immobilisation techniques, capsule release mechanisms, and the fundamental understanding of microbial biomineralisation.

This research translates fundamental principles of biomineralisation and microbial-mediated processes into tangible applications for heritage stone conservation. The practical focus is twofold. First, it aims to design and optimise a suitable biopolymer coating for sustained encapsulation of microorganisms. This involves exploring various technologies like 3D bioprinting, extrusion, electrospraying, and tailored polymerisation chemistry to achieve a robust and durable encapsulation matrix. The second step involves the selection, cultivation, and incorporation of appropriate microorganisms, either encapsulated or immobilised, into the coating system. Diverse formulations are then rigorously assessed through *in vitro* and *in situ* performance measures, facilitating comparison with control samples to identify the most effective formulation for further investigation.

Once the optimal formulation is established, the research delves deeper into biomineralisation strategies. Comparative studies are conducted between heterotrophic and autotrophic microorganisms to evaluate the potential of utilising photosynthesis as a driving force for biomineralisation within the coating system. This aligns with the second research question, which explores how both chemical and photosynthetic approaches can be harnessed to extend the lifespan of protective limewash layers on heritage structures.

3.0 Introduction

Mitigating the impact of extreme weather on heritage buildings necessitates the development of novel materials to protect exterior surfaces. Several studies, investigating the strengthening of lime renders, have utilised additives such as glass, carbon fibres, or reinforced cement-based mixes, (Angiolilli et al., 2020). While these additives may augment material strength and ductility the additives also introduce brittleness or as in the case of synthetic additives, have a deleterious impact on vapour permeability by reducing porosity and interfering with the curing process of the material. Historically, lime has stood as a robust weather-resistant compound, but the escalating frequency and intensity of erosive climate forces are exerting unprecedented stresses on the weather performance of the render.

The ramifications of inaction in addressing this issue will extend to the degradation of noteworthy heritage structures which in turn, has the potential to impede heritage tourism resulting in adverse economic consequences for local communities, (Mosoarca et al., 2017). In the immediate term, conventional conservation practices may prove adequate in temporarily sustaining the structural integrity of these structures. However, should meteorological conditions become increasingly severe and impactful, the conventional conservation paradigm will likely prove insufficient, exposing heritage structures to a cumulative risk of deterioration. Consequently, it is imperative to develop and embrace new technological approaches necessitating the development and adoption of innovative solutions. The realisation of this imperative transformation in conservation practice hinges upon the receptivity of heritage institutions to embrace change and secure funding mechanisms to support the requisite research, (Sesana et al., 2018).

As elaborated upon in the main introduction, this chapter delves into the first practice component of the employed methodology. This component is preceded by a rigorous examination of relevant literature and design methodologies, which serves as the theoretical underpinning for the subsequent practical exploration. This approach ensures a well-grounded and informed engagement with the chosen practice, fostering a deeper understanding of its potential contributions to the research objectives.

3.1 Extreme weather impact on built heritage

For several centuries, historic edifices have steadfastly withstood exposure to environmental stresses. In the most recent decade, arguably the most formidable challenge, and existential threat, confronting the longevity of historic structures results from the shifting climate and exposure to the extreme meteorological phenomena. The advent of warmer, wetter summers, punctuated by periods of intense wind-driven salt-laden rain, coupled with an increase in the frequency of droughts and water-saturated exteriors foreshadows unsustainable surface moisture levels that penetrate exterior renders resulting in structural failure. A more stringent regime of repair and maintenance is imperative to counterbalance the accelerating degeneration of materials to keep pace with the evolving climate conditions. Failure to institute maintenance programmes will lead to burgeoning reparation costs and a profligate consumption of material and labour resources to the detriment of the performance of historic buildings.

The resulting deterioration and attrition of notable heritage buildings can exert repercussions on the local community. The impact can extend beyond the immediate structural aspects and reach into the social, cultural, economic, and historical dimensions of the community. Culturally, heritage buildings serve as landmarks and repositories of historical memory, the loss of which erodes the collective identity of a community and severs intergeneration connections. There is also a significant economic impact on local heritage tourism, (Mosoarca et al., 2017). Heritage tourism supports local

artisans, and services with overall reduced tourism revenue impacting businesses, jobs, and local authority revenues. In the near term, adhering to conventional methodologies for the conservation and upkeep of the building exteriors may suffice. In anticipation of more aggressive climate changes occurring, conventional conservation practices may prove to be inadequate. Embracing innovative technological approaches to heritage preservation necessitating the adoption of novel solutions may be challenging. The inherent institutional resistance to change and the quest for new funding resources to underpin the research are barriers which must be overcome in addition to the challenge of technological innovation, (Sesana et al., 2018).

3.2 The traditional use of lime in building repair

Lime, a crucial material in construction and heritage preservation is produced from burning calcium-based rocks, seashells, and corals, in a kiln. During this process at around 900°C of calcining, calcium carbonate converts into quicklime or calcium oxide and carbon dioxide. The calcium oxide is 'slaked' in water forming a lime hydrate. Depending on the amount of water used, this may be in the form of lime putty, air-slaked using the water in the atmosphere, or with dampened aggregate, the latter forming a 'hot mix'. The wet hydrate, lime putty or calcium hydroxide is also known as non-hydraulic lime due to its inability to set underwater and relies on CO_2 in the air to carbonate. This process of induration must occur above 5°C and at optimal moisture levels. The production of hydraulic lime involves limestones burnt with the inclusion of clay, the more clay, the stronger the hydraulic lime mortar. The clay compounds, containing silicates, aluminates, and iron oxides, become reactive during the process and bond with the calcium ions in the calcium hydroxide. Hydraulic lime is used where a degree of hardness is required in the construction, and it will even be set underwater. Compared to non-hydraulic lime, hydraulic lime has higher compressive strength and is less permeable to water vapour which may impair the movement of moisture vapour in traditional material structures.

The lime render when applied to a building remains susceptible to erosion from atmospheric pollutants, biological attack, and the impact of wind-driven rain. Applying a sacrificial surface finish to the lime render has been a widespread practice to safeguard the render from erosion and mitigate the need for costly repairs, and to protect underlying building materials which may be scarce or of inferior quality, thus contributing to the longevity of heritage structures.

3.3 The sacrificial limewash layer

Limewash is applied in successive, stratified layers incrementally forming a protective, porous layer of calcium carbonate atop lime render. This stratified approach serves the dual purpose of impeding the ingress of moisture while facilitating its egress from within the building fabric. The limewash layer operates as a sacrificial defence mechanism against inclement weather conditions, extending the lifespan of the underlying lime render. The addition of binders to the limewash augments substrate adhesion between the lime render and limewash (Forster et al., 2020; Rodríguez-Navarro, 2012; Zhao et al., 2015). Nevertheless, the presence of binders contained within the limewash may have unintended consequences including mould proliferation, alterations in compressive strength and compromised moisture permeability. Traditional binders such as tallow, linseed oil, casein, and a range of pozzolans added to limewash exert distinct and lasting effects on the long-term efficacy of the limewash layers.

Lime has been used over centuries as a sustainable, durable, and non-toxic building material. In nature, calcium carbonate ($CaCO_3$) assumes a comparable role forming several protective layers

notably in the shells of marine animals such as coccoliths and as cuticle reinforcement in crustaceans. Within prokaryotic microbial communities, organisms such as bacteria can be induced to generate biogenic precipitation of $CaCO_3$ as an outcome of metabolic interactions with inorganic compounds, underlining the utility of $CaCO_3$ in both the natural and built environments, (Boquet et al., 1973).

3.4 Development of self-repairing bioconcrete

The production of concrete comprises the amalgamation of cement, water, and aggregate. Cement acts as the binding agent, filling the gaps between aggregate particles. This results in a high compressive strength; but also, susceptibility to cracking, a cause of material degradation and a gradual decline in long-term durability. Cracking in concrete allows dissolved anthropogenic pollutants and other chemicals to penetrate the structure. Self-repairing cracks can occur due to the entry of water or moisture and hydrating cement in the form of calcium oxide (CaO) to form calcium hydroxide ($Ca(OH)_2$), which in turn reacts with atmospheric CO_2 to produce $CaCO_3$ filling the gaps with the concrete-compatible calcium deposit. The success of autogenous self-repair mechanisms is dependent on the presence of non-hydrated material available in the cement cracks, the presence of which may adversely affect the workability of the cement. Introducing fibres, non-hydrated cement, and other healing agents into the concrete mix is another form of treatment, though the release and combination of healing agents with water is unpredictable. The addition of fibres in larger quantities in an attempt to improve the available density of the agents, may reduce the mechanical properties of the concrete. Challenges posed by these approaches have led to the exploration of alternative technologies, such as leveraging cellular processes to induce targeted biomineralisation within micro-fractured building materials, (Seifan et al., 2016).

The introduction of microorganisms into concrete mixes has demonstrated the ability to facilitate microfracture repairs that occur post-curing, (Jonkers, 2021; Vijay et al., 2017). These repairs primarily rely on metabolic biochemical pathways, including the hydrolysis of urea, denitrification, and sulphate reduction, though the by-products of these pathways can lead to environmental hazards. An alternative metabolic pathway, employed by phototrophs, avoids the production of undesirable toxic metabolites. Phototrophs utilise light as an energy source to drive the primary metabolic pathway supporting $CaCO_3$ precipitation. For deeper fractures within the substrate, the success of phototrophic repair depends on the presence of environmental CO_2 and the availability of light as an energy source, which may not be feasible in the innermost regions of concrete structures, (Castro-Alonso et al., 2019).

This chapter adopts an experimental approach that addresses the issue by designing a novel solution to introduce $CaCO_3$-precipitating bacteria into limewash before its application to building surfaces. The inclusion of bacteria enhances the repair of surface microfractures as they occur, augmenting carbonate precipitation and reinforcing the robustness of the sacrificial limewash layer. When activated, the bacterial $CaCO_3$ deposits effectively seal microfractures, preserving the strength and integrity of the lime render by slowly increasing the $CaCO_3$ density which reinforces the protective sacrificial layer. This mechanism resists water ingress while retaining open porosity to enable moisture permeability and avert long-term water-induced structural deterioration.

Mixing and preparing lime for use in building applications imposes physical and chemical stresses on exposed live cells. There is a reduction in pore size as material density increases, temperature rises, and an alkaline pH as high as 13 can be reached. These conditions reduce the survival rate of unprotected cells within the lime unless a mechanism can be found to protect and gradually release the cells when the external conditions are less severe.

3.5 Cell encapsulation and encapsulated chemical payloads

One strategy to mitigate the impact of the stress on bacterial cells is by immobilising them in a nutrient or gel medium encapsulated by an outer protective barrier. The encapsulation of bioactive materials within an outer matrix serves to shield the contents from potential harm during various stages, such as production, transportation, and delivery ultimately extending the viability of the cells until they reach their intended release site. The process of encapsulation introduces several challenges when designing the outer protective structure, including considerations related to the material properties and composition. The objective of the material design is to facilitate the encapsulation process in a manner that supports cell viability. Additionally, a mechanism, either passive or active, must be incorporated into the encapsulation lifecycle to govern and target the release of the bioactive material, *Figure 10*.

The specific requirements of the encapsulation process are contingent on the nature and intended function of the contents within the capsule. Factors such as time sensitivity, duration, and the location of release must be accommodated, and the design should incorporate mechanisms to prevent premature content release. The choice of dispersal strategies is contingent upon the environment in which the encapsulated material is intended for delivery. Various triggers can be employed to signal the encapsulation material to initiate content release, including photo-stimulation, heat, chemical chelation, pH changes, magnetic fields, or ionic modulation. For more advanced applications, multi-layered and multi-compound encapsulation materials can be employed to enhance the precision of the release mechanism. It is essential for the release mechanism to withstand adverse physical and chemical forces in the environment and to avoid introducing toxicity into the surroundings as the capsule disintegrates and releases its contents. The use of a modifying chemical trigger, for instance, must be approached with caution to prevent contamination of the immediate environment and potential harm to delicate ecosystems. The specific nature of the release mechanism is dictated by the characteristics of the release site, with pre-programmed timed or condition-dependent release mechanisms being favoured due to their efficiency in resource utilisation and focused impact.

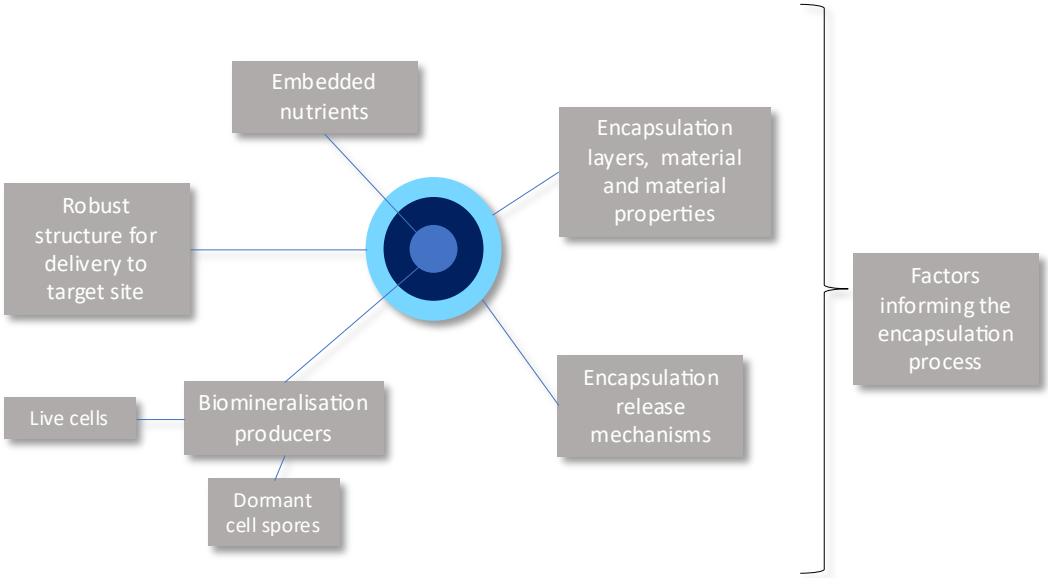


Figure 10: Multiple factors contribute to the success of an encapsulation process. Cells or spores entrapped within a hydrogel or enveloped by a polymer matrix must receive adequate nutrients to sustain metabolic

activities. Additionally, the encapsulation methodology must exhibit robustness, capable of withstanding and responding to the various mechanical and chemical environmental challenges that may threaten the integrity of the encapsulated cargo. The choice of capsule material must ensure compatibility with the surrounding environment and ideally be sourced from renewable origins, aligning with sustainable practices. The efficient delivery of microbial cells or spores to the designated target site, coupled with the control of timing and release of the encapsulated contents, must align with the intended application, ensuring optimal efficacy

3.6 Biopolymers

Polymers are large molecules constructed from smaller monomer units, arranged in a variety of covalently bonded structures. Monomers form two or more bonding sites, forming linear macromolecules. When three or more bonding sites are available, polyfunctional branched macromolecules emerge, developing into three-dimensional networks of branches and cross-links. The design of degradable polymers necessitates specific properties including robustness for targeted delivery, sustainability in development, and minimal environmental impact.

The four fundamental polymer structures linear, branched, cross-linked, and networked contribute to the structural and functional properties of both synthetic and natural polymers. Linear structures consist of long polymer chains interconnected by van der Waal or hydrogen bonds that can be disrupted by heat and subsequently reassembled. In contrast, branch polymers share a comparable polymer backbone, but with shorter chain structures which introduce interference, increasing the separation between the polymer backbones and altering the polymer density. The complex branching chains increase resistance to melting when heated. Cross-linked polymers involve several parallel polymer backbones linked by more robust covalent bonds. The degree of covalent linkage renders cross-linked polymers to thermoset, melting at higher temperatures. Network polymers feature complex three-dimensional linkages offering a high degree of structural integrity and a higher heat resistance.

Bio-based polymers are derived from renewable and biodegradable sources. Examples include alginates extracted from sources such as seaweed, brown marine algae, and bacteria, and cellulose obtained from industrial processes involving materials such as wood, cotton, flax, hemp, sisal, jute, and bamboo. Beyond plant and animal sources microorganisms serve as diverse and abundant sources of biopolymers. This broad spectrum of naturally produced biopolymers opens the immense potential for the biotechnological production of chemically and genetically tailored biopolymers that also align with environmental sustainability goals.

Bacteria synthesise various classes of biopolymers, either serving as virulence factors when generated by pathogenic bacteria or biomaterials when produced by non-pathogens. Biopolymers are derived from polysaccharides, polyamides, polyesters, and polyphosphates, all of which are found within bacterial biofilms and underpin the formulation of protective capsular materials around bioactive contents. Biopolymers apart from their immunological protective functions, enhance cellular adhesion, provide environmental defence mechanisms, store energy, and provide mechanical and chemical protection. While biopolymer compounds have diverse applications, biopolymer manufacture can present challenges to large-scale production.

This review centres on the encapsulation of carbonate-precipitating bacteria within biopolymer shells. The objective is to extend the durability and lifespan of limewash when used with lime-based construction products, acting as an enhanced sacrificial barrier, that resists erosion caused by changing weather patterns.

3.7 Microbial encapsulation

Microbial encapsulation involves packaging intact cells or spores within a polymer capsule, the size of which may be less than 1 µm. The process mobilises polymer materials to surround the bioactive material and the required nutrients to sustain and extend cell viability. Encapsulation polymers can be designed to respond to stimuli enabling targeted release of the encapsulated components at defined temporal or spatial coordinates.

The simplest encapsulation unit comprises three elements: a bioactive material suspended in nutritional gel and enclosed by the capsule material, *Figure 11*. The function of the capsule structure protect the contents, limit possible content loss, and extend cell viability. One approach to classifying cell entrapment revolves around the immobilisation of the cells, encompassing aggregation, adhesion, or adsorption within either inorganic or organic carriers. Biopolymers facilitate the infiltration of cells into the encapsulating matrix, anchoring the cells within the interstitial spaces, within suspended nutrients, or directly onto the matrix surface.

The attachment of cells occurs through mechanisms such as the secretion of adhesive polymers and covalent or ionic bonding between the cell membrane and the matrix wall. The integrity of the encapsulating barrier is important, supporting the navigation of the capsule through hostile physical and chemical environments while avoiding premature release. Hostile or toxic environmental conditions include oxygen stress, temperature extremes, dehydration, rapid changes in pH, and mechanical damage. In the absence of encapsulation, introducing cells directly into the environment with challenging parameters often leads to premature cell death, suboptimal population densities and limited success rates.

The wide range of possible applications for the encapsulation process, especially characterised by environmentally hostile environments such as alkaline pH found in lime-based products, opens opportunities to tailor an encapsulation design to improve the robustness of lime on historic structures.

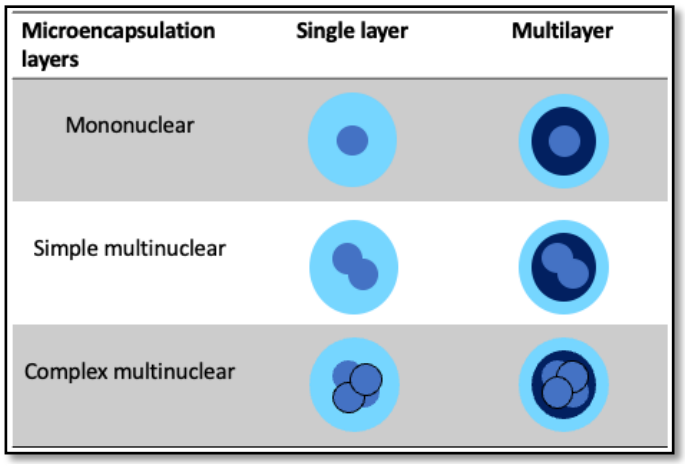


Figure 11: Design strategies for microencapsulation construction. Multilayer capsule design comprises several polymer layers capable of responding to fluctuating environmental conditions. A single core design may contain multiple contents including segregated nutrients, heterogenous cell types, spores, or contents release triggers. The number of cores and functions can increase the complexity of a single encapsulation. In combination, both designs produce structures capable of complex responses to environmental change

3.7.1 Encapsulation applications

Encapsulation serves to protect the capsule content from forces such as oxygen stress, dehydration, UV radiation or mechanical damage. While the encapsulation design is required to trigger site-specific capsule content release, the capsule may also need internal content separation barriers to prevent premature interaction between contents such as precursors and enzymes.

The successful metabolic activity of cells, when released from the capsule, relies on the contents surviving the journey to the delivery site. This can range from navigating corrosive stomach acid when delivering inoculants to the lower digestive tract, to surviving a high-alkaline pH as found in lime. Encapsulation can also protect cells from long-term storage either in dry, liquid, or frozen (-18°C) form which may be necessary to extend encapsulation as a viable industrial process allowing for medium-term storage and transportation.

One option is the encapsulation of cells into a dry powder for ease of handling using techniques such as spray, freeze and fluidized bed drying. Dry powders are an effective storage option but in powder form makes the controlled release of the contents challenging and may result in prematurely fracturing of the encapsulation coating. This may be avoided by incorporating hydrocolloidal beads that may provide a slower release of immobilising cells by adhering the cells to a controlled-release hydrogel matrix within the capsule. A hydrogel matrix can extend cell longevity due to the gradual release of water content from the gel, though the process has a shorter lifespan than dry powder encapsulation.

3.7.2 Designing encapsulating biopolymers

The choice of the encapsulation material is dependent on how the material responds to environmental factors such as the chemistry of the medium, temperature, pH, hydration, and fluidity. Biopolymers should be from renewable sources and environmentally sustainable for application with built heritage structures. The material must also encourage capsule content viability by providing a chemically stable internal environment and protective buffer for enclosed cells.

Inorganic materials such as ceramics, clay, glass, and silicates, have been successfully employed to immobilise cells. Inorganic materials possess inherent characteristics that facilitate the adsorption of organic compounds through covalent bonding sites, the chelation of metal compounds, or the formation of phase entrapment. It is important to note that when stored for extended durations, inorganic capsules may promote the formation of biofilms by the microorganisms contained within, fouling the adsorption surfaces of the material, and impeding the encapsulation process.

Encapsulated cells exhibit a heightened affinity and biocompatibility with natural biopolymers. Many biopolymer materials offer the advantage of forming three-dimensional matrices within the polymer structure providing a conducive environment for cells and surrounding nutrients. The level of protection offered by a biopolymer matrix is intricately linked to the rheology and kinetics of the polymer matrix, further underscoring the significance of the material choice.

3.8 Review of encapsulation technologies

Encapsulation technology extends across several industrial sectors, with each application tailored to specific needs and objectives. Nutritional encapsulation in foods, for example, is used to conceal odours or undesirable flavours and safeguard nutrients until they reach the designated absorption site in the human body. The release of the contents can be customised to be site-dependent or triggered by environmental cues such as pH levels, temperature variations, or the commonly employed reaction to water.

Incorporating bioactive compounds into various sectors, spanning from graphic design to pharmaceuticals, necessitates the adoption of diverse encapsulation techniques. Each technique is designed in consideration of the materials to be used, the intended location of deployment, and the specific application, as summarized in *[Appendices: Table 24]*.

3.9 Renewable and sustainable biopolymers – structure, source, and encapsulation options

Polymers encompass a diverse class of predominantly organic compounds, both natural and synthetic. Polymer chemistry primarily consists of carbon and hydrogen atoms augmented with other non-metallic elements notably oxygen, nitrogen, sulphur, and silicone. The carbon atoms form a backbone structure to which various chemical groups attach such as hydroxyl groups, leading to robust molecular bonds within and between the carbon chains.

In contrast, biopolymers are produced from natural sources, through the synthesis of biological materials or direct biosynthesis by living organisms. The natural origin, widespread availability, biocompatibility, biodegradability, and non-toxicity nature have laid the foundation for extensive biopolymer applications in fields such as nutrition, pharmaceuticals, and medicine.

Classifications of biopolymers can be approached from a number of angles. Firstly, they can be categorised based on their chemical composition, such as sugar, starch, and cellulose. Secondly, the source of origin serves as another classification criterion, differentiating between natural, synthetic, or microbial biopolymers. Lastly, structural classification is determined through the examination of their monomeric units, including polysaccharides, proteins, and polynucleotides

The structural composition of biopolymers varies depending on source of origin, and exposure to environmental factors which can impact their structural integrity and functionality. Biopolymers are predominantly characterised by their biocompatible and biodegradable chemical composition regarded as unlikely to lead to environmental toxicity. There are exceptions as exemplified by the biotechnological synthesis of non-biodegradable polymers from renewable sources like polythioesters. These polymers are synthesised by microorganisms but resist microbial degradation, challenging the paradigm of biodegradability, (Steinbüchel, 2005).

3.9.1 Polysaccharide biopolymers

Sources of polysaccharide biopolymers are diverse and include chitosan derived from the outer skeleton of shellfish, alginate sourced from brown seaweed, gellan gum produced by the bacterium *Sphingomonas elodea*, carrageenan obtained from red seaweed, and xanthan gum synthesised by the bacterium *Xanthomonas campestris*. Several of these biopolymers are excreted by bacteria and denoted exopolysaccharides, representing high-viscosity, long-chain sugar monomer polymers. In addition to sugar residues, the chemical backbone of exopolysaccharides incorporates several

chemical moieties such as phosphates, pyruvates, and acetates, (Vu et al., 2009). Bacterial exopolysaccharides manifest considerable functional diversity and offer promising potential for biotechnological applications. The limited availability of genomic data on exopolysaccharide production presently limits the optimisation of industrial applications, though the comparatively compact prokaryotic genomes offer further opportunities for future research into renewable polysaccharide production.

Several polysaccharide biopolymers can be modified by alteration or replacement to their hydroxyl groups, providing a wide range of additional functionalities (Dassanayake et al., 2018). The source and type of polysaccharide influences the properties displayed such as gelling potential, mechanical, chemical and degree of polymerisation, creating the potential for an extensive range of biopolymer applications. Among the numerous polysaccharide biopolymers available, several have been extensively researched, chemical structures identified, and functions studied. An overview of the source, structure, and function of several commonly encountered biopolymers is summarised in *[Appendices: Table 25]*.

3.9.2 Bacterial extracellular polymeric synthesis (EPS)

Biopolymer demand has substantially increased as they are biodegradable, biocompatible and have wide application across most industries. The demand continues to grow as new applications are sought to replace petrochemical polymers. Microbial polysaccharides are replacing traditional polysaccharide sources as they do not require purification during their extraction and can be biosynthesised within bioreactors ensuring a steady and reliable supply. In many instances, microbial biopolymers can be produced using waste materials as a carbon source for polymer chain biosynthesis.

Many bacterial biopolymers are extracellular, secreted from the cell to form a protective biofilm matrix. These biofilms enable diverse bacterial infections, providing immobilised cells within the matrix with environmental protection and facilitating their participation in biogeochemical processes contributing to nutrient cycling and bioremediation, (Flemming & Wuertz, 2019; Yadav, 2018). Biofilms provide nutrient-rich extracellular polymeric substances (EPS) serving as ideal incubators for fostering microbial cell diversity, (Penesyan et al., 2021).

The EPS serves as a conduit for transportation and communication among immobilised cells within the matrix. Concentration gradients of oxygen, nutrients, pH, and metabolites give rise to differential conditions within the matrix. The matrix forms an intercellular ecosystem, functioning as a multicellular structure and facilitating a chemical signalling system between cells known as quorum sensing. The chemical signals act as a mechanism for communication which facilitates autoinducers to regulate gene expression for complex processes such as sporulation, (Davies et al., 1998; Donlan, 2002; Rivas et al., 2005; Ruiz et al., 2008; Rutherford & Bassler, 2012; Von Bodman et al., 1998; Waters & Bassler, 2005). Quorum sensing and intercellular chemical signals within the EPS as observed in *Bacillus subtilis* also evokes cell differentiation within the cellular community, (López et al., 2009; Marlow et al., 2014; van Gestel et al., 2015; Veening et al., 2008)

EPS consists of biopolymers and complexes of proteins, polysaccharides, nucleic acids, and humic substances. The interplay between these chemical constituents results in a three-dimensional architectural matrix containing spaces that accommodate cells, safeguarding them from environmental stresses such as dehydration and fluctuations in pH. The EPS matrix varies depending on the cell type producing the polymeric substances. Functionally the EPS can be classified into several categories, primarily structural, sorptive, surface-active, redox-active, and nutritive, all of

which are important factors when considering biopolymer encapsulation. These properties endow EPS with the capacity to resist desiccation, biocides, inorganic pollutants, and UV radiation, (H. C. Flemming & Wingender, 2010).

Mechanically the polymer matrix serves as a protective shield, withstanding chemical toxins, mitigating the impact of mechanical stress, and forming a barrier against antimicrobial agents that might disrupt the cell community. Within the matrix immobilised cells metabolically adjust, resulting in slower cell growth. The reduction in cell activity serves to limit the damage inflicted by environmental toxins or antimicrobial agents that could potentially penetrate the polymeric matrix exterior.

The extraction of EPS biopolymers for use in cellular encapsulation presents intriguing possibilities for creating a non-hostile cellular environment which can shield cells and enhance their metabolic performance. Consequently, artificial biopolymer encapsulation may invoke a metabolic response with periods of dormancy and persistence thereby extending the encapsulated cell viability when exposed to antimicrobial stress.

3.9.3 Cellular mechanisms involved in extracellular polymeric production

Bacterial polysaccharides found within the EPS exhibit characteristics based on the bacterial species responsible for the EPS production. The EPS originating from Gram-negative bacteria is generally neutral or polyanionic. In contrast, Gram-positive bacteria will produce an EPS that is cationic. The polysaccharide composition indicates a dynamic synthesis process linked to changes in the environmental conditions, *Figure 12*, (Flemming & Wingender, 2001; Mayer et al., 1999; Ruiz et al., 2008; Waters & Bassler, 2005).

Bacterial polysaccharides are mostly synthesised intracellularly with the exceptions of dextran and levan, *[Appendices: Table 26]*. Both homopolysaccharides are synthesised extracellularly and are secreted into the extracellular space where enzymes facilitate their polymerisation. The formation of mucoid colonies serves as an indicator of high concentrations of secreted exopolysaccharides. (Rana & Upadhyay, 2020). The production of bacterial exopolysaccharides is orchestrated through four distinct cellular mechanisms, each playing a pivotal role in the biosynthesis process. These mechanisms include the ATP-binding cassette (ABC) transporter pathway (Martinez et al., 2020; C. Yang et al., 2021; Zaynab et al., 2021), the synthase pathway (Rana & Upadhyay, 2020), the Wzx/Wzy dependent pathway (Rana & Upadhyay, 2020; Schmid, 2018), and a fourth pathway which synthesises the extracellular polysaccharide using a single sucrase protein (Nanjani & Soni, 2012).

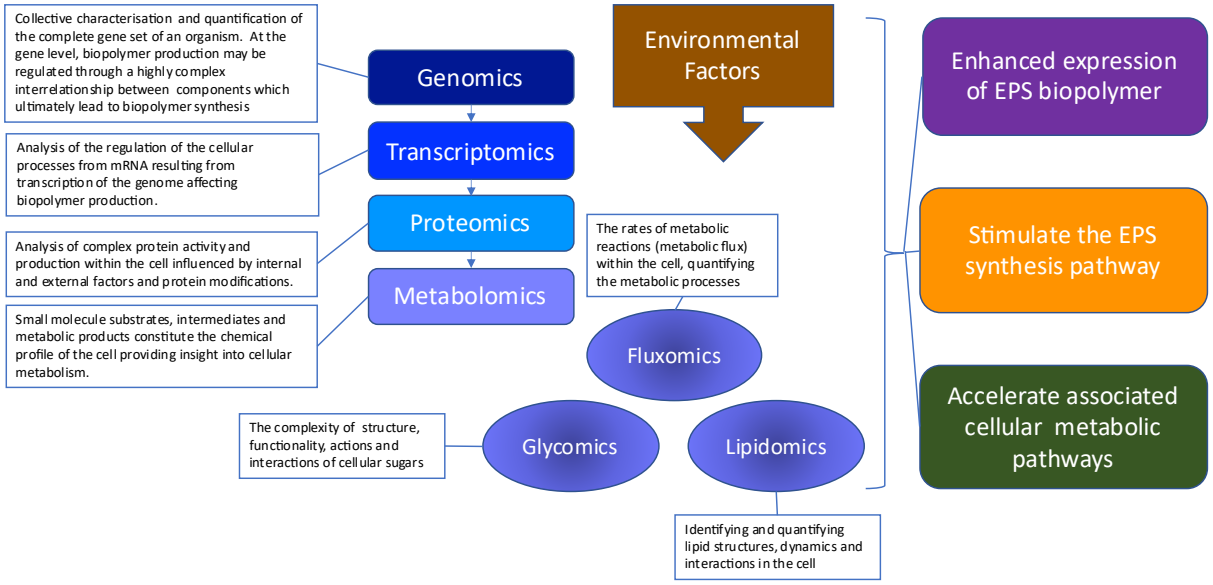


Figure 12: Cellular mechanisms involved in the production of bacterial EPS. The identification of specific genetic sequences responsible for encoding the biosynthetic machinery for EPS in the bacterial genome offers a pathway for enhancing or over-expressing EPS production. Environmental factors such as pH, temperature, ratio of carbon to nitrogen (C/N), availability and source of carbon, and oxygenation also exert an influence over EPS synthesis through these mechanisms, (Staudt et al., 2011). An optimal C/N ratio for exopolysaccharide expression is reported as 10:1, (Xiaotong et al., 2012)

3.9.4 Microbial producers of extracellular polysaccharides

Within the EPS exopolysaccharides that reside within the biofilm, (O'Gara, 2007) are found in embedded proteins, (O'Neill et al., 2008) and extracellular DNA, (Mann et al., 2009). Major producers of exopolysaccharides include bacteria *Sphingomonas, Pseudomonas, Acetobacter, Escherichia, Bacillus, Xanthomonas campestris, Zymomonas, Pseudomonas, Mycobacterium, Corynebacterium, Erwinia,* and *Azotobacter*, in addition to several halophilic archaea, (Czaczyk & Wojciechowska, 2003; Delattre et al., 2016; Rana & Upadhyay, 2020). Biopolymers are readily obtained from the extraction of exopolysaccharides, a process economically more advantageous than obtaining from non-microbial biopolymer sources, *[Appendices: Table 26]*.

The extraction and purification of exopolysaccharides are contingent upon the source and location of the biopolymers. Capsular polysaccharides are intracellular and therefore less accessible as they are bound to the cell. In contrast, extracellular polysaccharides are electrostatically attached to the cell surface making removal more economical, (Sugumaran & Ponnusami, 2017). Extraction from either the cell-wall complex or the polysaccharide capsule around the cells can be executed using enzymatic or chemical processes, (Jindal & Singh Khattar, 2018; Linton et al., 1991; Pace & Righelato, 1980; Smith & Pace, 1982; Sutherland, 2008). The polysaccharide yield is maximised by ensuring a non-alkaline extraction process which avoids glycoprotein cleavage preventing the unwanted co-extraction of proteins, (Slodki & Cadmus, 1978). A cost-effective, efficient, and non-invasive biopolymer extraction can be achieved using 1.5M NaCl solution on both Gram-positive and Gram-negative bacteria, (Chiba et al., 2015). Further refinement in the separation of protein and polysaccharide components can be achieved using molecular mass separation utilising a discontinuous electrophoretic system.

3.9.5 Protein-based biopolymers

Proteins serve as prominent biopolymers with diverse origins, encompassing both botanical, such as soy, and zoological sources like wool, silk, gelatine, and collagen. In the context of biotechnology and material science, their biocompatibility and enzymatic degradability are subject to modulation, primarily through techniques like crosslinking. Crosslinking enhances the structural integrity of proteins, rendering them useable for applications necessitating robust structural support, such as the bioprinting of protein matrices, (Nagarajan et al., 2019).

Historical narratives trace the inclusion of proteins into cementitious mixes as documented and recorded in ancient palm leaf texts at Padmanabhapuram Palace in India (Shivakumar & Selvaraj, 2020), the inclusion of casein protein admixtures (Brzyski et al., 2021), and the introduction of bovine blood and egg white into lime mixes (Sayre, 1976). The historical narratives suggest these organic materials would improve the binding between lime particles, diminishing pore size and preserving the material porosity to manage moisture dynamics, (Thirumalini et al., 2018).

Several natural proteins of non-microbial origin are readily available from incorporation as pozzolans, a choice of artisans to improve the texture, workability, adhesion, and performance of lime-based construction materials, *[Appendices: Table 27].*

3.9.6 Gel and polymer capsule formation

A gel forms when a charged polymer is introduced to an oppositely charged multivalent counter-ion. The process to generate this biocompatible matrix is relatively straightforward and inexpensive. A commonly employed gel is alginate, composed of sequenced 1,4-linked β-D-mannuronic and α-D-guluronic acid residues. Divalent cations (Ca^{2+}) can induce gel formation by binding to the guluronic residues forming electronegative cavities, the more abundant the guluronic residues the stronger the gelation. Calcium chelators such as phosphate or lactate, or displacement cations such as sodium or magnesium will hinder the formation of a stable gel.

Chitosan, a polyglucosamine polysaccharide similar to alginate in stability, may interact with cell membranes, limiting its utility in cell encapsulation. Pectin, a linear polymer of 1,4 α-D-galacturonate, binds to calcium ions to form a gel. A pectin gel is more resistant to chelation by competitive ions and, when combined with alginate, produces a robust pectate-alginate hybrid, (Tóth et al., 1989).

The process of creating thermally stable gel beads involves introducing a warm polymer solution into cold water, though very high polymer temperatures can negatively impact cell viability during encapsulation. Lower-temperature polymers offer a solution to this issue but may result in less resilient capsules that dissolve at lower temperatures. Research into designing temperature-sensitive triggers for capsule materials at specific temperatures is warranted. To enhance polymer capsule robustness, polymers can be mixed with other biopolymers such as locust bean gum or taurine, (Guiseley 1989, Audet, 1989, Cairns 1986, Murano 1998).

The size and solubility of the gel matrix within a newly formed gel capsule dictate its surface porosity, which, in turn, influences permeability. Several methods can be employed to control permeability. One approach is to introduce an outer membrane around the capsule, offering greater control over the exchange of substances between the contents within the capsule and the surrounding environment. This may introduce performance issues raising challenges related to

biocompatibility, biosustainability, and potential impairment to the capsule release mechanism, possibly affecting cell viability. Early studies have utilised poly-L-lysine as a high molecular weight polyamine coating, with secondary coatings of polymers such as alginate, (O'Shea 1984), polyethyleneimine, (Sun and O'Shea 1985, poly-L-ornithine, poly-L-glutamate, (Burgarski 1993), and chitosan, (McKnight 1988). Blending biopolymers or co-extruding a hydrophobic polymer with a hydrogel can enhance biopolymer resistance to degradation and slow down cell release from the gel capsule, (Dupuy, 1988). Another straightforward method involves adding additional outer layers by co-extruding cell-laden droplets of gel into a coating polymer, such as through interfacial coacervation, passively coating an additional layer on the capsule surface with an oppositely charged polymer.

The exterior composition of the exopolysaccharide capsule plays a critical role in determining its functional characteristics. Surface-active exopolysaccharides exhibit amphiphilic properties, featuring both hydrophilic and hydrophobic attributes, which influence the chemical reactivity of the polysaccharide. Each polysaccharide component facing the exterior environment governs the sorptive, surface-active, structural, and redox-active functionality of the capsule's exterior, (Kanamarlapudi & Muddada, 2017). Electroactive bacteria such as *Escherichia coli* can exhibit redox-active properties, which are useful for attracting and decontaminating environmental pollutants, including uranium, arsenate, and silver ions, (Freitas et al., 2011; Kang et al., 2014).

The structural benefits imparted by exopolysaccharides are important in protecting against external forces that could damage the cells within the gel matrix, and for improving water retention by extending periods of hydration and viability of the contents within the capsule.

3.10 Biopolymer capsule structure and content release mechanisms

The encapsulation polymer structures establish a selectively permeable polymeric barrier, segregating the interior contents from the surrounding environment. Variations in the choice of biopolymer materials exert a pronounced influence on the structural robustness, porosity, characteristics, and overall functionality of the encapsulated entity and its internal constituents. The primary objective of the encapsulation procedure is to sustain the encapsulated contents within a transient ecosystem conducive to cellular viability and proliferation. The retention of live cells within the capsule is subject to a finite temporal constraint, necessitating the efficient delivery and subsequent release of the contents.

3.10.1 Types of encapsulation structures

Encapsulation structures are predominantly capsules, microcapsules, or nanotubes, each of which serves as a secure enclosure for live cells, spores, or bioactive compounds. The selection of the most suitable structure is contingent upon the specific requirements of the intended application. Enhancements can be made to the polymer material to facilitate the efficient diffusion of gases, delivery of nutrients, and the removal of metabolic waste from the contents within the capsule.

To augment the properties of the polymer, modifications such as the introduction of secondary polymer structures, the integration of stimuli-responsive elements, or the incorporation of 'intelligent' or smart materials, capable of executing limited programmed responses, can be implemented, *Table 5*.

Table 5: Three examples of cell delivery vehicles which modify the encapsulating polymer, controlling content delivery and capsule content release

Encapsulation vehicle	Construction and composition	Properties	Advantages and disadvantages	References
Nanotube embedded polymers	Carbon nanotubes are embedded into the polymer matrix. The contents can be released from the nanotube using one of several mechanisms such as rupturing the nanotube with a laser or heating the contents which expand and are forced out of the tube.	The nanotubes will heat or rupture when absorbing infrared radiation between 700 to 1100 nm. Nanotubes range from 100 µm to 1000 µm in size.	Warming is achieve using molecular vibration, but the vibration or heat may cause cell death before the cells can be released. Sunlight is sufficient as a heating source to trigger nanotube content release though as a mechanism it is unpredictable in temperate climates.	(Iijima, 1991; Pastine et al., 2009; Prakash & Chang, 1995; Rodes et al., 2014)
Microcapsules with embedded chromophores	Chromophores embedded into the microcapsule polymer shell can be reversibly triggered by light to alter their structural configurations, such as switching between hydrophilic or hydrophobic properties.	Light sensitive chromophores such as azobenzenes undergo conformational change when exposed to light, changing their dipole moment, altering polymer permeability.	Polymer embedded chromophores can increase or decrease the polymer porosity and altering contents release. Studies are limited to chromophore embedded in synthetic polymers.	(Alfieri et al., 2021; Marder et al., 1997; Tao et al., 2004)
Microcapsules with embedded 'smart' materials	Smart materials are self-actuating in response to external stimuli. Smart materials are important features of 3D additive manufacturing, based on the work by Skylar Tibbits, Self-Assembly Lab, MIT, 2015.	Cellulose in conjunction with gels, copolymers, and nanoparticles, can incorporate smart responses to external stimuli such as pH, temperature, light, electricity, and mechanical forces. Blended 'smart' cellulose as the encapsulating polymer will respond to external stimuli, by changing porosity, altering capsule shape and triggered content release.	Cellulose is nontoxic, mechanically robust, biocompatible, and hydrophilic. The biopolymer demonstrates a high sorption capacity and relative stability over a broad temperature range.	(Correa et al., 2015; Klemm et al., 1998; Qiu & Hu, 2013)

3.10.2 Capsule release mechanisms

The release of content from capsules can be initiated through the manipulation of polymer morphology. Release triggers are able to be site-specific, stage-specific, or responsive to external stimuli such as pH, temperature, ionic strength, magnetic fields, light, irradiation, or osmotic shock, (Zhang et al., 2012). The release mechanism primarily arises from either the degradation of the polymer structure or the incorporation of trigger elements into the polymer matrix.

Polymer degradation can occur through either biotic or abiotic mechanisms. Biotic degradation commences with microorganisms catalysing the breakdown of chemical bonds within the polymer through enzymatic action. This enzyme-catalysed polymer degradation is analogous to the processes observed in proteins and polysaccharides. In the degradation of proteins, proteases effectively catalyse the unfolding of protein structures into amino acids, which can subsequently be further degraded to generate CO_2 or methane and water. Polysaccharide degradation similarly involves enzymatic activity targeting the glycosidic bonds of polysaccharides, leading to the production of smaller sugar units. In contrast, abiotic degradation relies on non-enzymatic physical and chemical environmental processes, including the natural degradation of polymer molecule chains. In cases where biotic or abiotic triggers are employed to dismantle the polymer, the mechanism must facilitate the on-demand degradation of the polymer. Despite its complexity, there exist notable examples of targeted polymer degradation for the controlled release of pharmaceuticals or food additives. An illustrative example is the degradation of pectin within tablets entering the large intestine, which is catalysed by colonic microbial-derived enzymes, resulting in the dissolution of pectin and the release of the drug, (Morales-Medina et al., 2022).

Incorporating trigger elements into the polymer, such as carbon nanoparticles and chromophores, can sensitize the capsule to external stimuli, enabling a response to light at specific wavelengths. These stimuli and trigger releases are designed to minimise any harm to encapsulated cells within the polymer matrix, as highlighted in *Table 5*. Given the broad spectrum of light, it is possible to accommodate multiple trigger points at different wavelengths. The selection of wavelengths can be made with consideration for ease of focus and minimal interference with the cellular processes within the encapsulated cells. However, a key challenge in utilizing light as a trigger is the limited accessibility to the delivery point of the capsule contents. Furthermore, it is crucial to avoid high-energy wavelengths that could induce photo-vibration in biomolecular structures, potentially leading to heating and premature degradation of the biopolymer.

3.11 Biopolymer availability and selection

The selection of an appropriate biopolymeric material, whether it be a polysaccharide or protein-based polymer for encapsulation necessitates careful consideration of several critical factors. These factors encompass polymer availability, biocompatibility, physical and chemical robustness, responsiveness to external stimuli, and compatibility with the encapsulation manufacturing process. Of particular significance is the requirement for these polymers to exhibit high levels of physical and chemical resilience, as this is imperative for their utilisation in an environment characterised by high alkalinity, such as a lime solution ($Ca(OH)_2$).

Among the abundant naturally occurring biopolymers, alginate and cellulose stand out as prominent choices as encapsulation materials due to their sustainability, ease of gelation, biocompatibility, biodegradability, and non-toxic nature.

Alginate, for instance, readily undergoes gelation when exposed to divalent cations, most notably Ca^{2+}. The resulting hydrogels can be further cross-linked to establish three-dimensional structural compartments. The alginate hydrogel compartments fulfil the role of delivery vehicles for cells, facilitating their safe transportation while providing a naturally moist environment. This is of importance for targeting treatment sites required in both pharmaceutical drug delivery and environmental applications.

Cellulose, in comparison, offers a diverse range of physical forms, including crystalline structures, fibrils, and hydrogels, each endowing the polymer with a wide spectrum of chemical, mechanical,

and biological properties that support effective encapsulation. In particular, cellulose sulphate stands out as a robust capsule polymer that retains its integrity across a wide pH range. Simple co-extrusion expressed through a vibrating nozzle has captured up to 5 million bacteria per capsule in 0.7 mm droplets embedded in cellulose sulphate (Gunzburg et al., 2020).

Sourcing alginate and cellulose from natural sources, such as seaweed and plants, presents a challenge of impurity removal. Such impurities, which include heavy metals, endotoxins, and organic compounds like hemicellulose and lignin, must be effectively eliminated from the raw extractions. Failure to do so could jeopardize the viability of encapsulated cells. An alternative approach to circumvent this issue involves the acquisition of these biopolymers through bacterial synthesis, eliminating the need for the cumbersome and costly impurity removal process.

While the abundance and inherent eco-friendliness, including renewability, biocompatibility, and biodegradability, position cellulose as a strong contender for replacing petroleum-based polymers, transitioning towards widespread cellulosic biopolymer substitution remains subject to challenges. Overcoming these obstacles in the coming years is crucial for unlocking the full potential of this environmentally sustainable material. Key areas demanding attention include the development of cost-effective, and time-efficient methods for all stages of cellulosic biopolymer production. This encompasses streamlining cellulose isolation from lignocellulosic sources, tailoring cellulose into suitable forms, and optimising polymerisation processes. The abundant, highly flexible scope offered by cellulose deserves further investigation as one of two potential encapsulation biopolymers along with alginate.

3.11.1 Bacterial Cellulose, physical and chemical properties

The mechanical, structural, thermal, and fluidic attributes of cellulose, an abundant and sustainable resource, render it highly suitable for applications in building construction. Among the various cellulose materials, bacterial cellulose nanofibers stand out as one of the most mechanically resilient organic substances. When cellulose fibres are fragmented into nanoscale constituents, they give rise to fibrillated cellulose. Fibrillated bacterial cellulose nanofibers can achieve dimensions as small as 5 nm, contrasting to plant cellulose, which averages around 100 µm, supporting bacterial cellulose as a useable encapsulation biopolymer (Li et al., 2021).

The density of the hydroxyl groups on the cellulose fibrils facilitates inter and intra-molecular hydrogen bonding conveying robust mechanical properties to the fibrils. The structural arrangement of the fibrils further strengthens the mechanical properties, supporting a high-strength, lightweight cellulose structure, which can be further improved by bonding the hydroxyl groups to other polymers (Biswas et al., 2017).

Fibrillated bacterial cellulose can be economically sourced from bioreactor cultures, (Shoda & Sugano, 2005; Wang et al., 2019). Chemical modification to the surface functional groups of the cellulose molecules modifies the cellulose membrane pore size. The reduction in pore size combined with water binding within the matrix, ensures resilience to desiccation. The modified porosity within the hydrogel structure maintains the movement of gases and nutrients enabling long-term support to encapsulated cells within the matrix, (Drachuk et al., 2017; Sun et al., 2018).

Crosslinking between cellulose fibrils confers elastic and mechanically robust properties to the hydrogel, while enabling the retention of nutrients and moisture (Oyen, 2014; Peppas et al., 2000). Modifications to the crystalline bonds through etherification synthesise cellulose ether derivatives

altering the solubility and other physicochemical properties such as biodegradability and hydrophilicity, extending the range of applications (Bhaladhare & Das, 2022; Kabir et al., 2018; Klemm et al., 2005; Rose & Palkovits, 2011; Sannino et al., 2009). Ether derivatives of cellulose have excellent water retention capacity and are utilised in the cementing process in oil extraction. During drilling for oil, fluid circulation loss is a serious problem and injecting cement improves the oil recovery process. Cellulose and cellulose derivatives termed cellulosics, commonly take this role as fluid loss additives maintaining the required rheological properties of the cement (K. Liu et al., 2021).

3.11.2 Sustainable synthesis of renewable bacterial cellulose

Cellulose sourced from bacteria is produced at a much lower energy cost and mitigates the risk of environmental pollution compared to the cellulose extraction process from wood (Brown Jr, 1999). Cellulose exhibits excellent mechanical and chemical properties and similar to alginate, demonstrates a capacity to retain water.

The biosynthesis of cellulose occurs utilising carbon available from the surrounding medium, which is polymerised into single, linear ß-(1-4)-glucan chains through the catalytic action of enzymes found within the cytoplasmic membrane, *Figure 13*. These chains traverse through pores in the outer membrane and reach the exoplasm, where they undergo assembly into sub-fibrils between 1 to 15 chains in length. These sub-fibrils undergo progressive crystallisation culminating in the formation of microfibrils a step in the progressive assembly toward forming cellulose fibres, (Bureau & Brown, 1987).

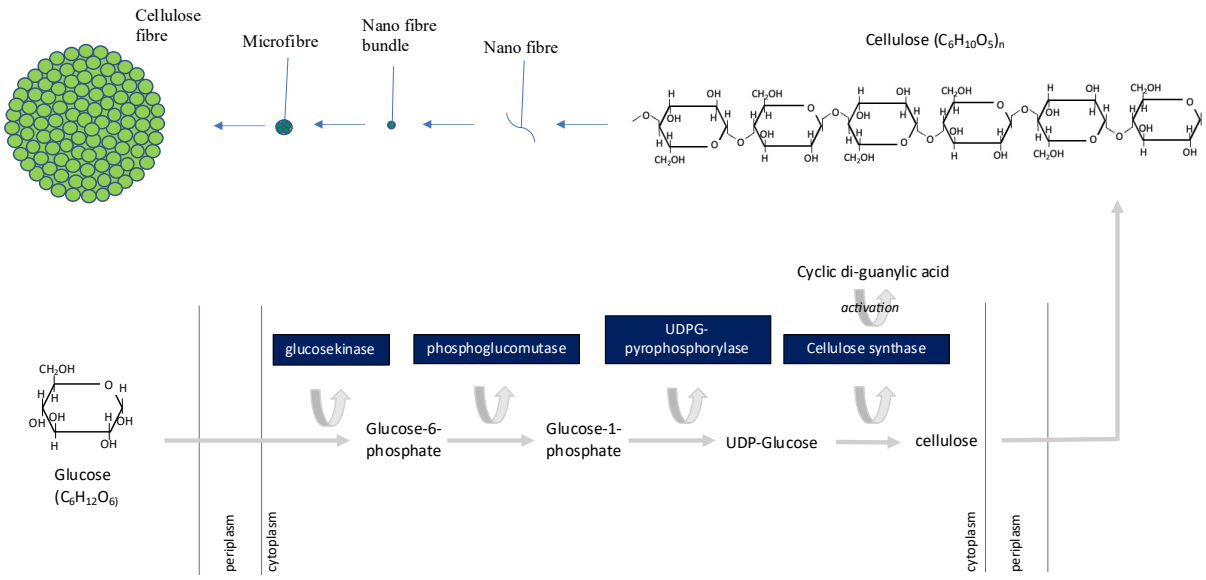


Figure 13: The biochemical pathway of cellulose synthesis in Acetobacter xylinum. The cellulose yield can be enhanced by utilising modified A. xylinum strains to attenuate the ketogluconate pathway, diverting glucose from forming ketogluconate. The diversion of the glucose carbon source to the above pathway augments a higher cellulose output. Nuclear magnetic resonance (NMR) of bacterially synthesised cellulose reveals two polymeric structures, cellulose I and II, the latter being more thermodynamically stable through greater hydrogen bonding *(Poddar & Dikshit, 2021)*

Bacterial cellulose production is an eco-friendly and renewable process that can utilise various organic fermentation media as carbon sources, (Velásquez-Riaño & Bojacá, 2017). Industrial and human waste can serve as viable carbon substrates, offering diverse sugars, nitrogen, and essential nutrients, (Horue et al., 2021). Other carbon sources include tobacco waste (Ye et al., 2019), and landfill waste, which if left to deteriorate are material contributors to CO_2 and CH_4 greenhouse gas generation. Landfill waste is composed of approximately 70% organic matter providing an ample carbon source to generate a fermentation medium (Dahiya et al., 2018).

Efficiency in bacterial cellulose production is fundamentally governed by the characteristics of the bacterial strain employed. *Gluconacetobacter hansenii*, a well-studied bacterial cellulose producer, is recognized for its efficiency. Nevertheless, this strain's slow growth leads to reduced cellulose production and a proclivity for spontaneous mutation into non-cellulose-producing strains. An approach to address these limitations involves the development of stable bacterial cellulose biosynthesis systems by recombinant expression using co-expression plasmids, pBCS and pCMP, of the bacterial cellulose synthase operon (*bsc*ABCD) and the upstream operon (*cmcax, ccp Ax*) introduced into *Escherichia coli.* The resultant dense fibre network is approximately 1000-3000 µm in length and has a diameter of 10-20 µm. The use of plasmids for recombinant expression into different strains of *E. coli* offers a basis for comparison to assess which strain is most able to bear the metabolic load on *E. coli* facilitating an efficient commercial production of cellulose. The comparative production by recombinant expression in *E. coli* is more advantageous for bacterial cellulose production than that found in the genetic source *G. hansenii* (Buldum et al., 2018). Within *Acetobacter,* cellulose synthase encodes three (*acs*AB, *acs*C, and *acs*D) or four (*bcs*A, *bcs*B, *bcs*C and *bcs*D) subunits (Kawano et al., 2002; Lee et al., 2014). Within the operon *bcs*A and *bcs*B encode and regulate cellulose synthesis whereas *bsc*C is likely to form the protein membrane pores which allow cellulose secretion and *bcs*D is responsible for the formation of the cellulose nanofibrils. The upstream operon also plays a crucial role in enhancing cellulose synthesis and ensuring the extracellular transportation of the cellulose chains.

Modification to newly synthesised bacterial cellulose can occur *in situ*, with the addition of exogenous molecules at the onset of the fermentation process, (Cacicedo et al., 2016), or *ex situ*, where modifications are introduced after the synthesis and purification, (Ul-Islam et al., 2021). Modifications change the cellulose fibre composite density, fibre porosity, and chemical functionality. The range of bacterial cellulose nanocomposites produced through these methods is extensive, featuring the inclusion of graphene nanosheets, metal oxides and carbon nanotubes. These materials are gradually replacing petroleum-derived plastics and opening new horizons in flexible electronics. The incorporation of remote-sensing chemical triggers and time-release compounds presents opportunities for further research in time-release encapsulation applications thereby enhancing the development of traditional materials.

3.11.3 Bioreactor design for bacterial cellulose production

The long-term production and availability of bacterial cellulose necessitates the implementation of an industrial process proficient in culturing bacteria and the efficient and economical extraction of cellulose. Bioreactors offer a controlled environment wherein the biochemical processes crucial to the production of natural products from cellular metabolism are cultivated and harvested. More recently, bioreactors have assumed an increasingly important role in providing source polymers for the bioprinting of cellular structures including tissue and organ biosynthetics.

One challenge for commercial development for the biosynthesis of cellulose is the impact of mechanical agitation on the bacterial culture. Stirring or other forms of movement are often

employed to ensure that the producing cells receive an adequate supply of oxygen and essential nutrients. Overly agitating the producing cells results in cellulose-producing bacteria converting into non-cellulose-producing mutants (Jamsheera & Pradeep, 2021). More recent advances in bioreactor design have enabled the scale-up of commercially produced bacterial cellulose, reducing the effect of mutation conversions into non-cellulose producers, and overcoming the increasing viscosity of the incubation fluid due to the cellulose accumulation, *[Appendices: Table 28]*.

3.11.4 Post-synthesis biopolymer strengthening

Following the synthesis of bacterial cellulose, the biopolymer can undergo further reinforcement through a process known as cross-linking. Cross-linking can be accomplished by the application of physical force, exposure to chemical stimuli, or subjecting the cellulose molecule to radiation, forming links between the polymer chains, *Table 6,* (Zainal et al., 2021). These processes create a hydrogel, a three-dimensional hydrophilic polymeric network structure into which cells can be incorporated through either covalent or physical attachment of the cells to the gel. The control of cell release from the hydrogel is achieved by facilitating cell release through expanding or contracting the gel, modifying the gel pH, and temperature, or altering the gel ionic strength.

Table 6: Methods for cross-linking polymer chains to form hydrogels. The rigidity of the gel matrix is determined by the density and strength of the bonds, with each cross-linking technique conferring varied responsive properties into the hydrogel *(Bhaladhare & Das, 2022)*. The hydrogel structure can be tailored during the cross-linking process by altering the density, biodegradability, mechanical strength, chemical reactivity, and porosity

Physical Crosslinking	**Chemical Crosslinking**	**Radiation Crosslinking**
Physical crosslinking occurs with macromolecular entanglement, ionic attraction, electrostatic interactions, hydrogen bonding or hydrophobic interactions (Akhtar et al., 2016). Physical crosslinking is usually reversible. It occurs during freeze-thaw and photo-initiator treatments. Adding polyvinyl alcohol to the cellulose nanocrystal during freeze-thaw improves the compression performance, reducing the gel porosity, (Butylina et al., 2016). Physical cross-linking is utilised for biopolymer applications in tissue engineering, tissue implants and drug delivery systems.	Chemical cross-linking occurs between covalent bonds, linking cellulose polymer chains with chemical crosslinked agents (Hu et al., 2019). Chemical cross-linking is irreversible, which results in the production of robust hydrogels. Citric acid is an inexpensive, nontoxic, hydrophilic, crosslinking agent (Gyawali et al., 2010; Stone et al., 2013). forms strong hydrogen bonds, improving water absorption and hydrogels with thermal stability at room temperature (Ghorpade et al., 2016, 2017) Covalently cross-linked hydrogels are highly stable and resistant to environmental pressures. Cell release control mechanisms can include the use of modified covalent bonds. Chemical cross-linking is utilised in biomedical, agriculture and pharmaceutical product development.	Gamma, ultraviolet and electron beam radiation invoke polymer cross-linking (Mizera et al., 2012). Replacing potentially toxic chemical cross-linking agents with radiation treatment enables designs of hydrogels for use in environments sensitive to chemical pollutants. Applications for radiation crosslinking are found in the food, biomedical and environmental sectors.

Despite their individual advantages in bioengineering applications, cellulose and alginate gels display divergent mechanical properties. Cellulose gels, while abundant and biocompatible, tend towards brittleness and limited rupture strength. Conversely, alginate gels boast elasticity and high breaking points. A promising approach, therefore, lies in the creation of composite gels that leverage the strengths of both polymers. By strategically combining cellulose and alginate, researchers can fine-tune crucial parameters like rupture strength, Young's modulus, and environmental stability, tailoring the resulting material to specific application demands. This strategy opens exciting avenues for bioengineering innovations by allowing for customized control over mechanical properties within a readily available, biocompatible scaffold.

3.12 Bacterial biomineralisation

Active bacterial biomineralisation is the phenomenon wherein bacteria secrete inorganic minerals utilising energy from the cell. Biomineral formation is a consequence of cellular metabolism incorporating selective cations and contingent on specific environmental conditions for the mineralisation to occur. The most common biominerals produced are calcium carbonate and calcium phosphate. In the natural world, these minerals are combined with biopolymers collagen or chitin to synthesise load-bearing structures such as bones and shells. The bacterial-induced mineralisation and subsequent mineral formation arise from the manipulation of the chemical environment surrounding the organism brought about by the metabolic activity of the cell.

Biomineralisation occurs across diverse environments where microbial metabolic processes lead to the production of $CaCO_3$. Several bacterial species have demonstrated the ability to generate optimal carbonate deposition and are outlined in *Table 7*. Biomineralisation is usually biologically controlled, biologically induced, or biologically mediated, (Achal & Mukherjee, 2015; Weiner & Dove, 2003).

Table 7: Bacteria demonstrating optimal calcium carbonate biomineralisation and deposition. Chemotrophic metabolic by-products from metabolic activity in precipitating bacteria may result in surface staining due to the formation of ammonia, nitrites, and hydrogen sulphide. Staining affects the limestone pore size, failing to prevent water entry or managing moisture movement out of the building

Microorganism	**$CaCO_3$ deposition**	**References**
Bacillus cereus	Identified *in-situ* generating a surficial protecting coat – *biocalcin*- from bacterial carbonatogenesis on Saint Médard Church. The coating improved resistance to water entry through bio-carbonation without any deterioration apparent on the building surface. Long-term issues can occur with the formation of endospores and biofilms on the building surface which may result in unsightly discolouration.	(Castanier, le Métayer-Levrel, et al., 1999; le Métayer-Levrel et al., 1999; Orial, 2000; Orial et al., 1993)
Myxococcus xanthus	Precipitation of $CaCO_3$ was produced down to a depth of >500 µm. Biofilm formation was not observed.	(Rodriguez-Navarro et al., 2003; Tiano et al., 1999)
Micrococcus sp. *Bacillus subtilis*	Calcium carbonate bio-precipitation reduced water entry on the building surface. Discolouration occurred, caused by bacterial metabolic by-products and fungal staining. Attempts to further improve $CaCO_3$ deposition by the introduction of organic matrix macromolecules from *Mytilus californianus* shells were unsuccessful resulting in low carbonate yields.	(Tiano et al., 1999)
Bacillus sp. Bacillus sphaericus	$CaCO_3$ microbial precipitation successfully reduced water absorption into limestone by around 50%.	(Dick et al., 2006)

The precipitation of $CaCO_3$ is regulated by several factors, including the concentration of calcium ions, the availability of dissolved carbon, the pH of the surrounding medium, and the presence of nucleation sites (Hammes & Verstraete, 2002). Incorporating microbially derived $CaCO_3$ into carbonate-based materials like concrete and lime has been reported to yield various beneficial effects, including reduced shrinkage during curing, enhanced resistance to freeze-thaw cycles and improved permeability (Achal et al., 2015; Bang et al., 2010; de Muynck et al., 2008, 2010; Ghosh et al., 2005; Jonkers, 2007, 2021; Jonkers & Schlangen, 2009; Wiktor & Jonkers, 2011).

One approach to include microbials into cementitious materials introduced spores of *B. sphaericus* into modified alginate-based hydrogels. *B. sphaericus* is an alkali tolerant ureolytic, decomposing urea forming ammonium (NH_4^+) and carbonate (CO_3^{2-}). In a high calcium environment this leads to the deposition of $CaCO_3$. The inoculated hydrogel was incorporated into concrete with the objective of autonomously repairing cracks and long-term maintenance of the substrate. The hydrogel immobilising the spores to provide a medium for the cells, when active, to precipitate around 70% $CaCO_3$ in weight, in and on to the hydrogel matrix. Measuring and comparing oxygen consumption between free spore bacterial applications and hydrogel immobilised spores provided a comparison for the potential to hydrogel immobilise carbonating bacteria in concrete applications (Wang, Snoeck, et al., 2014).

Numerous studies have employed several methodologies to evaluate the formation of microbial $CaCO_3$ deposition on stone surfaces. A summary of these approaches is presented in *Table 8*. Each of the studies concludes that microbial $CaCO_3$ deposits effectively reduce water ingress into the substrate layer without hindering moisture movement unlike the application of synthetic sealants.

Table 8: Overview of a range of methodologies applying and measuring the effectiveness of bacterial calcite surface deposits

Purpose of, and approach to the study	**Microbial source and inoculum composition**	**Evaluation of calcite formation**	**Reference**
Protection of building facades The application bacteria in a nutritional medium are applied directly to a limestone surface The 'biological mortar' consisted of the bacterial nutrient mix and limestone powder. The surface was repeatedly soaked with a standard nutritional medium	The study identified >100 different carbonatogenic strains isolated from carbonate-producing environments	Biocalcin formation was observed using a scanning microscope. As carbonated surfaces obscure new calcite formation an inert non-carbonate surface (brick) provided the control surface The study evaluated the enumeration of bacterial populations, measurement of surficial permeability and changes to the surface roughness by imprint moulding	(Le Métayer-Levrel et al., 1999)
Reinforcement of weathered porous calcareous stones A saturated calcium bicarbonate solution and calcite nanoparticles provide the Ca^{2+} and CO_3^{2-} ions. The solution was delivered onto	Organic matric molecules (OMM), aspartic acid-rich proteins control the biomineralisation of calcite. OMMs controlled the nucleation of $CaCO_3$ crystals within the weathered calcareous stone pores	Porosity, stone cohesion (determined by a drilling resistance measuring system), water uptake and hardness were assessed before and after treatment	(Tiano et al., 1999, 2006) (Gaggero & Scrivano, 2016; Tiano & Pardini, 2004; Vandevoorde et al., 2009)

the surface of porous stones the surface using a fine spray The study applied polypeptides to control $CaCO_3$ crystal formation	The stone was sprayed using $Ca(HCO_3)_2$ in a low concentration of polyaspartic acid to inhibit crystallisation forming in the solution A supersaturated solution of $CaCO_3$ was formulated using $CaCl_2$ and $(NH_4)_2CO_3$	New calcite crystal formation was evaluated using a calcein spray ($C_{30}H_{26}N_2O_{13}$) and measuring the fluorescence under UV light (495/515 nm).	
Brushed application of a saturated solution containing cultured cells in water Sterilised stone bioclastic limestone samples were inoculated with 4 ml of a B_4 culture (calcium acetate, 0.25%; yeast extract, 0.4%; dextrose, 0.5%; pH 8) and placed on a V-shaped glass rod in sterile Petri dishes soaked in sterile distilled water, incubated at 28°C for 15 days	Overnight cell cultures 6 x 10^6 cells cm^{-2} of *Micrococcus* sp. wild strain (BC434) and prototrophic *Bacillus subtilis*, (PB19) were brushed in several applications onto the upper stone surface Every 24 hours, the bacteria were coated with 1 ml of sterile B_4 nutrient medium	Bacterial samples showed a 60% reduction in water absorption using a capillary water absorption test soaking the samples in distilled water for 20 minutes	(Tiano et al., 1999)
Bio-deposition of $CaCO_3$ layer by *Bacillus* sp. Five strains of *Bacillus sphaericus* and one strain of *Bacillus lentus* were tested for ureolytic-based $CaCO_3$ precipitation Bacillus sp. was isolated from a biocatalytic ureolytic calcification reactor, suspended in a sterile 8.5 g l-1 NaCl solution, diluted and plated on precipitation agar. Optimal bacterial $CaCO_3$ deposition compared the differential precipitates by sample as measured by water absorption rate	Limestone samples inoculated with 1% of each *Bacillus* strain were incubated in airtight sterilised jars with liquid medium (3 g l^{-1} Nutrient Broth comprising 2.12 g l^{-1} $NaHCO_3$ and 10 g l^{-1}urea, and double deionised water) Positive colonies were selected based on microscopic visualisation of crystal formation over 10 days	7.5 g l^{-1} $CaCl_2$ added in two phases precipitated $CaCO_3$ onto biofilms formed on the limestone surface Deposited calcite crystals were visualised using a scanning electron microscope. Differential bacterial precipitates were measured by rate of water absorption. A saturation curve reached a constant 64% value after two days providing comparisons of water-repellent calcite formation	(Dick et al., 2006)
Induced carbonate biomineralisation using *Myxococcus xanthus* Investigates $CaCO_3$ precipitation and if additional protection is achieved without blocking, porous limestone or deleteriously effects moisture movement *Myxococcus xanthus*, a gram-negative, non-pathogenic aerobic soil bacterium can	*M. xanthus* was precultured in liquid medium CT (1%[wt/vol] Bacto Casitone and 0.1% [wt/vol] $MgSO_4.7H_2O$ in a 10 mM phosphate buffer, pH 6.5) and applied to the limestone	Weight change and X-ray diffraction determined the amount and composition of newly formed carbonate Texture and penetration depth of the carbonate production was determined by scanning electron microscopy Epitaxial growth on pre-existing calcite confirmed the formation of carbonate crystals during the first 10 days with minimal effect on moisture movement	(Chekroun et al., 2004; Rodriguez-Navarro et al., 2003)

induce carbonate precipitation in porous limestone The bacterium has no dormant spore phase which avoids unwanted metabolic products			
Bacterial carbonate precipitation forming an alternative surface treatment Investigated measures to protect concrete and lime surfaces against water damage, avoid pore blockers and eliminate environmentally incompatible repellents. Incorporating organic or inorganic protective coatings result in secondary issues such as differential thermal expansion coefficients or surface toxin production.	Cement mortar prisms were prepared and cured 28 days prior. Cultures utilised liquid nutrient culture medium of 3 g l^{-1} nutrient broth powder, 2.21 g l^{-1} $NaHCO_3$ and 10 g l^{-1} urea at 28 °C Prisms were immersed for 24 h in 0.3 and then 0.6 L of *B. sphaericus* stock culture prior to submersion in the nutrient solution	*Bacillus sphaericus* as an agent for carbonate precipitation was compared to organic and inorganic water repellents. Bacterial culture type and medium composition influenced $CaCO_3$ carbonation and crystal morphology Water penetration was measured using capillary water suction and weight change, and $CaCO_3$ morphology scanned using an electron microscope	(de Muynck et al., 2008, 2010)

Biologically controlled mineralisation occurs within one of two cellular locations. In extracellular mineralisation cations passively diffuse out of the cell into the surrounding exopolysaccharides. Intracellular mineralisation involves creating a vesicle inside the cell and isolating minerals within the space, *Table 9*. The vesicle and mineral are later secreted from the cell. An exception is *Achromatium oxaliferum* which precipitates calcite crystals into the intracellular space without the formation of vesicles (Head et al., 2000).

Biologically induced mineralisation precipitates minerals as a by-product resulting from the metabolic activity of the microorganism and interaction with the ions in the surrounding environment.

Biologically mediated mineralisation results from the interaction between organic and inorganic compounds independent of biological activity.

Table 9: Biomineralisation resulting from either extracellular or intracellular mechanisms (De Yoreo, 2003; Giuffre et al., 2013). The process may be triggered by HCO_3^- related pH changes (Chekroun et al., 2004; Dupraz et al., 2009; Görgen et al., 2021; Hoffmann et al., 2021; Perito et al., 2014), or gene mediation (De Wever et al., 2019; Mansor et al., 2015). Biodeposition of $CaCO_3$ is dependent on five factors, the pH of the environment, the availability of dissolved inorganic carbon, the availability of nucleation sites, the bacterial type, and the composition of nutrients available (Hammes & Verstraete, 2002)

Biomineralisation	Extracellular	Intracellular
Mechanism	Extracellular Mechanisms (Achal et al., 2015)	Intracellular Mechanisms (Benzerara et al., 2014; Cam et al., 2015; Head et al., 2000)
Energy Cost	Passive	Active

Process	Biomineralisation occurs with extracellular organic molecules	Biomineralisation occurs either from cellular metabolic activity or Mediated through gene expression
Precipitation	Biomineral precipitation triggers at the saturation index of the extracellular solution. Ca^{2+} activity and CO_3^{2-}, HCO_3^- activity increases while the energy threshold for nucleation to occur decreases	
Factors influencing mineralisation	**Biologically influenced mineralisation** results from bacterially manufactured organic polymers (glycoproteins and polysaccharides) in the EPS serving as nucleation sites and supported by a surrounding saturated ion solution	**Metabolic-induced mineralisation** occurs chemically as an increase in HCO_3^- ions modify the pH a shift which alters the CO_3^{2-} kinetics in the surrounding cell environment triggering mineralisation or **Gene controlled mineralisation** mediates aspects of expression of the nucleation and precipitation processes, such as performance of cell membrane ion pumps

3.12.1 Conditions leading to calcium carbonate biodeposition

Conditions driving the generation of sufficient concentrations of calcium and carbonate ions trigger $CaCO_3$ precipitation. This is illustrated when the ion activity product (IAP) exceeds the solubility constant (K_{so}) defining the saturation state (Ω), (Morse, 1983). Outlined in *Figure 14*, if Ω >1, precipitation will occur as the system is oversaturated, equation 14(a). As the amount of dissolved inorganic carbon available is dependent on the temperature and pressure of CO_2, the constants governing dissolution of CO_2 in water at 25°C and 1 atm, are included in the equation 14(b), (Stumm & Morgan, 1981).

(a)

$$Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_3$$

$$\Omega = a(Ca^{2+})\, a(CO_3^{2-})/K_{so} \quad \textit{with}$$

$$K_{so\ \text{calcite, } 25°C} = 4.8 \times 10^{-9}$$

(b)

$$CO_{2(g)} \leftrightarrow CO_{2(aq)}\ (pK_H = 1.468)$$

$$CO_{2(aq)} + H_2O \leftrightarrow H_2CO_3^*\ (pK = 2.84)$$

$$H_2CO_3^* \leftrightarrow H^+ + HCO_3^-\ (pK_1 = 6.352)$$

$$HCO_3^- \leftrightarrow CO_3^{2-} + H^+\ (pK_2 = 10.329)$$

$$\textit{With}\ H_2CO_3^* = CO_{2(aq)} + H_2CO_3$$

(c)

$$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O$$

$$Ca^{2+} + HCO_2^- + OH^- \rightarrow CaCO_3 + 2H_2O$$

$$2HCO_2^- \leftrightarrow CO_2 + CO_3^{2-} + H_2O$$

Figure 14: Equations outlining the dynamics of calcium carbonate precipitation. (a) $CaCO_3$ precipitation occurs when the ion activity product IAP is > the solubility constant (K_{so}). If the saturation state, Ω, is > 1 and the constants governing dissolving CO_2 in water (25°C and 1 atm) are given, the system is oversaturated, and precipitation will occur. By altering these parameters, microorganisms influence $CaCO_3$ precipitation, (Hammes & Verstraete, 2002). (c) dissolved CO_2 is normally in equilibrium with HCO_3^- and CO_3^{2-}, whereby the removal of CO_2 from the equation increases the pH resulting in $CaCO_3$ precipitation, (Dhami et al., 2014)

3.12.2 Photoautotrophic biodeposition of calcium carbonate

Photosynthetic microorganisms found in high calcium environments in marine and freshwater systems can trigger calcite precipitation. Within aqueous environments, photoautotrophs partner with heterotrophs, microorganisms which mediate $CaCO_3$ precipitation through reduction and oxidation reactions, utilising available urea, nitrates, sulphates, sulphides, and methane, (Arp et al., 2001; Plée et al., 2010; Zhu & Dittrich, 2016). Photosynthetic microorganisms have played a substantial role in the precipitation of carbonate contributing almost 70% of the carbonate rocks on the planet. Notably, the annual whiting events observed in North American lakes and subtropical seas are a consequence of the activities of cyanobacteria, (Altermann et al., 2006; Thompson et al., 1997).

3.12.2.1 Phototrophs – cyanobacteria and algae

Cyanobacteria exhibit a remarkable capability to precipitate $CaCO_3$ through mechanisms associated with photosynthesis. This precipitation process is initiated by the uptake of inorganic carbon during photosynthesis, which leads to an elevation in the pH of the surrounding environment. The properties of the outer cell membrane can also play a role in triggering this phenomenon. Cyanobacteria have also been observed forming $CaCO_3$ intracellularly. Bacterial viruses can induce cyanobacteria to lysate causing calcification due to carbonate dominating the inorganic carbon species in the environment. Virus particles such as viral capsids in the lysate may also stimulate precipitation to occur, (De Wit et al., 2015; Pacton et al., 2014; Peng et al., 2013; Xu et al., 2019).

Algae and cyanobacteria are primary microorganisms engaged in photosynthesis and related photoautotrophic processes leading to the precipitation of calcium carbonate, *Figure 15*. HCO_3^- enters the cytosol of the organism through the membrane where carbonic anhydrase catalyses the reaction to disassociate HCO_3^- into CO_2 and OH^-. The production and movement of hydroxyl ions into the immediate environment around the cell causes an increase in pH. If calcium ions are present in the surrounding environment, the increase in pH will encourage the formation of calcium carbonate crystals. By altering these precipitation parameters microorganisms can affect the precipitation of $CaCO_3$ (Hammes & Verstraete, 2002). Dissolved CO_2 is normally in equilibrium with HCO_3^- and CO_3^{2-}, whereby the removal of CO_2 from the equation contributes to an increase in the pH resulting in $CaCO_3$ precipitation, *Figure 14(c)* (Dhami et al., 2014).

Two metabolic pathways have been successfully combined by cyanobacteria, oxygenic photosynthesis and respiration, pathways which can occur simultaneously. In bacteria and algae, the primary production for assimilating inorganic carbon into organic carbon occurs via the ribulose biphosphate (RuBP) or Calvin-Benson cycle, though genomic studies have identified the existence of other autotrophic carbon fixation pathways within the ecosystem (Hügler & Sievert, 2010).

Within the thylakoid membrane, protein complexes play a crucial role in catalysing the respiratory and photosynthetic electron transport chain. In most cyanobacteria, photosynthetic electron transport primarily transpires in the thylakoid membrane, while the respiratory electron flow occurs in both the thylakoid and cytoplasmic membranes. Redox-active components such as the plastoquinone pool and the cytochrome b_6f complex are instrumental for both photosynthesis and respiration within the thylakoid membrane. Additionally, electron carriers like plastocyanin and cytochrome C_6 are found within the thylakoid lumen. The photosynthetic electron chain and respiratory electron flow are reviewed in detail in the literature (Andersson & Barber, 1996; Bendall & Manasse, 1995; Cooley et al., 2000; Gantt, 1994; Hippler et al., 1998; Kaneko et al., 1996; Mullineaux, 2014; Ort & Yocum, 1996; Schmetterer, 1994; Whitmarsh, 1998).

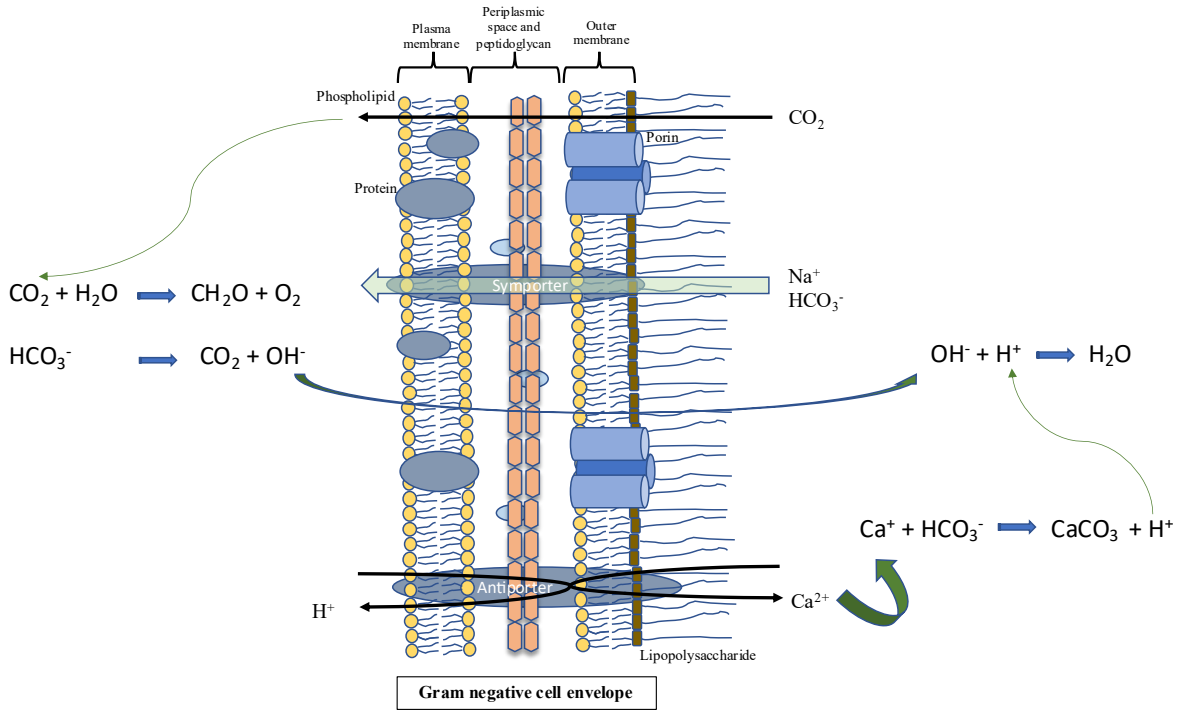


Figure 15: Movement of ions across a Gram-negative cell envelope illustrating photosynthesis-driven carbonate precipitation. Components of Gram-negative bacteria consist of a plasma membrane, periplasmic space, which contains a covalently linked to the outer membrane rigid peptidoglycan layer. Gram-positive cell walls are typically thicker (Madigan et al., 2019). The HCO_3^- and OH^- exchange process across the cell membrane produces an increase in the pH resulting from the OH^- in the immediate environment around the outer membrane. A symporter protein transports in Na^+ and HCO_3^- while CO_2 diffuses into the cell. As a result of the higher pH value and high calcium ion concentration, $CaCO_3$ crystals form at nucleation sites around bacteria with electronegative cell membranes and associated exopolymeric compounds (Hammes & Verstraete, 2002).

3.12.2.2 Chemotrophic bacteria

Chemotrophic microorganisms engaged in the biomineralisation of calcium carbonate employ various metabolic processes, including urea hydrolysis, ammonification of amino acids, denitrification, and sulphate reduction, as sources of energy. These metabolic activities result in the generation of environmentally harmful by-products, such as ammonia, nitrites, nitrous oxide, and hydrogen sulphide, which, upon interaction with oxygen, give rise to elemental sulphur or oxidised

sulphur species, (Castro-Alonso et al., 2019). prolonged exposure to these toxic by-products contributes to the corrosion of lime-based surfaces, including concrete, (O'Connell et al., 2010).

Both phototrophic and chemotrophic bacteria have developed survival strategies to endure harsh environmental conditions. They achieve this resilience by encapsulating their cellular DNA and critical biochemical processes within environmentally resistant spores. The remarkable resistance properties employed by bacterial spores to chemical and physical agents have been the subject of extensive investigation by the inclusion of spores into cement renders, which remain dormant until water enters through as the concrete matrix cracks, (Ertelt et al., 2021).

3.12.3 Bacterial spore formation

The bacterium *B. subtilis* replicates by binary fission every 30 minutes in an ideal non-stressful environment. When subject to deteriorating surroundings the cells experience metabolic stress, initiating the sporulation regulatory pathway. Within 10 hours, the bacterium sporulates and forms an endospore.

The spore is a repository for a replica of the bacterial DNA and essential nutrients encased within a dehydrated shell-like structure, extending prolonged viability in a dormant state. The structure contains high concentrations of calcium bound to dipicolinic acid (pyridine-2,6-dicarboxylic acid), a compound which plays a role in bacterial endospore heat resistance, (B. Setlow et al., 2006). The spore maintains a low water content containing a saturation of small acid-soluble proteins of the α/β type. These proteins protect the DNA from wet and dry heat, desiccation, and toxicity, and diminish the sensitivity of the DNA to UV radiation, (Cortezzo & Setlow, 2005; Nicholson et al., 2000, 2005; Popham et al., 1995; P. Setlow, 2003, 2006)

The formation of the endospore is a complex bacterial process that has been extensively studied in *B. subtilis*. The master regulator initiating sporulation in this bacterium is the DNA-binding protein Spo0A a conserved element in spore-forming bacteria. The sequential activation of the sporulation regulators results in the expression of over 500 genes, (Errington, 2003; Kroos, 2007; Molle et al., 2003).

Sporulation initiates asymmetric cell division leading to the emergence of two cell types, a smaller forespore housing a copy of the chromosome, and a mother cell which surrounds the forespore. This process results in the formation of two membranes with a surrounding peptidoglycan cortex. Over 70 different proteins coat the forespore membrane surface. Once the mother cell undergoes lysis, the new spore is released, (De Hoon et al., 2010).

Once formed the spore is capable of resisting heat, radiation, oxidation, and desiccation for up to hundreds of years. The spore can promptly initiate DNA replication and cellular growth upon the spore membrane receptors detecting nutrients and water.

3.13 Lime-based substrates

The use of concrete following the adoption of portland cement as a replacement for lime has become widespread throughout the world due to its technical superiority and high compression strength. The long-term viability of concrete, however, also presents challenges. Concrete exhibits limited tensile capacity rendering it susceptible to cracking. Issues encompassing thermal expansion,

thermal conductivity, chemical vulnerability to contaminants, such as sulphates, spalling due to stray currents, and corrosion to the steel rebar when inadequately shielded from sodium chloride, further complicate its long-term usage.

On a global scale, 30 billion tons of concrete is produced each year, and the demand is growing. The carbon footprint for concrete is at least 8% of anthropogenic CO_2 emissions. The demand and manufacture of concrete are anticipated to persist for the foreseeable future. Consequently, mitigating the carbon footprint associated with concrete necessitates innovative technological advancements for CO_2 capture during the manufacture process and novel strategies for carbon sequestration, (Editorial, 2021).

Structures built prior to 1880 featuring solid renders, are most likely constructed using lime mortar and lime render finishes. Lime-based materials demands lower energy consumption, are fired at lower temperatures, and readily absorb CO_2 during the hardening process. The surge in interest to utilise lime for historic repairs and new construction projects is primarily driven by its environmentally friendly profile.

3.13.1 Lime additives

Additives or admixtures have been incorporated into lime-based products with the aim of enhancing their working properties. These enhancements draw upon a wealth of historical knowledge accrued over centuries of practical application and, more recently, through comprehensive research endeavours aimed at elucidating the underlying chemical mechanisms governing their effectiveness. The primary objectives of these efforts revolve around the refinement of lime renders and mortars imbuing them with enhanced plasticity and workability. The desired attributes facilitate the development of greater rigour to withstand freeze-thaw cycles, and water entry while permitting gradual curing and progressive strength development through CO_2 inclusion. Among the additives employed, surfactants play an important role, manifesting as both positively and negatively charged ions. These ions align themselves toward either the outside surface or inner render thereby providing adhesion between the molecules on the surface of the render.

The integration of organic additives into lime products can be traced back to the construction of the Xianyang Palace in Xi'an, China where the use of pig blood as an additive was documented, (Zhao et al., 2014). Incorporating proteins in the form of blood, milk products, or albumin is purported to improve lime workability, strength, and resilience to adverse weather conditions, (Falkjar, 2019; Fang et al., 2015; Pahlavan et al., 2018). Under the direction of Thomas Telford, the construction mortar for the Pontcysyllte Aqueduct (1805), part of the Llangollen Canal, designated a Scheduled Ancient Monument of National Importance, included the blood from bulls. There exists an optimal additive composition in conjunction with lime, as excessive additive content has been demonstrated to reduce mortar compression and flexibility, (Mydin, 2017; Zhao et al., 2015).

Within natural shell structures, polar amino acids such as serine, cysteine, asparagine, glutamine, and lysine play an important role in $CaCO_3$ biomineralisation, (Zhang et al., 2022). These amino acids contribute to the stabilisation of amorphous $CaCO_3$ formation, promoting the formation of polymorphs, namely vaterite and aragonite. This results in an enhancement of the compression and flexibility of the lime, (Khan et al., 2021).

3.13.2 Preparing and applying limewash

Limewash presents as an aesthetically attractive surface treatment, enhancing architectural structures and providing a safeguard for the protection of historically significant buildings. It is comparably inexpensive and can be applied by artisans or homeowners alike. Limewash is versatile for application over a variety of substrates such as lime plaster, render, earth walls, and timber. It can be applied over old or existing limewash if the structural integrity of the finish is intact. It may also be applied to cement renders, though it requires pre-testing to determine if when employed it requires additives for improved adhesion. Caution is advised when employing additives such as tallow and casein as they have the potential to reduce porosity and foster undesired microbial growth.

As limewash is applied to a surface, it accumulates in thickness with each coat. The underlying coat must be an absorptive surface, as underlying layers of paint will prevent the adhesion and limit the performance of the lime. Limewash is made in one of two ways, using quicklime, or lime putty. The use of ordinary bagged lime in applications which dry too rapidly or are applied excessively thickly will result in deficient adhesion and a powdery lime finish.

Limewash derived from slaked lime or lime putty is diluted with water to attain a consistency resembling milk or single cream, approximately 20% lime to 80% water. The consistency is important, as if mixed too thick it will crack on drying. Many layers, up to five or more, of a thin wash are superior to fewer and less durable thicker coats. During the application process, a limewash brush of soft to medium stiffness serves to hold the limewash and act as a trigger to release cells from encapsulation.

On initial application, limewash is translucent, becoming opaque as it undergoes the drying process on the underlying substrate. This transformation signifies a conversion of calcium hydroxide to calcium carbonate. To facilitate this transformation, the underlying surface is kept damp, not wet, and the temperatures are regulated within the ranges 45°F (7.2°C) to 60°F (15.6°C), aligning with the average weather temperatures during the more temperate months of the year.

3.14 PRACTICE: Investigating biopolymer encapsulation strategies for enhanced weather resistance in limewash: A dual environment approach

Abstract

The escalating severity of the effects of climate change poses a significant threat to heritage buildings, particularly through the accelerated deterioration of their exterior lime render and mortar. This critical challenge for heritage owners and conservation professionals is further compounded by rising costs of both direct materials (lime, building materials) and indirect support resources (scaffolding, skilled labour). This economic reality accentuates the urgent need for innovative and cost-effective conservation strategies.

This study proposes a novel approach to address this issue by incorporating encapsulated biomineralising bacteria into a prepared limewash solution. The objective is to leverage the natural biomineralisation properties of these bacteria to enhance the weather performance of the protective limewash layer. As the limewash is applied, the encapsulated bacteria become embedded within the material, producing calcite crystals that strengthen its internal structure. This bio-based technique has the potential to significantly extend the lifespan of lime render and mortar, reducing the frequency and cost of repair interventions while aligning with sustainable preservation practices. Further research is necessary to optimise its application and long-term performance, but this innovative approach holds promise for safeguarding heritage structures in the face of a changing climate.

The central focus lies in evaluating the efficacy of biopolymers as encapsulation materials, safeguarding and sustaining bacterial growth within the limewash matrix. The ultimate objective is to achieve medium-term biomineralisation, manifested as an increase in the robustness of calcium carbonate ($CaCO_3$) within the limewash, while concurrently maintaining or enhancing its key performance criteria: porosity, absorption, and surface cohesion.

The study adopts a multifaceted approach, considering both autotrophic and heterotrophic bacterial strains. This diversity allows for the exploration of carbon dioxide capture potential during biomineralisation, with autotrophic bacteria utilising sunlight for ATP generation within their cells. A rigorous methodology is employed to assess the impact of various factors on the aforementioned limewash properties. Bacterial type, introduction method, biopolymer material, and encapsulation/immobilisation mechanics are all systematically evaluated. This assessment is conducted *in situ* on an exposed coastal island site in Scotland, complemented by comparative *in vitro* experimentation using limewash blocks. This dual approach ensures a comprehensive understanding of how each factor influences the robustness of the limewash outer layer, directly correlating to its suitability in mitigating the erosive effects of extreme weather events.

The anticipated outcomes of this research hold significant value for heritage owners and conservationists. The successful implementation of biomineralising bacteria in limewash offers a practical and sustainable solution for preserving heritage buildings under the duress of a changing climate. Furthermore, the in-depth investigation of biopolymer types, chemical formulations, and encapsulation processes will contribute valuable knowledge to the field of heritage conservation, informing best practices for this innovative preservation approach. The interdisciplinary nature of this research, encompassing biological, material science, and chemical perspectives, promises to advance the field of heritage conservation and pave the way for the sustainable preservation of historical structures.

3.14.1 Introduction

Limewash, a traditional and environmentally sustainable surface coating, relies on its formulation for the attainment of specific properties and enduring efficacy. In comparison with synthetic paints, limewash has a low-impact environmental footprint and is breathable, facilitating moisture dissipation and precluding issues such as mould and decay. Its distinctive, textured finish imparts a timeless aesthetic and has historically safeguarded heritage structures for centuries.

The inherent fire-resistant attributes of limewash render it a commendable choice as a fire-retardant coating for both residential and commercial buildings. Over time, limewash undergoes abiotic carbonation, absorbing CO_2 from the atmosphere, thereby elevating the density as calcium carbonate forms. This intrinsic process normally enhances the long-term resilience of the coating.

However, a substantial challenge confronting the sustained use of limewash as a protective surface coating lies in its resilience against erosive forces linked to climate change. Accelerated erosion of the limewash surface not only escalates maintenance expenditures but also diminishes the CO_2 sequestration advantage, leaving underlying materials, usually rendered, vulnerable to damage from dampness, biological colonisation, and infestation. Over time such vulnerabilities pose a tangible threat to the structural integrity of the building.

Changing climatic conditions have resulted in a surge in the erosive impact of extreme weather phenomena on the facades of heritage structures. Breathable materials such as lime, when subject to acidified rain, wind-driven salt-laden storms, longer protracted cycles of water saturation and arid spells are vulnerable to accelerated erosion. To address this challenge, the development of bio-enhanced limewash as a protective layer is proposed. This innovative approach aims to augment abiotic calcium carbonate deposition while preserving material porosity and resistance against climatic forces.

The methodological underpinning of this research involves the envelopment of bacterial cells within biologically derived polymers. These bacterial structures are created to enhance physical stability, providing a protective microenvironment for cell survival, and control cell release. Microfluidic devices are used to facilitate the generation of microcapsules containing several hundred bacteria encased within a biopolymer liquid, hardening due to a combination of solvent evaporation and the provision of a polymer cross-linking agent. This technique delivers high-throughput encapsulation with the potential to develop the concept on an industrial scale. The released encapsulated cells create crystalline deposits of calcium carbonate around nucleation sites surrounding the cell membrane, increasing the calcium carbonate density without blocking the fine pore structure in the lime. The approach merges material science, chemistry and microbiology resulting in a platform with multiple applications. The focus of this study is on building conservation and further contributing to new knowledge in heritage science.

Complying with conservation practice on lime-rendered heritage buildings requires limewash to be maintained and reapplied regularly to mitigate exterior erosion and prevent the underlying render from water damage. Limewash provides a waterproof yet vapour porous external surface which acts as a sacrificial layer over lime render, a technique successfully used by artisans on heritage buildings. The regular replacement of weather-eroded limewash prolongs the integrity of the lime render and the building structure.

Changes in weather patterns are increasing the frequency of wind-driven rain and drought cycles resulting in the water saturation of building exteriors. Limewash requires reapplication, depending on geographic location, after five to ten years. The increased weather intensity due to climate

change has reduced the maintenance frequency in exposed and coastal locations to less than three years.

Within the literature, the utilisation of bacteria has been explored predominantly as a means of microbially induced calcium carbonate precipitation within concrete. The harsh alkaline environment and mixing methods required to prepare concrete limit bacterial survival rates and once the concrete has been set it restricts bacterial access to nutrients, water, and oxygen.

In contrast, limewash is applied to the exterior of lime renders making the available of water and nutrients more accessible. This study proposes methods for effective techniques to introduce microorganisms into limewash. By comparing the direct addition, immobilisation, and encapsulation of bacteria utilising biopolymer structures into limewash, the impact on the porosity, cohesion, and water absorption of the limewash can be assessed to determine an optimal bio-formulation to address weather-driven erosion.

3.14.2 Microbially induced calcium carbonate precipitation

Microbial biomineralisation is influence by the concentration of available calcium, the availability of dissolved inorganic carbon, pH, and the presence of nucleation sites, (Hammes & Verstraete, 2002). Nucleation sites include bacterial cell membranes and polymeric substances found around the cell structures. In conjunction with supportive environmental conditions and bacterial strains, the crystal morphology of $CaCO_3$ formation on nucleation sites can be observed around the exterior of the cell.

The two microorganisms selected for this study, *Bacillus sphaericus*, and *Synechococcus elongatus* Nageli, (PCC 7942) nucleate $CaCO_3$ around the cell membrane. The study investigates how available energy sources drive $CaCO_3$ formation, while sustaining cell resilience using immobilisation and encapsulation techniques to create protective biopolymer structures. *Synechococcus* is a rapidly growing, unicellular autotrophic cyanobacterium found in oceanic surface and freshwater environments, whereas *B. sphaericus* is a mesophilic, aerobic Gram-positive bacterium that occurs worldwide in soil and aquatic habitats. Live cells rather than spores were used in the study to encourage immediate microbial $CaCO_3$ formation to accommodate the rapid application of limewash layers. Interestingly, dead cells remain capable of crystallisation by becoming nucleation units encouraging $CaCO_3$ crystallisation, a phenomenon that still can be considered as 'mineralisation through biological influence', (Dupraz et al., 2009).

3.14.3 Comparing alginate and cellulose biopolymers

The process of encapsulation used in this study requires the cross-linking polymerisation of sodium alginate and sodium carboxymethyl cellulose to form biopolymer protection around the bacterial cells. Each of the biopolymers was compared during and post-synthesis to evaluate the effectiveness and robustness of the extruded biopolymer shell. A similar process of immobilisation was compared by forming a cross-linked alginate hydrogel and the effectiveness of the hydrogel was evaluated by measuring the degree of adherence of incubated bacterial cells to the hydrogel surface.

Sodium alginate is a sustainably sourced, high-absorbent, polymeric flocculant harvested from the cell wall of *Sargassum* or *Turbinaria* brown algae (Viswanathan & Nallamuthu, 2014). Alginate, a

polysaccharide present in the cell walls of brown algae, predominantly exists as calcium, magnesium, and sodium salts. However, only sodium alginate exhibits water solubility, facilitating its extraction from the algal biomass. This process typically involves treatment with a hot alkali solution, followed by dilution and filtration to remove insoluble residues. Subsequent acidification precipitates the alginate, which is then redissolved upon the addition of alcohol and sodium carbonate. Notably, the resulting sodium alginate is insoluble in the alcohol-water mixture, enabling its straightforward extraction. Finally, the extracted alginate undergoes drying and grinding to yield sodium alginate powder. A clear viscous solution forms when sodium alginate is dissolved in water. The addition of calcium chloride to sodium alginate solution generates a replacement reaction, displacing the sodium ions associated with the alginate with calcium ions, forming a calcium alginate polymer gel *(chemical equation.1)*. The displaced sodium combines with the chloride ions to form sodium chloride which remains in solution and can be disposed of safely.

$$2NaC_6H_7O_6 + CaCl_2 \rightarrow 2NaCl + C_{12}H_{14}CaO_{12} \qquad (1)$$

Calcium alginate gel has interesting physical properties. It is hydrophilic as a result of negative charges on the alginate and yet the ionic gelation of calcium alginate is not water-soluble. This results from the gel-forming from layers of negatively charged alginate molecules interconnected by calcium ions Ca^{2+}, *Figure 16*.

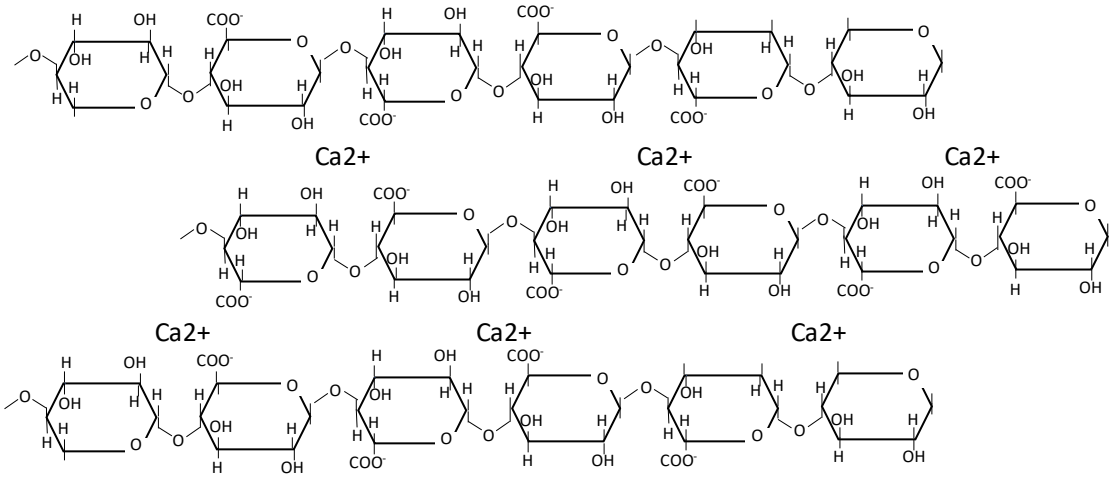


Figure 16: Chemical structure of calcium alginate. Alginate molecules are negatively charged COO^- providing a charge attraction to the Ca^{2+} ions layered between each alginate chain, resulting in the formation of a solid gelatinous hydrogel structure. A large amount of water is locked between the chains when the calcium and alginate form the polymer

Sodium carboxymethyl cellulose, a polyanionic biopolymer, results from the reaction of cellulose with chloroacetic acid and sodium hydroxide forming several active hydroxyl and carboxyl groups (Thakur & Thakur, 2014). It is a water-soluble polyanion which polymerises with poly-diallyl-dimethyl-ammonium chloride (pDADMAC) facilitating extrusion and encapsulation similar to the Ca^{2+}/alginate encapsulation process. The chemical synthesis of sodium carboxymethyl cellulose is relatively straightforward sourcing cellulose from wood pulp, and chloroacetic acid is a derivative of

vinegar and is a relatively safe biosynthetic. PolyDADMAC is also not toxic to humans and is formulated by reacting allyl chloride with dimethylamine, then undergoing a radical polymerisation with an organic peroxide functioning as a catalyst.

In this study, both the Ca^{2+}/alginate and CS/pDADMAC polymers were individually incorporated into two encapsulation techniques, bio-extrusion, and electrospraying. The biopolymer capsules produced from each technique were evaluated to observe the structural and chemical stability of the biopolymer shells. The process of hydrogel immobilisation requires the alginate polymer to be chemically cross-linked to form a robust 3D hydrogel matrix. Once formed, a process which takes a few seconds, the hydrogel matrix and adsorbed bacterial cells, a process that takes 16 hours, can be cut, or blended to the required size for inclusion in the limewash.

The impact of introducing bacterial-linked biopolymers into the limewash and the effect the biopolymer and biomineralising bacteria exerted on the relative robustness of the lime was measured using three criteria, porosity, a measure of moisture permeability, cohesion, an indicator of how surface strength resists erosion, and resistance to water absorption.

3.14.4 Advantage of microbially induced $CaCO_3$ precipitation in limewash when compared to self-healing cement studies

Ordinary portland cement (OPC) was introduced during 19th century Britain and provided the advantages of affordability, durability, and versatility. The high compressive strength of OPC was accompanied by a weakness in the concrete to develop cracks, which if untreated, may cause structural failure. The formation of microcracks in concrete has led to several initiatives to utilise synthetic binders as a flexible repair material, which reduces water entry into the concrete, but with a disadvantage in that it lacks the comparative compressive strength. Another drawback to using cement is the generation of carbon dioxide, pollutants, and energy usage during the production process. Initiatives to address these issues include encouraging researchers to investigate new approaches to remediate or even replace concrete for use in construction. Traditional products like lime in conjunction with the development of new biopolymer compounds are unlikely to replace concrete but may provide a reduction in its use and encourage the development of new bio-compounds from industrial waste.

There are several advantages to developing construction materials made with bio-products. Natural bioproducts are usually environmentally compatible and require a fraction of the energy to produce. The utilisation of microorganisms as biochemical factories to synthesise new products may also have the advantage of removing harmful pollutants and sequestering ions that would otherwise lead to oxidative and reductive environmental damage.

Unlike concrete repairs, in which attempts to fill fractures and cracks present practical challenges which may be deep inside the substrate, microbially induced $CaCO_3$ in limewash is easily applied across the limewash surface. This is lower cost and does not require artisan or trade skills for its application over lime render. If the bio-limewash is more resilient than currently available limewash products, the potential to reduce building ownership costs, maintenance schedules and the long-term CO_2 footprint of the property is compelling.

3.14.5 Questions raised by the research

Research in microbial biosynthesis prompts several questions regarding the advancement of encapsulation technology for the integration of bacteria to biomineralise and augment limewash.

In the context of the two research questions in this thesis, this practice element poses two sub-questions:

First, what is the impact of introducing natural biopolymers and biomineralising bacteria on the porosity, strength, and water resistance of limewash?

Second, what is the efficacy of technologies that enable bacterial viability in limewash to enhance limewash curing?

3.14.2 Materials and Methods

3.14.2.1 Bacterial preparation.

The experimental procedures involve the cultivation of two distinct microorganisms, *Synechococcus elongatus* Nageli PCC 7942, and *Bacillus sphaericus*, both obtained from ATCC (American Type Culture Collection). These methodologies adhere to standard practice in microbial cultivation.

The inoculation process involves aseptically introducing *S. elongatus* into Medium BG-11 broth, which has been sterilised by autoclaving at 120°C for 15 minutes. Subsequently, agar culture plates were prepared using BG-11 nutrient agar *[Appendices: Table 48].* Cultures were subjected to a controlled environment, maintained at 26°C, under a 'Sansi' grow light with a light density of 70 µmol S^{-1} m^{-2} for 14 days. Cultures of *S. elongatus* in BG-11 broth, subjected to the same conditions, were positioned in a slanted orientation to optimise gas exchange and augment exposure to light.

B. sphaericus was cultivated in liquid nutrient broth. The broth composition comprised 3 g L^{-1} nutrient broth powder, 2.21 g L^{-1} $NaHCO_3$, and 10 g L^{-1} urea, diluted to a final volume of 1 litre with distilled water. Incubation of the broth cultures occurred at 37°C for 16 hours, allowing the attainment of an OD_{630} turbidity reading of 1.4 for each culture as determined by Colony-Forming Units (CFUs) serial dilution counts.

The meticulous attention to aseptic technique, selection of appropriate growth media, and control of incubation conditions of these methodologies ensure the reproducibility and reliability of the experimental outcomes.

3.14.2.2 Immobilisation in Alginate Hydrogel

To prepare the alginate hydrogel, 1 g of calcium chloride ($CaCl_2$) was dissolved in 30 ml of distilled water. Once dissolved, 6 ml of the solution was slowly added, while stirring to a solution of 1.17 g sodium alginate dissolved in 50 ml of distilled water, at a temperature of 30°C. The resulting gelatinous calcium alginate was thoroughly washed in distilled water and allowed to stand for two days to facilitate the removal of excess moisture.

The prepared calcium alginate hydrogel was placed into a sealed jar and autoclaved at 120°C for 15 minutes, *Figure 17*. The hydrogel was aseptically sectioned into 2 mm thick segments and placed into 5 ml aliquots of each bacterial culture suspended in liquid broth. The hydrogel and *B. sphaericus* broth amalgamation was then incubated for 16 hours at 37°C after which it was transferred to refrigeration at 5°C until required. To measure bacterial cell viability, a section of the hydrogel was plated, and colonies were counted.

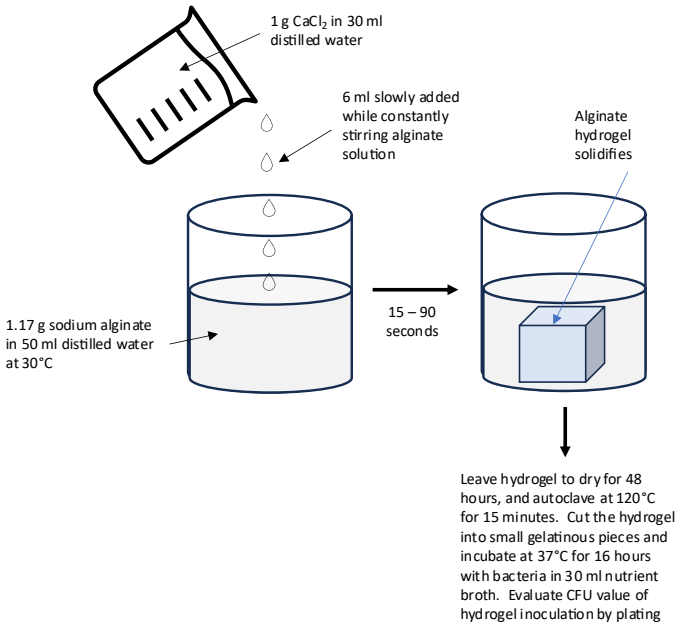


Figure 17: Immobilising bacteria by adsorption onto an alginate hydrogel. The hydrogel protects cells from mechanical force and supplies water, oxygen, and nutrients from the absorbed nutrient broth

3.14.2.3 Encapsulation

Bacterial broth containing either *S. elongatus* or *B. sphaericus* was diluted to achieve an OD_{630} turbidity reading of 1.4, forming the cell and nutrient solution to be enclosed within capsules during the encapsulation process. Live bacterial counts were established from a serial dilution standard line equation and plated for colony counting to determine the respective colony-forming unit values (CFU).

The encapsulation process utilised a coaxial needle style C 21G (inner)/28G (outer) 50,50,0 (SKE Research Equipment, Milan). Each of the liquid components was supplied from separate 50 ml syringes and delivered to the coaxial needle by an Inovenso, IPS series 5, dual syringe (Inovenso Teknoloji Ltd., Istanbul), enabling control over the encapsulation flow rate.

3.14.2.4 Biopolymer-hardening solutions used in the encapsulation process

An alginate polymer, 2 wt% sodium alginate was dissolved in distilled water and autoclaved at 120°C for 15 minutes. The resultant alginate solution was subsequently cooled to 25°C and 30 ml of this solution was introduced into a sterile 50 ml syringe. An inherent advantage of alginate as an

encapsulating polymer lies in its capacity to create a naturally moist environment, thereby fostering prolonged cell viability. This attribute is due to the water-retention capabilities of alginate, while cross-linking establishes structural compartments that serve the dual purpose of retaining additional water and a 3D matrix to enclose cells.

The second polymer in the study, sodium carboxymethyl cellulose was formulated with 2 wt% sodium carboxymethyl cellulose dissolved in distilled water and autoclaved at 120°C for 15 minutes. After cooling to 25°C, 30 ml of the carboxymethyl cellulose solution was dispensed into a separate sterile 50 ml syringe.

Bacterial cells were uniformly suspended in 60 ml of nutrient medium and incubated until reaching an OD_{630} of 1.4 equating to a CFU value of 10^6 cells mL^{-1}. The resulting bacterial cell suspension in each respective nutrient liquid medium was then introduced into sterile 50 ml syringes.

The capsule hardening solution for sodium alginate comprised a 50 ml solution of 2M $CaCl_2$, autoclaved at 120°C for 15 minutes and subsequently cooled to room temperature.

The capsule hardening solution for sodium carboxymethyl cellulose consisted of a 50 ml solution of 60 g L^{-1} pDADMAC, autoclaved at 120°C for 15 minutes and allowed to cool to room temperature.

3.14.2.5 The extrusion process

The 3D extrusion methodology utilised in this study demonstrates design flexibility and economic viability associated with the production of 3D extruded bioencapsulated components. The effectiveness of the extrusion process, hinges upon several determinants, encompassing the dimensions and quality of the required output, the nature of materials employed, and the scale and complexity of the extrusion apparatus.

In the context of most additive manufacturing procedures, object fabrication occurs through the sequential deposition of layers of polymer material, thereby incrementally constructing the overall form of the printed object. The biopolymer extrusion process, devised for the present investigation, represents an adaptation from conventional additive technology. It involves the 3D encapsulation of a liquid constituent within a biopolymer coating, the rapid solidification of which is achieved through cross-linking long-chain polymerisation, resulting in the encasing of the contents within a protective outer capsule.

Critical to the success of encapsulating live bacterial cells in the course of this study are the polymer formulations, the transition from liquid to solidified biopolymer and the mechanism to achieve low-stress extrusion. Alginate, primarily derived from seaweed, and cellulose, extractable from various plant sources, meet the environmental and physical criteria as suitable candidates based on these criteria, with the ability to sustain live cells, for application in limewash on conservation structures.

3.14.2.6 Alginate biopolymer capsules

The encapsulation process utilises a coaxial needle interconnected through autoclaved and sterile silicone tubes to each of the two prepared syringes affixed to the modulated delivery pump, *Figure 18*. The central outlet of the coaxial needle is linked to the syringe containing the cell/nutrient medium, while the outer coaxial needle is connected to the syringe containing the biopolymer.

Extrusion on the dual syringe pump operated at an optimised rate of 50 µL min^{-1}. Polymer-encapsulated cell droplets were extruded into a 2M $CaCl_2$ solution and the capsule wall solidified at room temperature taking approximately 30 minutes. The resultant capsules, centrifuged at 1200 rpm, and washed in sterile distilled water, were suspended in 80 ml of sterile nutrient broth. The capsules and nutrient broth were refrigerated for storage at 5°C.

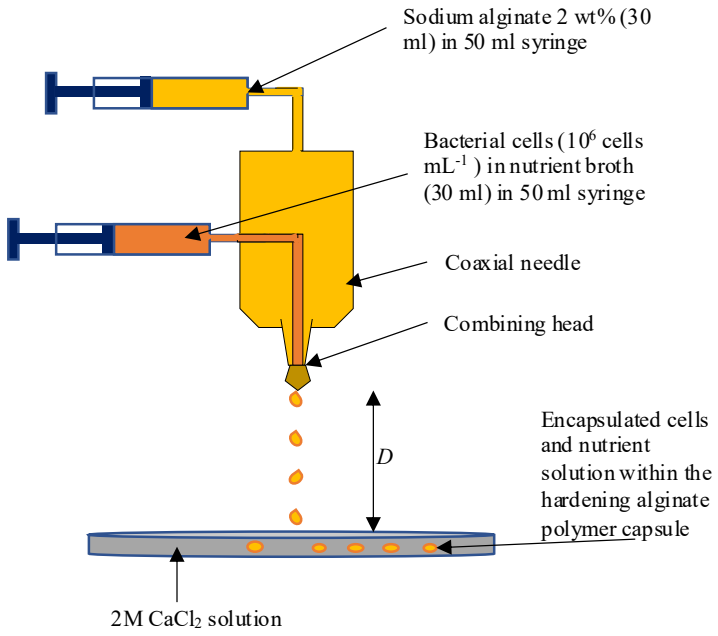


Figure 18: Extrusion of alginate-encapsulated bacterial cells in nutrient broth into a solution of 2M calcium chloride**.** The polymer and the cell solution are contemporaneously sprayed from coaxial jets to form the capsule, a process termed coextrusion. Several factors determine the coaxial diameter, porosity, and structure such as fluid viscosity, concentration of solutes, type of biological material, temperature and distance, D. Within the coaxial needle, the cell fluid is surrounded by the biopolymer forming a polymer sheath before the capsule exits the coaxial needle. If D is > 3 cm, the capsule fails to form due to impact forces when entering the hardening solution

3.14.2.7 Cellulose biopolymer capsules

The coaxial needle affixes through autoclaved sterile silicone flexible tubes configured identically to the extrusion process outlined in section 3.14.4.6. Two syringes, previously prepared, were introduced into the delivery pump, *Figure 19*. The outlet of the coaxial centre needle was linked to dispensing the bacterial nutrient medium, while the exterior coaxial assembly was connected to the syringe containing the cellulose biopolymer. The dual syringe pump was set to an optimal extrusion speed of 25 µL min^{-1}. Subsequently, droplets of polymer-encapsulated cells were extruded into a solution of 60 g L^{-1} pDADMAC. The capsule wall hardened at room temperature taking approximately 30 minutes. Following extrusion, the capsules underwent centrifugation at 1200 rpm and were washed in sterile distilled water. The capsules and nutrient broth were refrigerated for storage at a temperature of 5°C.

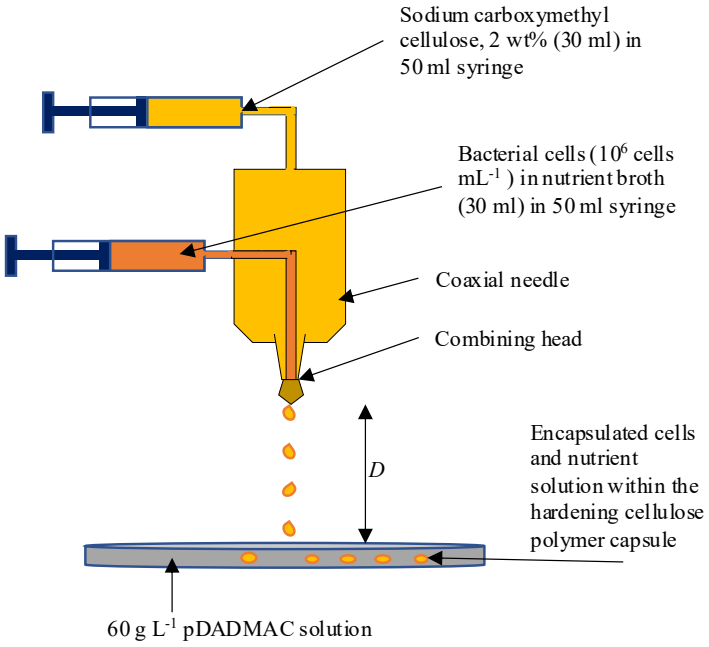


Figure 19: Extrusion of cellulose-encapsulated bacterial cells in nutrient broth into a solution of pDADMAC. Droplets exuded at 25 µL min^{-1} from the coaxial needle falling under gravity into the 60 g L^{-1} pDADMAC solution in the collecting base. By simple co-extrusion, up to 5 million bacteria can be captured per capsule expressed. The introduction of a vibrating nozzle enables the formation of stable 0.7 mm droplets of bacteria, (Gunzburg et al., 2020)

Modulating the flow rate through the coaxial needle appears not to influence droplet size, exerting influence primarily on the frequency of the droplet generation. A reduction in alginate concentration within the polymer capsule demonstrates a discernible trend towards smaller droplet sizes, likely attributable to variations in surface tension. Conversely, the propensity for capsule rupture increases with diminished alginate concentrations, a consequence of decreased structural robustness stemming from fewer cross-links in the polymer, (Chaurasia et al., 2017; Ching et al., 2017; Martins et al., 2017). This phenomenon has been quantified for capsules formed with 2.0% alginate exhibiting rupture below a threshold force of 6.0 N, a force magnitude inferior to that exerted by a fingertip, (Said Ismail et al., 2021). Microscopic examination of the capsules assessed the capsule wall integrity coupled with an evaluation of the viability and proliferation of the average bacterial cell population. A microtiter assay served to evaluate and confirm the viability of the encapsulated cells when stored at room temperature.

3.14.2.8 Electrospraying

Electrospraying involves a microencapsulation technique generating minute liquid droplets from a small needle aperture within an electric field. The process leverages electrostatic forces induced by the application of a high voltage between the liquid, typically a polymer, and a receiving substrate or collecting fluid. The formation of a 'Taylor cone' facilitated by the electrical charge is due to the forces from the charge exceeding the surface tension forces, delivering a spray of droplets toward the collecting base. The resultant droplet size and delivery rate can be modulated based on factors such as the voltage differential, needle diameter, which determine the production of micro or nano droplets.

3.14.2.9 Electrosprayed alginate biopolymer capsules

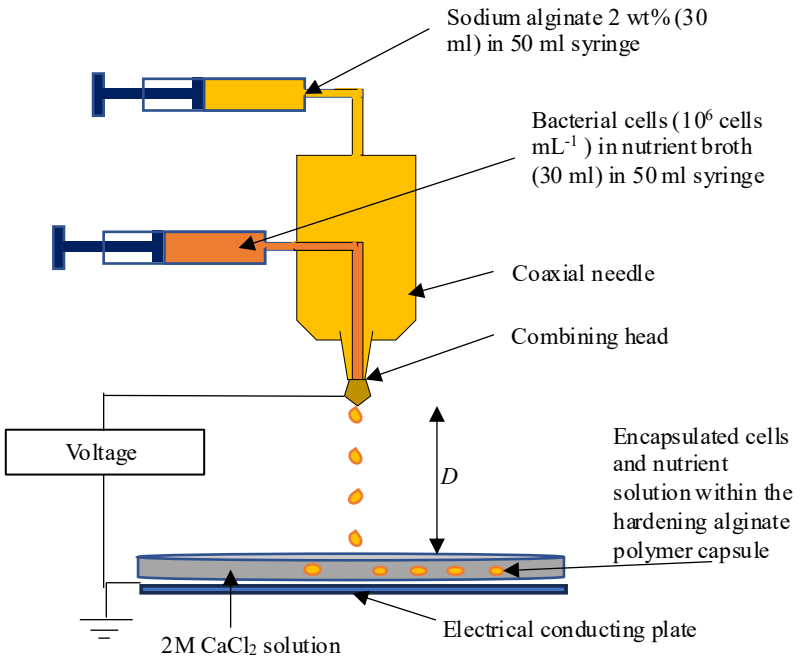


Figure 20: Electrosprayed extrusion of alginate-encapsulated bacterial cells in nutrient broth, into a solution of calcium chloride. The droplets exude from the coaxial needle head and become highly charged and produce a repelling force against the surface tension of the polymer. As the electric potential energy approaches the value of the surface energy, the shape of the droplet is distorted forming a Taylor cone. The critical voltage at which this occurs is dependent on several factors including the surface tension of the solution, the diameter of the droplet and the electric field strength. The charge holds the sheath in place as the newly formed coaxial capsule is pulled toward the oppositely charged (or earthed) base plate. Electrospraying produces smaller droplets the diameter of which is also determined by the solution concentration and the applied voltage. The contemporaneous spraying of the biopolymer and cell solution from two coaxial jets is termed electro-coextrusion

The coaxial needle is connected via autoclaved sterile silicone flexible tubes to two of the prepared syringes inserted into the electronically controlled delivery pump, *Figure 20*. The coaxial centre-needle outlet is connected to deliver the bacterial nutrient medium syringe, and the alginate-containing biopolymer syringe is connected to the exterior needle of the coaxial needle. The high-voltage unit (30kV, Genvolt HV Output, positive polarity) is attached across the coaxial needle and the conducting sheet, placed beneath the collecting dish solution. The extrusion speed on the dual syringe pump was operated at an optimal 50 µL min^{-1}. Polymer-encapsulated cell droplets were electrosprayed into 2M $CaCl_2$ and left to harden at room temperature for 30 minutes. The formed capsules were centrifuged at 1200 rpm and washed in sterile distilled water then suspended in 80 ml of sterile nutrient broth, refrigerated at 5°C. The high voltages used in the study required each piece of electrical equipment to be earthed to prevent feedback currents from damaging the devices.

3.14.2.10 Electrosprayed cellulose biopolymer capsules

The arrangement to electrospray cellulose polymer capsules follows a similar procedure to the alginate electrospray method. The alginate polymer is replaced with sodium carboxymethyl cellulose and the $CaCl_2$ hardening solution is replaced with 60 g L^{-1} pDADMAC fluid.

3.14.2.11 Evaluating post-extrusion survival rates

The assessment of bacterial cell survival after encapsulation or immobilisation procedures involved the utilisation of plating and subsequent enumeration of colonies. Enumeration of encapsulated bacterial cells and fragments within bacterially immobilised hydrogel matrices was achieved through the plate count method, and the quantification was expressed as colony-forming units (CFU mL^{-1}). A plate was deemed countable when exhibiting a colony range between 30 and 300, as fewer than 30 colonies would yield a sample size inadequately representative for accurate analysis. Conversely, counts surpassing 300 presented challenges in precise enumeration without the assistance of an automated counting system. The determination of colony count in the original bacterial solution was ascertained through agar plating serial dilution. The calculation involved considerations of the colony count, the total dilution factor of the enumerated plate, and the volume of the cultured medium plated, as represented by *Equation 2* and *Equation 3*. The encapsulation survival yield (ESY) is a measurement of the number of surviving cells post-encapsulation. ESY is calculated at different periods and graphically represented by *Equation 4*.

$$Dilution\ factor\ = \frac{Final\ volume\ (diluted\ volume + stock\ volume)}{Volume\ of\ the\ stock\ transferred} \quad (2)$$

$$CFU\ mL^{-1}\ = \frac{Number\ of\ colonies\ counted\ \times\ dilution\ factor}{Volume\ of\ culture\ plated} \quad (3)$$

$$ESY\%\ = \frac{Number\ of\ viable\ colonies\ after\ encapsulation}{Number\ of\ viable\ colonies\ before\ encapsulation}\ \times\ 100 \quad (4)$$

Encapsulation CFU mL^{-1} is counted from the incubated plate colonies formed from the source dilution at factor 10^6. Counting is conducted before encapsulation and following the encapsulation process over five hours. ESY evaluates the effect of the encapsulation process, including the effectiveness of the biopolymer capsule, on cell viability.

3.14.2.12 In vitro installation and assessment

Evaluating limewash curing within a controlled laboratory environment, alongside the examination of the impact of bacterial and biopolymer encapsulation on the curing process, presents both advantages and disadvantages. Among the advantages are the heightened precision in limewash preparation with and without bacterial additions, tight control over environmental conditions throughout and after application, and the creation of a curing environment unaffected by atmospheric pollutants and unpredictable weather events. Conversely, the drawbacks associated

with a laboratory setting encompass the stringent regulation of temperature, humidity, and light, as well as the absence of the influence of weather patterns on the limewash surface. This absence may lead to accelerated drying of the limewash, potentially compromising both abiotic and biotic curing processes. It is imperative to consider these factors in the analysis and comparison of results obtained *in vitro* versus those observed *on-site*.

3.14.2.13 Preparation of lime render units for in vitro analysis

Experimental lime units or blocks were designed to establish a test area measuring 28 mm x 18 mm x 3 mm, affixed to a foundational support structure with dimensions of 33 mm x 40 mm x 22 mm. The foundational substrate consisted of NHL 3.5 hydraulic lime base render (Conserv®, binder BS EN 459-1:2015), while the upper testing layer featured NHL 3.5 hydraulic lime finishing coat render (Conserv®, binder BS EN 459-1:2015, colour Elm Cragg). The criteria for material selection for the units prioritized attributes such as 100% natural sourcing, breathability, and sustainability, devoid of additives, plasticisers, or cement.

The production of these units involved the use of a mold, employing a lime-to-sand ratio of 1:2.5, combined with tap water to achieve the desired consistency. Following a curing period of 21 days, the units were removed from the mold and allowed an additional 7 days for air-drying at room temperature. A concluding lime coat, reaching a depth of 3 mm, was applied to the upper surface of each unit. Subsequently, the units, featuring the finishing lime coat, underwent a further 21-day curing process at room temperature, *Figure 21*.

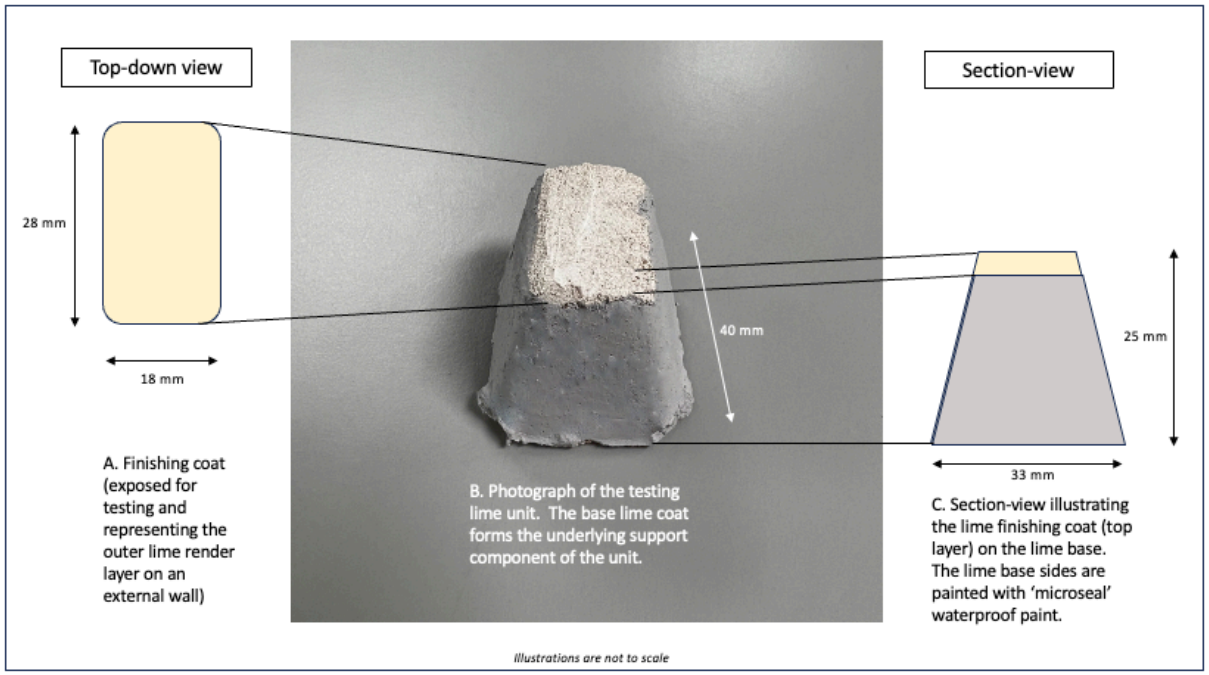


Figure 21: Dimensions of the testing units were designed to evaluate microbial biomineralisation, ascertain the viability of encapsulated cells, and conduct performance comparisons between the extrusion and electrospraying encapsulation methodologies. Additionally, these units served as a platform for appraising the effectiveness of alginate-hydrogel immobilised cells. The base unit is fortified with waterproof paint to prevent moisture ingress thereby isolating the experimental surface, measuring approximately ±504 mm²

3.14.2.14 Limewash preparation

The limewash is prepared by adding lime: water to a ratio of 50:50, slowly mixing in additional lime powder toward a lime: water ratio of 20:80, resembling a single cream consistency. The prepared limewash is divided into several containers and when required for application, mixed with the sample formulations as illustrated in *Table 10*.

The investigation, conducted to enhance efficiency and resource utilisation, was bifurcated into two sequential phases. In Phase 1, the impact of biopolymer composition and encapsulation/immobilisation methods on *B. sphaericus*, a resilient bacterium amenable to overnight culture, was scrutinised. Building upon the insights garnered in Phase 1, Phase 2 concentrated on refining the encapsulation protocol deemed most efficacious for assessing the second microorganism, *S. elongatus*, a cyanobacterium. *S. elongatus* has a longer incubation period than *B. sphaericus*, its growth conditions necessitating a more protracted and intricate culture arrangement.

Table 10: Phase 1 - Limewash/bacterial cell formulations derived from B. sphaericus, in non-encapsulated, encapsulated, and immobilised states. Encapsulation employs two distinct biopolymer matrices, alginate, and cellulose, which are assessed and contrasted as efficacious biopolymer bases for encapsulated bacteria. Test samples 4, 5, and 6 are hybrid formulations base layered with lime/alginate hydrogel. This layer is subsequently overlaid with either planktonic or encapsulated biopolymer formulations. Sample formulations are applied over three days in laboratory conditions. An identical experimental procedure and formulations is applied to both in vitro and in situ installations. Phase 2, investigating biomineralisation in S. elongatus is identical for the Control and Test Sample 3 utilising encapsulated-alginate biopolymer

n = number of limewash layers n^{*a} *= number of limewash layers applied first*	Limewash only	*B. sphaericus* – non-encapsulated	*B. sphaericus* – encapsulated – alginate biopolymer	*B. sphaericus* – encapsulated – cellulose biopolymer	*B. sphaericus* – immobilised – alginate hydrogel
Control	5				
Test sample 1		5			
Test sample 2			5		
Test sample 3				5	
Test sample 4		3			2^{*a}
Test sample 5			3		2^{*a}
Test sample 6				3	2^{*a}
Test sample 7					5

3.14.2.15 Preparation of inoculated limewash samples

Each of the test samples outlined in *Table 10* were prepared as follows:

Test sample 1: 10% v/v nutrient broth containing 10^6 bacterial cells mL^{-1} was added to the prepared limewash, mixed thoroughly, and applied to the test area. Approximately 3 hours was allowed between each of the 5 applications.
Test samples 2 - 6: 10% v/v nutrient broth containing 30% (v/v) encapsulated bacterial cells were added to the prepared limewash, mixed thoroughly, and applied to the test area. Approximately 3 hours was allowed between each of the 5 applications.

Test sample 7: 30% v/v nutrient broth containing 100% (v/v) hydrogel containing immobilised B. sphaericus cells was added to the prepared limewash, mixed thoroughly, and applied to the test area. Approximately 3 hours was allowed between each of the 5 applications.

3.14.2.16 On-site Installation and Assessment

The site of the experimental wall section is situated on the sheltered east coast of the island of Jura at the southern end of Small Isles Bay at a latitude: *55° 49' 59.99" N Longitude: -5° 55' 59.99" W*. The wall section is located on the east gable of a concrete-over-stone rendered building at approximately 1.52 m from ground level and unencumbered by human or vehicle traffic, *Figure 22*.

Figure 22: The building, aspect, and location of the experimental wall section. The section is north-east facing. The top layers of concrete were removed from the wall section, exposing the original stone beneath prior to the application of new lime render

3.14.2.17 On-site Preparation

The old concrete wall render was removed to dimensions measuring 900 mm x 500 mm x 40 mm, revealing the underlying stone substrate. A single levelling coat of lime render was applied onto the exposed stone to provide robust adhesion for subsequent lime render applications.

To prevent rapid moisture evaporation from the base lime coat, the underlying stone was soaked with water. Following the base coat application, as the base coat dried, a resilient surface skin formed permitting two successive lime render layers to be applied, denoted as the 'scratch and float coats'. Adequate intervals for initial wetting and drying were observed between the successive applications.

Each of the lime render strata was composed of Conserv NHL 3.5 hydraulic lime render adhering to BS EN 998-1:2016 standards, incorporating a medium coarse aggregate and blended sands conforming to BS EN 13139-1:2015, and lime binder in accordance with BS EN 459-1:2015. The

constituent elements were mixed with tap water, following the guidelines stipulated in the product instructions.

Due to unseasonably high temperatures and arid climatic conditions, the levelling coat dried rapidly requiring periodic spraying with water to prevent premature contraction encouraging the development of microcracks. The final layer, designated as the 'fine' lime layer, culminated in the application of a finishing or topcoat utilizing Conserv NHL 3.5 hydraulic lime render BS EN 998-1:2016, blended with tap water following the product instructions.

The curing period for the entirety of the constructed wall segment spanned 40 days and was completed as illustrated in *Figure 22 and 23*.

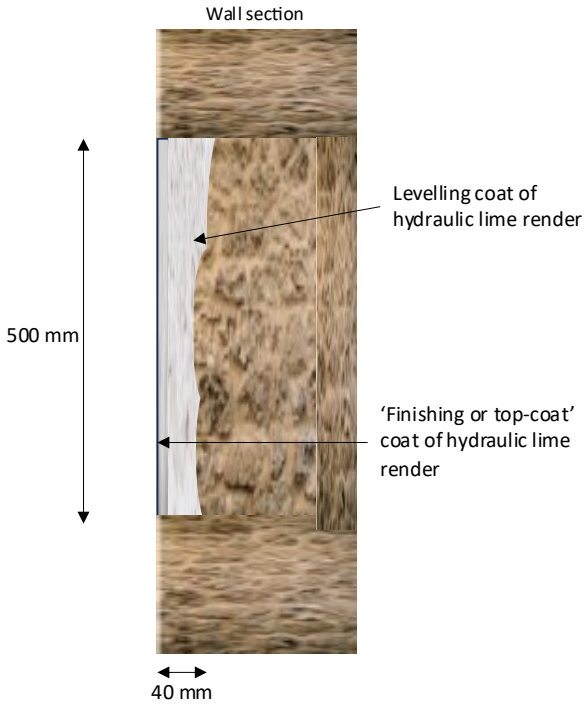


Figure 23: Wall cross-section displaying each of the lime layers. The total thickness of the section is ±40 mm. The fine topcoat was ±5 mm thick. During layering the surface was sprayed with water to avoid the material drying thereby preventing contraction and subsequent cracking

3.14.2.18 Limewash preparation

To formulate the standard limewash solution, a combination of hydraulic lime and water is employed in a ratio of 50:50. Additional water is incorporated as necessary to attain a final lime-to-water ratio of approximately 20:80, yielding a consistency akin to single cream. The resultant limewash was subsequently distributed into several containers, each formulated as indicated in *Table 11*. The formulations emulate the limewash applications utilised for the laboratory test units for phase 1.

During the application phase, each sample was applied to the designated wall section across defined 120 mm x 500 mm strips, as illustrated in *Figure 24*. The pH of the limewash registered at 11.1, the

relative humidity at 80% and air temperature of 18°C. Relative humidity and temperature were monitored using a Protmex HT607 Temperature Humidity meter high precision digital hygrometer and pH using an Apera SX610 ±0.1 accuracy, 0-14 pH meter.

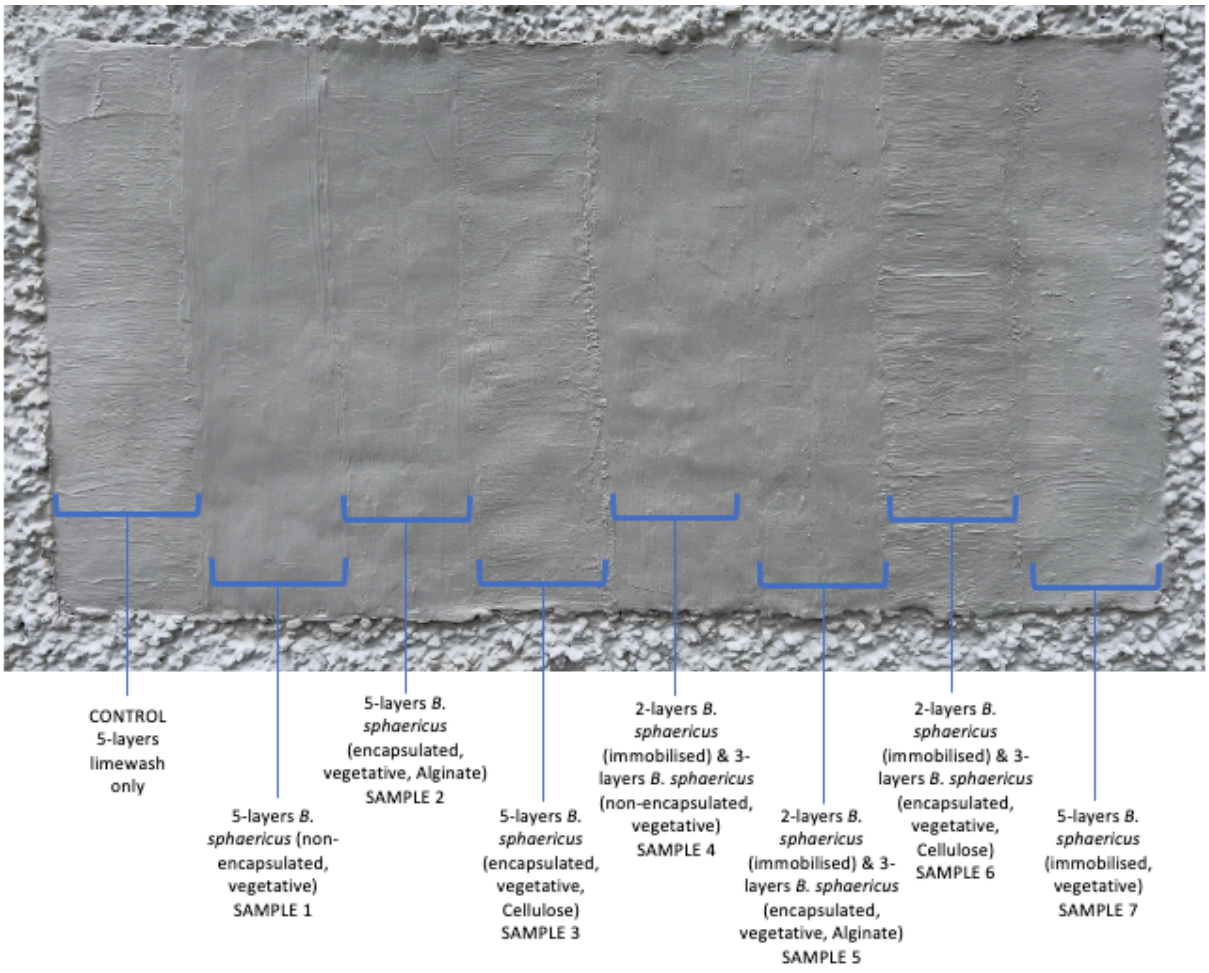


Figure 24: The wall section map outlines the areas for the application of each limewash formulation outlined in Table 10. Each section measures ±120 mm x 500 mm. The term 'Immobilised' denotes vegetative cells affixed to an alginate hydrogel, 'Alginate' refers to vegetative cells encapsulated within sodium alginate/$CaCl_2$ extruded polymer shell, 'cellulose' designates vegetative cells encapsulated within a sodium carboxymethyl cellulose/pDADMAC extruded polymer shell. The control is limewash only. Each section is textured in contrast to the next to facilitate visual differentiation

3.14.2.19 Measuring the effects of immobilisation and encapsulation on porosity, cohesion, and absorption of the limewash layer

The chosen methodologies for assessing alterations in the limewash formulations necessitated accuracy, portability, ease of administration and reproducibility in both field and laboratory environments. The three measures for evaluation across each lime formulation are porosity, cohesion, and absorption, chosen as key performance criteria indicating the degree of robustness of limewash.

3.14.2.19.1 Porosity

Porosity serves as a quantitative measure of open capillary pores within the crystal lattice of limewash. This parameter is subject to the influence of various factors, including the type of binder employed, the binder-to-aggregate ratio, aggregate dimensions, and the extent of compaction during the application onto the wall surface. Capillary pores confer the moisture permeable attributes to lime and can exhibit diameters spanning from 0.1 to 100 µm. The formation of these pores is contingent upon the water-to-binder ratio and the carbonation-to-hydration ratio. Pores facilitating the permeation of liquids or gases, are predominantly situated proximal to the lime surface, hence the terminology of 'open' pores (Maria, 2010; Mehta & Monteiro, 2014; Papayianni & Stefanidou, 2006).

Various techniques, both direct and indirect, are employed to measure porosity. Direct microscopic analyses, encompassing stereo binocular, petrographic, or scanning electron microscopy, prove more suited for *in vitro* assessments, necessitating the desiccation of the sample. Conversely, indirect methodologies, such as pressurised devices or mercury-based intrusion porosimetry, require the extraction of samples from the site for subsequent laboratory testing. In light of the constraints associated with remote, on-site conditions in this study, Alizarin Red stain emerges as a viable approach for porosity determination within the limewash layer. Alizarin Red, an anionic dye exhibiting an affinity for cationic metals like calcium, is applied by brushing 0.1 ml of a 0.025M solution onto the surface, followed by *in situ* air-drying of the sample. Subsequent excision from the limewash layer enables measurement to ascertain the depth of stain penetration. Where necessary, optical microscopy is employed to determine the Alizarin Red depth and staining characteristics, to verify the dimensions and morphology of calcite crystals, if formed.

3.14.2.19.2 Cohesion

Surface cohesion is appraised by examining the integrity and durability of the limewash surface layer. The evaluation entails the application of peeling tape of dimensions 25 mm x 15 mm, to the surface, and gauging the ability to remove the surface layer. Cohesion, in this context, serves as an indicative measure of the bonding strength between lime particles in the surface limewash. To conduct this assessment, segments of peeling tape are sectioned, weighed, and applied with consistent pressure onto the designated test surface area. Following a 15-second adhesion period, the tape is subsequently removed and reweighed. The weight variation serves as an indicator of the extent of surface material loss onto the tape, providing a quantifiable metric for the strength of bonding between the constituent particles of the limewash surface.

3.14.2.19.3 Absorption

Absorption levels detected at the limewash surface serve as an indicator of the water-resistance exhibited by the wash. Enhanced resistance to water saturation correlates with heightened protection for the underlying lime render and stonework, thereby mitigating the risk of long-term water-induced damage. The method employed to quantify water uptake relies on capillary water absorption, employing the contact sponge method as illustrated in *Figure 25.* The procedural framework outlined in the UNI 11432:2011 standard involves utilising a sponge with pre-defined dimensions, saturated with water until reaching its saturation point. The water-saturated sponge is pressed against the limewash surface at a standardised pressure. The quantification of water absorption is determined by assessing the disparity in the weight of the sponge before and after a

60-second application period (Gaggero & Scrivano, 2016; Ribeiro et al., 2022; Vandevoorde et al., 2009).

To ensure consistency in saturation, the sponge is submerged beneath the surface of the water, maintained at room temperature, undergoing repeated compressions to expel trapped air from within the sponge. Following a five-minute soaking period, the sponge is placed in a horizontal position on a Perspex slope, then inclined at an angle of 20° for 60 seconds, enabling the drainage of excess water before weighing. Subsequently, the sponge, held in the holding apparatus depicted in *Figure 25*, is promptly applied to the designated wall or lime block section, following which it is reweighed.

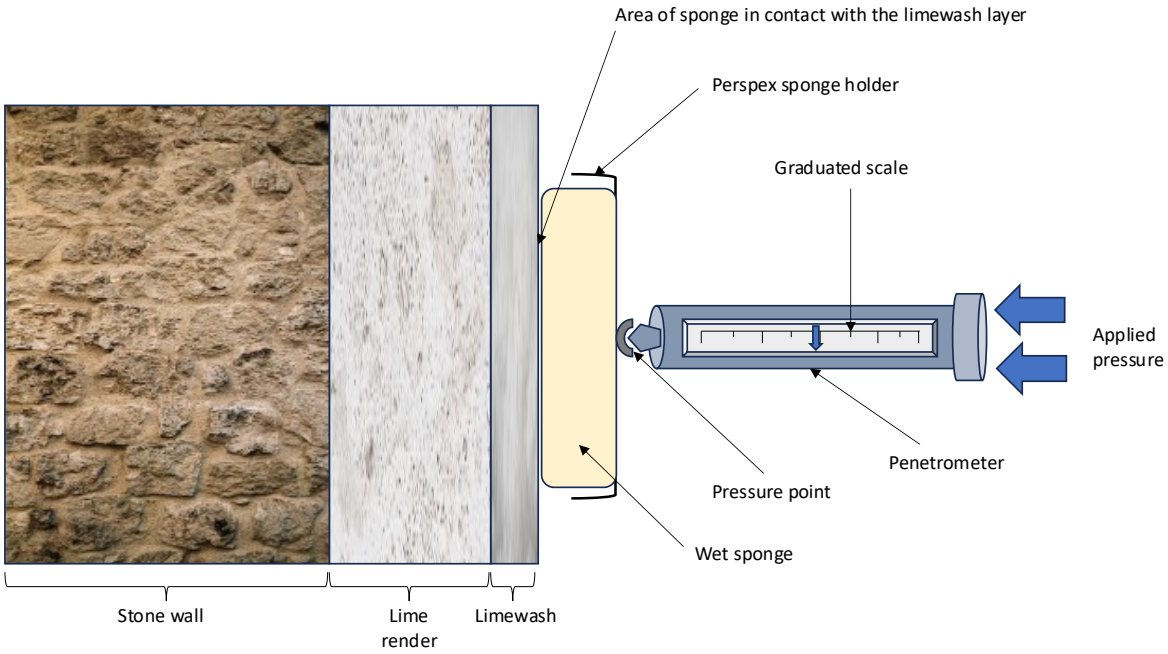


Figure 25: Applying the sponge apparatus to the wall. The image above illustrates the relationship between the underlying lime render and outer limewash layers. The wet sponge is retained within the Perspex holder and a consistent pressure is applied across the limewash surface. The force is consistently maintained, and measured using a pocket penetrometer, for 60 seconds. An identical approach is used to assess absorption on the in vitro lime units

The contact sponge method represents a non-invasive, in-situ approach enabling comparable assessment of water absorption by the limewash surface. This involves the systematic reweighing of the sponge, with subsequent computation of water uptake from the sponge, quantified in g mm^{-2}. Uniform pressure is achieved by utilizing sponges of identical density, affixed to a non-absorbent substrate such as Perspex, with positioned at the centre a pocket penetrometer, through which consistent pressure is exerted. In the course of this investigation, a load of 0.45 kg was applied to the interface connecting the sponge surface and the limewash section in direct contact with the sponge.

3.14.3 Results

3.14.3.1 The impact of alginate and cellulose biopolymer encapsulation on cell viability

The assessment of cell viability necessitates the use of reliable assays that can assess the variations in the encapsulation efficiency, stability of the encapsulating material and the impact of the encapsulation process on the microbial physiology.

The assessment of colony-forming units (CFU) over time is a method to gauge cell viability. This requires the enumeration of CFUs quantified as forming viable colonies under specific growth conditions.

Colony forming units (CFU) in the nutrient stock solution were measured before encapsulation and over 6 hours following the encapsulation process. *Figure 26* compares the colony-forming unit count indicating viable *B. sphaericus* cells in (a) baseline (not encapsulated), (b) encapsulated in alginate biopolymer and (c) encapsulated in cellulose biopolymer.

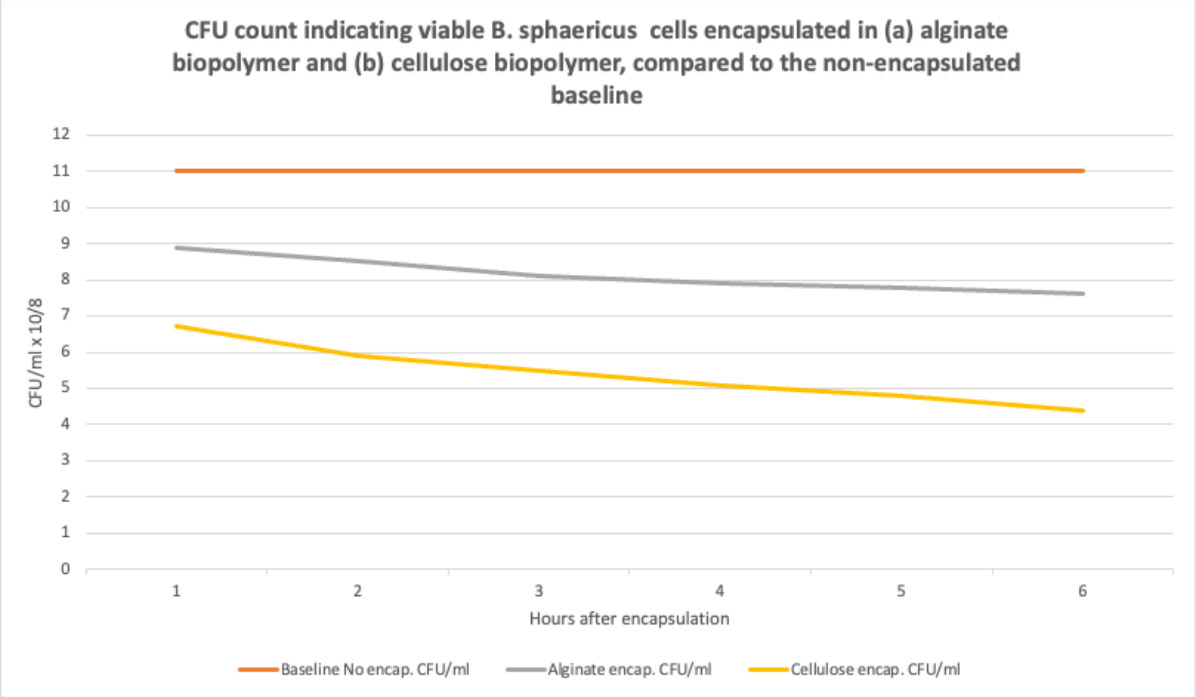


Figure 26: The impact of alginate and cellulose biopolymer encapsulation on cell viability. A lower CFU starting point for the alginate and cellulose biopolymer results as compared to the baseline is indicative of the volumetric impact of the biopolymer shell on reducing the cell count as compared to a liquid broth baseline comparison (red). Data located in *[Appendices: Table 29]*

To assess the decrease in the colony percentage count over the 6 hours, *Figure 27* illustrates the CFU mL^{-1} as a percentage of the CFU value taken at the start of the experiment.

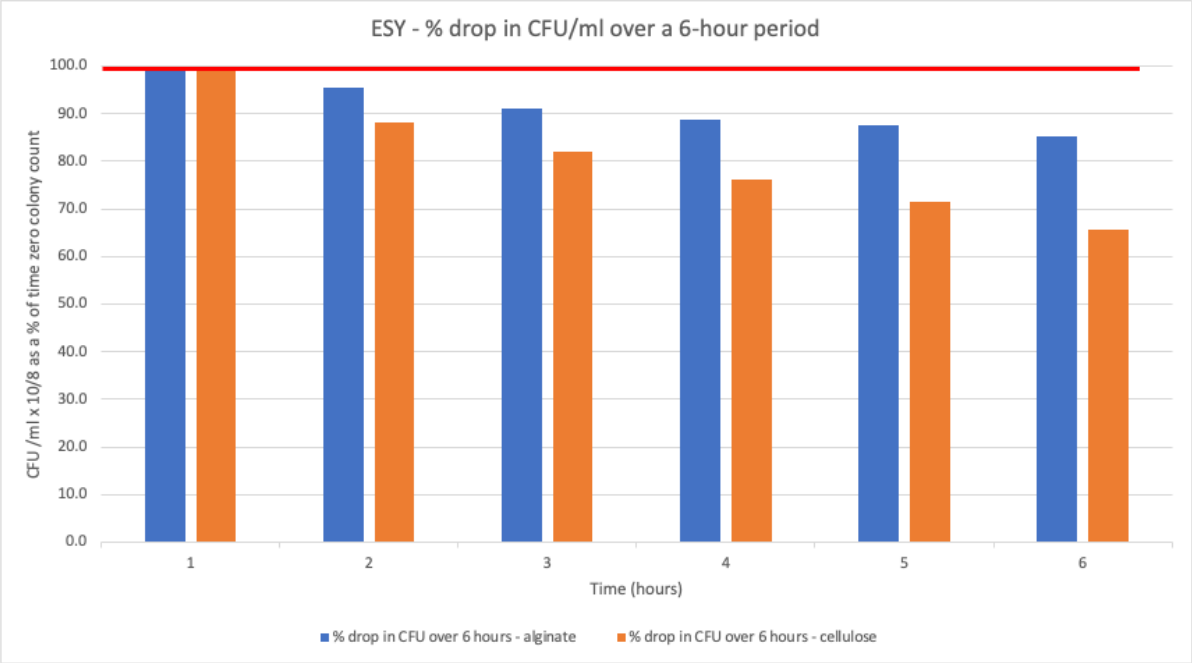


Figure 27: The *B. sphaericus* encapsulation survival yield (ESY) is a measure of the number of surviving cells post-encapsulation. Encapsulations CFU mL^{-1} were counted from the colonies formed on the inoculated agar plate, at a dilution factor of 10^6 before encapsulation and following the encapsulation process and monitored over six hours to evaluate the impact of the encapsulation process on cell viability. Data located in *[Appendices: Table 29]*

3.14.3.2 Comparative CFUs resulting from B. sphaericus inoculated alginate hydrogel

The alginate hydrogel fragments inoculated with *B. sphaericus*, underwent agar plating to quantify the formation of colony-forming units per millilitre, (CFU mL^{-1}). This assessment estimates the CFU count resulting from the immobilisation of *B. sphaericus* within the alginate hydrogel in comparison to the baseline count. *Figure 28* illustrates the comparative analysis of CFU count between *B. sphaericus* cells immobilised in the alginate hydrogel and the bacterial viability observed over 6 hours of immobilisation.

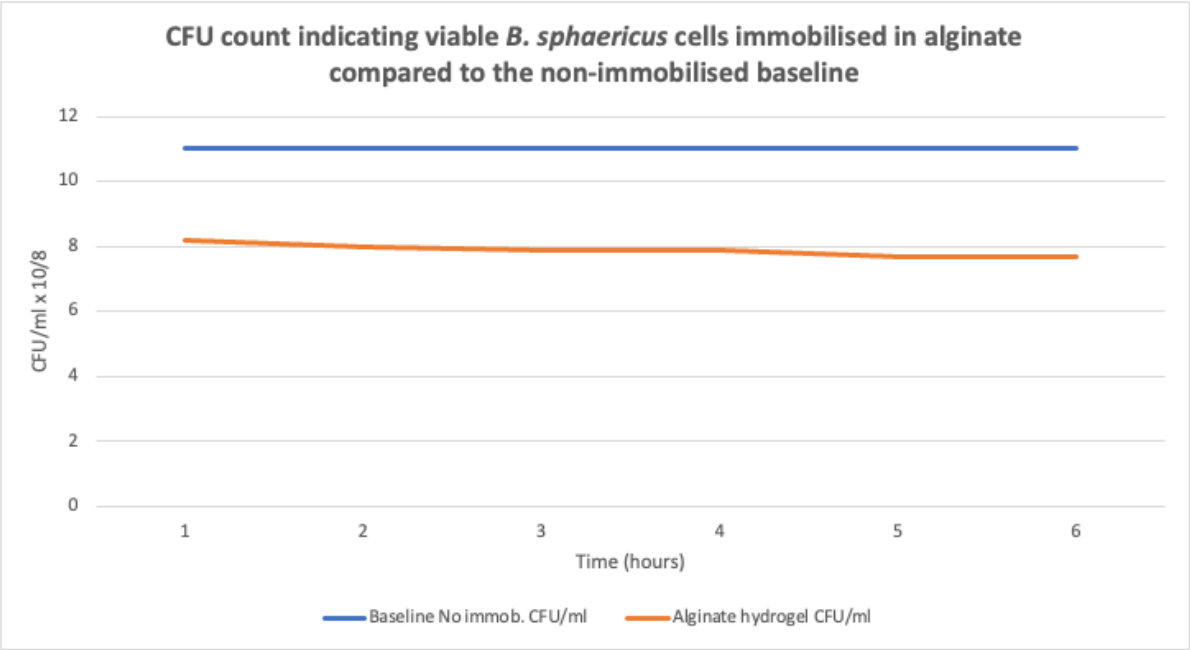


Figure 28: The CFU count for viable, immobilised *B. sphaericus* cells adhered to alginate hydrogel compared with viable non-immobilised cells, over 6 hours. Data located in *[Appendices: Table 30]*

The immobilisation survival yield (ISY) is a measurement of the number of surviving cells post-immobilisation. *Figure 29* illustrates the CFU mL^{-1} as a percentage of the time zero CFU value over the 6 hours.

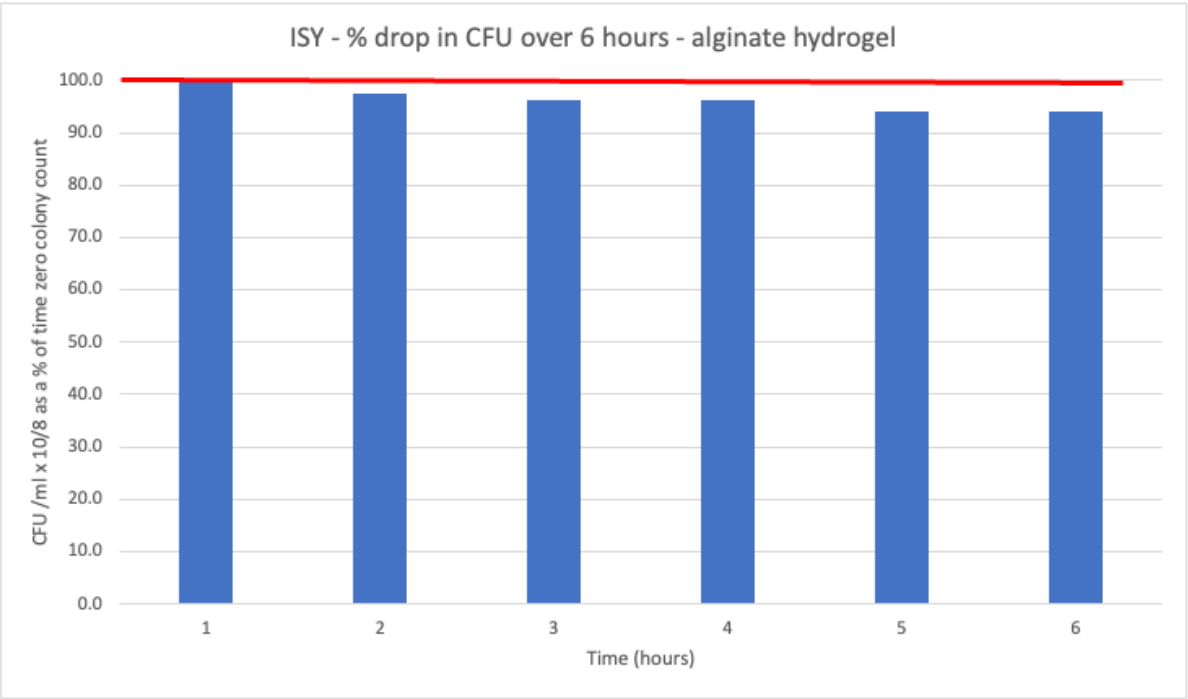


Figure 29: The immobilisation survival yield (ISY) counted from the plate colonies formed from the serial dilution - factor 10^6 before immobilisation and post-hydrogel immobilisation over six hours. The graph illustrates the impact of the alginate hydrogel on cell viability. Data located in [*Appendices: Table 30*]

3.14.3.3 In vitro and in situ results

The tabulated results for the (*in situ*) limewash wall sections and the (*in vitro*) lime units are found in the *[Appendices: Tables 31-39].*

Wall section A serves as the control consisting of 5 layers of limewash. The description of the bacteria, biopolymer, and method for insertion into the limewash is outlined in Table 11 for each of the sections B through H.

Table 11: Description of the formulations for each limewash layer as applied to the wall sections A-H. Identical formulations were utilised for the laboratory lime units. The control allocated to section A, comprises limewash void of bacterial or biopolymer additions. Wall sections B through H are formulated with bacterial, biopolymer or hydrogel components. In the instances when two different formulation layers are applied to the same section, (sections E, F, G) the layers are applied sequentially as outlined in Table 10

Section	Description
A	Control (Sample 1)
B	5-layers *B. sphaericus* (non-encapsulated) Sample 2
C	5-layers *B. sphaericus* (encapsulated) Alginate, Sample 3
D	5-layers *B. sphaericus* (encapsulate) Cellulose, Sample 4
E	2-layers *B. sphaericus* (immobilised), 3-layers *B. sphaericus* non-encapsulated, Sample 5
F	2-layers *B. sphaericus* (immobilised), 3-layers *B. sphaericus* , Alginate encapsulated, Sample 6
G	2-layers *B. sphaericus* (immobilised), 3-layers *B. sphaericus* , Cellulose encapsulated, Sample 7
H	5- layers *B. sphaericus* , (immobilised), Sample 8

Eight limewash samples were applied to assess their properties on different wall sections. The control sample 1 consisted of 5 layers of standard limewash. Samples 2, 3, and 4 contained non-encapsulated, alginate-encapsulated, and cellulose-encapsulated bacteria, respectively, applied in 5 layers. Samples 5, 6, and 7 had an initial 2 layers of base limewash with alginate hydrogel and immobilised bacteria, followed by 3 layers of non-encapsulated, alginate-encapsulated, or cellulose-encapsulated limewash, respectively. The final sample (8) consisted of 5 layers of alginate hydrogel with immobilised bacteria.

3.14.3.4 Porosity results

Images illustrating 60-second spot testing of Alizarin Red on each wall sample, sections A through H are illustrated in *Figure 30*. Profile sections were removed around the spot-tested limewash to enable measurement of the depth of Alizarin Red penetration into the wash and render. Measurements, in mm, were undertaken on-site and confirmed in the laboratory under an optical microscope.

Figure 30: The impact of Alizarin Red dye onto sections A – H, applying 0.1 ml of Alizarin Red (0.025M). The dye readily penetrated the limewash through open pores. Reduced pore accessibility to the dye resulted in horizontal spread on the surface of the limewash observed on sections E, F, and G

Phase 1

The results for the porosity measurements for *B. sphaericus* on wall sections A through H are plotted in Graph **A**, *Figure 31*, indicating the depth into the limewash open pore structure stained by Alizarin Red.

Comparative data for *B. sphaericus* inoculated lime units is displayed in Graph **B**, *Figure 31*.

Phase 2

The results for lime units comparing the control (limewash only) and alginate-encapsulated *S. elongatus* are displayed in Graph **C**, *Figure 31*.

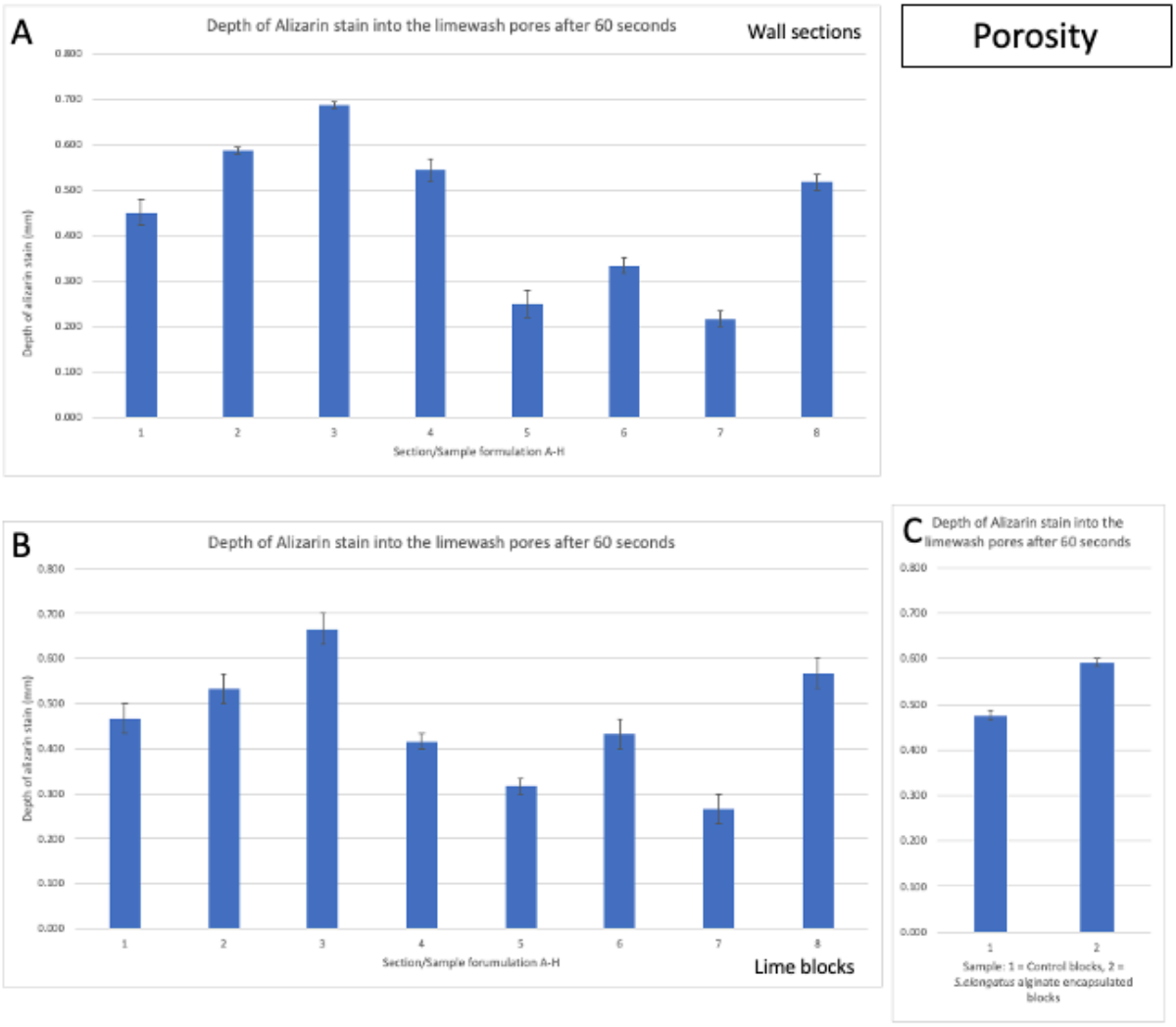


Figure 31: The impact of bacterial and biopolymer inclusion on limewash porosity assessed using a 60-second application of 0.1 ml Alizarin Red serving as a metric for open-porosity of the limewash. The standard error bars convey the extent of data dispersion around the mean value. Graph **A** illustrates porosity measurements taken on the wall sections A through H. Graph **B** delineates porosity measurements applied to the lime units. Graph **C** compares the impact on porosity for lime units of alginate biopolymer encapsulated *S. elongatus* (2) with the lime-only control formulation (1). Data located in *[Appendices: Table 31; Table 32; and Table 33]*

Statistical review of the data displayed in Figure 31

Standard error bars with minimal, or no overlap suggest a reasonable difference between the compared results. To confirm the significance of the difference, the study utilised a null hypothesis significance test between the control and alginate encapsulated *B. sphaericus* cells to determine if the results are from chance alone, or if the difference is significant. The null hypotheses and alternative hypotheses for graphs figures 31, 32 & 33 are noted in the appendices. It is common for biologists to use 5% as the critical significance level which is expressed in the tables below as alpha (α) at 0.05.

By rejecting (H_1), the null hypothesis (H_0) indicates the results are significant and the data pattern cannot be accounted for by chance alone.

Test material	Porosity results comparing the control to the alginate encapsulated *B. sphaericus*	Porosity results comparing the control to the alginate encapsulated *S. elongatus*
Lime blocks (*in vitro*)	P = 0.0209 P is therefore significant; the null hypothesis is rejected indicating there is a significant difference between the results	P = 0.0001 P is therefore highly significant; the null hypothesis is rejected indicating there is a significant difference between the results
Wall sections (*in situ*)	P = 0.0741 P is therefore suggestive only; the null hypothesis may or may not be accepted indicating and questions any significant difference between the results	

3.14.3.5 Cohesion results

Tests for cohesion serves as a quantitative assessment of the structural integrity or robustness of the external stratum of the limewash, indicating resistance to material displacement from the underlying strata. The quantification of solid mass removed through the tape-mediated peeling is expressed in grams per 250 square millimetre (g 250 mm^{-2}). This metric is derived from the comparative analysis of the predetermined weight of the peeling tape before and after its application to either the wall or the section of the lime block. The comparison of these outcomes is visually delineated in the graphical representations found in *Figure 32*.

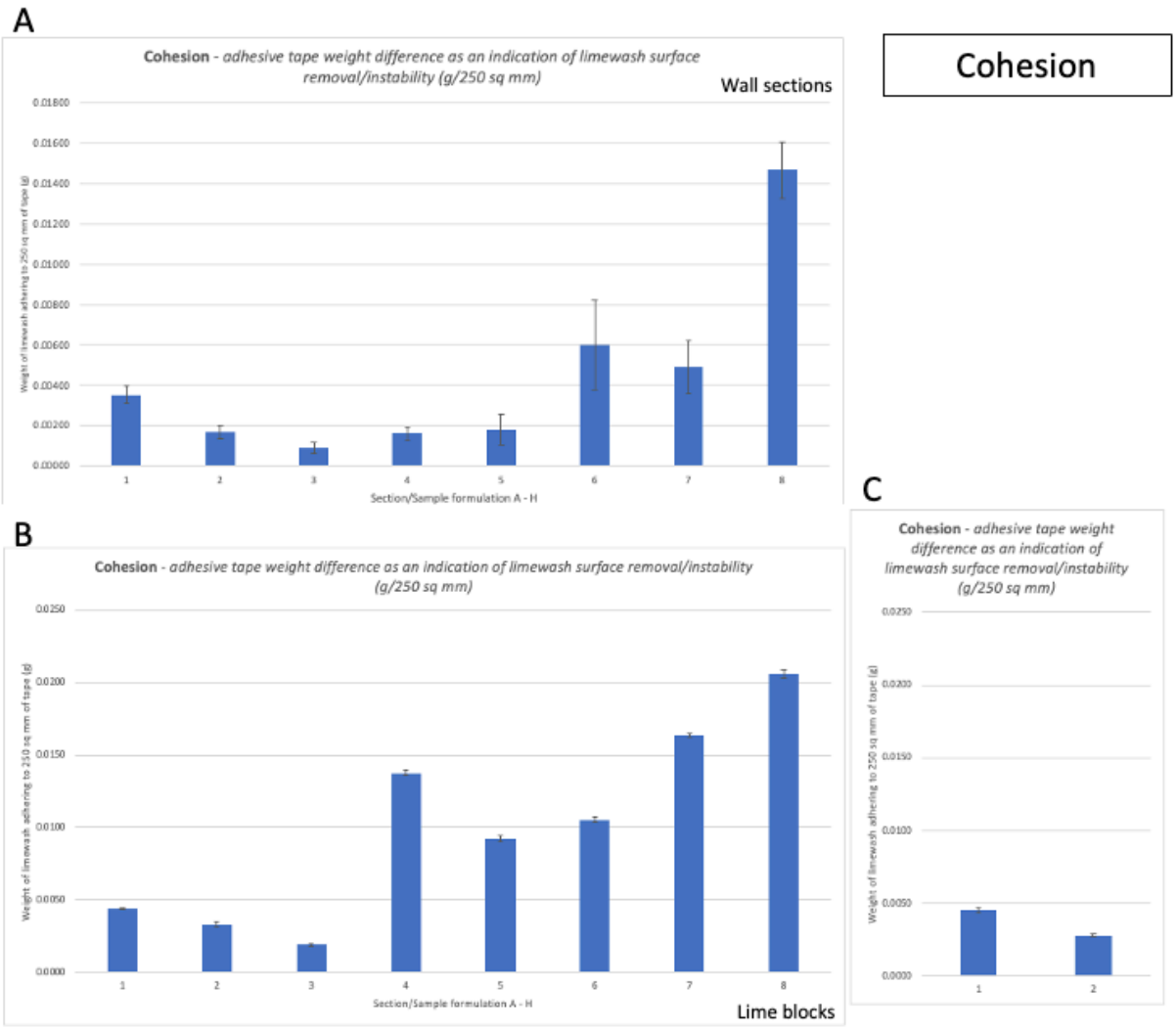


Figure 32: Impact of biopolymer and microbial inclusion on limewash cohesion. Graph **A** presents cohesion on *in-situ* wall section measurements. Graph **B** displays cohesion outcomes derived from measurements on corresponding lime units. Graph **C**, based on the experimental outcomes from Graphs **A** and **B**, compares cohesion measurements on control and alginate-encapsulated *S. elongatus* limewash on lime units. The standard error bars communicate the degree of spread of the data around the mean value. Data located in *[Appendices: Table 34]; Table 35; and Table 36]*

Statistical review of the data displayed in Figure 32

Standard error bars with minimal, or no overlap suggest a reasonable difference between the compared results. To confirm the significance of the difference, the study utilised a null hypothesis significance test to between the control and alginate encapsulated *B. sphaericus* cells to determine if the results are from chance alone, or if the difference is significant. It is common for biologists to use 5% as the critical significance level.

By rejecting (H_1), the null hypothesis (H_0) indicates the results are significant and the data pattern cannot be accounted for by chance alone.

Test material	Cohesion results comparing the control to the alginate encapsulated *B. sphaericus*	Cohesion results comparing the control to the alginate encapsulated *S. elongatus*
Lime blocks (*in vitro*)	P = 0.0385 P is therefore significant; the null hypothesis is rejected indicating there is a significant difference between the results	P = 0.0001 P is therefore highly significant; the null hypothesis is rejected indicating there is a significant difference between the results
Wall sections (*in situ*)	P = 0.0003 P is therefore highly significant; the null hypothesis is rejected indicating there is a significant difference between the results	

3.14.3.6 Water absorption – contact sponge method

A water saturated sponge was held for 60 seconds at a sustained pressure of 0.45 kg cm^{-2}. During the wall section assessment, the ambient temperature was 13°C and the relative humidity was 75%. The temperature was 18°C and the relative humidity 65% for the lime unit absorption measurement. The results are adjusted to g cm^{-2} for data comparison. *Figure 33* illustrates the data graphs for the test samples.

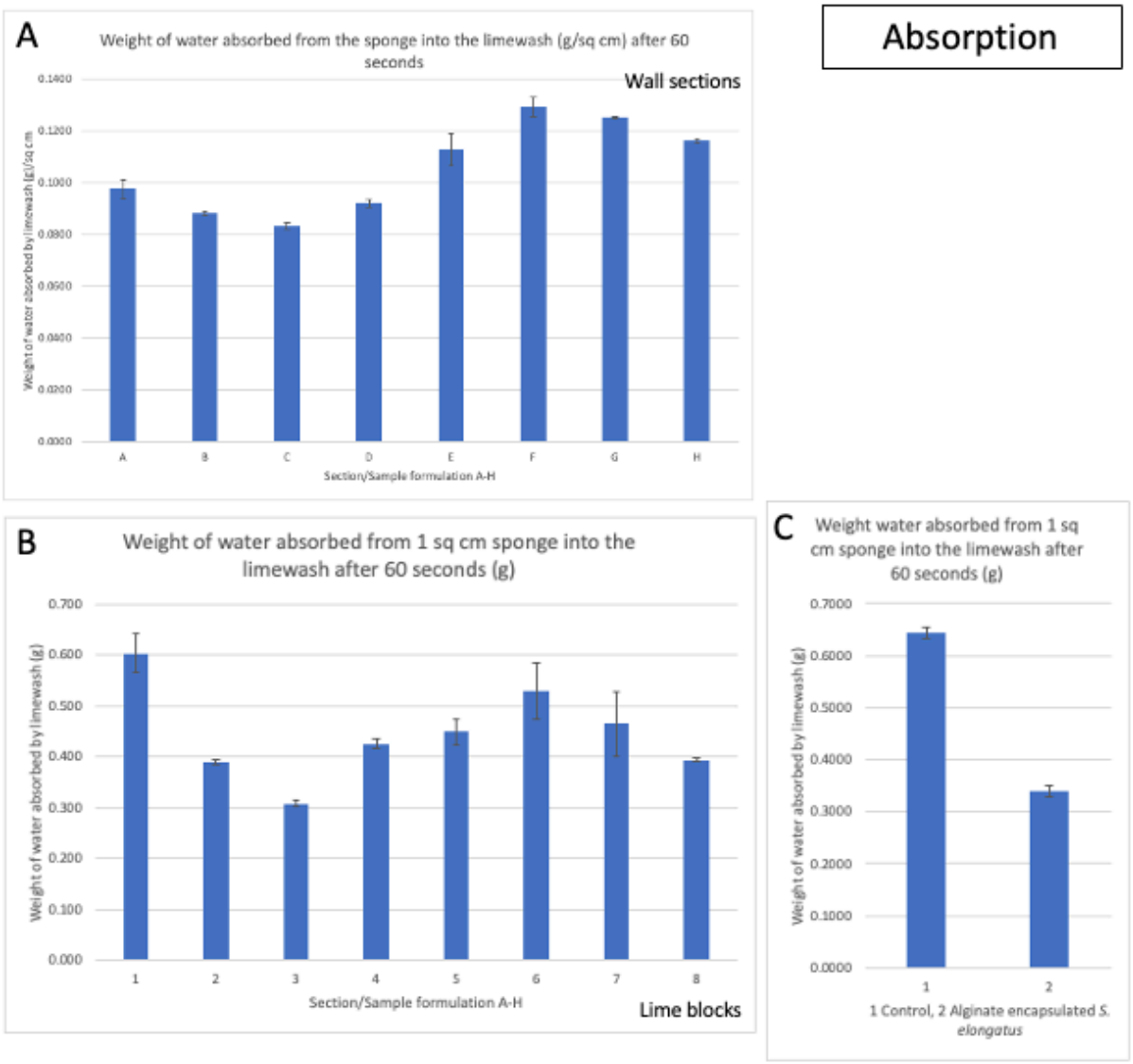


Figure 33: Impact of biopolymer and microbial inclusion on the water absorption properties of limewash. Absorption is a measure of the mass of water (g) absorbed by the limewash sample per sq. cm after 60-second contact between the sponge and lime wash surface. Graph **A** displays the results from the *in-situ* wall sections. Graph **B** charts the results from the *in vitro* lime blocks. The standard error bars communicate the degree of spread of the data around the mean value. Data located in *[Appendices: Table 37; Table 38; and Table 39]*

Statistical review of the data displayed in Figure 33

Standard error bars with minimal, or no overlap suggest a reasonable difference between the compared results. To confirm the significance of the difference, the study utilised a null hypothesis significance test between the control and alginate encapsulated *B. sphaericus* cells to determine if the results are from chance alone, or if the difference is significant. It is common for biologists to use 5% as the critical significance level.

By rejecting (H_1), the null hypothesis (H_0) indicates the results are significant and the data pattern cannot be accounted for by chance alone.

Test material	Absorption results comparing the control to the alginate encapsulated *B. sphaericus*	Absorption results comparing the control to the alginate encapsulated *S. elongatus*
Lime blocks (*in vitro*)	P = 0.0190 P is therefore significant; the null hypothesis is rejected indicating there is a significant difference between the results	P = 7.017E-08 P is therefore extremely significant; the null hypothesis is rejected indicating there is a significant difference between the results
Wall sections (*in situ*)	P = 0.0136 P is therefore significant; the null hypothesis is rejected indicating there is a significant difference between the results	

3.14.4 Analysis and Discussion

The study was premised upon two research sub-questions relating to the erosive atmospheric factors impacting limewash, exacerbated by the escalating frequency and unpredictability attributed to climate change. These questions are:

What is the impact of introducing natural biopolymers and biomineralising bacteria on the porosity, strength, and water resistance of limewash?

What is the efficacy of technologies that enable bacterial viability in limewash to enhance limewash curing?

The initial query hypothesises the potential ramifications of introducing biomineralising bacteria aligned with natural biopolymers on fundamental characteristics of limewash. The subsequent query scrutinises the efficacy of technologies to incorporate microbials into limewash prior to curing. To address these inquiries, the study examined the influence of two biomineralising microorganisms, namely bacterium *B. sphaericus* and cyanobacterium *S. elongatus* upon their introduction into limewash. This encompassed the examination of two naturally occurring biopolymers employed for the construction of protective structures through processes of encapsulation and immobilisation. The culmination of the study involved a comparative analysis of the effectiveness of three technological methodologies, coaxial extrusion, coaxial electrospraying and hydrogel formation. The integration of these outcomes facilitated the derivation of conclusions aimed at addressing the research questions put forward.

3.14.4.1 Survival yield for alginate and cellulose encapsulated cells

The quantification of colony-forming units (CFUs) serves as a metric for viable microbial cells within a given sample. The growth of CFUs over time offers a dynamic perspective on cell viability. Initially, a lag phase as cells adapt to their medium, may be observed. Subsequent phases represent active cell division, and exponential growth, eventually leading to a stationary phase denoting equilibrium between cell division and cell death. The last phase results in cell death due to nutrient exhaustion and raised concentration of metabolic waste products.

The encapsulation survival yield (ESY) is defined as the number of surviving cells post-encapsulation. In the quantification encapsulation, CFU mL^{-1} was counted from incubated plate colonies cultured from a serial dilution factor 10^6, yielding 30 – 300 colonies per plate. The assessment was performed

both pre- and post-encapsulation over six hours. The objective is to evaluate the impact of the encapsulation process on cell viability.

The purpose of the quantification is to ensure that cells are viable in sufficient numbers in encapsulated or immobilised states, enabling cell survival during the extrusion, collection, and transportation stages. The study posits that successfully encapsulated cells within a nutrient solution can survive the manufacturing process and successful medium-term storage at a temperature of 5°C until shipment. At this temperature cell metabolism reduces, mitigating metabolic stress within the capsule and temporarily arresting growth.

Figure 26 compares cell viability pre- and post-encapsulation over 6 hours measured at room temperature. The baseline (red) serves as the control indicating no discernible reduction in CFU count over the 6 hours for cells suspended in a nutrient solution.

The bacterial cells encapsulated within the alginate capsules demonstrated a marginal reduction in viability at encapsulation with a gradual decline in CFU count from 8.8 to 7.8 x 10^8 CFU mL^{-1}. The initial reduction from 11 x 10^8 CFU mL^{-1} suggests a combination of the extrusion stress during encapsulation, potential inhibitory effects upon contact with the $CaCl_2$ solution as the pre-formed capsule enters the hardening solution, and the influence of the capsule polymer in the plating solution.

A decrease in cell viability is evident within the cellulose capsules, following a pattern similar to the alginate-encapsulated cells, exhibiting a gradual reduction in viability over the 6 hours. The lower CFU viability in cellulose biopolymer capsules may be attributed to a weaker cellulose capsule wall structure or pDADMAC leakage into the cellulose capsule before hardening.

During the encapsulation process, alginate capsules promptly solidified forming a distinct opaque biopolymer shell, with sustained integrity during collection. In contrast, cellulose capsules upon exposure to the pDADMAC polymer hardening solution remained transparent, evolving into a semi-opaque state after 15 minutes. By visual observation, it is estimated the cellulose capsule formation had approximately an 80% success formation rate at extrusion compared to the alginate capsule success rate, closer to 95-98%.

Comparative analysis of encapsulation survival yield, expressed as a percentage of the initial extrusion CFU count, *Figure 27,* confirms a lower cell death reduction (15%) in alginate biopolymer capsules compared to cellulose biopolymer capsules (35%) over the 6 hours.

3.14.4.2 Survival yield for alginate hydrogel immobilised cells

B. sphaericus cells immobilised on an alginate hydrogel substrate exhibited sustained viability throughout a 6-hour post-immobilisation duration, *Figure 33*. The observed reduction in CFU counts relative to the non-immobilised baseline mirrors a similar trend as in the alginate and cellulose encapsulation process. The utilisation of $CaCl_2$ for polymerisation is a factor which may be implicated in the potential reduction of live cell viability upon adsorption onto the hydrogel surface. The subsequent removal of the cells from the hydrogel for plating, a requisite step for CFU enumeration may further contribute to a reduction in CFU results compared to the baseline.

The sustained cellular viability during the 6-hour post-immobilisation interval may be explained by the hydrogel attribute of retaining water and nutrients from the bacterial broth. Hydrogels, known for their gradual and consistent release of water and soluble nutrients, afford a protective zone to

the adsorbed cells ensuring their physical integrity and sustenance. This is substantiated by the maintained immobilisation survival yield at a level exceeding 90% at the termination of the 6-hour assessment period, *Figure 29*.

3.14.4.3 In vitro lime block analysis

The unit samples were maintained at laboratory controlled temperature and relative humidity conditions from the limewash formulation to unit application through to analysis. The controlled setting provided stable conditions for the systematic observation and quantification of the performance of each formulation on the lime units.

The sustained environmental uniformity diverging from in-situ weather conditions could impact the metabolic response of microorganisms used in the study. Specifically, the low relative humidity and the absence of rain may pose a constraint on the capacity of encapsulated and immobilised cells to actively metabolise within the limewash. Consequently, this could impede or even preclude the formation of Ca^{2+} nucleation sites. A daily spraying regime was implemented on the lime units to simulate an authentic rain cycle. It is important to underscore that this mitigation strategy, while introducing an element of variability is unrepresentative of the nuanced weather cycles experienced in-situ. The interpretation of the results should, therefore, be tempered by an awareness of the limitations inherent in the experimental design, *Table 12*.

Table 12: (Top bar - Section) – displays a qualitative summary of the lime units incorporating *B. sphaericus,* based on the three criteria measured to assess the ranked limewash formulation performance (Middle bars – Porosity, Cohesion, Absorption). The limewash formulations equate the formulations to wall sections/graph numbers and provide the formulation descriptions

Lime Units

Section	Below optimal Performing						Optimal Performance	
Porosity	G	E	D	F	A	B	H	C
Cohesion	H	G	D	F	E	A	B	C
Absorption	A	E	D	G	H	B	F	C

Section	Graph No.	Limewash formulation description
A	**1**	Control
B	**2**	5-layers *B. sphaericus* (non-encapsulated)
C	**3**	5-layers *B. sphaericus* (encapsulated) Alginate
D	**4**	5-layers *B. sphaericus* (encapsulate) Cellulose
E	**5**	2-layers *B. sphaericus* (immobilised), 3-layers *B. sphaericus* non-encapsulated
F	**6**	2-layers *B. sphaericus* (immobilised), 3-layers *B. sphaericus*, Alginate encapsulated
G	**7**	2-layers *B. sphaericus* (immobilised), 3-layers *B. sphaericus*, Cellulose encapsulated
H	**8**	5- layers *B. sphaericus*, (immobilised), Sample 8

3.14.4.4 In situ wall section analysis

The in-situ wall sections denoted as A to H, installed on the Isle of Jura, faced a North-East orientation, rendering them susceptible to the impact of prevailing weather conditions. Notably, during the initiation of the investigation, the installation of the limewash formulations on the wall section was conducted under uncharacteristically warm, dry, and sunlit weather conditions. This necessitated the consistent hydration of the wall section to avert premature drying, shrinkage, and

the formation of fissures within the wash. The comparative performance of each formulation of *B. sphaericus* on the wall sections is presented in *Table 13*.

Table 13: (Top bar - Section) – displays a qualitative summary of the lime sections incorporating *B. sphaericus*, based on the three criteria measured to assess the ranked limewash formulation performance (Middle bars – Porosity, Cohesion, Absorption). The limewash formulations equate the formulations to wall sections/graph numbers and provide the formulation descriptions

Wall Sections

Section	Below optimal Performing						Optimal Performance	
Porosity	G	F	E	A	H	D	B	C
Cohesion	H	G	D	F	E	A	B	C
Absorption	F	G	H	E	A	D	B	C

Section	Graph No.	Limewash formulation description
A	**1**	Control
B	**2**	5-layers *B. sphaericus* (non-encapsulated)
C	**3**	5-layers *B. sphaericus* (encapsulated) Alginate
D	**4**	5-layers *B. sphaericus* (encapsulate) Cellulose
E	**5**	2-layers *B. sphaericus* (immobilised), 3-layers *B. sphaericus* non-encapsulated
F	**6**	2-layers *B. sphaericus* (immobilised), 3-layers *B. sphaericus*, Alginate encapsulated
G	**7**	2-layers *B. sphaericus* (immobilised), 3-layers *B. sphaericus*, Cellulose encapsulated
H	**8**	5- layers *B. sphaericus*, (immobilised), Sample 8

The formulation C, comprising alginate-encapsulated *B. sphaericus*, exhibited superior performance compared to control (A) across all three evaluated parameters. This observation implies that *B. sphaericus* not only survived the application to the wall section but also initiated biomineralisation, thereby inducing a biotic augmentation of $CaCO_3$ in the abiotic curing of the limewash. The introduction of planktonic cells in nutrient broth directly into the limewash before application (B), yielded superior outcomes across the three assessed parameters, surpassing the outcomes for the control (A). This outcome was unexpected on two fronts: firstly, considering the susceptibility of cells to the initially elevated pH of the limewash, and secondly, the bacteria exhibited superior performance in comparison to both cellulose encapsulation and hydrogel immobilisation.

This outcome prompts inquiries into the impact of the biopolymer on limewash curing, the resilience of the cellulose capsules during application, and the influence of the hydrogel on limewash curing and adhesion. The previous assessment of cell survival yields indicates no hindrance to cell viability for alginate or cellulose biopolymers used in encapsulation or immobilisation. Consequently, a plausible explanation may infer that the physicochemical properties of cellulose during application are inferior to alginate, and alginate in a hydrogel form exerts an influence over limewash curing which hinders the curing. This explanation is substantiated by the suboptimal performance of the hydrogel and cellulose co-layered sections F & G. Further corroborating evidence is provided by the diminished effectiveness of both alginate-encapsulated bacteria (C) and non-encapsulated planktonic cells (B) when co-layered in a 2-layer, 3-layer formulation with a hydrogel.

3.14.4.5 Comparative analysis between the in vitro lime units and in situ wall section results

3.14.4.5.1 Porosity

Table 14: The limewash formulation demonstrating the optimal porosity performance is **C**, comprising 5 layers of limewash containing **alginate encapsulated *B. sphaericus***. The two formulations within the top three optimal porosity performances are **B**, comprising 5 layers of **non-encapsulated *B. sphaericus*** and **H**, 5 layers of **immobilised *B. sphaericus* in alginate hydrogel**. Formulations **C, B, H** and **D** showed greater porosity on the porosity scale than the control, A in the wall section comparisons. G displayed the lowest porosity ranking in both the lime units and the wall section.

Lime units

Porosity	G	E	D	F	A	B	H	C

Wall section

Porosity	G	F	E	A	H	D	B	C

The encapsulation of *B. sphaericus* within alginate matrices conferred the highest degree of protection and cell viability throughout the duration of the study. This is substantiated by the superior performance observed in porosity metrics, a result from the retention and enhancement of open pores within the limewash layers, *Table 14*. While the optical assessment of Alizarin Red revealed marginal differentials in porosity measurements among the formulations, the statistically significant distinction between the alginate-encapsulated bacterium and the control is noteworthy.

Sustained porosity serves as a quantifiable indicator of the moisture exchange between the building exterior and the underlying render layers. This parameter assumes critical importance in addressing concerns such as dampness, rising moisture, the egress of internal moisture from the building envelope, and the regulation of moisture accumulation in interstitial spaces.

Despite variations in ranking across lime units, the introduction of planktonic cells (B) into the limewash and the incorporation of bacterially adhered hydrogel (H) each engendered an optimal level of performance. It is possible a proportion of cells survived direct introduction into the alkaline limewash, somewhat diluted and less aggressive than lime render or concrete, and likely cell fragments from dead bacteria continued to provide nucleation sites for $CaCO_3$ formation. A similar scenario is conceivable for the hydrogel outcome; however, the failure of the hydrogel to manifest improved cohesion or adsorption implies that it is the interaction with the hydrogel, rather than live cells, that contributed to the enhanced limewash porosity.

3.14.4.5.2 **Cohesion**

Cohesion, denoting the extent of adherence within the surface layer and its resistance to erosion, constitutes the key parameter if porosity and absorption, on assessment, demonstrate acceptable performance ranges. The results of cohesion within the wall section were congruent with the lime unit outcomes. The control layer denoted as (A) serves as the benchmark, and any formulation yielding a substandard cohesion outcome compared to A necessitates rejection, *Table 15*.

Table 15: The cohesion results are identical for both the lime block and wall section testing. The optimal cohesion performance is **C**, comprising 5 layers of limewash containing **alginate-encapsulated *B. sphaericus***. The two formulations within the top three optimal cohesion performances are **B**, comprising 5 layers of **non-encapsulated *B. sphaericus*** and **A,** the **control.** All other formulations when measuring cohesion, performed at a level below optimal when compared to the control

Lime units								
Cohesion	H	G	D	F	E	A	B	C
Wall section								
Cohesion	H	G	D	F	E	A	B	C

Optimal cohesion performance, denoted as C, was observed in the case of alginate-encapsulated *B. sphaericus* and formulation B, the introduction of planktonic cells to the limewash. This indicates both formulations facilitate the accumulation of Ca^{2+} ions around nucleation sites for the generation of additional $CaCO_3$ molecules. While not quantified in the present study, it is plausible that the protective alginate layer enveloping the bacterial nutrient medium extends the calcium carbonating process within the limewash beyond the immediate nucleation of planktonic cells and cell membrane fragments.

The superior cohesion exhibited by formulations C and B establishes a foundational framework for enhancing the longevity of limewash against erosive weather forces, thereby retarding the sacrificial role of the limewash over the underlying render.

3.14.4.5.3 **Absorption**

The observed variance in *Table 16* for the control (A) may plausibly be attributed to an anomalously elevated ambient temperature during the preparation of the wall sections. In the absence of biopolymer or nutrient cell augmentations, the application of pure limewash would likely have undergone rapid desiccation, potentially resulting in microfractures within the lime matrix and subsequently amplifying water absorption characteristics. This explication may also be extrapolated to lime units subjected to low relative humidity and possessing a reduced scaled surface area, thereby facilitating accelerated desiccation in the absence of water-retaining biopolymer additions.

Table 16: The water absorption findings revealed a significant incongruity between the lime units and the respective wall sections. Alginate-encapsulated *B. sphaericus* (C) demonstrated superiority as the preeminent performer in comparison with alternative formulations, while the introduction of planktonic bacterial cells to the limewash (B) exhibited optimal performance

Lime units								
Absorption	A	E	D	G	H	B	F	C
Wall section								
Absorption	F	G	H	E	A	D	B	C

3.14.4.6 Comparative analysis of the optimal performance limewash formulations

In the context of formulations B to H, each introduces a biotic component with the overarching objective of augmenting the $CaCO_3$ density of limewash in comparison to the control, denoted as A. As outlined in the introduction to the chapter, this strategic augmentation seeks to extend the durability of limewash. The evaluation of durability in this study hinges upon three critical criteria: porosity, cohesion, and absorption. These parameters collectively serve as benchmarks for assessing the efficacy of the formulations and the impact this will have on extending the overall lifespan of limewash as a protective coating.

3.14.4.6.1 Formulation B – planktonic addition of *B. sphaericus* in nutrient medium to limewash

Formulation B exhibited optimal efficacy across all three evaluation criteria. This superior performance may be attributed to the capacity of dead cell fragments serving as nucleation sites for crystallisation within the limewash, facilitated by the retained capacity of cell membranes to assimilate Ca^{2+} cations, (Dupraz et al., 2009).

Biologically influenced mineralisation, serves as the primary contribution by this phenomenon, extending the biomineralisation process, thereby augmenting performance relative to the control group. A similar outcome was replicated on the primary façade of the Angera Cathedral in Italy. This involved the application of bacterial cell fractions, dissolved in $CaCl_2$, onto the surface, succeeded by the administration of a supersaturated calcium bicarbonate ($Ca(HCO_3)_2$) solution to furnish calcium ions and CO_2. Notably, this investigation elucidated the genesis of novel crystals within the pores of the stone substrate, yielding a discernible reduction in water absorption by as much as 6.8%, (Perito et al., 2014).

3.14.4.6.2 Formulation C, alginate encapsulated *B. sphaericus* in limewash

Encapsulation of *B. sphaericus* within the alginate biopolymer (C) consistently exhibited optimal performance across all three criteria, surpassing the control group in both *in situ* and *in vitro* assessments. The results demonstrated by formulation C, indicate augmentation and evidence of open porosity, thereby enhancing the moisture management capability of the limewash. Notably, this improvement did not compromise its absorption characteristics concerning water saturation resistance.

The literature varies on the length of time for microbially induced carbonate precipitation to form, one reason for the divergence arises from variations in the different mediums utilised for measurement and observation. Biomineralisation can manifest within a relatively short timeframe of 72 hours, depositing calcium carbonate crystals exhibiting morphological transformations from ellipsoidal to rhombic structures. (Gu et al., 2022). This phenomenon, observed through scanning electron microscopy, implies that bacteria can serve as nucleation sites for calcium carbonate formation from the initial day of incubation. However, owing to the diminutive size of the rhombic structures, their perceptibility may be delayed until the seventh day or later. It is noteworthy that crystal formation may be less conspicuous in the short term when bacteria are shielded by encapsulation.

3.14.4.6.3 Formulation D, cellulose encapsulated *B. sphaericus* in limewash

There is disparity in formulation D, lime unit performance in comparison with the wall sections, with porosity and saturation, recorded below optimal. The cellulose-encapsulated *B. sphaericus* formulation consistently exhibited inferior performance compared to formulation C, alginate-encapsulated and planktonic formulation B.

The diminished performance may be attributed to a rapid desiccation of the blocks, leading to failure of the cellulose as an encapsulating agent, to regulating water retention and release. This may explain the improved performance of the cellulose-encapsulated formulation on wall sections which were exposed to frequent rainfall during the study.

During the processes of extrusion and electrospraying, the cellulose capsules exhibited a prolonged formation in pDADMAC solution in comparison to the alginate counterparts in $CaCl_2$. Furthermore, the conversion rate of fully formed cellulose capsules extruding into the pDADMAC was notably lower, around 70-80% formation rate, in stark contrast to the 98% conversion rate observed for alginate capsule formation.

This discrepancy indicates a proclivity for cellulose to form a weaker biopolymer shell relative to alginate. Additionally, the cellulose biopolymer capsules demonstrated a tendency to coagulate within the pDADMAC solution, necessitating continuous rotation of the collecting plate to prevent premature fusion of cellulose capsules before the polymerisation process attained complete solidification.

3.14.4.6.4 Comparisons and discussion on formulations E, F, G and H

The outlier within the formulations denoted on the wall sections and lime units as E, F, G, and H, exhibited suboptimal performance, is formulation H. Formulation H distinguishes itself as an immobilising matrix for *B. sphaericus*, thereby facilitating adherence to the hydrogel matrix. A salient attribute of alginate hydrogel resides in its capacity to sequester water during the polymerisation process, gradually dispensing both water and associated nutrients over an extended time span, which can extend to several months. This inherent property likely contributed to the formulation H limewash attaining optimal performance on lime units, effectively mitigating the desiccative influence of the laboratory atmosphere, and releasing water and nutrients for the bacterial cells to remain viable thereby enhancing porosity and saturation characteristics.

Concomitantly, the wall segment of hydrogel-based formulation H exhibited some porosity performance, albeit saturation levels deviated from the optimal range. Plausible possibilities posit that the timed release of water from the hydrogel, sustained limewash porosity, whereas the wall segment, subject to ambient humidity and rainfall, experienced a dampening effect on hydrogel water release, resulting in suboptimal saturation performance.

The hydrogel-infused section H demonstrated suboptimal cohesion for both wall and block segments. Paradoxically, the favourable impact of hydrogel-mediated water management on limewash porosity appears to compromise the overall structural integrity relative to alternative formulations. It is conjectured that, over a protracted trajectory spanning several months, the gradual water release might foster prolonged biotic and abiotic CO_2 fixation within the limewash, culminating in a long-term resilient surface. However, the duration of the study precludes conclusive validation of this hypothesis, and the survivability of the initially softer hydrogel under sustained environmental extremes for building surfaces remains a subject of contention.

Sections E, F, and G featured composite limewash layers, with the foundational two layers comprising hydrogel-limewash formulation, juxtaposed with the subsequent three layers either non-encapsulated or encapsulated with alginate or cellulose. The composite limewash layers consistently demonstrated suboptimal performance. The inclusion of these composite layers, guided by the anticipation that the underlying slow-water-releasing hydrogel layers would augment the external bacterial layers, whether encapsulated or not, with a view to achieving optimal cohesion, porosity, and saturation performance, proved unsuccessful. Results imply that the hydrogel layers may have impeded the initial curing process, leading to compromised adherence of limewash layers to the underlying lime render, thereby resulting in suboptimal cohesion, porosity, and saturation characteristics.

3.15 PHASE II: Inclusion and performance of alginate encapsulated *S. elongatus* in limewash

The outcomes derived from phase I experimentation utilising *B. sphaericus* across diverse biopolymer formulations have provided instructive insights for evaluating the incorporation of cyanobacterial *S. elongatus* into alginate-encapsulated limewash. *S. elongatus,* a unicellular cyanobacterium, exhibits rapid autotrophic growth contingent upon sunlight availability and an appropriate nutrient profile. Importantly, *Synechococcus* is not associated with harmful algal blooms or freshwater toxins. This cyanobacterium, a freshwater photoautotroph, adeptly sequesters atmospheric CO_2 to build energy reserves. The capacity to sequester atmospheric CO_2, coupled with the absence of environmentally toxic metabolic by-products, typical of heterotrophs, renders *S. elongatus* a sustainable option for augmenting biomineralised limewash.

Figures 31, 32, and *33* delineate the performance differences between alginate-encapsulated *S. elongatus* and the control, contextualised against the benchmarks established during phase I, utilising *B. sphaericus* formulations. In each figure, graph C respectively illustrates statistically significant positive outcomes for porosity, cohesion, and absorption, contrasting favourably with the control, and mirroring the performance of alginate-encapsulated *B. sphaericus*.

In summary, both *B. sphaericus* and *S. elongatus*, when encapsulated in alginate, exhibit biomineralisation capability within limewash, with *B. sphaericus* demonstrating marginally superior performance. However, the advantages of incorporating either microorganism into lime-based materials are evident. Limewash, characterised by its diluted alkaline nature, is a receptive substrate for alginate-encapsulated *S. elongatus*, leveraging daylight availability, atmospheric CO_2 sequestration capability, and the absence of toxic metabolic by-products during mineralisation. In regions with limited sunlight, such as winter in the northern hemisphere, B. sphaericus may emerge as a more robust alternative, foregoing atmospheric CO_2 sequestration for broader applicability in harsher environmental conditions.

3.16 Addressing the research questions

The investigation into the impact of incorporating biomineralising bacteria, aligned with natural biopolymers on the porosity, strength, and water resistance of limewash has yielded findings which support limewash biomineralisation as a viable study for improving the outer surface robustness and longevity. The integration of live *B. sphaericus* or *S. elongatus* into the limewash matrix has demonstrated improvements in the short-term curing process when compared to abiotic curing. Optimal performance above the standard limewash control was discerned across the selected key parameters—porosity, cohesion, and absorption, results which challenge the view that the ostensibly inhospitable nature of the strongly alkaline limewash matrix would induce rapid cell death.

Results on planktonic inclusion into the limewash indicate that despite a higher proportion of cell death, the retained capacity of deceased cells to function as crystallisation nuclei in the liquid medium is a function of the ability of cell wall fragments to uptake cations such as Ca^{2+}, (Dupraz et al., 2009). Further exploration into the temporal dynamics of Ca^{2+}-mediated membrane activity could shed light on the duration and extent to which deceased cells contribute as crystallisation nuclei.

The utilisation of alginate-immobilised *B. sphaericus*, however, diverged from expectations that the adsorption of bacterial cells onto the nutrient hydrogel would protect and hydrate thereby sustaining bacterial nucleation of calcium carbonate, an outcome not substantiated. Instead, the hydrogel presence impaired open porosity and limited resistance to absorption, outcomes resulting in observed weakening of the adhesion of limewash layers to the underlying render, compromising the cohesive integrity of the outer layer. The incorporation of underlying layers of *B. sphaericus* hydrogel formulation with various encapsulation strategies failed to provide the anticipated hydrating and supportive nutrient underlay, resulting in suboptimal performance for each formulation.

Contrastingly, alginate-encapsulated *B. sphaericus* demonstrated optimal performance across all evaluated criteria—porosity, cohesion, and absorption. The alginate biopolymer displayed robust polymerisation during extrusion, forming a protective capsule around the bacterial cells. Notably, these encapsulated bacteria exhibited longevity throughout the manufacturing, storage, and delivery processes, even when transported over a considerable distance at ambient temperature. In contrast, cellulose-encapsulated cells exhibited lower robustness during manufacture, resulting in a slower and less effective polymerisation process. The coagulation of cellulose polymer capsules further reduced the surface area available for bacterial release upon application to the render surface. Comparative assessments of in vitro cell viability over the initial six hours post-manufacture underscored the superior robustness of alginate-encapsulated cells compared to their cellulose-encapsulated counterparts.

Drawing upon the outcomes from phase I wherein the assessment of bacterium *B. sphaericus*, transpired that the alginate biopolymer encapsulation emerged as the most effective biomineralisation vehicle, an analogous approach was adopted to evaluate phototropic cyanobacteria, *S. elongatus*. Commensurate outcomes were observed, underscoring the imperative role of UV light furnishing the energy requirements for the biomineralisation in *S. elongatus*.

In consideration of these findings, the investigation into the effectiveness of technologies facilitating bacterial viability in limewash for augmenting the curing process, emerges as a complex and multifaceted undertaking. The interaction among bacterial encapsulation, biopolymer selection, and subsequent effects on crucial performance parameters demonstrated in this study, presents an opportunity for a thorough exploration to elucidate the underlying mechanisms that may catalyse a broader application for bio-enabled surface protection.

The synergy of these technologies with chemobiological variables, albeit multifaceted, imparts several key insights. The generation of encapsulated three-dimensional structures through the application of coaxial bioprinting techniques affords a spectrum of dimensions for capsule construction, contingent upon factors such as coaxial extruding needle diameter, biopolymer type, concentrations of biopolymer and hardening agent, and the speed of bioprinting. The physical dimensions of the coaxial nozzle can impose limitations on the extent of capsule size reduction via bioprinting extrusion methods. Nevertheless, further reduction in capsule size and enhanced capsule production can be achieved through the introduction of a voltage differential between the extruding nozzle and the collecting fluid. The size and speed of the capsules exhibit a direct proportionality to the applied voltage, necessitating stringent safety protocols for both associated machinery and operators in the presence of the requisite 30 kV differential. Electrospraying smaller biopolymer capsules, with minimal impact on the encapsulated microorganisms, holds promise for refining and enhancing the performance of bio limewash.

3.17 Conclusion

The investigation was prompted by the erosive impact of increased frequency and intensity of weather patterns on limewash surfaces. The goal was to fortify the erosive resistance of limewash, which involved the preservation of distinctive lime attributes, including porosity and resistance to saturation. The positive consequence is a reduction in the frequency of exterior maintenance mitigating the carbon footprint associated with material degradation, labour, material transportation, and ancillary activities such as the conveyance and assembly of scaffolding. Of note is the correlated decrease in costs, rendering the initiative both environmentally and economically efficacious.

The research explored two focal areas: the impact of biopolymer-protected bacteria proficient in biotic biomineral formation and the identification of technology conducive to the embedding and viability of bacteria within the limewash solution.

Two microbial options, *B. sphaericus* and *S. elongatus*, were identified in the literature as facilitators of biotic calcium carbonate formation. The latter is a photosynthetic cyanobacteria capable of sequestering atmospheric CO_2.

The encapsulation of *B. sphaericus* or *S. elongatus* within alginate matrices offers a methodological approach to augment the biomineralisation process of limewash, as confirmed through statistical analyses comparing the limewash control to the experimental formulation.

Encapsulation within a calcium alginate biopolymer delivered a robust 3D bioprinted protective shell. This allowed for the introduction of either *B. sphaericus* or *S. elongatus* into mixed limewash formulations, with minimal compromise to cell viability. The alginate biopolymer yields a resilient, cost-effective, and sustainable resource that gradually decomposes into non-toxic constituents.

The alginate biopolymer safeguards the encapsulated microorganism when introduced into the alkaline limewash. Mechanically applying the limewash formulation to the wall ruptures the alginate capsules, thereby instigating the biomineralisation process.

Two bioprinting technologies underpinned the encapsulation process: 3D extrusion printing and 3D electrospraying. While both methodologies yielded intact capsules suitable for limewash integration, the latter exhibited a notable advantage in generating smaller capsule diameters at an accelerated rate. This was attributed to the energy supplied by the applied voltage differential between the printing nozzle and the collecting base. Importantly, cell viability remained comparable for both encapsulation techniques.

This study successfully demonstrated the encapsulation of *B. sphaericus* and *S. elongatus* within an alginate biopolymer. The encapsulation process demonstrated effective biomineralisation, augmenting porosity, enhancing cohesion, and improving water absorption characteristics. Consequently, these enhancements collectively fortified the resilience and durability of the limewash. *S. elongatus* is most suitable for limewash application within the late Spring to early Autumn timeframe, contingent upon latitudinal considerations. Both microorganisms are optimally deployed under temperate climatic conditions, precluding weather extremities.

Longer-term studies will afford opportunities to observe ongoing fortification of limewash durability, ensuring congruence with conservation and environmental principles.

3.18 Next steps for the study

The investigation has affirmed the potential to enhance the durability of limewash against erosive climate-induced weather fluctuations through the incorporation of calcium carbonate biomineralising bacteria, within a 3D bioprinted alginate-based biopolymer matrix.

To progress towards a commercially viable formulation, it is imperative to undertake further study, ensuring alignment with environmental and conservation practices. Areas warranting further study include understanding user preferences for building hues which will require coloured additives into the limewash. Understanding the effect of coloured pigments on bacterial viability and the consequential influence on calcium carbonate formation at nucleation sites requires a broad study of available limewash pigments.

During the study, an encapsulated biopolymer-to-limewash ratio of 10:90 was applied, based on initial experimentation, whereas more optimal ratios may be applicable and need further investigation.

Over several years, the limewash may change its properties depending on longer-term alterations in the chemobiological components of the formulated limewash. This includes investigating any repercussions of incorporating alginate biopolymers on the propensity for organic growth to colonise building surfaces and ensuring bacterial metabolism does not cause changes in the limewash patina due to long-term chemical interactions.

Subsequent discussions on prospective commercialisation and large-scale production must consider:

The viability of provisioning a nutrient additive featuring encapsulated viable cells to commercially available limewash. Stock preservation at 5°C to attenuate metabolic activity is plausible, albeit considerations must encompass refrigeration costs and inclusive delivery packaging within the economic paradigm. The finite viability of encapsulated cells at ambient temperatures, owing to metabolic depletion of nutrients and escalating cytotoxic metabolic by-products, must be factored into assessments.

There are several developmental opportunities to explore in the area of bacterial inclusion. By forcing the bacterial cells to sporulate, spores which are highly resistant to adverse conditions, introduced into alginate capsules, may be freeze-dried. Similar to freeze-drying foods, the spore-containing capsules may have an extended shelf life and provide a ready solution to storage and transportation issues which face live encapsulated alginate capsules. Further investigation is needed to assess the readiness of encapsulated spores to rehydrate in limewash and the time necessary for the bacteria to emerge from the spores and participate in biomineralisation together with the percentage viability yield following undergoing freeze-drying and long-term storage. The technological intricacies underpinning the freeze-drying of the formulation necessitate further exploration with regard to its impact on microbial longevity and biomineralisation performance. This entails designing forced sporulation, discerning reanimation conditions, conducting longevity investigations, and evaluating the ramifications of the process on the biopolymer polysaccharide chain architecture.

A second opportunity builds synergy with the second practice component of this study, the extraction and isolation of secondary metabolites, *Chapter Four*. Introducing microbial secondary metabolites during bioprinting into existing or supporting capsules as natural biocides introduces antimicrobial agents to prevent unwanted microbial growth on the limewash surface. This approach mitigates the combined effect of anthropogenic pollutants, the presence of carbon and nitrogen

additives which with warmer wetter weather encourages unwanted organic growth, deteriorating the patina of the building surface.

This roadmap for further investigation serves to address critical gaps in understanding encapsulation and biomineralisation in limewash, laying the groundwork for the development of a robust and sustainable solution with broad applicability in conservation practices.

CHAPTER FOUR: Antimicrobial impact of lichen secondary metabolites on climate-accelerated biodeterioration

Chapter Abstract

Biodegradation poses a significant threat to the longevity and integrity of building structures. Microbial colonisation, fuelled by readily available water, favourable environmental conditions, and nutrient sources, accelerates the deterioration of both exterior and interior surfaces. Climate change exacerbates this challenge by increasing moisture saturation, humidity levels, and erosive forces, further jeopardising the structural stability of buildings. Moreover, temperature fluctuations alter microbial growth patterns and metabolite production, potentially leading to increased occupant infection rates and inflammatory responses. Even non-pathogenic microbes must adapt their biochemical arsenal to survive and compete for resources in this changing landscape, an adaptation which may provide potential sources for antimicrobial agents.

The incorporation of organic materials into traditional building products, as discussed in Chapter Three, introduces a potential carbon source for colonising organisms, promoting biodeterioration of the substrate. Therefore, it is crucial to develop bioprotective strategies that target colonising organisms responsible for biodegradation while leaving beneficial bacterial inclusions, such as those involved in biomineralisation, unharmed. This necessitates the identification and extraction of naturally occurring antimicrobial compounds with selective activity.

This chapter explores the potential of non-pathogenic organisms, such as lichen, to biosynthesise a diverse array of pharmaceutically active compounds capable of inhibiting the growth and colonization of human pathogens on building surfaces. Secondary metabolites extracted from two lichen species, *Ochrolechia parella* and *Ramalina siliquosa*, sourced from island and coastal locations in the Northern UK, were evaluated for their antimicrobial efficacy against both pathogenic and biodegenerative microbes, *Staphylococcus aureus* and *Penicillium chrysogenum*. The lichen species selected share the same environmental habitat as the target microbes, potentially enhancing the specificity and effectiveness of their secondary metabolites.

The extraction and isolation of these secondary metabolites offer a more environmentally sustainable alternative to conventional chemotoxic or mechanical treatments for removing moulds and bacteria from building materials. This approach is less likely to induce allergic reactions or inflammatory responses in building occupants compared to harsh chemical agents.

The research findings demonstrate that isolated secondary metabolites from *Ochrolechia parella* and *Ramalina siliquosa* exhibit potent antimicrobial activity against *Staphylococcus aureus* and *Penicillium chrysogenum*. Eight out of the twenty-one isolated secondary metabolite extracts displayed significant antimicrobial potential. Moving forward, this study recommends employing advanced analytical techniques, such as Mass Spectrometry and Nuclear Magnetic Resonance, to identify the isolated bioactive compounds. Comparing the ion fragmentation patterns of the isolated metabolites with known secondary products from *O. parella* and *R. siliquosa*, can potentially identify existing compounds with targeted antimicrobial properties. For metabolites with no existing matches, sequencing the chemical structure offers the exciting possibility of discovering novel secondary metabolites with valuable antimicrobial properties.

This research paves the way for the development of bio-based antimicrobial strategies for building materials, promoting a more sustainable and occupant-friendly approach to managing microbial colonisation and biodegradation in the built environment.

4.0 Introduction

Chapter Four delves into the identification of novel lichen-based antimicrobials as natural deterrents against climate-driven biodeterioration as a result of microbial colonisation.

As elaborated upon in the main introduction, this chapter delves into the second practice component of the employed methodology. This component is preceded by a rigorous examination of relevant literature and design methodologies, which serves as the theoretical underpinning for the subsequent practical exploration. This approach ensures a well-grounded and informed engagement with the chosen practice, fostering a deeper understanding of its potential contributions to the research objectives.

Lichens represent a notably rich source of secondary metabolites with the potential to unveil previously unexplored applications in the pharmaceutical industry. Nearly 1000 secondary metabolites, predominantly specific to lichen, have been identified. These compounds exhibit pharmaceutical potential as antimicrobials, antioxidants, antivirals, anti-inflammatories, and cancer treatments. The unique relationship between the lichen photobiont and mycobiont is only partially understood in terms of how this symbiotic partnership contributes to a diverse array of secondary products. These partnerships confer an extended lifespan to the lichen, with the photobiont shielding against the detrimental effects of UV light, and the mycobiont countering the impacts of dehydration and damage from adverse climate conditions.

The synthetic design, development and manufacture of antimicrobial agents is time-consuming and costly in comparison to microbial bio-manufacturing. Microorganisms have evolved highly efficient manufacturing processes developing biosynthetic pathways to repurpose primary metabolites. The chemical logic governing the formation of the C-C and C-N bonds within secondary product scaffolds along with the enzymes orchestrating synthesis, characterise the synthesised products. Predicting the outcomes of modifications is contingent upon discerning patterns of reactivity. The efficiency of the biosynthetic pathway for secondary products can be enhanced by manipulating external growth conditions, supplying alternative precursor building blocks or modifying the cellular manufacturing process through alterations to the gene sequence. Genes responsible for biosynthetic enzyme production are frequently clustered on the genome, co-located with resistance and export genes, and exhibit coordinated transcription in one or more operons. Activator or repressor signalling molecules respond to diverse environmental and cellular conditions acting in concert with primary metabolic pathways, modifying the secondary metabolite synthesis pathway.

The yield of secondary active compounds derived from biological sources is typically low. Solvent extraction protocols though time and solvent-intensive, are the predominant methods for obtaining secondary product extracts restricting the discovery of natural products for pharmacological development. An urgent need exists to devise cost-effective methods for the extraction and identification of bioactive natural products. While solvent extraction protocols dominate, the choice of solvent becomes central for optimal extraction. The diverse range of secondary products produced by individual lichens necessitates meticulous separation, isolation, and testing to evaluate the pharmaceutical potential of these high-yield compounds. This study investigates solvent extraction, isolation, and purification techniques to assess the antimicrobial properties of secondary metabolites from the lichens *Ochrolechia parella* and *Ramalina siliquosa* on the nosocomial pathogen, *Staphylococcus aureus* and mould *Penicillium chrysogenum.* Alongside *Aspergillus, Cladosporium* and *Stachybotrys chartarum,* both *S. aureus* and P. chrysogenum can cause acute toxicity, even death. In addition to conventional microtiter assays to determine antimicrobial activity, this study also references the use of mass spectrometry as a method to identify the

chemical signatures of extracted antimicrobial secondary products, though this study focuses on identifying antimicrobial activity.

4.1 A review of lichen secondary metabolites

The use of biological materials sourced from herbal and alternative folk medicines date as far back as 60,000 years in Iraq, with written records some 8,000 years ago in China and 5,000 years in Sumer. Despite a millennium of practice, the absence of empirical data was met with scepticism by the scientific community. The resurgence of interest in alternative natural sources, driven by the escalating challenge of antimicrobial drug resistance, is contributing to the exploration of unconventional avenues in antimicrobial research, and to future profitability within the pharmacological sector (Pan et al., 2014).

Traditionally, lichens have been employed for medicinal, culinary, perfumery, and colouring textiles. In the last five decades, a growing number of compounds with pharmaceutical potential have been isolated from various lichen species. Notable among these is 'Chharila,' a blend of *Parmelia, Usnea longissima, Ramalina subcomplanata* and *Heterodermia tremulans*, utilised as an astringent and laxative in India (Shukla et al., 2010).

Over a thousand bioactive products derived from secondary metabolic pathways in lichen have been identified. Microbial secondary metabolites exhibit promising results serving as valuable pharmaceutical agents with antimicrobial, antioxidant, antiviral, anti-inflammatory, and anticancer properties. This study focuses on two coastal lichen species, *Ochrolechia parella* and *Ramalina siliquosa*, situated in extreme weather conditions across the UK, grow predominantly on stone surfaces. Both lichens, competing for nutrients on similar substrate territories in damp coastal environments, are subject to intricate interactions and competition with other microorganisms.

Lichens, composite organisms featuring a mutually beneficial relationship between fungi and algae or cyanobacteria, constitute a unique symbiotic partnership. Recent research indicates a more complex symbiotic lichen framework involving bacteria and algae, capable of producing a broader spectrum of pharmaceutical products than initially posited (Bates et al., 2011).

Within this partnership, the mycobiont plays a primary role in protecting the photobiont from ultraviolet (UV) light exposure and desiccation during extended droughts, while also absorbing mineral nutrients from the underlying surface or from dissolved atmospheric salts (BeGora & Fahselt, 2001; McEvoy et al., 2006, 2007; Nybakken & Julkunen-Tiitto, 2006; Solhaug et al., 2003). Photobionts, constituting 90% of lichen, are eukaryotic, whereas cyanobionts (10%) are prokaryotic cyanobacterial organisms. Photobionts perform photosynthesis, generating nutrients and precursor compounds, and fix atmospheric nitrogen to produce ammonia, a precursor for organic nitrogen compounds utilised by the mycobiont.

Taxonomically, saxicolous lichens are classified as epilithic based on their mode of attachment to the substrate and endolithic based on their mode of entry into the substrate, *Figure 34*. *O. parella* is an epilithic crustose lichen, the medulla of which maximises the attachment to the rock; *R. siliquosa* is a fruticose lichen, attached to the substratum at a single point.

The bacterial relationship in lichen is an opportunity for further exploration and may hold potential to identify new sources of antimicrobial, antioxidant, and pharmaceutically active agents (Kim et al., 2014). The diverse bacterial communities associated with lichens are gaining increased attention from researchers, aiming to evaluate the genes involved in secondary metabolite production with

the prospect of enhancing expression or modifying chemical pathways, (González et al., 2005; Grube et al., 2009).

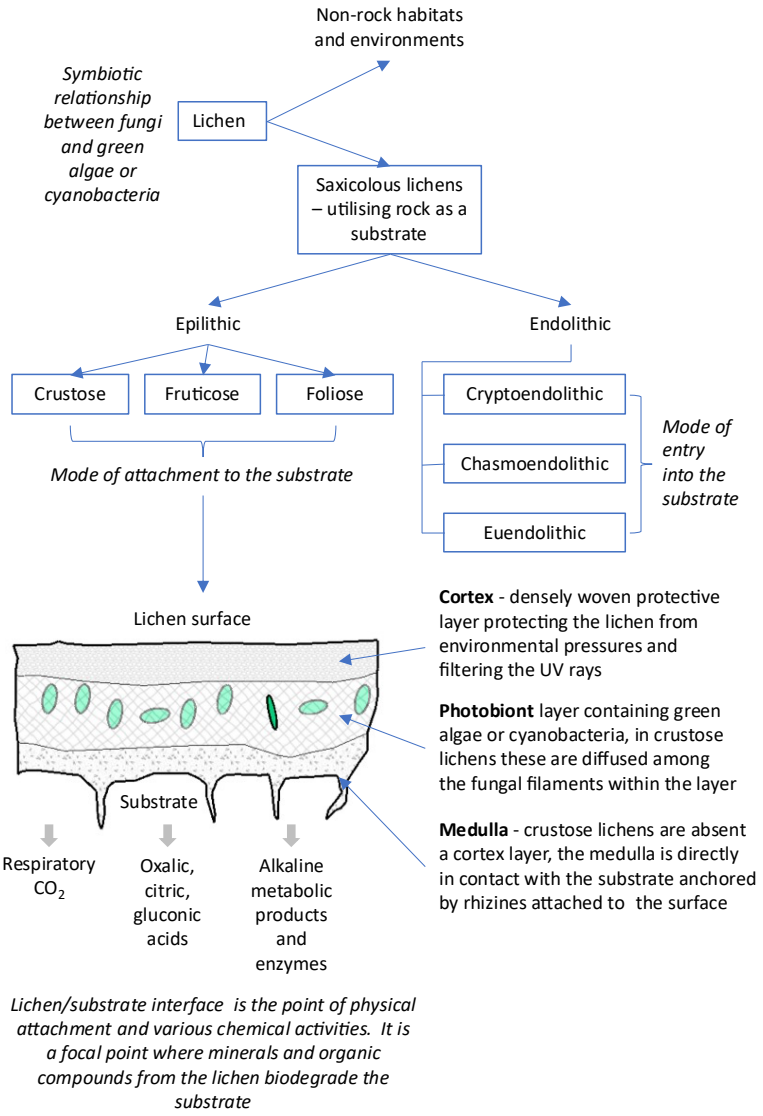


Figure 34: The taxonomy of saxicolous lichens and the mode of attachment of a crustose lichen (O. parella) to a substrate. The photobiont is protected below the cortex layer. The lichen interface with the substrate is anchored using rhizines, a site for several biochemical activities with the environment

The classification of lichen encompasses three distinct types: crustose, foliose, and fruticose, each delineated by the composition of the lichen thallus. The thallus, comprising an upper and lower cortex, an algal or cyanobacterial layer, and a medulla, exhibits species-specific variations in arrangement. Crustose lichens lack the lower cortex in their thallus structure. In contrast, foliose lichens encompass all layers and anchor themselves to substrates through rhizines. Fruticose lichens are characterised by a distinctive thallus structure enveloping a robust central cord, securing the lichen to the substrate. Photobionts occupy approximately 7% of the total thallus volume, though variations in bacterial and algal content exist, contingent upon the diversity of the bacterial component.

The ecological niche occupied by lichen reveals a remarkable diversity of nutrient acquisition strategies employed by lichen within their habitats. Notably, lichen utilise a plethora of mechanisms to extract essential nutrients from their substrate, as depicted in *Figure 35*.

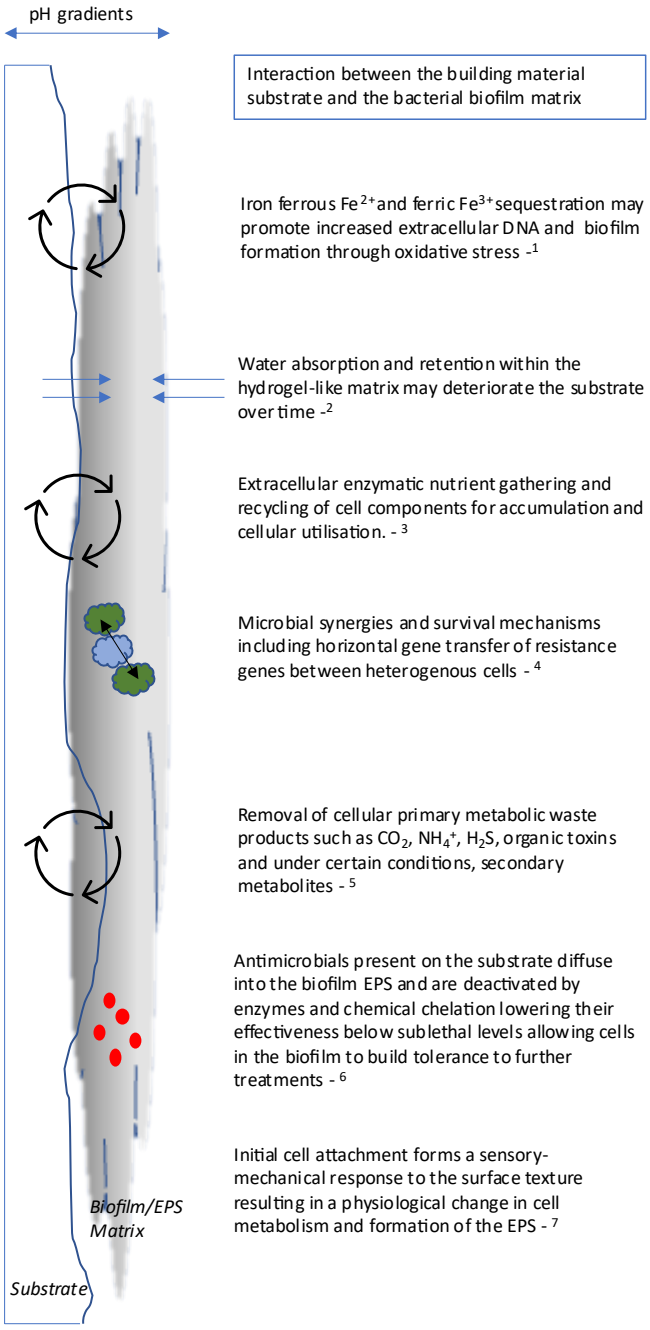


Figure 35: Diagram illustrating metabolic activities within the lichen and between the lichen, the environment, and the substrate. [1] - (Oh et al., 2018), [2] - *(Schmitt & Flemming, 1999)*, [3] - *(Flemming & Wingender, 2001)*, [4] - *(Molin & Tolker-Nielsen, 2003)*, [5] - (Guiamet et al., 2013), [6] - (Billings et al., 2015), [7] - (Carniello et al., 2018). Lichens are stable, and ecologically obligate, consisting of the mycobiont and one or more photoautotrophic organisms, algae, or cyanobacterium. This complex ecosystem supports lichens across a wide range of habitats. Mycobionts cultured without the photobiont may still produce secondary products, though the metabolite profile may differ, (Brunauer et al., 2007)

Lichen maintain a high level of independence on readily available environmental nutrients, while demonstrating sensitivity to changes in surrounding environmental conditions, having a marked effect on their growth and survival. Overall, their ability to thrive in diverse habits is demonstrative of how secondary products are used by lichen as an effective defence against adverse factors including competition from other lichen, bacterial attack and as a deterrent to encroaching plant and animal life.

Lichen produces two categories of metabolites, primary and secondary. Primary metabolites, chiefly comprising proteins, lipids, polysaccharides, carotenoids, and vitamins, originate in the photobiont and mycobiont. Their water solubility facilitates extraction during metabolic studies. Conversely, secondary metabolites, constituting up to 30% of the dry weight of a lichen, exhibit stability but poor water solubility, necessitating extraction using organic solvents. The medulla is a common deposition site for secondary products, many of which exhibit pigmentation, potentially contributing to the UV-filtering capabilities of lichen. (Crawford, 2019; Goga et al., 2020).

The production and metabolic investment in synthesising secondary metabolites prompt inquiry into the purpose of this diverse array of organic molecules. Although not directly participating in primary metabolic functions, a discernible connection between major primary metabolic pathways is observed. This linkage contributes to the availability of numerous precursor molecules, thereby facilitating their processing in secondary metabolic pathways, *Figure 36*.

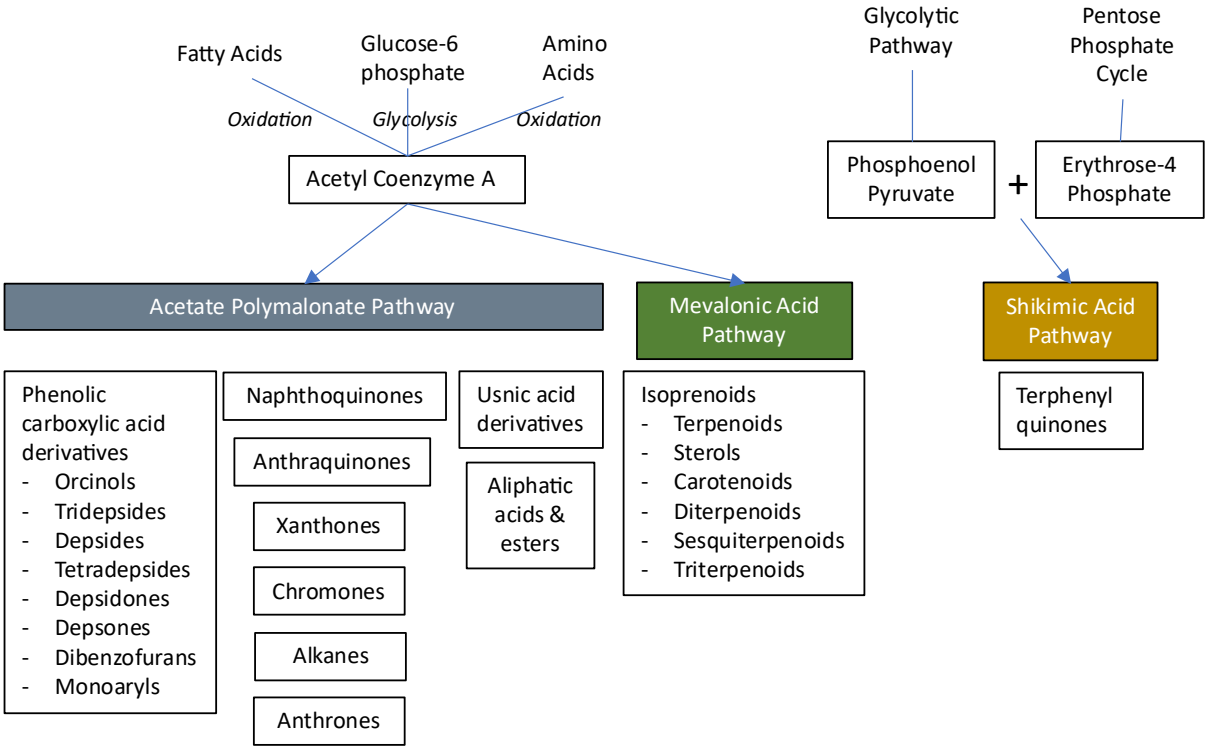


Figure 36: Three major cycles involved in the production of secondary metabolites, the acetate polymalonate pathway, the mevalonic acid pathway and the shikimic acid pathway. Each class of secondary product – small organic molecules <1500 Daltons) follows a set of chemical steps which enables the synthesis of the structural and functional group complexities using primary metabolic compounds and defines the final architectures

Secondary products are structurally divided into six main classes though within these are a wide range of subclasses and variants. These main classes are polyketides, stable framework peptides, isoprenoid/terpenoids, alkaloids, nucleosides and phenylpropanoids. The structural frameworks operate to a chemical logic which allows the formation of rules concerning the synthesis of the building blocks, the class structures of C-C and C-N bonds and the related enzymes which characterise their manufacture. This set of chemical logic sets the framework rules to allow the predictions as to how newly discovered secondary products may react, (Bertrand et al., 2018).

One of the main secondary product classes synthesised by lichens is polyketides, a family of modified aromatic polyphenols. The acetate polymalonate pathway is the major pathway through which many of the secondary metabolites are produced including lichen acids, mainly the primary β-orcinol derivatives, depsides, tridepsides, tetradepsides, depsidones, benzofurans, aryls, usnic acid and derivatives, polyphenolics, xanthones, and anthraquinones primarily derived from precursors malonyl CoA and acetyl CoA. The shikimic acid pathway is mainly responsible for producing pulvinic acid derivatives and a variety of disaccharides and polysaccharides, from precursors such as amino acids, especially phenylalanine and polyols, which are produced as intermediates in the primary metabolic pathways. The mevalonic acid pathway gives rise to the production of terpenoids, including diterpenoids, triterpenoids, sesquiterpenoids, carotenoids and sterols from precursor compounds, mainly acetyl CoA. These secondary compounds imbue lichens with a characteristic presentation and smell, (Tetali, 2019).

Lichens may also employ biochemical strategies to protect some proteins and peptides from recycling cellular processes by turning them into longer-lived low molecular weight secondary products. These strategies include the generation of cyclic peptides and modification of side chains set to resist enzymatic degradation of peptide bonds. These peptide-based secondary products are manufactured on non-ribosomal peptide synthetase frameworks, (Payne et al., 2017). The polyketide and non-ribosomal peptide synthesis are both constructed along a manufacturing assembly line following similar chemical logic which has facilitated bacterial hybrid manufacturing lines. A representation of a polyketide manufacturing line is shown in *Figure 48*, building the final product as the initial precursor progresses along the modular assembly units.

The prominent array of secondary products derived from a singular chemical framework, namely the Δ2 and Δ3 isomers of isopentenyl diphosphate, encompasses the valuable bioproducts known as isoprenoids/terpenoids. These compounds play integral roles in secondary metabolism, involving signal transduction, reproduction, communication, and defence mechanisms. Their indispensability for survival is underscored by their burgeoning significance in the pharmaceutical, biofuel, and nutraceutical industries, (Tetali, 2019).

Nitrogen atoms, while not pivotal to the polyketide, nonribosomal peptide, and isoprenoid families, constitute crucial components within the alkaloid families, forming at least one nitrogen atom enclosed within a heterocyclic ring. Alkaloids, therefore, employ primary amino acids as fundamental building blocks in the alkaloid synthesis process and for the establishment of functional groups that engage with biological targets.

Lichens adeptly employ a limited pool of primary metabolic building blocks and enzyme families in the construction of a wide spectrum of end-product architectures and functional group arrays. This efficiency underscores the capacity of microorganisms to generate diverse scaffold structures.

Each lichen species manifests a distinctive secondary metabolite profile, (Mitrović et al., 2011). Lichen secondary products exhibit distinctive attributes compared to many pharmaceuticals; for instance, certain metabolites can impede cancer cell proliferation without inducing secondary tumours over extended periods, in contrast to contemporary chemotherapy regimens. Consequently, lichen secondary products assume a noteworthy role in the realm of naturally derived bioactive clinical and environmental treatments, serving as foundational chemical structures for the synthesis of potent analogues. Notwithstanding, although a wealth of compounds has already been discovered, their bioavailability, scope and mechanism of action remain unclear in the literature, (Zhao et al., 2021).

Lichens strategically employ secondary products to exert allelopathic effects on other microbial species, particularly when the availability of preferred substrates becomes contested. Nevertheless, the intricacies of the symbiotic relationship between the photobiont and mycobiont within lichens remain inadequately elucidated, hindering conclusive determination of whether this symbiosis confers a competitive advantage for lichens against non-lichen organisms. Additionally, it remains unclear whether there exist preferred symbiotic relationships that favour the establishment of substrate dominance.

4.1.1 Ochrolechia parella

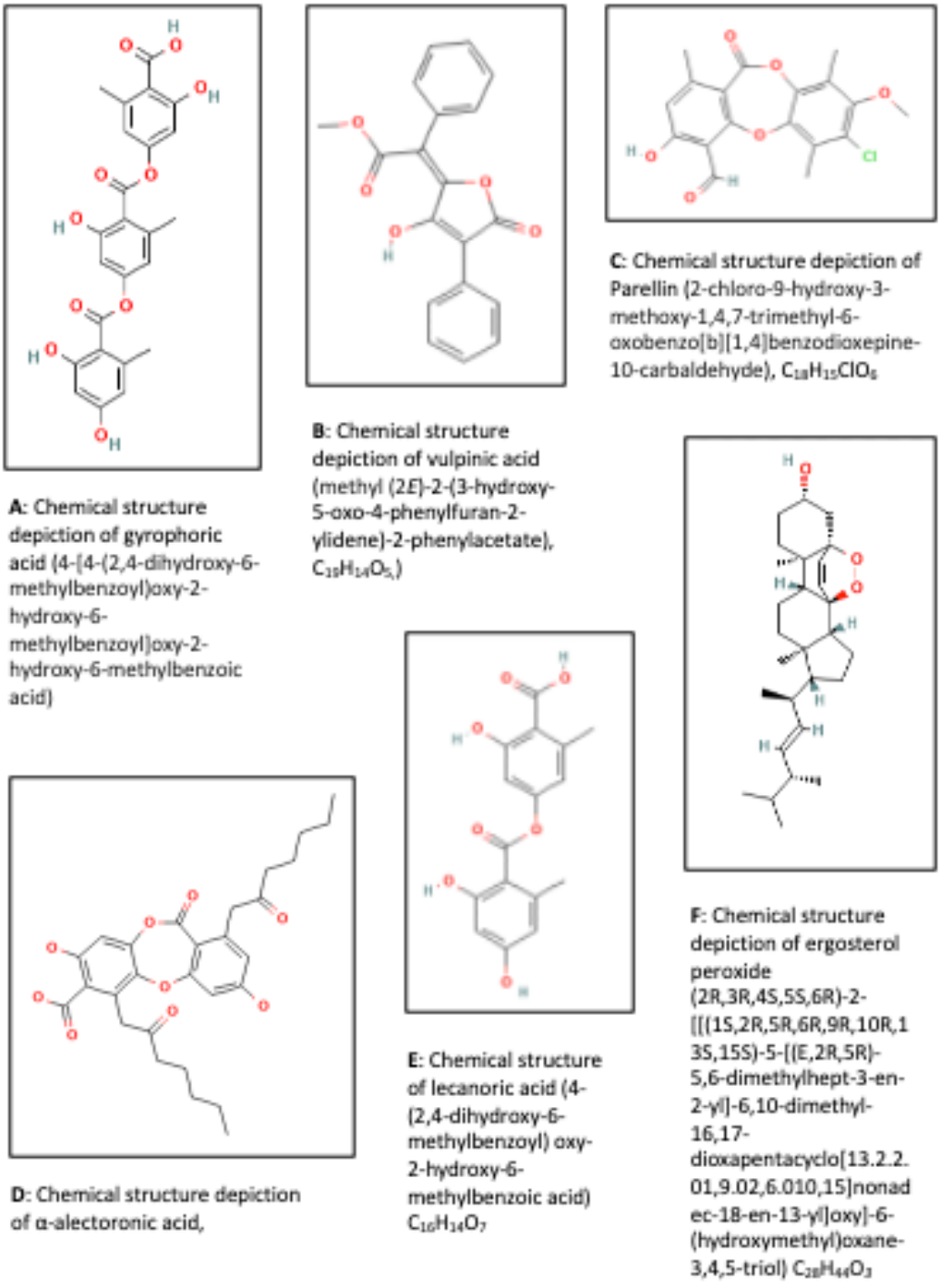
Ochrolechia parella, a robust crustose lichen, thrives in habitats characterised by extreme environmental conditions, notably cold temperatures, and high moisture levels with salinity. The adaptability of *O. parella* is evident in its occurrence in both the Arctic and Antarctic regions, predominantly on substrates composed of siliceous or siliciferous rock. *Figure 37.*

Figure 37: *Ochrolechia parella*. *Ochrolechia* species consists of a crustose thallus which is occupied by a chlorococcoid photobiont which is classified as Chlorophyceae - of the genus Trebouxia. The thallus of *O. parella* is grey with a white prothallus forming large patches or morphol substructures, resembling growth rings ranging from a smooth to a warted appearance and displaying an apparent contiguous physical structure. (Source: Image by author)

The secondary metabolites synthesised by *O. parella* constitute a distinctive assemblage of natural organic compounds, bearing diverse and significant biological implications, (Calcott et al., 2018). These compounds, extracts and bioactive substances confer multiple health benefits in the research fields of cancer therapeutics, mitigation of antioxidant stress and inflammation and combating diseases such as diabetes, *Table 17*.

Table 17: Literature sources quoting lichen secondary metabolite pharmaceutical activity produced by O. parella. Mechanisms of action of the secondary compounds remain an area for further study. The assumed targets of action include inhibition of cell wall synthesis, efflux pumps, plasma membrane integrity, DNA/RNA protein synthesis, cell division and mitochondrial function

Lichen Compound	Pharmaceutical activity	References
Gyrophoric acid *(Figure 38A)*	Antimicrobial, anticancer, antioxidant, antidiabetic	(Bačkorová et al., 2011; Cardile et al., 2017; Mohammadi et al., 2022; Plsíkova et al., 2014)
Vulpinic Acid *(Figure 38B)*	Antimicrobial, anticancer, stem cell control/ osteogenesis & adipogenesis, oxidative-stress therapy, herbivore antifeedant	(Bačkorová et al., 2011; Cansaran-Duman et al., 2021; Koparal, 2015; Lauterwein et al., 1995; Varol et al., 2016)
Lecanoric Acid *(Figure 38E)*	Antitumour, antioxidant, antibacterial, antifungal, antidiabetic, anticancer, anti-inflammatory, probiotic growth stimulant	(Bogo et al., 2010; Gaikwad et al., 2012; Gomes et al., 2003; Honda et al., 2010; Luo et al., 2009; Thadhani et al., 2011, 2015)
Parellin *(Figure 38C)*	A heavily methylated chlorinated depsidone awaiting biological evaluation	(Calcott et al., 2018; Millot et al., 2007)
α-Alectoronic acid *(Figure 38D)*	Bactericide	(Elix et al., 1974; Elix & Stocker-Wörgötter, 2008; Farkas et al., 2021; Latkowska et al., 2015)
Ergosterol peroxide *(Figure 38F)*	Anticancer, antimicrobial, cytotoxic, immunosuppressive, antioxidant	(He et al., 2018; Kim et al., 1999; Merdivan & Lindequist, 2017; Nowak et al., 2022; Yodsing et al., 2017)



A: Chemical structure depiction of gyrophoric acid (4-[4-(2,4-dihydroxy-6-methylbenzoyl)oxy-2-hydroxy-6-methylbenzoyl]oxy-2-hydroxy-6-methylbenzoic acid)

B: Chemical structure depiction of vulpinic acid (methyl (2*E*)-2-(3-hydroxy-5-oxo-4-phenylfuran-2-ylidene)-2-phenylacetate), $C_{18}H_{14}O_5$,)

C: Chemical structure depiction of Parellin (2-chloro-9-hydroxy-3-methoxy-1,4,7-trimethyl-6-oxobenzo[b][1,4]benzodioxepine-10-carbaldehyde), $C_{18}H_{15}ClO_6$

D: Chemical structure depiction of α-alectoronic acid,

E: Chemical structure of lecanoric acid (4-(2,4-dihydroxy-6-methylbenzoyl) oxy-2-hydroxy-6-methylbenzoic acid) $C_{16}H_{14}O_7$

F: Chemical structure depiction of ergosterol peroxide (2R,3R,4S,5S,6R)-2-[[(1S,2R,5R,6R,9R,10R,13S,15S)-5-[(E,2R,5R)-5,6-dimethylhept-3-en-2-yl]-6,10-dimethyl-16,17-dioxapentacyclo[13.2.2.01,9.02,6.010,15]nonadec-18-en-13-yl]oxy]-6-(hydroxymethyl)oxane-3,4,5-triol) $C_{28}H_{44}O_3$

Figure 38: Identified lichen secondary products sourced from *O. parella*. (National Center for Biotechnology, 2022).

The symbiotic relationship between bacteria and lichen offers a promising avenue for further investigation, holding the promise of uncovering novel reservoirs of antimicrobial, antioxidant, and pharmaceutically active agents (Kim et al., 2014). The diverse bacterial communities intricately associated with lichen have garnered increased attention, prompting researchers to explore the genetic underpinnings of secondary metabolite production, (González et al., 2005; Grube et al., 2009).

Notwithstanding these advancements, a comprehensive exploration of the impact of multiple bacterial communities within lichen species on secondary metabolite production remains an area for further scientific discourse.

4.1.2 Ramalina siliquosa

The Ramalina genus encompasses more than 240 species, sharing a comparable phenotype characterised by the inclusion of an algal source facilitating photosynthesis. This process yields organic compounds vital for the lichen metabolic pathways, culminating in the synthesis of various secondary products, predominantly polyphenolic, including depsides and depsidones. *Ramalina siliquosa* is prevalent along the Atlantic coast spanning from Portugal to northern Norway and extending eastward to Finland and the Russian shores and dominates seashore rocks and walls in northern Britain, *Figure 39*. Initial investigations revealed the production of hypoprotocetraric acid and regional variations in related depsidones norstictic, stictic, salazinic and protocetraric acids (Culberson, 1967).

Figure 39: *Ramalina siliquosa*, also known as sea ivory, is a branched lichen found on siliceous rocks and stone walls on coastlands around the UK and Iceland, (where it is a protected species). It is found above high-tide demarcation and tolerates salt-water spray. The prongs produce spore-producing bodies and form part of the diet of sheep in extreme weather locations such as Shetland and North Wales (Dobson, 2018; Tyler-Walters & Hiscock, 2023). (Author's image)

Chemical diversity among Ramalina chemotypes manifests in distinct concentrations of usnic acid, accompanied by varied biological activities, such as antibacterial or antioxidant properties, arising from disparate biosynthetic pathways. Despite comprehensive documentation of lichen secondary metabolites, ample prospects persist for systematic screening to discern specific antimicrobial and

antioxidant properties. Recent studies, underscore the inhibitory potential of lichen secondary metabolites against *Aspergillus* species, demonstrating protolichesterinic acid against *A. flavus*, lecanoric acid against *A. fumigatus* and orsellinic acid against *A. niger* with inhibitory effects commensurate with commonly used antifungal agents (Furmanek et al., 2019).

The close relationship between the cyanobacteria and the lichen biont significantly influences the biosynthesis of metabolites resulting from this symbiosis, thereby shaping the secondary product profile, (Calcott et al., 2018). This complex symbiotic relationship between cyanobacteria and lichen intricately intertwines their metabolic pathways, creating significant challenges in pinpointing the origins of secondary metabolites within the resulting organism, (Ranković & Kosanić, 2015).

While the solubility of most secondary metabolites in water is low, exceptions include lichen-manufactured polyphenols atranorin and usnic acid, (Fernández-Moriano et al., 2015). Organic solvents again emerge as effective extraction agents for these secondary products.

Less than 50% of *Ramalina* species have been subject to publications identifying chemical or biological activity from compound extracts. Usnic acid is the most scrutinized extract, with in-vitro assessments highlighting its inhibitory effects on biological activities. Ongoing investigations, reveal several other extracts demonstrating antimicrobial, antitumor, and anti-inflammatory properties, signifying the untapped potential of *Ramalina* as a source of diverse bioactive molecules, (Moreira et al., 2015).

Within the wide-ranging chemical profile of *R. siliquosa*, primary and secondary products coexist, with the latter exhibiting bioactive properties of interest to this study. Depsides, depsidones, fatty acids, sterols, and monocyclic aromatic compounds dominate the chemical profile, and the structures of select primary compounds are elucidated in *Figure 40*.

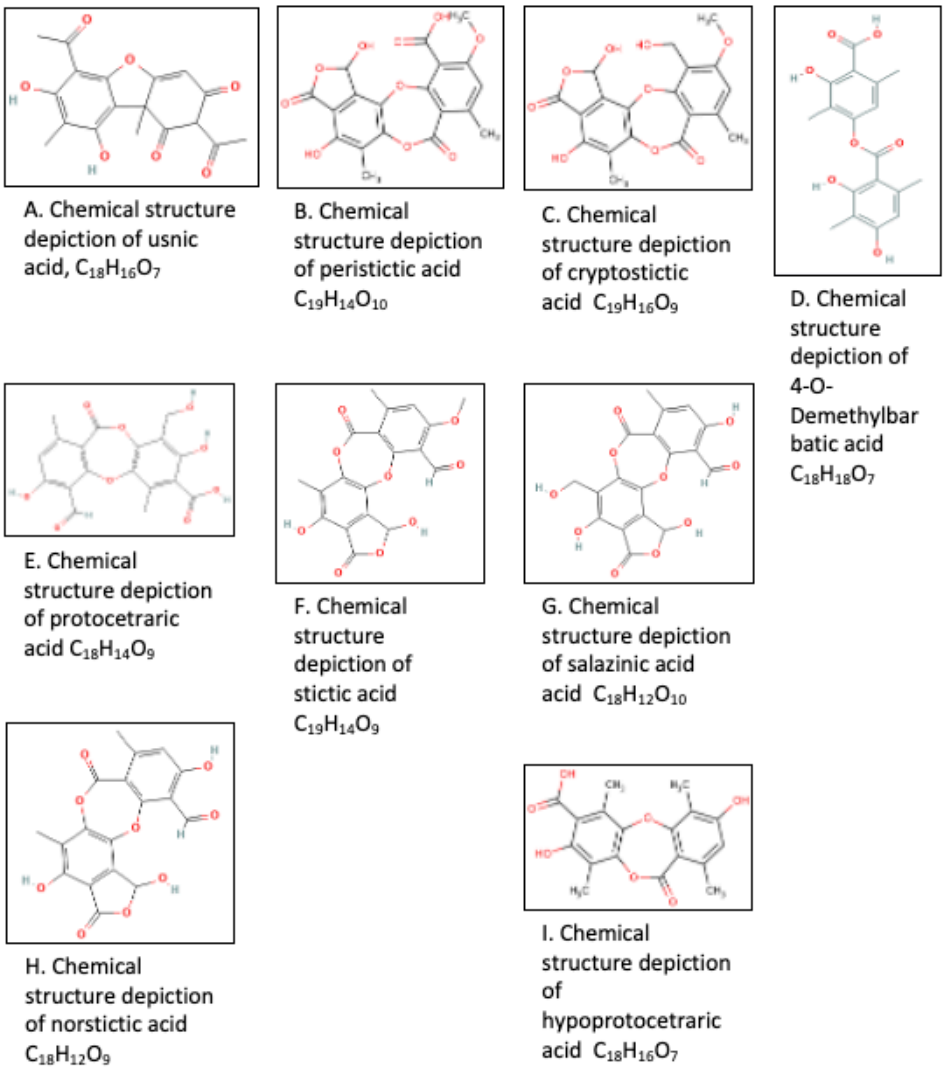


Figure 40: Identified lichen secondary products sourced from *R. siliquosa*, (Parrot et al., 2013). Chemical structures - (National Centre for Biotechnology, 2022)

Usnic acid, isolated in 1834, stands as one of the earliest secondary compounds derived from lichen and is variably distributed across *Ramalina* species. Certain species yield derivatives such as usimine A, usimine B, usimine C, usninic acid, and iso-usnic acid, each presenting a spectrum of bioactive effects, (González et al., 1992; Kim et al., 2018; Lee et al., 2010; Paudel et al., 2011). The antimicrobial activity of usnic acid or phenolic derivatives, exerts its effects by modifying protein structures in target cells and inducing apoptosis, (Cansaran et al., 2007; Ingólfsdóttir, 2002). Norstictic acid and protocetraric acid exert antimicrobial activity on several bacteria and fungi, at concentrations of between 3.3 – 6.6 μg $25\mu L^{-1}$, though usnic acid appears to show the best results exerting minimum inhibitory concentration (MIC) at concentrations of between 0.39 – 3.1 μg $25\mu L^{-1}$, (Tay et al., 2004).

The diverse bioactivities exhibited by R. siliquosa as biological sources of metabolites encompass antibiotic, antitumour, antimutagenic, antiviral, allergenic, inhibitory effects on plant growth and enzyme activity, and antimicrobial properties. Notwithstanding, research into the antifungal effects of lichen secondary products, particularly against building moulds and bacteria responsible for

biodeterioration, remains limited. The resurgence of interest in natural sources of antimicrobial agents, given the rise in antibiotic-resistant pathogens, emphasises the economic potential of identifying, mapping metabolic pathways, extracting, and enhancing secondary products from microbial sources compared to synthetic alternatives.

4.2 Climate-affected biodeterioration of traditional materials

There are divergent perspectives regarding the impact of microbial growth on historic structures, particularly whether it manifests as biodeteriorative or bioprotective, (Liu et al., 2022). The challenge is that bacterial colonisation on stone structures readily forms a protective biofilm. This biofilm serves multifaceted functions, including thwarting detachment from the substrate, constituting a biochemical safeguarding matrix, and sustaining hydration during arid periods. The heterogeneity of the biofilm facilitates the incorporation of diverse planktonic microbial organisms into this protective configuration, endowing the community with adaptability to a spectrum of environmental conditions, encompassing extreme weather events. This adaptability is attributed to the metabolically supportive matrix and the resilience of the biofilm against both physical and chemical attacks. A consequence of the metabolic processes is the infliction of physical and chemical damage as a result of the organic acids, release of cations, salt recrystallisation and mechanical adhesion to the material surface.

An alternative viewpoint discussed in Chapters Two and Three is that the robust tolerance of the biofilm atop the stone substrate is inherently bioprotective, shielding against weather erosion and the corrosive effects of anthropogenic pollutants that may lead to delamination of the stone. The degree of abiotic weathering particularly in response to heightened occurrences of extreme weather events on stone exteriors, serves as a metric for determining the bioprotective nature of the biofilm layers. However, unless the erosive forces and frequency of extreme weather events pose a substantial threat to abiotic stone weathering, the visual blemishes caused by biofilm formation and the potential damage from acidic and cationic metabolic waste products persist.

Biochemical mechanisms underpinning stone deterioration and discolouration are associated with the production of both organic and inorganic acids. Fungal hyphae contribute to physical degradation by infiltrating micro-spaces generated through climate-induced erosion in the stone, thereby destabilising the overall structural integrity. The synergistic action of hyphal penetration and acid-induced dissolution of the stone facilitates the ingress of water and salts into newly formed micro-fractures, culminating in the delamination of the stone surface, particularly during freeze/thaw cycles.

Climate fluctuations also exert discernible effects on exposed wooden structures. In locations characterised by a predominant use of wood in construction, such as Iceland and Norway, fungal decay emerges as a significant concern. Despite timber being lauded for its eco-friendly attributes, being renewable and a carbon sink, regions even subject to permafrost, such as the Arctic, witness wood succumbing to fungal decay, notably perpetrated by species like *Leucogyrophana mollis* thriving in polar conditions, (Mattsson et al., 2010). Fungal rot proves to be a pertinent issue even in extreme cold conditions. Over time, heritage wooden structures in polar settings exhibit deterioration, albeit at a measured pace, as fungi adeptly adapt to the severe cold, cyclically transitioning between dormancy and rethawing each summer, (Björdal & Dayton, 2020; Duncan, 2007). The global diversity of fungal species, estimated between 1.5 to 12 million, underscores the capacity of certain species to depolymerize wood, removing cellulose or lignin and inducing catastrophic decay, (Bhunjun et al., 2022; Blanchette, 2000).

4.3 The impact of climate and environmental changes on secondary metabolite production

The increase in secondary metabolites discovered and attributed to lichenized fungi, coupled with empirical indications of their susceptibility to environmental changes, presents a salient avenue for exploration of the processes underpinning their biosynthesis and regulatory mechanisms. Current investigations into the impact of environmental stimuli on lichen secondary metabolite production have generally operated under the premise that alterations in culturing conditions can effectively mimic natural environmental stresses. This allows employing targeted methodologies for in-depth scrutiny of lichen growth dynamics, secondary product synthesis, and the environmental impact within a wide range of habitats, *Figure 41*.

A diverse range of fungal secondary metabolites emanates predominantly from a select set of biochemical pathways, notably the mevalonic acid pathway, malonate pathway, and shikimic acid pathway, *Figure 36*. Manipulating available precursors within these pathways has emerged as a focal point, primarily oriented towards elucidating the production of novel antimicrobial agents. However, this approach equally holds promise for discerning the influence of environmental changes on nutrient supply availability. The ongoing exploration through next-generation sequencing of prokaryotic genomes promises continued insights into the repercussions of environmental conditions, particularly that manifest in extreme environments, on genomes, transcriptomes, proteomes, and the resultant modulation of secondary metabolite production and composition.

This study adopts a deductive approach to investigate the interplay between environmental changes and the production and potential modification of lichen secondary metabolites. Given the established premise that genetic adjustments or metabolic shifts in moulds and bacteria enhance their survival, it stands to reason that similar adaptations likely occur in other organisms thriving in analogous environments, such as lichens. Facing the pressure of securing long-term occupancy of their substrate, lichens must adjust their internal mechanisms to optimise competition. Hence, it is hypothesised that the production and maintenance of antimicrobial lichen secondary metabolites serve as a strategic response to the microbial challenge posed by competing species within the same niche.

Environmental alteration can directly influence cellular processes, driving adaptive responses illustrated by the interplay between UV light intensity and lichen pigment synthesis. Increased UV-B radiation arising from ozone depletion triggers enhanced pigment production in lichen as a photoprotective mechanism, effectively absorbing excess light. The combined threats of climate-induced ozone depletion and anthropogenic pollution suggest a continued weakening of the atmospheric shield, potentially leading to a sustained upregulation of lichen pigmentation. This phenomenon exemplifies how environmental changes directly modify cellular activity, shaping adaptive responses that dictate the ability of lichen to cope with changing stimuli.

The manufacturing processes and functional attributes of lichen secondary metabolites often remain elusive, providing inconclusive insights into the nexus between environmental conditions and production outcomes. Discussions relating to the impact of climate on lichen secondary metabolism predominantly orbit around variables such as pH, temperature, and UV radiation fluctuations. The adaptability of lichen across a spectrum of extreme environments is evident in the myriad of lichen species thriving in diverse global climates. Nevertheless, the impact of climate-induced alterations on lichen metabolic chemistry is relatively underexplored, despite discernible correlations between the production of specific secondary metabolites and lichen tolerance to environmental changes.

The response of lichen to environmental fluctuations is underscored by the activation of distinct gene clusters, thereby facilitating a flexible range of secondary metabolite synthesis, (Brunauer et al., 2007). These environmental dynamics have engendered analogues and derivatives within the secondary synthesis pathways, endowing lichen with a competitive advantage and exerting inhibitory effects over competitors vying for shared spatial niches on stone substrates, (Dayan & Romagni, 2001).

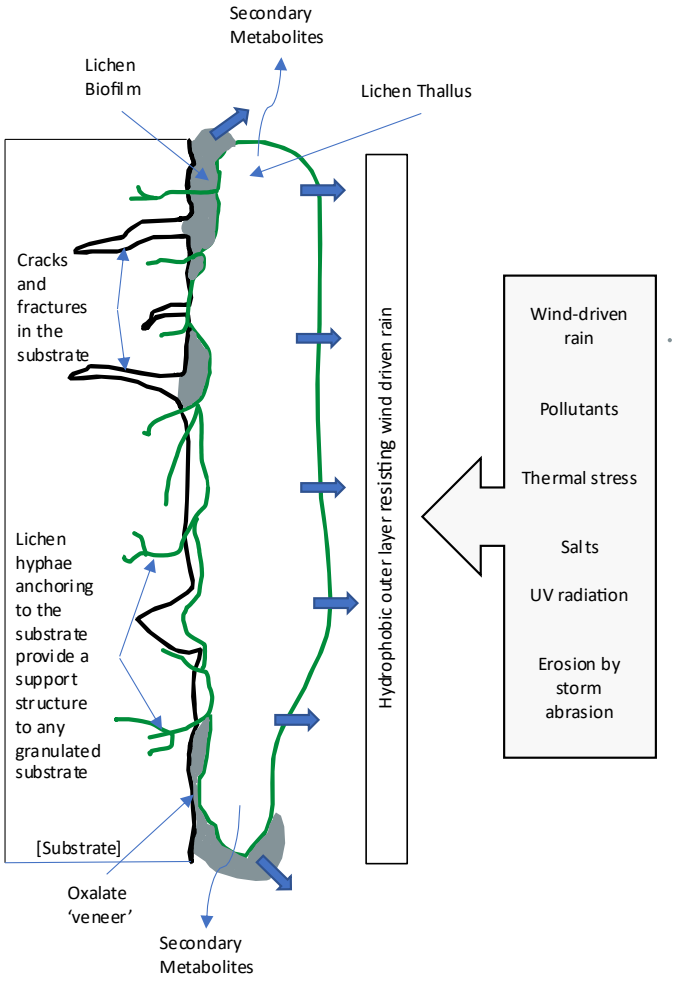


Figure 41: Climate and environmental factors, inclusive of anthropogenic pollutants, exert influence upon the outermost layer of surface lichen. The hydrophobic nature of the lichen exterior imparts some resistance to water-soluble environmental substances; however, this resistance may diminish due to surface abrasion, acid attack, and the presence of organic pollutants, thereby compromising the protective layer. The reliance of the lichen on cyanobacteria or algae residing within the upper structural layers is integral for shielding against UV light. An inherent peril to these protective mechanisms lies in prolonged desiccation periods of the lichen structure, which diminishes the protective envelope that the lichen extends to its pigment-producing bacterial or algal microbial associates

Efforts to establish a correlation between metabolite concentration and luminosity have yielded inconclusive findings, (Armaleo et al., 2008; Bjerke et al., 2005; McEvoy et al., 2007; Millot et al., 2007; Stephenson & Rundel, 1979; Werner Bjerke & Dahl, 2002). Millot et al, (2007), postulate that

the duration of light exposure may elicit either inhibitory or stimulatory responses contingent upon the specific secondary product, with heightened levels observed during the summer months. Furthermore, the synthesis of lichen compounds exhibits dynamic fluctuations in response to various environmental variables, including the proportion of UV-A and UV-B exposure (e.g., usnic acid), substrate pH, moisture levels, temperature (e.g., gyrophoric acid, 4-O-dimethylbarbatic acid), drought conditions, geographical location, elevation (rhizocarpic acid), and seasonal variations, (Bjerke et al., 2003, 2005; Werner Bjerke & Dahl, 2002).

The potential for the identification of novel sources of antimicrobial active agents resides within the intricate relationship between lichens and bacteria, (Kim et al., 2014). The increasing attention directed towards the diverse bacterial communities associated with lichens underscores a growing interest among researchers in assessing the genetic underpinnings of secondary metabolite production. This scrutiny aims to induce overexpression and modifications in synthesis processes, facilitating the incorporation of novel functional groups and the potential discovery of antimicrobial agents, (González et al., 2005; Grube et al., 2009).

The flexibility of lichen secondary metabolite production suggests a need for a comprehensive exploration of genomic regulation. The abundance of genes encoding secondary metabolites appears to surpass the catalogued compounds, hinting at the prospect that lichens have evolved an array of genes to orchestrate secondary metabolite production adaptively, responding to ecological and environmental stressors dynamically, (Deduke, Timsina, & Piercey-Normore, 2012).

4.4 Building material biodeterioration and the impact of fungal and bacterial organic acids

Organic acids synthesized by fungal, bacterial, and algal microorganisms have been implicated in the biodeterioration of architectural materials, (Gaylarde & Morton, 1999; Ortega-Calvo et al., 1991; Warscheid & Braams, 2000). The structure and chemical synthesis by fungi, known for their adeptness in the biodegradation process, readily degrade wooden, gypsum, concrete, mortar, and brick structures, while also compromising wallpapers, paints, and interior furnishings, *Figure 42*. The filamentous nature of fungi enables the utilisation of mycelium to infiltrate materials, employing various enzymes and metabolites in the chemical breakdown of substrates, particularly in the presence of water, (Pandey & Kiran, 2020).

Chemical acids generated by mycelium encompass citrate, malate, itaconate tartrate, gluconate, fumarate, oxalate, and succinate. These organic acids possess the capacity to extract available cations from the substrate, (Dörsam et al., 2017; Magnuson & Lasure, 2004). The combination of acidic assault and cation removal precipitates material weakening, with prolonged exposure resulting in structural failure, (Warscheid & Braams, 2000). A survey of 62 filamentous fungi isolated from 42 structures identified 40 strains exhibiting the production of acidic metabolites. *S. chartarum*, isolated from gypsum board and wallpaper, exhibited pronounced acid activity. The determination of acid activity involved quantification employing an acid activity coefficient (Q), estimated using bromocresol purple on growth medium. The manifestation of acidity zones around colonies was discerned by a yellow hue, and measurements of colony diameter (dc) and yellow zone diameter (dy) were undertaken. The acid activity coefficient (Q) was computed using the formula:

$$Acid\ activity\ coefficient, Q = dy - dc\ (mm) \quad (5)$$

Several *Penicillium* sp., *P. brevicompactum, P. expansum, Penicillium chrysogenum* and *S. chartarum* measured an acidity coefficient Q range between 1.32 +/- 0.05 and 2.83 +/- 0.01. The most

significant alterations in pH occurred during the initial day of the stationary growth phase, reflecting substantial organic acid production on mortar, followed by gypsum and wallpaper. The qualitative nature of organic acids produced appears contingent upon the type of building material, with mortar exhibiting heightened acid production potentially linked to its initial higher pH stimulating fungal acidogenesis, (Gutarowska & Czyżowska, 2009).

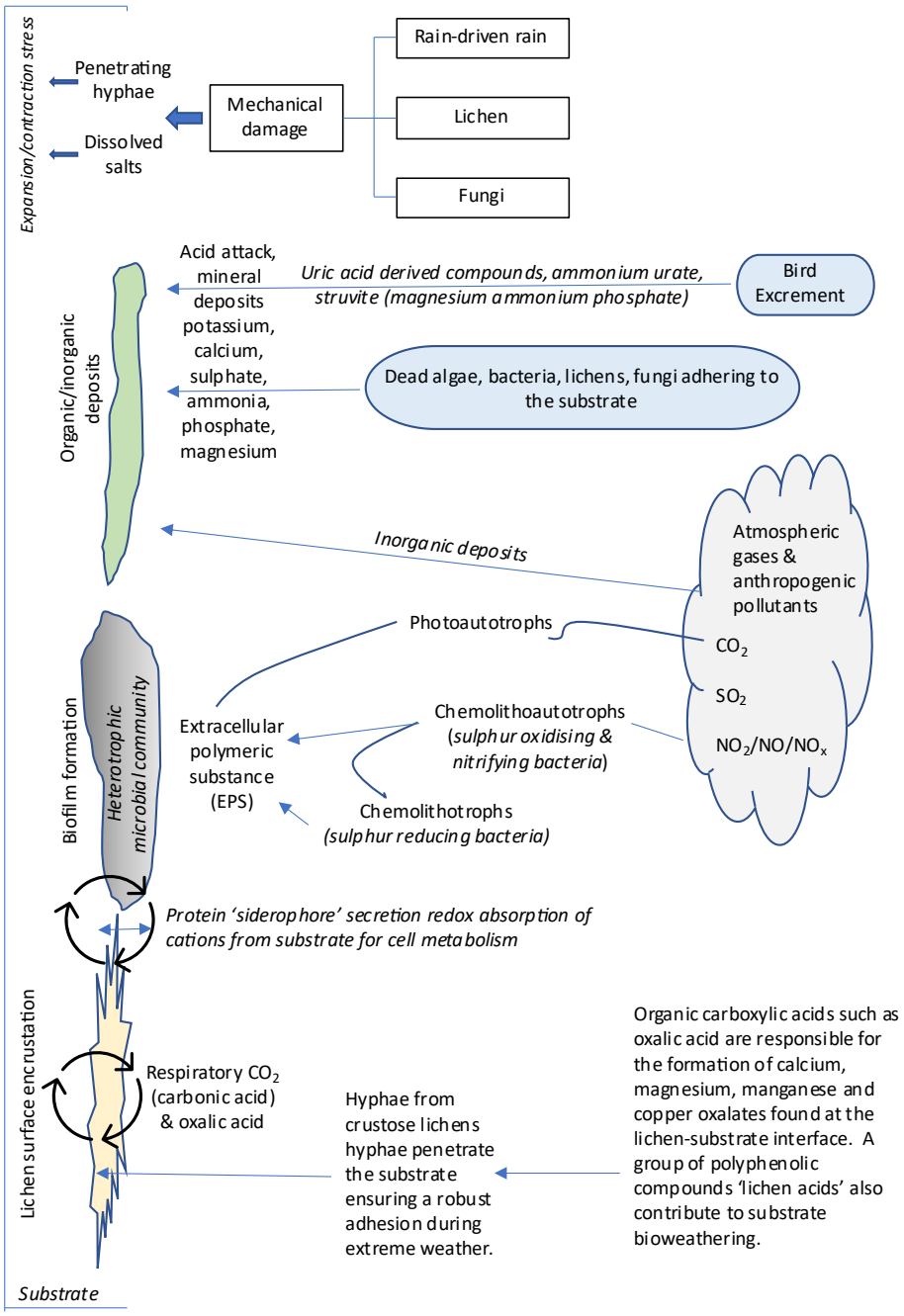


Figure 42: The impact of environmental conditions on substrate bioerosion mechanism. Crustose lichens comprise three distinct layers: the upper cortex layer, housing pigmentation; the central layer containing algal or cyanobacterial components; and the medulla, employing hyphae for substrate attachment. The formation of polyphenolic compounds at the lichen-substrate interface, plays a pivotal role in substrate surface erosion, thereby contributing essential cations for cellular metabolism, *(Adamo & Violante, 2000)*. Substrate surface erosion, a consequence of abiotic and biotic influences, encompasses mechanical damage induced by wind-

The formation of soluble salts arising from the interaction between various organic acids and substrate minerals, including Ca, Fe, Al, Mg, K, and Na salts, renders them susceptible to removal from architectural materials under the influence of rain, humidity, or condensation prompted by meteorological events. In certain scenarios, insoluble salts, exemplified by oxalic acid on calcium carbonate yielding calcium oxalate, are generated, leading to the degradation of building materials and the formation of an aesthetically undesirable patina attributed to fungal pigments on the material surface. The pronounced aggressiveness of mould infestation in damp environments is explicable through the diverse array of exoenzymes biosynthesised by fungi, including cellulases, glucanases, keratinases, and mono-oxygenases, in conjunction with low a_w values denoting the measure of unbound free water available for microbial growth.

On wallpaper surfaces, organic acids initiate the hydrolysis of polymer polysaccharides, particularly cellulose present in both wallpaper fibres and adhesive paste, resulting in material weakening and discolouration. Variations in moisture levels pose a serious threat to art objects within domestic and museum settings, necessitating treatment methods devoid of long-term deleterious effects on the fabric of the objects. Additionally, the presence of fungal spores and toxic metabolic by-products poses health risks to both museum personnel and artefact conservationists.

Despite the limited nutrient availability in stone substrates, fungi flourish in humid conditions induced by wind-driven rain and elevated temperatures. Epilithic fungi colonise the rock surface, while endolithic fungi inhabit microcracks within the stone, both exhibiting heightened metabolic activity conducive to fungal acid erosion and cation extraction.

Addressing fungal colonisation involves a restricted repertoire of physical and chemical methods contingent upon the composition and strength of the material harbouring organic contaminants and the desired treatment outcome for any residual patina. Traditional conservation methods, including the judicious selection of disinfectants based on mould type and chemical composition, present practical challenges, and limitations, (Allsopp et al., 2004). An alternative approach involving high doses of gamma radiation exceeding 10-25 kGy proves effective in fungal eradication, yet logistical difficulties in irradiating architectural recesses, operator health risks, and potential substrate damage necessitate careful consideration, (Shathele, 2009). Chemical treatments, encompassing liquid biocides, fumigation with methyl and ethylene bromide, or products containing slow-release formaldehyde or ammonium compounds, pose health and environmental risks. Ethanol, a comparatively less toxic fungitoxic solution, may exhibit efficacy with prolonged application, though its sufficiency in most restoration scenarios remains uncertain, (Nittérus, 2000).

Four prevalent fungi, namely *Cladosporium, Aspergillus, Penicillium*, and *Stachybotrys*, commonly referred to as moulds, collectively contribute to the degradation of traditional materials. The evolving climate may exacerbate mould contamination in repurposed commercial historic buildings, posing an escalating challenge. Given the potential health hazards associated with mould exposure, particularly in susceptible individuals, the legal implications for employers are likely to intensify, underscoring the need for comprehensive mitigation strategies. A comprehensive understanding of the distinct material deterioration mechanisms employed by various mould and bacteria species residing in damp pre-1919 buildings is crucial for formulating effective treatment strategies and containing biological risks. This study specifically investigates the interactions of four dominant moulds on traditional materials – *Aspergillus, Cladosporium, Stachybotrys, and Penicillium* – commonly found in such environments. Additionally, *Staphylococcus aureus*, an opportunistic

bacterial pathogen with an increasing prevalence in older, damp residences, particularly those harbouring animals, is included to evaluate the efficacy of lichen-derived antimicrobial secondary metabolites against this diverse microbial community. Through this multifaceted approach, the study aims to identify potential antimicrobial solutions for safeguarding the integrity of historic structures against deterioration mechanisms which have been outlined in Chapter Two.

4.4.1 *Cladosporium* moulds

Cladosporium is a genus of mould encompassing numerous species which can be found in both indoor and outdoor building surfaces, *Figure 43*.

Figure 43: The proliferation of *Cladosporium* moisture-laden wooden periphery, impervious to pre-emptive application of ostensibly 'mould resistant' commercial paint. Symptoms manifested by individuals exposed to *Cladosporium* include but are not limited to, headache, fatigue, breathing difficulties, chest pains and inflammation of the eyes. The emergence of this mould signifies water incursion or dampness. (Author's image)

The species is not confined to colonising building materials. The intrinsic nature of traditional materials to retain moisture coupled with the accretion of generations of surface nutrients stemming from prior surface treatments and anthropogenic contaminants furnishes a nutritional foundation conducive to rapid colonisation. *Cladosporium* is discernible by a dark-green to black pigmentation and typically develops a colony of hyphae giving rise to conidiophores bearing conidia spores. In ordinary circumstances, the mould is nominally pathogenic, whereas an individual with a compromised immune system, asthma or respiratory allergies may develop respiratory complications. Remedial interventions include acetic acid, hydrogen peroxide and chlorine (bleach), or in severe cases chemical treatments with pronounced environmental toxicity.

4.4.2 *Aspergillus* moulds

Aspergillus is identified by flask-shaped fruiting bodies called conidiophores, producing conidia a similar structure found in *Cladosporium* manifesting in either black or yellow-green, *Figure 44*.

Conidia disseminate spores from the mould into the atmosphere containing mycotoxins and volatile organic compounds that compromise indoor air quality.

Figure 44: *Aspergillus* is black on the surface and slightly white or yellow underneath. Other species such as *Aspergillus flavus* is yellow-green, with a brown underneath. Outbreaks of nosocomial aspergillosis occurs mainly amongst the most severely immune hospital patients, (Author's image)

Adverse health effects for individuals sensitive to *Aspergillus* spores typically manifest when a disturbance to the surface hosting the mould, such as during renovation or maintenance, occurs. Building materials fostering *Aspergillus* growth function as reservoirs for this infectious agent, which is liberated and typically enters the body via the pulmonary tract or through skin wounds.

Aspergillus spores from *Aspergillus flavus* and *Aspergillus parasiticus* prevalent on warm, damp surfaces -especially those comprised of cellulose, such as damp wood and wallpaper – constitute the principal source of a family of toxins, called aflatoxins. Prolonged exposure to aflatoxins, whether via inhalation through the pulmonary tract or ingestion through contaminated food exposed to fungal sporulation, is correlated with the development of liver cancer.

The ability of *Aspergillus* to degrade plant cell wall polysaccharides is accomplished through the production of a wide range of polysaccharide-degrading enzymes. This yield benefits within the textile, paper and pulp industries supplementing conventional chemical and mechanical processing. These enzymes are very effective against heteropolysaccharides such as hemicellulose, pectin, and cellulose, each contributing to rigid plant-based structures. The production of the cellulolytic enzymes by aspergilli is a substantial contributor to cellulose-based biodeterioration of traditional materials, (de Vries & Visser, 2001).

4.4.3 Stachybotrys chartarum

Stachybotrys chartarum is a black slimy mould that is common on the exterior and interior of buildings. It is slow growing, competing poorly with other moulds but proliferates in damp, dark, uncolonized areas of human habitation. It grows rapidly on cellulose-based materials such as paper and paper-covered plasterboard, *Figure 45*. It readily spreads through sporulation when the source

material is dry, causing the spores to be aerosolised causing further contamination and material biodeterioration and is a danger to human health.

Figure 45: *Stachybotrys chartarum* growing on damp wood. An association between acute idiopathic pulmonary haemorrhage amongst infants and *Stachybotrys* is thought possible, (Author's image)

At low doses, the effects are mildly neurotoxic causing headache, nausea, muscle aches, pains, and fatigue. As the exposure continues, the immune system may be negatively affected reducing the resistance to other infections. The symptoms *S. chartarum* is linked to have been termed 'sick building syndrome' though the literature is relatively ambiguous on the severity of impact on human hosts. Under normal conditions, *S. chartarum* requires water to thrive, and hidden leaks in roof spaces and walls may conceal contaminated areas until the damage becomes catastrophic through material failure. Climate change increases the opportunity for a rise in water entry into buildings through rising dampness, increased condensation, overflowing gutters, down pipes inadequate for the increased water volume and rain-saturated exterior renders, mortars, and stone surfaces. The easiest approach to control the mould is to control the moisture. Modern chemical control uses bleach, though the chlorine solution may not remove the residues which are toxic and allergenic and may cause additional toxicity and damage to traditional surfaces.

4.4.4 Penicillium chrysogenum

A member of the *Penicillium* genus, a blue-green fungus, ranks among the three most prevalent fungi, alongside *Aspergillus* and *Cladosporium*, in indoor environments exposed to moisture or within structures compromised by water ingress, *Figure 46*. While it is improbable to be a direct etiological agent of diseases, this fungus is proficient in synthesizing various compounds, such as Penicillin Roquefort Toxin (PR-toxin), a mycotoxin with the capacity to impede protein synthesis in the liver, potentially through interference with cellular transcription, (Moulé et al., 1978). This mycotoxin may induce acute toxicity by augmenting blood vessel permeability, consequently

diminishing blood volume, thereby causing direct and indirect harm to vital organs such as the lungs, kidneys, liver, and heart, (Chen et al., 1982).

Figure 46: Penicillium chrysogenum growing on an internal plaster wall in a damp environment in a heritage-at-risk building in Scotland. It reproduces through spores or conidia which are carried by air currents to new damp colonisation sites in the building. The airborne spores are implicated as human allergens due to the presence of serine proteases as major allergenic proteins. (Author's image)

Individuals inhabiting moisture-laden buildings with *P. chrysogenum* proliferation are predisposed to manifest allergic symptoms upon inhalation of the mould spores. Functioning both as an allergen and a pathogen, the growth of *P. chrysogenum* poses a hazard to individuals with compromised immune systems, (Knutsen et al., 2012). *P. chrysogenum* serves as a source for several secondary metabolites, encompassing roquefortines, cyclic hydrophobic tetrapeptides, siderophores, penitric acid, w-hydroxyemodin, chrysogenin, chrysogine, sesquiterpene, and sorbicillinoids. The diverse array of compounds produced by the mould facilitates a broad-spectrum chemical assault on the surrounding environment and host substrate, (Guzmán-Chávez et al., 2018).

The mould proliferates readily on damp or water-damaged materials, which serve as primary nutrient sources for its growth. Such materials include plasterboard, wallpaper, starch adhesives, synthetic paints, and accumulations of internal pollutants and dust. In persistently damp indoor environments, *Penicillium* may also colonise leather products, clothing and furniture, and extend its presence to perishable items such as dairy products, fruits, and vegetables. Consequently, the organism poses a dual threat, not only to immunocompromised individuals but also to the structural integrity of indoor materials, leading to destructive decay through the decomposition of organic substances, (Perrone & Susca, 2017).

4.4.5 Staphylococcus aureus

In contrast to fungal organisms, such as *Aspergillus, Cladosporium, Stachybotrys*, and *Penicillium* species, *Staphylococcus aureus (S. aureus*) stands out as a Gram-positive commensal bacterium with the potential to transform into an opportunistic pathogen, eliciting infections in various soft tissues and bloodstream, leading to sepsis, *Figure 47*. This transformation is facilitated by the synthesis of

enzymes and surface proteins, inducing cytolytic effects that culminate in inflammation and toxic shock. *S. aureus* demonstrates proficiency in horizontal gene transfer, acquiring genes resistant to a spectrum of antibiotics, thereby evolving into a state of total resistance against many pharmacologically manufactured antimicrobial agents. The combination of heightened virulence, attributable to surface proteins tightly binding the bacterium to host cells, enabling a high degree of invasiveness, and rapid dissemination contribute to its pathogenicity. The global impact of bacterial and fungal antimicrobial resistance on morbidity is substantial.

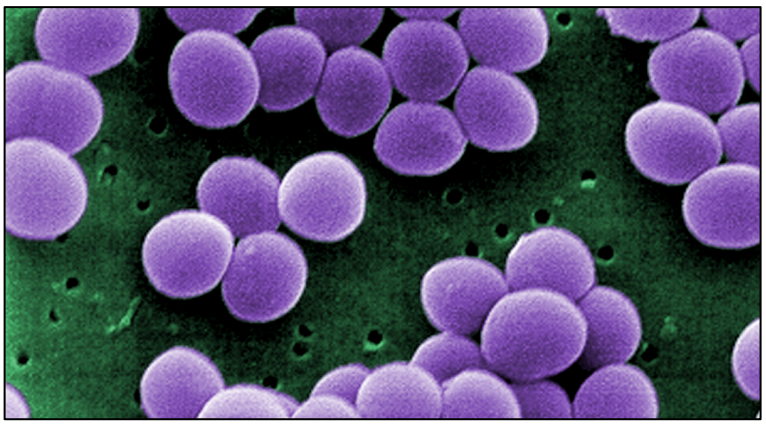


Figure 47: Scanning electron microscopic image of *S. aureus* taken from a vancomycin resistant culture, 2001. (*Source: Centre for Disease Control, public domain, photo credit J.H.Carr*)

The acquisition of antibiotic resistance poses a significant challenge for individuals exposed to *S. aureus.* Airborne transmission becomes particularly consequential in environments where the bacterium may not be conspicuously detectable, such as within wall and ventilation cavities. The prevalence of pathogenic and multi-resistant strains intensifies when buildings are situated in close proximity to animal farms, (Gandara et al., 2006; Madsen et al., 2018; Moon et al., 2014b).

S. aureus has a high survival rate in adverse conditions, enduring prolonged periods of drought. Upon reactivation in moist and damp conditions, it becomes highly contagious to exposed hosts. The frequency of such damp and dry conditions is anticipated to rise with climate changes, alongside increased water penetration into building infrastructures.

Temperature variations associated with climate change also contribute to the distribution of antibiotic resistance. Studies indicate that a temperature increase of 10 °C correlates with a respective rise in antibiotic resistance of 4.2%, 2.2%, and 2.7% for common pathogens like *Escherichia coli, Klebsiella pneumoniae,* and *Staphylococcus aureus*. This data implies that the threat of antibiotic resistance may be significantly underestimated as environmental conditions and suboptimal housing performance are influenced by temperature elevations resulting from climate change, (MacFadden et al., 2018).

4.5 Biosynthesis of secondary metabolites

Synthetically manufacturing complex antimicrobial agents is time consuming and expensive in comparison to the manipulation of organisms for intracellular bio-manufacture the biochemical

products. Microorganisms have evolved highly efficient manufacturing processes developing biosynthetic pathways that repurpose primary metabolites. The chemical logic that builds up the C-C and C-N bonds within secondary product scaffolds and the enzymes involved in the synthesis characterises the resulting products and predicts modifications based on reactivity patterns. Yield efficiency and chemical modifications of the secondary product biosynthetic pathway can be achieved through the alteration of external growth conditions, provision of alternative precursor building blocks, or direct modification of the cellular manufacturing process utilising genetic modification to the gene sequence. Genes responsible for the biosynthetic enzyme production are frequently clustered on the genome, often with resistance and export genes, and exhibit coordinated transcription in one or more operons. Activator or repressor signalling molecules responsive to various environmental and cellular conditions, work in conjunction with primary metabolic pathways stimulating related physiological modifications such as sporulation.

The five structurally distinct subclasses of polyketide natural products are constructed with a similar logic based on principles derived from fatty acid biosynthesis. Polyketides exhibit remarkable structural diversity and can be isolated from bacteria, plants, and fungi. Their structure comprises a continuous backbone of carbon atoms which may be configured into rings, accompanied by numerous functional groups such as ketones, alcohols and alkenes attached. Notable examples include erythromycin and oxytetracycline, both recognised antibiotics.

Polyketides are assembled by polyketide synthase enzymes with four identified classes. Type I synthases, prevalent in bacteria are proteins with multiple active enzyme sites, each operating once during the assembly line cycle. Type I iterative synthases found in fungi and some bacteria, feature multiple active enzyme sites each capable of functioning during multiple chain extension cycles. Type II synthases found in bacteria, consist of mutually supportive single active sites on small proteins acting collaboratively in secondary metabolite assembly. Type III synthases, found in plants and some bacteria are compact proteins catalysing all necessary steps.

Non-ribosomal peptides are assembled from amino acid units, without restriction to the 20 proteinogenic units, encompassing over 100 different amino acids. Structurally, these peptides can be linear with free N- and C- termini, cyclic joining the N- and C- termini or through the linkage of a side chain, typically to the C-terminus. Examples include vancomycin an antibiotic and cyclosporin, an immunosuppressant.

Ribosomal peptides are composed of amino acids, undergo enzymatic modification after translation on the ribosome. Thiostrepton is one of the first known ribosomal peptide antibiotics.

Aminoglycosides represent extensively modified carbohydrate ring structures that incorporate amine-substituted cyclohexane rings. The hydroxy groups within these structures are subject to substitution by amines or ketones, accompanied by the addition of methyl groups to carbon, nitrogen, or oxygen atoms within the ring system, as exemplified by the antibiotic streptomycin.

Terpenes, terpenoids, or isoprenoids constitute a diverse category of cyclic secondary metabolites characterised by a hydrocarbon framework featuring alkenes, ethers, ketones, alcohols, esters, and carboxylic acids as functional groups. Illustrative instances encompass monoterpene, such as menthol, taxol—an anticancer agent, and sesquiterpene artemisinin—an anti-malarial agent.

4.5.1 Genomic and proteomic modifications, deletions, and restructuring of secondary metabolite synthesis

Several natural product types are synthesized through a modular assembly of enzymes, each module dedicated to a specific fragment in the synthesis process. The orchestration of these metabolic processes is governed by secondary metabolism genes, which, when modified or deleted, can induce alterations in the enzyme pathway, leading to the formation of novel secondary product structures, *Figure 48*. This schematic, representative of a single type I polyketide synthetase module, delineates the interconnection between genes encoding proteins, housing a series of enzyme domains. These domains, organized in recurring modules, facilitate the stepwise chain extension cycle, ultimately culminating in the production of the secondary metabolite. Upon completion of the chain extension across the modules, the thioesterase domain facilitates the folding of the carbon chain, promoting the reaction between the terminal hydroxyl (-OH) group and the thioester link, resulting in the formation of a cyclic ester, subsequently liberated from the polyketide synthase.

Similarly, the manipulation of the synthesis pathway can be achieved by rearranging the order of enzymes, thereby inducing modifications in the arrangement of structural groups and properties within the secondary product.

Biosynthesis can be directed by altering the precursors upon which the organism relies for the secondary metabolite pathway. Exploiting the structural resemblance between precursors and the adaptability of enzymes in the synthesis pathway, the cell can be directed to generate novel metabolites.

The emergence of new secondary products in nature is facilitated through mutagenesis, wherein a naturally occurring mutant cell undergoes alterations in a biosynthetic step, enabling the organism to produce secondary metabolites from alternative precursors. Mutagenesis serves as the foundation for creating synthetic secondary metabolites by mutating and isolating cells capable of altering precursor selection due to changes in biosynthetic steps or enzymes in the manufacturing process. Genomics has revolutionized the study of secondary metabolic pathways, allowing for the manipulation of genes through insertion, modification, or deletion, eliminating the need to rely on fortuitous mutagenesis, (Anyaogu & Mortensen, 2015; Scharf & Brakhage, 2013; Tsunematsu et al., 2013; Yaegashi et al., 2014). Advancing further in the pursuit of new secondary metabolites involves the cloning of individual biosynthetic genes, incorporating them into new codes within an organism, thereby generating novel biosynthetic enzymes and unprecedented secondary products.

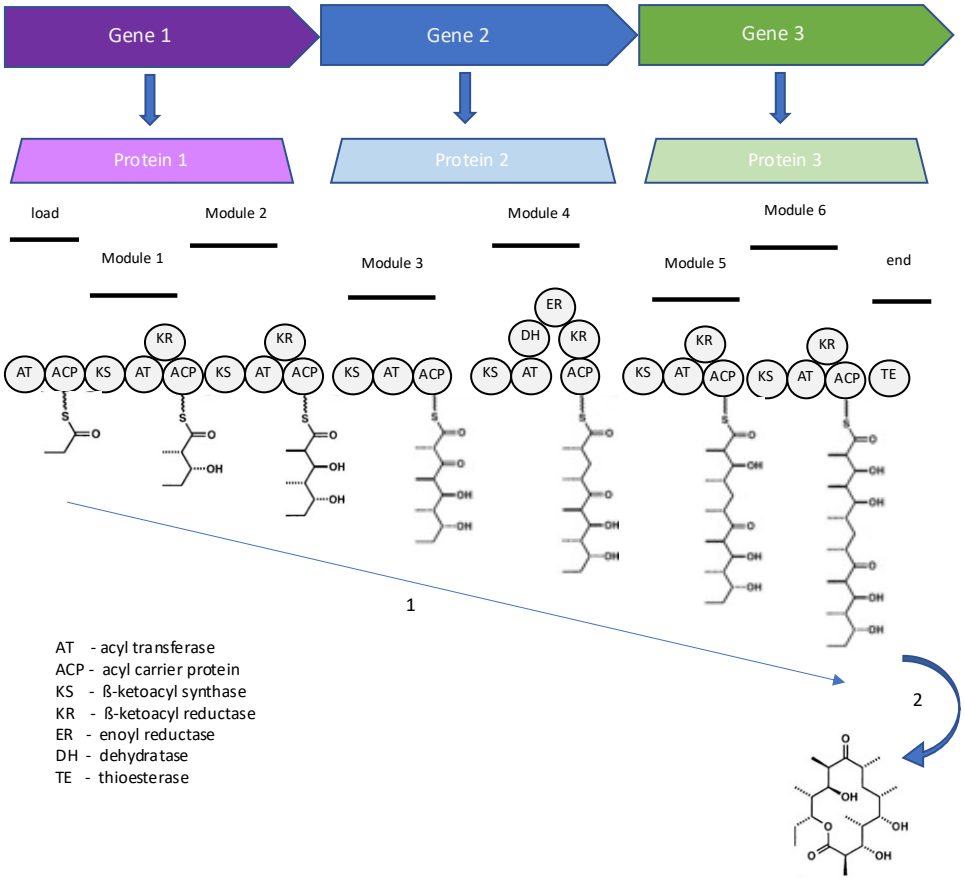


Figure 48: Diagrammatic representation of the function of the polyketide synthase involved, in this example, in the biosynthesis of erythromycin. Three genes 1 – 3, code for 3 proteins 1 – 3, and enzyme domains are arranged in repeating groups or modules. Each module contains a set of domains which carry out an aspect of the single chain extension (1) from the starter unit to the thioesterase domain which folds the carbon chain (2) to produce a cyclic ester which is then released

Following the liberation of the synthesized secondary product by polyketide synthetase, various biochemical post-modifications become feasible. These modifications encompass the oxidation of C-OH bonds, epoxidations of C=C bonds, additional folding to generate cyclic structures (apart from those facilitated by the thioesterase), and methylations at the O and C atoms.

Predominantly, lichen polyketide metabolites emanate from the fungal partner, although the influence of algal or cyanobacterial carbohydrate production on polyketide synthesis is plausible, (Elshobary et al. 2016). Despite the diversity in lichen polyketide production, the cultivation of lichen, due to slow growth, poses a formidable challenge. The advent of gene knockout and heterologous expression techniques has facilitated an in-depth scrutiny, leading to the elucidation of 81 polyketide synthase gene sequences, their genomic localisation within lichen genomes, and the assignment of biosynthetic pathway predictions derived from these gene clusters (Bertrand & Sorensen, 2018). Conventionally, the taxonomic nomenclature of the mycobiont mirrors that of the symbiotic unit.

Among the 81 identified polyketide synthase gene sequences from lichenizing fungi, 38 belong to the type I non-reducing category. These proteins are generally large, featuring multiple enzyme active sites. The foundational architecture of non-reducing lichen entails a starter acyl carrier protein

transacylase (SAT), a ketosynthase (KS), an acyltransferase (AT), an acyl-carrier protein (ACP), a product template domain (PT), and a terminal domain comprising either thioesterase (TE), claisen cyclase (CYC), or reductase (R), (Staunton & Weissman, 2001; Weissman, 2015). Of note, The type I non-reducing PKS architecture in lichens encompasses only the SAT-KS-AT-PT-ACP-TE domains, resembling those implicated in the biosynthesis of aromatic polyphenols.

This biosynthesis structure applies in the formation of usnic acid, a common lichen secondary product, the carbon atom for which originates from acetate while two methyl groups derive from S-adenosylmethionine, (Abdel-Hameed et al., 2016).

Lichen used in traditional medicine was reviewed as early as 1921 and more recently by Crawford, 2015, and Upreti et al. 2007, although not comprehensive reviews, they provide an opportunity for further research, particularly in bioinformatic studies, (Crawford, 2019; Smith, 1922; Upreti & Chatterjee, 2007)

A viable approach to delineate the functions of uncharted genes associated with secondary products involves the application of stimuli to induce metabolite production. Environmental alterations exert a profound influence on lichen secondary metabolism, with factors such as the availability of UV light, the origin of carbon, and desiccation during periods of drought being pertinent examples, (Brunauer et al. 2007; Deduke, Timsina, and D. 2012; Elshobary et al. 2016). This technique has been used to induce *C. grayi* to produce grayanic acid, enabling the related gene cluster to be identified, (Culberson & Armaleo, 1992). Investigating the impact of stressors arising from changes in environmental factors on organisms necessitates innovative field-based methodologies to comprehend the intricate interplay between climate fluctuations, activation or inhibition of biosynthetic genes, transcriptional expression, and secondary metabolite synthesis.

4.5.2 Secondary metabolite production in *O. parella*

The symbiotic association between photosynthetic algae or cyanobacteria within lichen and the biosynthesis of secondary metabolites presents significant research potential in the quest for novel bioactive agents. This potential is paralleled by the secondary product generation resulting from co-associated bacteria (Calcott et al., 2018).

The availability of data on lichen secondary metabolite production from the literature is limited, reviewing a relatively limited number of lichen species. In this instance, the pharmacological properties of several *O. parella* secondary metabolites have been researched and summarised in *[Appendices: Table 40]*.

Gyrophoric acid (GA) a secondary product with a molecular formula $C_{24}H_{20}O_{10}$, *Figure 38A*, modulates several cellular pathways relating to cardiovascular disease, diabetes, and cancer. This compound is a polyaromatic depside with functional carboxyl and hydroxyl side groups that can target specific enzymatic active sites. Gyrophoric acid has been shown to affect topoisomerase 1, compromising cell viability and resulting in apoptosis. Beyond potential applications in cancer treatment, these cytostatic effects may extend to exert broader control over cell growth and differentiation, (Cardile et al., 2017; Mohammadi et al., 2022).

The chemical structures of gyrophoric and vulpinic acids (VA), *Figure 38B*, have been recognized for their role as ultraviolet filters. In human keratinocytes, both compounds, at non-toxic doses below 400 µM, exhibit *in vitro* photoprotective properties, preventing cytotoxicity, apoptosis, and

cytoskeletal degradation. This outcome holds promise for the development of future UV-protective products, (Lohezic-Le Devehat et al., 2013; Varol et al., 2016). The mechanism of GA-mediated apoptosis is unclear; however, parallels with other lichen metabolites, such as usnic acid, suggest similarities as apoptosis markers, (Goga et al., 2019).

In addition to its capacity to circumvent cytotoxic and apoptotic effects induced by UV light, GA demonstrates effective antimicrobial activity against various bacterial and fungal pathogens, coupled with robust antioxidant protection, (Ranković et al., 2008).

4.5.3 Secondary metabolite production in *R. siliquosa*

There exist 246 distinct species within the *Ramalina* genus worldwide, displaying considerable diversity in polyketide production (Moreira et al., 2015b). This variability encompasses the synthesis of β-orcinol depsides and depsidones, wherein usnic acid stands out as the predominant cortical compound, (Esslinger, 2021)

In studies of *Ramalina siliquosa* complex found in Brittany, ten main compounds were identified. In *Table 18*, the first six entities delineate the chemotypes – primarily depsidones, a type of polyphenolic compound composed of two or more monocyclic aromatic units linked by an ester group. The presence of these compounds play a role in distinguishing the differentiation between *R. siliquosa* species, (Parrot et al., 2013).

Table 18: Literature sources quoting pharmacologically active chemical secondary extracts identified from *R. siliquosa*. An undefined grouping of 22 minor compounds, such as connorstictic acid, peristictic acid, conhypoprotocetraric acid, variolitic acid, gangaleoidin, physodic acid, and coquimboic acid have also been identified requiring further study

Secondary Product	Description and properties	References
(1) Salazinic acid	A depside with a lactone ring, the presence or absence of which can help distinguish between species of lichen. It has been shown to have antimicrobial properties *in vitro* and may be a potent modulator of key signalling pathways in colorectal cancer cells. It also serves as an antioxidant and photo protectant. The salazinic acid content of *R. siliquosa* has been shown to increase as the annual mean temperature increases and can be higher on south-facing stone.	(Fagnani et al., 2022; Hamada, 1982; Manojlović et al., 2012)
(2) Peristictic acid	A β-orcinol depsidone, possible action as a superoxide anion scavenger with comparative potency to ascorbic acid and low human cell toxicity.	(Elix & Wardlaw, 2000; Ismed et al., 2017)
(3) Cryptostictic acid	A depsidone with a high radical and superoxide scavenging abilities.	(Bay et al., 2020; Gadea et al., 2017)
(4) Protocetraric acid	Broad spectrum antimicrobial properties against pathogenic microbes including SARS-CoV-2 and selective antifungal activity.	(Fagnani et al., 2022; Nishanth et al., 2015)
(5) Stictic (scopuloric) acid	Demonstrates antioxidant, cytotoxic and apoptotic effects *in vitro*.	(Lohézic-Le Dévéhat et al., 2007; Pejin et al., 2017)
(6) Norstictic acid	A selective allosteric transcriptional regulator functioning as an anti-cancer agent, targeted antibacterial activity and can reduce some tumour growths. Environmentally involved in metal homeostasis by forming pH-dependent metal complexes affecting intracellular metal uptake.	(Garlick et al., 2021; Hauck et al., 2010)

(7) Hypoprotocetraric acid	A secondary compound demonstrating bactericidal properties and effective within a period of 0-6 hours from kinetic time kill assay studies.	(Culberson, 1965; Culberson & Hale, 1973; Neeraj et al., 2011; Parrot et al., 2013)
(8) 4-O-demethylbarbatic acid	Demonstrates antibacterial and anti-cancer properties and linked to the formation of atranorin (a depside) in lichen as a precursor compound.	(Kim et al., 2021; LaGreca et al., 2020; Micheletti et al., 2021; Punniyakotti et al., 2023)
(9) Usnic acid	An abundant lichen metabolite with antibiotic properties against *S. aureus*, *enterococci*, and *mycobacteria*. The secondary product has ultraviolet absorption and preserving properties. Usnic acid is toxic and tools such as encapsulation release systems are required to overcome this pharmacological obstacle to fully exploit the antitumour, antiviral and antiparasitic properties.	(Cocchietto et al., 2002; Lauterwein et al., 1995; Macedo et al., 2021)

4.6 Effects of lichen secondary metabolites on biofilm formation and microbial growth

Most microorganisms comprising bacteria and fungi, develop protective communities referred to earlier in the text as biofilms (Bridier et al., 2017). Biofilms are instigated by the attachment of planktonic cells to surfaces, stimulating the production of extracellular polymeric substance (EPS). The EPS serves as a substrate facilitating cellular adherence, endowing resistance against external physical and chemical threats, and fostering a collective co-metabolic existence within the community, (López et al., 2010). The environmental rigours, including alterations in nutrients, temperature extremities, desiccation, pH fluctuations, as well as physical and UV-induced threats exerted upon biofilms confer protective advantages in diverse ecological, industrial, and medical environments, (Flemming et al., 2016). These advantages pose formidable challenges to human endeavours, manifesting as environmental encrustations, conduit obstructions, the formation of slime and supporting the proliferation of pathogenic entities on for example, crucial medical apparatus such as catheters and within water purification systems. The biofilm provides a template for the development of phenotypic variants, in part a result of horizontal gene transfer, within the cooperative network of bacteria. The EPS is adept at providing physical protection to cells contained in the matrix and provides a substantive barrier to hinder the entry of antimicrobial compounds (Bridier et al., 2017).

The biofilm EPS initially forms a conditioning layer on the attachment surface comprising of polysaccharides and proteins serving as a robust scaffold to attract additional cellular affixation. Subsequent planktonic cells adhere to the preconditioned surface, impelled by the negative charge of the outer membrane, bonding through hydrophobic, electrostatic, and van der Waals forces. These initially feeble forces are subsequently fortified by the EPS, evolving into a supportive matrix wherein cells and extraneous materials aggregate. As the EPS accumulates proteins, nucleic acids, and lipids, it develops into a three-dimensional, charged, and hydrated structure, proficient in sustaining a nutrient-rich environment for the enclosed cellular consortium. This matrix, facilitating chemical diffusion, nutrient provisioning, metabolic toxin dispersion, and waste removal, engenders cellular communication within the biofilm community. The fortifying influence of the matrix persists until diffusional inefficiencies prompt biofilm disengagement, inducing network fragmentation and affording opportunities for fresh colonisation.

Biofilms confer heightened resilience to various environmental stressors upon microorganisms ensconced within the EPS matrix, affording protection against deleterious conditions that would typically compromise or exterminate planktonic cells.

Removing the biofilm presence from architectural substrates poses formidable challenges, typically necessitating interventions of a physical or chemical nature. However, these removal methodologies may render the substrate structurally compromised, rendering it susceptible to subsequent biological and chemical assaults. Leveraging natural antimicrobial agents for biofilm attenuation or eradication encompasses strategies such as inhibiting persister cell formation, a phenomenon wherein cells assume a state of diminished growth, bolstering tolerance to antimicrobial interventions, or impeding pivotal steps in microbial primary metabolic pathways. Disruption or impairment of the biofilm structure presents an opportune moment to expose biofilm-embedded bacteria to antimicrobial agents. Strategies encompassing the inhibition of biofilm production, coupled with the role of antimicrobial agents, open avenues for the exploration of secondary products derived from lichens, with potential anti-biofilm and antimicrobial attributes, (Beloin et al., 2014).

PRACTICE: Assessment of antimicrobial efficacy of secondary metabolites from lichen *Ochrolechia parella* and *Ramalina siliquosa* against deteriorative growth of mould *Penicillium chrysogenum* and bacterium *Staphylococcus aureus*

Abstract

The diverse bioactive potential of lichen-derived secondary metabolites, encompassing antimicrobial, antioxidant, antiviral, and anti-inflammatory activities, is well-established. However, despite the recognised potential of lichens as reservoirs of these valuable compounds, research exploring the impact of climate-driven weather extremes on their secondary metabolite production and antimicrobial profiles, particularly in the context of construction materials, remains limited. This chapter addresses this critical gap by investigating the extraction, isolation, and antimicrobial evaluation of secondary metabolites from two coastal lichen species, *Ochrolechia parella* and *Ramalina siliquosa*, known for their resilience to extreme weather conditions. The study aims to assess their potential as sustainable and eco-friendly antimicrobial agents for combating biodeterioration on traditional building materials.

The investigation delves into the largely unexplored potential of coastal lichens as a source of sustainable and effective antimicrobial agents for the protection of traditional building materials. Resilient lichens, *Ochrolechia parella* and *Ramalina siliquosa*, thrive in harsh coastal environments, suggesting a unique metabolic profile with potentially valuable antimicrobial properties. Through careful extraction, isolation, and evaluation of their secondary metabolites, the study aims to identify bioactive compounds with potent activity against *Penicillium chrysogenum* and *Staphylococcus aureus*, two major microbial contributors to biodeterioration in buildings.

By unearthing bioactive antimicrobials from natural sources, this research establishes a foundational chemical framework with twofold purpose: guiding microbial biosynthesis and facilitating in vitro chemosynthesis of commercially viable antimicrobial agents. This research not only offers a promising approach to combating the increased microbial growth observed on traditional surfaces due to climate-induced environmental changes, but also contributes to the development of environmentally friendly antimicrobial solutions while seeking to establish a foundation for future antimicrobial development in the construction industry.

4.7 Introduction

The colonisation of buildings by microorganisms can lead to surface coverage, loss of original traditional materials, and irreversible changes to the substrate where microorganisms flourish. The nature of the physico-biological nature of the building is highly dynamic and subject to continuous changes under influences from the environment. Even minor shifts in microclimatic conditions can disrupt the balance between the environment and the build surface, triggering biodegenerative processes. Understanding the interactions is crucial to preventing damage and providing insight into environmentally focused restoration and conservation treatments.

Lichen *O. parella* and *R. siliquosa* grow in coastal and island locations across the north of Scotland, tolerating extreme weather conditions, the latter providing a food source for grazing animals during the winter. The deterioration of exterior building elements leading to cracks and water entry provides ideal conditions for proliferation on the interior surfaces of the building such as wallpaper, post-1950s plasterboard and adhesive pastes. These internal surfaces comprise organic compounds including diverse nutrients such as cellulose, and other carbon sources providing a stable nutritional anchorage for rapid microorganism growth and dissemination.

The practice section pertains to the exploration of how innovative configurations in microbial biosynthesis can propel traditional building conservation forward amidst the backdrop of climate change, particularly by alleviating the biodeterioration of built heritage. The biochemical processes involved in the biodegradation orchestrated by moulds and bacteria residing on both interior and exterior surfaces manifest environmentally induced responses, giving rise to the formation of initial biofilm colonies and subsequent microbial proliferation. The pace of biodeterioration gains momentum enhanced by the availability of nutrients, water, and warmth.

This study operates on the premise that less deleterious microorganisms when subjected to analogous environmental stimuli, undergo similar stimulation for growth and engage in competitive interactions with moulds and bacteria. Competition is enacted through the production of secondary metabolites serving as antimicrobial agents to the targeted microorganism. The core hypothesis suggests that by successfully identifying antimicrobial secondary metabolites that may provide competitive advantage over other microbial colonisation, a framework is established to derive antimicrobial synthetic analogues designed from sustainable and natural sources. These frameworks, in turn, serve as templates for the formulation of natural conservation treatments. The cultivation of a library of antimicrobial secondary products emerges as a promising bioconservation strategy. This strategy is designed to enhance ongoing maintenance and repair efforts, aimed at mitigating climate-accelerated bioerosion in the context of heritage preservation.

4.7.1 Challenges for Secondary Metabolite Research

The investigation into the production of secondary metabolites by communities is challenging when cultivating and sustaining lichens within laboratory environments. Lichens, characterised by slow growth rates spanning 0.01 to 64 mm per annum, predominantly gravitate towards the lower end of this spectrum. The protracted lifespan coupled with languid growth poses a conundrum for *in vitro* experimentation, as alterations in environmental parameters exert substantial impacts on metabolic pathways and ensuing chemical profiles, (Deduke, Timsina, & Piercey-Normore, 2012). The obligate symbiotic nature of lichens further complicates endeavours to segregate the photobiont from the mycobiont for the progression of comparative genetic investigations. Despite these challenges, innovative methodologies have yielded the sequencing and registration of more than 80 polyketide

synthase (PKS) gene clusters in lichens, inclusive of commonplace accessory genes like cytochrome p_{450} and FAD-dependent oxidases, as registered on GenBank, (Gunawardana et al., 2021).

Usnic acid emerges as the focal point among bioactive secondary metabolites derived from lichens. Its molecular representation $C_{18}H_{16}O_7$; 2,6-diacetyl-7,9-dihydroxy-8, 9b-dimethyl-1, 3(2H, 9ßH) - dibenzofurandione, has limited solubility in water which necessitates systematic exploration of optimal solvents for extraction. Ethanol, posited as a viable alternative to the commonly employed acetone, has exhibited pharmaceutical relevance. Nevertheless, the selection of a solvent requires consideration beyond efficiency, encompassing safety, health, and environmental implications, (Ahmad et al., 2017).

While acetone is a predominant solvent for lichen secondary product extraction as illustrated in the extraction of gyrophoric acid from *Umbilicaria hirsuta* (Goga et al., 2019), diverse solvents with varying polarities present the prospect to extract a broader spectrum of bioactive compounds. Post-solvent extraction, filtration followed by vacuum evaporation is typically employed to separate bioactive compounds from the solvent. The scrutiny of competitive interactions among lichens has led to methodological advancements, such as the exploration of non-random associations between thalli groups to discern coexistence patterns. The nearest neighbour model stands out as a promising predictor of degrees of competition in distinct lichen communities, spotlighting synthesised products for potential antimicrobial scrutiny, (Armstrong & Welch, 2007; Bhattacharyya et al., 2016)

Thin layer chromatography (TLC) serves as a methodology for segregating and assessing the presence of secondary lichen products within extractions and may be augmented by the incorporation of mass spectrometry as a complementary technique supporting product identification, (Le Pogam et al., 2017). Micro extracts from lichen fragments can be chromatographed utilising a two or three-eluent system, with resulting compound spots assigned relative front (R_F) values for comparison to known lichen substances. Although a straightforward procedure, the determination of R_F values is time-consuming and resource-intensive. Preparative TLC, leveraging a macro scale with adsorbent silica, expeditiously isolates individual compounds within lichen extracts, facilitating rapid purification with minimal eluent volumes. Detectable zones extracted from the silica plate enable the isolation of reasonably pure compounds from the chromatogram (Wing & Bemiller, 1972).

The synergistic combination of preparative TLC and mass spectrometry processes has found extensive application in the analysis of secondary products, contributing to metabolic gene knockout and gene cluster identification across diverse organisms, (Bertrand & Sorensen, 2018; Medema et al., 2015; Nepal & Wang, 2019; Rutledge & Challis, 2015). Nevertheless, secondary products are complex consisting of an array of molecules displaying varying properties. Isolating and ionising specific molecules can be challenging as several dominant ions may mask molecules of interest. The complex spectra resulting from unknown compounds can be challenging to decipher as spectral databases are unlikely to be sufficiently comprehensive to identify these novel complex compounds, an opportunity for future researchers.

The assessment of minimum inhibitory concentrations of lichen secondary products impacting bacterial growth is effectively conducted by utilising 96-well plates and serial dilution protocol. Determining the minimum concentration at which bacterial growth stops or is prevented and evaluating potential inhibition of biofilm formation constitutes a straightforward yet resource-intensive procedure, (Andrews, 2001; Ben-David & Davidson, 2014).

4.7.2 Materials and Methods

4.7.2.1 Lichen Secondary Product Collection and Extraction

O. parella and *R. siliquosa* samples were collected from North facing, heritage stonework at an extreme weather site at Yell, Shetland, 60°34'21.9"N 1°11'20.9"W, *Figure 49*. Approximately 150g of each specimen was collected, air-dried, and stored in sealed bags for transportation.

Figure 49*: R. siliquosa* (left*)* and *O. parella* (right) prior to their retrieval from colonised stonework situated on a geographically isolated and climatically challenging coastal island within the North Sea region. Both lichen species exhibit prolific growth at the collection site, demonstrating resilience to environmental factors

Samples of mould and bacteria were collected from three locations within the premises, at a site where water damage and the proliferation of microorganisms was apparent on the surfaces, *Figure 50*. Care was taken to ensure collection sites were free from animal and bird contamination.

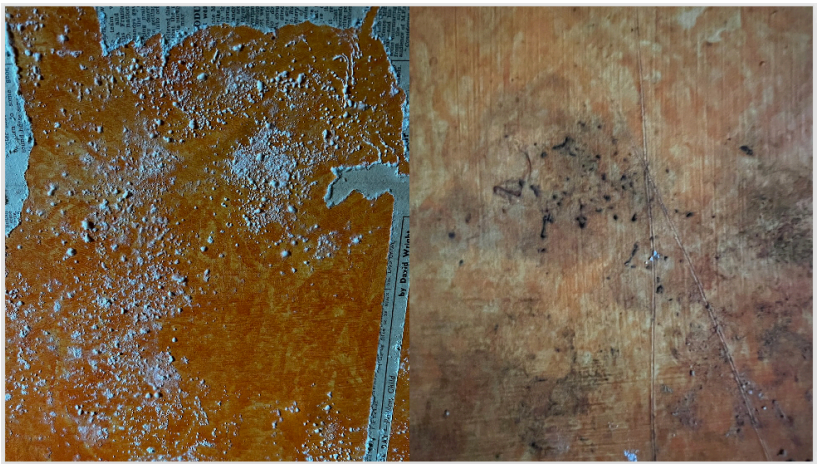


Figure 50: *Samples of mould collected from damp surfaces of interior walls. White mould, situated on damp wallpaper (left of image), was identified as the source of a distinctive odour, commonly accompanied by a confluence of mould species such as Cladosporium, Penicillium, and Aspergillus. On the right, the presence of black mould exemplifies Stachybotrys proliferation. The images illustrate the manifestation of mould growth on*

damp wallpaper, thriving due to the abundance of carbon and a diverse array of nutrients present within the wallpaper, underlying paste, and the wall substrate

4.7.2.2 Sampling methods

Several microbial sampling methods are commonly utilised including swabbing – a sterile swab collects the sample and is suspended in nutrient medium – impaction – collecting and blowing sample air across a solid surface – and direct plating – pressing nutrient agar directly onto the microbial sample area. For the purposes of this study two methods were applied, needle sampling and adhesive tape.

Needle sampling involves the aseptic extraction of minute biological samples from the material surface, thereby minimising intrusion, and damage to the underlying substrate. The utility of this method is constrained by the limited scope of analyses applicable to the extracted samples. The paucity of information regarding the distribution of the sample and its relationship to neighbouring microorganism colonies restricts comprehensive analysis. Nonetheless, the method excels in species identification while mitigating the impact of extraction on the material.

Conversely, the adhesive tape method entails transferring information from surface microorganisms to a glass slide for microscopic staining and identification. The width of the tape facilitates a more expansive representation of the sample and its surrounding colony, providing an accurate depiction of spatial relationships between organisms. The effectiveness of the method relies on selecting an adhesive strength that minimally damages surface laminations, especially when climatic or pollutant-induced delamination has occurred. Colonies, sourced from interior *Penicillium chrysogenum* and *Staphylococcus aureus*, were cultivated on agar plates commensurate to the nutrient profile of the organism. The adhesive tape-affixed samples were placed on the agar plate surface.

Microorganisms that breach material surface layers, frequently cause delamination or crumbling. The meticulous extraction and collection of biomasses attached to moist or crumbling material using both these methods and sterile instruments can be undertaken with minimal disruption to the microbiome colonising the test area.

Culturing microorganisms on media plates tailored to their specific nutrient requirements is integral. *Staphylococcus aureus* thrives on standard nutrient agar or nutrient broth, while *Penicillium chrysogenum* flourishes on DG18, *[Appendices: Table 41]* or malt extract agar, *[Appendices: Table 42]*. Incubation periods vary; optimal growth conditions for *P. chrysogenum* occur at 23°C and pH 3-4.5, with a potential duration of up to three weeks. The bacterium in this grouping, *S. aureus*, readily proliferates on nutrient agar or in nutrient broth at 36°C, typically achieving growth overnight.

In supplement to specimens procured from the in situ site, laboratory-purified samples of *S. aureus* and *P. chrysogenum* were acquired from ATCC as samples in reserve in the event of issues with collected sample viability.

4.7.2.3 Solvent selection and usage

Solvents exhibit inherent characteristics of polarity, either polar or non-polar. The determination of the polarity of a solvent is contingent upon the evaluation of its dielectric constant. Polarity, a fundamental attribute, manifests in various physical properties, notably impacting surface tension

and solubility. For instance, water, exemplifying a strongly polar solvent, manifests a dielectric constant of 80 at 20°C, underscoring its polarity. Conversely, solvents attaining dielectric constants below 15 are deemed nonpolar. In comparison, the dielectric constant for methanol is 32.6 and ethanol 24.3 at 20°C. The dielectric constant is defined as the ratio of the electric permeability of the material to the electric permeability of free space. It is expressed as a function of temperature. The higher the numerical value of the constant the greater the ability of the solvent to dissolve salts.

The significance of polarity is particularly evident in the extraction of compounds from organic sources, wherein the polar nature of solvents plays a decisive role. Organic compounds such as sugar, alongside inorganic entities like sodium chloride (NaCl), exhibit strong polarity, rendering them soluble solely in highly polar solvents like water.

Efficiency in solvent extraction within organic samples is contingent upon various factors, including particle size, solvent to solid ratio, extraction temperature, and duration. Each of these parameters influences the process of extracting chemical products from organic matrices. Within the realm of protic solvents, the propensity to donate protons (H^+) to solutes through hydrogen bonding is a defining characteristic. Water (H_2O), an omnipresent protic solvent, serves as a notable example.

Conversely, polar aprotic solvents lack an acidic proton while retaining their polar nature. Exemplifying this category, acetone (C_3H_6O), engages with strong acids and bases, demonstrating miscibility with water and methanol.

In the context of solvent selection for extraction studies, secondary metabolites, characterised by reduced polarity, necessitate less polar solvents. In this study, methanol is chosen as the solvent, given its capacity to facilitate the solubility of a wide range of secondary metabolites. Conversely, primary metabolites such as carbohydrates and proteins, displaying a predilection for water solubility, are less likely to be extracted from the lichen source during the methanol extraction process.

4.7.2.4 Solvent extraction procedure

From the air-dried specimens gathered, 50 grams of lichen are ground utilising a pestle and mortar, aiming to optimise the surface area accessible to the solvent. Subsequently, 200 ml of methanol is introduced into a stoppered flask, initiating an extraction process within an agitator, lasting 48 hours. Employing a vacuum filtration pump and filtration apparatus, each specimen is filtered to eliminate solid debris from the resultant extract. The obtained extracts are subjected to evaporation within a vacuum evaporation chamber, facilitating the determination of the weight of the desiccated extract sample for each lichen *[Appendices: Table 43]*

The extraction yield (6) for each lichen is defined as:

$$Yield\ \% = \frac{Mass\ of\ metabolite\ in\ extract}{Mass\ of\ the\ dried\ lichen\ sample} \times 100 \quad (6)$$

Using the standard dilution equation (7), a solution of each extract was formulated to a concentration of 10 mg ml^{-1} in methanol. The formula is used to calculate an unknown quantity

where two solutions are proportional, where C_1V_1 is the concentration or amount and the volume at the start of the dilution and C_2V_2 is the concentration or amount and volume required on completion of the dilution.

$$C_1 \times V_2 = C_2 \times V_2 \quad (7)$$

4.7.2.5 Assessment of antimicrobial activity of the extracted lichen compounds

Three different agar plates were prepared, DG18 agar and Malt Extract Agar for *P. chrysogenum*, and Nutrient Agar for *S. aureus*. DG18 agar serves as an optimal medium for general-purpose mould propagation, characterised by a reduced water content relative to other agars. This characteristic impedes the rapid proliferation of fungal spores and mitigates bacterial growth, thereby minimising potential sources of interference and contamination.

Each agar plate underwent inoculation with *P. chrysogenum* and *S. aureus*, respectively, prior to the introduction of sterile cellulose discs laden with either the control substance (methanol) or the lichen extract in methanol. Four sterile cellulose discs were prepared for each agar plate. Of these, one disc was inoculated with 2 µl of methanol, representing the control, with an explicit placement on each plate. The remaining three discs were individually impregnated with 2 µl of lichen extract, allowed to dry, and subsequently positioned onto the agar plate, *[Appendices: Figure 76]*.

Distinct incubation periods were instituted for each agar plate. *S. aureus* underwent a 24-hour incubation at 36°C, while *P. chrysogenum* was optimally cultivated at 23°C under a pH range of 3-4.5, with an extended duration of up to three weeks.

4.7.2.6 Separating, identifying, and isolating individual lichen extracts

The presence of a range of secondary metabolites within an organic solvent such as methanol must be verified to confirm extraction was effective and to inform the appropriate technique to isolate the number of secondary products in the extract. The technique utilised to isolate and identify the presence of secondary products in the extract is thin layer chromatography.

4.7.2.6.1 Confirming the presence of multiple extracted compounds

Thin layer chromatography (TLC) is a separation technique for quantitative and qualitative analysis. TLC stands as a pivotal separation technique employed in both quantitative and qualitative analyses. The method involves the application of a thin layer of a stationary phase, typically silica gel, onto an inert substrate, such as an aluminium plate. Functioning as the mobile phase, a liquid solvent transports the samples under examination across the plate, effecting the separation of the sample into distinct compound components

It is crucial to note that the mobile and stationary phases exhibit disparate properties. The stationary phase is characterised by high polarity. In this particular investigation, the mobile phase consists of a solvent mixture comprising dichloromethane (dielectric constant 8.93 at 20°C) and methanol, which, in contrast to the stationary phase, manifests non-polar attributes. After the separation process, the

visual detection of spots, that is, the visual representation of deposited compounds, is facilitated under ultraviolet light.

Quantification is achieved by determining the ratio of the distance travelled by the substance relative to the baseline of origin, divided by the total distance covered by the mobile phase. This ratio is termed the retardation or retention factor, denoted as, R_f. The schematic representation of the chromatographic separation and identification of compounds within an unknown extract is illustrated in *Figure 51*.

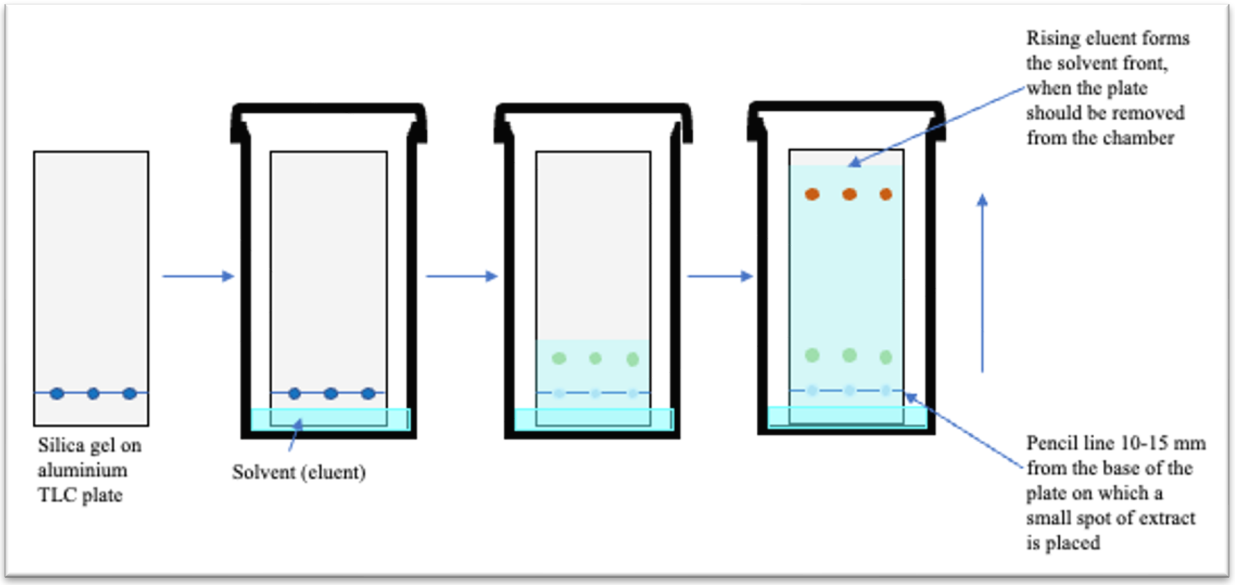


Figure 51: Fabricating a Thin-Layer Chromatography (TLC) plate, wherein the plate, inscribed and bearing a minute aliquot of the extract, is introduced into a sealed glass jar containing an eluent comprised of 10% methanol and 90% dichloromethane. The eluent ascends the silica gel matrix affecting the migration of compounds inherent to the extract. The Relative front (R_f) value is ascertained by evaluating the distance traversed by the extracted compound from the baseline and the extent covered by the solvent front after the process. The completed plates can be viewed [Appendices: Figure 78]

Procedure:

TLC plates, commercially procured and composed of silica gel affixed to an inert binder of calcium sulphate on an aluminium substrate, are, due to the fragile composition of the plate, carefully labelled. A fine pencil demarcation, situated approximately 10-15 mm from the base of the plate, establishes the line of origin. This mark designates the area where the analyte will be deposited for subsequent analysis.

A micro-capillary tube is used to apply a minute aliquot of the extract to the baseline. Subsequent to application, the solvent undergoes controlled evaporation to preclude interference with the mobile phase. Multiple samples are consecutively deposited on the baseline, maintaining adequate spatial separation to prevent samples blending.

Within a screw-top separation chamber housing a strip of filter paper extending from the rim to the base, a solvent mixture comprising 10% methanol and 90% dichloromethane is introduced to a

depth not surpassing 10 mm. The filter paper ensures a saturated solvent environment for the TLC, facilitating optimal separation.

Subsequent to the assembly of the TLC plate into the separation chamber, the solvent ascends the plate, carrying components of the eluate. Extraction is terminated when the solvent advances approximately 15 mm from the top of the plate.

The apex of the advancement of the solvent, termed the solvent front, is lightly delineated with a pencil. Post-extraction, the TLC plate undergoes visualisation under ultraviolet light, and the observed sample deposits or spots are outlined using pencil markings.

Thin layer chromatography encounters limitations dictated by the prerequisite for the solubility of the target component in the mobile phase. The R_f, calculated from the chromatograph, is recorded. In this instance the Relative front Rf determined from the chromatograph was recorded though not used for identification purposes due to limitations of the procedure when comparative compounds are either not yet identified (as in this study) or not available.

4.7.2.6.2 Application of Preparative Thin-Layer Chromatography for the Separation and Isolation of Constituents from Lichen Species O. parella and R. siliquosa

Preparative thin-layer chromatography (PTLC) represents a methodology employed for the concurrent separation, isolation, and extraction of minute quantities of compounds, thereby effecting the purification of samples derived from the initial extract. Analogous to thin-layer chromatography (TLC), PTLC constitutes a separation technique utilising a stationary phase, typically involving a more substantial coating of silica on either glass or aluminium. The mobile phase, exploiting the polar attributes of the solvent, facilitates the segregation of components within the mixture. While PTLC may lack the sensitivity characteristic of high-performance liquid chromatography, its adaptability and efficiency render it a valuable method for purifying minute amounts of compounds.

Procedure:

Employing multiple plates, a dot coding applied to one of the upper corners of each plate serves as a means of identification for individual plates and avoiding plate contamination.

Extracts exhibiting antimicrobial activity are subjected to PTLC, with each extract allocated to a separate plate. This process facilitates the separation of active compounds within the extract, purifying the samples for subsequent identification, either by confirming Rf value comparisons, through mass spectrometry, or the assessment of bioactive antimicrobial of compounds.

The eluent, composed of 90% dichloromethane and 10% methanol, is applied to sit below the straight line on which the extract is deposited. It is imperative that the extract is thoroughly dried before introducing the plate and sealing the container, *Figure 52*. A fine dotted line, drawn 1 cm from the top of the plate, serves as an indicator signifying the completion of the process.

When the solvent reaches the upper dotted line, the plate is carefully extracted, it is subsequently allowed to air-dry in a well-ventilated environment.

Under UV light, the plate reveals the compound lines which are carefully demarcated using the fine point of a scalpel or a soft pencil, ensuring the preservation of the integrity of the silica layer. Once

marked, the plate is carefully scraped, with each silica segment transferred into individually labelled glass falcon tubes *[Appendices: Figure 79]*.

To each falcon tube, 5 ml of methanol is introduced, followed by vortex agitation for 15 seconds, a procedure repeated five times. Centrifugation is then employed to compact the silica within the falcon tubes.

The extract segment undergoes filtration and is subsequently stored in small glass screw-top bottles. The use of glass is imperative throughout the procedure to preclude exposure to plastics, which may introduce minute particles of contaminants.

The mass of the secondary product extracted within the methanol is determined by evaporating the methanol samples prior to reconstituting the solutions in up to 1 ml of methanol to standardise the concentrations. The resultant samples are stored at 5°C.

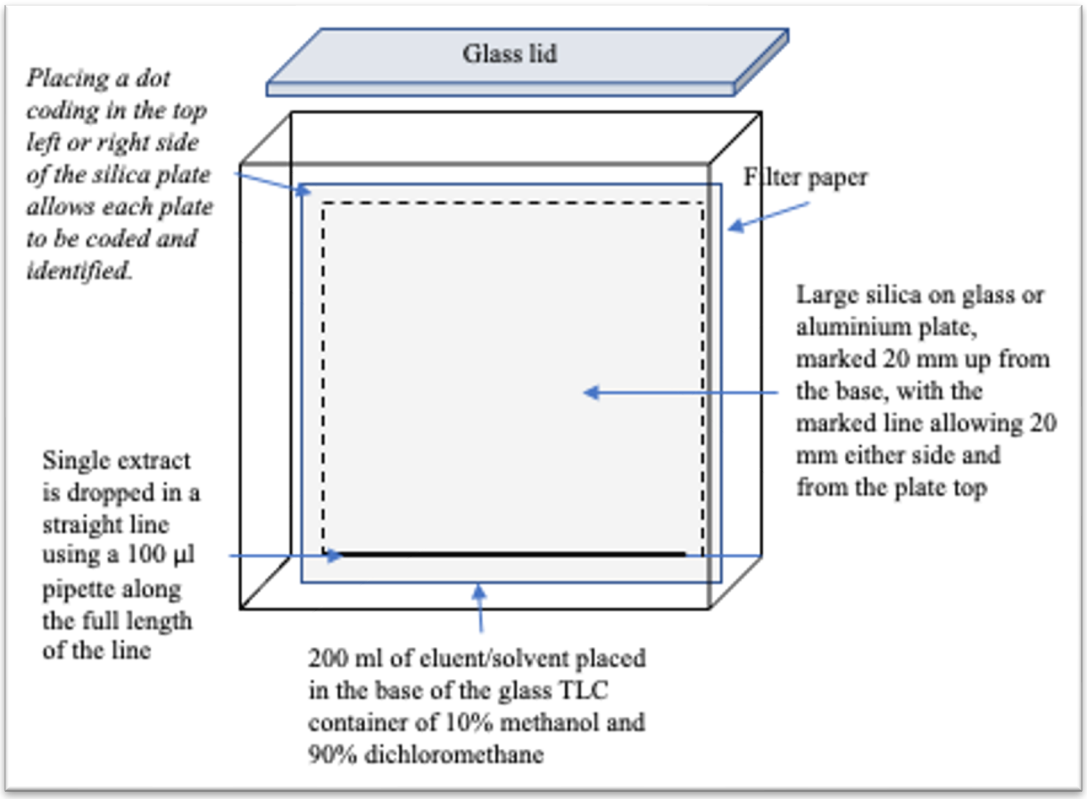


Figure 52: Preparatory TLC plate within the solvent container. Filter paper is included to maintain a consistent solvent saturation. A 20 mm margin is allowed around the plate to mitigate contamination or damage from handling the plate

4.7.2.6.3 Investigating the inhibitory effect of preparatory TLC extracts from *O. parella* and *R. siliquosa* on microbial growth

Procedure

Agar media plates were prepared by the aseptic pouring of autoclaved warm agar into sterile petri dishes. Three different sets of agar plates were prepared, DG18 agar and Malt Extract Agar, for optimal growth of *P. chrysogenum*, and Nutrient Agar to cultivate *S. aureus*.

The respective plates were inoculated with each of the biodeteriorating microbes, *P. chrysogenum* and *S. aureus* from liquid broth cultures with the addition of one cellulose disc per plate containing the control (methanol).

Four aseptically prepared cellulose discs were allocated to each agar plate. One cellulose disc on each plate received 2 µl of methanol as the control, while the remaining three discs were spot-inoculated with 2 µl of lichen extract, allowed to aseptically dry, and subsequently introduced onto the agar surface.

The incubation parameters for each microbial strain varied, with *S. aureus* subjected to a 24-hour incubation period at 36°C and *P. chrysogenum* optimal at 23°C for a duration of up to 3 weeks.

4.7.2.6.4 Measuring the effect of bioactive extracts in microtiter bioassay serial on *S. aureus* and *P. chrysogenum* growth

Microtiter assays represent a quantification approach for determining the concentration of an active antimicrobial compound, offering a convenient and cost-effective means to assess potency. This method involves a sequential dilution of the original sample, maintaining a constant dilution factor (a tenfold decrease in concentration for each step) to create a series of progressively less concentrated samples. The application of a dilution factor of 10 facilitates the precise measurement of extremely low substance concentrations, particularly advantageous in evaluating the antimicrobial efficacy of secondary metabolites.

Within antimicrobial susceptibility testing, broth dilution stands as a fundamental method. This procedure entails the preparation of ten-fold dilutions of the antimicrobial agent in a liquid growth medium within a 96-well microtitration plate. Each well receives microbial inoculum, prepared in the same medium following dilution of a standardised microbial suspension adjusted either to 0.5 McFarland scale or with turbidity set to 1.6 ± 0.5 at 630 nm using a spectrophotometer. Subsequent to thorough mixing, the microtitration plate undergoes incubation under conditions conducive to the growth of the test microorganism.

Various potency metrics, such as the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), offer insights into the bioactivity of antimicrobial agents. The MIC, defined as the lowest concentration inhibiting visible microorganism growth after overnight incubation, serves as a critical parameter (Andrews, 2001). Conversely, the MBC is the antimicrobial concentration preventing all bacterial cell growth. In the context of initial assessments of secondary metabolite antimicrobial activity, the MBC may not be applicable due to insufficient concentrations. The study primarily concentrates on inhibitory concentrations influencing visible growth, determined through optical density measurements of bacterial turbidity. The MBC or minimum fungicidal concentration (MFC), also known as the minimum lethal concentration (MLC), represents the concentration needed to eliminate 99.9% of the final inoculum after a 24-hour incubation, determined through sub-culturing samples onto agar plates to ascertain surviving cell counts (CFU mL^{-1}).

Until the MIC is attained, the antimicrobial agent concentration is deemed non-inhibitory (NIC), as it does not constrain bacterial growth. Beyond the NIC threshold, the MIC triggers observable, and measurable reductions in bacterial growth. As the compound concentration escalates, it may achieve the MBC, halting all bacterial growth. MIC determination employs a semi-quantitative test providing an approximate value for the lowest secondary metabolite concentration needed to impede the growth of the selected bacteria or mould. The microtiter method assesses sample turbidity, a property influencing optical density measurements, generating data for graphical representations of inhibitory effects.

The turbidity of the initial microbial solution is gauged before use in well-plates, adjusted within the appropriate nutrient medium to an approximate OD of 1.6 ± 0.5 at 630 nm. Each 96-well plate adheres to the format depicted in *Figure 53 and Figure 54*, featuring two control rows for baseline comparisons. Row H serves as the control for absorbance under normal bacterial growth conditions, while Row A gauges the absorbance of microbial growth influenced solely by the addition of methanol, assessing potential impacts of the methanol solvent used in lichen sample extracts on microbial growth.

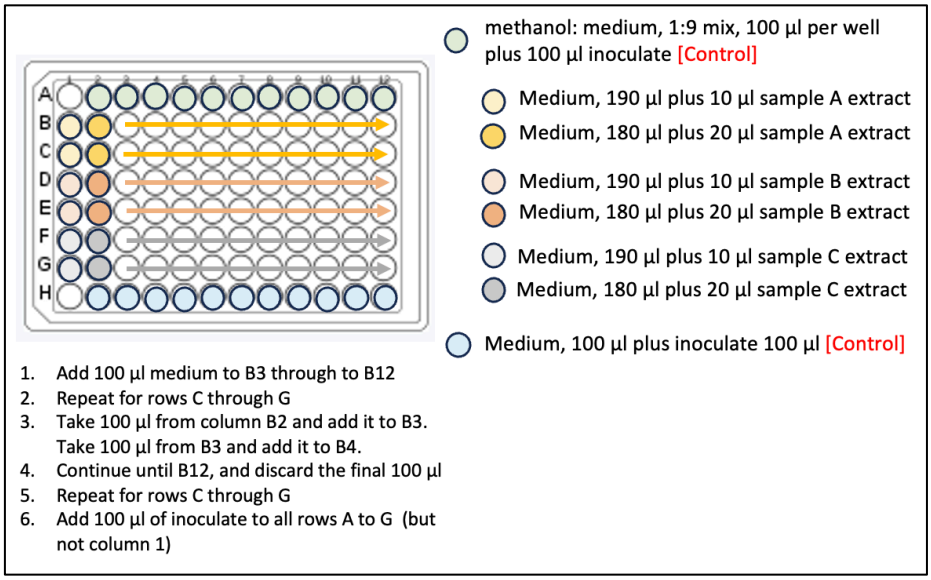


Figure 53: The spatial disposition of 96-well plates established for the investigation. Measurement of unimpaired microorganism growth absorbance is conducted along row H. The evaluation of the impact stemming from the inclusion of methanol in the sample additives (methanol exclusively) on absorbance measurements of microorganism growth is delineated within the control row A. Serial dilutions begin from an extract concentration of 100 µg ml^{-1} and diluted 10-fold between dilution 1 (100 µg ml^{-1}) through to sample dilution 11 (1.00E-08 µg ml^{-1}). Note: Data recorded for graphical and bar graph analysis, wells 2 – 12 correspond to serial dilutions 1-11 on both analysis graphs. The extract concentration in well 2 initiates the serial dilutions with 100 µg ml^{-1} as the base

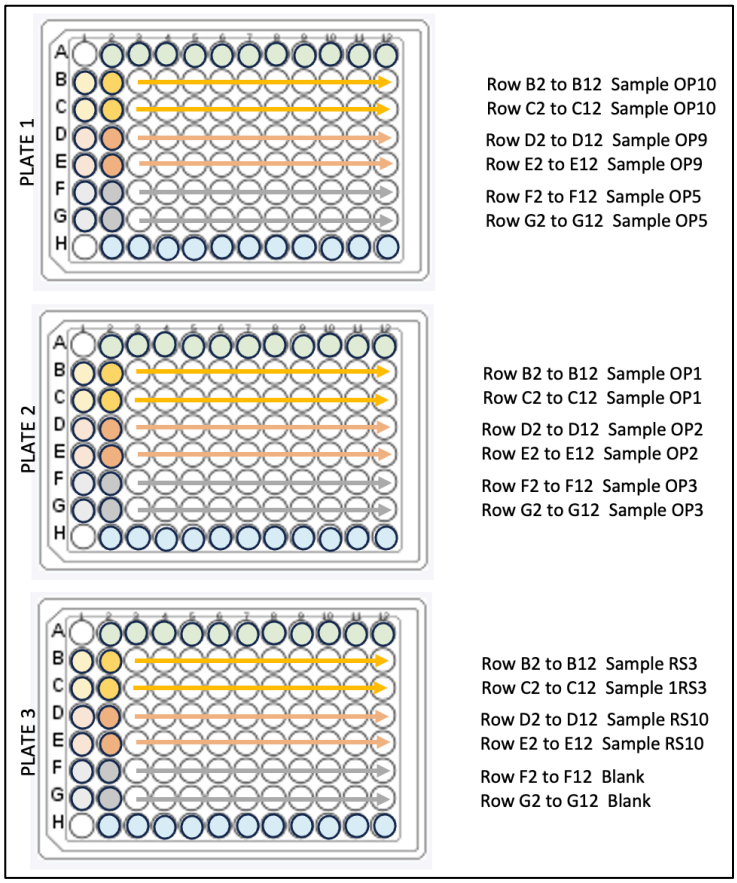


Figure 54: 96-well plate layout for plates 1 to 3**.** Layout for plates 1-3 analysing the antimicrobial impact of extracted and purified samples *O. parella* 1,2,3,5,9,10, and *R. siliquosa* 3 and 10

4.7.3 Results

4.7.3.1 Solvent extraction

The extraction yield for the two lichen species collected from the coastal site location was *R. siliquosa* at 2.84% and *O. parella* at 5.45%, *[Appendices: Table 43]*

4.7.3.2 Assessment of antimicrobial activity exhibited by the extracted lichen compounds

Antimicrobial activity in both the *R. siliquosa* and *O. parella* extracts resulted in antibacterial interference, resulting in zones of inhibition of microbial growth, across all three plates. Each plate incorporated a control disc comprising of methanol only, to ascertain the absence of inhibitory effects on microbial growth attributable to methanol, *[Appendices: Figure 76]*. No zones of inhibition were discerned around the control discs on any of the test plates. The diametric measurements of the inhibition zones varied with dimensions ranging from 8 mm observed on the *P. chrysogenum* plates to 11 mm indicated on the *S. aureus* plates. The observed zones of inhibition serve as indicators of the inhibitory effects of compounds derived from each lichen isolate on the targeted microbial species, *[Appendices: Figure 77].*

4.7.3.3 Separating and identifying individual compounds from lichen methanol extracts

The preparatory plates facilitated chromatographic separation for the individual lichen extracts. Assessment of the separation quality on preparatory TLC plates involves gauging the distance and sharpness between the extract fronts as delineated in *Figure 55 and Figure 56*. Limitations inherent in the technique have the potential to obfuscate the demarcation between each constituent within the extract. In the event of such occurrences, similar properties are likely to manifest between adjacent isolates on the preparatory plate during the antimicrobial evaluation. Consequently, the lichen extract may be separated using different polarity solvents prior to isolation on the preparatory plate.

Figure 55: Preparatory TLC plate separating extracts obtained from *O. parella.* Variations in the line-front are common, reflecting imperfections in the silica layer varying resistance to the eluent as it rises upwards, depositing component compounds

Figure 56: Preparatory TLC plate separating extracts obtained from *R. siliquosa*. Variations in the line-front is common, reflecting imperfections in the silica layer varying resistance to the eluent as it rises upwards, depositing component compounds

In addition to silica variations on the plate, separation variations may also occur depending on the appropriateness of the solvent to each isolate and changes to the temperature within the chromatography chamber.

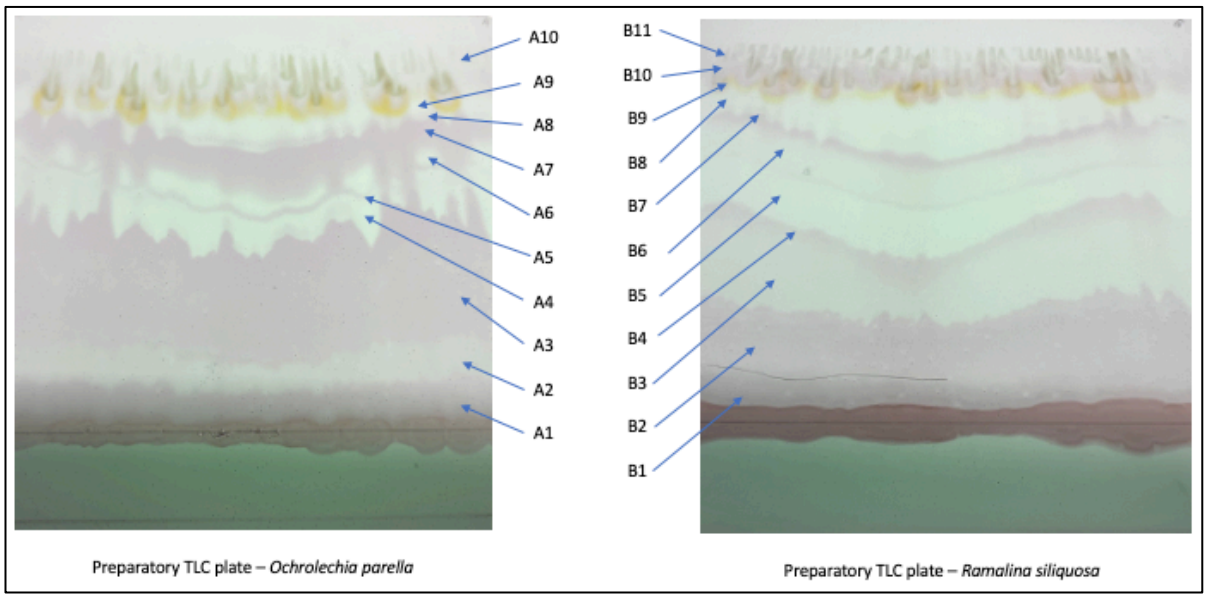


Figure 57: Colour variations denote separation zones on the TLC preparatory plate, discernible when subjected to short-wave ultraviolet light. The distinctive striations of individual compounds are observable and

assigned sample codes on the respective lichen plates. Specifically, codes A_{1-10} correspond to ten discreet isolates originating from the extract of lichen *O. parella*; while codes B_{1-11} pertain to eleven distinct isolates derived from the lichen *R. siliquosa*. The silica area between striations can be extracted from the plate facilitating the isolation of purified compounds isolate from the lichen extract

Compound separation for *O. parella* and *R. siliquosa* yielded distinct striation bands on each chromatography plate facilitating the extraction of individual bands for subsequent analysis. Similar in the retention factor R_f fronts corresponding to A_1/B_1, A_2/B_2, A_4/B_5, B_8/B_9 suggest there may be similarity in secondary products obtained from both lichens, *Figure 55 and 56*. To validate this hypothesis, a recommended approach involves conducting chromatography runs with pure samples of known secondary products under identical conditions and subsequently comparing the R_f values of these reference samples with those obtained from the lichen extracts. This verification process was deferred attributed to the cost and resource commitments associated with the comprehensive identification of the numerous extracted samples. The mass of silica plus secondary product extract obtained from each silica zone from the preparatory TLC plates in *Figure 57* is tabulated and displayed graphically *[Appendices: Table 44]*.

4.7.3.4 Inhibitory effect of purified extracts from O. parella and R. siliquosa on microbial growth

The zone of inhibition delineates a region devoid of microbial proliferation observed proximal to a treated disc on an agar plate, indicating the impact of the test compound on microbial growth. The magnitude of the zone of inhibition is indicative of the susceptibility of the microorganism to the antimicrobial agent *[Appendices: Table 45].*

4.7.3.5 Antimicrobial inhibitory effects by O. parella and R. siliquosa extracts on the growth of S. aureus

Cellulose discs each impregnated with a different extract per disc are positioned onto nutrient agar plates inoculated with *S. aureus* designated plates 33 to 40. Following an overnight incubation period, the presence or absence of zones of inhibition surrounding each disk is delineated as in *Figure 58*.

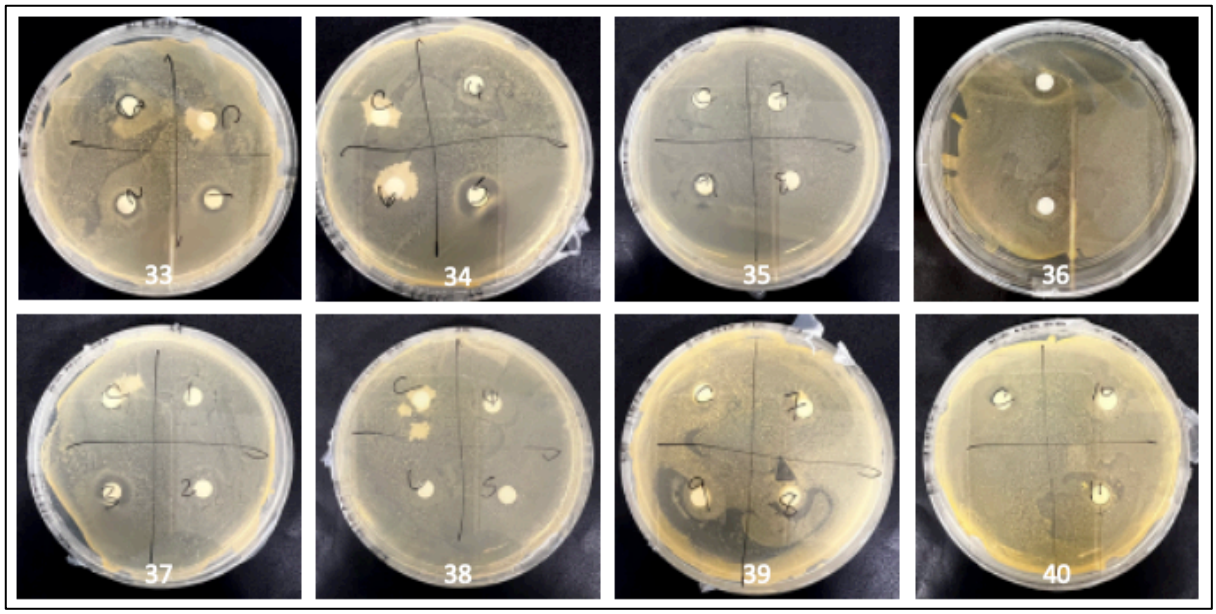


Figure 58: Plates 33 – 36 *(O. parella* secondary metabolite extracts, samples 1-10*: S. aureus* grown on nutrient agar) and Plates 37 – 40 *(R. siliquosa* secondary metabolite extracts, samples 1-11: *S. aureus* grown on nutrient agar). The formation of inhibitory halos indicates antimicrobial activity. Zones of inhibition are observed on Plate 33 (sample 1: 9mm), (sample 2: 8 mm), (sample 3: 8 mm), Plate 34 (sample 5: 13 mm), Plate 35 (sample 9: 11 mm), Plate 36 (sample 10: 11 mm), Plate 37 (sample 3: 12 mm)

4.7.3.6 Antimicrobial inhibitory effects by O. parella and R. siliquosa extracts on the growth of P. chrysogenum

The extract-treated cellulose discs positioned on *P. chrysogenum* inoculated agar plates 1 to 16 are illustrated in *Figure 59*.

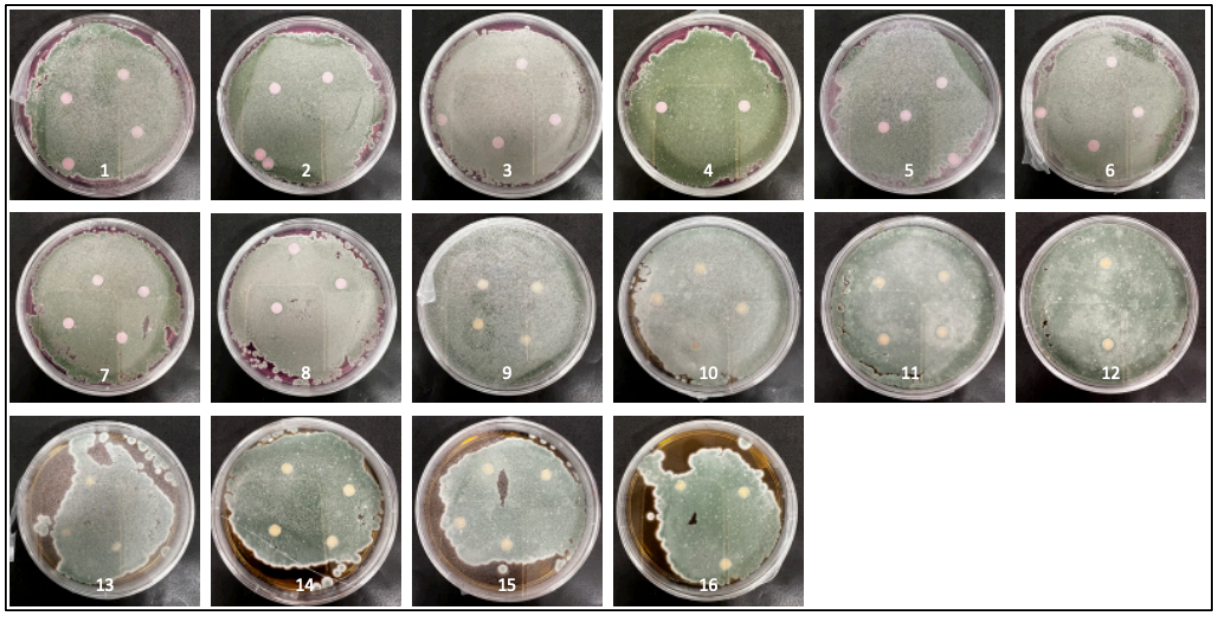


Figure 59: Plates 1 – 4 *(O. parella* secondary metabolite extracts, samples 1-10: *P. chrysogenum* grown on DG18 agar), plates 5 – 8 *(R. siliquosa* secondary metabolite extracts, samples 1 - 11: *P. chrysogenum* grown on DG18 agar), plates 9 – 12 *(O. parella* secondary metabolite extracts, samples 1-10, grown on medium malt extract agar), plates 13 – 16 *(R. siliquosa* secondary metabolite extracts, samples 1-11: *P. chrysogenum* grown on medium malt extract agar). Zones of inhibition are only observed on Plate 8 (sample 10: 11 mm)

The agar disc-diffusion methodology proves unsuitable for ascertaining the minimum inhibitory concentration (MIC) due to the inherent limitation of accurately quantifying the diffusion of the antimicrobial agent within the encompassing agar medium. Nonetheless, the disc-diffusion approach offers distinct advantages in terms of its simplicity, cost-effectiveness, and utility in assessing the efficacy of various microorganism-antimicrobial agent combinations. Furthermore, it furnishes a straightforward framework for the interpretation of successful outcomes. A summary of the secondary metabolite extracts on cellulose discs recording any antimicrobial action resulting in inhibition zones around the discs are presented in *Table 19*.

Table 19: Summary observations and measurements of inhibition zones resulting from sample lichen extracts from *O. parella* and *R. siliquosa*. The recorded samples successfully demonstrated inhibitory effects on *S. aureus* and *P. chrysogenum* growth

Lichen secondary metabolite samples	***S. aureus***	***P. chrysogenum***
O. parella		
Sample 1	9 mm	-
Sample 2	8 mm	-
Sample 3	8 mm	-
Sample 5	13 mm	-
Sample 9	10 mm	-
Sample 10	11 mm	-
R. siliquosa		
Sample 3	12 mm	-
Sample 10	-	11 mm

4.7.3.7 Microtiter Results based on Turbidity

The analytical framework assessing the antimicrobial impact of isolated secondary metabolites on microbial growth provides a systematic approach interpreting microtiter results derived from serial dilutions of antimicrobial agents. The parameter determining minimum inhibitory concentration (MIC) values indicates the lowest concentration of an antimicrobial agent at which visible microbial growth is inhibited.

The systematic analysis follows a structured approach to presenting the microtiter results from the serial dilutions. Data is collected from each well in the microtiter place, the concentration of the antimicrobial agent and the corresponding microbial growth or inhibition observed and recorded *[Appendices: Table 46-53]*

Line graphs represent and inspect the relationship between antimicrobial concentration and microbial growth in the form of sample-concentration response curves. The analysis seeks to identify trends and patterns in the data that may reveal dose-dependent inhibitory effects.

Graphs based on the optical density readings for decreasing concentrations of respective secondary products are presented in *Figures 60* through to *Figure 67,* below. Absorbance values are displayed as optical density (OD), measured at OD 630 for all samples. The turbidity measurement of microbial cultures is widely used to determine the cell number of growing microorganisms in a culture. OD ranges vary between 580 and 650 depending on the optical set up on the photometer by the manufacturer. This fact OD values vary depending on the instrument is relevant restricting comparison of results to reference or literature values.

Serial dilutions begin from an extract concentration of 100 µg ml^{-1} and diluted 10-fold between dilution 1 (100 µg ml^{-1}) through to sample dilution 11 (1.00E-08 µg ml^{-1})

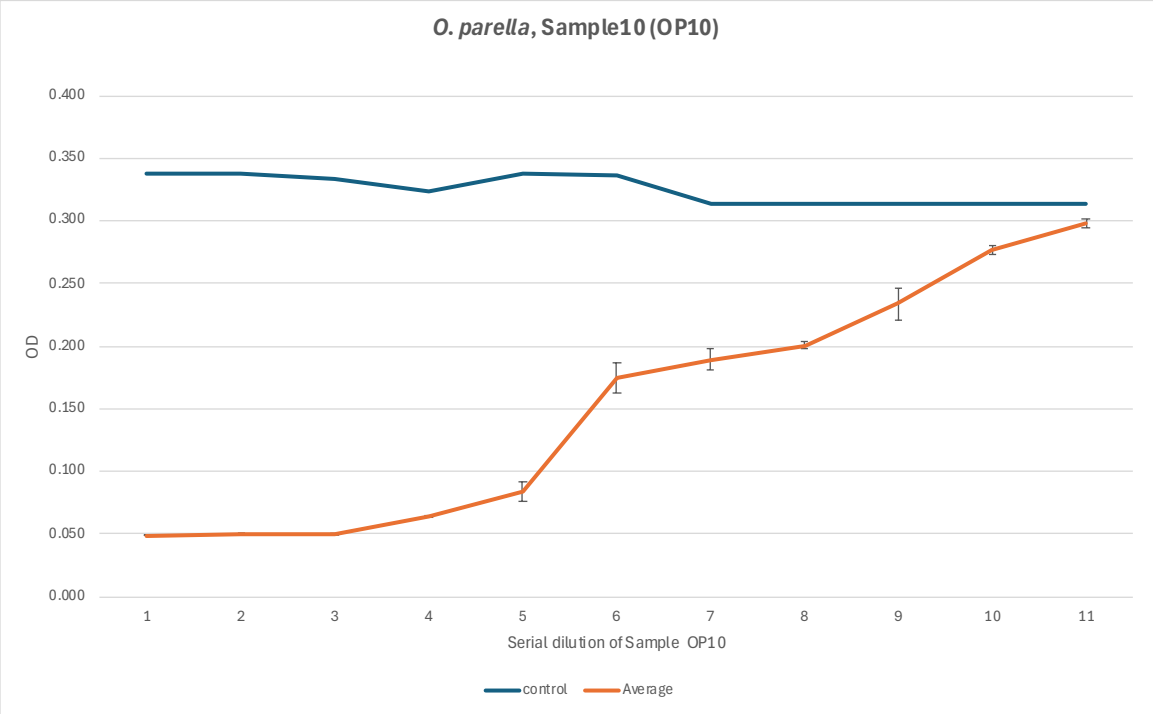


Figure 60: Graph plotting the antimicrobial effect of sample 10, extracted from *O. parella. S. aureus* bacterial growth is inhibited at concentrations in wells 1,2,3. The inhibitory effect decreases as the extract is diluted between cells 3 and 11. Data is based on turbidity measurements at OD 630 [Appendices: Table 46]

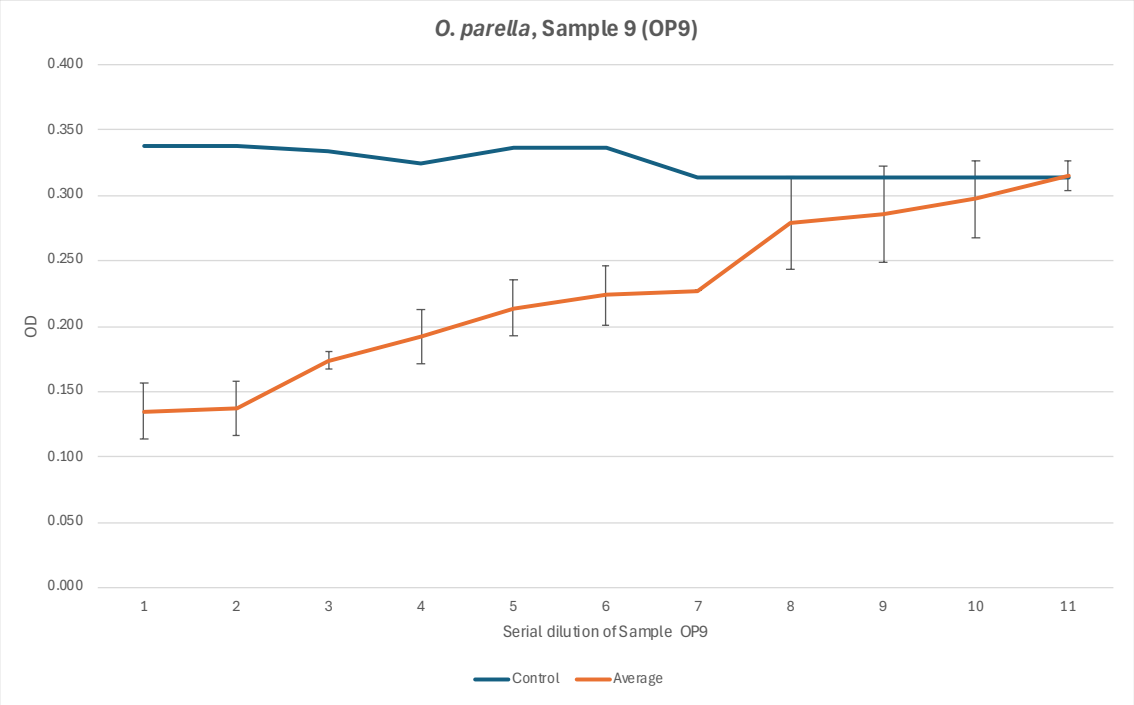


Figure 61: Graph plotting the antimicrobial effect of sample 9, extracted from *O. parella*. *S. aureus* bacterial growth is inhibited at concentrations in wells 1 and 2. The inhibitory effect decreases as the extract is diluted between cells 2 and 11. Data is based on turbidity measurements at OD 630 [Appendices: Table 47]

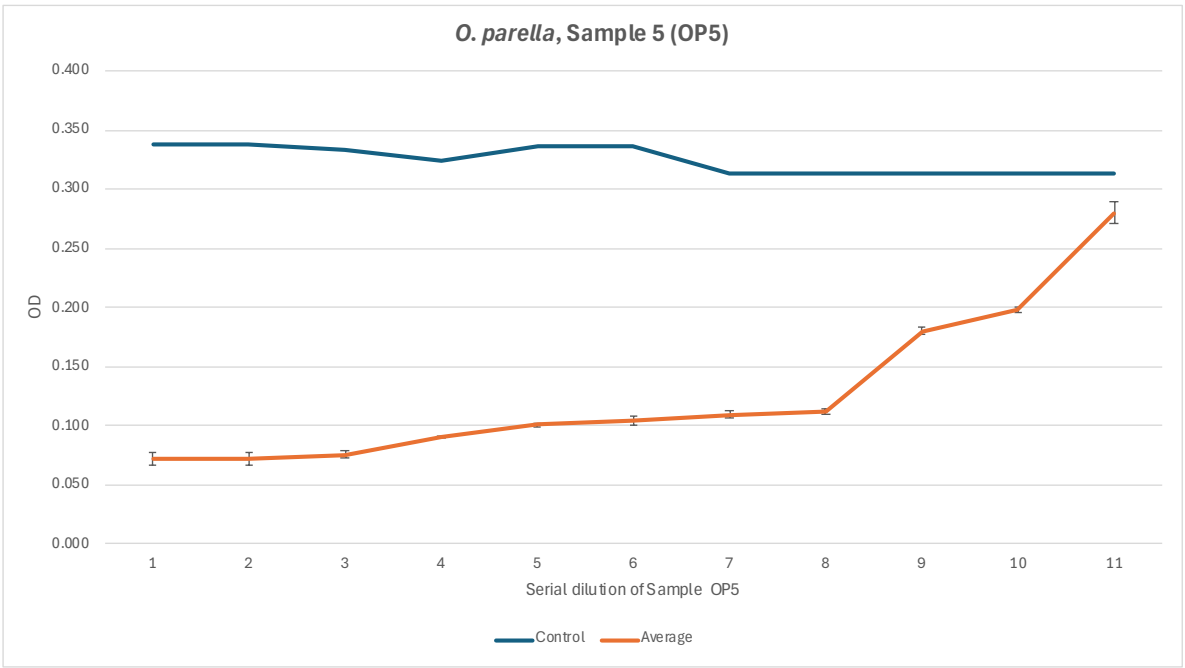


Figure 62: Graph plotting the antimicrobial effect of sample 5, extracted from *O. parella*. *S. aureus* bacterial growth is inhibited at concentrations in wells 1, 2 and 3. The inhibitory effect of the sample on bacterial growth is most pronounced between cells 8 and 11. Data is based on turbidity measurements at OD 630 [Appendices: Table 48]

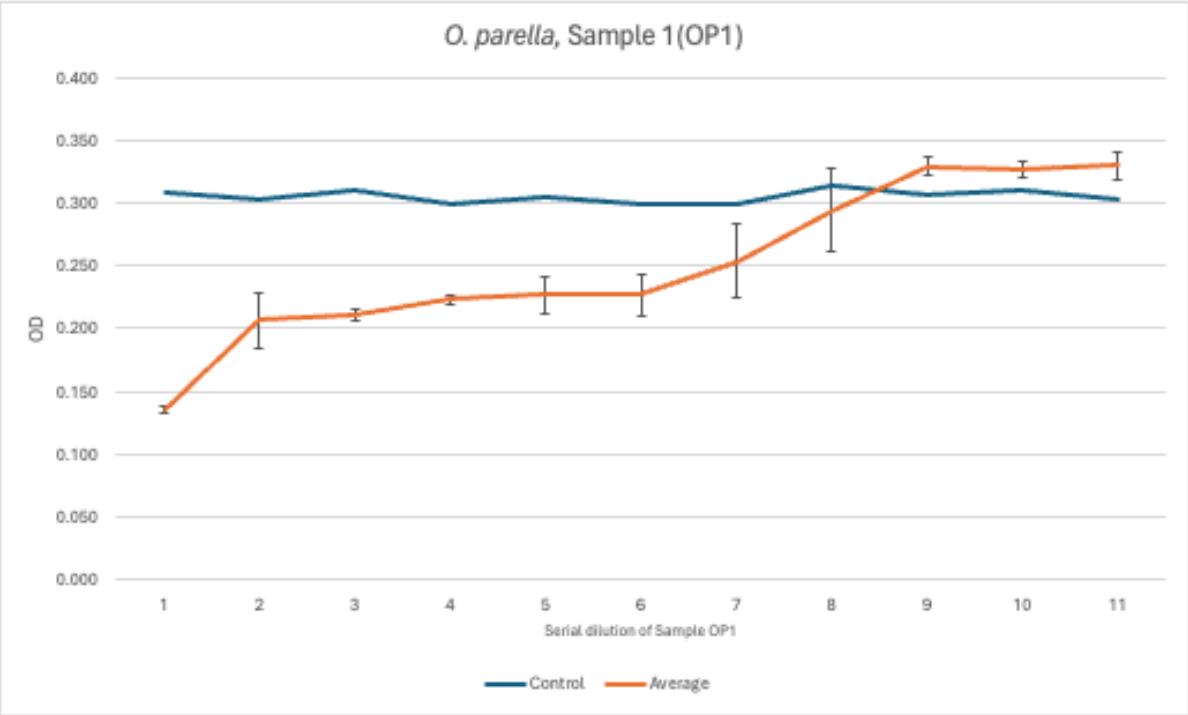


Figure 63: Graph plotting the antimicrobial effect of sample 1, extracted from *O. parella*. *S. aureus* bacterial growth is inhibited at well 1 concentration. A strong inhibitory effect of the sample on bacterial growth continues through to cell 8. There appears slight microbial growth stimulation in wells 9 to 11. Data is based on turbidity measurements at OD 630 [Appendices: Table 49]

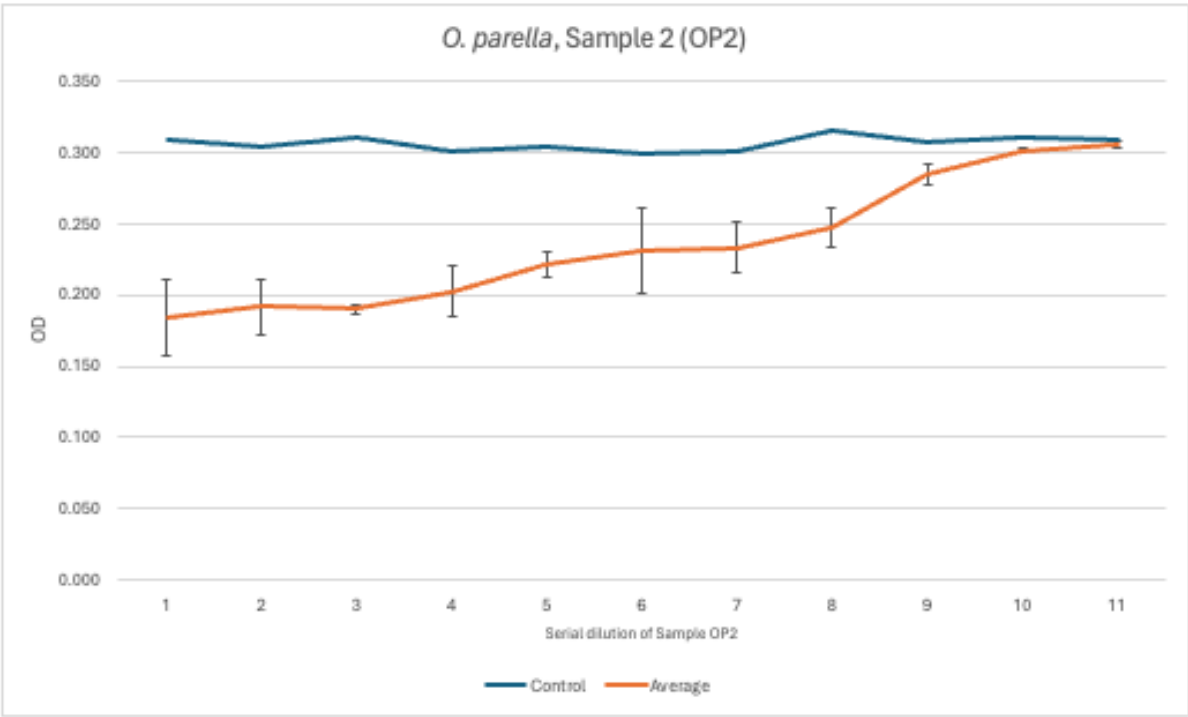


Figure 64: Graph plotting the antimicrobial effect of sample 2, extracted from *O. parella*. *S. aureus* bacterial growth is inhibited from well 1. The effect gradually reduces between well 1 and well 11. Data is based on turbidity measurements at OD 630 *[Appendices: Table 50]*

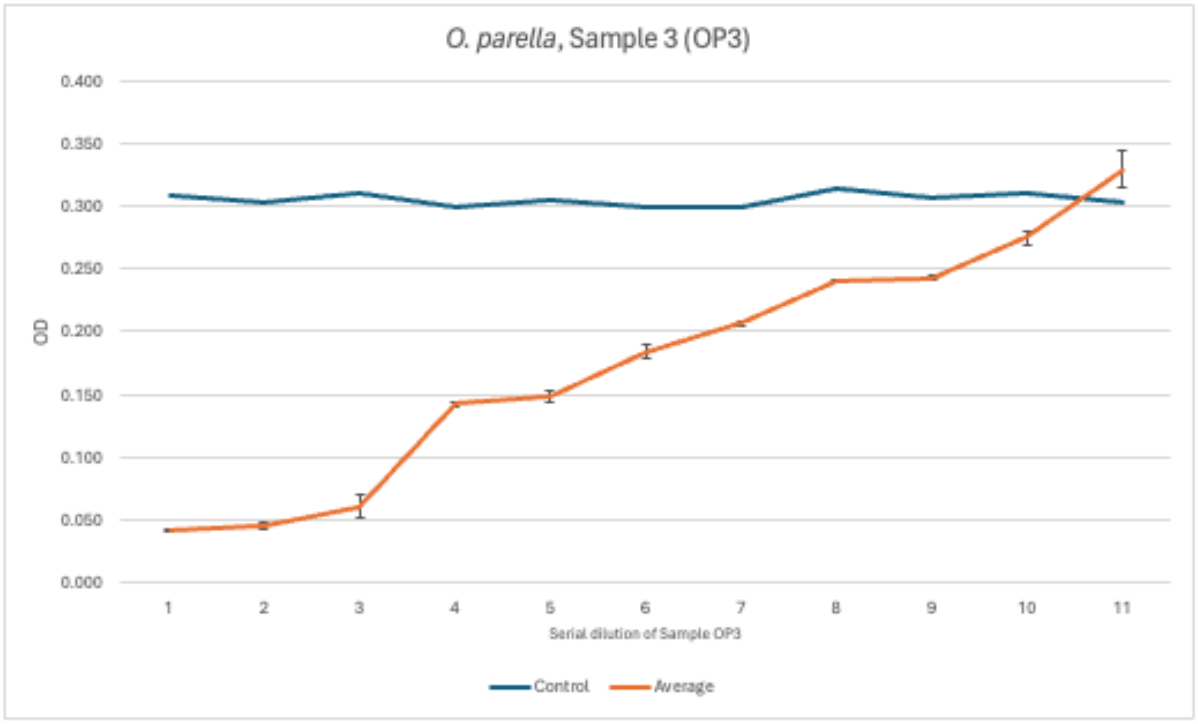


Figure 65: Graph plotting the antimicrobial effect of sample 3, extracted from *O. parella*. *S. aureus* bacterial growth is inhibited from well 1 and 2. The effect gradually reduces between well 3 and well 10. Growth exceeds the control in well 11. Data is based on turbidity measurements at OD 630 [Appendices: Table 51]

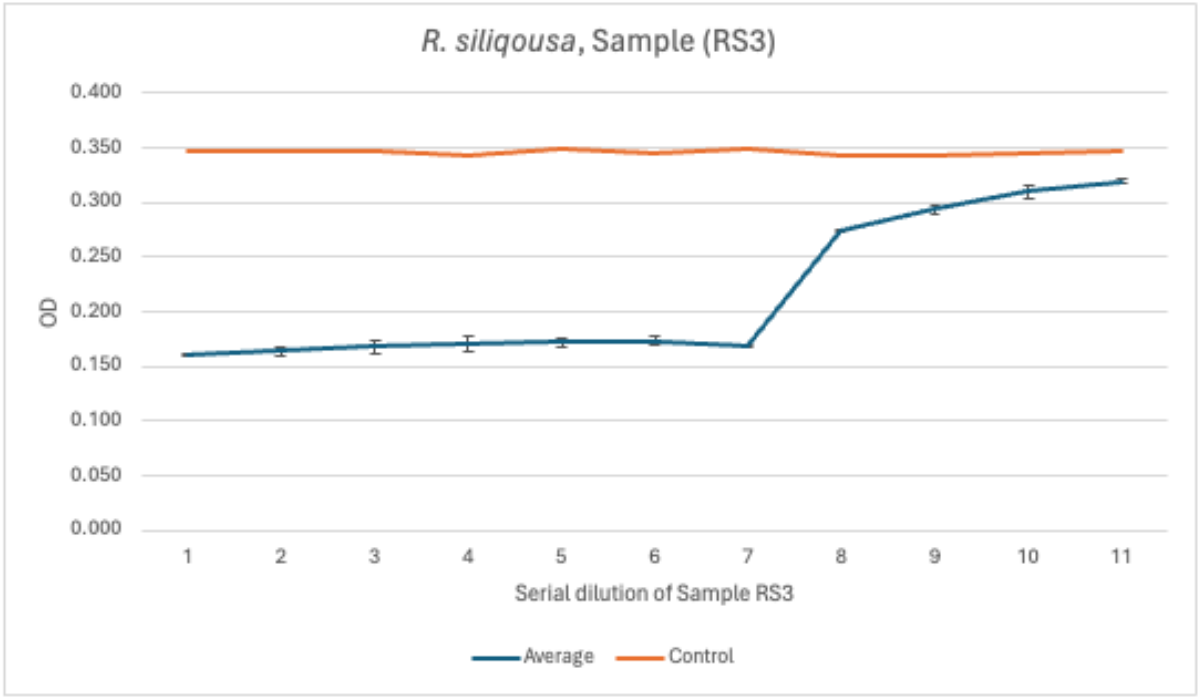


Figure 66: Graph plotting the antimicrobial effect of sample RS3, extracted from *R. siliquosa*. *S. aureus* bacterial growth is inhibited between wells 1 to 7. The effect gradually reduces between well 7 and well 11. Data is based on turbidity measurements at OD 630 [Appendices: Table 52]

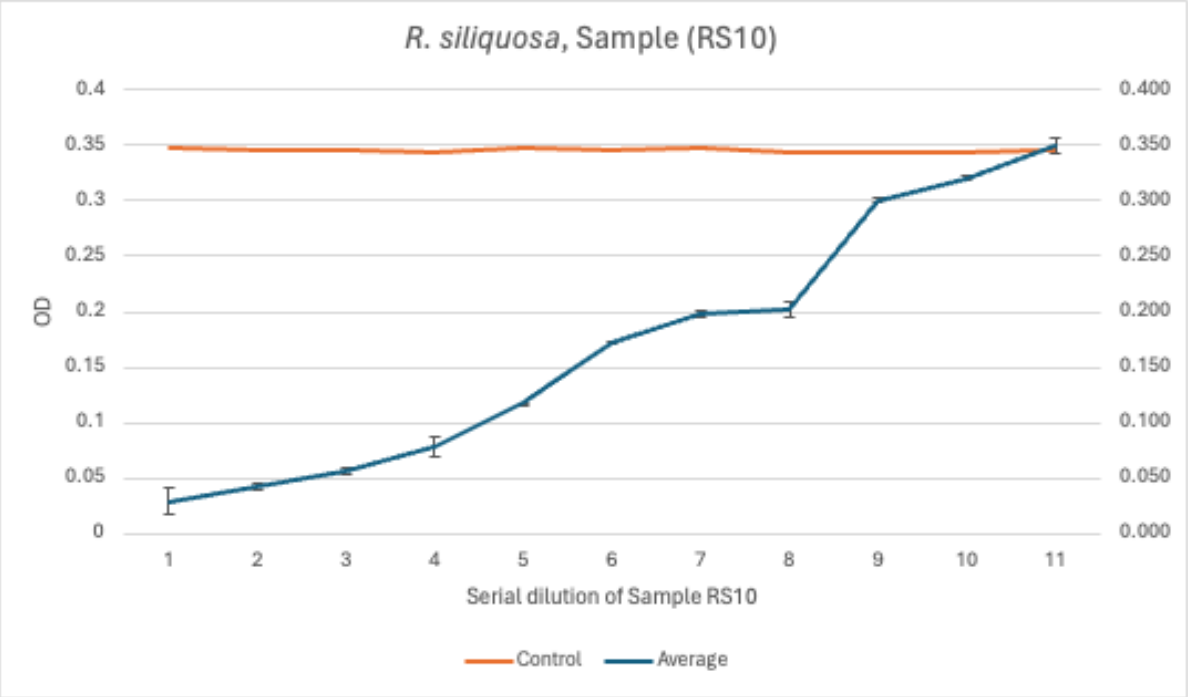


Figure 67: Graph plotting the antimicrobial effect of sample RS10, extracted from *R. siliquosa*. *P. chrysogenum* fungal growth is inhibited between wells 1 to 10. Data is based on turbidity measurements at OD 630 *[Appendices: Table 53]*

Standard error bars displayed on *graphs 60 to 67* are indicators as to the degree of dispersion exhibited by the data around the mean value. Low spread implies the data clusters around the mean, a more extensive spread signifies greater variability from the mean. An additional determinant of the standard error spread pertains to the reliability of the mean value in accurately representing the dataset. A reduced standard error spread corresponds to heightened data reliability, although it is imperative to note that larger error bars do not inherently invalidate the dataset.

In instances where the standard deviation error bars exhibit escalating degrees of overlap, it may suggest that the observed differences lack statistical significance. Such an inference necessitates confirmation through rigorous statistical testing. Conversely, non-overlapping standard deviation error bars indicate a potential significance in differences, though this observation does not conclusively establish statistical significance. In the present experimental context, the constrained sample size poses challenges in conducting a statistically robust analysis, rendering it arduous to draw definitive conclusions.

To enhance the inferential precision of the analysis, a more extensive sample size ('n') is desirable, as it would yield narrower error bars and more accurate estimations of true population values. For each error bar in the aforementioned graphs, 'n' is limited to 2, underscoring the experimental constraints. The discernible trends in the graphs, coupled with the observed error bar overlaps, suggest a discernible pattern of action for each antimicrobial agent, though with larger sample sizes the degree of confidence, determined by establishing probability values, will affirm statistical significance.

4.7.4 Analysis and Discussion

The identification of antimicrobial properties in natural sources, exemplified by the exploration of tree bark extracts in Peru, has paved the way for the development of biocidal solutions with enhanced sustainability. While challenges regarding long-term efficacy, potential degradation, and higher initial production costs remain, the inherent specificity and environmental compatibility of natural antimicrobials position them as promising candidates for sustainable biocontrol strategies.

Supporting the initial observations of inhibition zones using disc diffusion assays, six extracts derived from *O. parella* demonstrably exhibited antimicrobial activity against *S. aureus*. Initial concerns regarding the potential identity of secondary metabolites in extracts OP1, OP2, and OP3, arising from their adjacency on the preparative TLC plate, were refuted by the distinct antimicrobial profiles displayed by each extract at equivalent strengths. Minimum inhibitory concentrations (MICs) for all extracts ranged between 1 and 100 µg ml^{-1}, *Figure 68*. As anticipated, decreasing extract concentrations resulted in diminished antimicrobial effects, transitioning from complete inhibition to decelerated microbial growth without complete eradication, as evidenced by residual growth in wells.

Secondary metabolite effect on growth of *S. aureus*

Disc Diffusion zone of inhibition	*Sample concentration*	µg/ml										
		1.00E+02	1.00E+01	1.00E+00	1.00E-01	1.00E-02	1.00E-03	1.00E-04	1.00E-05	1.00E-06	1.00E-07	1.00E-08
	Sample no. (well)	1	2	3	4	5	6	7	8	9	10	11
	Sample extract											
9 mm	**OP1**											
8mm	**OP2**											
8mm	**OP3**											
13mm	**OP5**											
10mm	**OP9**											
11mm	**OP10**											
12mm	**RS3**											
0	**RS10**											

Secondary metabolite effect on growth of *P.chrysogenum*

Disc Diffusion zone of inhibition	Sample concentration	µg/ml										
		1.00E+02	1.00E+01	1.00E+00	1.00E-01	1.00E-02	1.00E-03	1.00E-04	1.00E-05	1.00E-06	1.00E-07	1.00E-08
	Sample no. (well)	1	2	3	4	5	6	7	8	9	10	11
	Sample extract											
0	**OP1**											
0	**OP2**											
0	**OP3**											
0	**OP5**											
0	**OP9**											
0	**OP10**											
0	**RS3**											
11mm	**RS10**											

Colour key:
Red - MIC, growth appears fully inhibited
Orange - some inhibition of growth but not MIC
Light green - not statistically different to the control minimal to no growth inhibition
Dark green - results indicates very low concentrations of extract may have a stimulatory effect on growth

Figure 68: Summary analysis of the antimicrobial effects of lichen extracts from *O. parella* and *R. siliquosa*. These effects are categorised as MIC, slowed growth, and minimal effects on growth, on two microorganisms, *S. aureus* and *P. chrysogenum*. Extract concentrations are in serial dilution and presented in µg ml^{-1}

While the *O. parella* secondary metabolites exhibited no antimicrobial response against *P. chrysogenum, R. siliquosa* extract RS10 demonstrated a dose-dependent inhibitory effect. At the highest concentration tested (100 µg/ml), RS10 significantly suppressed *P. chrysogenum* growth. However, this effect diminished rapidly with decreasing extract concentration, becoming undetectable below 10^{-5} µg ml^{-1}. These findings suggest that RS10 contains potential antifungal compounds whose activity is concentration-dependent.

This contrasts with the observed activity against *S. aureus*, where only one *R. siliquosa* extract, RS3 displayed any inhibitory effect. Notably, the MIC range for RS3 against *S. aureus* was considerably wider, 100 - 10^{-4} µg ml^{-1}, compared to the MIC for RS10 against *P. chrysogenum* at 100 µg ml^{-1}. This implies that the bioactive compounds within RS3 are potent even at lower concentrations of 10^{-5} µg ml^{-1} making it the most potent antimicrobial in the testing samples against *S. aureus*.

Overall, these results highlight the differential antimicrobial potential of secondary metabolites within *O. parella* and *R. siliquosa*, with distinct efficacies and concentration specificities observed against *S. aureus* and *P. chrysogenum*.

The results suggest several points about the distinctness and activity of the lichen extracts. Despite their proximity on the preparatory TLC plate, extracts OP1, OP2, and OP3 displayed differential antimicrobial profiles, indicating unique chemical compositions rather than contamination between layers. Similarly, extracts OP9 and OP10 exhibited distinct activity patterns. Notably, extract RS10 demonstrated antimicrobial effects against the mould *P. chrysogenum*, while *O. parella* lacked such activity.

However, the application of secondary metabolite antimicrobials necessitates careful consideration of potential adverse effects on building occupants. Human bodies harbour diverse ecosystems of beneficial microorganisms, known as microbiomes, that play crucial roles in host defence, including the gastrointestinal microbiome. Consequently, risk assessment is paramount before employing antimicrobials on building interior surfaces. Broad-spectrum or targeted antimicrobials can disrupt these microbiomes, potentially leading to serious physiological consequences. Nevertheless, the extracts derived from non-toxic lichens found on and around buildings minimises risks at low concentrations. Nonetheless, compliance with health and safety protocols requires thorough toxicological screening of any secondary metabolites to assess their potential impacts on human occupants.

When assessing health concerns, determining the minimal effective dose in product development, relies on establishing the minimum inhibitory concentration (MIC) of the antimicrobials. While various methods exist, this study employs the standardised dilution technique to quantify in vitro antimicrobial activity. This method measures the lowest concentration inhibiting microbial growth, as defined by visual turbidity measurements in µg ml-1. EUCAST guidelines are adopted to ensure uniform result evaluation.

The discovery of antimicrobial properties within natural sources on which this study is based has been foundational for the development of modern biocides, antioxidants and diverse pharmacological applications. While challenges such as long-term efficacy, potential degradation, and higher initial production costs need to be addressed, the specificity and environmental compatibility of natural antimicrobials make them promising candidates for sustainable biocontrol.

Lichens, with their remarkable adaptability to diverse environments marked by varied pH, temperature, ultraviolet light, and water saturation cycles, offer a compelling source of such products. While the metabolic processes underpinning this robustness remain elusive, secondary metabolites play a key role in conferring environmental tolerance. This suggests flexibility in lichen species and provides an insight that relates to them activating different gene clusters depending on the environment.

One of the two lichen investigated, *O. parella*, exhibits environmentally dependent production of secondary metabolites, particularly with respect to light intensity. This necessitates further investigation to optimise harvesting periods and understand the environmental impacts on both the availability and potency of these valuable compounds throughout the year.

This research argues against the indiscriminate use of concentrated, broad-spectrum synthetic chemical treatments. Instead, it proposes identifying synergistic combinations of compatible secondary metabolites capable of targeting a wider range of organisms. This approach offers the

potential for effective antimicrobial activity at lower doses, thereby mitigating environmental contamination risks.

Determining the minimal effective dose relies on establishing the minimum inhibitory concentration (MIC) of the antimicrobials. While various methods exist, this study employs the standardised dilution technique to quantify in vitro antimicrobial activity. This method measures the lowest concentration inhibiting microbial growth, as defined by visual turbidity measurements in µg ml-1. EUCAST guidelines are adopted to ensure uniform result evaluation.

By focusing on MIC levels of natural antimicrobials and targeting specific interactions, these results based on *O. parella* and *R. siliquosa* extracts, pave the way for a more sustainable approach to biocontrol, minimizing environmental impact and potentially enhancing efficacy through synergistic combinations.

In the evaluation of the efficacy of secondary metabolites for use in conservation, comparative assessments against synthetic alternatives establish important benchmarks. Natural agents may degrade faster due to their organic nature, requiring more frequent application, and their initial extraction and purification can be expensive, increasing conservation costs. However, long-term benefits are likely to emerge. The abundant supply of organic precursors combined with potentially lower research and development costs for natural products compared to complex, and potentially environmentally toxic, synthetic alternatives can ultimately prove advantageous.

Another advantage is how advances in genetic sequencing have fuelled a renewed interest in secondary metabolites. Identifying gene clusters responsible for their biosynthesis is a necessary step to commercialisation. Though slow lichen metabolism poses challenges, gene editing techniques and computational *in silico* approaches offer tools to accelerate unlocking their potential. By harnessing the power of genetic technology and a nuanced understanding of microbial resistance, full potential of these nature-derived solutions can be unlocked for a sustainable and healthy built environment.

Operationalising this strategy for application in the construction industry demands a deep understanding of antimicrobial mechanisms to address biodeterioration and its interaction with climate change. This knowledge can pave the way for constructing natural product combinations that not only enhance effectiveness but also impede microbial adaptation and resistance development. This is particularly effective in construction when dealing with a range of materials and biodeterioration accelerated by external factors such as more frequent extreme weather events.

Antimicrobial efficacy hinges on interference with key cellular processes, including cell wall synthesis, membrane integrity, nucleic acid synthesis, ribosomal function, and folate pathways. Dissecting these mechanisms has been integral to designing effective antimicrobial combinations and mitigating microbial adaptation and resistance.

Cell wall composition dictates susceptibility - bacteria rely on peptidoglycan, while fungi possess chitin-reinforced walls and ergosterol-rich membranes. This disparity translates to contrasting resistance profiles, providing some insight into the contrasting antimicrobial results of secondary metabolites from *R. siliquosa* and *O. parella* demonstrated in this study. Bacterial agents generally target peptidoglycan assemblies, whereas antifungal drugs disrupt ergosterol synthesis, leading to impaired membrane fluidity and integrity.

Delving deeper into resistance, bacterial mechanisms are better understood, involving plasmid and phage-mediated genetic exchange. Fungal mechanisms, however, remain shrouded in obscurity due to the lack of standardized DNA transfer assessment methods.

Among bacterial targets, the cell wall stands out. Antimicrobials can hamper cell wall synthesis, disrupt ion pumps and efflux mechanisms, or destabilise peptidoglycan, ultimately rendering the cell vulnerable to osmotic lysis.

Ribosomes and their associated protein synthesis machinery are another crucial battleground. Antibiotics can bind ribosomal RNA, disrupt messenger RNA reading frames, or inhibit ergosterol-synthesizing cytochrome P_{450} enzymes in fungi, effectively halting protein production.

Nucleic acid metabolism offers another avenue for intervention. Antimicrobial penetration through the cell wall allows targeting of enzymes critical for DNA replication and repair, causing deoxyribose strand breaks that stall replication and transcription.

Bacteria possess additional vulnerabilities in the form of growth-specific metabolic pathways. Targeting enzymes involved in folate conversion, nucleotide biosynthesis, or chemical signalling pathways can stall bacterial growth. Identifying the specific mechanisms affected by individual secondary metabolites is crucial when developing synthetic improvements from naturally sourced lead compounds for crafting effective antimicrobial combinations.

In conclusion, a nuanced understanding of antimicrobial mechanisms, particularly their interplay with diverse cell wall structures, and their influence on resistance development is key to crafting effective antimicrobial regimens. By harnessing the power of genetic technology and a nuanced understanding of microbial resistance, full potential of these nature-derived solutions can be unlocked for a sustainable and healthy built environment based on a vast range of microbial secondary metabolites.

4.7.5 Conclusion

The study tackles the research questions through a novel microbial biosynthesis-based bioremediation strategy aimed at alleviating climate-exacerbated biodeterioration in built heritage, offering a potential solution for preserving cultural assets. By focusing on two coastal lichen species, *Ochrolechia parella* and *Ramalina siliquosa*, exposed to increased extreme weather events, the study explores their potential as sources of antimicrobial secondary metabolites for combatting biodeteriorative microbes.

Through solvent extraction and thin-layer chromatography, eight secondary metabolites exhibiting antimicrobial activity were identified from a total of 21 extracts. Notably, six extracts from *O. parella* and one from *R. siliquosa* inhibited the growth of *Staphylococcus aureus*, while only one extract from *R. siliquosa* displayed activity against *Penicillium chrysogenum*. The remaining 13 extracts, despite lacking visible inhibition against the tested microbes, may possess bioreactivity towards unassessed bacteria or moulds.

These findings suggest that both lichen species harbour bioactive secondary metabolites with potential for application in treating biodeterioration on damp historic building surfaces. Further research is warranted to identify the specific bioactive compounds using techniques like comparative R_f TLC, mass spectrometry, and nuclear magnetic resonance. Characterisation of their chemical

structures and functional groups will pave the way for synthesising synthetic analogues, facilitating the commercialisation of bioremediation strategies for protecting heritage structures from climate-induced biodeterioration.

The investigation into lichen-derived antimicrobial metabolites extends beyond the realm of built heritage preservation. These environmentally sustainable agents hold promise for broader applications in environmental pharmacology, aligning with the growing emphasis on sustainable natural products. Additionally, the study underscores the importance of investigating the interplay between environmental stressors and biopositive secondary metabolites in lichens, potentially leading to the discovery of novel therapeutic agents for various diseases.

4.8 Chapter Summary

This chapter delves into the intersection between climate change, the biodeterioration of traditional materials by microbes, and the realm of secondary metabolites. It unpacks the multi-faceted dilemma of climate-driven material decay, showcasing the intriguing potential of microbial-derived compounds as a sustainable solution for safeguarding built heritage.

The chapter contextualises by exploring how climatic shifts, primarily rising temperatures, humidity, and precipitation, orchestrate the biodeterioration of traditional materials by microorganisms, particularly moulds and bacteria. It investigates how these environmental fluctuations influence the growth and activity of biodeteriorating microorganisms, focusing on their impact on traditional materials.

Environmental fluctuations, including those induced by climate change influence the production of secondary metabolites by microorganisms. These biosynthesised compounds are then brought potential to protect biomaterials or influence microbial competition is explored.

Shifting focus to the vulnerability of traditional materials in buildings, the chapter dives deep into the specific challenges of biodeterioration in this context. The types of microorganisms involved, the mechanisms of decay, and the environmental factors that exacerbate the problem are examined.

Lichen secondary metabolites, emerges as potential antimicrobial treatments for material deterioration. The chapter explores how these defensive compounds can inhibit biofilm formation and microbial growth, paving the way for potential applications in safeguarding materials.

Finally, the chapter delves into the practicalities of assessing the antimicrobial efficacy of secondary metabolites extracted from specific lichen species, such as *Ochrolechia parella* and *Ramalina siliquosa*. Their effectiveness against pathogenic organisms like *Penicillium chrysogenum* and *Staphylococcus aureus* is studied in detail.

CHAPTER FIVE: Bioenhancement and built heritage conservation: Future direction for the research

Chapter Abstract

The concluding chapters of this study present a critical review of two key themes: the opportunity to create innovative bioprotective solutions, and the urgent need to delve deeper into the impact of climate-driven biodeterioration on built heritage. This review is undertaken with careful consideration of the implications arising from the research findings presented in Chapters Three and Four.

The well-documented changes in global temperature, the increasing frequency and intensity of extreme weather events, and the ongoing research detailing the effects on glaciers and global ice reservoirs all point towards a stark reality: these phenomena will undoubtedly have an existential impact on every facet of daily life. Simply continuing to apply and replicate past strategies for built heritage conservation may prove insufficient in the face of the significant climate-related challenges that buildings will be forced to endure.

This presents a unique challenge and, simultaneously, an exciting opportunity. The development of new and adaptable technologies lies at the heart of this endeavour. These technologies must seamlessly weave together the reliability of past practices with cutting-edge material science and innovative manufacturing techniques. By doing so, researchers can build a broader and more robust portfolio of solutions for protecting our built heritage.

Informed by the stringent requirements of heritage conservation science, this chapter outlines a framework for the future direction and potential avenues for further research building upon the foundations laid in chapters three and four. This framework not only seeks to safeguard cultural treasures but also explores opportunities to unlock economic and environmental value across a wide range of industry applications.

5.0 Bioenhancement, built heritage and climate change

The intersection of climate change and the bioconservation of built heritage is a complex and crucial issue, which this study has taken a crucial and multifaceted approach, identifying gaps in existing knowledge while researching bioprotective options to address several of the challenges.

Based on the loss and potential threat by climate change impacting numerous built heritage sites, the consequence of inaction erodes the context and meaning of these sites, disrupting intergenerational transmission of traditional knowledge and practices, and erasing valuable knowledge systems embedded in these sites. Without singular focus, mitigation and adaptation measures by local authorities and heritage associations can divert resources and attention away from heritage preservation, especially when economic hardships threaten maintenance budgets and building upkeep. The speed of deterioration of heritage resources due to the increased frequency in extreme weather events will lead to neglect and further vulnerability of valuable cultural assets.

Conversely, a focus on built heritage can become a tool for climate action. As discussed, traditional building techniques often ground and embody sustainable principles. Studying and incorporating these practices, such as lime exterior surfaces and sacrificial limewash coatings, into novel bio-driven preservation research can promote climate resilience.

During national and local economic hardship with multiple demands for basic services on departmental budgets, opportunities to engender community engagement utilising heritage as a tool for climate action are imperative. Heritage sites can serve as focal points for climate education and local engagement, encouraging traditional knowledge sharing and introducing new technological initiatives such as bioprotective surfaces and the use of natural antimicrobial agents with both occupant health and building resilient benefits, raising awareness, and inspiring communities to engage. Retaining and extending the use of heritage and pre-1919 buildings improves the prioritisation and allocation of resources in the face of limited funding.

Building sufficient numbers of quality dwellings remains a constant challenge for local and national authorities. The development of adaptation strategies enabling historical and pre-1919 structures to be retrofitted for energy efficiency and resilience demonstrate practical options for adaptation in existing settlements. The repurposing of older buildings mitigates the arguments for the need to develop new construction on green sites adjacent to high density locations and the cost of reforming brown industrial sites safe for residential buildings. Older buildings constructed with brick and stone are effective heat sinks and provide a solid surface for bioenhanced lime finishes and natural antimicrobial treatments on the building surfaces.

Remote coastal and island locations are particularly and unequally vulnerable, the impact of climate change is unevenly distributed. Indigenous communities such as found in Islands on the North and West of Scotland and other geographically vulnerable regions face disproportionate risk. In addition to enhancing community engagement, existing legal frameworks and conservation practices may not be adequate to address the complex threats posed by climate change. Indeed, this inadequacy may extend to opposition to novel technological adaptations of traditional conservation practices. Integrating climate resilience into heritage policies and local authority planning practices requires urgent attention and innovative approaches balancing the vulnerability of climate challenges with the potential of built heritage.

While the growing research on the impact of climate change on heritage tourism, more often a crucial financial contribution to the local economy, offers valuable insights, critical gaps remain. Owing to the diverse and geographic spread of historic structures, many of the existing studies, as

with this thesis, focuses on individual case studies, often highlighting dramatic threats like sea level rise or extreme weather events. This can be valuable for raising awareness but neglects broader systemic forces. A more holistic approach is needed, examining the interplay between climate change, global tourism trends, and local socio-economic dynamics. Further studies that move beyond singular site analyses are needed to explore how shifting climate patterns influence tourist flows, reshape destination branding, and exacerbate pre-existing vulnerabilities in tourism-dependent communities. Additionally, the field often overlooks the agency of local stakeholders. Local communities and cultural custodians possess rich knowledge of adapting to environmental change and should be recognised as active participants in shaping resilient heritage tourism futures. Filling these critical gaps with interdisciplinary research provides exciting opportunities for further research that can pave the way for sustainable and equitable heritage tourism in the face of a changing climate.

An outcome from this study is the identification of knowledge gaps and the limited supporting research on the specific impacts of climate change on different types of heritage. More data and scientific understanding are crucial for effective adaptation and mitigation strategies.

As the planet warms, rainfall intensifies and precipitation patterns alter, amplifying the biodegradation of traditional building materials. These changing conditions create optimal conditions for diverse microbial communities, accelerating their growth and degradation potential. As buildings become more frequently exposed to rainfall events and rising sea levels, the building materials, exposed to prolonged moisture, facilitate microbial access and ultimately the structural integrity fails. Altered climatic conditions are likely to lead to the emergence of new microbial species capable of degrading materials previously considered resistant, requiring new antimicrobial tools to address this threat from shifting biotic communities.

Wood is particularly vulnerable to microbial attack, increasing the decay rate, which is of particular concern in timber-framed buildings and historical structures. Though more durable, masonry is also susceptible to biodegration, a process enhanced by moisture and temperature fluctuations resulting in acidification, ion chelation, and mineral sequestration. These changes in the stone surface material leads to compromised structural integrity and loss of cohesion.

The challenges and considerations facing biodeterioration of built heritage relate to the limited long-term data on the combined effects of climate change and biodegradation. This makes it difficult to accurately predict traditional material lifespan under future climate scenarios. The proposal to bioenhance limewash by introducing biopolymer encapsulated biomineralising microorganisms is a new building practice requiring building conservation practices to accept material adaptation. However, the long-term sustainability of construction materials increasingly requires consideration of the biodegradability cycle of the proposed material. Bioenhanced limewash against this criterion is a viable strategy, composed of traditional materials, natural biodegradable polymers and bacteria commonly found in soil and lakes.

In the event temperature rises exceed 1.5°C, further points arise for consideration utilising biomaterials and natural antimicrobials in the construction industry. The viability of microorganisms such cyanobacteria and bacteria *S. elongatus* and *B. sphaericus* or lichens such as *O. parella* and *R. siliquosa* over long periods of extreme temperatures is unknown. Similarly, the role of temperature and moisture variations on these organisms in specific geographic regions.

The concept of bioenhancement for at-risk traditional building materials is for the preservation of cultural heritage and promotion of sustainable construction and maintenance practices. The increasing threat of climate change and its impact on traditional materials has spurred the search for

sustainable and resilient alternatives. Microbial biopolymer encapsulation proposed in this research, a bio-based technology, utilises the metabolic capabilities of microorganisms to produce protective coatings, emerging as a promising solution.

There are several opportunities and potential challenges which need to be addressed for the practical development of bioenhancement strategies.

The development of bioenhanced limewash offers an eco-friendly alternative to the application of synthetic water-resistant paints which are often reliant on energy-intensive and polluting processes. Utilising biomineralising bacterial and cyanobacterial resources which are renewable and capable of developing long-term protective surfaces align with green building principles and reduces the environmental impact. Enhancing traditional materials leverages unique properties and building sensitivities, such as moisture management. Bioenhanced materials and techniques can be tailored to specific material compositions, ensuring compatibility with the original performance of the building material while minimising the harmful environmental side effects. Compared to synthetic polymers, naturally sourced biopolymers are usually biodegradable, aligning with circular economy principles. The option to derive biopolymers from microbial synthesis extends the option of tailoring polymer structures which can be manipulated through microbial engineering, offering the potential to design coatings with specific functionalities like UV resistance, fire retardancy and water repellence. The replacement of bio-toxic treatments with microbially sourced antimicrobial agents to minimise harmful moulds and bacteria on building surfaces are likely to be less allergenic to occupants without compromising biocidal effects.

Compared to traditional restoration methods, bioenhancement can offer cost savings due to readily available or cultivated biological materials and potentially less labour-intensive processes. The development of natural biopolymer compounds for use in new conservation technologies are a readily available resource, the availability and lower cost making heritage preservation more accessible for communities with limited resources.

Bioenhancement, as demonstrated with alginate encapsulated limewash can improve the structural properties and longevity of traditional materials enhancing their resistance to weathering, decay, and mechanical stress. This extends the lifespan of historical structures and reduces the need for frequent interventions, lowering the carbon profile associated with material manufacturing, employed labour, equipment usage and repetitive maintenance schedules.

The field of bioenhancement and the development of new biomaterials is still in its infancy, with the potential to evolve offering possibilities for novel functionalities in traditional materials. In addition to bioenhanced surfaces and self-healing properties, the enhancement of thermal insulation through bio-based modifications and bio-photo-electric enhancements to solar powered generation of electricity can add value to historical structures, without impinging on heritage conservation values.

Though bioenhancement offer exciting possibilities for developing novel functionalities, the approach must consider the challenges to be overcome to be accepted in mainstream conservation practice. The long-term performance and durability of bio-enhanced materials remain under investigation. Extensive research and monitoring are needed to ensure the effectiveness of these methods over extended periods.

The incorporation of cyanobacteria into bioenhanced surface coverings, for example, must be monitored to avoid discolouration forming due to the photosynthetic pigments within the cells, and ensure discarded rainwater from the building surface avoids contaminating water supplies, rivers, and lakes. Similarly, the introduction of antimicrobial agents into building materials and biocides

necessitates careful consideration of potential health risks and unique allergenic reactions for occupants and the environment. Thorough safety assessments and regulations are crucial to ensure responsible implementation.

Climate change is driving significant shifts in microbial communities, potentially accelerating biodeterioration processes that damage materials and infrastructure. In this context, the antimicrobial properties, in this study derived from lichens *O. parella* and *R. siliquosa*, offer intriguing possibilities for sustainable bio-based protection. This study explored the extraction and isolation of a range of secondary metabolites from these sources, and critically examined the impact of these metabolites on climate-accelerated biodeterioration by the mould *P. chrysogenum* and pathogenic bacterium, *S. aureus*, considering their efficacy, environmental implications, and future directions.

The outcome from the study has shown promising results isolating a range of secondary metabolites from each lichen, capable of selectively inhibiting the growth and activity of *P. chrysogenum* and *S. aureus*. In this context, both lichens offer intriguing possibilities for sustainable biobased protection. These finding support similar studies in plant-derived terpenes found to inhibit wood-decaying fungi. However, the effectiveness of secondary metabolites varies depending on several factors such as the specificity of the secondary product against specific organisms targeted, and the nature of the material being protected.

Potentially the utilisation of secondary metabolites offers eco-friendly alternative to synthetic biocides, however it is crucial to consider possible interactions and environmental impacts. Some metabolites, if synthesised to give an invasive advantage to the organism could inadvertently introduce an ecological disruption in the surrounding environment.

One advantage of secondary metabolites as bioactive compounds delivering antimicrobial capabilities is the relative low concentrations required to inhibit growth. Commercially, the large-scale cultivation of selected organisms for their metabolite production requires careful planning to ensure sustainable practices and minimal resource depletion. It is highly likely many of the identified natural antimicrobials will become lead compounds for more effective or efficient secondary metabolites as a result of precursor or genetic modifications altering the chemical structure of the metabolite. In both natural and altered state, the environmental implications must be carefully considered. While caution is essential, the opportunity to explore synergistic combinations of different metabolites and developing targeted delivery systems will lead to enhanced efficacy and a reduction of synthetic chemical toxins currently used in material preservation.

Current bioenhancement techniques often lack standardised protocols which are required for the scaling up of production processes and for commercial viability and ease of adoption by the end-user. Integrating biological processes and materials into building restoration is likely to raise concerns among members of the public and heritage conservation professionals. Long-term studies, peer-reviewed empirical data and effective communication and education are crucial steps to address misconceptions and garner wider acceptance for bioenhancement technologies.

Concerns regarding ethical considerations must be considered as the use of biological agents raises concerns regarding potential ecological imbalances, and the unpredictable nature of adaptive processes such as mutagenesis, resulting in unintended consequences. The involvement of peer-reviewed research, sustainable resourcing, controlled test-applications, and long-term monitoring are essential to minimise environmental risks.

Designing bioenhancements for at-risk traditional building materials presents an exciting and promising approach for sustainable heritage preservation, capable of mitigating environmental

changes brought about by climate change. Addressing the challenges related to long-term efficacy, safety, standardisation, public perception, and ethical considerations is crucial for responsible and successful implementation. Through continued research, development, and open dialogue, bioenhancement has the potential to revolutionise heritage conservation and contribute to a more sustainable, climate-durable built environment.

5.1 Future direction for the research

The inescapable evidence for UK climate change has gained relevance within heritage conservation groups. Assessing the performance of historical buildings under such environmental pressures has become crucial for their long-term preservation. Drawing upon the experimental methods presented in the previous chapters, three strategic research directions are proposed that leverage future climate risk assessments to inform broader advancements in the built heritage sector and with wider application across the construction industry.

Innovative strategies are essential to combat physicochemical erosion, address the rising of biodeteriorating microorganisms colonising traditional materials, and prevent structural failure. This necessitates the development of conservation alongside robust technologies and compatible new and hybrid materials. Such technologies such demonstrate not only responsiveness to, but even the ability to anticipate detrimental environmental changes.

Across the UK and Europe, initiatives have been underway documenting the initial stages of the impact of climate change on building deterioration as well as assessing building vulnerability, and exploring adaptation strategies for cultural heritage, (Sabbioni et al., 2008; Sesana et al., 2018). While research analysing these threats on the surfaces of heritage structures is growing, studies proposing solutions, methods and tools remain relatively scarce.

Based on this shortfall, three research strategies are proposed as continuations from the discoveries emerging from the practice elements in this thesis. These strategies, which extend the biopreservation approach with potential for broader application in the construction industry, each target a specific aspect of climate-related degradation in traditional materials.

Bioprospecting, informed by the empirical findings of Chapter 4, seeks to harness secondary metabolites from microorganisms to combat destructive moulds and bacteria. Bioprinting organic inclusions, drawing upon the empirical findings of Chapter 3, aims to enhance material performance by introducing bioorganic elements through novel design technologies. Finally, the development of designer and hybrid biopolymers is proposed as a means to complement existing materials, create new hybrid compounds, and potentially replace failing traditional materials altogether.

This research agenda builds upon the established foundation of biopreservation while pushing the boundaries of its application, potentially leading to significant advancements in the field of sustainable built heritage conservation.

5.2 Bioprospecting secondary metabolite bio-deterrents

The resilience of the built environment hinges on its ability to withstand diverse threats. Fortunately, natural selection offers a potent arsenal in the form of secondary metabolites, organic compounds synthesised by living organisms beyond their core metabolic needs. These multifaceted molecules

empower sustainable and durable construction practices by offering UV protection, antimicrobial prowess, antioxidant shielding, and even biomineralisation facilitation.

One notable challenge faced by building materials is UV-induced photodegradation. Secondary metabolites like flavonoids produced by actinomycetes and polyphenols synthesised by recombinant bacteria can combat this issue. Their inherent UV-absorbing properties act as a shield, preventing surface damage and mitigating long-term sun exposure.

Another pervasive threat comes from microbial colonisers. These organisms unleash a destructive repertoire of toxins and exploit diverse degradation mechanisms. Fortunately, secondary metabolites like terpenoids exhibit potent antimicrobial activity. Their incorporation into building materials like limewash, wood preservatives, and wallpaper adhesives offers a natural defence against fungal decay and algal biofilm formation.

But the benefits of secondary metabolites extend beyond direct antagonism. Certain compounds, such as terpenes, tannins, flavonoids, and saponins, possess exceptional antioxidant properties. Integrating these substances into coatings or as additives to existing building products can not only neutralise free radicals and reduce oxidative damage but also degrade into environmentally harmless compounds over time.

Unveiling the remarkable potential of secondary metabolites in construction requires unlocking the vast natural resource bank. Initiatives like The LOTUS Initiative for Open Natural Products, documented in The Natural Products Atlas, play a crucial role in cataloguing and mapping these valuable tools. Maintained by researchers at Simon Fraser University, this collaborative effort allows for comprehensive exploration of secondary metabolite research, (van Santen et al., 2019). Furthermore, specialised databases focusing on the bioactivity of these compounds within the construction industry can hold economic and environmental benefits, (Singab et al., 2022).

Investing in this untapped field, currently hindered by underfunding, could yield innovative products with transformative consequences. Biomineralisation techniques and environmentally compatible biocides derived from secondary metabolites have the potential to combat climate-induced building failures and safeguard the health of occupants.

5.2.1 Developing bioactive compounds

The reliance on natural bioactive compounds as inspiration for synthetic analogues has recently undergone a paradigm shift in biochemical engineering. The focus has moved from simply replicating naturally sourced bioactive compounds to surpassing it, pushing the boundaries of potency and efficacy. This is particularly evident in antimicrobial development, where researchers are transitioning from solely characterising isolated active elements to harnessing the power of molecular biology. This direct targeting of specific receptors on pathogen cells bypasses the laborious process of bioactivity-guided fractionation, paving the way for the design of novel, highly effective antimicrobials with greater efficiency.

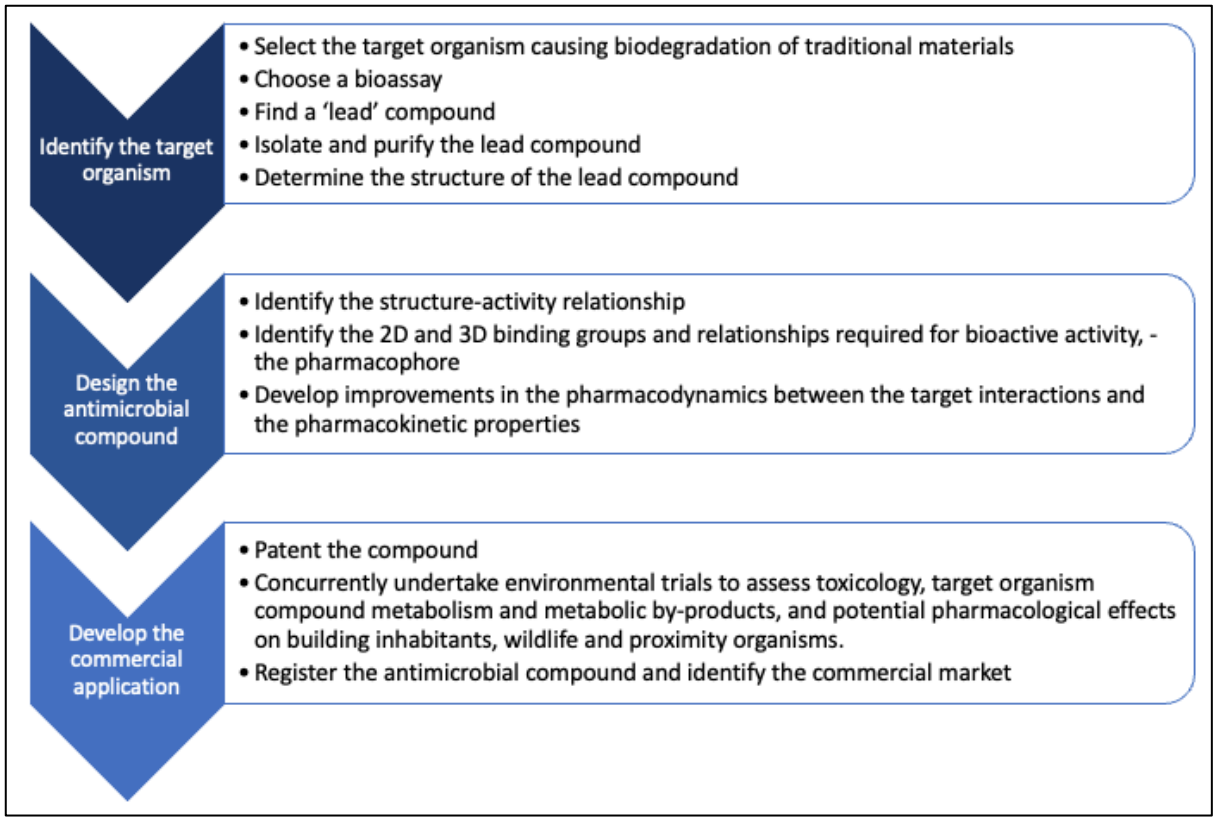


Figure 69: Discovery, design, and development steps for commercial development of natural antimicrobial compounds, (Patrick, 2017). Lichen often produces several secondary metabolites which act in combination to increase efficiency, detected during environmental trials. The above process may take several years to successfully deliver a commercial product

While extraction and purification of naturally occurring antimicrobials from the biosphere remains a critical initial step, it constitutes only the first glimpse into their full potential. Unlocking the next level lies in deciphering the intricate pathways connecting the molecular structure of these compounds to their target receptors within the organism. This deeper understanding paves the way for the design of synthetic agonists and antagonists, crafted to either mimic or disrupt the effects of the natural compound. Agonists, upon binding to their specific receptors, emulate the actions of the original molecule, while antagonists occupy the binding site, blocking receptor activation and subsequently the biological response. This intricate dance between agonist and antagonist can be further illuminated by techniques like nuclear magnetic resonance spectroscopy (NMR). NMR provides invaluable insights into the identification of epitopes, small molecules that bind to specific regions of a binding site, laying the groundwork for the development of lead compounds. By visualising the 3D structure of the natural compound and its interactions with target receptors, researchers can glean crucial information for designing next-generation antimicrobials with enhanced specificity and potency, pushing the boundaries of antimicrobial development.

5.2.2 Exploiting secondary product mechanisms of antimicrobial action

The traditional focus on mould and bacterial degradation of materials often overlooks the fundamental dissimilarities in their underlying cellular machinery, potentially impeding the

development of effective antimicrobial strategies. Unlike bacteria, classified as prokaryotes with relatively simplistic structures, moulds and fungi are eukaryotes possessing distinct organelles like mitochondria and a defined nucleus. This eukaryotic complexity necessitates targeting diverse structures and metabolic processes compared to their prokaryotic counterparts, as exemplified in *Table 20*. A singular approach to antimicrobial control may therefore prove insufficient due to the inherent disparities in fungal and bacterial cellular organization and function. Future research holds promise in delving deeper into these distinctions to design more precise and efficient strategies for material preservation against microbial assault.

Table 20: Mould and bacterial antimicrobial targets. The cell wall and cell membrane are the primary barriers utilised by moulds against antimicrobial agents. The mould cell wall contains glucan and chitin and is a target for antimicrobial compounds, though to be effective, many of these agents often demonstrate non-discriminating adverse effects due to the doses required and potential cytotoxicity of the compounds. The cell wall and plasma membrane of all microbials are primary targets for bio-toxic agents as they form the primary layer of defence. Examples of pharmaceutical agents targeting each of the pathways are indicated in brackets

Bacteria (prokaryotic)	Mould (eukaryotic)
Damage metabolic pathways (antimetabolites)	Inhibit cell wall synthesis (Echinocandins)
Inhibit cell wall synthesis (Penicillin)	Disrupt cell membrane functionality (Polyenes, Azoles)
Disrupt the plasma membrane (Polymyxins)	Damage fungal DNA and protein synthesis
Impede protein synthesis (Tetracyclines)	Impeding metabolic and signal pathways
Prevent transcription and translation of DNA/RNA or the formation of essential proteins and enzymes (Fluoroquinolones)	Disrupting the production of fungal virulence factors suppressing growth

The impact of low-dose antimicrobial agents with sustained growth-inhibitory effects on both pathogenic bacteria and moulds warrants further investigation. One promising avenue lies in the synergistic potential of combining low-dose antimicrobial agents. Such combinations present a greater challenge for pathogens to develop resistance, as the simultaneous targeting of multiple essential pathways diminishes the probability of successful compensatory mutations. This vulnerability stems from the sheer number of affected individuals within the microbial population, effectively reducing the odds of a single organism acquiring adaptive mutations across all targeted processes.

In the quest for synergistic antimicrobials, natural sources offer a compelling alternative to synthetic compounds. Their long history of safe use and established ecological integration suggest potentially minimal disruption to natural ecosystems compared to novel synthetic drugs. Medical folklore abounds with examples of naturally-derived synergies, such as the honey-vinegar blend known as oxymel. While neither honey nor vinegar individually exhibits potent antimicrobial activity, their combination in an oxymel formulation demonstrably suppresses the growth of key pathogens like *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Evidence suggests this synergism arises from the concerted impact of the organic acids in vinegar and the diverse secondary metabolites in honey on distinct cellular targets, leading to metabolic disruption and ultimately, cell death, (Harrison et al., 2023).

Efficient extraction and analysis of secondary metabolites are crucial to unlocking the full potential of natural synergies. Tailoring extraction processes to match the polarity of target compounds and employing sophisticated separation and analytical techniques are essential. Rapid characterisation of chemical structures can be achieved using high-throughput spectrometry, which, coupled with metagenomic and metatranscriptomic analysis and chemoinformatic tools, can illuminate the *in vivo* production pathways and mechanisms of action of these metabolites. The integration of machine learning algorithms enables the development of accurate predictive models based on mined bioinformatics data. Genomic signatures associated with enhanced secondary metabolite production can be identified using these models, facilitating the assessment of potential environmental impacts, particularly in the context of building material deterioration.

5.3 Bioprinting organic inclusions

The integration of live cells within biopolymer matrices into building materials using 3D bioprinting offers a transformative approach to architecture. This technology enables controlled delivery and dynamic adaptation of embedded biological elements within construction materials, developing bio-integrated architecture and its applications in the built environment.

The next generation in bioprinting is evolving from additive manufacturing principles developed for 4D printing. The "fourth dimension" signifies the ability of the printed object to adapt to external stimuli or pre-programmed cues. This introduces responsive designs in the form of smart or intelligent materials, initially exemplified by self-folding structures first introduced by Skylar Tibbet who heads the Self-Assembly Laboratory at MIT. (Chu et al., 2020). Selecting appropriate materials for 4D printing poses challenges. The material must fulfil application requirements, exhibit desired shape memory, and adapt to layered additive manufacturing.

The majority of 4D Smart material research has focused on synthetic polymers based on the comparative ease when building into the structure environmental sensor and trigger mechanisms to alter the molecular designs.

Selecting appropriate smart biomaterials for 4D bioprinting poses several crucial challenges. The chosen material must not only fulfil application requirements but also exhibit desired shape memory or physicochemical property changes in response to a desired stimulus. Furthermore, adapting this smart biopolymer to layered additive biomanufacturing opens new possibilities for innovative protein and enzyme layering within polymer structures, paving the way for bio-responsive materials. This approach transcends passive release mechanisms, enabling controlled delivery and dynamic adaptation of embedded bio-elements. Genetically modified organisms could biosynthesise new and responsive polymer structures, paving the way for bio-integrated architecture.

While an intriguing concept, the nascent field of 4D bioprinting for architectural applications faces diverse hurdles in material selection and stimuli-responsive design. Biomaterial compatibility, intricate layering of protein/enzyme functionality, and genetic engineering of bespoke polymers necessitate stringent considerations. Transcending passive release mechanisms, is an approach which promises dynamic adaptation of embedded bio-elements, opening doors for bio-integrated architecture. Encapsulation of biomineralising cyanobacteria within alginate biopolymer capsules arguably represents a simple but effective example. Two different external release mechanisms can release the bacterial load from the capsule, the physical application using a brush, causing the polymer capsules to rupture, and moisture from precipitation on the limewash surface, triggering

the swelling of the biopolymer and contents release. Both mechanisms are simple examples but effective mechanisms which are triggered to release the contents at the most appropriate time.

Environmentally responsive materials hold intriguing possibilities. Piezoelectric polymers, for example, can transduce pressure into electricity, potentially powering sensors or harvesting ambient energy, (Mahapatra et al., 2021), while liquid crystalline elastomers react to stimuli with mechanical actuation, converting kinetic energy into usable electricity within novel biopolymers, (Brighenti et al., 2020).

Shape-memory polymers, chromogenic materials, magnetorheological materials, and photoactive polymers offer diverse functionalities, including manipulating morphology, controlling encapsulated material release, undergoing structural alterations, and influencing surface emissivity. Shape-memory polymers and their composites respond to diverse stimuli, manipulating morphology, controlling encapsulated material release, or undergoing structural alterations under light, (Dayyoub et al., 2022). Chromogenic smart materials, readily formed from hydrogels or polymers, reversibly change colour in response to various triggers, influencing surface emissivity (Lampert, 2004). Magnetorheological materials, incorporating nanoferromagnetic fillers, possess tuneable rheological properties under magnetic fields, (Lu et al., 2021). Photoactive polymers find applications in controlled release systems and potentially solar energy conversion, (Kondo, 2020).

Driven by the need for sustainable heritage maintenance, bio-responsive smart biopolymers and bespoke additive manufacturing hold transformative potential. Self-assembling and damage-healing structures could significantly reduce maintenance costs and empower restoration projects. Combining high-resolution 3D scanning with bioprinting offers a transformative approach to intricate repair and replacement of heritage artifacts. This bespoke technology enables customised assembly, minimises material waste, and paves the way for incorporating bioprotective or self-repairing components and opens opportunity for new business development in design and 3D digital heritage artifact libraries available to conservationists.

The integration of these technologies requires addressing challenges like complexity, convergence of materials science and computer engineering, comprehensive testing, and cost barriers. Bioprinting for architectural applications presents a paradigm shift in the field. This technology offers a sustainable and adaptable approach to construction, with promising applications in heritage conservation and beyond. Future research and development hold the key to unlocking the full potential of this transformative technology.

5.4 Designer and hybrid biopolymers - integrating traditional and novel materials in sustainable heritage conservation

The preservation of our built heritage necessitates a multifaceted approach that embraces both the application of new technologies to existing materials and the development of enhanced traditional materials. While the development of innovative compounds for contemporary structures holds significant promise for reducing the carbon footprint of the construction industry, traditional materials remain a cornerstone of heritage conservation. Exploring the synergies between established methods and cutting-edge advancements paves the way for a more sustainable and effective approach to protecting the architectural legacy.

Polymer science plays a crucial role in this endeavour. Understanding the fundamental structure and properties of polymers, categorised into thermoplastics, plasticisers, fibres, elastomers, and thermosets, is essential for their application in conservation practices. The unique characteristics of

each category, dictated by the degree and type of interchain bonding, provide a diverse toolbox for material modification and repair, *Figure 70*.

Thermoplastics, for example, exhibit a reversible softening upon heating, offering opportunities for controlled manipulation and reshaping. Their properties, influenced by factors like crystallinity and glass transition temperature, can be further tailored through the addition of plasticisers. However, concerns regarding plasticiser migration and environmental impact necessitate a cautious approach to their use in heritage contexts.

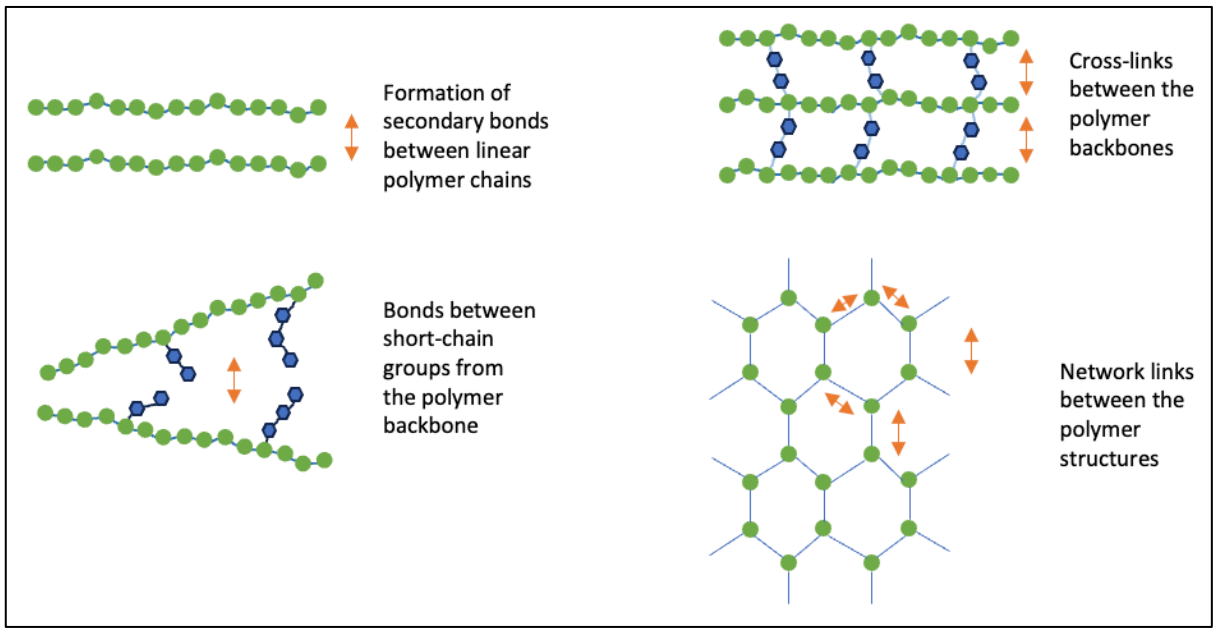


Figure 70: Four basic structures form the polymer characteristics and determine the strength and flexibility. The intra-polymer and intra-polymer structural characteristics define the rigidity of the structure and the brittleness or ductile nature

Polymer fibres, on the other hand, offer exceptional strength and flexibility due to their ordered crystalline structure. These properties make them ideal for reinforcing weakened building elements and creating composite materials with superior mechanical properties. Elastomers, conversely, possess remarkable elasticity but lack permanent shape retention. This characteristic find application in shock absorption and vibration dampening, potentially offering protection to heritage structures from environmental stresses. Thermoset polymers, with their irreversible curing upon heating, provide another avenue for material consolidation and strengthening. Biopolymers, such as cellulose and alginate, present exciting possibilities in this realm. As demonstrated in chapter three their inherent sustainability, combined with the addition of lime, leads to hybrid materials with improved self-healing and calcium carbonate nucleation capabilities. This synergy between the elastic properties of biopolymers and the superior compressive strength of lime holds promise for the restoration and reinforcement of historic structures.

The ecological advantages of biopolymers are undeniable. Their low-energy footprint, renewability, and minimal health risks make them compelling alternatives to synthetic materials in conservation applications. However, challenges remain concerning their mechanical properties, water solubility, and production costs. Continued research and development in biopolymer science, driven by their

biodegradability, non-toxic nature, and diverse physicochemical properties, offer promising solutions for overcoming these limitations.

The construction industry presents a vast potential market for biopolymers, synthetic biopolymers, and biopolymer-hybrid materials. By leveraging tools like molecular design, advanced omic technologies, and designer polymer chemistry, biopolymer research can be steered towards developing novel materials for repair and replacement in heritage contexts. This innovative approach, if embraced, can ensure the enduring legacy of built heritage while minimising environmental impact and promoting sustainable practices.

5.5 Chapter summary

The integration of bioprotective technologies into the preservation of traditional materials offers a credible strategy for biodeterioration mitigation, yet significant hurdles persist. Bio-based approaches leverage the metabolic capabilities of microorganisms to combat biodegradative agents, presenting several advantages in the face of climate change: environmental sustainability, reduced toxicity, and potential self-healing properties. Biomineralisation and biofilm biopolymers formed by beneficial bacteria act as physical barriers against destructive microbes and environmental threats, while enzymes or antimicrobial secondary compounds produced by these organisms directly target and neutralise biodeterioration agents. Additionally, the possibility of bioremediation of already degraded materials adds another exciting dimension to this approach.

One key facet is the potential of genetically modified (GM) microorganisms, which adds a layer of complexity to the intricate interplay of opportunities and challenges. Targeted genetic manipulation of microbes presents compelling opportunities. Engineered organisms can be equipped with enhanced bioremediation capabilities, enabling them to target and degrade harmful biodeteriogens more effectively. Moreover, the production of specific antimicrobial compounds or the expression of surface functionalities that inhibit colonisation by destructive microbes can be achieved through genetic manipulation, potentially leading to more durable and efficacious bioprotective treatments. This could revolutionise the field of biotechnology by promoting the use of sustainable and environmentally friendly methods for material preservation.

However, translating the theoretical benefits of bioprotection into practical applications for traditional materials necessitates addressing several critical challenges. Firstly, the long-term efficacy and durability of bio-based treatments require rigorous investigation. Environmental factors, material compatibility, and potential interactions with existing preservation methods need careful consideration. Secondly, the potential for unintended consequences, such as the disruption of desirable microbial communities or the introduction of allergenic or pathogenic agents, necessitates thorough risk assessment and mitigation strategies.

The potential consequences of integrating GM microorganisms into bioprotective strategies necessitates a critical appraisal of the potential challenges. Public perception and regulatory hurdles surrounding the use of GM organisms remain significant, and robust risk assessment protocols are crucial to ensure the safety and environmental compatibility of genetically altered microbes. Potential unintended consequences, such as the horizontal transfer of engineered genes to native microbial communities or the emergence of resistant biodeteriogens, require careful consideration and mitigation strategies. Moreover, the ethical implications of manipulating living organisms for human benefit must be thoughtfully addressed within the context of bioprotective applications.

Finally, the economic feasibility of bioprotective technologies, particularly compared to established methods, demands further consideration. Scalability, production costs, and application methods all influence the economic viability of these approaches.

By exploring the conceptualisation and fabrication of biological and physicochemical organic compounds, bioprotective technologies offer the potential for sustainable, renewable, and robust improvements in the durability of conventional materials. This advancement represents a significant stride towards the preservation of built heritage and its adaptation to the challenges of climate resilience.

CHAPTER SIX: Conclusions

Built heritage faces increasing threats from climate change, with traditional materials experiencing accelerated biodegradation due to complex interactions between biochemical, physical, and ecological factors. Traditional repair methods struggle to keep pace with the intensifying threats of climate change, bioinspired and physicochemical organic compounds offer a revolutionary path towards sustainable, self-healing, and adaptable materials for built heritage preservation. At the start of this study, this thesis posited that the strategic design and fabrication of these compounds, drawing inspiration from the resilience shown by biological systems, can demonstrably enhance the durability and lifespan of conventional materials, enabling built heritage to not just survive, but thrive, in the face of climatic extremes.

On taking this thesis position, the research unfolded within a framework constructed by posing two synergistic questions:

The first questions the mechanisms of biodegradation. How do climate change factors such as temperature, humidity, and precipitation, impact the interactions between biochemical, physical and material properties, and ecological elements that drive the biodegradation of traditional construction materials? In what way do these key biodegradation pathways involved in biomineralisation and bioerosion threaten specific heritage materials under different climate scenarios?

The second questions whether through bioremediation and the biosynthesis of sustainable materials, innovative solutions can counter the erosive threat. To what extent can targeted microbial biosynthesis strategies be used to design and fabricate novel bio-based materials for bioremediation and protection of built heritage? How can these bio-inspired materials be tailored to exhibit desired properties such as water resistance, antimicrobial activity and self-healing while ensuring compatibility with specific historical materials and conservation ethics?

The strengths of the research in supporting the thesis

As discussed in the introductory chapter, the earlier comprehensive literature review illuminated critical knowledge gaps that have shaped the conceptual framework and research direction of this thesis. By examining the intersections of climate change, biodeterioration, biomineralisation, bio-delivery systems, and sustainable biocides, this study has identified experimental evidence in subsequent chapters supporting a multidisciplinary approach to address the challenges facing cultural heritage preservation.

While existing research has made significant strides in understanding the impact of climate change on heritage materials, several limitations have been identified. The predominant focus on environmental and material sciences has overlooked the potential of biological sciences to inform the development of effective conservation strategies. Additionally, the lack of development of standardised methodologies for biodeterioration assessment had hindered accurate analysis and comparison of biomineralisation processes.

Furthermore, research on biomineralisation has primarily concentrated on concrete, neglecting the specific needs of traditional materials used in built heritage conservation. The development of biodegradable delivery systems for targeted application within construction materials represents a significant opportunity to advance bio-based preservation techniques. By bridging the gap between the pharmaceutical and construction industries, this research has sought to develop innovative and sustainable solutions for the protection of cultural heritage.

The presented argument for the thesis makes a compelling case for exploring bio-based solutions in built heritage conservation. By leveraging various bio-inspired strategies, this research proposes new avenues to counter the damaging effects of climate change on historical materials and holds immense potential to revolutionise conservation practices, ensuring the longevity and authenticity of built heritage. However, a deeper analysis reveals areas requiring further critical evaluation before fully endorsing the claims made, an evaluation which is discussed in the latter part of this chapter.

The strength of the thesis is supported by a comprehensive research approach that incorporates diverse areas like microbial activity, climate change, and traditional material vulnerabilities, offering a holistic perspective on biodegradation challenges. The proposed applications, such as erosive-resisting limewash and lichen-derived biocides, showcase the potential of bio-based solutions for combating biodeterioration. Within a wider context emphasising sustainability, carbon sequestration, and reduced reliance on harmful chemicals the outcome of this research strengthens the arguments for bio-based approaches.

The earlier chapters sought to address the two posited questions in detail, providing empirical evidence to support the central thesis claim. Chapters one through four delve into the theoretical and practical aspects, while chapters three and four showcase two practical applications that exemplify the potential of bio-based solutions in built heritage conservation.

The opening chapter of this investigation navigates the interplay between climate change and the biodegradation of traditional materials. It lays the groundwork for an examination of how environmental perturbations induced by a warming planet influence the breakdown of historical and cultural artefacts. To this end, it delves into the fundamental mechanisms of microbial biodegradation operating within these materials, illustrating the interaction between microbes and their substrates. Subsequently, the chapter performs an analysis of how climate change is central to amplifying or dampening the tempo of degradation processes.

Moving beyond material loss, the chapter illuminates the potential for improved traditional material preservation to translate into environmental health benefits. By curbing biodegradation, occupant exposure to hazardous microbial by-products – a consequence of active breakdown – and the impact of microbial virulence factors, can be significantly reduced. Furthermore, the chapter introduces the notion of biodegradation and bioprotection as potentially interchangeable entities, depending on the lens through which they are viewed. This conceptual reframing suggests that climate change may be subtly tipping the scales in this delicate biological tug-of-war, potentially opening doors for the development of innovative bioprotection strategies tailored to safeguard cultural heritage in the face of a changing climate.

This chapter serves as a springboard, propelling the reader into a deeper exploration of this multifaceted issue. It paves the way for subsequent chapters to dissect the specific vulnerabilities of diverse traditional materials – wood, paper, textiles and stone – while analysing the climatic factors influencing their biodegradation. Ultimately, this investigation aspires to not only unveil the complex interplay between climate and material decay but also to illuminate the potential for harnessing biodegradation, reimagined as a force for preservation, in the fight against the burgeoning challenges of a changing climate.

The second chapter introduces the concept of innovative bioprotective strategies for safeguarding traditional materials. It highlights the issue of limewash degradation on historical lime-rendered edifices, attributing it to the erosive effects of wind-driven precipitation. Subsequently, the chapter presents a proposition for incorporating biomineralising microbial cells into the limewash

composition as a potential means to bolster its density and weather resilience. A comparative analysis is then undertaken, juxtaposing the metabolic pathways implicated in bacterial calcium carbonate precipitation. This analysis probes and subsequently addresses concerns surrounding the longevity of cellular life within the highly alkaline conditions of lime and the potential for mechanical damage during lime preparation. The discourse culminates in the exploration of diverse microbial inclusion strategies aimed at preserving cell viability within a cementitious matrix.

Building upon the exploration of lime in the prior chapter, a cornerstone material in pre-1919 architecture, chapter three delves into the development of a revolutionary self-repairing limewash. This research diverges from existing approaches by presenting a novel framework: biomineralisation of the protective outer layer on lime render buildings, empowered by photosynthesis as the driving force for calcium carbonate precipitation within the limewash matrix.

The chapter extends this perspective by proposing an experimental design that critically compares encapsulation and immobilisation technologies using biopolymers as support matrices for biomineralising organisms. Subsequently, a practical study assesses the efficacy of the proposed biomineralisation strategy, both *in vitro* and i*n situ*.

Ultimately, the research demonstrates that photosynthesis-driven biomineralisation of limewash, utilising cyanobacteria encapsulated within alginate biopolymers, can effectively mitigate weather-induced erosion while simultaneously offering environmental benefits. These benefits include the sequestration of atmospheric carbon dioxide and a reduced carbon footprint due to decreased biodegradation and less frequent surface maintenance.

While incorporating biopolymers like organic carbon into inorganic compounds like limewash offers advantages for building materials, it introduces the potential for biocolonisation by microorganisms on building surfaces. Chapter four explores the extraction and isolation of secondary metabolites from lichen communities as a potential solution for biofilm control, specifically in light of climate change.

The study recognises that climate change, with its associated increases in temperature and moisture, affects not only the colonisation dynamics of benign microorganisms like lichen but also creates favourable conditions for the rapid growth and colonisation of biodegrading moulds and bacteria. Therefore, it hypothesises that lichen, in response to these changes, will produce secondary metabolites with antimicrobial properties to compete with and inhibit the growth of harmful organisms on the building surface.

The practical study focuses on lichen species *O. parella* and *R. siliquosa*, sourced from the same location as the biodegrading organisms. It demonstrates that these lichens synthesise potent antimicrobial secondary metabolites that effectively prevent and inhibit bacterial and mould growth. The chapter concludes that once the structural and functional configurations of these metabolites are identified, they hold considerable promise as lead compounds for developing enhanced, synthetic antimicrobial agents for building surface protection. This finding complements the previous chapter on biomineralised limewash, offering a potentially synergistic solution for preventing unwanted colonisation on the biomineralised surface.

The study underscores the necessity for additional investigations to pinpoint the most efficacious naturally derived secondary metabolites for the development of novel biocides. This emphasis extends to a thorough evaluation of the inhibitory mechanisms at play against select Gram-positive and Gram-negative bacteria, alongside eukaryotic moulds, to guarantee compatibility with biomineralisation-associated organisms. These findings pave the way for further delving into the

intricate interplay between climate shifts, biodegradation processes, and the latent potential of microbial biosynthesis for bioremediation applications and the sustainable development of materials in the context of built heritage conservation.

Areas for further critical analysis

While the *in vitro* and initial *in situ* studies are promising, the long-term efficacy, particularly the durability of bio-based solutions in diverse environmental conditions needs thorough assessment, essential to ensuring solutions perform reliably across various climates and weather patterns. Introducing novel microorganisms and bioactive compounds therefore necessitates careful consideration of the potential unintended consequences on non-target organisms and ecosystem dynamics.

Both of these initiatives present a need for a nuanced understanding of the complexity within this field of research. The introduction of microorganisms into environmental frameworks such as heritage structures and the use of microbially synthesised antimicrobial agents present potential trade-offs between the different approaches. With careful bioprotective strategies leveraging ongoing discussions about potential limitations and unpacking opposing viewpoints, the outcomes will strengthen the argument and provide valuable insights to further these solutions.

There are also considerations in the genetic modification of microorganisms which raises ethical concerns that require open discussions and transparent approaches to ensure public trust and acceptance. Collaboration between conservation scientists, ethicists, policymakers and the public is essential to address concerns and responsibly refine acceptable stakeholder solutions.

To achieve widespread adoption, there are scalability and accessibility hurdles which will determine the economic feasibility of these solutions particularly in resource-limited settings and deserves further exploration. A contributory factor toward adoption is demonstrating the long-term compatibility of bio-based solutions with different historical materials and conservation practices.

Therefore, while the thesis offers a structured and promising argument for employing bio-based solutions in built heritage conservation, a critical assessment reveals the need for further rigorous research addressing long-term efficacy, potential unintended consequences, ethical concerns, and practical applications. Only through in-depth analysis and responsible development can the immense potential of bio-inspired solutions be harnessed for the sustainable preservation of cultural heritage in a changing climate.

In defence of the thesis, bio-inspired approaches have been demonstrated by this study as capable of the sustainable and effective protection of built heritage in the face of climate change challenges. Resistance to adopting new bio-based practices can be offset by careful selection and control of microbes, mitigating unintended consequences. Ongoing research can address durability and efficacy concerns through the tailoring of bio-inspired materials which are compatible with specific materials and practices, as demonstrated by the use of lime in this study.

In conclusion, bioprotective technologies hold immense promise for mitigating biodeterioration in traditional materials. While the arguments are compelling, it is important to acknowledge the potential limitations and opposing viewpoints which when addressed over the long term strengthen the argument and further valuable insights into the approach. Acknowledging the multifaceted relationship between biodegradation, climate change, and environmental health paves the way for innovative and sustainable approaches. It is important to acknowledge that this research is an ongoing process. Recognising both the limitations and the progress allows for a balanced and

constructive conversation about the future of bioprotective technologies in heritage conservation. By considering these critical perspectives, this research can contribute significantly to advancing the field of bio-based conservation and ensure the responsible and effective application of these technologies for safeguarding heritage structures for future generations.

Glossary

A

Abiotic	Non-living physical or chemical parts of the environment that affect living organisms and the functioning of ecosystems.
Acidolysis	A chemical reaction similar to hydrolysis where an acid replaces the role of water
Allelopathic	Methods to chemically inhibit the growth of one organism by another due to the release of substances acting as growth inhibitors
Alpha pyrones	An unsaturated cyclic chemical compound with the molecular formula $C_5H_4O_2$
Andrastin A	Isolated from Penicillium spp. Andrastin A is a complex molecule and farnesyltransferase inhibitor a potentially useful secondary metabolite against tumour proliferation
Apoptosis	Programmed cell death occurring within the normal growth and development lifecycle of an organism
ATP-binding cassette transporter pathway	Multiple subunits forming a transport system superfamily belonging to large, ancient gene families. They utilise the energy of ATP to translocate substrates across membranes
Autotrophic	An organism that can synthesise organic substances from inorganic substances such as carbon dioxide.
a_w	Symbol for water activity, or the amount of free water available. The optimum a_w value for the growth of a microorganism is usually in the range of 0.99 to 0.98 on a scale of 0 – 1.0. S. aureus is the most a_w tolerant pathogen with an a_w of 0.86

B

Bacteraemia	Refers to the presence of bacteria in the bloodstream
Biofilm	An extracellular polymeric matrix produced by an assemblage of microbial cells attaching the colony to a surface, primarily composed of polysaccharides. The biofilm performs physical, chemical and transport services for the movement of compounds between, into and out of the colony
Biophilic design	A building industry term to connect the building occupant with the environment through the construction of 'biophilic spaces'
Bromocresol purple	Bromocresol purple is a dye of the triphenylmethane family and a pH indicator

C

Chelation	Bonding of ions and molecules to metal ions by a chelator often acting as a sequestering agent.
Chemolithotrophs	Uses inorganic reduced compounds in energy-producing reactions, a process involving oxidation of inorganic compounds linked to the synthesis of ATP
Chromophore	A molecule which absorbs a particular wavelength of light thereby emitting a colour
Citreohybridonol	A secondary metabolite with a molecular weight of 500.6 g mol^{-1} associated with respiratory tract infections from Penicillium spp. in damp buildings

D

Decarboxylation	The chemical reaction removing a carboxyl group from a compound releasing carbon dioxide
Diprotic acid	Acids capable of donating two protons, examples include sulphuric acid (H_2SO_4) and carbonic acid, (H_2CO_3)

Discontinuous electrophoretic system	A technique for separating proteins according to size and charge, a type of gel electrophoresis
Dyspnoea	A shortness of breath or breathlessness

E

Endocarditis	
Endoliths	Bacteria that live immediately beneath the stone surface using the substrate to protect the cell from UV radiation
Endophthalmitis	An inflammation of the inner coats of the eye resulting from intraocular colonisation by infectious agents such as *Penicillium*
Extracellular polymeric matrix	A key element comprising the biofilm matrix, composed primarily of polysaccharides, water, and small amounts of proteins and DNA. Purified versions can be extracted to form microbial biopolymers

F

G

Glass transition temperature	Thermoplastics are polymers that soften when heated and become firm when cooled, a point called the 'glass transition temperature' T_g. The point at which the polymer melts is referred to as the 'melt transition temperature' T_m, a measure that increases with the crystallinity of the polymer

H

Haemosiderin	A complex of partially digested ferritin and lysosomes functioning as a metalloprotein iron storage complex, commonly found at sites where haemorrhage occurs
Halophilic	Organisms which are defined by their affinity for high saline environments, found within hypersaline ecosystems
Heteropolysaccharide	Polysaccharides which contain two or more different monosaccharide units.
Heterotrophic	
Homopolysaccharide	Polysaccharides made up of a single type of sugar monomer, for example, cellulose.
Horizontal gene transfer	The lateral movement of genetic information between different cell genomes, introducing DNA or RNA into the target cell, either as new genes or replacing existing genes.
Humic substances	Coloured organic compounds formed naturally during long-term decomposition and transformation of biomass residues.

I

Lead Compound	A chemical compound with pharmacological or biological activity that has some therapeutic use but has a suboptimal structure that requires modification to fit better to the target. The lead compound chemical structure serves as the starting point for chemical modification to improve the potency and selectivity or new compounds.
Isochromans	Heterocyclic compounds and their derivatives, used extensively to produce therapeutically related applications

J

K

Keratinase	Proteolytic enzymes that digest keratin, possessing a wide range of temperature and pH ranges that allow a complete degradation of complex proteins.
Keratitis	Inflammation or irritation of the cornea

Koninginins	A series of secondary products found in *Trichoderma harzianum* and *Trichoderma koningii* and *P. corylophilum* in damp buildings

L

M

Meroterpenoids	A chemical compound with a partial terpenoid structure often with diverse bioactivity potential like anticancer, and anti-inflammatory abilities
Mesophiles	Organisms that grow in moderative temperatures, flourishing in temperatures between 20°C and 45°C such as *Staphylococcus aureus*. The optimum temperature for growth is human body temperature of 37°C.
Microbiocenosis	A group of interacting organisms living as a biotic community alongside the physical environment supporting the community
Mutagenesis	The process by which the genetic information of an organism alters due to a mutation which may cause naturally or as a result of exposure to mutagens

N

Nosocomial	Refers to infections which originate in a hospital often relating to opportunistic pathogens

O

Omics	Several areas of biological study of the entire complement of biomolecules or a molecular process usually ending in -omics, such as genomics, transcriptomics, proteomics, or metabolomics
Operon	A single DNA unit comprising a cluster of genes controlled by a single promoter
Opportunistic pathogen	An infectious pathogen normally commensal in the body, but triggers to cause disease when the host immune resistance is weakened
Otomycosis	A fungal infection which affects one or both ears usually in tropical environments

P

Petrographic microscopy	Used for optical analysis of thin sections of rocks and minerals
Phase change materials (PCMs)	Compounds which absorb or release heat as they undergo a physical change, such as from solid to liquid, or liquid to gas. Organic PCMs include petrochemical products, lipids, and sugar alcohols, often used with encapsulation technologies
PCR (analysis)	Polymerase Chain Reaction tests are accurate diagnosis tools to detect DNA or RNA of organisms by amplifying the DNA sequences incorporating the use of primers and a DNA polymerase
Phototrophs	Organisms that use light as a primary source for metabolism through photon capture to produce organic compounds. Notably, not all phototrophs are photosynthetic. Phototrophs can be autotrophs (an organism producing compounds using carbon and light energy or inorganic chemical reactions) or heterotrophs (take their energy from other sources of organic carbon)
Planktonic	Free living microorganisms which have not formed or joined a biofilm
pKa	A measure of the strength of an acid on a logarithmic scale, useful in comparing the strengths of different acids
Psychrophiles	Microorganisms which exist in extreme cold and can grow in temperatures from 0°C to 5°C
Pulmonary haemosiderosis	Repeated episodes of intra-alveolar bleeding resulting in accumulation of hemosiderin and the development of pulmonary fibrosis and severe anaemia

Q

Quorum sensing	Gene expression regulation in response to fluctuations in cell-population density using chemical signal molecules called autoinducers proportional to cell density
R	
rRNA (16S)	16S ribosomal RNA is a component of the 30S subunit of a prokaryotic ribosome. The genes coding for it are used in reconstructing phylogenies due to the slow rates of evolution of this gene region and therefore highly conserved.
S	
Sepiolite	A naturally occurring clay mineral of sedimentary origin composed of hydrous magnesium silicate.
Sesquiterpene phomenone	
Spalling	The process of delamination of stone surfaces due to the accumulation of salts degrading the stone
speleothems	A geological formation by mineral deposits that accumulate in caves. They are commonly found in calcareous caves due to carbonate dissolution reactions
Stromatolitic	A layered sedimentary formation created mainly by photosynthetic microorganisms such as cyanobacteria and sulphate-reducing bacteria
T	
Thermophiles	Microorganisms that grow at temperatures of 55°C or higher
Tunicate	A marine invertebrate capable of biosynthesising cellulose
Taylor's cone	A structure which forms, due to the application of a large voltage, creating a charged solution as it exits an electrospinning or electrospraying needle a result of electrostatic repulsion sufficient to offset the surface tension of the liquid
U	
Upregulation (of genes)	A cellular process triggered by an internal or external signal resulting in increased expression of one or more genes usually resulting in higher productions of the encoded proteins
V	
W	
Wzx/Wzy dependent pathway	In bacteria, the majority of cell-surface polysaccharides are produced by this pathway, including spore coatings, exopolysaccharides, and heteropolymeric antigens.
X	
Y	
Z	

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APPENDICES

The following appendices are referenced by the main body of the thesis as follows:-

[Appendices: Figure/Table: X]

where *X* is the figure or table number

Supporting documentation:

Support data for Practice components in chapters Three and Four

Health and Safety support documentation

- DSEAR Risk Assessment – COSHH Appendices 2 (Acetone, methanol, dichloromethane)
- COSHH Risk Assessment Form (*O. parella* and *R. siliquosa* extracts, and microbial stock, including *Synechococcus sp.* and solvents, nutrients, electrical equipment and chemicals utilised in the study) – Biosciences Laboratory Approvals
- COSHH Risk Assessment Form (*O. parella* and *R. siliquosa* extracts, and solvents, utilised in the study) – Chemistry Laboratory Approvals
- Technical hazard sheet – Limewash
- Technical hazard sheet – Hydrated lime
- Risk Assessment – Task Analysis – on-site, Isle of Jura
- COSHH Risk Assessment Form – on-site, Isle of Jura

Published paper

Novel biodesign enhancements to at-risk traditional building materials
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PRACTICE – support data

Note: Summary graphs and statistical results - definitions

For assessing the variability of different samples in relation to each other **standard error** comparisons are appropriate, in this instance in evaluating whether samples are different or correlated due to chance alone or chance plus something else.

A minimal or no overlap between the standard error bars for comparative data suggests that the samples under review are unlikely to have come from the same population. Standard error bars are indicative and suggest correlation or difference, greater confidence in the outcome can be achieved by conducting a two-tailed test against a null hypothesis significance test.

A **null hypothesis significance test** helps decide whether chance alone can account for apparent patterns in the data.

A **two-tailed test** is used when the alternative hypothesis is non-directional, stating that populations are different.

The **P value** reflects the probability of getting a value for the statistic equal to, or more extreme than, the one calculated in the null hypothesis significance test if the null hypothesis is true. P is utilised in this study to establish a comparative significance level as follows.

The below grading interpretation of P values enables the use of a grading system to indicate the degree of significance. This system is derived from comparing the P value range to the chosen value of 0.05 which has been chosen as the benchmark indicating significance and the interpretation of P values is defined below.

Range of P Values	Interpretation used in this study
>0.05	Non-significant
0.10 – 0.05	Suggestive
0.05 – 0.01	Significant
0.01 – 0.001	Highly significant
<0.001	Extremely significant

Table 21: Methods of microbial biodeterioration. Planktonic bacteria, biofilms, and fungal organisms collectively engage in the deterioration of building materials through a multifaceted approach comprising physical, chemical, and biological mechanisms. Biofilms and lichens represent consortiums of microorganisms that firmly adhere to surfaces, culminating in the formation of a protective matrix composed of extracellular polymeric substances. Biofilms exhibit remarkable adaptability for surface attachment and subsequent biodegradation of substrates. This adeptness is accomplished through the generation of corrosive by-products, notably organic acids, and sulphur compounds, which instigate chemical reactions corroding building materials such as metals and concrete. Biofilms actively produce an array of enzymes that expedite the decomposition of intricate organic compounds. Notable examples include proteases, which are instrumental in the degradation of proteins found in wood or synthetic paints, and cellulases, which catalyse the breakdown of cellulose present in wood or paper. Inside the confines of building interiors, fungal organisms are proficient in deteriorating natural fibres, including cotton, linen, silk, and wool. This degradation process manifests as structural weakening, discolouration, and ultimately fabric disintegration

Micro-organism	**Method of biodeterioration**		
	Mechanical/physical	**Chemical**	**Biological**
Bacteria – planktonic and pre-biofilm stage	Unattached planktonic bacteria exhibit limited mechanical erosive impact on their immediate environment until they undergo aggregation, resulting in the formation of biopolymers and subsequent adhesion to adjacent surfaces.	Planktonic cells will excrete metabolites, acids, and chelating agents impacting the chemical properties of adjacent materials, heightening porosity, and weakening the mineral matrix.	Endoliths, are a group of bacteria that thrive immediately beneath stone surfaces, effectively utilizing the substratum as a protective shield against the deleterious effects of intense UV radiation. The formation of dark patinas, arising from the secretion of oxalates, serves to augment the screening of UV radiation. If the stone undergoes rapid cycles of heating and expansion, this process can lead to potential delamination of the uppermost layer of the stone.
Bacterial biofilms	During colonisation, bacteria engage in the production of polysaccharides and proteins, culminating in the formation of electrostatic, hydrophobic, and van der Waals adhesive attachments, (Flemming et al., 2016; López et al., 2010). Once this attachment occurs, the resulting three-dimensional extracellular polymeric substance (EPS) assumes highly adhesive properties, effectively anchoring the bacterial colony to the substrate, (Zhao et al., 2023). The EPS is central to attracting secondary colonisers into the matrix, particularly photosynthetic microorganisms that contribute to the carbon	The chemical composition and surface characteristics of stone influence the growth of biofilms. This influence is evident when comparing the varying rates of biofilm colonisation on stones with differing porosities, such as limestone and marble. The availability of bioessential elements like calcium (Ca), iron (Fe), and magnesium (Mg) serves as a crucial nutrient source for the biofilm development. In addition to mineral-derived nutrients, organic debris, including remnants from prior growth on the stone, such as lichen and previous biofilms, bird	Microbial cells residing within a biofilm undergo metabolic adjustments in response to the availability of substrate nutrients. This phenomenon is manifested as alterations in their metabolic activities. The extracellular polymeric substance (EPS) is central in facilitating communication and establishing a network conduit for the transportation of nutrients and disposal of metabolic waste products. The network conduits within the biofilm serve to promote a broader distribution of organic acids onto the substrate, thereby enhancing the absorption

	production capacity of the biofilm. Temperature fluctuations lead to the expansion and contraction of water within the biofilm, generating mechanical stress upon the adjacent substrate.	excrement, and deposits of anthropogenic pollutants, can contribute additional nutrients in the form of nitrogen and phosphorus. These organic inputs further support the successful stone surface colonisation of biofilms. Cell metabolism leads to the dissolution of carbon dioxide (CO_2) found present in water held by the biofilm which leads to a reduction in pH at the interface between the substratum and the biofilm. This lowering of pH accelerates the chemical weathering of the underlying rock.	of essential nutrients. The biofilm also functions as a platform for the horizontal transfer of genetic material between heterogeneous microbial species. In particular, this genetic exchange includes the potential transmission of antimicrobial-resistant genes, which can bolster the biodeterioration capacity of the EPS and bolster the resistance of cells in the biofilm to biocidal treatments. The presence of enhanced gene structures within the biofilm community supports the enzymatic biodegradation of various substrates, encompassing stone, cement, and wood. The interplay of microbial activities within the biofilm is a subject of significant academic interest and underscores the multifaceted role of biofilms in various ecological and industrial contexts.
Fungi, lichen, and algae	Fungi and lichen extend hyphae into the microfracture crevices within a substrate, thereby inducing delamination and increasing the porosity of the material. This process is intensified through the incorporation of mineral salts assimilated from the substrate into the lichen thallus, which, in response to fluctuations in temperature and moisture content, undergoes cycles of expansion and contraction, consequently widening the microcracks. Ongoing physical weathering increases the ingress of water into the larger fractures present in the substrate. This, in turn, promotes the proliferation of biological organisms and contributes to	The substratum pH influences biological colonisation and is dependent on the pH range tolerance of the organism. The pH is an indication of the quantity of H^+ and OH^- ions which form at the extremes of the pH scale and are damaging to cellular processes. Most fungi prefer slightly acidic substrates whereas cyanobacteria and several algae are more tolerant of alkaline conditions (Caneva et al., 2008). Fungi and lichens weather rocks through a variety of chemical mechanisms, including: **Organic acid production:** Fungi and lichens produce a variety of	Fungi, lichen, and algae contribute to the biodeterioration of heritage structures through both mechanical and chemical mechanisms including by: **Pigment Production**: These organisms produce pigments that permeate the stone, resulting in the staining of surfaces. The pigments result in a difficult to remove patina rendering the stone more vulnerable to damage, particularly from solar radiation and mechanical conservation procedures. **Moisture Augmentation**: The microorganism presence on the stone surfaces elevates the moisture content through the continuous absorption

	erosional processes driven by the action of water. Lichen display an exceptional capacity for water retention which contributes to the deterioration of building materials. The thallus is well-suited to absorb and retain moisture, offering partner cyanobacteria or algae cells a reliable water source during periods of drought.	organic acids, including oxalic acid, citric acid, and gluconic acid. These acids dissolve minerals in rocks, causing structural weakness. **Chelation:** Some organic acids can chelate metal cations, binding to the cations and preventing the cations from bonding to the minerals in the rock. This weakens the rocks and make them more susceptible to weathering. **Oxidation:** Fungi and lichens oxidize minerals in rocks, causing them to break down.	of ambient moisture. The increase in moisture content serves as an ideal medium for the proliferation of additional organisms, including mosses and vascular plants. The colonisation by secondary organisms can lead to substantial harm to the stone by affecting joints, causing cracks, and contributing to the delamination of the stone surface. **Enzymatic Action**: Fungi, including lichen, are prolific secretors of a diverse array of enzymes, notably cellulases and ligninases. Each of these enzymes play a role in the decomposition of organic compounds such as lignin and cellulose, which are commonly found in wood. Consequently, this enzymatic breakdown weakens the structural integrity of the wood, ultimately culminating in its disintegration.

Table 22: The impact of organic acids on biodegradation. Comparison of the acidity and structure of microbially produced organic acids which cause the degradation of traditional materials. Oxalic acid is the least corrosion due to the protective calcium oxalate which forms a seal over the substrate though this lowers the porosity and hampers the management of moisture in the material. The most corrosive of the acids is citric acid (Bertron et al., 2009; Dyer, 2017; Larreur-Cayol et al., 2011)

Organic Acid	**Chemical Profile**	**Examples of organisms** *F=Fungi, B=Bacteria, L=Lichen, A=Algae*	**Substrate biodegradation**	**References**
Fusaric $C_{10}H_{13}NO_2$	Fusaric acid, characterised as a mycotoxin, exhibits notable antimicrobial properties, rendering it a potential candidate for the control of bacterial strains, including *Bacillus* species. The Intrinsic resistance observed in various microorganisms is conjectured to be attributed to the existence of a fusaric acid tripartite efflux pump within the cellular architecture. Fusaric acid has an acidity pK_a value of 1.13	*Fusarium* sp. (F)	*Fusarium* sp. are the main producers of fusaric acid. Several bacteria can produce enzymes which degrade fusaric acid as part of their normal metabolic processes.	(Bacon et al., 1996, 2006; Hu et al., 2012)
Acetic $C_2H_4O_2$	Bacterial strains that synthesise monocarboxylic acetic acid are employed in the industrial synthesis of vinegar. These microorganisms catalyse the oxidation of ethanol and exhibit remarkable resilience to an acidic environment. Even at minimal concentrations of 0.5 %wt, acetic acid can manifest toxic properties. The bacterial resistance to acetic acid is attributed to a multifaceted array of mechanisms, including the involvement of proteins such as aconitase and a hypothesized ABC-transporter. The pKa of acetic acid stands at 4.75.	*Fusarium* sp. (F), *Aspergillus* sp. (F), *Acetobacter* sp. (B), *Gluconobacter* sp. (B), *Komagataeibacter* sp. (B) *Desulfosporosinus* sp. (B), *Clostridia* sp. (B)	The susceptibility of metal pipelines, fuel storage tanks, and the mechanical deterioration of historical architectural structures due to Microbiologically Influenced Corrosion (MIC) concerns the industrial sector. Acetic acid emerges as a key contributor to this phenomenon, amplifying the corrosive nature of these materials while simultaneously acting as a potent electron donor to facilitate microbiota metabolism and MIC processes. Notably, acetic acid's deleterious impact extends to the dissolution of calcium hydroxide within cementitious mixes, resulting in the removal of protective layers and exposing more surface area to acid-mediated corrosion. Furthermore, this acid interacts with various hydrates, including aluminates, persistently producing soluble salts, such as calcium acetate monohydrate. The presence of these soluble salts, in turn, impairs the	(Liaud et al., 2014; Mariam Ninan et al., 2020; Nakano & Fukaya, 2008; Sowards et al., 2014; Sterflinger, 2000)

			structural integrity of the substrate and elevates its porosity, thereby exacerbating the vulnerability to MIC-related corrosion.	
Citric $C_6H_8O_7$	Citric acid exhibits an aggressive reactivity in the context of deteriorating cementitious paste. This organic compound manifests as a triprotic acid, capable of furnishing three protons per molecular when dissociating dissociation, resulting in three distinct pKa values. Specifically, these pKa values are measured at 3.1, 4.7, and 6.4, a progression mirroring the augmented negative charge associated with the successive donation of protons.	*Fusarium* sp. (F), *Aspergillus* sp. (F), *Penicillium frequentans* (F)	The pronounced degradation of clay silicates, micas, and feldspars found in both sandstone and granite, as well as calcite and dolomite present in limestone, can be attributed to the presence of citric acid. Salt precipitation occurs on the surface of the substrate, forming calcium (calcium citrate tetrahydrate), aluminium and iron complexes. These compounds are susceptible to detachment, thereby exposing the underlying surface to subsequent deteriorative processes.	(De La Torre et al., 1993; Fomina et al., 2007; Jestin et al., 2004; Liaud et al., 2014; Rashid et al., 2004; Sterflinger, 2000)
Glyoxylic $C_2H_2O_3$	Glyoxylic is one of the C_2 carboxylic acids. It has an acidity pK_a of 3.83.	*Fusarium* sp. (F)	A comparatively feeble acid exhibiting a deleterious effect on the structural matrix inherent in cement compositions through a process known as acidolysis, resulting in the dissolution of the solid phase.	(Sterflinger, 2000)
Oxalic $C_2H_2O_4$	Oxalic acid is produced by several organisms notably fungi and bacteria. Fungi are widespread producers of oxalic acid, while numerous bacteria incorporate this compound into their metabolic pathways. Oxalic acid has an acidity pK_a of 1.25.	*Aspergillus* sp. (F)	As the cementitious material is degraded by the microorganism it forms calcium oxalate monohydrate forming a surface protective layer. Oxalic acid appears the least aggressive to cementitious materials, forming a surface covering which seals the pores in the substrate.	(Chuang et al., 2007; Dutton & Evans, 2011; Fomina et al., 2007; Jestin et al., 2004; Liaud et al., 2014; Palmieri et al., 2019; Rashid et al., 2004; Sterflinger, 2000)
Formic CH_2O_2	Formic acid, a small molecule, impedes the proliferation of microorganisms and can potentially induce cytotoxic effects. These effects may arise from its ability to induce oxidative stress and hinder the process of protein biosynthesis. It has an acidity pK_a of 3.75.	*Aspergillus* sp. (F), *Cladosporium* sp. (F)	Formic acid is a relatively weak acid. It is detrimental to the cementitious matrix through acidolysis causing dissolution of the solid phase.	(Liaud et al., 2014; Sterflinger, 2000; Zeng et al., 2022)
Fumaric $C_4H_4O_4$	Fungal metabolism synthesises fumaric acid via two distinct pathways: the tricarboxylic acid cycle, predominantly confined to the mitochondrial metabolic cycle, and the	*Aspergillus* sp. (F), *Cladosporium* sp. (F) *Saccharomyces cerevisiae* (F)	A relatively weak acid. It is detrimental to the cementitious matrix through acidolysis causing dissolution of the solid phase.	(Jestin et al., 2004; Liaud et al., 2014; Sterflinger, 2000)

	reductive tricarboxylic acid cycle, operating within the cytosol. The latter pathway is primarily accountable for the extracellular production observed in filamentous fungi. Fumaric acid exhibits an acidity level characterised by a pKa of 3.03.			
Gluconic $C_6H_{12}O_7$	Gluconic acid has an acidity pK_a of 3.6 and consists of a six-carbon chain with five hydroxyl groups, terminating in a carboxylic acid group. The gluconate anion can chelate $Ca^{2+,}$ Fe^{2+}, K^+ and Al^{3+} metals.	*Aspergillus* sp. (F), *Penicillium* sp. (F) *Acetobacter* sp.(B) *Gluconobacter* sp. (B) *Aureobasidium* sp. (F) *Saccharomyces* sp. (F)	A relatively weak acid. It is detrimental to the cementitious matrix through acidolysis causing dissolution of the solid phase.	(Chuang et al., 2007; Fomina et al., 2007; Liaud et al., 2014; Rashid et al., 2004; Sterflinger, 2000)
Glyoxylic $C_2H_2O_3$	Glyoxylic acid, with the chemical formula HOOCCHO, is a two-carbon organic compound that belongs to the family of α-ketoacids. It is a highly reactive compound due to the presence of a carboxyl (–COOH) and an aldehyde (–CHO) functional group within its structure. The structure of the molecule comprises a carbonyl group (C=O) and a carboxylic acid group (–COOH) connected to a two-carbon chain. Glyoxylic acid is an essential component in the glyoxylate cycle, which is a variation of the tricarboxylic acid cycle found in certain microorganisms, including bacteria and plants. It has an acidity pK_a of 3.3.	*Aspergillus* sp. (F)	Glyoxylic acid, a relatively weak acid is involved in various chemical reactions, such as acidolysis and can condense with primary amines to form imines. Glyoxylic acid can undergo decarboxylation to yield glyoxal.	(Sterflinger, 2000)
Itaconic $C_5H_6O_4$	Itaconic acid, also known as methylene succinic acid, is a dicarboxylic organic acid. Itaconic acid is characterised by a double bond between two adjacent carbon atoms and two carboxylic acid functional groups. This metabolite has antimicrobial properties and can disrupt the citric acid cycle in invading pathogens, hindering their growth. Itaconic acid has two steps in how it dissociates resulting in an acidity pK_a of 3.84 and 5.55.	*Aspergillus* sp. (F) *Ustilago maydis* (F)	A relatively weak acid. It is detrimental to the cementitious matrix through acidolysis causing dissolution of the solid phase.	(Liaud et al., 2014; Sterflinger, 2000; van der Straat et al., 2014)
Succinic $C_4H_6O_4$	Succinic acid is recognised as one of twelve bio-based building blocks, as designated by the U.S. Department of Energy, owing to its potential to facilitate the advancement of the bio-based economy. The	*Aspergillus* sp. (F)	Succinic acid is highly aggressive and generates calcium salts within a cementitious mix such as the compound calcium succinate trihydrate, an insoluble salt. Degradation	(Fomina et al., 2007; Liaud et al., 2014; Mariam Ninan et al., 2020; nova-Institut GmbH,

	molecular architecture of succinic acid presents a viable alternative to phthalic anhydride and adipic acid in applications involving plasticizers and polyurethanes, thereby offering promising avenues for the development of biobased polymers, notably exemplified by polybutylene succinate. As a diprotic acid it has an acidity pK_a of 4.21 and 5.64.		within the matrix is zonal, forming an amorphous outer degraded zone made of aluminium and iron and an inner zone with the principates of the calcium salt.	2018; Rashid et al., 2004)
Pyruvic $C_3H_4O_3$	Pyruvic acid is a simple α-ketoacid with a carboxylic acid and ketone functional group. It has an acidity pK_a of 2.45.	*Aspergillus* sp. (F)	A relatively weak acid. It is detrimental to the cementitious matrix through acidolysis causing dissolution of the solid phase. Pyruvic acid may play a role in fostering the precipitation of calcium carbonate when mixed into a cementitious matrix which includes *Bacillus* sp. It may do so by serving as a carbon source.	(Jestin et al., 2004)
Propionic $C_3H_6O_2$	Propionic acid, also known as propanoic acid, is a naturally occurring carboxylic acid with the chemical formula CH_3CH_2COOH. It is a simple organic compound consisting of three carbon atoms, six hydrogen atoms, and two oxygen atoms. Propionic acid is a liquid at room temperature and has a pungent, unpleasant odour. Propionic acid has material science applications. It is used in the synthesis of various compounds, including propionate-based plastics and resins. It is miscible with water and possesses qualities like other carboxylic acids such as formic and acetic acids. It has an acidity pK_a of 4.88.	*Aspergillus* sp.(F) *Propionibacterium* sp. (B) *Coprothermobacter platensis* (B)	It displays limited antimicrobial properties against several moulds and bacteria. It is a medium strength acid biodegrading lime and concrete surfaces.	(Gonzalez-Garcia et al., 2017; Jestin et al., 2004; Liaud et al., 2014)
Butyric $C_4H_8O_2$ + Isobutyric acid $C_4H_8O_2$	Butyric acid, also known as butanoic acid, is a short-chain fatty acid. butyric acid is a carboxylic acid, consisting of a four-carbon alkyl chain terminated by a carboxyl group. It is a saturated fatty acid, meaning that it lacks double bonds in its carbon chain. The presence of the carboxyl group allows it to exhibit acidic properties, as it can release protons (H+) in aqueous solutions. As a carboxylic acid it reacts with bases and will corrode metals.	*Aspergillus* sp. (F) *Clostridium butyricum* (B)	Found on cementitious surfaces, the fungal microbes are the most common producing butyric acid at a comparable strength with acetic acid and forms complexes in aqueous solution with elements Ca, Al, Fe and Si. Butyric acid, with its strong odour, has been historically associated with the preservation of cultural heritage items, such as books, manuscripts, and	(Dyer, 2017; Jestin et al., 2004; Liaud et al., 2014)

	Both butyric acid and the isomer Isobutyric acid have an acidity pK_a of 4.84.		textiles. The acid can be released during the decomposition of certain materials and can pose a threat to the preservation of these artifacts.	
Ascorbic $C_6H_8O_6$	A water-soluble furan-based lactone of 2-ketogluconic acid, forming a mildly acidic solution. Ascorbic acid has applications in the field of material science. It is often used as a reducing agent in chemical processes and as a preservative to prevent the oxidation of food products. It has an acidity pK_a of 4.17.	*Aspergillus* sp. (F) *Galdieria partita* (A)	Ascorbic acid biosynthesis proposed by Fu et al., (2020) factors as a major photoprotective process in red algae.	(Fu et al., 2020; Liaud et al., 2014)
Lactic $C_3H_6O_3$	Lactic acid is a soluble α-hydroxy propionic acid belonging to the carboxylic acid family. There are two enantiomers of lactic acid, namely L-lactic acid and D-lactic acid, with L-lactic acid being the predominant form in biological systems. Lactic acid can be polymerized to form polylactic acid (PLA), a biodegradable and bioactive polymer. It has an acidity pK_a of 3.86.	*Aspergillus* sp. (F), *Cladosporium* sp. (F) *Lactobacillus* sp. (B)	Lactic and acetic acids cause dissolution and leach acid-susceptible constituents out of cementitious materials. The loss of the leached minerals causes an increase in capillary porosity, a loss of cohesiveness and failure in the strength of the material.	(Dyer, 2017; Liaud et al., 2014; Sterflinger, 2000; Zhang et al., 2023)
Malic $C_4H_6O_5$	Malic acid is a 2-hydroxybutanedioic dicarboxylic acid produced with a chemical structure consisting of two carboxyl groups (-COOH) attached to an alkane chain which contributes to a sour taste when added to foodstuffs. It is an important component in the Calvin Cycle and Citric Acid Cycle. It has an acidity pK_a of 3.4.	*Aspergillus* sp. (F)	A relatively weak organic acid though may be detrimental to the cementitious matrix found in building structures caused by acidolysis which dissolves the solid phase. The formation of the acid salts may lead to expansion and contraction within the cement structure forcing the creation of microfractures.	(Liaud et al., 2014; Mariam Ninan et al., 2020)
Tartaric $C_4H_6O_6$	It is found naturally in many fruits and incorporated into food groups and baking products as antioxidant E334. It has two dissociation constants with an acidity pK_a of 2.98 and 4.34.	*Aspergillus* sp. (F)	Tartaric acid is used as a retardant on cement-free mortars and performs as a superplasticiser, extending the mortar fluidity by up to 2 hours in calcium sulphoaluminate cements. In a cementitious mix, tartaric acid is moderate to low in aggressive attack, forming insoluble calcium tartaratetetrahydrate which forms a moderate protective layer.	(Coppola et al., 2018; Liaud et al., 2014; Mariam Ninan et al., 2020)
Malonic $C_3H_4O_4$	It is a dicarboxylic acid of medium strength which is characterised by two carboxyl (COOH) functional groups located at the ends of a carbon	*Penicillium* sp. (F)	Malonic acid is a precursor for several chemical compounds, which can form protection against UV light, corrosion, and	(Sterflinger, 2000)

	chain. Its structural formula can be represented as HOOC-CH2-COOH. The presence of two carboxyl groups imparts important chemical properties and reactivity to malonic acid. It is a building block chemical in B vitamin synthesis. It is highly hydrophobic and virtually insoluble in water. It has an acidity pK_a of 2.85.		oxidation. Isolated it has few applications and the compound is relatively unstable.	
Tenuazonic $C_{10}H_{15}NO_3$	Tenuazonic acid is a secondary metabolite belonging to the class of tetramic acids and is a mycotoxin which acts as an inhibitor on ribosomal protein synthesis. It contains a tetramic acid moiety, which is a four-membered ring with an amide group, and an isoprenyl side chain with properties that are phytotoxic and cytotoxic. It has an acidity pK_a of 3.5.	*Penicillium* sp. (F) *Alternaria* sp. (F) *Chaetomium* sp. (F) *Aspergillus* sp. (F) *Ulocladium* sp.(F) *Paecilomyces* sp. (F)	Fungal specimens isolated from infested building materials are usually found on water damaged sections of gypsum board, wallpaper, wood, and chipboard. Symptoms include allergy, fatigue, skin, and mucous membrane irritation. Visually fungal growth on building materials can vary from discolouration to large biomass growths and cottonlike colonies. Surface discolouration indicates decay and material solubilisation.	(Nielsen et al., 1999; Sterflinger, 2000)

Table 23: The impact of environmental changes on colonising microbial populations. Environmental changes invoked by fluctuations in the climate and the impact on microbial populations and responses on the deterioration of traditional materials

Environmental factors	Implications of the microbial response	References
Temperature increases and fluctuations	Microorganisms exhibit susceptibility to fluctuations in temperature, which can influence the growth kinetics and the concomitant production of organic acids. An increase in the density of a microbial population serve to foster horizontal gene transfer between species, with implications on the processes of organic matter decomposition ecosystems. This phenomenon exerts a discernible impact on the efficacy of the carbon cycle. An additional long-term consequence effects the structural integrity of edifices, encouraging the dissolution of salts and the formation and subsequent expansion of microfractures within building substrates. Variations in temperature and relative humidity can induce an escalation in the precipitation of moisture within interior spaces, leading to the accumulation of moisture on wall coverings, wood surfaces, and wall finishes encouraging the colonisation by mould and bacteria, thereby fostering their adhesion, proliferation, and the attendant biodegradation of the underlying substrates.	
Altered precipitation patterns	Climate change-induced alterations in precipitation patterns result in modifications to moisture levels and the saturation of building render. These fluctuations in moisture content influence the development and functionality of microbial communities. Specifically, elevated moisture levels create conducive environments for microbial proliferation, leading to enhanced production of organic acids and an increased demand for nutrients derived from the substrate. During periods of intense rainfall, building render and mortar joints become oversaturated, thereby promoting the growth and penetration of hyphae into the substrate, ultimately expediting material deterioration.	
Changes to pH	The interplay between organic acid-producing microorganisms and the pH of the immediate surroundings is dynamic and environmentally significant. The extent of pH modulation is linked to alterations in climatic conditions that either facilitate or impede the proliferation of microorganisms. Among the various climatic factors, changes in the atmospheric concentration of carbon dioxide play a key role, contributing to the phenomenon of rain acidification. Acidification, in turn, influences the pH of lime or cement-based materials, ultimately promoting the accelerated dissolution of their alkaline salt components. Such environmental shifts have the potential to foster the abundance and diversity of organic acid producers within the ecosystem. Generally, complexation reactions, the reaction of elements in the substrate with different organic acids, occurs when metal ions have been released into solution following initial acidolysis. The elements commonly encountered in lime, hydraulic lime, and cement matrices, namely calcium (Ca), aluminium (Al), magnesium (Mg), and iron (Fe), exhibit varying propensities to form salts with organic acids, though aluminium and iron salts tend to display relatively lower solubility. In particular, calcium ions exhibit a notable affinity for binding with a range of organic acids, excepting tartaric,	(Bertron et al., 2009; Larreur-Cayol et al., 2011; Mariam Ninan et al., 2020)

	oxalic, and citric acids. This proclivity for solubilization results in leaching, a phenomenon that becomes more pronounced as rain saturation increases. Consequently, the enhanced leaching of calcium ions contributes to the degradation of lime and concrete structures. The extent of degradation is further contingent on the properties and solubilities of the calcium salts formed in the process. For instance, calcium acetate ($Ca(C_2H_3O_2)_2$), calcium propionate ($Ca(C_3H_5O_2)2$), and calcium lactate ($Ca(C_3H_5O_3)_2$) are soluble in water. Other Ca-organic acid salts, such as calcium citrate ($Ca_3(C_6H5O_7)_2$), calcium malate ($C_4H_4CaO_5$) and calcium oxalate (CaC_2O_4), are sparingly soluble. These differential solubilities contribute to the complex chemical dynamics that underlie the intricate relationship between organic acid-producing microorganisms, environmental pH, and the degradation of lime, hydraulic lime, and concrete structures.	
Environmental changes invoking phase changes in microbial community composition	Climate changes have the capacity to induce shifts in the composition and diversity of microbial communities. Microorganisms participate in intricate ecological networks, wherein modifications to the community structure can influence the production of organic acids and secondary metabolites. Fluctuations in environmental factors such as temperature, moisture levels, and nutrient availability can tip the balance in favour of specific microbial taxa, thus potentially modulating the dynamics of organic acid production. On a broader geographical scale, alterations in climate patterns may exert adverse effects on the functional roles played by microbial communities involved in carbon and nutrient recycling processes. This, in turn, may contribute to the establishment of positive feedback loops elevating atmospheric carbon and methane levels.	(Gutarowska & Czyżowska, 2009; Komar et al., 2023)

Table 24: An overview of encapsulation technologies employed across industry sectors. Not all encapsulation techniques are suitable for live cell inclusion due to processing stresses which reduce cell viability. Electrospinning, extrusion, emulsification and coacervation have proven successful for the encapsulation of live cells

Encapsulation technique	Description	Reference
Spray chilling/freeze-drying and cooling	Spray cooling, chilling and spray drying are similar immobilisation processes. The core material is dispersed in a liquified coating and atomised into chilled air, solidifying the capsule. This process is ideal for a material such as oil, (melting point around 32°C). Freeze-dried probiotics encapsulated in liquified lipids form beads between 75 -300 µm. Modifications to the melting point of the capsule material controls content release when temperature variation provides the trigger point. Spray drying is simple, fast, scalable, and cost-effective and used for bioactive ingredients such as vitamins, probiotic living cells, antioxidants, and enzymes. Spray drying and freezing live cells does reduce cell viability which can be improved using cryogenic protectants prior to freezing, though more costly and energy inefficient. Freeze drying compared to spray drying is faster and cheaper while achieving higher cell viability rates.	(Bakry et al., 2016; Celli et al., 2015; Jafari & McClements, 2017; Sarabandi et al., 2018)
Coacervation *Figure 71*	Coacervation is a technique that can address storage, light, oxygen, or heat sensitivity issues which threaten to deactivate the capsule contents. The process of coacervation occurs as a pH-sensitive ionic attraction between charged ions. Gelatin and Arabic gum are biodegradable coacervation compounds which change electrostatic attraction between ions based on changes in pH. Using gelatin as a coacervation material for bacterial cell capsules is not effective due to the relatively high temperatures (60°C) required to dissolve the compound. Gelatin may also be unacceptable as an unsustainable animal product. Gum Arabic, a complex polysaccharide predominantly composed of arabinogalactan and glycoprotein (1%) exhibits low-energy solubility and can more easily form protective capsules which are supportive of a live-cell coacervation process.	(Devi et al., 2016; Kruif et al., 2004; Muhoza et al., 2022; Napiórkowska & Kurek, 2022)
Fluid bed coating	Fluid bed coating keeps in motion a solid form of the core material. The liquid coating is sprayed through a nozzle, circulating the compound to be encapsulated through the high velocity air, depositing an atomised coating material on the core surface. The process is most effective for encapsulating solid materials. The coating thickness, level of protection and speed of capsule decay can be controlled by extending the period of treatment.	(Coronel-Aguilera & San Martín-González, 2015; Guignon et al., 2002; Teunou & Poncelet, 2002)
Liposome entrapment	Liposomes possess an aqueous core surrounded by a phospholipid membrane which spontaneously forms when phospholipids and an aqueous medium are mixed. The core contents of the capsule are efficiently contained suspended and insoluble in the surrounding phospholipid medium.	(da Silva Malheiros et al., 2010)
Rotational suspension separation	Rotational suspension separation suspends the core in the capsule material dropping it on to a rotating disk. The encapsulated particles spin off-the-disk which can then be either dried or chilled for storage and transportation.	(Mishra, 2019)

Extrusion *Figure 72, Figure 73*	Extrusion mixes a dense liquid core with the capsule material, forcing the mix into a dehydrating liquid. Depending on the extrusion technique, the hardened material may then be fractured into similar size pieces and dried. The larger size of extruded capsules may not be suitable for all applications. Extrusion can also be used as a microencapsulation process extruding microorganisms through a needle to form droplets which fall into a hardening solution. The needle diameter controls the bead size, the distance of the fall into the hardener influences the shape. Alginate, a linear heteropolysaccharide, is a biopolymer extrusion material formulated from *D*-mannuronic and *L*-guluronic acid the exact proportions of which vary depending on the algal source. Divalent calcium cations preferentially bind to the *L*-guluronic acid forming an extruded gel. A cell suspension therefore can be added to a sodium alginate hydrocolloidal solution which will harden when dropped into a $CaCl_2$ solution. The ionically cross-linked calcium alginate gel forms a biocompatible three-dimensional matrix sphere around the cell suspension. The alginate concentration can vary from 0.6% with 0.3M $CaCl_2$, to 1-2% alginate with 0.05 to 1.5M $CaCl_2$. Increasing the sodium alginate concentration, needle diameter and the proportion of *L*-guluronic acid reduces the bead size. Extrusion is a low-cost technology which can support a cell survival rate up to 95%. Bead size by extrusion may reach diameters of 2-5 mm. Modifying the droplet extrusion parameters can produce pendent droplets to a continuous stream by adjusting the jet velocity, the liquid surface tension and density, and the nozzle diameter. A coaxial air jet around the needle can increase the velocity drop which has a flow rate limit of no more than 30 mLh^{-1}.	(Huguet et al., 1994; Jankowski et al., 1997; John et al., 2011; Krasaekoopt et al., 2003; Smidsrød & Skjåk-Bræk, 1990)
Emulsification *Figure 74*	A small volume of cell-polymer suspension, the discontinuous phase, is added to a large volume of vegetable oil, the continuous phase and homogenised to form an emulsion. Insoluble polymer gel particles are formed by cross linking the polymer within the oil phase. The size of the encapsulated particles is controlled by the agitation speed of the solution. Adding emulsifiers like sodium lauryl sulphate, lower the surface tension reducing the capsule size down to 25 μm. Other additives can support the emulsification process like carrageenan, a marine algal polysaccharide, cellulose acetate phthalate, a polymer insoluble at a pH of 5 or lower, and chitosan, derived from crustacean shells. Carrageenan encapsulation beads are formed at higher temperatures and stabilised by the addition of potassium ions in the form of potassium chloride. Calcium ion enriched alginate added at a slightly acidic pH of 6.5 prior to the emulsion formation forms gel beads tolerant of carbonate environments. Alginate stability is fragile in the presence of phosphate, lactate and citrate which act as calcium ion chelating agents, weakening the gel encapsulation which may provide a design mechanism to control contents release. Scaling up emulsion production is expensive compared to extrusion due to the inclusion of oil. Microorganism survival rates range between 80 – 95%, and encapsulated bead size diameters are smaller than by extrusion, between 25 μm to 2 mm.	(Abang et al., 2012; Audet & Lacroix, 1989; Krasaekoopt et al., 2003; Poncelet et al., 1995; Smidsrød & Skjåk-Bræk, 1990)
Inclusion complexation	Encapsulation takes place at the molecular level using cyclodextrins which contain a hollow, hydrophobic centre, and a hydrophilic outer membrane surface. Molecules of lower polarity replace the water	(Alonso et al., 2014)

	molecules in the centre of the cyclodextrin causing the complex to precipitate out of the liquid as the solubility decreases.	
Coaxial electro-spraying and electrospinning *Figure 75*	Coaxial electrospraying is an electrohydrodynamic process producing multilayer microparticles with coaxial electrified jets. Coaxial electrospinning and electrospraying delivers single or double step encapsulation of water-soluble bioactive agents including live cells, into nanofibers or nanocapsules. Encapsulated cell storage stable at room temperature - with 40-day viability - and 130 days at 5ºC has been achieved. Cells remain viable in calcium alginate microbeads as small as 300 µm Small diameter beads are produced by extruding the polymer solution through an electrostatically charged needle. The collecting plate is grounded or has an opposite electrostatic charge. The extruding droplet from the needle tip accumulates a charge and is pulled toward the collecting plate. The droplet diameter is controlled by reducing needle diameter, increasing electrostatic potential, solvent evaporative volatility, reducing drop distance, diluting polymer concentration, and reducing the flow rate.	(Arpagaus, 2019; Jiang et al., 2014; Rostamabadi et al., 2020; Tapia-Hernández et al., 2017); (Hallé et al., 1994)

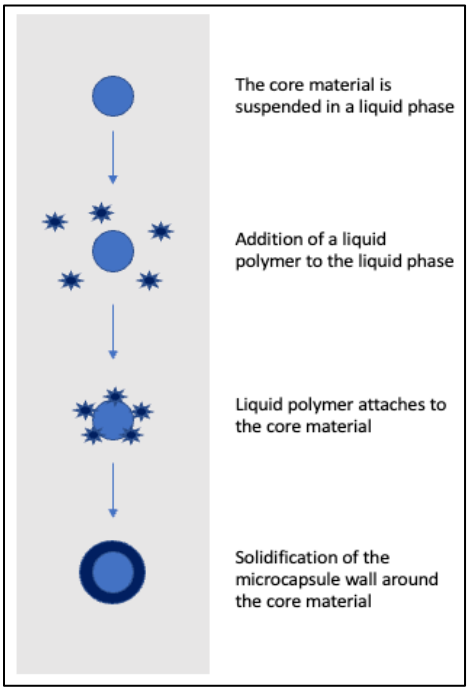

Figure 71: Coacervation process: Diagram outlining the steps in the coacervation process forming a microcapsule wall around the core material. The core material is suspended in a liquid phase separated from the polymeric solution which surrounds the core. The coating, such as the polysaccharide complex, solidifies, encapsulating the core material. The two liquid phases separate into a concentrated colloidal phase, the coacervate, and a second diluted colloidal phase. Similar to emulsification, coacervation follows four stages, an initial suspension of the core material in the liquid phase, secretion of the coacervate liquid phase, deposition of liquid polymer around the core material and the firming of the microcapsule wall

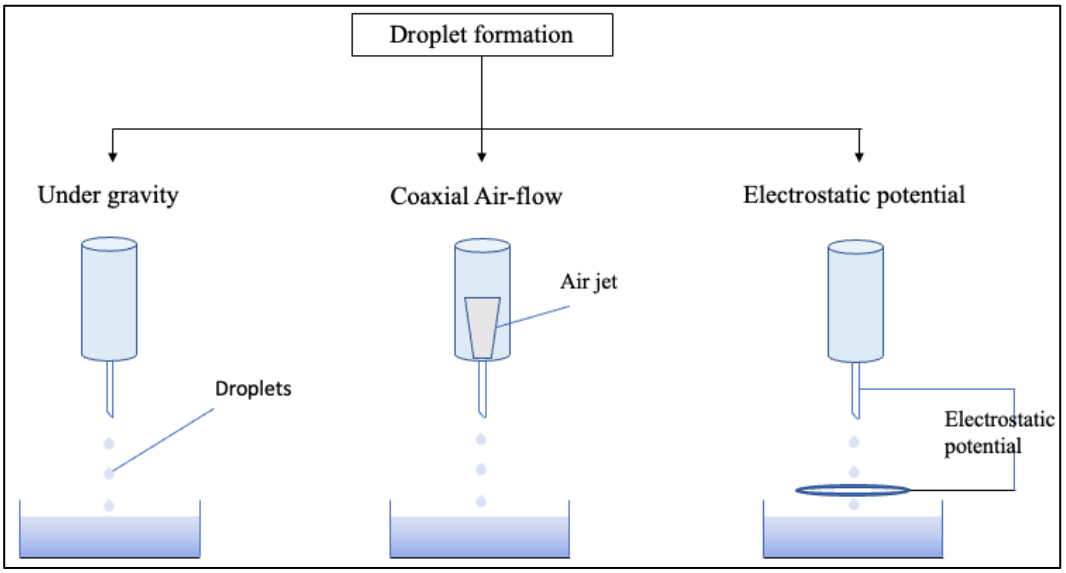

Figure 72: Three types of extrusion mechanisms. (i) Droplets form under gravity from the dispensing nozzle. A low-cost technique but subject to nozzle blockage. (ii) Adding a coaxial air-jet increases droplet delivery speed and reduces the capsule size. (iii) Applying an electrostatic potential increases the dispensing force. Droplet characteristics are determined by the potential difference, polymer viscosity and nozzle diameter

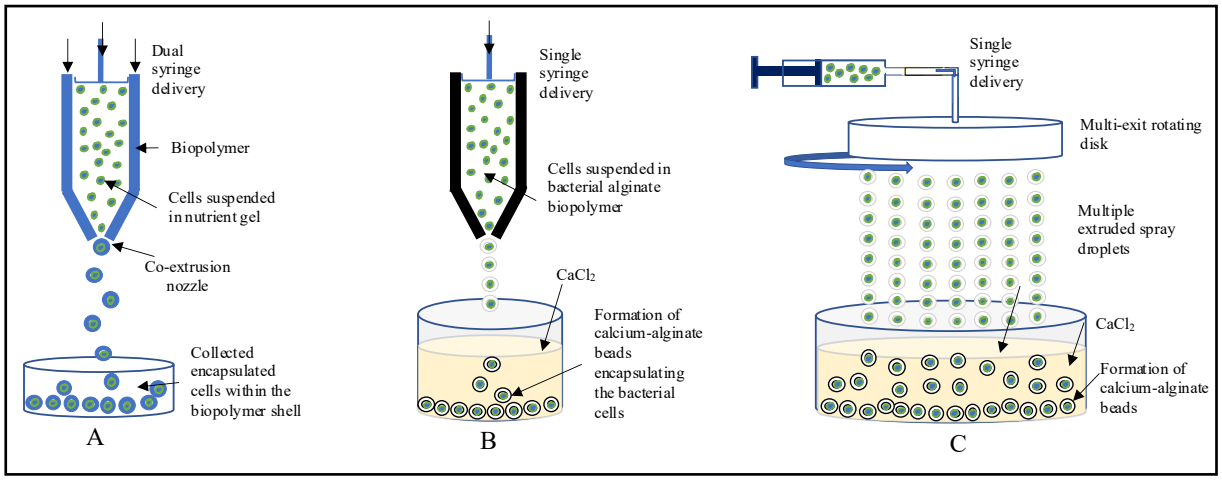

Figure 73: Low cost, high-volume encapsulation techniques. These extrusion processes are designed to maintain relatively low stress on the capsule contents to deliver high cell viability post-encapsulation. In (B) and (C), cells are suspended in a nutrient hydrocolloid solution such as alginate or cellulose. In (A) a dual syringe delivery separates the biopolymer from the nutrient/cell solution, which are combined in a co-extrusion nozzle. The biopolymer solution evaporates on exiting the nozzle and the capsule falls into a collecting dish. (B) and (C) drop the unformed capsules into a hardening solution of multivalent cations, in this instance calcium chloride, triggering the polymer to form a cross linked three-dimensional matrix around the cells. (C) uses a rotating disk with multiple extrusion nozzles to improves the optimal cell inclusion per droplet and increases the rate of production of formed beads. Extrusion encapsulation has demonstrated high cell viability compared to other methods discussed in Table 24

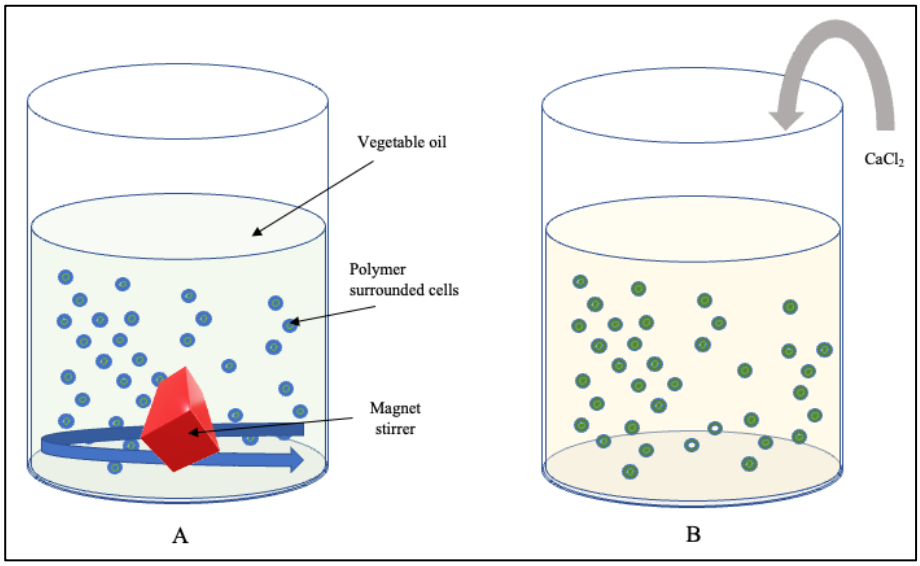

Figure 74: Encapsulation by emulsification – (A) cell droplet dispersion by stirring in oil (emulsifying), (B) addition of $CaCl_2$ hardens the polymer forming the encapsulated bacterial cells. Capsules retain a liquid core. The technique is based on the continuous phase (vegetable oil) and the discontinuous phase (cell-polymer suspension), the speed of stirring homogenises the liquids forming a water-in-oil suspension cross-linking the water-soluble polymer with $CaCl_2$

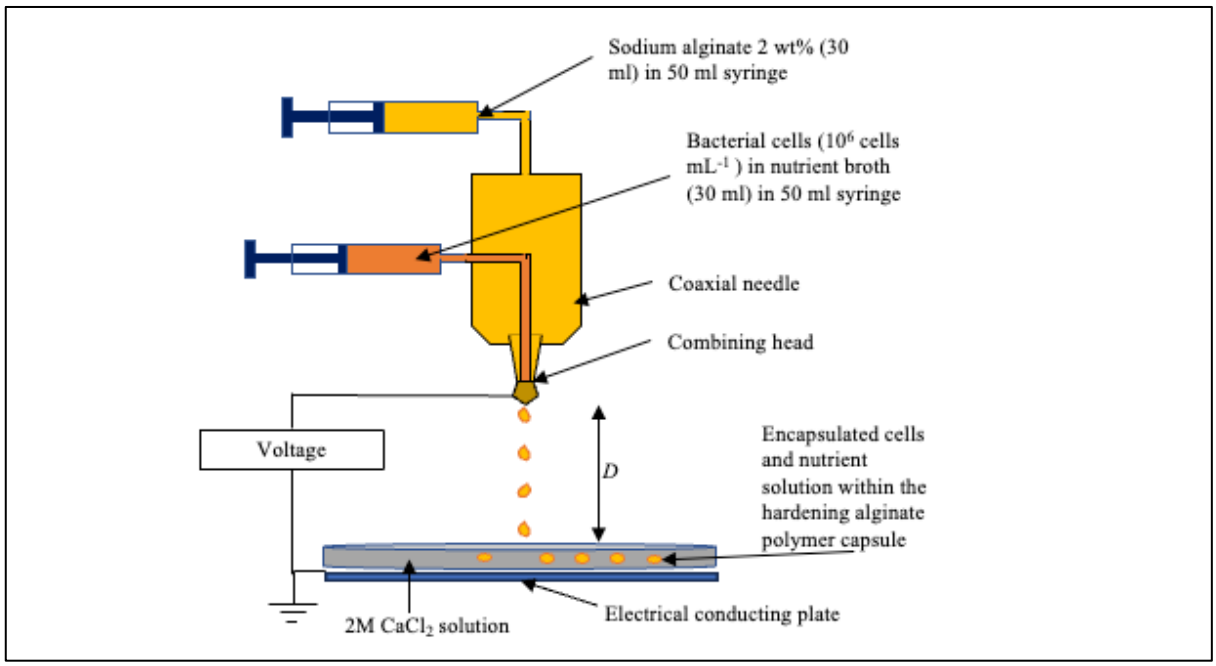

Figure 75: Coaxial electrospraying: Droplets exuded from the coaxial needle become highly charged and produce a repelling force against the surface tension of the polymer in the droplet. This produces smaller droplets which propel toward the charged collecting base, increasing the speed for evaporation of any volatile solvents in the droplet mix. Sodium alginate and the cell/nutrient solution are contemporaneously sprayed from the coaxial jet. This process is termed electro-coextrusion. Coaxial capsule composites demonstrate stability and high porosity. Coaxial diameter is determined by fluid viscosity, solute concentration, the inclusion and type of biological material, the applied voltage, room temperature and the distance, D, through which a solvent may evaporate. If the optimum distance D is exceeded, the capsule breaks on hitting the hardening solution and fails to form a viable unit

Table 25: An overview of several key biopolymer polysaccharides and amino-polysaccharides sourced from plants, animals, and microorganisms

Polysaccharide	Source	Structure and function	Reference
Agar	Agar is obtained from the *Rhodophyceae* class of red algae. Agar is a low-cost renewable biopolymer source that can substitute for oil-derived plastic-based food packaging.	Seaweed-derived alginate composition varies depending on the area of the plant it is harvested and the growth conditions. Agar is a thermo-reversible hydrocolloid and can repeatedly transform between a highly viscous gel and a low viscous solution, unlike alginate in which gelation is irreversible. Pure agar is brittle, with high moisture permeability and poor thermal stability. Biopolymer blends improve agar functionality such as antimicrobial, hydrophobic, and higher plasticity properties. Agar is composed of agaropectin and agarose, the latter a linear polysaccharide of repetitive units of D-galactose and 3-6, anhydro-L-galactose, linked by alternating α-(1-3) and β-(1-4) glycosidic bonds.	(Armisén, 1991; Martínez-Sanz et al., 2019; Mostafavi & Zaeim, 2020; Rehm & Valla, 1997; Remminghorst & Rehm, 2006)
Alginate	Alginate is a collective term for the biopolymer sourced from marine brown algae *Phaeophyceae,* brown seaweeds such as species of *Ascophyllum*, and seaweeds *sargassum* and *turbinaria* and the red algae, *Rhodophyceae.* Alginate is also produced by *Azotobacter vinelandii* and several *Pseudomonas* species which produce a similar polymer structure to alginate.	Alginate is an irreversible hydrocolloid material. It can be set to form strong and impressionable material using sodium alginate and aqueous calcium sulphate ($CaSO_4$), forming calcium alginate and sodium sulphate (Na_2SO_4). This is a fast reaction which can be slowed by the addition of trisodium phosphate (Na_3PO_4). Sodium or calcium alginate added to concrete mortar as a replacement for synthetic superabsorbent polymers (SAP) removes the risk of decreased mortar strength commonly found with SAP applications. Alginates consist of linear copolymer units of β-(1-4) linked d-mannuronic acid and β-(1-4) linked l-guluronic acid and can form a three-dimensional matrix to provide a supportive environment to encourage cellular growth.	(Abbas & Mohsen 2020; Arab et al., 2021)
Carrageenan	A family of linear sulphated anionic polysaccharides extracted from red seaweeds such as *Chondrus crispus*.	Carrageenan is made up of alternating 3-linked β-D-galactopyranose (G-units) and 4-linked α-D-galactopyranose (D-units) or 4-linked 3,6-anhydro-α-D-galactopyranose (DA-units). The biopolymer is used in delivery systems of bioactive ingredients in the food industry based on its flexibility to form several delivery structures including hydrogels, emulsions, microcapsules, and nanotubes. Kappa (κ)-carrageenan has been investigated as a viscosity modifying admixture in cement-based materials indicating a mixture of 0.5% (κ)-carrageenan showed the highest compressive strength with a beneficial effect as a superabsorbent agent on shrinkage of low water-to-cement concrete.	(Boukhatem et al., 2021; Dong et al., 2021)
Cellulose	An abundant supply of cellulose biopolymer material sourced from	Cellulose is a long-chain highly polymerised molecule, the degree of polymerisation differing based on the methods employed for its	(Klemm et al., 2005; Peresin et al., 2010; Rinaudo, 2006)

	plants, microorganisms, and tunicates, a marine invertebrate capable of biosynthesising cellulose.	extraction. It is a linear homopolymer of β-D glucose monomers covalently lined together by 1-4 glycosidic bonds. Cellulose comprises of several fibres bound with extensive hydrogen bond linkages. This multi-layered assembly is formed from fibrils, and cellulose molecules. The biopolymer is highly crystalline, hydrophilic, though insoluble in water and most common solvents. The degree of crystallisation and fibre-arrangement is determined by the cellulose source. Cotton fibre is approximately 80% crystalline, compared to wood cellulose at around 50% crystalline.	
Chitin	One of the most abundant amino-polysaccharide biopolymers. Chitin is found in exoskeletons, insect cuticles and fungal cell walls providing strength and structural support. It is extracted from the shells of crabs, shrimps, and lobsters.	A linear polymer with acetamide or amine functional groups attached to C_2 of the glucose monomer and linking 2-deoxy-D-glucopyranose together by β(1-4) glycosidic bonds. Extensive intra and inter-polymer chain hydrogen bonds which results in highly crystalline microfibril formation. Chitin is a tough biopolymer, structurally comparable to cellulose and functionally comparable to the fibrous protein keratin. It is highly insoluble in water and most solvents and forms strong biopolymer coatings resulting in it being adopted in several industrial processes. When combined in an organic matrix with calcium carbonate, it forms a tough composite.	(Chen et al., 2015; Ravi Kumar, 2000; Rinaudo, 2006)
Chitosan	A derivative of chitin formed due to partial deacetylation. It can be made by treating shells comprising of chitin with sodium hydroxide. Commercially the degree of deacetylation of chitin ranges from 60-100%.	Chitosan can be dissolved in weak acids with pH of 6.5 or less, such as hydrochloric acid, and organic acids such as formic, acetic, oxalic, and lactic acids. Chitosan when added as a coating on alginate beads, forms a membrane around the alginate strengthened by the positively charged chitosan amino groups with negatively charged carboxylic acid alginate groups. Beads prepared with a high molecular weight chitosan provide encapsulated cells with a higher survival and viability due to the thicker bead membrane. This has been demonstrated in simulated gastrointestinal conditions. Similarly, poly-L-lysine can form strong polymer complexes with alginate providing similar encapsulation coating properties. Chitosan is soluble in water.	(Ravi Kumar, 2000; Rinaudo, 2006; Chávarri et al., 2010; Cui et al., 2008)
Inulin	Inulin is a plant polysaccharide found in more than 36,000 plant species, though it is mainly extracted from chicory. It is an energy storage polysaccharide and is found in roots and rhizomes.	Inulin is soluble in water creating an osmotic switch as it alters its osmotic potential by adjusting the level of inulin polymerisation. It is a heterogenous collection of linked fructose polymers which can form a three-dimensional gel network ideal for use in emulsions and spray-drying encapsulation. Inulin is a combination of fructose and glucose molecules forming polydisperse β(2-1) fructan. A glucose molecule is usually found at each end of the fructose chain linked by α(1-2) bonds.	(Dobre et al., 2008; Goëlo et al., 2020; Phelps, 1965)
Pectin	Pectin is a high molecular weight polysaccharide that contributes to plant cell	Pectin is a polymer with a linear structure comprising a large number of galacturonic acid monomer units linked via α-(1→4)-glycosidic bond forming the polymer backbone.	(Mohnen, 2008; Rehman et al., 2019; Rosales & Fabi, 2023)

	wall structure and is commonly associated as a gelling agent. It is a complex structural and functional polysaccharide.	In addition to its structural role, pectin regulates cell-cell adhesion and intracellular signalling molecules. In addition to applications in the food and pharmaceutical industries, pectin can bind to tumours and reduce growth and cancerous cell migration. It is used to form stable nanostructures to protect, aid absorption and improve the availability of active compounds.	
Pullulan	Pullulan is produced aerobically from starch by the polymorphic fungus *Aureobasidium pullulans*. It is an economically viable polymeric source material made from feedstock fermentation.	Pullulan is a water-soluble, non-toxic, strictly linear glycosic polysaccharide consisting of three glucose units connected by α-(1→4)-glycosidic bonds, connected to each other by an α-1,6 glycosidic bond. It is used in a wide variety of applications including cosmetic products, food additives (inhibits fungal growth), blood plasma substitute and adhesives. It is readily soluble in water, has excellent water retention and low viscosity.	(Cheng et al., 2011; Kraśniewska et al., 2019; Singh et al., 2008)
Starch	Starch is an abundant, renewable biopolymer that has applications across industrial sectors including food, textiles, and pharmaceuticals. It can be extracted from numerous plant sources such as potato (*Solanum tuberosum*), corn (*Zea mays*) and wheat (*Triticum aestivum*).	Starch is a homopolymer comprising amylose and amylopectin. Amylose molecules may have up to ten branches, whereas amylopectin has α-glucose units in its polymeric structure linked with (1→4) glycosidic and α (1→6) bonds. Due to brittleness, starch is best utilised as a biopolymer blend, the application guiding the blend design. Starch based biopolymers are non-toxic and biocompatible, and their biodegradable properties are replacing petrochemical plastics. Starch ethers have been used as additives for hydraulic binders such as cement and lime.	(Datta, 2011; Falcão et al., 2022; Gamage et al., 2022)

Table 26: Sources of biopolymers extracted from microbial biofilms. Bacterial biopolymers have diverse physicochemical and rheological properties derived from the biological building blocks (Tang et al., 2012). Microbial polymer biosynthesis is more economically competitive than non-microbial sources as the extraction and purification processes are lower cost, (Llamas et al., 2012). Pullulan, curdlan, dextran, and cellulose are homopolysaccharide polymers which differ in linkage bonds compared to heteropolysaccharides, affecting their chemical behaviour, synthesis, and industrial applications

Microorganism	Polysaccharide	Chemical structure	Properties	References
Lactobacillales (*Lactobacillus* sp.), *Leuconostocaceae* (*Leuconostoc* sp.), *Leuconostoc mesenteroides*, *Streptococcaceae* (*Streptococcus* sp.)	Dextran $C_{18}H_{32}O_{16}$	Homopolysaccharide, high molecular weight, backbone structure contains glucose with multiple α-(1,6)-linkages. The arrangement of side chain linkages varies between bacterial strains. It is a water-soluble glucan which is highly biodegradable and biocompatible.	Commercial producers *L. mesenteroides* and *L. dextranicum* produce a matrix gel which can strain out macromolecules. It is widely used in clinical applications such as wound care and drug encapsulation as it is non-toxic.	(Hussain et al., 2017; Lapasin, 2012; Naessens et al., 2005; Rehm & Valla, 1997)
Lactobacillus sp. (*L. rhamnosus, L. kefir, L. kefiranofasciens*)	Kefiran ($C_{12}H_{24}O_{11}$)	A capsular water-soluble branched glucogalactan polysaccharide. The exopolysaccharide is produced by lactic acid bacteria.	A non-toxic polysaccharide with antibacterial, antifungal and antitumour properties. Kefiran can enhance gel viscosity and viscoelasticity, chosen for applications requiring scaffolds or encapsulation. Demonstrates antimicrobial activity	(Rehm et al., 2009; Micheli et al., 1999; Moradi & Kalanpour, 2019)
Rhodospirillales, Sphingomonadales, (*Sphingomonas elodea*) *Acetobacter*, (*Acetobacter xylinum*), *Agrobacterium, Pseudomonas, Rhizobium, Achromobacter, Aerobacter, Azotobacter, Sarcina ventriculi, Escherichia, Pseudomonas elodea,*	Cellulose, $(C_6H_{10}O_5)_n$	Cellulose is a linear homopolysaccharide of monosaccharide glucose with 1,3 β- and 1,6 β glycosidic linkage. Unlike plant sourced cellulose, bacterial cellulose has an ultrafine nanofibrous network structures. Cellulose is highly hydrophilic.	The purity of bacterial cellulose is utilised in non-toxic food applications and biomedical formulations. It has a high tensile strength, flexible, a high modulus of elasticity, shear-thinning capacity, and chemical stability. It is also non-toxic and porous, and biocompatible with cell insertions.	(Garrity et al., 1984; Jonas et al., 1998; Klemm et al., 2001)
	Gellan	Gellan is a linear anionic heteropolysaccharide with tetrasaccharide repeating molecules of β-D-glucose, -D-glucuronic acid and α-L-rhamnose. At high temperatures – around 120°C it forms a transparent gel. Gelation depends on the gellan concentration, ionic	Gellan is a stable, elastic gel producing a consistent viscosity matrix in which to suspend cells. Applications are within the food and biomedical industry. Cells can be entrapped using gel beads by extrusion or emulsification and warm gellan gum. Gellan is suitable for immobilisation of thermophilic	(Fialho et al., 2008; Rehm & Valla, 1997; Lapasin, 2012)

		strength, and the use of divalent stabilising cations.	microorganisms that can tolerate temperatures up to 60°C. Citrate or metaphosphate decrease the gel formation temperature thereby accommodating heat sensitive microorganisms.	
Sphingomonas sp. Alcaligenes	Welan ($c_7H_8N_2O_3$)	Water-soluble biopolymer composed of tetrasaccharide backbone with side chains of either L-rhammose or L-mannose substitute on C_3 of the 1,4-linked glucose structure comprising of D-glucose, D-glucuronic acid and L-rhammose.	Welan, due to its stability and viscosity at higher temperatures, has pseudoplastic applications in the food, biomedicine, and industrial sectors such as cement production. It is stable across the pH range and in the presence of calcium.	(Kaur et al., 2014; Chandrasekaran et al., 1995; Vu et al., 2009)
Alcaligenes faecalis, Alcaligenes faecalisvar, Sinorhizobium meliloti	Curdlan $(C_6H_{10}O_5)_n$	Low molecular weight comprising of β-1,3-linked glucose residues, linear, water-soluble linear homopolysaccharide with 1,3 β- and 1,6 β glycosidic linkage.	Curdlan when heated above 55ºC forms an elastic gel. It is non-toxic and can be used in food and pharmaceuticals and bioremediation. Exhibits anti-tumour, anti-HIV, immunomodulatory effects. Poor solubility in water, thermally stable and non-toxic.	(Chandrasekaran et al., 1995; Freitas et al., 2011; Gummadi & Kumar, 2005; Hussain et al., 2017; Lapasin, 2012; Rehm & Valla, 1997)
Pseudomonadales, Pseudomonas aeruginosa, Azotobacter chroococcum, A. vinelandii	Alginate $(C_6H_8O_6)_n$	Linear hetero-polysaccharide of D-mannuronic and L-guluronic acid the composition varies by microorganism source. Guluronic acid content determines the gelling performance. Porosity is reduced by modifying the alginate with additives or coating the beads with polymer. Alginate is a key component of desiccation resistant cysts in *Azotobacter*.	Utilised in several industrial and pharmaceutical applications including cell encapsulation. Enzymes are used for a controlled time-release. It is non-toxic and biocompatible. Increasing the Na^+/Ca^{2+} ratio using chelating compounds such as anti-gelling cations Na^+, Mg^{2+}, phosphate, citrate, or lactate, triggers encapsulated content release.	(A Rehm et al., 2009; Ertesvåg & Valla, 1998; Krasaekoopt et al., 2003; López-Pliego et al., 2018; Mandal et al., 2006; Peteiro, 2018; Rowley et al., 1999)
Xanthomonadales, Xanthomonas campestris	Xanthan $C_{35}H_{49}O_{29}$	High molecular weight anionic hetero-polysaccharide comprising a glucose-unit main chain and side chain trisaccharide of α-D-mannose and acetyl group. It has a β 1,4-linked D-glucose backbone and substituted alternately	The stabilisation properties of Xanthan enables additives to be suspended in the high viscosity polysaccharide gel. Xanthan is used in the cosmetic, health, industrial, and pharmaceutical industries.	(Hussain et al., 2017; Rehm & Valla, 1997)

		with every second glucose residue is a trisaccharide chain. It is water soluble, thermally stable with limited electrical conductivity.		
Aureobasidium pullulans	Pullulan $(C_6H_{10}O_5)_n$	Linear repeating copolymer homopolysaccharide with 1,3 β- glucan and 1,6 β-glycosidic linkage	Pullulan is a water-soluble gum produced aerobically by fungus *A. pullulans*. Used in the medical, pharmaceutical, lithographic, and food industries.	(Freitas et al., 2011; Sugumaran, 2017; R. S. Singh et al., 2008)
Zymomonas mobilis, Bacillus subtilis, Hallomonas, Psuedomonas, Streptococcus, Mycobacterium, Gluconobacter	Levan $(C_{18}H_{32}O_{16})_n$	A homopolysaccharide of fructose units. The enzyme levansucrase breaks the sucrose bond between glucose and fructose, polymerising the units. Fructose composition is a-typical for a biopolymer.	Solubility is temperature dependent in water and oil. It is insoluble in several organic solvents such as methanol and ethanol. The molecular branching and hydroxyl groups provides the biopolymer with cohesive strength	(Freitas et al., 2011; Öner et al., 2016)
Streptococcus zooepidemicus	Hyaluronic acid $(C_{14}H_{21}NO_{11})_n$	An exopolysaccharide with repeating units of D-glucuronic acid and N-acetyl-D-glucosamine. It is a hydrophilic polymer holding large amounts of water due to hydrogen bonds between the carboxyl and N-acetyl groups.	Hyaluronic acid demonstrates non-immunogenic, non-inflammatory, bacteriostatic, and biodegradable properties. It is not mechanically stable under stress limiting applications to the cosmetic and drug industries.	(Hussain et al., 2017; Morra, 2005; Pitarresi et al., 2009)

Table 27: Proteins as bioactive constituents for storage and conveyance. Numerous proteins have utility for storage, conveyance, and the encapsulation of bioactive constituents. In their native conformation, proteins often lack the requisite robustness, but may be modified to tune their performance as encapsulating agents. This adaptability arises from the intricate folded structures and coiled geometries characterising proteins that are sensitive to environmental factors, such as pH, ionic strength, and subtle variations in temperature. These factors induce alterations in protein hydrophobicity and surface charge, affecting the biopolymer encapsulation coatings. Protein sourced from animal, plant or microbe materials can improve mechanical properties of lime-based material. A challenge to utilise pure protein biopolymers as encapsulation structures, is the vulnerability of the protein molecules to denaturing, resulting from the complex folded protein structure limiting applications away from mechanically stressful environments. It is an area for further research to extending the robustness of proteins and leverage synergies by using proteins as additives to polysaccharide biopolymers

Protein	Chemical Structure	Properties	References
Gelatin	A water-soluble biopolymer produced denaturing collagen in animal bones and skin. It consists of large numbers of glycine, proline, and 4-hydroxy proline residues. Gelation, is a reversible transformation, occurring when the gelatine is cooled below 30 - 35°C.	The amphoteric nature of gelatin interfaces with anionic polysaccharides such as gellan for use in encapsulation. Gelatin becomes positively charged when the pH falls below the isoelectric point. The robust gelatin protection around a cationic polysaccharide can extend the resistance to adverse environmental conditions.	(Deshmukh et al., 2017; Klemm et al., 2005; Krasaekoopt et al., 2003, 2006)
Albumins	A water-soluble family of globular animal proteins. The protein is sensitive to environmental stress, but albumin conjugates have improved functionality. It is found in the serum of mammals and is synthesised almost exclusively in the liver. α-lactalbumin, a major whey protein in milk consists of 123 amino acids in a globular tertiary structure with two lobes forming a calcium-binding site. The protein can exist either calcium bound, or calcium depleted.	Albumins denature on heating. Readily available in pure form, water soluble, biodegradable, and mostly biocompatible. Biopolymer particles can be formed by denaturing globular proteins using a change in pH to encourage repulsion between protein molecules and promote unfolding. Other albumin types include ovalbumin and whey protein. Low viscosity, high solubility, emulsify and form films. A pectin matrix coated with whey increases the protective layer surrounding encapsulated cells. Whey proteins are used in extrusion methods of cell encapsulation.	(Barone et al., 2020; Gerez et al., 2012; Matalanis et al., 2011; Weinbreck et al., 2010; Ying et al., 2012)
Soy	Soy protein is a hydrophilic globular protein consisting of polypeptide chains of protein polymers with disulphide and hydrogen bonds forming 3D structures. The source is widely available and renewable. Calcium ions cause cold-set gelation useful for heat-sensitive cell encapsulation.	The soy protein carrier may be useful for live cell delivery and release within acid to alkaline environments. Soy protein compared to other proteins is economically competitive for large scale production making it one of the most easily available biodegradable polymers. It has a low water resistance which may be improved by developing soy bio-composite	(Chhavi et al., 2017; Q. Liu et al., 2015; Maltais et al., 2010; Yew et al., 2011)
Fibroin	An insoluble protein found in insect silk. Its primary structure consists of a reoccurring amino acid sequence $(Gly\text{-}Ser\text{-}Gly\text{-}Ala\text{-}Gly\text{-}Ala)_n$ in layers of antiparallel beta sheets. Fibroin – albumin polymer blends can produce biopolymer structures with diameters averaging 200 nm.	Biocompatible, biodegradable, and anti-microbial properties. The biopolymer is thermally stable. Silk fibroin can form porous three-dimensional structures and is currently used in three-dimensional printing and tissue engineering.	(Buitrago et al., 2018; Nguyen et al., 2019)
Caseins	Casein proteins, a family of phosphoproteins are isolated from milk. It forms a gel-like structure with the addition of acids, calcium, or	It is found in milk as a suspension due to its relatively hydrophobic property. Useful proteins to build structures with further functionality with other	(H. Chen et al., 2015; Song et al., 2022)

	enzymes. Zein-casein composite nanocapsules are well established as spray-dried complexes.	biopolymers. Utilised within the food and pharmaceutical industries to form encapsulated systems to protect anthocyanins in food products.	
Gliadin and zein	Gliadin is made up of single-chain polypeptides linked by intramolecular disulphide bonds. It has low solubility in aqueous solution excepts at high pH. Zein is a prolamine rich protein found in the endosperm of corn kernels. It is a hydrophobic and thermoplastic material.	Hydrophobic proteins which form biopolymers suitable for encapsulation of active ingredients. Zein is only soluble in aqueous ethanol. Zein fibres have been produced by electrospinning. It can be processed into bioplastic polymers for use in microencapsulation. The main commercial focus is toward delivery of biopackaged ingredients in the food and pharmaceutical industry.	(Neo et al., 2013; Torres-Giner et al., 2010)

Table 28: Bioreactor systems. A system comparison of bioreactor advances and techniques for commercial production of bacterial cellulose. Bacterial cellulose production optimally occurs at pH 6.5. The production of secondary metabolites gluconic, lactic and acetic acid inhibits cellulose production when contaminating the culture medium and must be mitigated within the bioreactor for optimal cellulose delivery. The energy consumption required to run the bioreactors can also be a limiting factor in bacterial cellulose production, however, the growing demand for high-grade bacterial cellulose will encourage the scaling up of more efficient cellulose production technologies

Bioreactor System	**Properties**	**References**
Static culture bioreactor	Bacterial cellulose cultures are strain-dependent, with production taking from ten days to six weeks. Simply constructed static vessels use coconut water, added sugar and as the nitrogen source, ammonium sulphate. This category of reactor yields 5 g L^{-1} in less than a month.	(Iguchi et al., 2000; Lee et al., 2014)
Stirred culture bioreactor	The accumulation of cellulose in the culture increases the viscosity-producing gradients which restrict the nutrient and oxygen supply to the cellulose-producing bacteria. The cellulose yield is dependent on the stirring speed inside the tank, which increases the available oxygen. Over a six-day period, the cellulose yield can range from 1.08 to 1.71 5 g L^{-1}. Adding molasses hydrolysed by sulphuric acid provides the carbon source yielded 5.3 g L^{-1} over 72 hours. The addition of agar, or insoluble microparticles such as clay, reduces shear stress and also increases cellulose production. Increasing the bacterial cellulose cell concentration increases cellulose production as a quorum response, which restricts normal metabolism and increases cellulose synthesis.	(Bae et al., 2004; Bae et al., 2005; Dudman, 1959; Kouda et al., 1996; Stepanov & Efremenko, 2018)
Airlift bioreactors	A simple, easy-to-use design though less suitable for viscous cultures results in a lower cellulose production. The energy used by this method compared to a stirred tank reactor is considerably less. Modifications to the reactor design to improve oxygen transfer plus the addition of agar reportedly produced a yield of 6.8 g L^{-1}.	(Choi et al., 2009; Kim et al., 2012)
Aerosol bioreactors	The supply of oxygen and carbon are limiting factors on the production of bacterial cellulose within a bioreactor. In this design an aerosol reactor attaches bacteria to a rotating drum improving access for the bacteria to the nutrient flow. To overcome the increased build up and viscosity caused by cellulose hampering the drum rotation, nutrients are sprayed from an aerosol onto the drum, producing up to 9 g of dry cellulose per day. Pellicles form at the highest oxygen and nutrient concentration on the top of the liquid media encouraging bacterial growth.	(Hornung et al., 2007)
Membrane bioreactor	A membrane bioreactor achieves a static environment, the membrane forming a large, shallow area on which to promote cellulose production. A hydrophilic membrane with a pore size of 0.45 µm allows nutrients, including oxygen rich air, to pass through one side of the membrane. Cellulose producing bacteria are located on the other side of the membrane. The newly produced cellulose diffuses through the membrane allowing for easier collection and a reduction in the cellulose extraction cost delivering a bacterial cellulose production of 0.4 g (dry mass) m^{-2} h^{-1}.	(Hofinger et al., 2011; Lee et al., 2014; Yoshino et al., 1996)
Horizontal lift reactor	Most bioreactors require the apparatus to be halted while the cellulose is extracted. A horizontal lift reactor cultures the cellulose while continuously extracting the cellulose which is produced along the length of the tank. This technique does not disturb the bacterial cellulose nanofibers present within the bacterial pellicle.	(Kralisch et al., 2010)

Rotary bioreactor	In the airlift and agitation bioreactor systems, the cellulose adheres to parts of the reactor hampering removal. Bacterial cellulose production is reportedly 2.75-fold higher in rotary bioreactor systems compared to statically produced cellulose from the same volume of media. A rotary bioreactor incorporates eight circular discs, redirecting cellulose adhesion, the discs which are part covered in culture medium, with the remaining part exposed to the air. Eight discs appear to be the optimal number to encourage efficient bacterial pellicle production.	(Serafica et al., 2002; Sharma et al., 2022)

Table 29: CFU count indicating viable *B. sphaericus* cells encapsulated in (a) alginate biopolymer and (b) cellulose biopolymer, compared to the non-encapsulated baseline (left). ESY - % drop in CFU/ml over a 6-hour period (right)

Time (hrs)	Baseline No encap. CFU/ml	Alginate encap. CFU/ml	Cellulose encap. CFU/ml
1	11	8.9	6.7
2	11	8.5	5.9
3	11	8.1	5.5
4	11	7.9	5.1
5	11	7.8	4.8
6	11	7.6	4.4

% drop in CFU over 6 hours - alginate	% drop in CFU over 6 hours - cellulose
100.0	100.0
95.5	88.1
91.0	82.1
88.8	76.1
87.6	71.6
85.4	65.7

Table 30: CFU count indicating viable *B. sphaericus* cells immobilised in alginate compared to the non-immobilised baseline (left). ISY - % drop in CFU over 6 hours - alginate hydrogel, (right)

Time (hrs)	Baseline No immob. CFU/ml	Alginate hydrogel CFU/ml
1	11	8.2
2	11	8
3	11	7.9
4	11	7.9
5	11	7.7
6	11	7.7

% drop in CFU over 6 hours - alginate hydrogel
100.0
97.6
96.3
96.3
93.9
93.9

Table 31: Porosity results – wall sections - *B. sphaericus*

Conditions at the time measurements were taken:

Temperature 14° C
Relative Humidity 76%

Porosity was measured using 0.025M solution of Alizarin Red, applied and air dried before depth of penetration measurements were undertaken. Three tests were conducted on each wall section. Results are in mm.

	Depth of stain 0-1 mm					
Wall Section	Test 1	Test 2	Test 3	Average	Standard Deviation	Standard error
A	0.50	0.40	0.45	0.450	0.050	0.029
B	0.60	0.58	0.58	0.587	0.012	0.007
C	0.68	0.70	0.68	0.687	0.012	0.007
D	0.55	0.50	0.58	0.543	0.040	0.023
E	0.20	0.25	0.30	0.250	0.050	0.029
F	0.35	0.35	0.30	0.333	0.029	0.017
G	0.25	0.20	0.20	0.217	0.029	0.017
H	0.50	0.55	0.50	0.517	0.029	0.017

Table 32: Porosity results – lime blocks - *B. sphaericus*

Conditions at the time measurements were taken:

Temperature 20° C
Relative Humidity 60%

Porosity was measured using 0.025M solution of Alizarin Red, applied and air dried before depth of penetration measurements were undertaken. Three tests were conducted on each lime block. Results are in mm.

	Depth of stain 0 - 1 mm					
	Test 1	Test 2	Test 3	Average	Standard Deviation	Standard error
A (Control)	0.50	0.40	0.50	0.467	0.058	0.033
B (non-encapsulation)	0.50	0.60	0.50	0.533	0.058	0.033
C (Encapsulation - alginate)	0.60	0.70	0.70	0.667	0.058	0.033
D (Encapsulation - celluose)	0.40	0.45	0.40	0.417	0.029	0.017
E (2 + 3 non-encap)	0.35	0.30	0.30	0.317	0.029	0.017
F (2 + 3 alginate)	0.50	0.40	0.40	0.433	0.058	0.033
G (2 + 3 cellulose)	0.20	0.30	0.30	0.267	0.058	0.033
H (hydrogel)	0.60	0.50	0.60	0.567	0.058	0.033

Table 33: Porosity results – Lime blocks – *S. elongatus*

Temperature 20° C
Relative Humidity 60%

Porosity was measured using 0.025M solution of Alizarin Red, applied and air dried before depth of penetration measurements were undertaken. Results are in mm.

Twelve lime blocks comprised the experiment for comparison with the results in section A 2. Six blocks formed the control (limewash only), and six blocks were treated with a limewash-encapsulate mix.

The control and alginate encapsulated *S. elongatus* comparisons were carried out on six control and six inoculated lime blocks.

	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Average	Standard Deviation	Standard error
A (Control)	0.500	0.450	0.500	0.450	0.450	0.500	0.475	0.027	0.011
B (Alginate encapsulated SE)	0.600	0.550	0.600	0.600	0.600	0.600	0.592	0.020	0.008

Graph A – Wall Sections (*B. sphaericus*) – Porosity – Statistical results

The null hypothesis compares the porosity results from the Control (1 or section A) and the highest performing formulation of alginate encapsulating *B. sphaericus* (3 corresponding to wall section C).

A two-tail t-test was carried out on the sample data. The difference between the results can be considered non-significant, therefore the null hypothesis must be accepted. This outcome concludes that the data for the porosity of moisture between Control A and Sample C is not significant as indicated by the non-overlap of the standard error bars.

Null hypothesis for data comparisons for *B. sphaericus* Control A and Alginate encapsulation C
H0 - The porosity of the limewash between the Control A and Alginate Encap. C is the same
H1 - The porosity of the limewash between the control A and Alginate Encap. C is different

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	0.45	0.68666667
Variance	0.0025	0.00013333
Observations	3	3
Pearson Correlation	-0.8660254	
Hypothesized Mean Difference	0	
df	2	
t Stat	-6.8005666	
P(T<=t) one-tail	0.01047287	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.02094574	
t Critical two-tail	4.30265273	

Degrees of freedom are 2
Alpha is 0.05, P is significant
The null hypothesis (H0) can be rejected

Graph B – Lime blocks (*B. sphaericus*) – Porosity – Statistical results

The null hypothesis compares the porosity results from the Control (1 or section A) and the highest performing formulation of alginate encapsulating *B. sphaericus* (3 corresponding to wall section C).

A two-tail t-test was carried out on the sample data. The difference between the results can be considered suggestive only and may consider the null hypothesis to be accepted. This outcome concludes that the data for the porosity of moisture between Control A and Sample C is only possibly significant as indicated by the non-overlap of the standard error bars.

Null hypothesis for data comparisons for *B. sphaericus* Control A and Alginate encapsulation C
H0 - The porosity of of the limewash between the control A and Alginate Encap. C is the same
H1 - The porosity of the limewash between the control A and Alginate Encap. C is different

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	0.46666667	0.66666667
Variance	0.00333333	0.00333333
Observations	3	3
Pearson Correlation	-0.5	
Hypothesized Mean Difference	0	
df	2	
t Stat	-3.4641016	
P(T<=t) one-tail	0.03708995	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.0741799	
t Critical two-tail	4.30265273	

Degrees of freedom are 3
Alpha is 0.05, P is therefore only suggestive
The null hypothesis (H0) may be accepted

Graph C – Lime blocks (*S. elongatus*) – Porosity – Statistical results

The null hypothesis compares the porosity results from the Control (1 or section A) and the highest-performing formulation of alginate encapsulating *S. elongatus* (B or 2).

A two-tail t-test was carried out on the sample data. The difference between the results can be considered highly significant, therefore the null hypothesis can be rejected. This outcome concludes that the data for the porosity of moisture between Control A and Sample C is highly significant as indicated by the non-overlap of the standard error bars.

Null hypothesis for data comparisons for *S. elongatus* Control A and Alginate encapsulation B

H0 - The porosity of of the limewash between the control A and Alginate Encap. B is the same

H1 - The porosity of the limewash between the control A and Alginate Encap. B is different

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	0.475	0.59166667
Variance	0.00075	0.00041667
Observations	6	6
Pearson Correlation	0.4472136	
Hypothesized Mean Difference	0	
df	5	
t Stat	-11.067972	
P(T<=t) one-tail	5.2442E-05	
t Critical one-tail	2.01504837	
P(T<=t) two-tail	0.00010488	
t Critical two-tail	2.57058184	

Degrees of freedom are 5
Alpha is 0.05, P is highly significant
The null hypothesis (H0) can be rejected

Note: Explanation for using a different control, excluding *S. elongatus* from wall section study and representation as separate data in Figures 31, 32, 33

Cyanobacterium *Synechococcus elongatus* is phototrophic and capable of extracting carbon dioxide from the atmosphere, forming calcium carbonate crystals around extracellular nucleation sites. The intention of the study was to include the encapsulated *S. elongatus* assessment at the same time as *B. sphaericus*. Unfortunately, the post-COVID impact delayed the availability of viable *S. elongatus* stock from UK or European suppliers. A further delay resulted from an unresponsive microbial batch supplied by the supplier from pre-COVID stock. The supply of a viable sample stock extended the practice element by 6-months. This presented an on-site location challenge. The Northern location of the Isle of Jura, Scotland, reduces available daylight and temperatures lower from August onwards, which would have negatively impacted the study or added a further 12-month delay. To mitigate the challenge, *S. elongatus* encapsulation in limewash evaluation was conducted *in vitro* addressing the seasonal environmental challenges. Based on the previous results from *B. sphaericus* on both wall sections and lime blocks, the cyanobacterium was evaluated within an alginate encapsulate.

Table 34: Cohesion results – wall sections - *B. sphaericus*

Five test strips of adhesive tape were cut, measured and weighed before and after the cohesion test on the respective wall section. The weight of lime removed by the adhesive tape from the surface of the section is adjusted to compare surface abrasion in g cm^{-2}

Wall Section	Test No.	Area of tape (sq mm)	Wt before (g)	Wt after (g)	Diff. (g)	1 sq cm adheres (g)	Standard Deviation
A	1	471.09	0.044	0.050	0.006	0.00127	
	2	331.91	0.031	0.034	0.003	0.00090	
	3	546.04	0.051	0.059	0.008	0.00147	
	4	471.09	0.044	0.051	0.007	0.00149	
	5	417.56	0.039	0.047	0.008	0.00192	
				Average	0.006	0.00141	0.00037

Wall Section	Test No.	Area of tape (sq mm)	Wt before (g)	Wt after (g)	Diff. (g)	1 sq cm adheres (g)	Standard Deviation
B	1	310.5	0.029	0.032	0.003	0.00097	
	2	471.1	0.044	0.047	0.003	0.00064	
	3	610.3	0.057	0.059	0.002	0.00033	
	4	492.5	0.046	0.048	0.002	0.00041	
	5	406.9	0.038	0.042	0.004	0.00098	
				Average	0.003	0.00066	0.00031

Wall Section	Test No.	Area of tape (sq mm)	Wt before (g)	Wt after (g)	Diff. (g)	1 sq cm adheres (g)	Standard Deviation
C	1	461.0	0.030	0.033	0.003	0.00065	
	2	481.8	0.045	0.045	0.000	0.00000	
	3	504.3	0.047	0.049	0.002	0.00038	
	4	417.6	0.039	0.041	0.002	0.00048	
	5	505.4	0.047	0.048	0.001	0.00022	
				Average	0.002	0.00034	0.00025

Wall Section	Test No.	Area of tape (sq mm)	Wt before (g)	Wt after (g)	Diff. (g)	1 sq cm adheres (g)	Standard Deviation
D	1	374.7	0.035	0.039	0.004	0.00107	
	2	471.1	0.044	0.047	0.003	0.00064	
	3	546.0	0.051	0.053	0.002	0.00037	
	4	417.6	0.039	0.041	0.002	0.00048	
	5	460.4	0.043	0.046	0.003	0.00065	
				Average	0.003	0.00064	0.00027

Wall Section	Test No.	Area of tape (sq mm)	Wt before (g)	Wt after (g)	Diff. (g)	1 sq cm adheres (g)	Standard Deviation
E	1	417.6	0.039	0.039	0.000	0.00000	
	2	578.2	0.054	0.058	0.004	0.00069	
	3	492.5	0.046	0.051	0.005	0.00102	
	4	353.3	0.033	0.039	0.006	0.00170	
	5	556.7	0.052	0.053	0.001	0.00018	
				Average	0.003	0.00072	0.00068

Wall Section	Test No.	Area of tape (sq mm)	Wt before (g)	Wt after (g)	Diff. (g)	1 sq cm adheres (g)	Standard Deviation
F	1	471.1	0.044	0.069	0.025	0.00531	
	2	492.5	0.046	0.062	0.016	0.00325	
	3	567.5	0.053	0.054	0.001	0.00018	
	4	417.6	0.039	0.044	0.005	0.00120	
	5	439.0	0.041	0.050	0.009	0.00205	
				Average	0.011	0.00240	0.00198

Wall Section	Test No.	Area of tape (sq mm)	Wt before (g)	Wt after (g)	Diff. (g)	1 sq cm adheres (g)	Standard Deviation
G	1	588.9	0.055	0.078	0.023	0.00391	
	2	546.0	0.051	0.056	0.005	0.00092	
	3	546.0	0.051	0.062	0.011	0.00201	
	4	417.6	0.039	0.046	0.007	0.00168	
	5	546.0	0.051	0.058	0.007	0.00128	
				Average	0.011	0.00196	0.00116

Wall Section	Test No.	Area of tape (sq mm)	Wt before (g)	Wt after (g)	Diff. (g)	1 sq cm adheres (g)	Standard Deviation
H	1	428.3	0.040	0.068	0.028	0.00654	
	2	364.0	0.034	0.061	0.027	0.00742	
	3	588.9	0.035	0.062	0.027	0.00459	
	4	503.2	0.057	0.080	0.023	0.00457	
	5	417.6	0.039	0.065	0.026	0.00623	
				Average	0.026	0.00587	0.00126

Average, standard deviation and standard error

Wall Section	Avg Limewash loss (g/sq cm)	Standard deviation	Standard error
A	0.00141	0.00037	0.0002
B	0.00066	0.00031	0.0001
C	0.00034	0.00025	0.0001
D	0.00064	0.00027	0.0001
E	0.00072	0.00068	0.0003
F	0.00240	0.00198	0.0009
G	0.00196	0.00116	0.0005
H	0.00587	0.00126	0.0006

Table 35: Cohesion results – lime blocks – *B. sphaericus*

Three test strips of adhesive tape were cut, measured and weighed before and after the cohesion test on the respective lime block. The weight of lime removed by the adhesive tape from the surface of the section is adjusted to compare surface abrasion in g cm^{-2}

(Weight in g/ sq cm)	1			2			3			Average	Standard deviation	Standard error
	Before	After	Diff	Before	After	Diff	Before	After	Diff			
A (Control)	0.0246	0.0290	0.0044	0.0245	0.0290	0.0045	0.0254	0.0298	0.0044	0.0044	0.00006	0.00003
B (non-encapsulation)	0.0216	0.0250	0.0034	0.0235	0.0265	0.0030	0.0203	0.0239	0.0036	0.0033	0.00031	0.00018
C (Encapsulation - alginate)	0.0221	0.0239	0.0018	0.0202	0.0223	0.0021	0.0220	0.0238	0.0018	0.0019	0.00017	0.00010
D (Encapsulation - celluose)	0.0225	0.0360	0.0135	0.0224	0.0364	0.0140	0.0212	0.0350	0.0138	0.0138	0.00025	0.00015
E (2 + 3 non-encap)	0.0200	0.0289	0.0089	0.0204	0.0297	0.0093	0.0263	0.0358	0.0095	0.0092	0.00031	0.00018
F (2 + 3 alginate)	0.0200	0.0309	0.0109	0.0217	0.0320	0.0103	0.0240	0.0344	0.0104	0.0105	0.00032	0.00019
G (2 + 3 cellulose)	0.0236	0.0398	0.0162	0.0224	0.0390	0.0166	0.0226	0.0389	0.0163	0.0164	0.00021	0.00012
H (hydrogel)	0.0222	0.0430	0.0208	0.0221	0.0421	0.0200	0.0210	0.0420	0.0210	0.0206	0.00053	0.00031

Table 36: Cohesion results – lime blocks – *S. elongatus*

Based on the previous work in section A 2 the alginate biopolymer was utilised as the preferred encapsulation polymer. The result below compares the control data to the data for alginate-encapsulated *S. elongatus*.

(next page)

(Weight in g)	1			2			3			4			5			6			Average	Standard Deviation	Standard error
	Before	After	Diff	Before	After	Diff	Before	After	Diff	Before	After	Diff	Before	After	Diff	Before	After	Diff			
A (Control)	1.5900	0.9000	0.6900	1.4770	0.8500	0.6270	1.3800	0.7400	0.6400	1.3900	0.7700	0.6200	1.5400	0.8900	0.6500	1.5100	0.8770	0.6330	0.6433	0.0251	0.0102
B (Alginate encapsulated)	1.4800	1.1050	0.3750	1.5200	1.2200	0.3000	1.4200	1.0800	0.3400	1.4400	1.1100	0.3300	1.4100	1.0500	0.3600	1.4300	1.0900	0.3400	0.3408	0.0258	0.0105

Table 36 Cohesion results – lime blocks – *S. elongatus*

Graph A – Wall Sections (*B. sphaericus*) – Cohesion – Statistical results

The null hypothesis compares the cohesion results from the Control (1 or section A) and the highest performing formulation of alginate encapsulating *B. sphaericus* (3 corresponding to wall section C).

A two-tail t-test was carried out on the sample data. The difference between the results can be considered significant, therefore the null hypothesis must be accepted. This outcome concludes that the data for the cohesion between Control A and Sample C is significant as indicated by the non-overlap of the standard error bars.

Null hypothesis for data comparisons for *B. sphaericus* Control A and Alginate encapsulation C
H0 - The cohesion of the limewash between the control A and Alginate Encap. C is the same
H1 - The cohesion of the limewash between the control A and Alginate Encap. C is different

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	0.00352221	0.00086209
Variance	8.4295E-07	3.8701E-07
Observations	5	5
Pearson Correlation	0.22001019	
Hypothesized Mean Difference	0	
df	4	
t Stat	6.01279495	
P(T<=t) one-tail	0.00192617	
t Critical one-tail	2.13184679	
P(T<=t) two-tail	0.00385234	
t Critical two-tail	2.77644511	

Degrees of freedom are 4
Alpha is 0.05, P is therefore significant
The null hypothesis (H0) can be rejected

Graph B – Lime blocks (*B. sphaericus*) – Cohesion – Statistical results

The null hypothesis compares the cohesion results from the Control (1 or section A) and the highest performing formulation of alginate encapsulating *B. sphaericus* (3 corresponding to wall section C).

A two-tail t-test was carried out on the sample data. The difference between the results can be considered highly significant, therefore the null hypothesis is rejected. This outcome concludes that the data for the cohesion results between Control A and Sample C is significant as indicated by the non-overlap of the standard error bars.

Null hypothesis for data comparisons for *B. sphaericus* Control A and Alginate encapsulation C

H0 - The cohesion of the limewash between the control A and Alginate Encap. C is the same

H1 - The cohesion of the limewash between the control A and Alginate Encap. C is different

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	0.0044	0.0019
Variance	3E-09	3E-08
Observations	3	3
Pearson Correlation	1	
Hypothesized Mean Difference	0	
df	2	
t Stat	38	
P(T<=t) one-tail	0.0003	
t Critical one-tail	2.92	
P(T<=t) two-tail	0.0007	
t Critical two-tail	4.3027	

Degrees of freedom are 2

Alpha is 0.05, P is therefore highly significant

The null hypothesis (H0) can be rejected

Graph C – Lime blocks (*S. elongatus*) – Cohesion – Statistical results

The null hypothesis compares the cohesion results from the Control (1 or section A) and the highest-performing formulation of alginate encapsulating *S. elongatus* (2 or B).

A two-tail t-test was carried out on the sample data. The difference between the results can be considered highly significant, therefore the null hypothesis must be accepted. This outcome concludes that the data for cohesion between Control A and Sample B is significant as indicated by the non-overlap of the standard error bars.

Null hypothesis for data comparisons for *S. elongatus* Control A and Alginate encapsulation B

H0 - The cohesion of the limewash between the control A and Alginate Encap. B is the same

H1 - The cohesion of the limewash between the control A and Alginate Encap. B is different

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	0.0045	0.0028
Variance	2E-07	6E-08
Observations	6	6
Pearson Correlation	0.382	
Hypothesized Mean Difference	0	
df	5	
t Stat	10.973	
P(T<=t) one-tail	5E-05	
t Critical one-tail	2.015	
P(T<=t) two-tail	0.0001	
t Critical two-tail	2.5706	

Degrees of freedom are 2

Alpha is 0.05, P is therefore highly significant

The null hypothesis (H0) can be rejected

Table 37: Absorption results – wall sections - *B. sphaericus*

Conditions at the time measurements were taken:

Temperature	13° C
Relative Humidity	75%
Each measurement was conducted over	60 seconds

The contact sponge dimensions are 44 x 55 x 0.06 mm. Five tests were conducted on each wall section. The results were calculated for a 1 cm^{-2} wall section. Results are in g cm^{-2} s^{-1}

Wall Section	Test No.	Wt before (g)	Wt after (g)	Diff. (g)	1 sq cm of wall section absorbs(g)	Water absorption g/cm2/S	Standard Deviation
A	1	11.415	8.829	2.586	0.10686	0.00178	
	2	11.238	8.744	2.494	0.10306	0.00172	
	3	11.021	8.617	2.404	0.09934	0.00166	
	4	11.026	8.882	2.144	0.08860	0.00148	
	5	11.099	8.901	2.198	0.09083	0.00151	
				Average	0.0977	0.00163	0.00013

Wall Section	Test No.	Wt before (g)	Wt after (g)	Diff. (g)	1 sq cm of wall section absorbs(g)	Water absorption g/cm2/S	Standard Deviation
B	1	11.254	9.074	2.180	0.09008	0.00150	
	2	10.950	8.805	2.145	0.08864	0.00148	
	3	10.731	8.608	2.123	0.08773	0.00146	
	4	11.024	8.885	2.139	0.08839	0.00147	
	5	10.998	8.898	2.100	0.08678	0.00145	
				Average	0.0883	0.00147	0.00002

Wall Section	Test No.	Wt before (g)	Wt after (g)	Diff. (g)	1 sq cm of wall section absorbs(g)	Water absorption g/cm2/S	Standard Deviation
C	1	10.650	8.609	2.041	0.08434	0.00141	
	2	10.943	8.862	2.081	0.08599	0.00143	
	3	9.948	8.063	1.885	0.07789	0.00130	
	4	10.888	8.854	2.034	0.08405	0.00140	
	5	11.055	9.009	2.046	0.08455	0.00141	
				Average	0.0834	0.00139	0.00005

Wall Section	Test No.	Wt before (g)	Wt after (g)	Diff. (g)	1 sq cm of wall section absorbs(g)	Water absorption g/cm2/S	Standard Deviation
D	1	10.629	8.311	2.318	0.09579	0.00160	
	2	10.402	8.289	2.113	0.08731	0.00146	
	3	10.885	8.648	2.237	0.09244	0.00154	
	4	11.001	8.787	2.214	0.09149	0.00152	
	5	10.985	8.709	2.276	0.09405	0.00157	
				Average	0.0922	0.00154	0.00005

Wall Section	Test No.	Wt before (g)	Wt after (g)	Diff. (g)	1 sq cm of wall section absorbs(g)	Water absorption g/cm2/S	Standard Deviation
E	1	10.962	8.260	2.702	0.11165	0.00186	
	2	10.362	7.784	2.578	0.10653	0.00178	
	3	10.561	7.257	3.304	0.13653	0.00228	
	4	10.826	8.189	2.637	0.10897	0.00182	
	5	11.099	8.672	2.427	0.10029	0.00167	
				Average	0.1128	0.00188	0.00023

Wall Section	Test No.	Wt before (g)	Wt after (g)	Diff. (g)	1 sq cm of wall section absorbs(g)	Water absorption g/cm2/S	Standard Deviation
F	1	10.537	7.050	3.487	0.14409	0.00240	
	2	10.494	7.508	2.986	0.12339	0.00206	
	3	11.022	8.002	3.020	0.12479	0.00208	
	4	10.980	7.872	3.108	0.12843	0.00214	
	5	11.005	7.934	3.071	0.12690	0.00212	
				Average	0.1295	0.00216	0.00014

Wall Section	Test No.	Wt before (g)	Wt after (g)	Diff. (g)	1 sq cm of wall section absorbs(g)	Water absorption g/cm2/S	Standard Deviation
G	1	10.563	7.508	3.055	0.12624	0.00210	
	2	10.028	7.006	3.022	0.12488	0.00208	
	3	11.019	7.965	3.054	0.12620	0.00210	
	4	11.026	7.982	3.044	0.12579	0.00210	
	5	10.888	7.901	2.987	0.12343	0.00206	
				Average	0.1253	0.00209	0.00002

Wall Section	Test No.	Wt before (g)	Wt after (g)	Diff. (g)	1 sq cm of wall section absorbs(g)	Water absorption g/cm2/S	Standard Deviation
H	1	10.484	7.631	2.853	0.11789	0.00196	
	2	10.754	7.966	2.788	0.11521	0.00192	
	3	10.654	7.817	2.837	0.11723	0.00195	
	4	11.026	8.189	2.837	0.11723	0.00195	
	5	10.872	8.101	2.771	0.11450	0.00191	
				Average	0.1164	0.00194	0.00002

Average, standard deviation and standard error

Wall Section	Average water absorption (g/sq cm/S)	Standard Deviation	Standard error
A	0.00163	0.00013	0.0001
B	0.00147	0.00002	0.0000
C	0.00139	0.00005	0.0000
D	0.00154	0.00005	0.0000
E	0.00188	0.00023	0.0001
F	0.00216	0.00014	0.0001
G	0.00209	0.00002	0.0000
H	0.00194	0.00002	0.0000

Table 38: Absorption results – lime blocks - *B. sphaericus*

Conditions at the time measurements were taken:

Temperature	20° C
Relative Humidity	60%
Each measurement was conducted over	60 seconds

The contact sponge dimensions are 10 x 20 x 0.06 mm. Testing was conducted on six lime blocks. The results are calculated in g cm^{-2} s-1

(Weight in g/sq cm)	1			2			3			Average	Standard Deviation	Standard error
	Before	After	Diff	Before	After	Diff	Before	After	Diff			
A (Control)	1.594	0.957	0.637	1.475	0.945	0.530	1.577	0.933	0.644	0.604	0.064	0.037
B (non-encapsulation)	1.391	1.003	0.388	1.518	1.134	0.384	1.441	1.042	0.399	0.390	0.008	0.005
C (Encapsulation - alginate)	1.433	1.133	0.300	1.485	1.181	0.304	1.459	1.139	0.320	0.308	0.011	0.006
D (Encapsulation - celluose)	1.540	1.102	0.438	1.656	1.225	0.431	1.471	1.064	0.407	0.425	0.016	0.009
E (2 + 3 non-encap)	1.360	0.945	0.415	1.540	1.107	0.434	1.510	1.012	0.498	0.449	0.044	0.025
F (2 + 3 alginate)	1.638	1.002	0.636	1.512	1.019	0.493	1.470	1.013	0.458	0.529	0.095	0.055
G (2 + 3 cellulose)	1.648	1.068	0.580	1.396	1.037	0.358	1.460	1.004	0.456	0.465	0.111	0.064
H (hydrogel)	1.538	1.139	0.399	1.533	1.147	0.386	1.541	1.144	0.397	0.394	0.007	0.004

Table 39: Absorption results – lime blocks – *S. elongatus*

Conditions at the time measurements were taken:

Temperature 20° C
Relative Humidity 60%
Each measurement was enacted over a 60-second interval.

The contact sponge dimensions are 10 x 20 x 0.06 mm. Testing was conducted on six lime blocks. The results are calculated in g cm^{-2} s-1

(next page)

(Weight in g)	1			2			3			4			5			6			Average	Standard Deviation	Standard error
	Before	After	Diff	Before	After	Diff	Before	After	Diff	Before	After	Diff	Before	After	Diff	Before	After	Diff			
A (Control)	1.5900	0.9000	0.6900	1.4770	0.8500	0.6270	1.3800	0.7400	0.6400	1.3900	0.7700	0.6200	1.5400	0.8900	0.6500	1.5100	0.8770	0.6330	0.6433	0.0251	0.0102
B (Alginate encapsulated)	1.4800	1.1050	0.3750	1.5200	1.2200	0.3000	1.4200	1.0800	0.3400	1.4400	1.1100	0.3300	1.4100	1.0500	0.3600	1.4300	1.0900	0.3400	0.3408	0.0258	0.0105

Table 39 Absorption results – lime blocks – *S. elongatus*

Graph A – Lime Wall Sections (*B. sphaericus*) - Absorption– statistical results

Null hypothesis for data comparisons for *B. sphaericus* Control A and Alginate encapsulation C

H0 - The absorption of water between the control A and Alginate Encap. C is the same

H1 - The absorption of water between the control A and Alginate Encap. C is different

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	0.09773554	0.083363636
Variance	6.1357E-05	9.91445E-06
Observations	5	5
Pearson Correlation	-0.0032608	
Hypothesized Mean Difference	0	
df	4	
t Stat	3.80234431	
P(T<=t) one-tail	0.00953259	
t Critical one-tail	2.13184679	
P(T<=t) two-tail	0.01906518	
t Critical two-tail	2.77644511	

Degrees of freedom are 4

Alpha is 0.05, P is therefore significant

The null hypothesis (H0) with caution can therefore be rejected.

Graph B – lime blocks (*B. sphaericus*) – Absorption - statistical results

The null hypothesis compares the absorption results from the Control (1 or section A) and the highest performing formulation of alginate encapsulating *B. sphaericus* (3 corresponding to wall section C).

A two-tail t-test was carried out on the sample data (below). The difference between the results can be considered significant, and the null hypothesis is rejected. This outcome concludes the data for the absorption of water between Control A and Sample C is significantly different as indicated by the non-overlap of the standard error bars.

Null hypothesis for data comparisons for *B. sphaericus* Control A and Alginate encapsulation C

H0 - The absorption of water between the control A and Alginate Encap. C is the same

H1 - The absorption of water between the control A and Alginate Encap. C is different

Comparing A (control) with C (Alginate encapsulated)

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	0.6037	0.30796667
Variance	0.00405223	0.0001128
Observations	3	3
Pearson Correlation	0.37351975	
Hypothesized Mean I	0	
df	2	
t Stat	8.46686311	
P(T<=t) one-tail	0.00683206	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.01366413	
t Critical two-tail	4.30265273	

P (for two tailed t-test) is 0.013.
Degrees of freedom are 2
Alpha is 0.05, P at 0.0136 is therefore significant
The null hypothesis (H0) can therefore be rejected.

Graph C – Lime blocks (*S. elongatus*) – Absorption - statistical results

The null hypothesis compares the absorption results from the Control (1) and the high-performing formulation of alginate encapsulating *S. elongatus* (3).

A two-tail t-test was carried out on the sample data (below). The difference between the results can be considered highly significant, and the null hypothesis is rejected. This outcome concludes the data for the absorption of water between Control A(1) and Sample B(2) is significantly different as indicated by the non-overlap of the standard error bars.

Null hypothesis for data comparisons for *S. elongatus* Control A and Alginate encapsulated B

H0 - The absorption of water between the control A and Alginate Encap. B is the same

H1 - The absorption of water between the control A and Alginate Encap. B is different

Comparing A (control) with B (Alginate encapsulated)

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	0.6433333	0.34083
Variance	0.0006303	0.00066
Observat	6	6
Pearson (	0.8202025	
Hypothes	0	
df	5	
t Stat	48.532188	
P(T<=t) o	3.509E-08	
t Critical	2.0150484	
P(T<=t) t	7.017E-08	
t Critical	2.5705818	

P (for two tailed t-test) is 7.01748E-08
Degrees of freedom are 6
Alpha is 0.05, P is therefore extremely significant
The null hypothesis (H0) can therefore be rejected.

Table 40: Secondary metabolites produced and known pharmaceutical activity by lichen *O. parella.* The assumed targets of action include inhibition of cell wall synthesis, efflux pumps, disruption of plasma membrane integrity, suppression of DNA/RNA protein synthesis, cell division and mitochondrial function

Lichen Compound	**Pharmaceutical activity**	**References**
Gyrophoric acid	Antimicrobial, anticancer, antioxidant, antidiabetic	(Bačkorová et al., 2011; Cardile et al., 2017; Mohammadi et al., 2022; Plsíkova et al., 2014)
Vulpinic Acid	Antimicrobial, anticancer, stem cell control/ osteogenesis & adipogenesis, oxidative-stress therapy, herbivore antifeedant	(Bačkorová et al., 2011; Cansaran-Duman et al., 2021; Koparal, 2015; Lauterwein et al., 1995)
Lecanoric Acid	Antitumour, antioxidant, antibacterial, antifungal, antidiabetic, anticancer, anti-inflammatory, probiotic growth stimulant	(Bogo et al., 2010; Gaikwad et al., 2012; Gomes et al., 2003; Honda et al., 2010; Luo et al., 2009; Thadhani et al., 2011, 2015)
Parellin	A heavily methylated chlorinated depsidone awaiting biological evaluation	(Calcott et al., 2018; Millot et al., 2007)
α-Alectoronic acid	Bacteriocide	(Elix et al., 1974; Elix & Stocker-Wörgötter, 2008; Farkas et al., 2021; Latkowska et al., 2015)
Ergosterol peroxide	Anticancer, antimicrobial, cytotoxic, immunosuppressive, antioxidant	(He et al., 2018; Kim et al., 1999; Merdivan & Lindequist, 2017; Nowak et al., 2022; Yodsing et al., 2017)

Table 41: Formula and method for the preparation of DG18 Agar (Dichloran 18% glycerol agar). The reagents in the table are introduced into distilled water, followed by stirring and heating to facilitate the dissolution of agar. The resultant solution is adjusted to a volume of 1000 ml with distilled water. Glycerol of analytical reagent grade, totalling 220 g, is incorporated, and the agar is subjected to sterilisation through autoclaving at 121°C for 15 minutes. Subsequently, the medium is cooled to 45°C, and plates are poured under aseptic conditions

Reagent	**Quantity**
Glucose	10.0 g
Bacteriological peptone	5.0 g
KH_2PO_4	1.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
Rose Bengal (5% aqueous soln., w/v)	0.5 ml
Dichloran (0.2% in ethanol, w/v)	1.0 ml
Chloramphenicol	0.1 g
Agar	15.0 g
Distilled water	1.0 litre

Table 42: Formula and method for the preparation of Malt Extract Agar (MEA). The reagents in the table are introduced into distilled water, followed by stirring and heating to facilitate the dissolution of agar. The resultant solution is adjusted to a volume of 1000 ml with distilled water. The agar solution is subjected to sterilisation at 121° C for 15 min. The medium is cooled to 45° C and the plates are then poured under aseptic conditions

Reagent	**Quantity**
Malt extract, powdered	20.0 g
Glucose	20.0 g
Peptone	1.0 g
Agar	20.0 g
Distilled water	1.0 litre

Table 43: Weight of dried compounds extracted using methanol solvent**.** Source lichen materials, *O. parella* and *R. siliquosa*, 50 g each, were separately crushed and extracted in methanol. The dried weight of the compounds extracted from each of the lichen was measured following solvent evaporation

Sample	Weight of container empty (g)	Weight of container with dried sample (g)	Weight of sample (g)
O. parella	162.965	165.691	2.726
R. siliquosa	135.677	137.098	1.421

Yield % for *O. parella* is 5.45% and for *R. siliquosa* is 2.84%

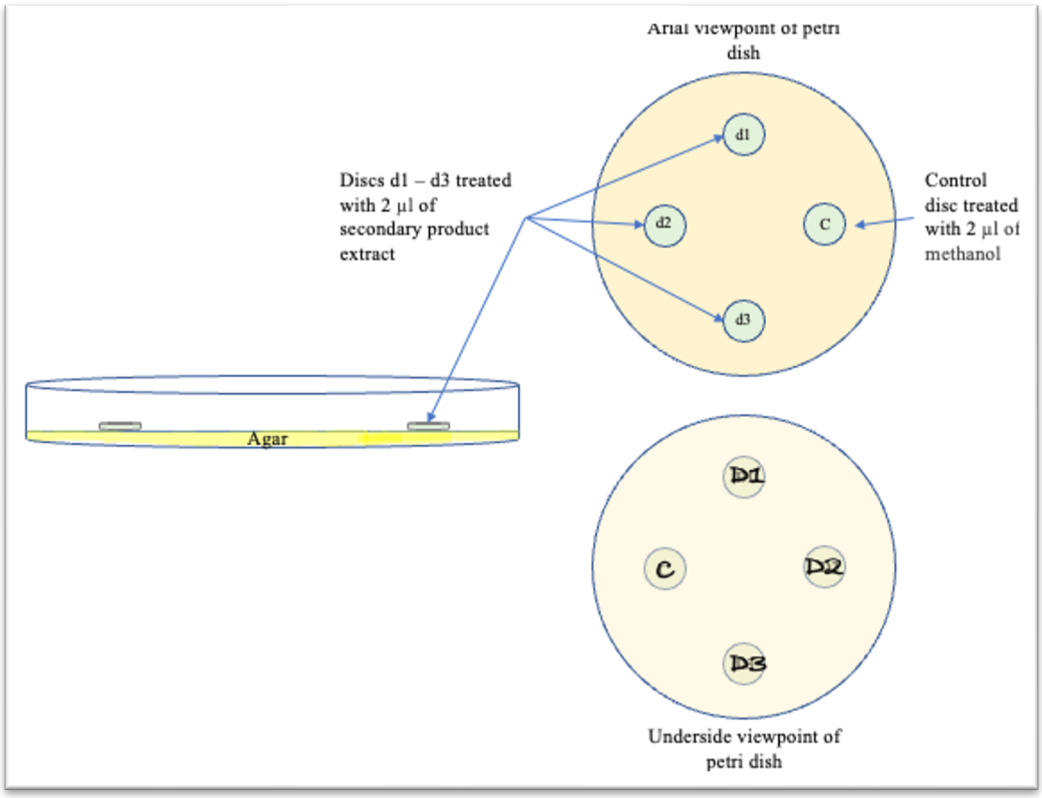

Figure 76: Assessing the antimicrobial properties of extracted lichen compounds. Disc assay to assess antimicrobial inhibitory effect of potential secondary metabolites within initial crude extracts against bacterium *S. aureus*, and mould *P. chrysogenum*. Post-incubation, zones of inhibition around the treated discs indicated antimicrobial properties inhibiting the growth of the microorganisms on the nutrient plates

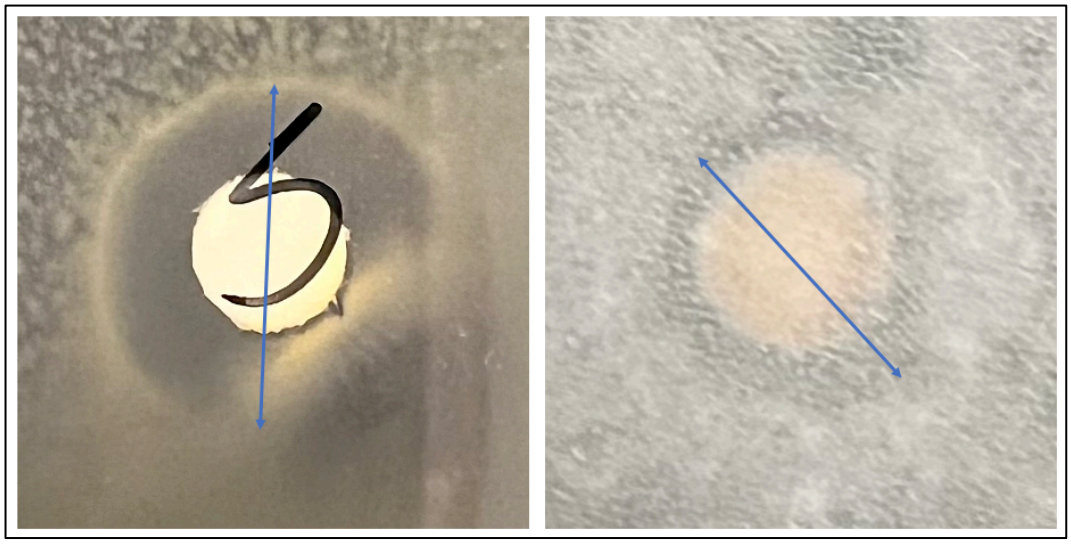

Figure 77: Examples of zones of inhibition obtained from initial assessments of antimicrobial properties of the extracted compounds from O. parella and R. siliquosa. Diameters for the zone of inhibition ranged from 11 mm (left) on S. aureus to 8 mm (right), on P. chrysogenum. The initial extracts of both lichen compounds contained antimicrobial agents

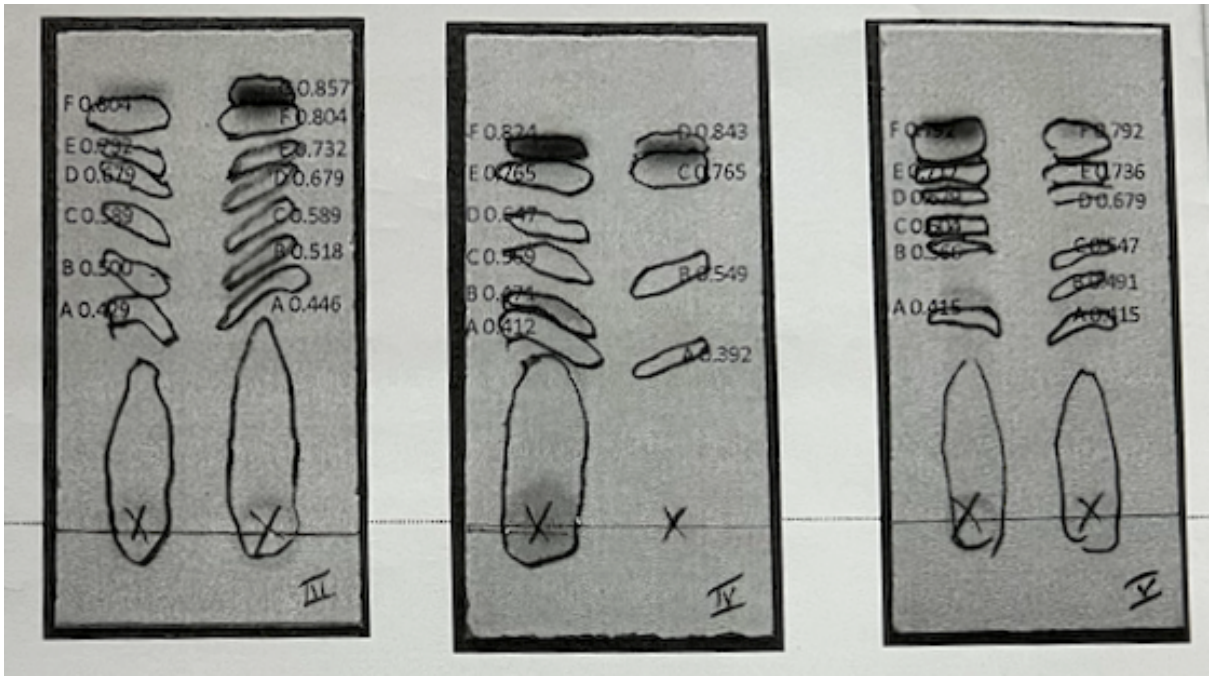

Figure 78: Thin-layer chromatography plates illustrating the separation and Rf calculations of secondary products identified in the extracts from *O. parella* and *R. siliquosa*. Procedure is repeated several times and reviewed and marked under UV light, to confirm product separation. The TLC plate study above confirmed the presence of several separate products in the samples, supporting moving to the process of separating, isolating, and extracting each potential secondary product compound using preparatory thin-layer chromatography

Figure 79: Outlining and extracting secondary metabolite segments from the preparatory TLC plate. The image below indicates a sample has been removed from the plate using a scalpel blade, revealing the underlying supporting aluminium sheet. The silica containing the isolated secondary product is weighed and stored in a small, labelled glass bottle. The procedure is repeated for each identified layer

Table 44: Comparative mass of silica containing secondary products extracted from the preparatory TLC plates A and B. The weight differentials between samples indicates the relative spread of the secondary product deposit as the solute progressed up the plate

Container label	Weight - empty (g)	Weight with silica-secondary product extraction (g)	Weight of slica-secondary product (g)
A1	19.951	20.159	0.208
A2	19.608	19.775	0.167
A3	20.723	21.458	0.735
A4	19.466	19.694	0.228
A5	19.796	19.833	0.037
A6	19.643	19.748	0.105
A7	19.330	19.564	0.234
A8	20.073	20.117	0.044
A9	19.148	19.272	0.124
A10	18.934	19.158	0.224
B1	20.041	20.178	0.137
B2	20.387	20.480	0.093
B3	19.098	19.374	0.276
B4	20.437	20.818	0.381
B5	18.944	19.061	0.117
B6	20.115	20.371	0.256
B7	19.198	19.446	0.248
B8	20.394	20.486	0.092
B9	20.462	20.795	0.333
B10	20.647	20.781	0.134
B11	20.815	21.031	0.216

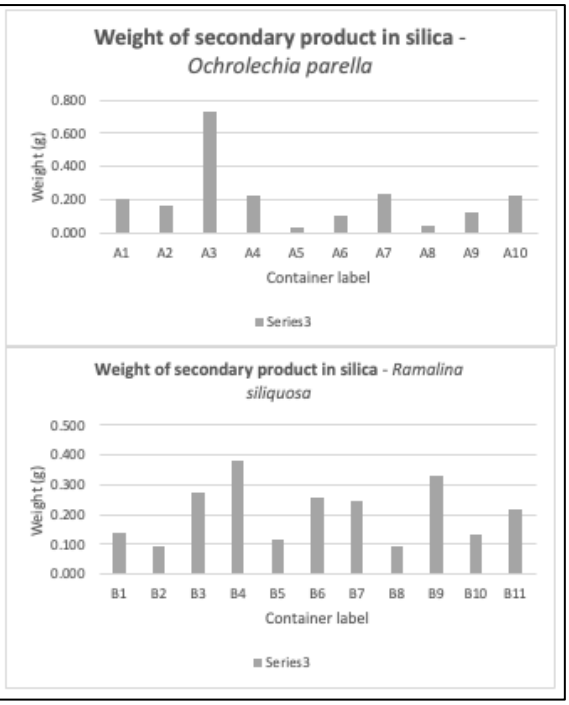

Table 45: The inhibitory effect of preparatory TLC extracts from *O. parella* and *R. siliquosa* on microbial growth for bacterium *S. aureus*, and mould, *P. chrysogenum*

Coding	Plate No.	Sample						
OP DG PC	1	C	1	x	2	x	3	x
	2	C	4	x	5	x	6	x
DG18	3	C	7	x	8	x	9	x
	4	C	10	x				
RSDGPC	5	C	1	x	2	x	3	x
	6	C	4	x	5	x	6	x
DG18	7	C	7	x	8	x	9	x
	8	C	10	11 mm	11	x		
OPMEPC	9	C	1	x	2	x	3	x
	10	C	4	x	5	x	6	x
ME	11	C	7	x	8	x	9	x
	12	C	10	x				
RSMEPC	13	C	1	x	2	x	3	x
	14	C	4	x	5	x	6	x
ME	15	C	7	x	8	x	9	x
	16	C	10	x	11	x		
OPNASA	33	C	1	9 mm	2	8 mm	3	8 mm
	34	C	4	x	5	13 mm	6	x
NA	35	C	7	x	8	x	9	**10 mm**
	36	C	**10**	**11 mm**				
RSNASA	37	C	1	x	2	x	3	12 mm
	38	C	4	x	5	x	6	x
NA	39	C	7	x	8	x	9	x
	40	C	10	x	11	x		

Key:

OP - *Ochrolechia parella*
DG - Medium DG18
PC - *Penicillium chrysogenum*
RS - *Ramalina siliquosa*
ME - Medium Malt extract
SA - *Staphylococcus aureus*
NA - Medium Nutrient Agar
1 - 10 Samples obtained from *Ochrolechia parella* preparatory TLC extraction
1 -11 Samples obtained from *Ramalina siliquosa* preparatory TLC extraction

Measurements:

x indicates no zone of inhibition observed
8 mm indicates e.g., an 8mm diameter zone of inhibition

Table 46: Microtiter Results based on Turbidity, (OD 630), for Sample OP10

Sample OP10	96-well plate columns					
Serial Dil.	Col A (cont)	Col B	Col C	Avg	Stand. Dev	Stand. Error
1	0.338	0.049	0.049	0.049	0.000	0.000
2	0.338	0.050	0.050	0.050	0.000	0.000
3	0.334	0.051	0.049	0.050	0.001	0.001
4	0.324	0.064	0.065	0.065	0.001	0.001
5	0.337	0.076	0.091	0.084	0.011	0.008
6	0.336	0.186	0.163	0.175	0.016	0.012
7	0.314	0.181	0.198	0.190	0.012	0.009
8	0.314	0.203	0.198	0.201	0.004	0.003
9	0.314	0.246	0.221	0.234	0.018	0.013
10	0.314	0.273	0.280	0.277	0.005	0.004
11	0.314	0.295	0.301	0.298	0.004	0.003

Table 47: Microtiter Results based on Turbidity, (OD 630), for Sample OP9

Sample OP9	96-well plate columns					
Serial Dil.	Col A (cont)	Col D	Col E	Avg	Stand. Dev	Stand. Error
1	0.338	0.114	0.156	0.135	0.030	0.021
2	0.338	0.116	0.158	0.137	0.030	0.021
3	0.334	0.167	0.181	0.174	0.010	0.007
4	0.324	0.172	0.213	0.193	0.029	0.020
5	0.337	0.235	0.193	0.214	0.030	0.021
6	0.336	0.246	0.201	0.224	0.032	0.023
7	0.314	0.227	0.227	0.227	0.000	0.000
8	0.314	0.243	0.314	0.279	0.050	0.036
9	0.314	0.249	0.322	0.286	0.052	0.037
10	0.314	0.268	0.327	0.298	0.042	0.030
11	0.314	0.304	0.326	0.315	0.016	0.011

Table 48: Microtiter Results based on Turbidity, (OD 630), Sample OP5

Sample OP5	96-well plate columns					
Serial Dil.	Col A (cont)	Col D	Col E	Avg	Stand. Dev	Stand. Error
1	0.338	0.077	0.066	0.072	0.008	0.006
2	0.338	0.078	0.066	0.072	0.008	0.006
3	0.334	0.079	0.072	0.076	0.005	0.004
4	0.324	0.090	0.091	0.091	0.001	0.001
5	0.337	0.102	0.099	0.101	0.002	0.001
6	0.336	0.101	0.108	0.105	0.005	0.004
7	0.314	0.112	0.107	0.110	0.004	0.003
8	0.314	0.114	0.109	0.112	0.004	0.003
9	0.314	0.177	0.183	0.180	0.004	0.003
10	0.314	0.196	0.201	0.199	0.004	0.003
11	0.314	0.271	0.289	0.280	0.013	0.009

Table 49: Microtiter Results based on Turbidity, (OD 630), Sample OP1

Sample OP1	96-well plate columns					
Serial Dil.	Col A (cont)	Col B	Col C	Avg	Stand. Dev	Stand. Error
1	0.308	0.138	0.132	0.135	0.004	0.003
2	0.303	0.229	0.185	0.207	0.031	0.022
3	0.310	0.215	0.207	0.211	0.006	0.004
4	0.300	0.227	0.22	0.224	0.005	0.004
5	0.304	0.242	0.212	0.227	0.021	0.015
6	0.299	0.244	0.210	0.227	0.024	0.017
7	0.300	0.283	0.225	0.254	0.041	0.029
8	0.314	0.327	0.261	0.294	0.047	0.033
9	0.307	0.322	0.337	0.330	0.011	0.008
10	0.310	0.334	0.320	0.327	0.010	0.007
11	0.302	0.341	0.319	0.330	0.016	0.011

Table 50: Microtiter Results based on Turbidity, (OD 630), Sample OP2

Sample OP2	96-well plate columns					
Serial Dil.	Col A (cont)	Col D	Col E	Avg	Stand. Dev	Stand. Error
1	0.308	0.210	0.158	0.184	0.037	0.026
2	0.303	0.211	0.172	0.192	0.028	0.019
3	0.310	0.187	0.193	0.190	0.004	0.003
4	0.300	0.184	0.220	0.202	0.025	0.018
5	0.304	0.230	0.213	0.222	0.012	0.009
6	0.299	0.201	0.260	0.231	0.042	0.030
7	0.300	0.251	0.215	0.233	0.025	0.018
8	0.314	0.261	0.234	0.248	0.019	0.014
9	0.307	0.291	0.277	0.284	0.010	0.007
10	0.310	0.299	0.302	0.301	0.002	0.002
11	0.309	0.303	0.307	0.305	0.003	0.002

Table 51: Microtiter Results based on Turbidity, (OD 630), Sample OP3

Sample OP3	96-well plate columns					
Serial Dil.	Col A (cont)	Col D	Col E	Avg	Stand. Dev	Stand. Error
1	0.308	0.042	0.043	0.043	0.001	0.000
2	0.303	0.048	0.043	0.046	0.004	0.003
3	0.310	0.051	0.070	0.061	0.013	0.010
4	0.300	0.144	0.140	0.142	0.003	0.002
5	0.304	0.144	0.153	0.149	0.006	0.005
6	0.299	0.189	0.178	0.184	0.008	0.006
7	0.300	0.209	0.204	0.207	0.004	0.003
8	0.314	0.239	0.242	0.241	0.002	0.002
9	0.307	0.245	0.241	0.243	0.003	0.002
10	0.310	0.269	0.280	0.275	0.008	0.006
11	0.302	0.314	0.344	0.329	0.021	0.015

Table 52: Microtiter Results based on Turbidity, (OD 630), Sample RS3

Sample RS3	96-well plate columns					
Serial Dil.	Col A (cont)	Col B	Col C	Avg	Stand. Dev	Stand. Error
1	0.347	0.159	0.162	0.161	0.002	0.002
2	0.346	0.159	0.168	0.164	0.006	0.005
3	0.346	0.162	0.173	0.168	0.008	0.005
4	0.343	0.164	0.177	0.171	0.009	0.006
5	0.348	0.168	0.176	0.172	0.006	0.004
6	0.345	0.169	0.178	0.174	0.006	0.004
7	0.348	0.169	0.168	0.169	0.001	0.001
8	0.343	0.272	0.274	0.273	0.001	0.001
9	0.343	0.298	0.289	0.294	0.006	0.005
10	0.344	0.304	0.315	0.310	0.008	0.006
11	0.346	0.317	0.322	0.320	0.004	0.003

Table 53: Microtiter Results based on Turbidity, (OD 630), Sample RS10

Sample RS10	96-well plate columns					
Serial Dil.	Col A (cont)	Col D	Col E	Avg	Stand. Dev	Stand. Error
1	0.347	0.041	0.017	0.029	0.017	0.012
2	0.346	0.046	0.039	0.043	0.005	0.004
3	0.346	0.054	0.060	0.057	0.004	0.003
4	0.343	0.070	0.087	0.079	0.012	0.009
5	0.348	0.117	0.118	0.118	0.001	0.000
6	0.345	0.172	0.174	0.173	0.001	0.001
7	0.348	0.194	0.201	0.198	0.005	0.004
8	0.343	0.195	0.209	0.202	0.010	0.007
9	0.343	0.302	0.298	0.300	0.003	0.002
10	0.344	0.322	0.319	0.321	0.002	0.002
11	0.346	0.343	0.356	0.350	0.009	0.006

Table 54: Formulation and method for the preparation of BG11 nutrient medium

Method

For 1 Litre Total Volume. **Note**: BG11 is light sensitive and may degrade. It should be stored in the refrigerator when not in use.

Liquid Medium:

1. To approximately 900 mL of distilled H_2O add the first 8 components in the order specified while stirring continuously.
2. Bring the total volume to 1 Litre with dH_2O.
3. Cover and autoclave medium to sterilize.
4. The micronutrients are added separately to a total volume of 1 Litre.
5. Add 1mL of the micronutrient solution to the 1L Macronutrient BG11 Broth

Agar Medium:

1. To approximately 400 mL of distilled H_2O add the first 8 components in the order specified while stirring continuously.
2. Bring the total volume to 500 mL with distilled H_2O.
3. In a separate container add 15 g of nutrient agar to 500 mL of distilled H_2O (final 1.5% w/v).
4. Cover and autoclave both solutions.
5. In a water bath allow both solutions to cool to 45-50 °C.
6. Add sterile Sodium Thiosulfate to the agar solution and mix well.
7. Combine both agar and liquid solutions, mix well.
8. Allow to cool then store at refrigerator temperature.

Formulation:

Macronutrients	**Mg L^{-1}**
$NaNO_3$	1500.00
K_2HPO_4	40.00
$MgSO_4.7H_2O$	75.00
$CaCl_2.2H_2O$	36.00
Citric Acid	6.00
Na_2CO_3	20.00
Na_2EDTA	1.00
Ferric ammonium citrate	6.00
Micronutrients	
H_3BO_3	2.86
$MnCl_2.4.H_2O$	1.81
$ZnSO_4.7H_2O$	0.222
$Na_2MoO_4.2H_20$	0.39
$CuSO_4.5H_2O$	0.079
$Co(NO_3)_2.6H_2O$	0.0494

Health and safety support documentation

This form is to accompany a COSHH risk assessment only and is not a stand-alone DSEAR risk assessment. Ensure that the above is taken into account in your COSHH assessment.

Section 1: Risk	
Risk of fire, explosion or energetic event *Describe how the activity can lead to fire, explosion or thermal runaway or cause an explosive atmosphere*	Acetone, methanol, and dichloromethane are volatile, flammable solvents which require care in storage, transport, and attentiveness as to any flames or sparking electrical machinery in the vicinity. Each of the three solvents are kept in capped glass bottles, in fire safe storage, and transportation limited to moving the bottle between the storage and the extraction fume unit in which experimentation takes place. The experimental work is kept isolated from any flames or electrical machinery and fumes extracted within the fume cupboard.
Ignition sources *What potential sources of ignition are present e.g. Bunsen burners, hot plates, ovens, furnaces or heating equipment?*	The experiment does not require the use of any source of ignition including Bunsen burners, hot plates, or heating equipment. The experiment will be kept isolated from any adjacent experiments in the workspace.
Section B: Specific DSEAR controls	
What steps have been taken to control releases at source or reduce the quantity of the dangerous substance stored or used to a minimum?	Acetone, methanol, and dichloromethane are common solvents and bottles of each chemical will be stored within a fire safe cupboard. Bottles will be opened, and contents accessed within the fume cupboard with active extraction only. Volumes of chemicals used within the experiments are low volume and sealed when used for solvency qualities, and are stored either in refrigerated units, or, in the fume/extraction cupboard. Preparatory chromatography will be limited to within the fume/extraction cupboard.
What steps have been taken to avoid releases that may lead to formation of explosive atmosphere under normal and accidental conditions?	Limiting the release of solvent gases to within the fume/extraction cupboard. The solvents are stable at room temperature and can be stored safely without pressure build up within a fireproof cupboard.

A full DSEAR risk assessment may be required if:		Yes	No
	The work activity involves the use or storage of pressurised flammable gas cylinders such as acetylene or hydrogen;		No
	Very large quantities of flammable substances are involved, for where the recommended limits for storage of flammable substances are exceeded (50 litres for extremely or highly flammable substances and flammable liquids with a flashpoint below the maximum ambient temperature of the working area, 250 litres for other flammable liquids with a higher flashpoint of up to 55°C.);		No
	The work activity involves the use of explosives;		No
	The work activity is still likely to create an explosive atmosphere even when using the controls specified above;		No
	The work activity involves the use of flammable/ oxidising/ explosive substances only and not substances hazardous to health as defined by COSHH.		No

Title	**Assessment of antimicrobial activity of lichen secondary metabolites *from Ochrolechia parella* and *Ramalina siliquosa* and assessing the impact of photoautotrophic biopolymer encapsulation on biomineralisation in limewash**
Assessor	**Peter Booth**

Substance(s) Information

Outline of Task/Method	A) Methanol solutions of secondary metabolites from *O. parella* and *R. siliquosa* are assessed for antimicrobial activity against *Penicillium corylophilum, Stachybotrys chartarum* and *Staphylococcus aureus* using disc assays and microtiter 96-well plate bioassays to establish minimum inhibitory concentrations. B) Overnight cultures of *Bacillus sphaericus* are immobilised into calcium alginate hydrogels and assessed for calcium nucleation within a limewash coating on lime blocks 40x30x20mm. C) Overnight cultures of *Synechococcus sp.* PCC8806 or 8807 or 7942 are encapsulated within a cellulose biopolymer capsule using (i) simple coaxial extrusion and (ii) coaxial electrospraying and assessed for calcium nucleation within a limewash coating on lime blocks 40x30x20 mm while exposed to sunlight/natural light. D) Calcium nucleation is assessed using Alizarin Red, adhesive tape and small sponges.
Location of Activity	*Microbiology 1J026*

Product/ Substance	Hazard	Form	Quantity Used or Stored	Duration of Exposure	Route of Entry	Frequency of Exposure	Workplace Exposure Limit UH Guidance EH40	Risk Level [1] (RAG rating)
Methanol CH_3OH CAS: 67-56-1 99.8%+	 GHS02,GHS06,GHS08 Acute Tox. 3 Dermal - Acute Tox. 3 Inhalation - Acute Tox. 3 Oral - Flam. Liq. 2 - STOT SE 1 *Hazard Statements* **H225 - H301 + H311 + H331 - H370** *Precautionary Statements* **P210 - P233 - P280 - P301 + P310 - P303 + P361 + P353 - P304 + P340 + P311**	Liquid	1 ml per vial of sample	Minutes. Exposure is always restricted to use within a ventilated fume cupboard. Gloves and eye covering must be worn when handling	Skin contact, inhalation, ingestion must be avoided.	Daily for the period of the project (10 days)	Long term exposure limit (8-hr TWA reference period) 200 ppm. Short term exposure limit (15-min reference period) 250 ppm	Low
1% Anigene	Working solution is non hazardous	Liquid	200 mL spray bottle of 1% working solution	Minutes 1 - 5	Skin contact, inhalation eye contact, ingestion	Daily as and when required	None	Low
DG18 Agar $(C_{12}H_{18}O_9)_n$ CAS:9002-18-0 KH_2PO_4 CAS: 7778-77-0 99%+ $MgSO_4.7H_2O$ CAS: 10034-99-8	Potassium Phosphate – non-hazardous, dust mask precaution Magnesium Sulphate heptahydrate (Epsom salts) – non-hazardous 4,5,6,7-Tetrachloro-2',4',5',7'-	Solid/ liquid gel	250 ml of the DG18 agar mix	Minutes. Exposure is always restricted to use within a ventilated fume cupboard. Gloves	Inhalation	1 – 2 Hours for 2 days	There are no long-term exposure limits. Dichloran and Chloramphenicol concentrati	Low

[1] Risk level is based on the likelihood of exposure and the severity of the health effects. For example, a high-risk substance could cause serious health effects and the level of exposure makes it likely that these effects could occur. The risk level will determine the level of controls required to control exposure.

Rose Bengal (5% aqueous) $C_{20}H_2Cl_4I_4Na_2O_5$ CAS:632-69-9 95%+ Dichloran glycerol agar $C_6H_4Cl_2N_2O_2$ Chloramphenicol $Cl_2CHCONHCH(CH_2OH)CH(OH)C_6H_4NO_2$ CAS: 56-75-7	tetraiodofluorescein disodium salt, Acid Red 94, Bengal Rose B sodium salt, Rose Bengal sodium salt - Non-hazardous  Dichloran glycerol agar – non-hazardous  Chloramphenicol $C_{11}H_{12}Cl_2N_2O_5$ H317, H318, H350, H351, H360, H361, P203, P261, P264+P265, P272, P280, P302+P352, P305+P354+P338, P317, P318, P321, P333+P313, P362+P364, P405, and P501			and eye covering must be worn when handling to prevent contamination			on is less than 1 in 10,000	
Malt extract agar (glucose, peptone, agar)	Non-hazardous	Solid/ liquid	500 ml	Minutes. Exposure is always restricted to use within a ventilated fume cupboard. Gloves and eye covering must be worn when handling to prevent contamination	Inhalation	1-2 hours over 2 days	None	Low
Nutrient Broth (glucose, peptone, sodium chloride, yeast extract)	Non-hazardous	Solid	250 ml	Minutes. Exposure is always restricted to use within a ventilated fume cupboard. Gloves and eye covering must be worn when handling to prevent contamination	Inhalation	1 -2 hours over 2 days	None	Low

Sodium alginate CAS: 9005-38-3 homopolymeric blocks of 1->4-linked homopolymeric blocks of 1->4-linked sodium beta-D-mannuronate and sodium alpha-L-guluronate residues, covalently linked together in different sequences or blocks. The sodium salt of alginic acid.	Non-hazardous	Solid	500 gm	Hours	Non-toxic Recommender PPE eye covering and gloves	Daily for 10 days	None	Low
Cellulose sulphate CAS 9004-34-6	Non-hazardous	Solid	500 gm	Hours	Non-toxic Recommender PPE eye covering and gloves	Daily for 10 days	None	Low
Calcium chloride $CaCl_2$ CAS: 10043-52-4	 H319, P264+P265, P280, P305+P351+P338, and P337+P317	Solid	500 gm	Minutes Gloves and eye covering must be worn when handling	Causes serious eye damage/eye irritation. EPA identifies as of low concern used for experimental data	Daily for 2 days	None	Low
Poly diallyl dimethyl ammonium chloride $(C_8H_{16}ClN)_n$ CAS: 26062-79-3	Water soluble cationic polymer H412, P273 and P501 Harmful to aquatic life with long lasting effects	Liquid	1 L	Hours Gloves and eye covering must be worn when handling	Avoid accidental release into drains. Keep in suitable containers for technician disposal	Daily for 2 days	None	Low
Citric Acid $HOC(COOH)(CH_2COOH)_2$ 99.5%+ CAS: 77-92-9	 GHS07 H319-H335 P261 - P264 - P271 - P280 - P304 + P340 + P312 - P305 + P351 + P338	Solid	500 g	Hours Gloves, mask and eye covering must be worn when handling	Eye irrit. 2 - STOT SE 3, inhalation	Daily for 2 days	None	Low
Hydraulic lime render NHL 3.5 Premixed with natural English lime ($Ca(OH)_2$, CAS:1305-62-0) sand and aggregate	 GHS05, GHS07 H315, H318, H335 P261 - P264 - P271 - P280 - P302 + P352 - P305 + P351 + P338 According to regulations No 1272/2008 (CLP)	Solid	5 kg	Hours Alkaline resistant gloves, mask and eye covering must be worn when handling	Skin irritation, eye irritation, targets respiratory tract irritation	Hourly for 1 day	None	Low

Do any of the substances require a licence or notification to purchase? COSHH Tables	*No*		
Will the substance(s) or composition change during the activity and have these changes been considered during this assessment?	*No*		
Who will be Harmed and How?			
Who may be exposed to the substance(s)?	*Researcher, students, staff*		
What are the general consequences of exposure?	*Methanol is in very low volume and opened in ventilated fume hood using full PPE. Other compounds listed are non-hazardous. There are no consequences to the exposure, however care should be taken, as with any powders, not to inhale.*		
Do any of the substances pose additional risks to certain individuals *(e.g. expectant mothers)*	*No*		
Control Measures			
Does the substance(s) require authorisation to retain? *(i.e. is it used very infrequently, or age poses additional risks)* Consult your SBU safety contact if unsure.		Yes	No
			No
Can a less hazardous substance be used to do the same job? *(if you do not know, please contact your supplier for additional information)*		Yes	No
			No
Physical or Engineering Controls	*Local Exhaust Ventilation, glove box, goggles, lab coat, face mask as preventative against inhalation. Methanol compounds (1 ml per vial) will be stored within the refrigerator 3-5°C and accessed within the fume cupboard during usage.*		
Administrative Controls	*Signage on experiments left to culture overnight.*		
Personal Protective Equipment **Specify which type and when they are to be worn**	*Safety glasses, nitrile gloves, lab coat, and face mask as preventative against inhalation*		
Storage Requirements	*Methanol samples are 1 ml per sample and will be stored in standard refrigeration conditions when not in use in the fume cupboard. Other items above are non-combustible solids or liquids and non-hazardous*		
Transport of the substance(s)	*Substances will not be transported.*		
Disposal *(Refer to local procedures)*	Disposal must not be into an open waste system such as a sink. Disposal of each of the solvents will follow laboratory procedure keeping the solvents separate from other volatile interactions and disposed of without contamination of public drainage. The solvents must not be able to enter the public water supply. All chemicals will be collected in leak-proof containers and disposed of by the technical team. Fungal cultures *O. parella* and *R. siliquosa*, moulds *Penicillium corylophilum, Stachybotrys chartarum* and *bacterium S. aureus* and culture contaminated waste must be disposed in autoclave cans for autoclaving before disposal.		
Health Surveillance required? (UH Guidance)	*Does this substance(s) require any user health surveillance? if yes, please state which type & level and who it is conducted by:*	Yes	No
			No
Have persons undertaking this activity and using this substance(s) been provided with information and training in its use? *(Ensure that the findings in this risk assessment are communicated to all involved with this activity and that they are aware of the required controls).*		Yes	No
		Yes	
Emergency Procedures			

First Aid Measures **If substance(s) reacts with water this must be mentioned first** *(Refer to local procedures)*	Protocol requires following usual first aid measures which are: Call a first aider or Security. If ingested: Rinse mouth with water If inhaled: Move person into fresh air. If in contact with eyes: Flush eyes with water If in contact with skin: Wash off with plenty of water for 5-20 minutes, repeat up to twice more if necessary.
Fire Fighting Measures *(Refer to local procedures)*	Methanol is highly flammable and extinguished using the correct fire extinguisher (CO_2 or foam) or fire blanket. **Emergency procedure in case of fire:** Sound the alarm. Leave building by the nearest available exit. Report to assembly point no:9 which is outside LRC. Do not return to the building until authorised to do so. Do not use lifts. Call security on 01707 285555
Accidental Release Measures *(Refer to local procedures)*	**Bacterial cultures:** *Penicillium corylophilum, Stachybotrys chartarum, Staphylococcus aureus, Bacillus sphaericus, Synechococcus sp.* PCC8806 or 8807 or 7942. In case of spill, Wear nitrile gloves, wipe area with 70% IMS or 1% Anigene from outside to inside. Repeat the procedure until area is clean, collect all waste in biohazard bag. All the waste to go in autoclave cans. Minute volumes of secondary metabolites dissolved in methanol (below) from O. parella and R. siliquosa will be disposed of following methanol clean-up. Quantities of secondary metabolites are <0.001g ml^{-1}. **Non-hazardous chemicals:** **Calcium chloride:** (non-hazardous in low volumes) In case of spill, wear nitrile gloves, Soak with tissue and water, repeat the procedure until the area is clean, waste to go in general bin. **Citric Acid:** (non-hazardous in low volumes) In case of spill, wear nitrile gloves, Soak with tissue and water, repeat the procedure until the area is clean, waste to go in general bin. **Cellulose sulphate:** In case of spill, wear nitrile gloves, Soak with tissue and water, repeat the procedure until the area is clean, waste to go in general bin. **Sodium alginate:** In case of spill, wear nitrile gloves, Soak with tissue and water, repeat the procedure until the area is clean, waste to go in general bin. **Nutrient broth/malt extract agar**: In case of spill, wear nitrile gloves, Soak with tissue and water, repeat the procedure until the area is clean, waste to go in general bin. **Lime**: (mix of $Ca(OH)_2$, sand and aggregate), In case of spill, wear nitrile gloves, face covering and eye covering. Soak with tissue and water, repeat the procedure until the area is clean, waste to go in general bin **Hazardous Chemicals:** **Methanol spillage: Methods for Containment and Clean-up:** Contain and soak up spill with absorbent that does not react with spilled product. Contaminated absorbent poses the same hazard as the spilled product. Place used absorbent into suitable, covered, labelled containers for disposal. Flush spill area. Any spillage will be reported to laboratory staff. **Poly diallyl dimethyl ammonium chloride**: Contain and soak up spill with absorbent material. Contaminated absorbent poses the same hazard as the spilled product. Place used absorbent into suitable, covered, labelled containers for disposal. Flush spill area. Any spillage will be reported to laboratory staff. **DG 18 agar**: (non-hazardous in low volumes) however contain and soak up spill with absorbent material. Contaminated absorbent poses the same hazard as the spilled product. Place used absorbent into suitable, covered, labelled containers for disposal. Flush spill area. Any spillage will be reported to laboratory staff.

DSEAR Risk Assessment (Dangerous Substances & Explosive Atmospheres Regulations)

Is/are the substance(s) used subject to the Dangerous Substances & Explosive Atmospheres Regulations? i.e. flammable, explosive, compressed gas, corrosive to metal (HSE guidance) **If yes, then please contact your SBU Safety Adviser or the Health and Safety Team.**	Yes	No
		No

Are there any hazards not relating to substances that need to be considered for this activity?

Hazard	What harm	Who	Risk Level	Controls

Glassware handling	Cuts, spills	Researcher, students, staff	Low	Visual examination of glassware prior to use, discard damaged items, care using gloves to ensure firm, non-slip contact with glassware
Laboratory equipment	Cuts, spills, injury from inappropriate use of equipment	Researcher, students, staff	Low	Training on all electrical, electronic, moving, and analytical equipment to be provided prior to use and confirmed by laboratory staff supervision.
Repetitive handling	RSI	Researcher	Low	Provision of break periods to avoid stress from repetition and to retain alertness during repetitive procedures
Slips, trips, falls	Contusions, skeletal-muscular strains, or breakages	Researcher, students, staff	Low	Appropriate sensible/lab appropriate non-slip footwear, attention to any spillages which occur, use of wet-floor warning signs if spillages occur, mindful conduct when in the lab
Equipment: Positive polarity voltage generator, HV PSU AC Input 0-30kV 1.0A, 30W +ve output with remote interlock and remote interlock cherry switch safety features	Electrical shock	Researcher, students, staff	medium	Training on all electrical, electronic, moving, and analytical equipment to be provided prior to use and confirmed by laboratory staff supervision. Additional safety features purchased with the equipment using remote interlocks. Equipment will not be operated in the presence of other students, researchers, or staff other than the researcher/assessor. Equipment will be deactivated between experimental sessions. Safety screen will be erected between experimental equipment and operator. Non-conducting shoes and gloves will be used when operating the equipment. IEEE standard 510-1983 (IEEE Recommended Practices for Safety in High-Voltage and High- Power Testing) provides a working framework for establishing safe procedures and read in conjunction with local regulations and accepted codes of practice. The following excerpts are taken from IEEE 510 All ungrounded terminals of the test equipment or apparatus under test should be considered as energised. Common ground connections should be solidly connected to both the test set and the test specimen. As a minimum, the current capacity of the ground leads should exceed that necessary to carry the maximum possible ground current. The effect of ground potential rise due to the resistance and reactance of the earth connection should be considered. Precautions should be taken to prevent accidental contact of live terminals by personnel, either by shielding the live terminals or by providing barriers around the area.
IPS-13 double channel syringe pump with coaxial nozzle- A programmable double channel pump providing flow rates between 17.89 ml/min - 121.5 ml/min. (12V unit with multi-voltage adapter.	Electric shock	Researcher, students, staff	low	Training on all electrical, electronic, moving, and analytical equipment to be provided prior to use and confirmed by laboratory staff supervision.

Assessment of Risk		
Are all the controls detailed above currently in place?	Yes	No

	Yes	

If these controls are not in place or additional controls are required, please state action to be taken. Please note – the work CANNOT take place if adequate control measures are not in place.

Remedial Actions Required	By who?	By when?
Please state any further controls that have not been outlined in the assessment that may be required		

	Yes	No
Are all hazards to health adequately controlled with all control measures?	Yes	

Assessor Name	Assessor Signature	Date
Peter Booth		

Has an assessment been made previously? If so, please state the details of the assessment below:

Previously Assessed by	Date

The line manager or supervisor should sign below to show that the assessment is a correct and reasonable reflection of the hazards and of the control measures and actions required.

Line Manager/Supervisor Name	Line Manager/Supervisor Signature	Date

Lab or Workshop Manager/Health and Safety Adviser

Name	Job Title	Signature	Date

Date of Next Review	

Title	***Extraction and isolation of lichen secondary metabolites***
ssor	***Peter Booth***
Substance(s) Information	
Outline of Task/Method	*Ochrolechia parella and Ramalina siliquosa, common lichen found on stone walls and buildings in coastal locations, are the source for extraction using solvents acetone and methanol, subject to concentration using an evaporator, and identified, isolated, and extracted on TLC and Preparatory TLC plates using a methanol/dichloromethane elute. The extracted secondary metabolites are for use in biosciences.*
Location of Activity	*Chemistry Laboratory (to be allocated)*

Product/ Substance	*Hazard*	*Form*	*Quantity Used or Stored*	*Duration of Exposure*	*Route of Entry*	*Frequency of Exposure*	*Workplace Exposure Limit UH Guidance EH40*	*Risk Level [1] (RAG rating)*
Acetone CH_3COCH_3 CAS: 67-64-1 99.5%+	 GHS02,GHS07 *H225 Highly flammable liquid and vapor.* *H319 Causes serious eye irritation.* H336 May cause drowsiness or dizziness.	*Liquid*	*1,000 ml*	*Minutes. Exposure is always restricted to use within a ventilated fume cupboard. Gloves and eye covering must be worn when handling*	*Eye, lungs, skin. Fume exposure, skin contact and ingestion must be avoided.*	*Daily for the period of the project (10 days)*	*Long term exposure limit (8-hr TWA reference period) 500 ppm. Short term exposure limit (15-min reference period) 1500 ppm*	*Amber*
Methanol CH_3OH CAS: 67-56-1 99.8%+	 GHS02,GHS06,GHS08 *H225 Highly flammable liquid and vapor.* *H301 + H311 + H331 Toxic if swallowed, in contact with skin or if inhaled.* *H370*	*Liquid*	*2,000 ml*	*Minutes. Exposure is always restricted to use within a ventilated fume cupboard.*	*Skin contact, inhalation, ingestion must be avoided.*	*Daily for the period of the project (10 days)*	*Long term exposure limit (8-hr TWA reference period) 200 ppm. Short term exposure limit (15-min*	*Amber*

[1] Risk level is based on the likelihood of exposure and the severity of the health effects. For example, a high-risk substance could cause serious health effects and the level of exposure makes it likely that these effects could occur. The risk level will determine the level of controls required to control exposure.

	Causes damage to organs (Eyes, Central nervous system).			*Gloves and eye covering must be worn when handling*			*reference period) 250 ppm*	
Dichloromethane CH_2Cl_2 CAS: 75-09-2 99.8%+	 GHS07,GHS08 *H315 Causes skin irritation. H319 Causes serious eye irritation. H336 May cause drowsiness or dizziness.* *H351 Suspected of causing cancer.*	*Liquid*	*1,000 ml*	*Minutes. Exposure is always restricted to use within a ventilated fume cupboard. Gloves and eye covering must be worn when handling*	*Eye contact, skin exposure, inhalation and ingestion must be avoided as the compound can target the central nervous system*	*Daily for the period of the TLC and preparatory TLC activity (2 days)*	*Long term exposure limit (8-hr TWA reference period) 100 ppm. Short term exposure limit (15-min reference period) 200 ppm.*	*High*

Do any of the substances require a licence or notification to purchase? COSHH Tables	*Yes, University has licence.*
Will the substance(s) or composition change during the activity and have these changes been considered during this assessment?	*No*
Who will be Harmed and How?	
Who may be exposed to the substance(s)?	*Researchers and students jointly operating within the fume cupboard*
What are the general consequences of exposure?	***Acetone and methanol** are commonly used solvents and protective measures protecting the eyes, skin, and use of a fume cupboard to protect inhalation minimises exposure. Ingestion must be avoided. Exposure irritates the eyes, skin, and lung tissue.* ***Dichloromethane*** *H315: Causes skin irritation. H319: Causes serious eye irritation. H336: May cause drowsiness or dizziness.* ***H351: Suspected of causing cancer.*** *must not be exposed to eyes, skin and used exclusively within the fume cupboard. Ingestion must be avoided, and the risks noted that dermal or ingestion entry into the body can cause central nervous damage and is potentially carcinogenic.*

Do any of the substances pose additional risks to certain individuals (e.g. expectant mothers)	*These substances pose equal risk to all individuals.*		
Control Measures			
Does the substance(s) require authorisation to retain? (i.e. is it used very infrequently, or age poses additional risks) Consult your SBU safety contact if unsure.		*Yes*	*No* *No*
Can a less hazardous substance be used to do the same job? *(if you do not know, please contact your supplier for additional information)*		*Yes*	*No* *No*
Physical or Engineering Controls	*Provision of lab coat, goggles/eye protection, non-permeable gloves, and the sole use of an exhaust vented fume cupboard for all work where the solvents are exposed.*		
Administrative Controls	*There is access control to the laboratories. Training in use of extractor fume cupboards, use of PPE and handling of hazardous chemicals and biological risks has been undertaken.*		
Personal Protective Equipment ***Specify which type and when they are to be worn***	*Safety glasses, nitrile disposable gloves, lab coat used in conjunction with and exhaust ventilated fume cupboard*		
Storage Requirements	*Acetone- storage class code 3 - Flammable liquids* *Methanol-storage class code 3 - Flammable liquids* *Dichloromethane – storage class code 6.1D - Non-combustible, acute toxic Cat.3 / toxic hazardous materials or hazardous materials causing chronic effects. Stored in a metal, hazard labelled, lockable cupboard when not in use.*		
Transport of the substance(s)	*Transportation is not required*		
Disposal *(Refer to local procedures)*	***All chemicals used in this activity to be given to chemistry technical team for disposal.*** *Disposal must not be into an open waste system such as a sink. Disposal of each of the solvents will follow laboratory procedure keeping the solvents separate from other volatile interactions and disposed of without contamination of public drainage. The solvents must not be able to enter the public water supply.*		
Health Surveillance required? *(UH Guidance)*		*Yes*	*No* *No*
Have persons undertaking this activity and using this substance(s) been provided with information and training in its use? (Ensure that the findings in this risk assessment are communicated to all involved with this activity and that they are aware of the required controls).		*Yes* *Yes*	*No*
Emergency Procedures			
First Aid Measures ***If substance(s) reacts with water this must be mentioned first*** *(Refer to local procedures)*	*Protocol requires following usual first aid measures which are:* *Call a first aider or Security.* *If ingested: Rinse mouth with water* *If inhaled: Move person into fresh air.* *If in contact with eyes: Flush eyes with water* *If in contact with skin: Wash off with plenty of water for 5-20 minutes, repeat up to twice more if necessary.*		

Fire Fighting Measures (Refer to local procedures)	*Acetone and methanol are highly flammable and extinguished using the correct fire extinguisher (CO_2 or foam) or fire blanket.* ***Emergency procedure in case of fire:*** *Sound the alarm.* *Leave building by the nearest available exit.* *Report to assembly point no:9 which is outside LRC.* *Do not return to the building until authorised to do so.* *Do not use lifts.* *Call security on 01707 285555*
Accidental Release Measures (Refer to local procedures)	***Acetone/methanol spillage: Methods for Containment and Clean-up:*** *Contain and soak up spill with absorbent that does not react with spilled product. Contaminated absorbent poses the same hazard as the spilled product. Place used absorbent into suitable, covered, labelled containers for disposal. Flush spill area. Any spillage will be reported to laboratory staff.* ***Dichloromethane spillage****: In the event a dichloromethane chemical spill or leak occurs, several steps need to be taken:* 1. *Remove personnel from area.* 2. *Secure and control entrance to the area* 3. *If safe, stop or reduce the spill/leak.* 4. *Absorb liquids in a material such as dry sand earth, or a similar material, and place into sealed containers.* 5. *Ventilate area of spill or leak* 6. *Do NOT wash into sink or sewer.*

DSEAR Risk Assessment *(Dangerous Substances & Explosive Atmospheres Regulations)*		
Is/are the substance(s) used subject to the Dangerous Substances & Explosive Atmospheres Regulations? i.e. flammable, explosive, compressed gas, corrosive to metal (HSE guidance) ***If yes, then please contact your SBU Safety Adviser or the Health and Safety Team.***	*Yes*	*No*
		No

Are there any hazards not relating to substances that need to be considered for this activity?				
Hazard	*What harm*	*Who*	*Risk Level*	*Controls*
Glassware handling	*Cuts, spills*	*Researcher, students, staff*	*Low*	*Visual examination of glassware prior to use, discard damaged items, care using gloves to ensure firm, non-slip contact with glassware*
Laboratory equipment, including evaporator, filtration unit, weighing scales, UV lightbox	*Cuts, spills, injury from inappropriate use of equipment*	*Researcher, students, staff*	*Low*	*Training on all electrical, electronic, moving, and analytical equipment to be provided prior to use and confirmed by laboratory staff supervision.*
Repetitive handling	*RSI*	*Researcher*	*Low*	*Provision of break periods to avoid stress from repetition and to retain alertness during repetitive procedures*

Slips, trips, falls	*Contusions, skeletal-muscular strains, or breakages*	*Researcher, students, staff*	*Low*	*Appropriate sensible/lab appropriate non-slip footwear, attention to any spillages which occur, use of wet-floor warning signs if spillages occur, mindful conduct when in the lab.*

Assessment of Risk				
Are all the controls detailed above currently in place?			*Yes* *Yes*	*No*
If these controls are not in place or additional controls are required, please state action to be taken. Please note – the work CANNOT take place if adequate control measures are not in place.				
Remedial Actions Required		*By who?*	*By when?*	
No.				
Are all hazards to health adequately controlled with all control measures?			*Yes* *Yes*	*No*
Assessor Name	***Assessor Signature***		***Date***	
Peter Booth			*11 May 2023*	
Has an assessment been made previously? If so, please state the details of the assessment below:				
Previously Assessed by			***Date***	
The line manager or supervisor should sign below to show that the assessment is a correct and reasonable reflection of the hazards and of the control measures and actions required.				
Line Manager/Supervisor Name	***Line Manager/Supervisor Signature***		***Date***	
Lab or Workshop Manager/Health and Safety Adviser				
Name	***Job Title***	***Signature***	***Date***	
Date of Next Review				

1. Identification of the Substance and Company

Substance or preparation trade name: Limewash
Other Names: Colourwash, Whitewash
Company name & address:

Cornish Lime Company Ltd.
Brims Park,
Old Callywith Road,
Bodmin,
PL31 2DZ

Telephone: 01208 79779
Emergency telephone number: 999

2. Composition

Substance: Calcium Hydroxide + Water + inorganic pigments (optional)
% content: Various – dependent on age, contact us for more details.
CAS Number: 1305-62-0
Classification: Irritant/Corrosive (only corrosive to aluminium)
EINECS: 215-137-3

3. Hazards Identification

Irritating to skin and eyes, can cause chemical burns if not washed off. Risk of serious damage to eyes if not washed out – keep saline eyewash available when working with Lime. In case of eye irritation after washing seek immediate medical attention.

4. First aid measures

Skin contact: Pat dry the contaminated body surfaces in order to remove all traces of product. Wash affected skin area immediately with plenty of water. Remove contaminated clothing. If necessary seek medical advice

Eye contact: Rinse eyes immediately with plenty of water/saline solution and seek medical advice.

Ingestion: Clean mouth with water and drink afterwards plenty of water. Do NOT induce vomiting. Obtain medical attention.

5. Fire fighting measures

Suitable extinguishing media: The product is not combustible. Use a dry powder, foam or CO2 fire extinguisher to extinguish the surrounding fire. Use extinguishing measures that are appropriate to local circumstances and the surrounding environment.

Unsuitable extinguishing media: None.

Special hazards in fire: Avoid generation of dust. Use breathing apparatus. Use extinguishing measures that are appropriate to local circumstances and the surrounding environment

Required special protective equipment for fire-fighters: Use breathing apparatus

6. Accidental release measures

Personal precautions:

Keep unprotected persons away.
Avoid contact with skin, eyes, and clothing – wear suitable protective equipment (see section 8).

Environmental precautions: Contain the spillage. Cover area if possible to avoid unnecessary hazard. Avoid uncontrolled spills to watercourses and drains (pH increase). Any large spillage into watercourses must be alerted to the Environment Agency or other regulatory body.

Methods for cleaning: soak up the limewash with a suitable inert material and dispose of in a suitable container, wash away the minimum amount possible.

7. Handling and storage

Handling: Avoid contact with skin and eyes. Wear protective equipment (refer to section 8 of this safety data sheet). Do not wear contact lenses when handling this product. It is also advisable to have individual pocket eyewash. When handling buckets the usual precautions should be paid to the risks outlined in the Council Directive 90/269/EEC.
Avoid ingestion and contact with skin and eyes. General occupational hygiene measures are required to ensure safe handling of the substance. These measures involve good personal and housekeeping practices (i.e. regular cleaning with suitable cleaning devices), no drinking, eating and smoking at the workplace. Shower and change clothes at end of work shift. Do not wear contaminated clothing at home.

Storage: The substance should be stored under cool frost free conditions. Any contact with air should be avoided – retain the water layer on the surface of tubs etc. Keep away from acids, significant quantities of paper, straw, and nitro compounds. Keep out of reach of children. Do not use aluminum for transport or storage.

8. Exposure Controls

Control Parameters (dust – not applicable when wet):

SCOEL recommendation (SCOEL/SUM/137 February 2008; see Section 16.6):
Occupational Exposure Limit (OEL), 8 h TWA: 1 mg/m³ respirable dust of calcium dihydroxide
Short-term exposure limit (STEL), 15 min: 4 mg/m³ respirable dust of calcium dihydroxide
PNEC aqua = 490 µg/l
PNEC soil/groundwater = 1080 mg/l

Personal protection equipment:

Eye protection: Do not wear contact lenses. Wear tight fitting goggles with side shields, or wide vision full goggles. It is also advisable to have individual pocket eyewash

Skin protection: Since calcium dihydroxide is classified as irritating to skin, dermal exposure has to be minimized as far as technically feasible. The use of protective gloves (nitrile), protective standard working clothes fully covering skin, full length trousers, long sleeved overalls, with close fittings at openings and shoes resistant to caustics and avoiding dust penetration are required to be worn.

Environmental measures: Avoid releasing to the environment. Contain the spillage. Any large spillage into watercourses must be alerted to the regulatory authority responsible for environmental protection or other regulatory body.

For detailed explanations of the risk management measures that adequately control exposure of the environment to the substance please check the relevant exposure scenario, available via your supplier.

9. Physical and chemical properties

Appearance: White wet slurry
Odour: none
pH: 12-13
Boiling point: 100^{0}C
Melting point: N/A
Flashpoint: N/A
Explosive properties: N/A
Vapour pressure:N/A – water release will give a H_2O vapour pressure based on temperature
Relative density: 1.1Kg/L or higher
Solubility: 1844.9 mg/L (study results, EU A.6 method) – from lime powder form
Oxidising properties: N/A

10. Stability and reactivity

Conditions to avoid: Minimise exposure to air
Materials to avoid: Calcium dihydroxide reacts exothermically with acids to form salts. Calcium dihydroxide reacts with aluminium and brass in the presence of moisture leading to the production of hydrogen.

$$Ca(OH)2 + 2\ Al + 6\ H2O \rightarrow Ca[Al(OH)4]2 + 3\ H2$$

Hazardous decomposition products: None.
(Further information: Calcium dihydroxide reacts with carbon dioxide to form calcium carbonate, which is a common material in nature.)

11. Toxicological information

Acute toxicity: Calcium dihydroxide is not acutely toxic.
Oral LD_{50} > 2000 mg/kg bw (OECD 425, rat)
Dermal LD_{50} > 2500 mg/kg bw (OECD 402, rabbit)
Inhalation no data available
Classification for acute toxicity is not warranted.
(For irritating effects to the respiratory tract see below)

Excessive exposure may affect human health as follows:
Skin contact: Calcium dihydroxide is irritating to skin
Eye contact: Calcium dihydroxide entails a risk of serious damage to the eye
Inhalation/ingestion: From current data it is concluded that $Ca(OH)_2$ is irritating to the respiratory tract, this is less applicable when it is in a wet state.

Calcium dihydroxide is considered not to be a skin sensitiser, based on the nature of the effect (pH shift) and the essential requirement of calcium for human nutrition.

12. Ecological information

Acute pH-effect. Although this product is useful to correct water acidity, an excess of more than 1 g/l may be harmful to aquatic life. pH-value of > 12 will rapidly decrease as result of dilution and carbonation.

13 Disposal Considerations

Disposal of calcium dihydroxide should be in accordance with local and national legislation. Processing, use or contamination of this product may change the waste management options. Dispose of container and unused contents in accordance with applicable member state and local requirements.
The used packing is only meant for packing this product; it should not be reused for other purposes. After usage, empty the packing completely

14. Transport information

Calcium dihydroxide is not classified as hazardous for transport (ADR (Road), RID (Rail), IMDG / GGVSea (Sea).

15. Regulatory information

Authorisations: Not required
Restrictions on use: None
Other EU regulations: Calcium dihydroxide is not a SEVESO substance, not an ozone depleting substance and not a persistent organic pollutant.
National regulations: Water endangering class 1 (Germany)

16. Other Information

Data are based on our latest knowledge but do not constitute a guarantee for any specific product features and do not establish a legally valid contractual relationship.

Hazard Statements
H315: Causes skin irritation
H318: Causes serious eye damage
H335: May cause respiratory irritation

Precautionary Statements
P102: Keep out of reach of children
P280: Wear protective gloves/protective clothing/eye protection/face protection
P305+P351: IF IN EYES: Rinse cautiously with water for several minutes
P310: Immediately call a POISON CENTRE or doctor/physician
P302+P352: IF ON SKIN: Wash with plenty of soap and water
P261: Avoid breathing dust/fume/gas/mist/vapours/spray
P304+P340: IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing
P501: Dispose of contents/container in accordance with local/regional/national/international regulations

Risk Phrases
R37: Irritating to respiratory system
R38: Irritating to skin
R41: Risk of serious damage to eyes

TARMAC
A CRH COMPANY

Uniclass L621
/ CI/SfB / / Yq2 / /
March 2017

SAFETY INFORMATION

HYDRALIME

Health and Safety Information Prepared in accordance with Annex II of the REACH Regulation EC 1907/2006, Regulation (EC) 1272/2008 and Regulation (EC) 453/2019

1 IDENTIFICATION OF THE SUBSTANCE AND OF THE COMPANY/UNDERTAKING

1.1 Product identifier	
Substance name	Hydrated lime
Synonyms	Slaked lime, Air slaked lime, Building lime, Fat lime, Chemical lime, Finishing lime, Mason's lime, Calcium dihydroxide, Calcium hydroxide, Calcium hydrate, Lime, Lime water.
	Please note that this list may not be exhaustive.
Chemical name and formula	Calcium dihydroxide – Ca(OH)2
Trade name	Hydralime
CAS	1305-62-0
EINECS	215-137-3
Molecular Weight	74,09 g/mol
REACH Registration number	01-2119475151-45-0053

1.2 Relevant identified uses of the substance or mixture and uses advised against

Please check the identified uses in Table 1 of the Appendix of this SDS. Uses advised against: There are no uses advised against.

1.3 Details of the supplier of the safety data sheet

Tarmac Cement and Lime Ltd,
Portland House, Bickenhill Lane,
Birmingham B37 7BQ
Technical helpdesk: 0845 812 6323
Email: info-cement@tarmac.com

1.4 Emergency telephone

Emergency telephone number available during office hours: ***Tel 0845 812 6232***
Emergency telephone number available outside officehours: No

2 HAZARDS IDENTIFICATION

2.1 Classification of the substance

2.1.1 Classification according to Regulation (EC) 1272/2008

STOT Single Exp. 3, Route of exposure: Inhalation
Skin Irritation 2
Eye Damage 1

2.2 Label elements

2.2.1 Labeling according to Regulation (EC) 1272/2008

Signal word: Danger

Hazard pictogram

Hazard statement

H315: Causes skin irritation.
H318: Causes serious eye damage.
H335: May cause respiratory irritation.

Precautionary statements

P102: Keep out of reach of children.
P280: Wear protective gloves/protective clothing eye protection/face protection.

P305+P351+ P310: IF IN EYES: Rinse cautiously with water for several minutes. Immediately call a POISON CENTER or doctor/physician.
P302+P352: IF ON SKIN: Wash with plenty of soap and water.
P261: Avoid breathing dust / fume / gas / mist / vapours / spray.
P304+P340: IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P501: Dispose of contents/container to hazardous waste collection point

2.3 Other hazards
The substance does not meet the criteria for PBT or vPvB substance. No other hazards identified.

3 COMPOSITION/INFORMATION ON INGREDIENTS

3.1 Composition

Main constituent
Name: Calcium dihydroxide
CAS: 1305-62-0
*EINECS:*215-137-3

Impurities
No impurities relevant for classification and labelling.

4 FIRST AID MEASURES

4.1 Description of first aid measures
General advice
No known delayed effects. Consult a physician for all exposures except for minor instances.

Following inhalation
Move source of dust or move person to fresh air.
Obtain medical attention immediately.

Following skin contact
Carefully and gently brush the contaminated body surfaces in order to remove all traces of product.
Wash affected area immediately with plenty of water.
Remove contaminated clothing. If necessary seek medical advice.

Following eye contact
Rinse eyes immediately with plenty of water and seek medical advice.

After ingestion
Clean mouth with water and drink afterwards plenty of water. Do NOT induce vomiting.
Obtain medical attention.

4.2 Most important symptoms and effects, both acute and delayed
Calcium dihydroxide is not acutely toxic via the oral, dermal, or inhalation route. The substance is classified as irritating to skin and the respiratory tract, and entails a risk of serious damage to the eye. There is no concern for adverse systemic effects because local effects (pH-effect) are the major health hazard.

4.3 Indication of any immediate medical attention and special treatment needed
Follow the advice given in section 4.1

5 FIRE FIGHTING MEASURES

5.1 Extinguishing media

5.1.1 Suitable extinguishing media
Suitable extinguishing media: The product is not combustible. Use a dry powder, foam or CO2 fire extinguisher to extinguish the surrounding fire. Use extinguishing measures that are appropriate to local circumstances and the surrounding environment.

5.1.2 Unsuitable extinguishing media
Do not use water.

5.2 Special hazards arising from the substance or mixture
None

5.3 Advice for firefighters
Avoid generation of dust. Use extinguishing measures that are appropriate to local circumstances and the surrounding environment.

6 ACCIDENTAL RELEASE MEASURES

6.1 Personal precautions, protective equipment and emergency procedures

6.1.2 For non-emergency personnel
Ensure adequate ventilation
Keep dust levels to a minimum.
Keep unprotected persons away.

Avoid contact with skin, eyes, and clothing – wear suitable protective equipment (see section 8). Avoid inhalation of dust – ensure that sufficient ventilation or suitable respiratory protective equipment is used, wear suitable protective equipment (see section 8).

6.1.3 For emergency responders

Keep dust levels to a minimum.
Ensure adequate ventilation.
Keep unprotected persons away.
Avoid contact with skin, eyes, and clothing – wear suitable protective equipment (see section 8).
Avoid inhalation of dust – ensure that sufficient ventilation or suitable respiratory protective equipment is used, wear suitable protective equipment (see section 8).

6.2 Environmental precautions

Contain the spillage. Keep the material dry if possible. Cover area if possible to avoid unnecessary dust hazard. Avoid uncontrolled spills to watercourses and drains (pH rising). Any large spillage into watercourses must be alerted to the Environment Agency or other regulatory body.

6.3 Methods and material for containment and cleaning up

In all cases avoid dust formation.
Keep the material dry if possible.
Pick up the product mechanically in a dry way.
Use vacuum suction unit, or shovel into bags.

6.4 Reference to other sections

For more information on exposure controls /personal protection or disposal considerations, please check sections 8 and 13 of this safety data sheet.

7 HANDLING AND STORAGE

7.1 Precautions for safe handling

7.1.1 Protective measures

Avoid contact with skin and eyes. Wear protective equipment (refer to section 8 of this safety data sheet). Do not wear contact lenses when handling this product. It is also advisable to have individual pocket eyewash. Keep dust levels to a minimum. Minimise dust generation. Enclose dust sources, use exhaust ventilation (dust collector at handling points). Handling systems should preferably be enclosed. When handling bags usual precautions should be paid to the risks outlined in the Council Directive 90/269/EEC.

7.1.2 Advice on general occupational hygiene

Avoid inhalation or ingestion and contact with skin and eyes. General occupational hygiene measures are required to ensure safe handling of the substance. These measures involve good personal and housekeeping practices (ie, regular cleaning with suitable cleaning devices), no drinking, eating and smoking at the workplace. Shower and change clothes at end of work shift. Do not wear contaminated clothing at home.

7.2 Conditions for safe storage, including any incompatibilities

The substance should be stored under dry conditions. Any contact with air and moisture should be avoided. Bulk storage should be in purpose-designed silos. Keep away from acids, significant quantities of paper, straw, and nitro compounds. Keep out of reach of children. Do not use aluminium for transport or storage if there is a risk of contact with water.

7.3 Specific end use(s)

Please check the identified uses in Table 1 of the Appendix of this SDS. For more information please see the relevant exposure scenario in the Appendix, and check section 2.1: Control of worker exposure.

8 EXPOSURE CONTROLS /PERSONAL PROTECTION

8.1 Control parameters

SCOEL recommendation (SCOEL/SUM/137 February 2008):Occupational Exposure Limit (OEL), 8 h TWA: 1 mg/m³ respirable dust of calcium dihydroxide Short-term exposure limit (STEL), 15 min: 4 mg/m³ respirable dust of calcium dihydroxide PNEC aqua = 490 µg/lPNEC soil/groundwater = 1080 mg/l

8.2 Exposure controls

To control potential exposures, generation of dust should be avoided. Further, appropriate protective equipment is recommended. Eye protection equipment (eg, goggles or visors) must be worn, unless potential contact with the eye can be excluded by the nature and type of application (ie, closed process). Additionally, face protection, protective

clothing and safety shoes are required to be worn as appropriate. Please check the relevant exposure scenario, given in the Appendix.

8.2.1 Appropriate engineering controls

If user operations generate dusts or fumes, use process enclosures, local exhaust ventilation, or other engineering controls to keep airborne levels below recommended exposure limits.

8.2.2 Individual protection measures, such as personal protective equipment

8.2.2.1 Eye/face protection

Do not wear contact lenses. For powders, tight fitting goggles with side shields, or wide vision full goggles. It is also advisable to have individual pocket eyewash.

8.2.2.2 Skin protection

Since calcium dihydroxide is classified as irritating to skin, dermal exposure has to be minimised as far as technically feasible. The use of protective gloves (nitrile), protective standard working clothes fully covering skin, full length trousers, long sleeved overalls, with close fittings at openings and shoes resistant to caustics and avoiding dust penetration are required to be worn.

8.2.2.3 Respiratory protection

Local ventilation to keep levels below established threshold values is recommended. A suitable particle filter mask is recommended, depending on the expected exposure levels - please check the relevant exposure scenario, given in the Appendix.

8.2.2.4 Thermal hazards

The substance does not represent a thermal hazard, thus special consideration is not required.

8.2.3 Environmental exposure controls

All ventilation systems should be filtered before discharge to atmosphere. Avoid releasing to the environment. Contain the spillage. Any large spillage into watercourses must be alerted to the Environment Agency or other regulatory body. For detailed explanations of the risk management measures that adequately control exposure of the environment to the substance please check the relevant exposure scenario, available via your supplier. For further detailed information, please check the Appendix of this SDS.

9 PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on basic physical and chemical properties

Appearance	White or off white (beige) fine powder
Odour	odourless
Odour threshold	not applicable
pH	12.4 (saturated solution at 20 °C)
Melting point	> 450 °C (study result, EU A.1 method)
Boiling point	not applicable (solid with a melting point > 450 °C)
Flash point	not applicable (solid with a melting point > 450 °C)
Evaporation rate	not applicable (solid with a melting point > 450 °C)
Flammability	non flammable (study result, EU A.10 method)
Explosive limits	non explosive (void of any chemical structures commonly associated with explosive properties)
Vapour pressure	not applicable (solid with a melting point > 450 °C)
Vapour density	not applicable
Relative density	2.24 (study result, EU A.3 method)
Solubility in water	1844.9 mg/L (study results, EU A.6 method)
Partition coefficient	not applicable (inorganic substance)
Auto ignition temperature	no relative self-ignition temperature below 400 °C (study result, EU A.16 method).
Decomposition temperature	When heated above 580°C, calcium dihydroxide decomposes to produce calcium oxide (CaO) and water (H2O).
Viscosity	not applicable (solid with a melting point > 450 °C)
Oxidising properties	no oxidising properties (Based on the chemical structure, the substance does not contain a surplus of oxygen or any structural groups known to be correlated with a tendency to react exothermally with combustible material)

10 STABILITY AND REACTIVITY

10.1 Reactivity

In aqueous media Ca(OH)2 dissociates under formation of calcium cations and hydroxyl anions (when below the solubility).

10.2 Chemical stability

Under normal conditions of use and storage, calcium dihydroxide is stable.

10.3 Possibility of hazardous reactions

Reacts exothermically with acids. When heated above 580 °C, calcium dihydroxide decomposes to produce calcium oxide (CaO) and water (H_2O): $Ca(OH)_2 \rightarrow CaO + H_2O$. Calcium oxide reacts with water and generates heat. This may cause risk to flammable material.

10.4 Conditions to avoid

Minimise exposure to air and moisture to avoid degradation.

10.5 Incompatible materials

Reacts exothermically with acids to form salts. Reacts with aluminium and brass in the presence of moisture leading to the production of hydrogen.

$Ca(OH)_2 + 2Al + 6H_2O \rightarrow Ca[Al(OH_4)]_2 + 3H_2$

10.6 Hazardous decomposition products

None.

Further information: Calcium dihydroxide reacts with carbon dioxide to form calcium carbonate, which is a common material in nature:

$Ca(OH)_2 + CO_2 \rightarrow CaCO_3 + H_2O$

11 TOXICOLOGICAL INFORMATION

11.1 Information on toxicological effects

Calcium dihydroxide is classified as irritating to skin and the respiratory tract and it entails a risk of serious damage to the eye. The occupational exposure limit for the prevention of local sensory irritation and decrease of lung function parameters as critical effects is OEL (8 h) = 1 mg/m³ respirable dust.

Toxicity endpoints	Outcome of the effects assessment
Absorption	The primary health effect of calcium dihydroxide is local irritation due to a pH shift. Therefore, absorption is not a relevant parameter for the effects assessment.
Acute toxicity	Calcium dihydroxide is not acutely toxic.
Oral	LD50 > 2000 mg/kg bw (OECD 425, rat)
Dermal	LD50 > 2500 mg/kg bw (OECD 402, rabbit)
Inhalation	No data available. Classification for acute toxicity is not warranted. For irritating effects to the respiratory tract see below.
Irritation/ corrosion	*Eye irritation* Calcium dihydroxide entails a risk of serious damage to the eye (eye irritation studies (in vivo, rabbit). *Skin irritation* Calcium dihydroxide is irritating to skin (in vivo, rabbit) *Respiratory irritation* From human data it is concluded that Ca(OH)2 is irritating to the respiratory tract. Based on experimental results, calcium dihydroxide requires classification as irritating to skin [R38, Irritating to skin; Skin Irrit 2 (H315 – Causes skin irritation)] and as severely irritating to the eye [R41, Risk of serious damage to eye; Eye Damage 1 (H318 - Causes serious eye damage)]. As summarised and evaluated in the SCOEL recommendation (Anonymous, 2008), based on human data it is proposed to classify calcium dihydroxide as irritating to the respiratory system [R37, Irritating to respiratory system; STOT SE 3 (H335 – May cause respiratory irritation)].
Sensitisation	No data available. Calcium dihydroxide is considered not to be a skin sensitiser, based on the nature of the effect (pH shift) and the essentiality of calcium for human nutrition. Classification for sensitisation is not warranted.
Repeated dose toxicity	Toxicity of calcium via the oral route is addressed by upper intake levels (UL) for adults determined by the Scientific Committee on Food (SCF), being UL = 2500 mg/d, corresponding to 36 mg/kg bw/d (70 kg person) for calcium. Toxicity of Ca(OH)2 via the dermal route is not considered as relevant in view of the anticipated insignificant absorption through skin and due to local irritation as the primary health effect (pH shift). Toxicity of Ca(OH)2 via inhalation (local effect, irritation of mucous membranes) is addressed by an 8-h TWA determined by the Scientific Committee on Occupational Exposure Limits (SCOEL) of 1 mg/m³ respirable dust. Therefore, classification of Ca(OH)2 for toxicity upon prolonged exposure is not required.
Mutagenicity	Bacterial reverse mutation assay (Ames test, OECD 471): Negative. Mammalian chromosome aberration test: Negative. In view of the omnipresence and essentiality of Ca and of the physiological non-relevance of any pH shift induced by lime in aqueous media, lime is obviously void of any genotoxic potential. Classification for genotoxicity is not warranted.
Carcinogenicity	Calcium (administered as Ca-lactate) is not carcinogenic (experimental result, rat). The pH effect of calcium dihydroxide does not give rise to a carcinogenic risk. Human epidemiological data support lack of any carcinogenic potential of calcium dihydroxide. Classification for carcinogenicity is not warranted.
Toxicity for reproduction	Calcium (administered as Ca-carbonate) is not toxic to reproduction (experimental result, mouse). The pH effect does not give rise to a reproductive risk. Human epidemiological data support lack of any potential for reproductive toxicity of calcium dihydroxide. Both in animal studies and human clinical studies on various calcium salts no reproductive or developmental effects whatsoever were detected. Also see the Scientific Committee on Food (Anonymous, 2006). Thus, calcium dihydroxide is not toxic for reproduction and/or development. Classification for reproductive toxicity according to regulation (EC) 1272/2008 is not required.

12 ECOLOGICAL INFORMATION

12.1 Toxicity

12.1.1 Acute/Prolonged toxicity to fish
LC50 (96h) for freshwater fish: 50.6 mg/l
LC50 (96h) for marine water fish: 457 mg/l

12.1.2 Acute/Prolonged toxicity to aquatic invertebrates
EC50 (48h) for freshwater invertebrates: 49.1 mg/l
LC50 (96h) for marine water invertebrates: 158 mg/l

12.1.3 Acute/Prolonged toxicity to aquatic plants
EC50 (72h) for freshwater algae: 184.57 mg/l
NOEC (72h) for freshwater algae: 48 mg/l

12.1.4 Toxicity to micro-organisms e.g. bacteria
At high concentration, through the rise of temperature and pH, calcium dihydroxide is used for disinfection of sewage sludges

12.1.5 Chronic toxicity to aquatic organisms
NOEC (14d) for marine water invertebrates: 32 mg/l

12.1.6 Toxicity to soil dwelling organisms
EC10/LC10 or NOEC for soil macroorganisms: 2000 mg/kg soil dw
EC10/LC10 or NOEC for soil microorganisms: 12000 mg/kg soil dw

12.1.7 Toxicity to terrestrial plants
NOEC (21d) for terrestrial plants: 1080 mg/kg

12.1.8 General effect
Acute pH-effect. Although this product is useful to correct water acidity, an excess of more than 1 g/l may be harmful to aquatic life. pH-value of > 12 will rapidly decrease as result of dilution and carbonation

12.2 Persistence and degradability
Not relevant for inorganic substances

12.3 Bioaccumulative potential
Not relevant for inorganic substances

12.4 Mobility in soil
Calcium dihydroxide, is sparingly soluble, and so present a low mobility in most ground conditions.

12.5 Results of PBT and vPvB assessment
Not relevant for inorganic substances

13 DISPOSAL CONSIDERATIONS

13.1 Waste treatment methods
Disposal of calcium dihydroxide should be in accordance with local and national legislation. Processing, use or contamination of this product may change the waste management options. Dispose of container and unused contents in accordance with applicable member state and local requirements. The used packing is only meant for packing this product; it should not be reused for other purposes. After usage, empty the packing completely.

14 TRANSPORT INFORMATION
Calcium dihydroxide is not classified as hazardous for transport (ADR (Road), RID (Rail), IMDG / GGVSea (Sea).

14.1 UN-Number
Not regulated

14.2 UN proper shipping name
Not regulated

14.3 Transport hazard class(es)
Not regulated

14.4 Packing group
Not regulated

14.5 Environmental hazards
None

14.6 Special precautions for user
Avoid any release of dust during transportation, by using tight tanks.

14.7 Transport in bulk according to Annex II of MARPOL73/78 and the IBC Code
Not regulated

15 REGULATORY INFORMATION

15.1 Safety, health and environmental regulations/ legislation specific for the substance

Authorisations:	Not required
Restrictions on use:	None
Other EU regulations:	Calcium dihydroxide is not a SEVESO substance, not an ozone depleting substance and not a persistent organic pollutant.
National regulations:	Water endangering class 1 (Germany)

15.2 Chemical safety assessment

A chemical safety assessment has been carried out for this substance. An exposure scenario (ES) is available as Annex A to this MSDS. This Annex elates to the uses applicable to the Hydralime product

16 OTHER INFORMATION

Data are based on our latest knowledge but do not constitute a guarantee for any specific product features and do not establish a legally valid contractual relationship.

16.1 Hazard Statements

H315: Causes skin irritation.
H318: Causes serious eye damage.
H335: May cause respiratory irritation.

16.2 Precautionary Statements

P102: Keep out of reach of children.
P280: Wear protective gloves/protective clothing/ eye protection/face protection.
P305+P351: IF IN EYES: Rinse cautiously with water for several minutes
P310: Immediately call a POISON CENTER or doctor/physician.
P302+P352: IF ON SKIN: Wash with plenty of soap and water.
P261: Avoid breathing dust / fume / gas / mist / vapours / spray.
P304+P340: IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P501: Dispose of contents/container to hazardous waste collection point

16.3 Abbreviations

EC50: median effective concentration
LC50: median lethal concentration
LD50: median lethal dose
NOEC: no observable effect concentration
OEL: occupational exposure limit
PBT: persistent, bioaccumulative, toxic chemical
PNEC: predicted no-effect concentration
STEL: short term exposure limit
TWA: time weighted average
VPvB: very persistent, very bioaccumulative material

16.4 Key Literature references:

Anonymous, 2006: Tolerable upper intake levels for vitamins and minerals Scientific Committee on Food, European Food Safety Authority, ISBN: 92-9199-014-0 [SCF document] Anonymous, 2008: Recommendation from the Scientific Committee on Occupational Exposure Limits for calcium oxide (CaO) and calcium dihydroxide (Ca(OH)2), European Commission, DG Employment, Social Affairs and Equal Opportunities, SCOEL/SUM/137 February 2008

For futher information
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E-mail info-cement@tarmac.com
Customer services & sales
Tel: 0845 812 6300
E-mail customerservice@tarmac.com

University of Hertfordshire UH

School/SBU/ Department:	Creative Arts	Assessor Name:	Peter Booth
		Date:	28 May 2023

ACTIVITY TITLE/DESCRIPTION:

DEP Project – Microbial self-repairing building coating, a collaboration with industry partners UK Hempcrete Ltd and Whyte & MacKay Ltd. The test site is located at the Whyte & MacKay's Jura Distillery and focuses on a long-term option for building conservation. The project requires the staff member to travel (on public transport by train, ferry, and bus) to the Island of Jura site to complete the on-site preparatory work and subsequent observational testing.

The university will seek to utilise the developed product to lower traditional lime render coatings. The University Health and Safety Policy has been followed in the preparation of this risk assessment. (UPR HS08 version 09.1 effective from 5 April 2022)

IDENTIFY HAZARDS	WHO COULD BE HARMED & HOW		EVALUATE THE RISK AND DECIDE ON CONTROLS		RECORD YOUR FINDINGS AND IMPLEMENT THEM	
Hazards associated with the activity/task/Event? *What are the significant hazards with the potential to cause harm?* *Review the activity, location & people involved. Check equipment or manufacturer instructions. Check UH, Sector or HSE guidance.*	**Who could be harmed?** *Who is at risk from harm: Students, Staff, Visitors and/or Contractors?*	**How could they be harmed?** *Types of injury: Major or Minor Injuries from Lifting/Handling, Slips/Trips/Falls or Ill Health Effects.*	**What controls are currently in place/available to reduce the risk?** *Current control measures: Engineering Controls, Safe Operating Procedures, Local Rules, Training or Supervision.*	**What further action is necessary?** *Actions/additional controls required to reduce the remaining risks.*	**Remaining Actions?** Actions by **Who** and by **When?**	**Actions Completed?** Completed **(Y/N)**
Exposure to hazardous substances Hydraulic Lime 3.5 The CAS number for hydraulic lime is 1305-62-0 (Sigma Aldrich) PubChem ID 329752521.  GHS05,GHS07 **Hazard statements** H315, H318, H335. **Precautionary statements** P261, P264, P271, P280, P302, P352, P305, P351, P338	Researcher	Skin contact Inhalation Respiratory irritation Eye contact Ingestion	Researcher has appropriate training in handling and dealing with lime. The attached COSHH Risk Assessment and Safe Operating Procedure. All PPE equipment is to be worn in compliance with the RA and SOP. Which includes: Protective gloves, protective clothing, eye protection and face protection including the	Signage is created and notices to identify the work area which is excluded from public access. Area of access can only be approached using appropriate PPE.	Project Supervisor	

Hazard classifications Eye Dam.1 – Skin Irrit. 2 – STOT SE 3 A separate COSHH assessment for this compound is submitted.			wearing of a face mask to prevent inhalation. Procedures will be carried out in the outdoors in the open and mixing and pouring will take place in designated sections only. All contaminated disposable items and rubbish placed in "fast cast" bin and disposed of in accordance with local arrangements for hazardous waste. The researcher is trained in handling lime and lime based products including disposal.			
Exposure to microbial culture ASN III nutrient broth containing cellulose, Synechococcus sp. All three compounds listed above are not considered dangerous or placing users at-risk. Synechococcus sp. is a non-pathogenic cyanobacteria found in seawater and freshwater lakes.	Researcher	Spillage due to slips, trips or falls. Splashing due to mixing.	Liquid is in sealed containers which will be opened once and mixed with the hydraulic lime solution. During handling and mixing all PPE equipment will be work in compliance with the use of hydraulic lime - protective gloves, protective clothing, eye protection and face protection including the wearing of a face mask to prevent inhalation. Appropriate footwear is worn to prevent slips, trips, or injury. The researcher is trained in handling microbial cultures including disposal.			
Exposure to infection: COVID-19 https://herts365.sharepoint.com/sites/HealthSafetySustainability/SitePages/Infection-Control-Guidance.aspx	Researcher	Spread of Viral Potential - respiratory infection or infection from contaminated surfaces	Steps to reduce the spread of the respiratory disease: Adequate ventilation – project is conducted outside. Cleaning of tools and surfaces	Researcher is fully vaccinated including regular booster shots. Researcher carries a home testing covid kit for testing		

On-site, and during travel to and from site			On removal of gloves, good hand hygiene, Use of hand sanitiser during travel Avoiding people with symptoms Carry disposable face coverings during travel in case of suspected infected passenger. **Staff Guidance** infection control **Student/Visitor Guidance:** Coronavirus (COVID-19) advice Anyone who feels unwell or tests positive to stay at home and follow Government guidance. Government guidance	if suspected symptoms occur during travel.		
Manual Handling	Researcher	User injury, muscular, skeletal or impact related	Good manual handling practice. Training attended.	iHASCO manual handling training completed		
Slips/Trips/Falls (Note: this project does not require working at height)	Researcher	User injury, bruises, sprains	Good housekeeping practiced, Walkways and working areas to be kept clean and tidy	All spillages must be dealt with in accordance with the client's local health and safety policy		

Title	**Client project – Jura (Design Exchange Programme)**
Assessor	**Peter Booth**

Substance(s) Information

Outline of Task/Method	*Travel to client site on Isle of Jura, Scotland, to apply a limewash coating plus experimental formulation to 0.025 m² wall site, isolated, with signage, from the client's normal work activities. The application site is in open air, and at standing height level.*
Location of Activity	*Isle of Jura, Scotland*

Product/ Substance	Hazard	Form	Quantity Used or Stored	Duration of Exposure	Route of Entry	Frequency of Exposure	Workplace Exposure Limit UH Guidance EH40	Risk Level [1] (RAG rating)
Hydraulic lime render NHL 3.5 Premixed with natural English lime ($Ca(OH)_2$, CAS:1305-62-0) sand and aggregate	 GHS05, GHS07 H315, H318, H335 P261 - P264 - P271 - P280 - P302 + P352 - P305 + P351 + P338 According to regulations No 1272/2008 (CLP) *Product safety sheet attached*	*Solid/ liquid*	*5 kg*	Hours Alkaline resistant gloves, mask and eye covering must be worn when handling	Skin irritation, eye irritation, targets respiratory tract irritation	*One day, 2-3 hours*	*N/A*	*Low*
Formulated ASN III nutrient broth containing cellulose, Synechococcus sp.	Synechococcus sp. is a non-pathogenic cyanobacteria found in sea and freshwater sources. It does not have a health and safety sheet. A product sheet is attached. ATCC III Medium – product sheet attached – non-toxic. Cellulose CAS no 9004-34-6 classified as a non-hazardous substance according to Regulation (EC) No 1272/20 (safety sheet attached)	Liquid	500 ml	Hours Alkaline resistant gloves, mask and eye covering must be worn when handling	Non-pathogenic , non-toxic solution. pH neutral	One day, 2-3 hours	N/A	Low

Do any of the substances require a licence or notification to purchase? COSHH Tables	No
Will the substance(s) or composition change during the activity and have these changes been considered during this assessment?	The lime when applied will sequester carbon dioxide and convert into calcium carbonate (limestone). This substance is chemically inactive and does not pose a risk.

Who will be Harmed and How?

[1] Risk level is based on the likelihood of exposure and the severity of the health effects. For example, a high-risk substance could cause serious health effects and the level of exposure makes it likely that these effects could occur. The risk level will determine the level of controls required to control exposure.

Who may be exposed to the substance(s)?	Researcher. The area of application is isolated with hazard warnings which does not permit access to another party other than the researcher.		
What are the general consequences of exposure?	H315 causes skin irritation, H318 causes serious eye damage, H335 may cause irritation		
Do any of the substances pose additional risks to certain individuals *(e.g. expectant mothers)*	P102 – keep out of the reach of children		
Control Measures			
Does the substance(s) require authorisation to retain? *(i.e. is it used very infrequently, or age poses additional risks)* Consult your SBU safety contact if unsure.		Yes	No
			No
Can a less hazardous substance be used to do the same job? *(if you do not know, please contact your supplier for additional information)*		Yes	No
			No
Physical or Engineering Controls	*The product is not combustible nor toxic. It is not classified as hazardous for transport*		
Administrative Controls	The area is marked 'no access' with clear signage around the area of application. The researcher has been trained and certified using lime on building surfaces.		
Personal Protective Equipment **Specify which type and when they are to be worn**	S37: Wear suitable gloves S39: Wear eye/face protection **Eye protection**: Do not wear contact lenses. Wear tight fitting goggles with side shields, or wide vision full goggles. It is also advisable to have individual pocket eyewash **Skin protection**: Since lime is classified as irritating to skin, dermal exposure must be minimized as far as technically feasible. The use of protective gloves (nitrile), protective standard working clothes fully covering skin, full length trousers, long sleeved overalls, with close fittings at openings and shoes resistant to caustics and avoiding dust penetration are required to be worn.		
Storage Requirements	The hydraulic lime is in locked stored controlled by the client/partner on their work site, complying with the client's industry health and safety standards.		
Transport of the substance(s)	The hydraulic lime is stored and provided from the client/partner site and will not be transported. The nutrient/cellulose/microorganism solution will be transported via a specialist courier services such as Speedlink or PDQ specialist couriers in sealed containers with absorbent packaging and cellulose wadding.		
Disposal *(Refer to local procedures)*	Avoid releasing to the environment. Contain the spillage. Any large spillage into watercourses must be alerted to the regulatory authority responsible for environmental protection or other regulatory body. There will be no nutrient/cellulose/microorganism solution waste requiring disposal.		
Health Surveillance required? (UH Guidance)	*Does this substance(s) require any user health surveillance? if yes, please state which type & level and who it is conducted by:*	Yes	No
			No
Have persons undertaking this activity and using this substance(s) been provided with information and training in its use? *(Ensure that the findings in this risk assessment are communicated to all involved with this activity and that they are aware of the required controls).*		Yes	No
		Yes	
Emergency Procedures			
First Aid Measures **If substance(s) reacts with water this must be mentioned first** *(Refer to local procedures)*	The substance forms a hydrate with water which begins carbonation reducing the alkalinity of the substance. Usual first aid measures are: Call a first aider, there is one on -site. If ingested: Rinse mouth with water		

	If inhaled: Move person into fresh air – application is in the open. If in contact with eyes: Flush eyes with water If in contact with skin: Wash off with plenty of water for 5-20 minutes, repeat up to twice more if necessary.
Fire Fighting Measures *(Refer to local procedures)*	The product is not combustible. Use a dry powder, foam, or CO_2 fire extinguisher to extinguish the surrounding fire. Extinguishing measures will be used that are appropriate to local circumstances and the surrounding environment.
Accidental Release Measures *(Refer to local procedures)*	Contain the spillage. Cover area, if possible, to avoid unnecessary hazard. Avoid uncontrolled spills to watercourses and drains. Any large spillage into watercourses must be alerted to the Environment Agency or other regulatory body. In this instance only small volumes will be mixed in batch and do not pose a threat to the watercourse.

DSEAR Risk Assessment (Dangerous Substances & Explosive Atmospheres Regulations)

Is/are the substance(s) used subject to the Dangerous Substances & Explosive Atmospheres Regulations? i.e. flammable, explosive, compressed gas, corrosive to metal (HSE guidance) **If yes, then please contact your SBU Safety Adviser or the Health and Safety Team.**	Yes	No
		No

Are there any hazards not relating to substances that need to be considered for this activity?

Hazard	What harm	Who	Risk Level	Controls
Manual handling	*Potential muscular and skeletal injuries*	*Researcher*	*Low*	*Avoid carrying heavy bags of materials. Equipment is handheld and lightweight. Full PPE must be worn during carrying and mixing of liquids.*
Slips, trips, falls	Spillage of limewash or research formulation	Researcher	Low	Avoid carrying open liquids. Limewash formulation mixed on-site of application to avoid spillage. Application is at flat ground level and does not require a support structure.
Accidental release	Spillage of limewash or research formulation	Researcher	Low	Due to the small application area (0.025 m^2) The volumes are applied from small volume containers. Any spillage will be treated with water, avoiding the watercourses.
Emergency action- fire, first aid, security	Burns, first aid treatment for injury, compromised site security for materials	Researcher	Low	None of the compounds used are combustible or a fire risk. The site location is outside the building and away from any services to the building. The site has signage preventing access, and the population of the island at ±80 people present a low risk of intrusion. Materials prior to and after use will be stored in a locked external storeroom.

Assessment of Risk

Are all the controls detailed above currently in place?	Yes	No
	Yes	

If these controls are not in place or additional controls are required, please state action to be taken. Please note – the work CANNOT take place if adequate control measures are not in place.

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Please state any further controls that have not been outlined in the assessment that may be required	*None*	N/A

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Novel biodesign enhancements to at-risk traditional building materials

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Extreme weather conditions increase the frequency of regular maintenance on heritage buildings and cause erosion of traditional materials. Developments in bio-enhanced self-repair materials provide an opportunity to improve building performance and reduce the frequency of costly maintenance schedules. The microbial sequestration of carbon by bacteria, encapsulated and layered into several limewash coats, facilitates capturing atmospheric carbon and reduces carbon-generating maintenance regimes. The use of hydrogels, alginates and biofilm derived biopolymers as novel bacterial encapsulation and nutrient delivery vehicles is discussed and the opportunity to develop self-healing sacrificial limewash as a future research project. Microbial enhanced carbon-fixing limewash may also offer a broader application to improve the performance of sustainable materials such as hemp-lime bio-composites as a fast-forward projection of problems and solutions with these materials in the future.

KEYWORDS

carbon-fixing, limewash, hemp-lime, heritage, conservation, encapsulation

1 Introduction

The goal of this review is to improve the long-term CO_2 performance of lime substrates by applying a bioactive-carbonating limewash to protect the renders on heritage building which are at-risk from the effects of climate change. The objective is to propose a biological approach to extend limewash performance on building exteriors which are subjected to weather erosion and because of this approach improve long-term capture of atmospheric CO_2.

Extreme weather patterns generate global droughts, floods, wind-driven rain, changes in pH and biological attack (Table 1) which threaten heritage building longevity. Climate change is challenging existing conservation policy for listed heritage buildings such as potential conflicts with zero carbon programmes and insulation retrofits (Brimblecombe, Grossi and Harris, 2011).

This study examines the effect climate change will have on heritage buildings particularly those buildings on at-risk registers and in private ownership. Maintenance and upgrade programme costs for listed buildings are rising rapidly, driven by limited artisan skills, traditional material shortages and increasing

supplemental costs, such as building scaffolding and complying with health and safety regulations. A comparison of 30 listed churches in England revealed a range of expenditure for average cost of repairs in the region of £100–250 k while the speed of deterioration is so rapid such expenditure is insufficient to maintain building stability (Historic England, 2019). Without substantial changes to conservation policies, extreme weather events will ultimately result in a decline in built heritage assets. The development of more robust self-healing biomaterials such as bio-limewash, will help to address maintenance costs while improving the sequestering of atmospheric CO_2 and extending the longevity of heritage assets.

2 Background

Aware of the challenge, the United Kingdom government, directed by the 2008 Climate Change Act, publishes a climate change risk assessment (CCRA) every 5 years (UK Government, 2008). Legislation directed toward moderating greenhouse gases and their contribution toward solar heat reflection, is under development. The CCRA3 Risk Independent Assessment 2021, is a result of more than 3 years of work based on the latest scientific evidence from over 450 experts on weather-related hazards informing the CCRA3 Government Report. The CCRA3 Report has published several themed factsheets including how cultural heritage has been assessed and the types of action necessary to adapt to climate change risk.

Pre-1919 homes constitute approximately 20% of the total housing stock across the United Kingdom. Unlisted pre-1919 buildings more readily accommodate a retrofit intervention to reduce the carbon footprint (Hamot and George, 2021). In contrast, listed properties as defined in Planning Legislation (HM Government, 1997) are governed by less-agile heritage policies restraining the adoption of zero carbon emission goals and adaptive strategies to climate change.

Biodesigned applications offer a sustainable approach to address this challenge by enhancing traditional construction materials. This paper considers how lime render can be modified to extend built heritage lifespan and performance, reduce maintenance costs and lower CO_2 emissions. This review examines current microbe cementitious carbonation technologies and explores options to enhance the sacrificial role of limewash to slow down the speed of climate accelerated lime render deterioration.

Lime was originally selected on availability, aesthetic qualities, and a proven track record of preserving buildings (Carran et al., 2012). The microscopic structure of lime facilitates moisture permeability and when saturated forms a watertight outer skin limiting the accumulation of trapped water.

Ecologically sustainable, lime slowly absorbs CO_2 hardening while it carbonates. It has a lower embodied energy than concrete and the potential to embed atmospheric carbon for the lifetime of the building.

The increase in anthropogenic airborne pollutants and extreme weather erosion undermine the long-term longevity

TABLE 1 Environmental factors influenced by climate change and their impact on lime-based products.

Factors influenced by climate change	Challenge to lime-based products	References
Temperature	In 2021 the maximum temperature reached 32.2°C compared to the average hottest day during the period 1961—1990 of 31.4°C. In 2022, the maximum temperature was 40.3°C recorded at Coningsby, Lincolnshire and a new provisional temperature for Scotland was set at 38.7°C. Compared to extensive studies on the impact of higher temperatures on cement-based products, there is limited research on the impact on heritage materials such as lime other the effects of fire. Temperatures below 5°C and higher than 18°C restrict natural carbonation taking place	(RMetS, 2022) (Doleželová et al., 2018; Pachta, Triantafyllaki and Stefanidou, 2018)
Sea level	From the early 1900s sea levels have risen around the United Kingdom by 16.5 cm and the increase in levels is accelerating. In addition to coastal erosion, the frequent storm surges result in the generation of wind-driven salt-saturated rain. The Roman architect Vitruvius in his work *De Architectura* developed a salt-resistant lime mix to withstand the eroding effect of sea water by adding finely ground natural mineral marble powder in a ratio of 1 part lime to three parts pozzolan (volcanic ash). The growth of salt crystals within the lime reduces the tensile strength of the render. Pozzolans may effectively lower salt erosion but similarly reduce the tensile strength of the lime	Morgan, (1960)
Changes in pH	The increased absorption of CO_2 acidifies the ocean and ocean spray, while acid rain forms due to the sulphur dioxide and nitrogen oxide released from power stations and volcanoes. An increase in volcanic activity results from climate change as ice-loss reduces the pressure on the land mantle enabling volcanic venting	Tuffen, (2007)
Biological attack	An increase and extension of wet/dry weather cycles encourages fractures in the lime allowing moisture penetration. Microorganism and their biofilms retain moisture resulting enlarging the cracks and furthering erosion opening the lime to further invasion	Viles, (2002)

of lime-based products. The physical degradation of lime render and mortar arises from the chemical removal of the calcium ions by dissolved atmospheric acidic gases and by chemical substitution with sulphates and chlorides. As erosion occurs, spaces form in the lime providing damp niches for chemotrophs which produce toxic compounds of ammonia and nitrite salts and as they die form a nutrient base for other organisms. Traditionally, limewash is applied to extend the life of the underlying render and mortar by providing a sacrificial surface which is more easily repaired under a regular maintenance schedule, protecting the building, and reducing ongoing costs. Extreme weather events result in more intense wind-driven rain, halving the lifespan of the limewash layer. Doubling the frequency of the maintenance schedule is likely to be cost prohibitive and raise the carbon footprint of the building. A bio-enhanced limewash layer can reduce underlying lime render damage and can also provide a protective layer to a wider range of materials such as hemp-lime.

Hemp-lime can reduce or eliminate carbon emissions from conventional construction processes which can shorten the time taken to achieve net zero targets (Bharadwaj, Jankovic and Carta, 2021). As hemp-lime bio-composite material comes with negative embodied emissions of -108 kg CO_2/m^3 (Bevan and Woolley, 2008) resulting from sequestration of carbon dioxide in the hemp plant during its growth, the use of this material leads to a significant reduction in embodied emissions. Building performance improvements resulting from hemp-lime bio-composites are stable internal air temperatures and relative humidity. The inclusion of hemp-lime into historic building repairs is of growing interest for low energy consumption and occupant health in housing (Eberlin and Jankovic, 2014), as well as in non-residential projects where stable temperatures and relative humidity help with the preservation of museum artefacts (Leskard, 2022) or pharmaceutical products (Couch, Perry and Wilkes, 2014).

3 Literature review

Carbonates in varying forms of limestone account for nearly 42% of the total carbon on the planet, a significant portion of which is biogenic in origin (Zhu and Dittrich, 2016). Carbon sequestration by microbial CO_2 fixation is now recognised as an emerging and promising technology (Rossi et al., 2015). Photosynthetic microbes, such as cyanobacteria and microalgae, contribute to capturing CO_2 (Kumar et al., 2011). In addition to the environmental benefits, the commercial opportunities of exploiting environmentally beneficial microbial products are significant. Microbial biologics in 2015 were valued at US$ 277 billion, estimated to reach US$ 400 billion by 2025 (Grand View Research, 2017). Microbials contribute toward generated lime-concrete CO_2 micro-encapsulating pastes (Wang and Soens, 2014), biopolymers (Moradali and Rehm, 2020), biocides and biosurfactants (Fidanza and Caneva, 2019; Plaza and Achal, 2020), biofilm generated bioelectricity (Nealson, 2017), biofuels (Kumar et al., 2018) and brownfield site bioremediation (Megharaj and Naidu, 2017). The economic value of the carbon-fixing global market in the future is likely to be significantly greater than past estimates.

3.1 Lime render and weather erosion

Lime is produced from burning calcium-based rocks at a temperature of 900°C forming unstable calcium oxide (CaO) (Figure 1). Calcium oxide is "slaked" or hydrated with water, to form lime putty, or dampened aggregate to form "hot-lime." The addition of water to calcium oxide is violently exothermic producing thixotropic wet hydrate, traditionally preferred by artisans. Hydrated lime undergoes induration from atmospheric carbon dioxide at a temperature above 5°C and with a residual moisture level. The carbonation process absorbing atmospheric CO_2 occurs at 5 mm per month from the outer skin working inwards (Young, 2008).

It is the outer stone or render that is under threat from climate change (Table 1) causing extended drought and flood cycles, rain acidity (H_2SO_3, HNO_3) resulting in changes in pH, increasing temperature, biological attack, and storm force winds (Sabbioni, Brimblecombe and Lefevre, 2008). Wind driven rain and rainwater salination in coastal areas drive salts which accumulate in weathered microcracks. During periods of drought, salt crystallisation exerts significant pressure within the spaces in the lime resulting in material failure.

By the 19th century use of lime mortar declined as Portland cement, an easy to use, fast curing and high compression strength material, became widely used. Cement has two drawbacks, it can fracture and become brittle, and it is a major contributor to atmospheric CO_2 (Blankendaal, Schuur and Voordijk, 2014). Researchers are exploring ways to lower these CO_2 emissions due to the extensive use of concrete in construction, though the costs remain prohibitive (Scrivener, John and Gartner, 2018).

Lime in comparison is a time-consuming material as the application requires a build of several layers which is dependent on weather conditions and availability of competent skills. Inappropriate conservation treatments such as epoxy resins and $Ba(OH)_2$ solution are environmentally toxic and hamper moisture permeability which causes irreversible damage to the microstructure of the lime (Rodriguez-Navarro et al., 2003). Additives such as sealants and pozzolans attempt to enhance the adherence, waterproofing and antiseptic properties of the lime. Over the centuries the application of lime on buildings have included additives such as marine salt (1811), skimmed milk (1881), warm slaughterhouse blood mixed with stale beer (1883), flour (1887), sugar (1890), and molasses (1913) (Taliaferro, 2015). Artisans believed the addition of blood improved binding strength, weather resistance and carbonation. This

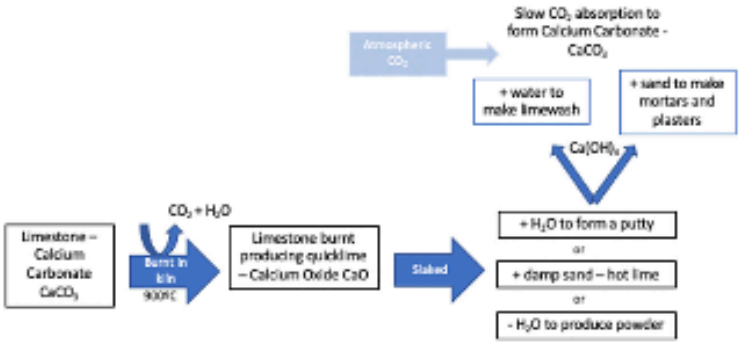

FIGURE 1
The lime cycle.

may be a result of blood protein hydrolysis within the alkaline environment (Fangquiang et al., 2015).

Concrete despite the ease of application and high compressive strength, is subject to cracking. Increased brittleness generates microcracks allowing water and pollutants to entry undermining long-term structural integrity. Thermal expansion between different component materials reduces material strength which over time leads to environmental pollution (de Muynck et al., 2008).

Early attempts to incorporate microbial repair mechanisms to strengthen concrete, involved casting the concrete with *Bacillus* spores and calcium lactate ($C_6H_{10}CaO_2$) as a nutrient embedded in clay pellets. As cracks form, water splits the pellets resuscitating the dormant bacteria. The bacteria then form calcium carbonate ($CaCO_3$) deposits, sealing the cracks (Jonkers, 2007; Wiktor and Jonkers, 2011). During this microbial repair process, the compressive strength of the concrete was noticeably improved (Bang et al., 2010). However, anaerobes such as ureolytic bacteria generate eco-toxic by-products such as ammonia while precipitating calcium carbonate through biomineralisation, thereby contributing to toxic run-off. In contrast, autophototrophic bacteria fix atmospheric carbon forming strong calcite layers within the cracks and avoid the production of toxic by-products (Zhu and Dittrich, 2016).

3.2 Mechanisms of bacterial precipitation of calcium carbonate

Microbial precipitation results from metabolic activities which are either heterotrophic (e.g., urea hydrolysis) or autotrophic. The negatively charged outer cell membrane binds divalent cations such as Ca^{2+}, resulting in the organism forming a crystal nucleation site. As urea hydrolyses into CO_2 and ammonia, bio-deposition increases both the surrounding pH and resulting carbonate concentration (Chahal, Rajor and Sidique, 2011). Active microbial $CaCO_3$ precipitation accelerates with cell metabolism at a faster rate than passive chemical precipitation (Stocks-Fischer, Galinat and Bang, 1999) demonstrated by improvements in CO_2 sequestration in *Chlorella* sp. and *Spirulina platensis* up to 46% (Ramanan et al., 2010).

Three autotrophic metabolic pathways are involved in bacterial calcium carbonate formation (Castanier et al., 1999), non-methylotrophic methanogenesis, anoxygenic photosynthesis and oxygenic photosynthesis (Figure 2). All three pathways use CO_2 as a carbon source and in the presence of calcium ions, produce a precipitation of calcium carbonate.

Photosynthesis is the principal contributor to the production of carbonate rocks (Altermann et al., 2006) such as cyanobacteria formation of stromatolitic carbonate speleothems in the photic zone of carbonate caves (Léveillé, et al., 2007). Photosynthesis leads to calcite precipitation by conducting an HCO_3^-/OH^- exchange across the cell membrane increasing the pH around the cells. By diffusion or *via* a symporter, CO_2 enters the cell wall (Espie and Kandasamy, 1992), the CO_2 is then synthesised into organic matter while bicarbonate is converted to CO_2 and OH^-, the latter released out of the cell increasing the pH of the external environment. Cyanobacteria are the only organism that utilise H_2O as an electron donor during photosynthesis and the degree of light intensity is critical for this photosynthetic pathway (Kumar et al., 2011). Low intensity light limits the biomass productivity whereas high intensity can cause photo-inhibition. (Rubio Camacho et al., 2003).

When microbial carbonate is formed, the carbonate adheres to the original material while retaining moisture permeability (Rodriguez-Navarro et al., 2003). Importantly, microbial $CaCO_3$ deposition conforms to conservation standards and does not alter the appearance of the stone (Jroundi et al., 2010).

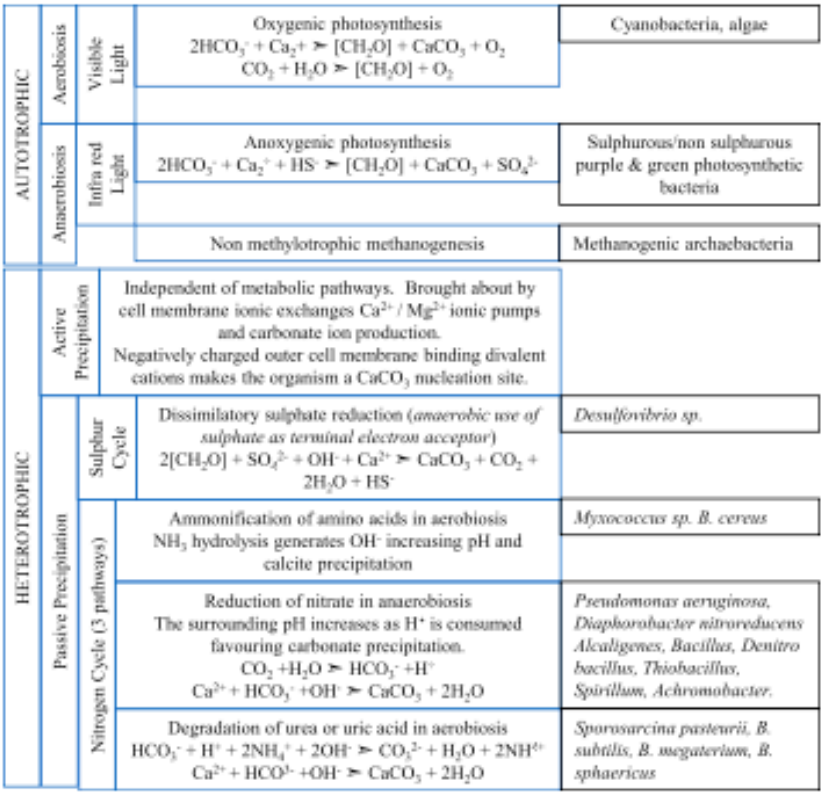

FIGURE 2
Comparison between autotrophic and heterotrophic bacterial production of $CaCO_3$. A redox generated high environmental pH is common across metabolic pathways. Autotrophic bacteria: Aerobiosis (Dupraz and Visscher, 2005), Anaerobiosis (Baumgartner et al., 2006; Reeburgh, 2007), Heterotrophic bacteria: Active Precipitation (Stocks-Fischer, Galinat and Bang, 1999), Passive precipitation (sulphur cycle) (Baumgartner et al., 2006; Braissant et al., 2007), (nitrogen cycle pathways) (Lee, 2003; Rodriguez-Navarro et al., 2003; Kavazanjian and Karatas, 2008; González-Muñoz et al., 2010; Jroundi et al., 2010; Achal and Mukherjee, 2015; Erşan, de Belie and Boon, 2015; Wei et al., 2015).

TABLE 2 Comparison of three methods employed to inoculate cement paste with bacteria.

	Direct application	Immobilisation	Encapsulation
Advantages	Simplest and cheapest method. Bacteria or spores are added on-site and the bacteria containing material is directly applied to the surface (Khaliq and Ehsan, 2016)	Enables the cells to tolerate the alkaline conditions of the cement paste and survive the mixing process. Immobilisation in sepiolite a hydrous magnesium silicate increases the viability of bacterial cells and extends the calcite precipitation fracture healing process (Bang, Galinat and Ramakrishnan, 2001; Seifan et al., 2018; Sandalci, Tezer and Basaran Bundur, 2021)	The encapsulate protects the bacterial cells or spores against the harsh alkaline environment and reduces damage from mixing and application. Encapsulation enables the introduction of nutrients into the capsule to extend bacterial performance (Oyen, 2014; Wang and Soens., 2014)
Disadvantages	The harsh alkaline environment and limited availability of nutrients results in a high cell mortality and extensive physical damage to any live cells (Jadhav et al., 2018)	Additional cost and off-site preparation of bacteria and immobilisation material. Antimicrobial qualities of immobilisation materials may reduce bacterial performance (Shaheen, Khushnood and Ud Din, 2018)	The discarded capsules may reduce the integrity of the concrete matrix undermining the benefits of the calcite precipitation. Thick capsule walls may impede cell resuscitation preventing the cells from entering the microfractures

TABLE 3 Applications utilising bacterial inclusion and encapsulation techniques employed.

Encapsulation application	Encapsulation technique	References
Food bio-products (Such as protection from oxidation, adverse chemical reactions, evaporation)	Spray drying, spray cooling, extrusion, co-crystallisation, coacervation	(da Silva et al., 2014; Poornima and Sinthya, 2017; Abd El Kader and Abu Hashish, 2020)
Phase change materials (PCMs)—organic, inorganic, eutectic (Protection from flammability, PCM agent separation, thermal instability)	Emulsions, electroplating, solvent evaporation, precipitation	(Milián et al., 2017; Gao et al., 2022)
In-situ biodegradation and bioremediation (Cell immobilisation for use in contamination sites)	Spray-drying, extrusion, freeze drying, electrospinning, coacervation, liposomes, ionic gelation, molecular inclusion	(Sarma, Pakshirajan and Mahanty, 2011; San Keskin et al., 2018; Bamidele and Emmambux, 2020; Guo et al., 2020; Valdivia-Rivera et al., 2021)
Drug delivery (Colon-targeted antitumour drugs—acid tolerant pectin polymers to release active drugs at site)	Hydrogels, pellets, microspheres, microsponges	Khotimchenko, (2020)
Enhanced construction materials (Concrete-strengthening enhancements)	Polymeric microcapsules incorporating chemical healing agents prepared by an oil-in-water dispersion mechanism based on an emulsion polymerisation technique. Sonification using a hydrophobic solution to generate microcapsules. Polymer encapsulation of bacterial spores *Bacillus sphaericus* using a melamine-based microcapsule system. Spores embedded in nutrient enriched hydrogels mixed directly into the mortar. Porous expanded recycled glass granules hold the spores and nutrients and trigger as the crack forms, promoting substrate repair	(Asua, 2002; Feng et al., 2008; Blaiszik et al., 2009; Wiktor and Jonkers, 2011; Wang et al., 2014; Souradeep and Kua, 2016; Zhang et al., 2021)

3.3 Methods for bacterial inclusion into a cementitious matrix

Bacterial inclusion into a cement or lime material follows three widely used methods, direct application, immobilisation, and encapsulation (Table 2) (Griño, Daly and Ongpeng, 2020). The simplest method directly applies live bacterial cells or spores with or without supporting nutrients to the concrete mix. Any micro spaces in the concrete fill with calcite precipitate, improving the overall compression strength (Ghosh et al., 2005). Due to the high alkaline environment, researchers have used alkaliphilic or alkali-tolerant strains that are capable of spore formation such as *Bacillus sphaericus*, a ureolytic, alkali-tolerant spore forming microbe. However, unprotected cells cannot endure the harsh environmental conditions and may not survive long enough to provide sufficient calcite repair (Wang and Soens, 2014; Li et al., 2019).

To improve live cell viability and precipitation, bacterial cells can be immobilised within a protective material. Bacteria immobilised within graphite nano-platelets and light weight aggregates can extend calcite precipitation up to 28 days (Khaliq and Ehsan, 2016). Other protective materials include limestone powder (Shaheen, Khushnood and Ud Din, 2018), iron oxide nanoparticles (Seifan et al., 2018), polyurethane (Bang, Galinat and Ramakrishnan, 2001) and sepiolite (Sandalci, Tezer and Basaran Bundur, 2021) which, subject to availability of nutrients, can extend viability for up to a year. A third method for inclusion into a cement or lime paste is to encapsulate the bacteria or spores within a biodegradable capsule providing a mechanical buffer during application and enclosed nutrients to extend cell viability.

3.3.1 Bacterial encapsulation

Encapsulation reduces the risk of physical or chemical damage to cells or spores prior to release into the cementitious matrix. The design of the capsule material must ensure encapsulation does not hinder carbonate precipitation, access to water, deteriorate the lime or cement matrix chemical profile nor reduce compression strength. Successful cell encapsulation becomes a function of surface texture, shell thickness and diameter (Joseph et al., 2010). Changes to the wet/dry curing environments can also influence the self-healing response of encapsulated cells (Wang et al., 2012). Microbial immobilisation using encapsulation is a more robust approach compared to solid or fluid microbe inclusion. For the process of encapsulation to be economic, consideration must include consistent evidence of microbial survival, longevity in transportation and ease of usage at the site of application.

3.3.2 Cell encapsulation technologies

The food, medical and environmental sectors utilise encapsulation for the introduction of targeted microbial cells as a means of extending the life and effectiveness of the microbes beneficial metabolic processes, with each technology adapted to its specific application (Table 3).

Bashan et al. (2002) inoculated soil using microbeads produced by a low-pressure spray of suspended bacterial culture in a highly nutrient liquid base mixed into an alginate solution. The resulting suspension expressed as small diameter droplets, which when sprayed through a calcium chloride solution hardened to form 100–200 µm containing colony forming units. The microbeads produced were viable and when added to wet or dry mediums could resist a standard

freeze-drying procedure. Within 15-days within a moist environment the microbeads successfully biodegraded within the medium.

This example illustrates just one of the methods used to immobilise and encapsulate at the micro level, others include flocculation, adsorption to surfaces, covalent bonding to a carrier, intercellular cross-linking, polymer-gel encapsulation, and matrix entrapment (Cassidy, Lee and Trevors, 1996). Each of these technologies require the selection of a polymer which will perform appropriately for the chosen application. There are a wide range of synthetic and natural polymers available. Natural biopolymers are more likely to be compatibility with environmentally sustainable goals than synthetics and better equipped to provide a supportive environment for microbial growth when used as the encapsulating medium.

3.4 Encapsulation polymers

3.4.1 Algal polysaccharides

Alginate and κ-carrageenan are two natural polymers, the guluronic acid in alginates for example, will readily form cross-linked polymer networks when exposed to Ca^{2+} ions. Alginates are linear polymers of β (1,4)—D-mannuronic acid and *α* (1,4)-L-guluronic acid monomers which are found in nature in varying configurations and displaying a wide range of properties (da Silva et al., 2014; Dhamecha et al., 2019). The cross-linked matrix encloses spaces which can entrap, protect, and immobilise cells. Calcium alginate has a thick, large pore alginate matrix ideal for bacterial occupation (Voo et al., 2016). Bacteria held within calcium alginate beads consistently generate calcite precipitation when compared to control groups (Soysal et al., 2020). As calcium alginate beads biodegrade, they provide sufficient time for a steady supply of nutrients and calcium ions for ongoing microbial carbonate precipitation. Microorganisms are encapsulated into the alginate using the traditional syringe method to produce the alginate beads which form in the range of 0.5–3.5 mm diameter (Lancy and Tuovinen, 1984). More advanced techniques can reduce the size of the beads down to 120 μm (Musgrave et al., 1983).

Algal polysaccharide beads increase the surface for cell attachment for encapsulated microorganisms allowing for a substantial increase in cellular metabolism. Alginate encapsulated *Saccharomyces cerevisiae* cells produced 80% more ethanol when compared to planktonic cells (Galazzo and Bailey, 1990).

Carrageenan produced by red algae such as *Chondrus crispus* offer a varied structural diversity composed of linear chains of β(1,3)-D-galactose and α(1–4)-D-galactose units which form a robust encapsulation gel (Perrechil et al., 2020). The encapsulation gel can be formed by extruding carrageenan and cell suspensions at a temperature of 42°C into a cold solution of potassium chloride. The risk of denaturing several of the temperature sensitive proteins within the cells can be mitigated through the addition of lotus bean or carob bean gum. This technique has been used for the large-scale production of encapsulated microorganisms for the treatment of contaminated soil sites (Hulst et al., 1985). Manufacturing techniques can generate industrial production capability of encapsulated alginate more than 24 lhr^{-1} using resonance nozzles, rotating disk atomisers, low pressure ultrasonic nozzles and parallel plate electrostatic droplet generators (Ogbonna et al., 1989; Stormo and Crawford, 1992). The alginate and carrageenan compounds improve encapsulation by providing chemical and mechanical stability, ensuring a more effective release of the capsule contents and protect the cells if exposed to freeze/thaw cycles (Poncelet et al., 1994; Malhotra and Basir, 2020; Sariyer et al., 2020).

3.4.2 Pectin

Like alginate, pectin is an anionic polysaccharide derived from plant cell walls and is composed of long sequenced partially methyl-esterified (1–4)-linked *α* D-galactosyluronic acid which forms a natural hydrogel with the addition of Ca^{2+} divalent ions (Yang, Mu and Ma, 2018). A simple hydrogel encapsulation technique incorporates the cell suspension with $CaCO_3$ and sodium alginate, which can be either extruded or applied as an emulsion (Liu, Xie and Nie, 2020). The thick stable wall of the pectin capsule exerts a controlled release and reducing the stress on the capsule contents.

3.4.3 Hydrogels

Initial encapsulation methods utilised a porous aggregate to encase the bacterial spores and nutrients (Jonkers, 2007). Hydrogels are a broader range of compounds which include alginate and pectin, and similarly consist of a hydrophilic gel of cross-linked polymer chains in which bacteria or spores are held and from which water is dispersed. Several hydrogels including calcium alginate provide a non-toxic, renewable natural source with properties such as a well-structured matrix and large pores, ideal for encapsulating bacterial cells (Voo et al., 2016). Hydrogel encapsulation mimics an intracellular environment by holding over 90% water (Oyen, 2014). The slow release of water held in the hydrogel matrix extends a protection to the cells from physical and chemical damage and provides water to facilitate metabolic $CaCO_3$ precipitation (Wang and Snoeck, 2014).

3.4.4 Bacterially generated biopolymers—Biofilms

A challenge for biopolymer immobilisation technology is their relatively low mechanical strength. Instability in the protective capsule will result in untimely release of bacteria and premature cell death. This can be addressed by reducing the size of the capsule to nano or micro encapsulation which demonstrates improved cell survival rates and cell lifespan (Jampílek and Králová, 2017; Prasad, Bhattacharyya and Nguyen, 2017).

TABLE 4 Introduction of additives to biopolymers for capsule performance improvement.

Additive	Encapsulation advantages	References
Clay minerals	• Improved capsule wall thickness, • Extended bacterial survival rate • Reduced UV damage • Controlled cell release	(Zohar-Perez et al., 2003; Liffourrena and Lucchesi, 2018)
Skimmed Milk	• Increases cell count • Faster release of cells from the capsule	(Yu et al., 2001; Bashan et al., 2002; Power et al., 2011)
Starch (alginate)	• Improves capsule matrix strength reducing physical stress • Reduces exposure to UV radiation	(Dunkle and Shasha, 1989; Jankowski, Zielinska and Wysakowska, 1997; Kim et al., 2005; Qi and Tester, 2019)
Chitin and chitosan	• Bioactive oligosaccharides improve resistance to pathogens and overall antimicrobial properties of the capsule	(Estevinho et al., 2013; Berger et al., 2014; Muzika et al., 2017; Nah and Jeong, 2021)
Humic acid	• Improved cell survival	Rekha et al. (2007)
Sugars	• Protection from osmotic pressures • Improved resistance to desiccation	(Morgan et al., 2006; Schoebitz, López and Roldán, 2013; San Keskin et al., 2018)
Proteins (hydrolysates, gelatine, albumin, elastin, casein, biofilm lectins)	• Enhance nutrient uptake by encapsulated cells • Bio-stimulants • Improved encapsulation rates • Improved linkage between microorganisms and exopolysaccharides	(Nesterenko et al., 2013; Elzoghby, Elgohary and Kamel, 2015; Colla et al., 2017; Casadesús, Polo and Munné-Bosch, 2019; Vejan et al., 2019; Valdivia-Rivera et al., 2021)

A key advantage provided by bacterial biopolymers is a three-dimensional space which can accommodate the microorganism. Bacteria produce four primary polymer classes, polysaccharides, polyesters, polyamides and inorganic polyanhydrides each expressing diverse properties. Microbial extracellular polymeric substance (EPS) or exopolymers, produced as a survival mechanism by bacterial cells, adhere to both hydrophobic and hydrophilic surfaces assisting in the formation of three-dimensional bacterial biofilm architectures (Decho and Gutierrez, 2017). As bacterial cells produce the biofilm it provides a highly effective barrier to toxic molecules. The barrier raises the minimum inhibitory concentration of cytotoxins compared to that needed to destroy planktonic cells. Biofilm biopolymers are more resistance to mechanical stress by utilising electrostatic and hydrophobic forces, offering structural protection to resist deforming forces (Billings et al., 2015).

3.5 Biopolymer enhancements

3.5.1 Additives

Introducing organic or inorganic additives to biopolymers during the encapsulation process can further enhance the physical properties for polymer encapsulation (Table 4). Additives are application specific, but if incompatible with the polymer may impede the encapsulation process (Viveganandan and Jauhri, 2000; Liu et al., 2015). The addition of the additive can enhance bio-carbonation and is an area for future research to advance encapsulation technologies. The addition of lectins -carbohydrate-binding proteins—to biofilm derived biopolymers improve the linkages between the bacterial cells and the exopolysaccharides in the capsule wall and improve the encapsulation success rate (Table 4).

3.5.2 Microfibres

The addition of microfibres together with the encapsulated bacteria to the lime medium can further advance the bio-carbonation process. The coupling effect between the added fibres loaded with encapsulated bacteria into the three-dimensional matrix of the fibre and the addition of calcium lactate as a precursor improves the efficiency and extends the bio-carbonation period (Luo, Qian and Li, 2015; Su et al., 2021). Environmentally compatible fibres such as cellulose also encourage the bacterial cells to produce EPS possibly by causing genomic or proteomic changes to the cells (Gupta, Kua and Tan Cynthia, 2017; Singh and Gupta, 2020).

3.5.3 Bacterial selection

The highest bio-carbonation efficiency can be determined by the encapsulation of the most appropriate non-pathogenic bacteria. Within a recent comprehensive review into the performance of several *Bacillus* sp. incorporated into concrete mixes, *Bacillus halodurans* demonstrated the highest efficiency in spore formation, survival, and calcium carbonate formation (Sri Durga et al., 2021).

4 Designing a limewash encapsulation technology

Souradeep and Kua, (2016) identified an eight-factor checklist to evaluate the effectiveness of a self-healing system in concrete substrates. Six of these factors can be adapted to evaluate the selection of an effective system for use in limewash encapsulation.

1. The capsule wall must be sufficiently robust to protect the capsule contents during mixing and sufficiently thin to trigger a timely release of the bacterial healing agent.
2. A uniform density of the capsules contained throughout the limewash will allow a consistent bacterial release across the application area.

3. The timing of the release of the healing agent must be sufficiently responsive to be available when and where the bio-carbonation is required.
4. As the capsules fracture and empty the bacterial contents, the capsule fragments must not impair the structural integrity of the limewash.
5. The release of the capsule contents must both maintain the viscosity of the limewash to allow for uniform distribution of the capsules and be sufficiently viscous for the bacteria to be retained at the point of application.
6. The survival rate of the bacteria directly relates to the stability and releasing mechanism of the capsule polymer. Spores provide a more robust bioactive content (available for up to 6 months) whereas active bacterial cells will respond immediately on release from the capsule but have a limited lifespan (Wiktor and Jonkers, 2011).

The merits of developing a bacterial-enabled limewash for use on lime render and lime composites are promising as a slower eroding limewash sacrificial layer when exposed to extreme weather events has environmental and economic benefits.

Based on this review, there are four areas which inform the design for limewash encapsulation and extend the natural carbonation process through bio-carbonation.

1. Selection of a micro or nano encapsulation technology of no more than 100–200 μm to protect the capsule contents from physical stress.
2. Development of an EPS derived biopolymer able to sustain living bacterial cells and trigger their timely release during the limewash application process.
3. Assess which additive, microfibre and bacterial species combine to maximise bio-carbonation as the limewash carbonates between applications to the stone or render.
4. Design a density formulation for the bacterial capsules which allows a uniform bacterial release which does not impair the characteristics of the limewash.

5 Next steps

Local authorities are bound by statutory obligations within heritage conservation policy that may limit local authority discretion to consider innovative alternatives within the consent process. Conservation principles are also challenged to acknowledge a key ambition of the United Kingdom Government set out by the Government Construction Strategy to systematically reduce carbon emissions. The resulting growing body of environmental legislation may be an opportunity for the introduction of new technologies and alternative conservation products to expand the portfolio of traditional materials needed to address extreme weather events. Any new conservation product must be subject to rigorous review resulting in the development of technical and safety data sheets, environmental product declarations and supported by building application guides.

Biodesigned materials for use within the construction industry can extend beyond enhanced traditional heritage materials, such as lime. This review recommends the advantages of bacterial encapsulated limewash and seeks to encourage ongoing investment in microbial self-repair technologies. The incorporation of microbial materials in building construction is likely to become a mainstream technology (Heveran et al., 2020).

No less important are the opportunities from this review to encourage development of alternative construction materials, such as hemp-lime bio-composites which can be protected by carbon sequestration layers. By extending the performance of microbe encapsulated limewash beyond listed buildings, developing innovative biodesigned active coatings to combat environmental pollutants and GHGs, could have global impact, environmentally and economically. The availability of genome databases and further investigation into secondary metabolic pathways will lead the way toward transformational microbial advances for environmental improvements for heritage buildings and within the wider construction industry.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

PB contributed to conception and design of the study. PB wrote the first draft of the manuscript. LJ wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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