Beyond the Brick

Collaborations with a Sensing Microbial System in the Built Environment

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Abstract

The environmental damage caused by buildings has become clear over the past two decades. Their construction and operation significantly worsened the climate crisis through enormous annual CO2 emissions. Rectifying this damage will require an ideological shift, one that involves working with invisible microscopic living systems. The very same living organisms that have helped shape the Earth's ecosystems over billions of years.

At present, designers have made efforts to reduce our dependency on carbon-intensive resources by integrating living organisms into the built environment using biomaterials. However, difficulties keeping organisms alive have reduced their implementation to mere fabrication tools. Emerging synthetic biology techniques present an opportunity to integrate organisms into the built environment through engineered living materials. These materials can self-assemble and maintain the embedded properties of microbes, such as self-healing and adaptive response capabilities. The design process focuses on shaping the conditions for their livelihood through the simultaneous design of form, matter, and microbe, exemplifying an organism-centric design process that spans across scales.

In this thesis, I propose that living materials offer a path to address the environmental repercussions of the built environment while also transforming how we inhabit and interact with buildings over their lifespan - achieved through a collaboration with microscopic living organisms. To this end, I explore the design and fabrication of a biocemented engineered living material through in silico, in vitro, and in vivo methods. I propose a design methodology driven by wet-lab experimentation and define design constraints for macro-scale applications. I then fabricate biocemented brick modules and demonstrate their ability to bind into larger assemblies. Lastly, I evaluate the microbial viability of the designed living material and demonstrate sensing and reporting capabilities on the biomineralized surface.

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There is no better place to be doing this work than here at MIT. The community has fostered an environment to investigate these ideas fully. My technical know-how was made possible thanks to Justin Buck, who opened up the biomaker space lab for this work. It was there he taught me invaluable wet-lab techniques when no others could take me on due to the pandemic. I will be forever grateful for all the incredible conversations in the most amazing basement lab. I also have to thank Erika Wirachman and the Grossman Lab for the last-minute conversations that allowed me to complete the moonshot of engineering an unfamiliar microbe. I owe a special thanks to Sandy Curth for joining me on this microbial journey, any undertaking is easier done together than alone.

I owe a lifetime of thanks to my parents, Aida and Jose, my first teachers. They taught me to walk and talk and become the person I am today. Thank you both for teaching me to never stop. I could not have done this without my partner Amir Behan whose dreams inspired me to start this journey. Your support, love, and laughter kept me together. I'd also like to dedicate this work to my grandparents. To my grandfathers - for giving me the confidence to always keep going and my grandmothers for showing me creativity and strength.

Lastly, a special thanks to the microbes, who are too unaware of deadlines.

Contents

1 Introduction

1.1 Introduction

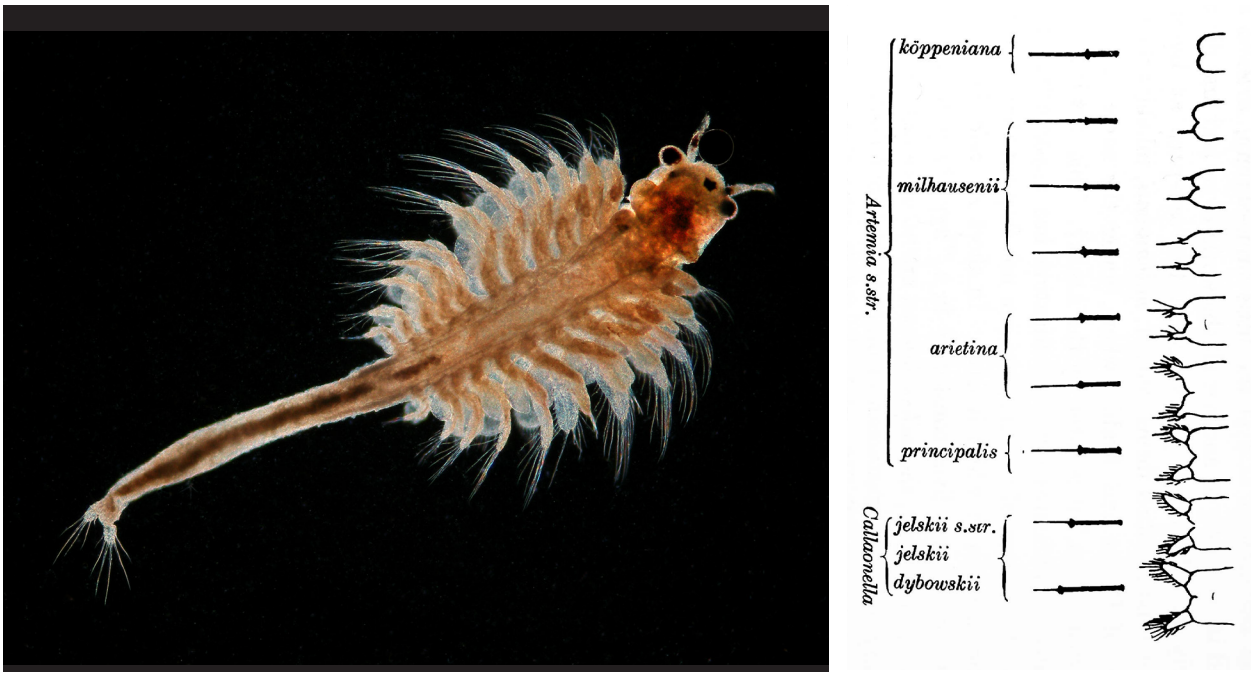

Figure 01 Brine-Shrimp Tail Morphology: Thompson's observation on the change in tail-segment and tail-fin morphology relative to salinity of water. Image of Brine-Shrimp - Reprinted from fishkeepingfolks.com, 2022 (Left). Thompsons diagram comparing tail-segments from more to less saline conditions - Reprinted from Thompson, 1992 (Right)

In 1917, D'Arcy Wentworth Thompson published the book "On Growth and Form." A biologist and mathematician, Thompson critiqued the emphasis on evolution as the primary determinant of the structure of organisms and instead proposed that physical and environmental forces played a central role. "We might call the form of an organism an 'event in space-time' and not merely a 'configuration in space,'" he claimed (Thompson, 1992). His hypothesis was based on the dynamic behavior he witnessed in the growth of organisms over time. He noted that growth could vary across the surface, forming gradients. It could also be affected by temperature and other physical conditions and could even be negative. These abilities result from the fact that living organisms contain embedded knowledge that allows them to continuously respond to physical, chemical, and biological variations in their environment. In a 1957 lecture, Francis Crick would

unravel the mechanism behind Thompson's observations by defining the central dogma of molecular biology, which describes the flow of information from sequences in DNA to the synthesis of proteins, thereby describing life as the flow of information, matter, and energy (Cobb, 2017).

DNA carries the instruction for life's development, survival, and reproduction (National Human Genome Research Institute, 2020). It confers living organisms with the ability to be resilient, dynamic, and directly confront a changing ecosystem. Organisms can also use their abilities to interact with and shape their habitats to promote their prosperity. This capacity is exemplified in the formation of relationships across species, also known as symbiotic relationships. For example, coral forms living skeletons that can grow over kilometers on the ocean floor due to a collaborative effort with photosynthetic algae (Ambrose, 2020). The algae supply nutrition to the coral in exchange for shelter and protection. Our bodies also rely on symbiotic relationships. For instance, our gut provides an ideal environment for a community of bacteria. The various species, collectively referred to as gut microbiota, help maintain bodily health by protecting against certain toxins and producing energy from foods that our body's cells cannot digest such as fiber (Microbiome Foundation, 2019). Both examples demonstrate a living composition that challenges the boundaries of individual organisms and the relationship between habitat and inhabitants. For example, corals grow in branchlike patterns to maximize the algae's exposure to sunlight (Ambrose, 2020). The needs of their collaborator drive their form. Likewise, our food cravings may be driven by gut microbiota to maintain a balance in bacterial populations (Alcock, 2014). Thus, these living systems are inseparable. Each specie's health and survival relies on the dynamic cooperation that formulates the greater living community.

Planet Earth can similarly be seen as a dynamic living macro-organism that also acts as a habitat for human presence. However, our current methods of construction and habitation, driven solely by human interest, do not follow mutualistic constitutions and have resulted in significant ecological damage. As of 2018, building construction and operation generate nearly 40% of annual global CO2 emissions (Architecture 2030, 2021). There are ongoing efforts to reduce the adverse effects of buildings by replacing carbon-

intensive and polluting materials such as concrete and steel with renewable resources made from living organisms such as wood and mycelium. However, biomaterials are not kept alive. Instead, they are rendered inert before integrating into buildings, resulting in the loss of capabilities such as self-healing and adaptive response. Emerging synthetic biology techniques present an opportunity to integrate living systems into the built environment through engineered living materials (ELMs). ELMs are materials in which microorganisms constitute an integral part by assembling the material and adding programmable functionalities such as the ability to sense and respond to environmental cues (Nguyen, 2018). As a result, ELMs present the possibility to address the existing challenges of construction and operation while also changing how we inhabit and interact with a building over its lifespan by creating systems of communication and collaboration between co-inhabitants. Ultimately, living materials could redefine buildings from static entities situation in a landscape to intimate, integrative, and dynamic living components of an ecosystem.

Several challenges associated with incorporating ELMs into the built environment include long manufacturing times, durability issues, weak mechanical performance, and decreasing microbial activity over time (Gilbert, 2021, Navarro, 2021, Srubar, 2021). The thesis addresses these issues through in silico, in vitro, and in vivo methods, providing a design methodology and wet-lab protocols for developing an ELM brick module. The following section of the proposal provides background information and a review of previous work related to the development of living materials. Section three describes the design methodology and fabrication techniques used to develop the ELM module. Subchapters in this section cover the making of various individual brick units, a joining technique for combining multiple units into larger assemblies, and an evaluation of microbial activity on the surface of ELMs. Finally, section four concludes by summarizing the contributions and discussing future work.

2 Background

2.1 Biomimicry

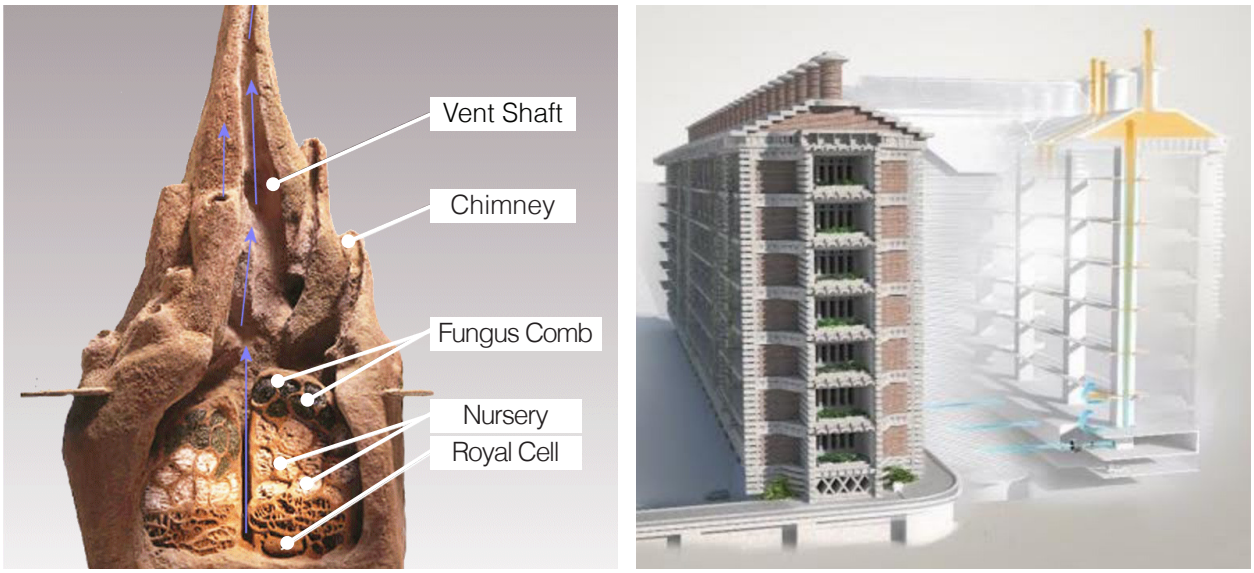

Figure 02 Eastgate Center Design through Biomimicry: Termite mound section showing passive ventilation cooling strategy - Reprinted from Ahmad, 2018 (Left). Section through Eastgate Center atrium mimicking termite mound cooling approach - Reprinted from asknature.com, 2022 (Right).

Designing living materials requires a different approach from previous biodesign methods such as biomimicry and biofabrication. Biomimicry is a design process inspired by biological solutions to functional challenges (Pawlyn, 2011). The approach presumes that nature has found the most efficient solution to minimize resource and energy consumption. For example, the design of the Eastgate Center in Zimbabwe incorporates a passive cooling system that was inspired by termites (Figure 2). Termites live in tall mounds that contain a series of vents through the top and sides. Hot air is drawn from the main chambers through vents by convection, pulling in cool air from lower levels, which maintains a desirable environment (Turner, 2008). Similarly, the Eastgate Center is self-cooling by venting hot air through the chimneys and pulling cool air from lower floors using fans. The building uses 35% less energy than an average building of similar size with air-conditioning (Pearce, 2016).

Designers have also turned to biomimicry to reduce the amount of material used for structural supports. For example, the designer Joris Laarman used a computer-aided optimization program to design the structure of a bridge over a canal as shown in Figure 03 (Laarman, 2015). The software was generated based on the principles of plants such as the Amazon water lily that use minimal resources by adding material according to stress concentrations (Pawlyn, 2016). Accordingly, central supports are thicker and branch into delicate tendrils to support the outermost extents which carry less structural load.

Figure 03 Laarman 3D Printed Bridge through Biomimicry: Amazon water lily structure - Reprinted from pangaeaproject.com, 2013 (Left). Elevation of 3D printed bridge mimicking the branching strategy for the bridge's structure - Reprinted from dezeen.com, 2021 (Right).

2.2 Biofabrication

Biomimicry focuses on structural efficiency and resource reduction. However, the process largely relies on copying forms from nature and does not necessarily address the sustainability associated with material composition or fabrication method. These topics have come to the forefront due to the rapid acceleration of global warming. Anthropocentric activity since the industrial revolution has led to a sevenfold increase in carbon dioxide emissions, from 5 billion tons per year in the mid-20th century to 35 billion tons per year by the end of the century (Lindsey, 2020, Luthi, 2008). If we do nothing, we will surpass 1.5C of global warming resulting in catastrophic and irreversible effects such as frequent extreme weather events, sea-level rise, and ecosystem loss (IPCC, 2018). Thus, biofabrication processes have emerged to directly address the issue by using living organisms to produce renewable replacements for high embodied carbon materials. The approach depends on finding organisms that can produce controllable material properties in vitro through guided growth or regulated environmental conditions.

Figure 04 Bolt Threads Mycelium Products: Mycelium Leather Footwear (Left), Mycelium Leather Handbag (Middle), Mycelium Leather Clothing (Right) - Reprinted from boltthreads.com, 2022

Bolt Threads is a company that makes use of mycelium to fabricate their sustainable leather products. Mycelium is the fine web of cells that form the root-like system of fungi (Stamets, 2005). The thin roots can grow around raw feedstock, intertwining and binding loose fragments together. Bolt Threads produces materials with mycelium by controlling ambient humidity. Their process uses mulch, air, and water in a vertical agriculture facility

powered by 100% renewable energy. Consequently, the fabrication of the sustainable leather consumes less land and emits fewer greenhouse gasses than raising livestock (Bolt-Threads, 2022). Figure 04 shows examples of products the company has produced using their mycelium leather, such as footwear, handbags, and clothing. The goal of the product was to provide consumers with the look and feel of leather but without the carbon footprint. The techniques have also scaled up in size to create bricks and panels for Architectural installations such as the Hy-Fi pavilion by the Living and the Growing Pavilion by Pascal Leboucq and Krown Design (Benjamin, 2014, Leboucq, 2022).

Figure 05 Fullgrown Chair: Process diagram for guided growth (Left), Finished chair (Right) - Reprinted from fullgrown.co.uk, 2022

Fullgrown is another company that utilizes biofabrication for the manufacturing of its products. The company grows chairs by guiding sapling growth, as shown in Figure 05. The process involves training, pruning, and grafting trees into the desired form over several years. “Like an organic 3D printing that uses air, soil, and sunshine as its source materials,” claims Fullgrown. Once the tree has achieved its desired form, it is cut down. The finished piece is fully functional and has the potential to last longer than conventionally fabricated chairs since it contains no joinery or adhesives that could weaken over time (Fullgrown, 2022).

Biofabrication focuses on the manufacturing process. It uses organisms to create renewable material alternatives with desirable properties and forms. The design process demands an understanding of the growth mechanisms used by biological organisms to

achieve a desired effect. Nonetheless, the organisms are killed at the end of the process and do not directly benefit from the implementation of the method.

2.3 Engineered Living Materials

Biomimicry approaches organisms as inspiration. Biofabrication regards them as manufacturers. However, living materials present a different paradigm - organisms as collaborators. Engineered living materials (ELMs) are materials composed of living cells that retain additional functionalities post-production (Gilbert, 2021). Current research has focused on small-scale prototypes made with bacteria, yeast, or fungi that have applications in medicine, electronics, and the built environment (Srubar, 2021). Unlike previous biodesign processes, the organisms are kept alive in the making and use of ELMs. Therefore, they maintain their abilities to sense, respond, and participate in their surroundings and contribute to the material's purpose by embedding their living capabilities including sensibilities to various stimuli and mechanical forces (Gilbert, 2021).

The image shown in Figure 06 of the Grand Prismatic Spring in Yellowstone National Park is an existing example of microbial sensing and response. Through colors, the microbes indirectly communicate information regarding the different conditional strata of the spring, including variances in temperature, depth, and exposure to sunlight. For instance, the green pigmentation is caused by the bacteria's production of chlorophyll, a pigment that helps the microbe absorb energy from the sun through photosynthesis (Quammen, 2016). As the pool becomes shallower towards the outer boundaries, the microbes are exposed to increasing UV from the sun's light. The bacteria, therefore, produce another pigment known as carotenoids which range from yellow to red, to mitigate the harmful effects of the UV exposure (National Park Service, 2022). However, there are some spring sections where microbes cannot survive, such as the pool's center. Temperatures in this section reach up to 190 Fahrenheit (Geiling, 2016). Without a bacterial population, the pool's center has a deep blue tone caused by the scattering blue wavelengths of light. Thus, the presence and pigmentation of the bacteria in the spring continuously communicate local temperature, depth, and UV exposure information.

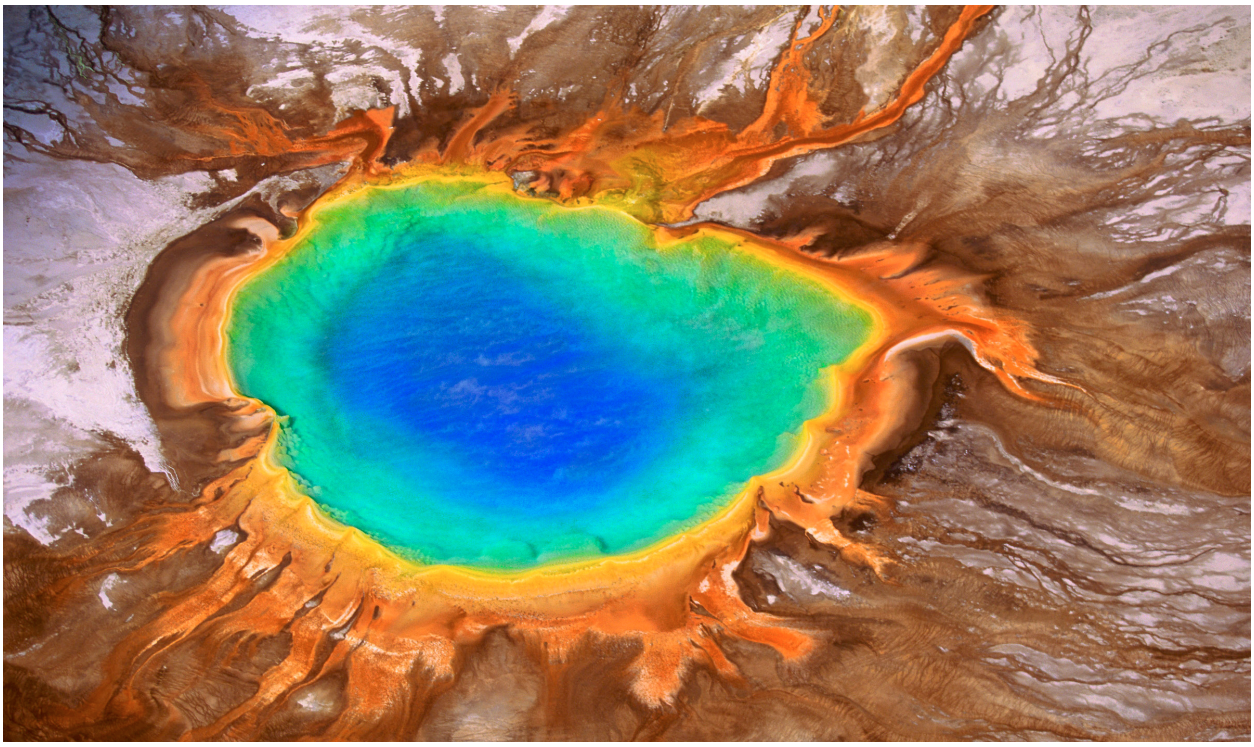

Figure 06 Grand Prismatic Spring: The colors of the spring are caused by microbial mats and their ranging exposure to temperature, sunlight, and UV - Reprinted from yellowstonenationalpark.com, 2022

Emerging synthetic biology techniques are extending the scope of microbial abilities by imbuing microorganisms with novel biological capacities. Synthetic biology uses molecular biology tools and techniques to forward-engineer cellular behavior (Cameron, 2014). The field uses approaches such as modularization, rationalization, and modeling to generate a catalog of genetic devices and biological modules to increase the accessibility and reproducibility of engineering biological systems (Khalil and Collins, 2010; Shetty, 2008). To achieve this goal, the field has established a common language known as SBOL: a standardized format for the electronic exchange of information on biological designs' structural and functional aspects (Sbol Standard, 2022). The field has also developed a collection of parts known as Biobricks. Biobricks are DNA sequences that conform to an assembly standard. Their modularity allows for the easy reconfiguration and assembly of diverse systems. The parts are cataloged in the Registry of Standard Biological Parts and now contain over 20,000 entries (iGEM, 2022). Another critical development was the creation of online platforms for information sharing, including OpenWetWare. The website promotes the sharing of information amongst researchers working in biological

engineering. It includes information on materials such as media, buffers, and common chemicals. The website also includes details on common in-vivo and in-vitro protocols and additional resources such as reference materials and lab start-up guides (Openwetware, 2017). Consequently, synthetic biology is increasingly becoming an accessible tool to non-biologists such as designers and architects, with implications for the design of products, spaces, and environments.

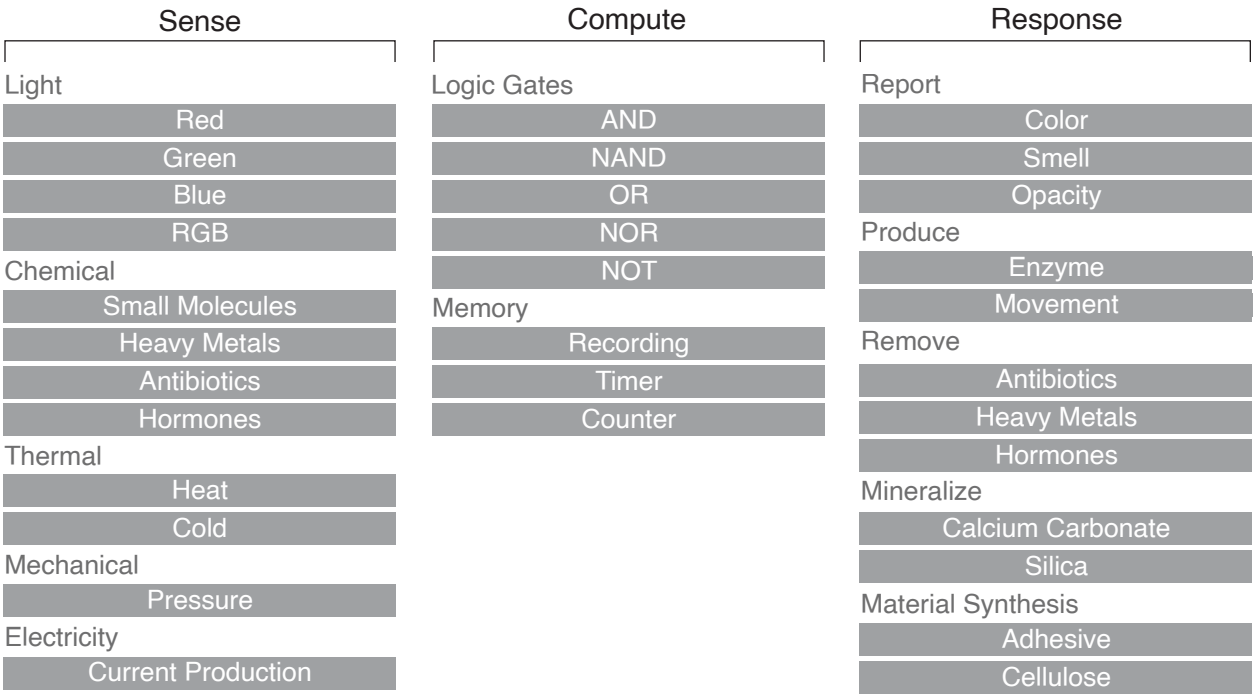

Figure 07 Microbial Sensing, Reporting, and Actuating Capabilities: A matrix of established genetic parts that can be combined to form living systems in bacteria.

A list of established genetic parts is shown in Figure 07. These parts can be combined to form a variety of living devices. For example, coupling light and chemical sensing with the AND gate and a color reporter would only produce color if both light and the specific chemical are sensed. Once the DNA sequence is determined, it can be encapsulated into a circular strand known as a plasmid, that facilitates the transfer of instructions into bacterial cells (Monroe, 2020). The instructions can be uptaken by microbes and enacted if they don't interfere with essential cell functions. Try as we might, microbes will not take on work with too heavy an energy load. Therefore, the genetic designs should take into

consideration the burden placed on the organism to perform additional tasks. This can be determined by measuring the percent reduction in the growth of the microorganism before and after genetic transformation (Muller, 2011). The task and associated load must therefore be considered in conjunction with the organisms selected to enact it.

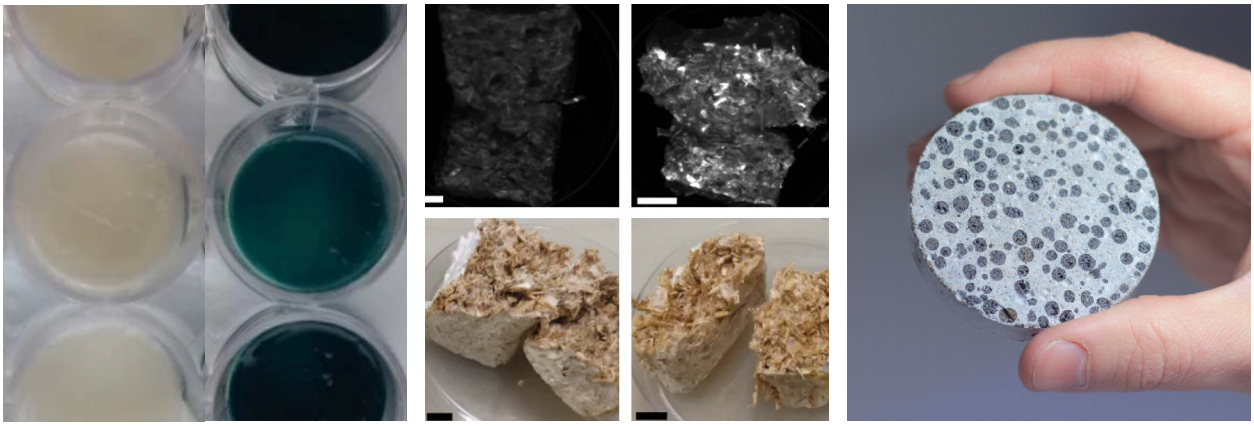

Figure 08 ELM Precedents: Syn-SCOBY bacterial cellulose - Reprinted from Gilbert, 2021 (Left), Fungal bacterial biocomposite - Reprinted from McBee, 2021 (Middle), Self-healing concrete - Reprinted from mastercivilengineer.com (Right)

ELMs have been made in collaboration with organisms such as bacteria, yeast, and mycelium and typically fall into one of three material categories: bacterial-cellulose, fungal composites, and biomineralized aggregate. Syn-SCOBY is a bacterial-cellulose living material project (Figure 08 Left). Bacterial cellulose is an ex-cellular product of vinegar bacteria that produces a small gel-like substance of almost pure cellulose (Iguchi, 2000). The advantages of bacterial cellulose include mild growing conditions and high material yield (Gilbert, 2021). The project uses a co-culture of bacteria and engineered yeast to create a living material that could sense and respond to antibiotics or hormones present in water streams. To achieve macroscopic structures, McBee et al developed a fungal-bacteria biocomposite (Figure 08 Middle). The mycelium was used to bind raw feedstock together and bacteria from the material's native microbiota was characterized and engineered to functionalize the material (McBee, 2021). The living system's proposed use included the production of protective molecules to reduce ultraviolet damage, the creation of on-demand products such as food and medicine, and the ability to react to pollutants, toxins, or chemical threats (McBee, 2021). Another emerging category of ELMs is microbial biomineralization of aggregate. The process uses bacteria to induce the formation of

calcium carbonate crystals that bind together loose sands or soils. Researchers at TU Delft have used the process to develop self-healing concretes by embedding microbes into concrete mixes (Figure 08 Right). The researchers have demonstrated the ability to seal cracks as large as .46mm wide by adjusting the concrete makeup and selecting robust microbes (Wiktor, 2011, Jonkers, 2007).

Designing ELMs requires a multiscalar approach. At the micro-scale, substrate selection is key. It affects cell distribution and the ability for biochemical reactions to take place (Srubar, 2021). Current substrate approaches include soft media such as agarose or alginate hydrogels, cellulose papers, fibers, or rigid matter such as sand, soil, and mortar concrete (McBee 2021, Rodrigo-Navarro, 2021, Tang, 2021). The macro-scale implications require geometric ingenuity. Conditional gradients can occur as the living material scales in size. For instance, implementing ELMs at building scales will result in living materials that cover large amounts of surface area and depths making it difficult to maintain temperature and oxygen consistency throughout. Designers must therefore develop geometric frameworks that minimize the variances that could inhibit the growth of microorganisms as the material is implemented for a specific use.

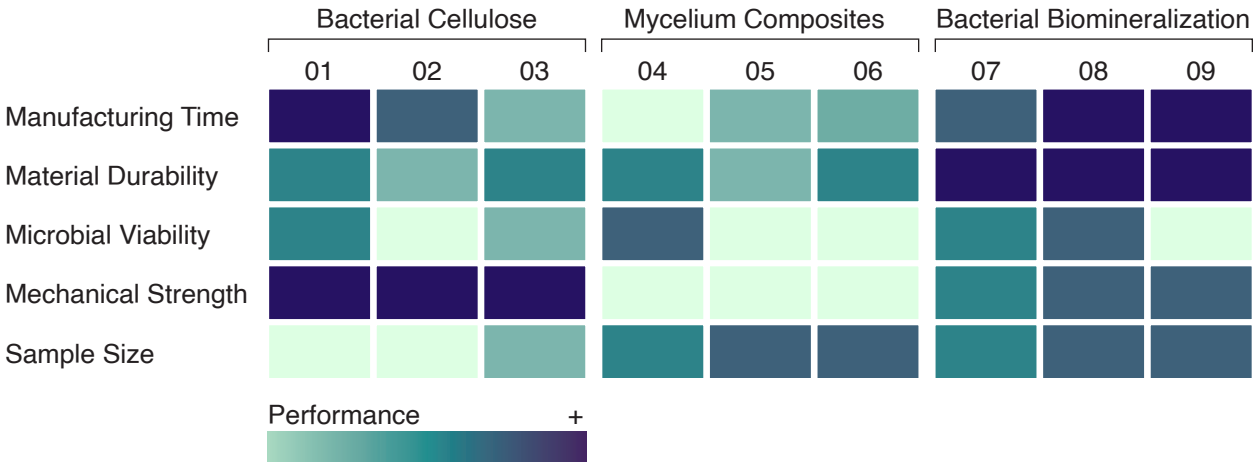

Figure 09 Organism System Fitness Map: Evaluation of different organism system's performance across common ELM challenges such as manufacturing time, material and microbial viability, mechanical strength, and produced sample size. 01:Syn-SCOBY (Gilbert, 2021), 02:Living Matter (Gazit, 2016), 03 :Photosynthetic Living Material (Balasubramanian, 2021), 04:Fungal Bacterial Biocomposites (McBee, 2021), 05:Mycotree (Heisel, 2017), 06:Hy-Fi Pavilion (Benjamin, 2014), 07:Regeneration of LBM (Heveran, 2020), 08:Bioconcrete (Bang, 2001), 09:Biomason (Biomason, 2022)

Several challenges must be addressed in order to integrate ELMs into large-scale built structures, including long manufacturing times, durability issues, weak mechanical performance, and decreasing microbial activity over time (Rodrigo-Navarro, 2021, Srubar, 2021, Tang, 2021). Figure 09 demonstrates how projects corresponding to the different organism systems perform across these obstacles. For instance, bacterial-cellulose projects demonstrate high tensile strengths ranging from 45 to 145 MPa (Gilbert, 2021, Balasubramanian, 2021). They also exhibit rapid growth, forming pellicles as soon as three days (Gilbert, 2021). However, the majority of samples produced have been small in size: only 10cm on average. On the other hand, mycelium has been used to successfully grow bricks and panels for habitable temporary structures such as the Hy-Fi and the Growing Pavilion (Benjamin, 2014, Leboucq, 2022). Yet, the living material is susceptible to drying out and can be easily contaminated by mold, making long term implementation difficult (McBee, 2021). Biomineralization offers the advantage of high strength and durability. The process has produced materials that achieve up to 30MPa in compressive strength, equivalent to standard concretes, with modest microbial viability - up to six months if spores are encased in the material (Al-Thawadi, 2008 Jonkers, 2007). The challenge with biomineralized substrate is the inconsistency in biocementation as depth is increased. To date, no one organism system has demonstrated robust functionality at or beyond a year.

2.4 Biomineralization

The thesis will explore living materials through microbially induced biomineralization due to it's durability, strength, and robustness. Microbially induced calcium carbonate precipitation (MICP) is a process that relies on soil-borne microbes to create an ideal environment for the formation of calcium carbonate crystals. The technique has been studied as a cement alternative (Stocks-Fischer, 1999, De Muynck, 2008), a restoration approach for ornamental stone (Orial, 1993), to repair cracks in concrete (Ramakrishnan, 2005, Seifan, 2016), to increase the strength of concrete (Bang, 2001), to remediate aquifers (Fujita, 2000, Warren, 2001), and to stabilize soils that tend towards liquefaction (DeJong, 2006).

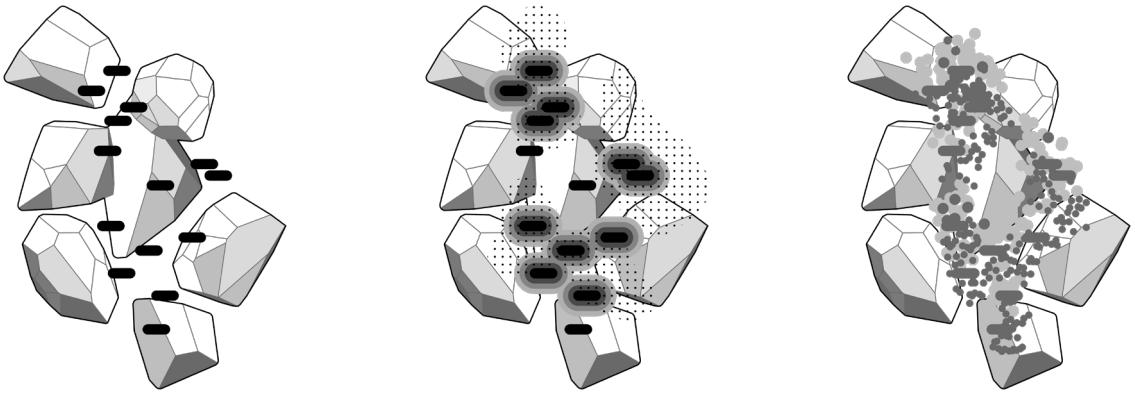

Figure 10 Biomineralization Process: Process of biocementation through the introduction of bacteria, calcium, and urea that results in calcite formation (Top) SEM images of calcium carbonate crystals fixing sand to a fiberglass strand - in collaboration with Alexander Curth, 2022 (Bottom)

The process, shown in Figure 10, begins when ureolytic microbes are introduced or cultivated in loose aggregate such as sand or soils. A solution of calcium and urea is then added which interacts with the urease enzyme produced by the bacteria. The enzyme breaks down the urea into carbonate and ammonia and increases the pH of the local environment. The pH increase allows for the carbonate to bond to the calcium ions which results in the generation of calcium carbonate crystals. The crystal matrix, shown in SEM images in Figure 10, surround and encapsulate loose granules of sand which creates the bond that holds the material together (Dejong, 2006, Gonzalez-Munoz, 2010, Stocks-Fischer, 1995). Denser matrices and specific crystal morphologies is thought to result in greater compressive strength (Al-Thawadi, 2008).

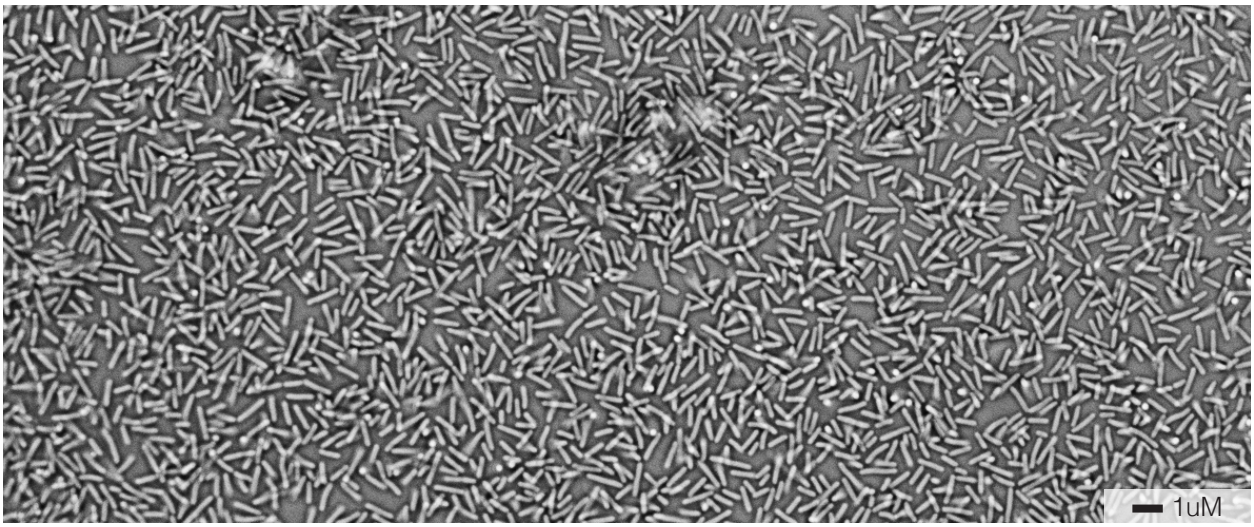

Figure 11 Sporosarcina pasteurii: Microscopy image of S.pasteurii, 40X

Biomineralized ELMs are best suited for incorporation into building components including structural elements. One advantage is the unconfined compression strength. Studies have reported an average compressive strength of 7MPa with up to 30MPa achieved (Rahman, 2020, Al-Thawadi, 2008). This is comparable to concretes used today which range in strength from a lean concrete at 5MPa to a standard concrete at 30MPa and above. The time frame for producing and curing the materials is also similar to current practices for concrete which can take seven days for an initial cure and up to 28 days to achieve full strength. Finally, key components such as calcium and urea can be supplied through indistial by-products such as egg shells and tofu waste water, allowing the process to be implemented at sizeable production scales (Castro-Alonso, 2019, Fang, 2019).

A popular biomineralizing organism is Sporosarcina pasteurii (S.pasteurii), shown in Figure 11. The microbe is resilient - withstanding dry environments, unfavorable temperatures, and inadequate nutrition by forming spores (Chuo, 2020, Wang, 2014). The bacteria also produce high amounts of the urease enzyme which is believed to result in better biocementation results (Castro-Alonso, 2019). Yet, to ensure the feasibility of a biomineralized ELM a couple of challenges need to be addressed. The first includes the entombing behavior observed through biomineralization, which can be seen in SEM imagery (Figure 10 Bottom Right). As the calcium carbonate crystals form they are attracted to the negatively charged walls . Figure 12 shows an example of this behaviour on a 90mm petri dish. A small calcium carbonate crystal is created if the cells are dispensed together. However, a distribution of the bacteria creates a layer of calcification across the entire petri dish surface. The behaviour demonstrates that the crystal growth is localized around the cells which encase the microbe over time. As the microbes becomes entombed in the crystals they loose access to oxygen. In this unfavorbale environment the bacteria begin to form spores which allow them to become dormant for up to six months (Jonkers, 2007). To mitigate this issue, biocementation could be completed in staggered cycles to ensure there is always a robust microbial population in the porous substrate. Another challenge of the process is the production of ammonia as a waste product which can lead to local eutrophication of freshwater sources. A possible way to attenuate this issue is through the incorporation of nitrogen-fixing bacteria in a co-culture.

Figure 12 Localized Calcification: Petri dishes with S.pasteurii demonstrating local calcification effects. Microbes centrally deposited (Left) Microbes distributed evenly across surface (Right)

MICP can also be used for bioremediation by replacing calcium with heavy metals such as lead, cadmium, and zinc (Ferris, 2004, Hamdan, 2011, Rajasekar, 2021). Heavy metals are a major source of concern because they do not biodegrade, are difficult to remove once they have contaminated biological tissue, and may be lethal (Ojuederie and Babalola, 2017). Exposure to heavy metals is detrimental to bodily health and can result in gastrointestinal and kidney disfunction, neurotoxicity, skin toxicity, immune system disfunction, and cancer (Balali-Mood, 2021, Mitra, 2022). The time of exposure and concentration play a significant role in determining the degree of toxicity. Yet, maximum established concentrations are low. For example, The Comprehensive Environmental Response Compensation and Liability Act establishes limits for lead at 0.015mg/L and cadmium at 0.005 mg/L (EPA, 2015). Without constant monitoring systems it is also difficult to determine exposure length.

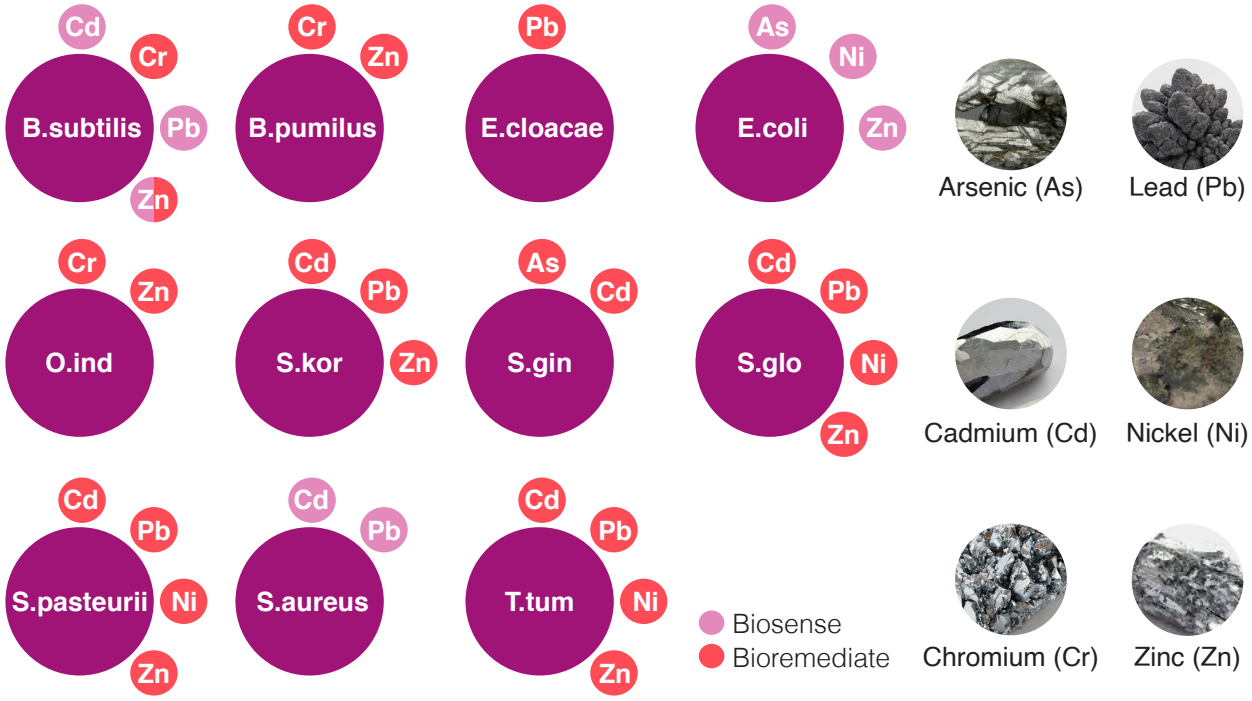

Figure 13 Bioremediation through Biocementation Palette: A palette of microbes that bioremediate heavy metals through biocementation as well as microbes that have demonstrated the ability to sense various heavy metals through genetic engineering. O.ind: Oindicireducens, S.kor: S.koreensis, S.gin: S.ginsengisoli, S.glo: S.globispora, T.tum: T.tumescens

Moreover, current remediation techniques are energy intensive. For instance, in-situ thermal remediation is a treatment that makes use of heat to vaporize and remove contaminants in soil and groundwater. The process heats the ground to temperatures near

800C and is conducted over the time span of months to years (EPA, 2021). Less energy intensive processes such as phytoremediation also face challenges. The technique uses plants to absorb heavy metals, but the process requires the removal and burning of plants making it costly as well as unreliable since heavy metals can be released back into the environment during natural decomposition (Ojuederie and Babalola, 2017).

An engineered living material comprised of biocementing microbes could address the challenges currently facing remediation techniques by offering a low-energy method for bioremediation with constant monitoring. The process is also promising because different organisms can be used to target a range of pollutant sources as shown in Figure 13 and the biological method is also on average 90-100% efficient at capturing pollutants in the span of a few days (Mugwar and Harbottle, 2016, Rajasekar, 2021). Lastly, not only are organisms able to remediate they can also sense these heavy metals at the range of concentration needed to prevent any harm to human health. Figure 14 shows the concentrations that are considered toxic against currently established sensing minimums for microorganisms.

Heavy Metal	Max Allowable Concentration	Organism Sensitivity
Arsenic	.010mg/L (Water)	20ug/L
Cadmium	.005mg/L (Water), 5ng/M^3 (Air)	0.8ug/L
Chromium	.100mg/L (Water)	50ug/L
Lead	.015mg/L (Water), .5ug/ M^3 (Air)	0.2ug/L

Figure 14 Critical Heavy Metal Concentrations and Sensitivities: Prevalent heavy metal maximum allowable concentrations matched to current sensing capabilities of engineered organisms - Data sourced from EPA, 2022, Kim, 2018, and Verma and Singh, 2005

The thesis will use heavy metal sensing as a case study for a biomineralized ELM. The living material would use color to report the presence of heavy metals as shown in Figure 15. These color signifiers would communicate to people the need to catalyze the biocementation process for the remediation of sensed pollutants. If the microbes are sustained through practices of care it could serve as a continuous monitoring system, constantly informing users on the actions needed to collaboratively improve the environment before toxins over accumulate.

Figure 15 Living Material Palette: Conceptual render of a colorful biocemented living material palette. Colors would correspond to the different pollutants sensed, creating bricks that communicate the presence of local contaminants provide information on ecosystem health.

3 Design

3.1 Overview

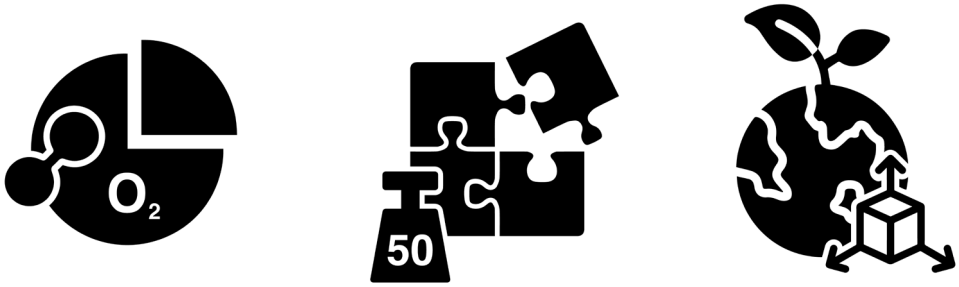

Figure 16 Stakeholders: The stakeholders for the biocemented living material include the microbe, the human, and the local environment.

To define the design constraints for the biocemented engineered living material the stakeholders were established (Figure 16). The stakeholders include the microbes which will contribute the biocementing, bioremediating, and sensing capabilities. The humans which will deploy and support the system over its use. And lastly, the ecosystems in which these systems will be incorporated. Each stakeholder brings to the design process a set of constraints to be considered which is addressed through a modular brick approach.

To define the microbial constraints, two microorganism species were selected including Sporosarcina pasteurii ATCC 11859 and Bacillus subtilis W168. The S.pasteurii 11859 strain would provide the biocementing and bioremediating capabilities of the living materials and the B.subtilis W168 would contribute the sensing and reporting abilities. Both microbes are soil-borne and can therefore tolerate conditions that resemble the typical application context. For them, access to oxygen is key. While both are facultative aerobes, they require oxygen to perform the biocementing and biosensing tasks (Jain and Arnepalli, 2019, Nakano and Zuber, 1998). The overall geometry of the brick must therefore maximize access to oxygen by increasing the surface area and limiting depths beyond 100mm which were found to be inadequate for oxygen permeability during wet lab experiments.

The human constraints are defined by the scale and weight of the module. Minimizing the number of parts will allow for easier assembly and disassembly of the living systems. However, this constraint must be balanced with the resulting increase in weight and scale. Due to the characteristics of the substrate, doubling the size of the module quickly escalates it's weight. Accordingly, the size of the module will be limited to a manageable weight and size that can be moved as needed while also remaining as large as possible to increase the bioremediation potential and reduce the number of parts.

Ecological limitations were also considered. Driving the constraints for this stakeholder is the production of ammonia as a waste product. Ammonia can cause eutrophication: a phenomenon that creates algae blooms in freshwater sources, consuming the majority of oxygen and resulting in the death of local fish populations (EPA, 2013). At the same time, increasing the bioremediation potential of the process would be beneficial for this stakeholder to support the health of the local ecologies. The modular system addresses these issues by providing a local scale of remediation. Applying this process across industrial scales would result in cytotoxicity to local environments. Therefore, the regional implementation allows for the modules to be deployed as needed with careful environmental impact monitoring. Additionally, in the event of leaching or eutrophication, the modular system could be disassembled and removed.

The following subchapters advance the design of the individual units through the lens of these stakeholder interests and limitations. Each section covers the corresponding study's design, methods, and results. Subchapter one goes over the design and fabrication of individual units, providing detail on the module design, mold design, and cementation process. Subchapter two describes the recommended method for combining multiple units into larger assemblies and provides examples of possible implementations in the built environment. Finally, subchapter three covers the incorporation of both microorganisms to create the biomineralized living material. This subchapter investigates the nutrition composition, viability of the sensing organism over time, and the assessment of a sensing and reporting genetic system.

3.2.1 Modular Unit Design

The design of the individual brick unit is described in Figure 17. The geometry is derived from a hexagon, a tiling geometry that contains the most area with the least perimeter and is also a structurally stable form (Asknature, 2021). To allow for easier assembly, registers are introduced by slightly pushing and pulling the perimeter. Depth is attributed to the module based on the pollutant source. A pollutant can be present in the surrounding context's air, soil, and water. In response, the module's surface and volume are increased in singular or multiple directions to increase contact with possible pollutant sources. The extensions could also be calibrated based on the source or concentration of the known pollutant and result in asymmetrical geometries at thresholds.

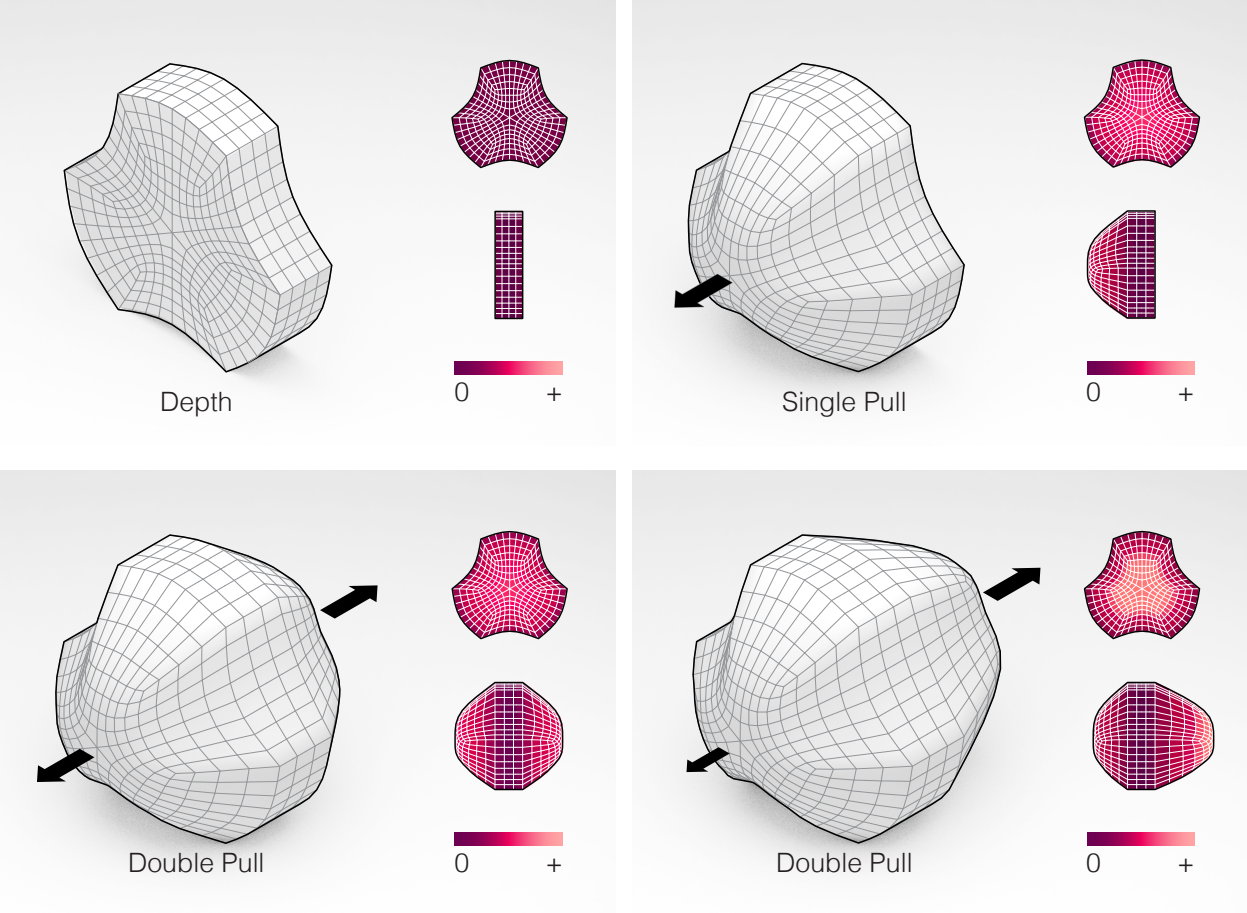

Figure 17 Design Logic: Depth added to the flat module based on pollutant source (Top Left). The module's surface and volume can be increased towards a singular direction (Top Right) or towards multiple directions (Bottom Left) depending on known contaminant source. The increase can also be calibrated based on pollutant concentrations (Bottom Right).

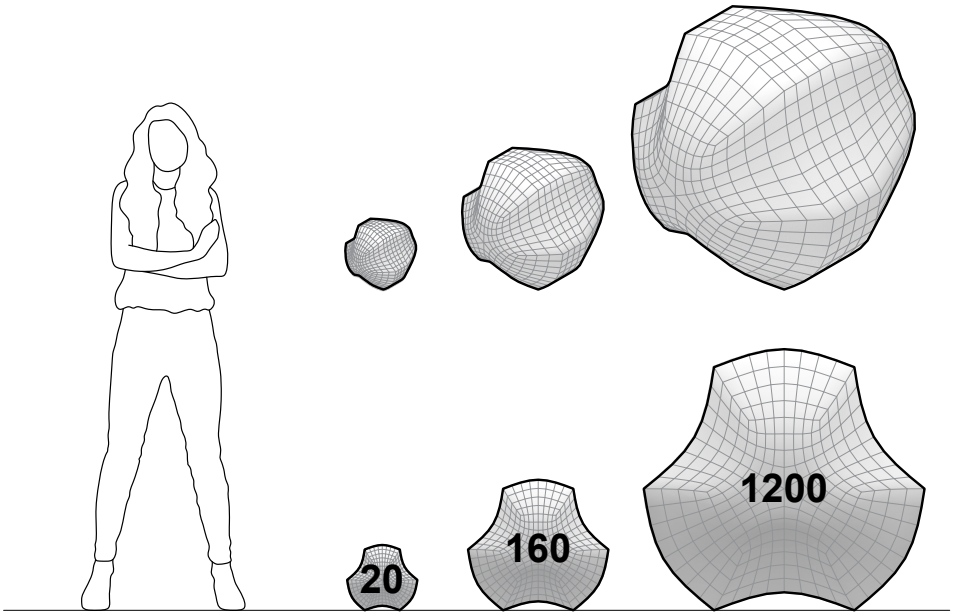

Figure 18 Scale and Weight Implications: Each doubling in scale results in an eight-fold increase on overall module weight.

The depth of the geometry was then analyzed at three different scales: 230mm, 460mm, and 920mm which were determined based on Figure 18. To analyze the oxygen availability, a voxel representation of the brick was constructed and the depth was analyzed in relation to the oxygen facing surface (Figure 19). Different techniques to remove material were tested depending on the percentage of voxels that lacked oxygen. The strategies included small indents to larger carve outs or holes for areas considered vastly deep. The modifications were then translated back to surface representations and their surface area and volumes were analyzed (Figure 20).

Design one resulted in a 12% surface area increase and a 10% decrease in volume. Design two gained 65% in surface area, but lost the largest amount of volume at 50%. Finally, design three resulted in a 94% increase of surface area with only a 14% decrease in volume. To further increase the surface area, grooves were added to the oxygen facing surface, shown in Figure 21. Future studies could help define additional constraints for the groove geometries through studies of fluid flow and their impact on the access to nutrition for the microbes and the erosion of the exposed surface.

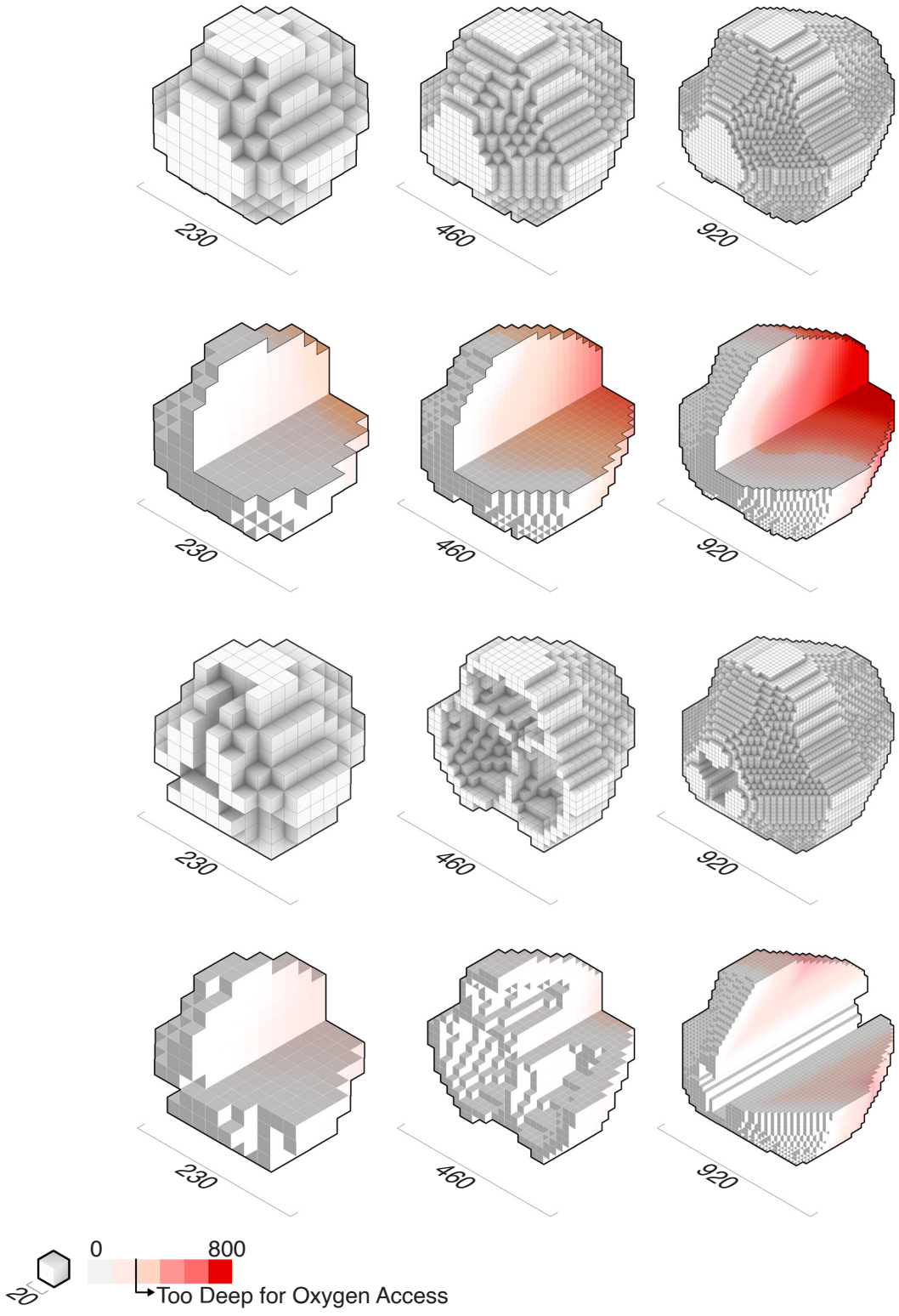

Figure 19 Oxygen Analysis: Voxel representations of the module at three scales were constructed and used to study oxygen availability based on depth. Portions beyond 100mm of the exposed face, colored in red, were deemed too far for microbial viability. All distances shown in mm.

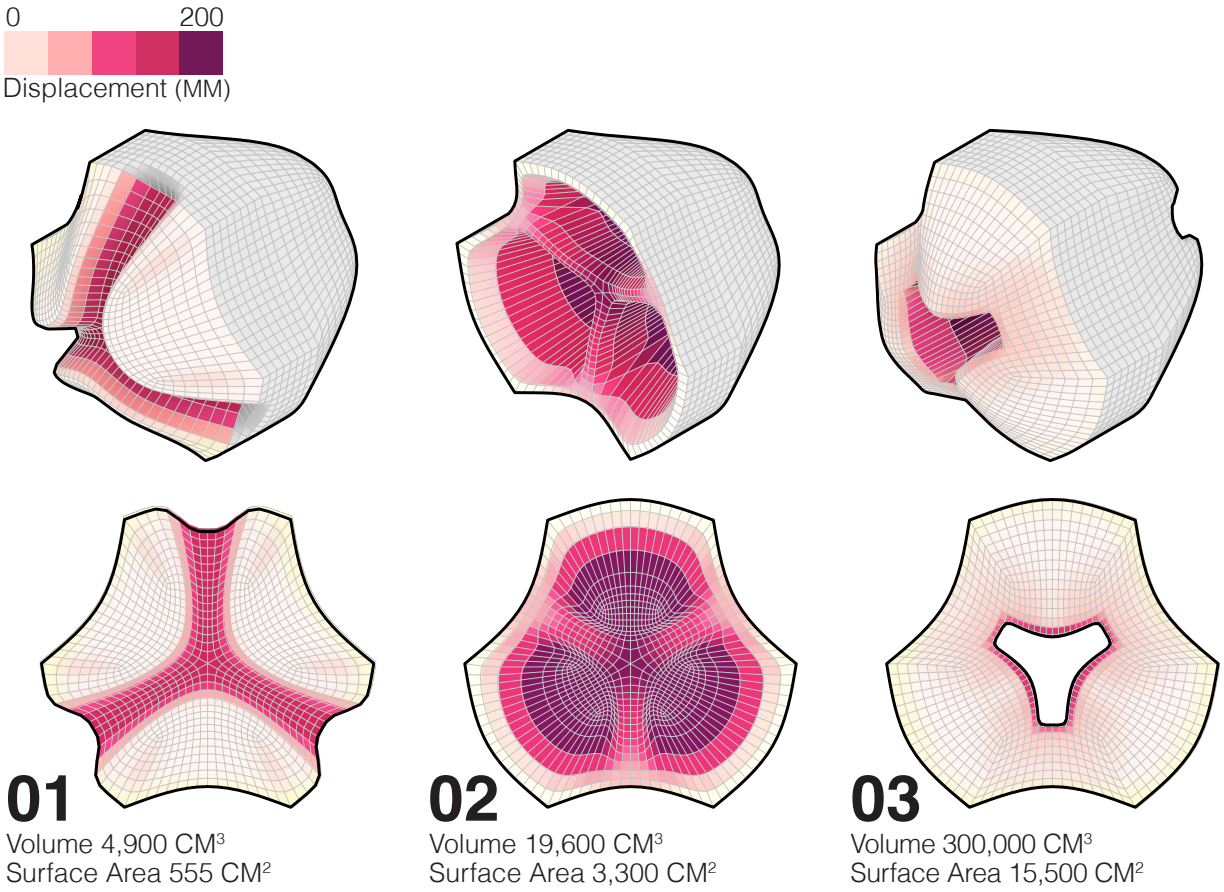

Figure 20 Surface Representation: A Surface representation of the three optimized designs including an indent (Left), a carve (Middle), and the introduction of an opening (Right).

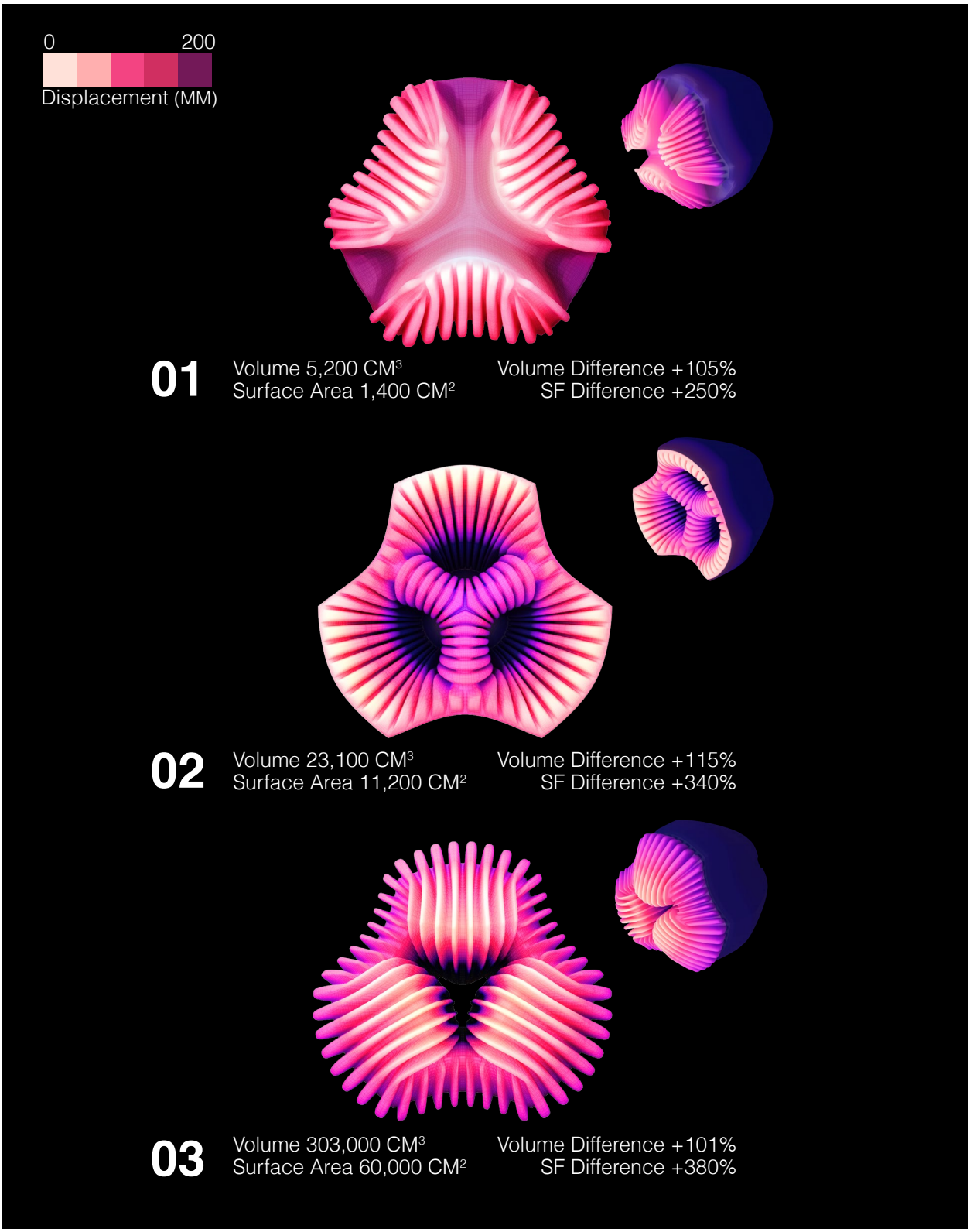

Figure 21 Grooves: Textured grooves were introduced to the oxygen facing surface to further increase the surface area and create pockets for the sensing microbe.

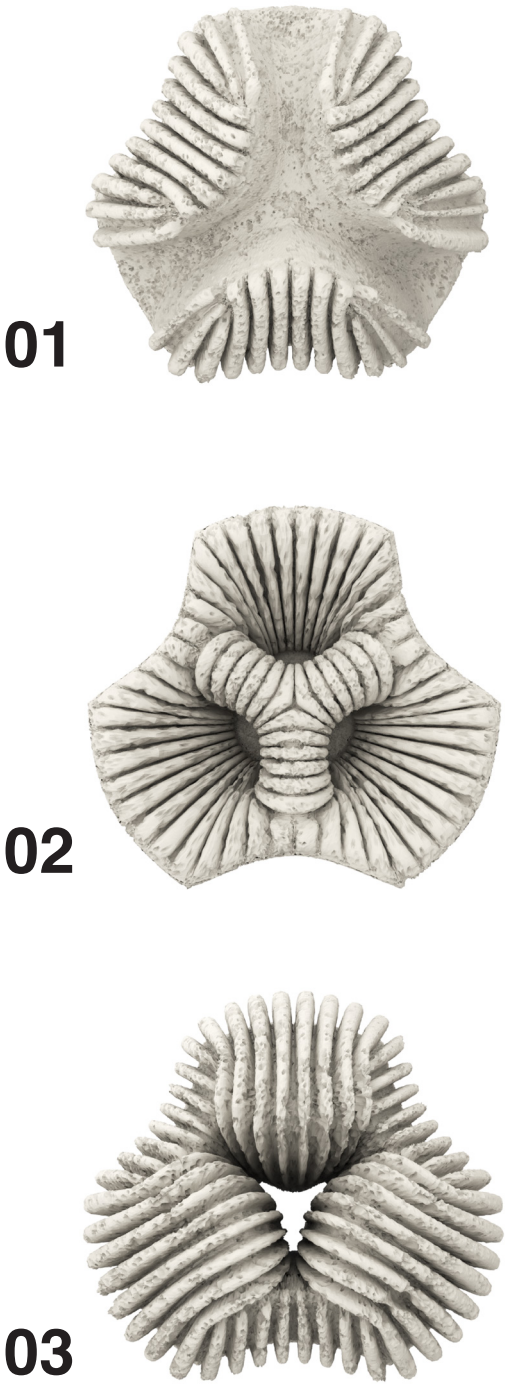

Figure 22 Microbial Pockets: The spaces between the grooves would be ideal protective pockets for the sensing microbes to inhabit.

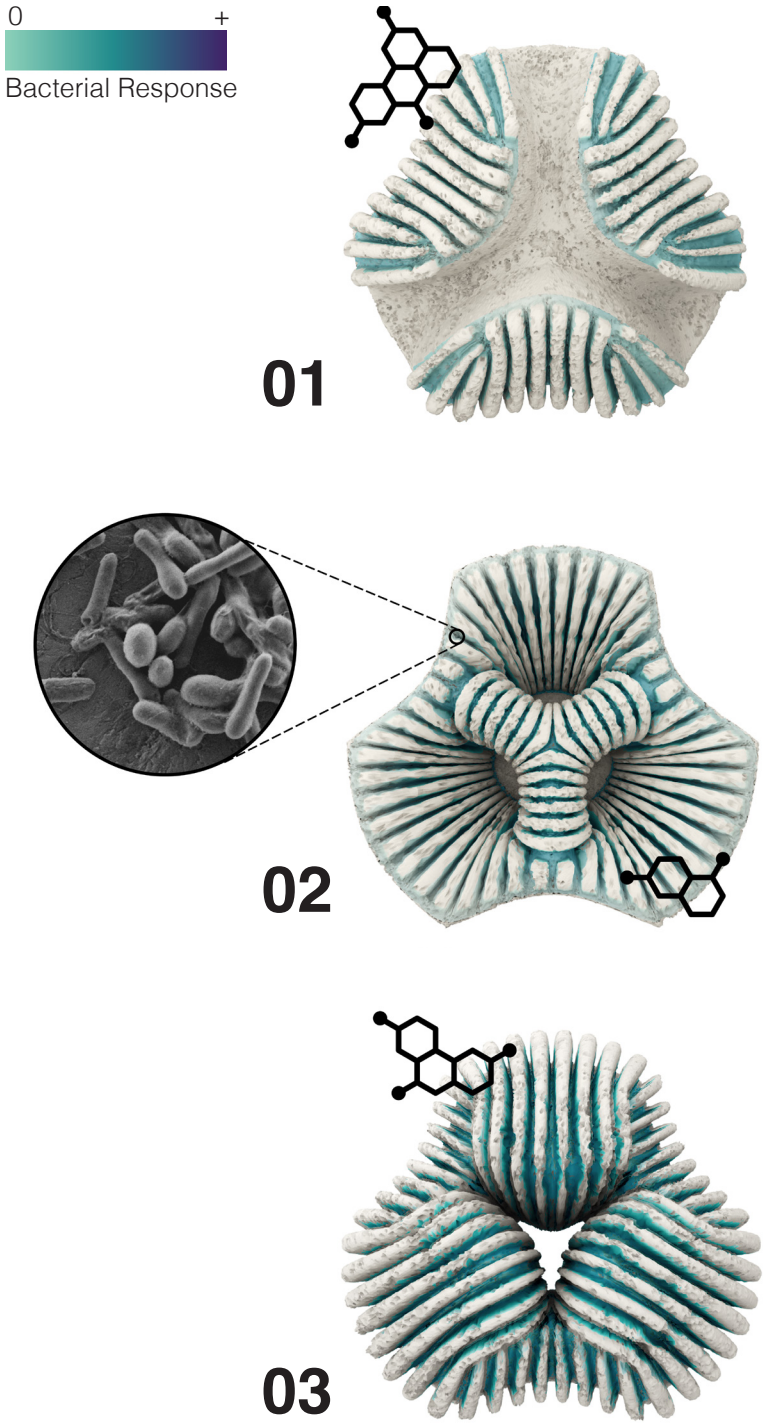

Figure 23 Microbial Sensing Pockets: In the presence of contaminants, the sensing microbes residing in the spaces between the grooves would express a color.

3.2.2 Modular Unit Methods

Bacterial Growth Media and Conditions. S.pasteurii ATCC 11859 was grown at 30C with aeration in a shaking incubator at 200rpm in the following media: 20g/L Yeast Extract and 10g/L Ammonium Sulfate. The pH was adjusted with 0.13M Tris Buffer to a pH of 9. Each item was autoclaved separately at 121C for 30 minutes before combining (ATCC, 2022).

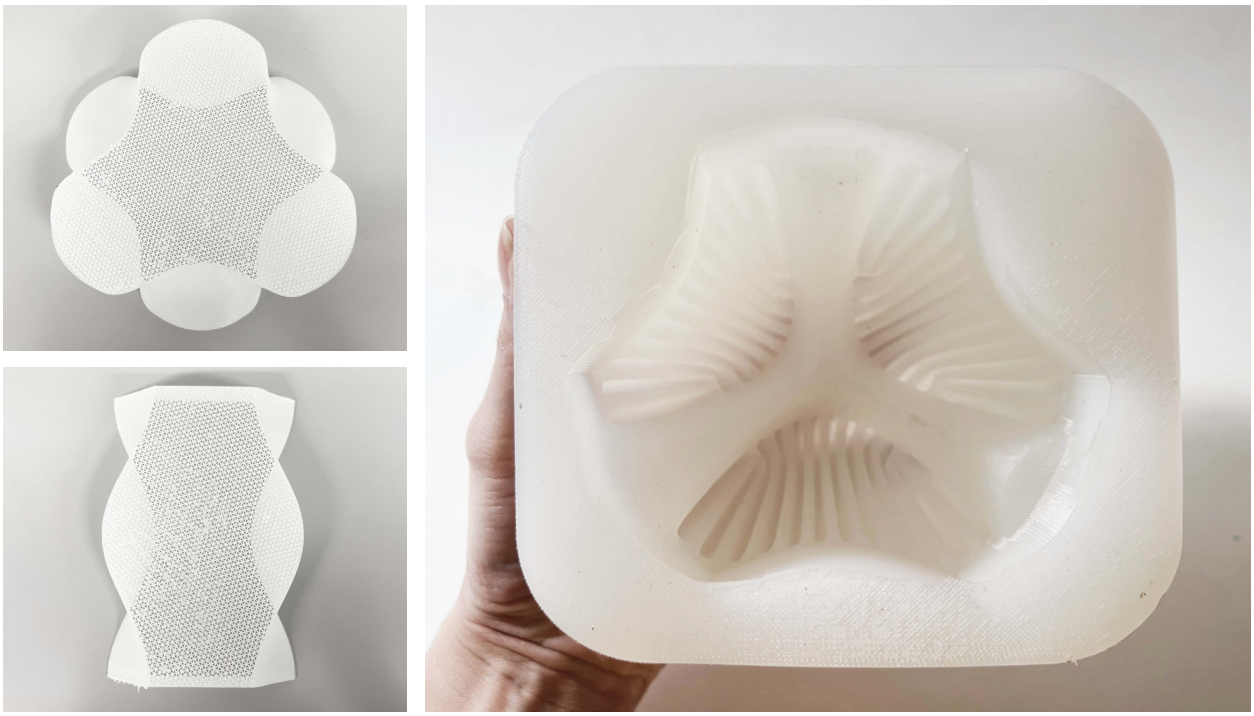

Figure 24 Mold Designs: Early iteration 3D printed PLA molds (Left) and silicone mold (Right).

Fabrication of PLA and Silicone Molds. The PLA molds were 3D printed using FilaCube Ivory White PLA on the Prusa i3 MK2S. All PLA mold geometries were modified with Prusa's slicer to create a permeable diamond grid bottom at 90% infill density. The silicone molds were created by 3D printing a positive in FilaCube Ivory White PLA. The 3D prints were then cast in Smooth-On Dragon Skin 10 - Fast Silicone Rubber to create the final molds. All molds were sterilized in an alcohol bath for 30 minutes and placed in a biosafety cabinet to dry before packing with sand and inoculating.

Inoculation of PLA and Silicone Molds. Coarse decorative sand and fine pure silica sand 70 mesh, were used for all biocementation experiments. The sand was sterilized by

placing in the autoclave at 121C for 30 minutes in a shallow aluminum container 9”x9” in size. After sterilization, molds were packed with the corresponding sand. All samples were vibrated by hand for about one minute to increase compaction of the sand granules. The samples were then inoculated with an overnight culture of S.pasteurii ATCC 11859 OD600 of 0.25-0.35 and 6mM Calcium Chloride (Al-Thawadi, 2008). The inoculated molds were incubated for 24-48 hours at 30C before biocementing.

Preparation of Biocement Solution. A 0.5M Calcium Chloride and Urea solution was used for all biocementation experiments. The solution consisted of 30g/L Urea, 10g/L Ammonium Chloride, 2.12g/L Sodium Bicarbonate, and 73.5 g/L Calcium Chloride (Liu, 2019).

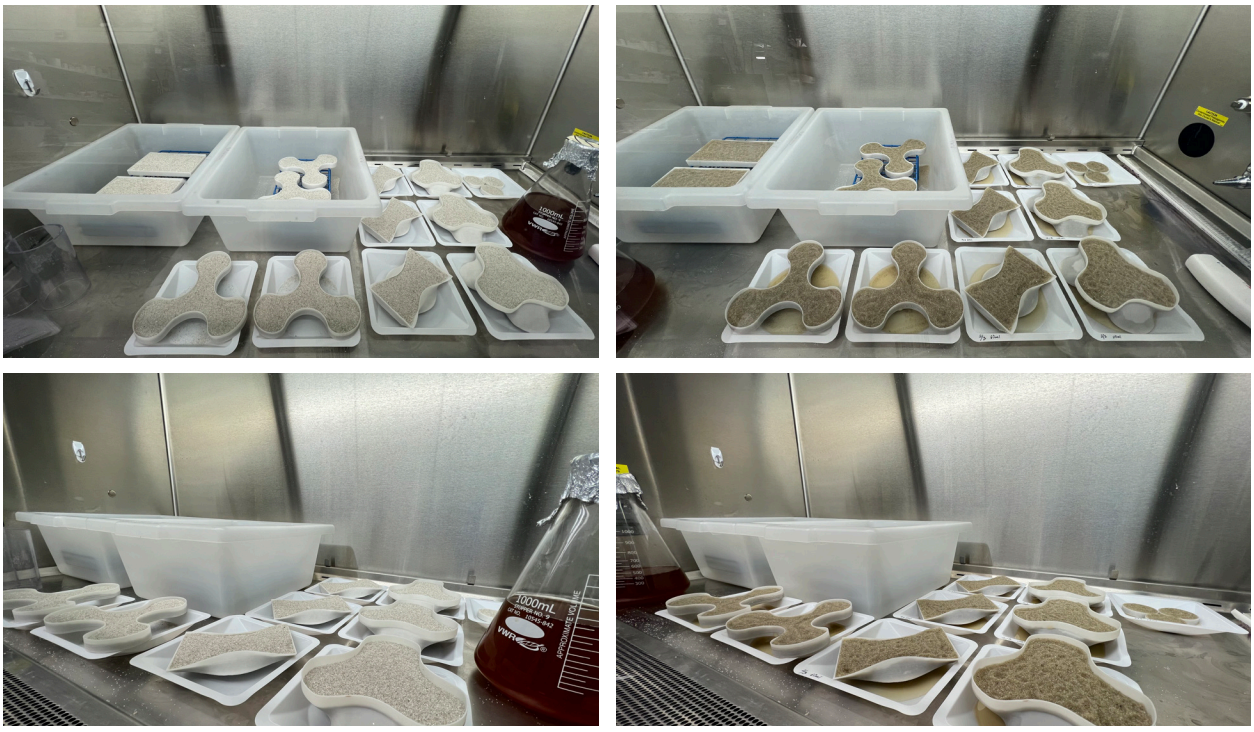

Figure 25 Biocementation: Biocementation process occuring in the biosafety cabinet. PLA molds filled with coarse sand (Left) and inoculated with bacteria and biocement solution (Right).

Biocementation of PLA and Silicone Molds. The molds were flushed with the biocement solution at an equivalent volume to that used for inoculation. Each mold was flushed four times with biocement solution over 48 hours and then incubated at 30C for 24 hours before leaving to dry in the biosafety cabinet at room temperature (25C).

3.2.3 Modular Unit Results

The initial results of the PLA molds are shown in Figure 26. The biocemented samples were released from the molds with crisp edges and were consistent across numerous samples. Adding a small lip on subsequent versions led to the easy removal of the PLA molds without any chipping.

Figure 26 Biocementation Results: Biocemented modules and objects using PLA molds.

Overhangs on early interlocking designs (Figure 27) repeatedly collapsed since it was challenging to evenly saturate sand with bacteria and biocement solution. Sharp corners were also susceptible to breaking during the mold removal process.

Figure 27 Interlocking: Biocemented interlocking modules.

Some samples bonded to the PLA mesh, as shown in Figure 28 (Bottom). This behavior is promising and suggests the possibility of creating a hybrid or composite biocemented substrate to increase the material's strength and durability. The material also took on the formal nuances of the casting process, such as maintaining the curvature from an uneven rack and the PLA mesh's texture, as demonstrated in Figure 28.

Figure 28 Resolution and Bonding: Biocementation results including crisp edges (Top Left), high resolution (Top Right), curvature from bowing formwork (Bottom Left), and bonding to the PLA (Bottom Right).

Silicone casting also proved successful (Figure 29) if openings were created to allow the solution to flow through. However, the process was less successful than the flat PLA molds because the silicone molds did not provide the same access to oxygen during biocementation. Lack of oxygen had the most significant effect on the grooves (Figure 30), which did not biocement since they were the furthest from the exposed face.

Figure 29 Casted Module: Biocemented three-dimensional module.

Figure 30 Casted Module with Grooves: Biocemented three-dimensional module with grooves.

3.3 Assembly

Using biocementation as an assembly method was inspired by phenomena observed in the lab, including a broken cylinder that re-cemented together when left for a few days on a lab workbench. To investigate different paths of reliably connecting modules, I explored binding units together with varying combinations of media, cement solution, bacteria, and sand.

3.3.1 Assembly Design

Flat modules were designed, biocemented, and used to test the assembly of several units. The tiles were placed within a PLA mold to hold the modules close during re-cementation. Eliminating significant gaps improves the chances of successfully joining the modules.

Figure 31 Tiles to be Assembled: A group of modules ready to be biocemented together.

However, the need for formwork would be inconvenient for large-scale applications or complex geometries. Integrating interlocking geometries could be an alternative to remove the necessity for temporary formwork. Implementations for the assembled system including are shown in Figure 32. The envisioned possibilities include planters for the local cultivation of contaminant-free food. Living columns that could provide outdoor shelter while remediating polluted air, ground, and water. Or bricks for building facades in highly polluted cities.

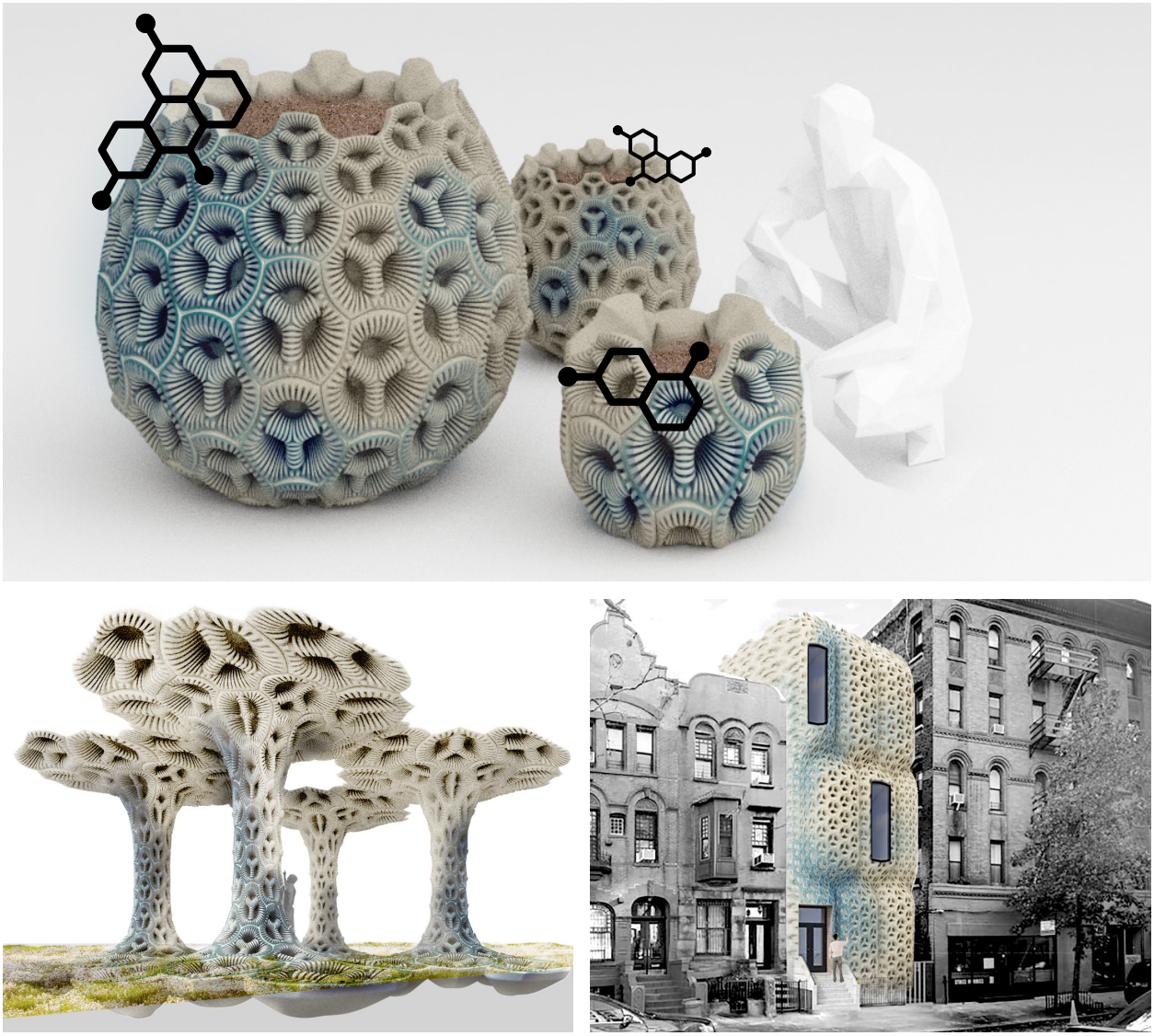

Figure 32 Living Assemblies: Various examples of the modules used in the built environment. At the object scale as a planter (Top), as a column and pavilion (Bottom Left), and as a brick facade system in urban environments (Bottom Right).

3.3.2 Assembly Methods

Assembly of Biocemented Units: Previously cemented tiles were set into PLA molds. For initial studies, sand was introduced into the seams to reduce the opening size, followed by bacteria and cement solution. The process followed the same biocementation method used to fabricate the individual tiles outlined in the unit methods section. The second iteration of the assembly tests removed the use of sand and tested assembling the tiles with only media, biocement solution, or bacteria and biocement solution. Next, the liquid solutions were added using a serological pipette until the solution fully saturated the seams. The process was repeated four times over 48 hours and incubated for 24 hours at 30C before leaving to dry.

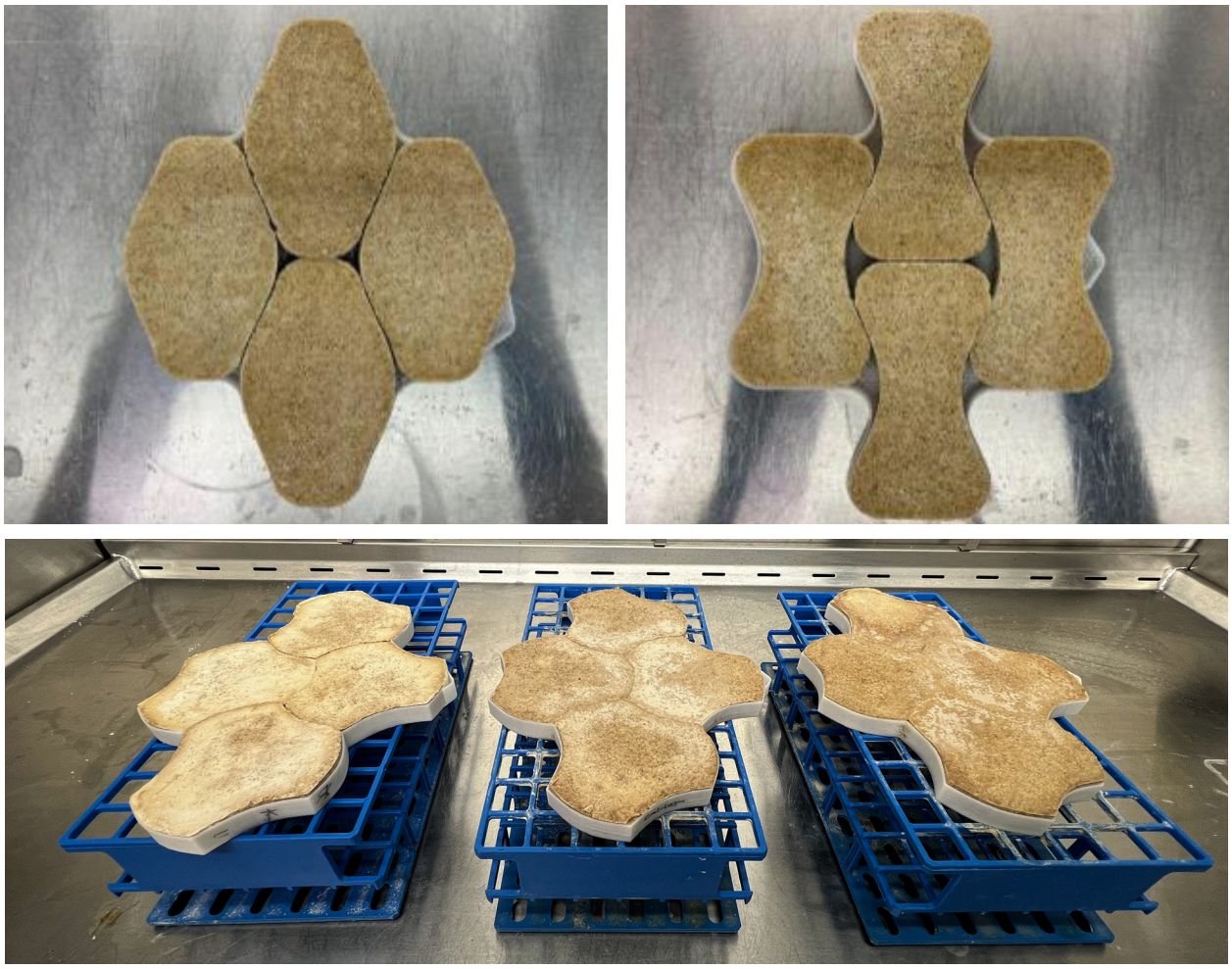

Figure 33 Biocementation: Tiles placed within 3D printed PLA molds to prevent movement while joining (Top) Biocemented tiles in molds (Bottom).

3.3.3 Assembly Results

Preliminary biocemented assemblies with the addition of sand, successfully joined together, as seen in Figure 34. However, the various geometries fared differently. For example, the convex hexagons (Figure 35 Top) were very rigid, while the concave hexagon broke after removing from their mold. In addition, a buildup of calcium expressed as a white

Figure 34 Biocemented Assemblies: Four units joined together with biocement. The areas that received extra rounds of biocement have begun to turn white.

Figure 35 Biocementation of Various Assembly Geometries: Different biocemented geometries including a convex hexagon (Top) and a concave hexagon (Bottom) were tested.

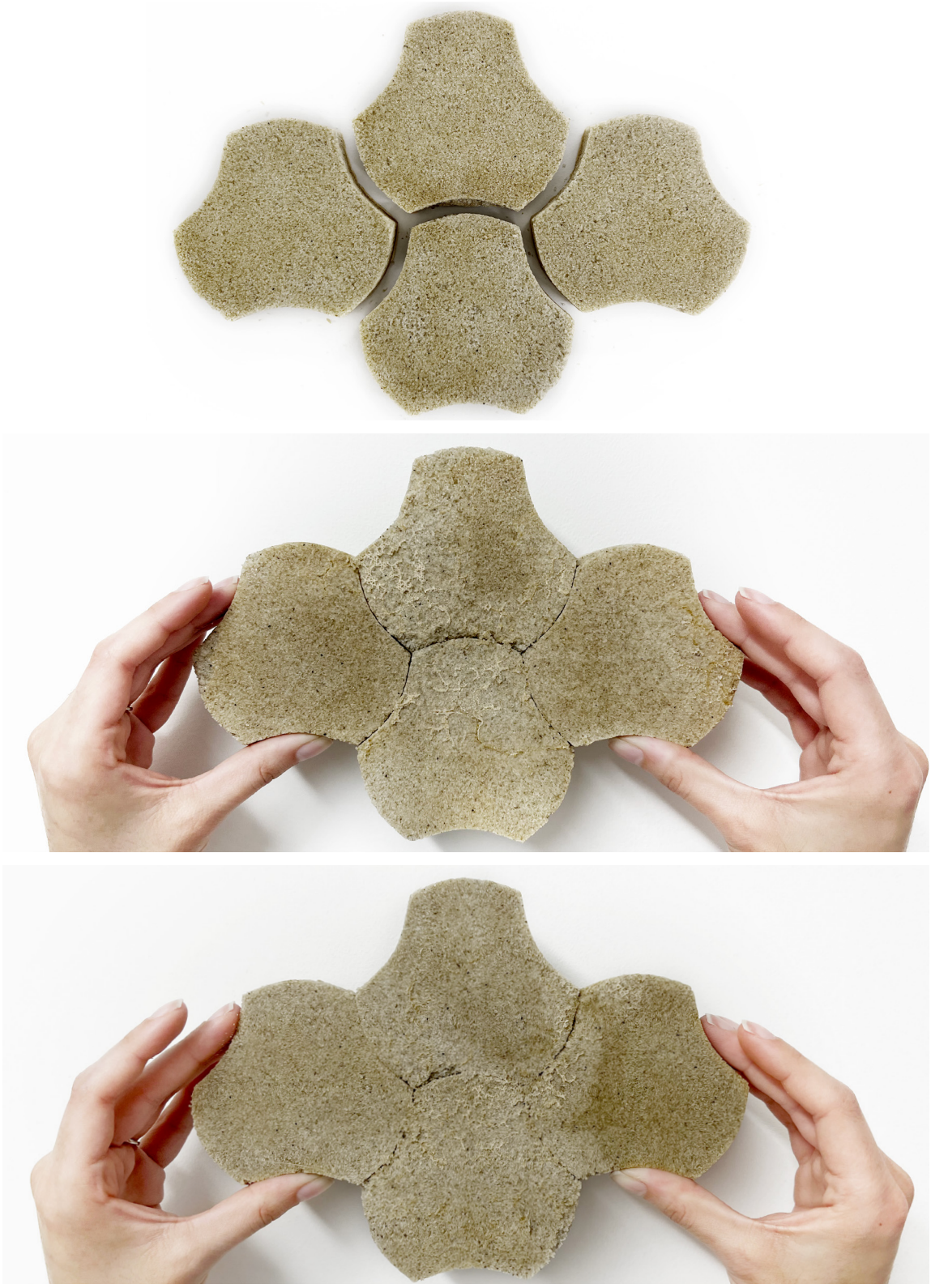

Figure 36 Biocementation with Different Solutions: Only media (Top), biocementation solution (Middle), biocementation solution and microbes (Bottom).

chalky layer was observed across all samples. This phenomena is not demonstrated in the second biocementation study (Figure 36) because the biocementation occured on the opposite face.

The second study focused on establishing the minimum requirement for biocementing several units together. The study tested varying solutions consisting of media, biocementation solution, or bacteria and biocementation solution. The results indicate that only supplying nutrition via a media solution is not enough to biocement the modules together (Figure 36 Top). The outcome was anticipated because of the lack of calcium - a crucial component for forming new calcite crystals. Assembly with bacteria and biocementation solution also yielded expected results. The method assembled the units into a rigid configuration but required the addition of freshly cultivated bacteria in addition to the biocementation solution (Figure 36 Bottom). In contrast, using only a biocementation solution yielded unexpected results. This method also led to the successful joining of units (Figure 36 Middle), suggesting that the microbial community remained alive after two weeks and could continue to play a role as a collaborator for biocementation.

3.4.1 Living Bricks Design

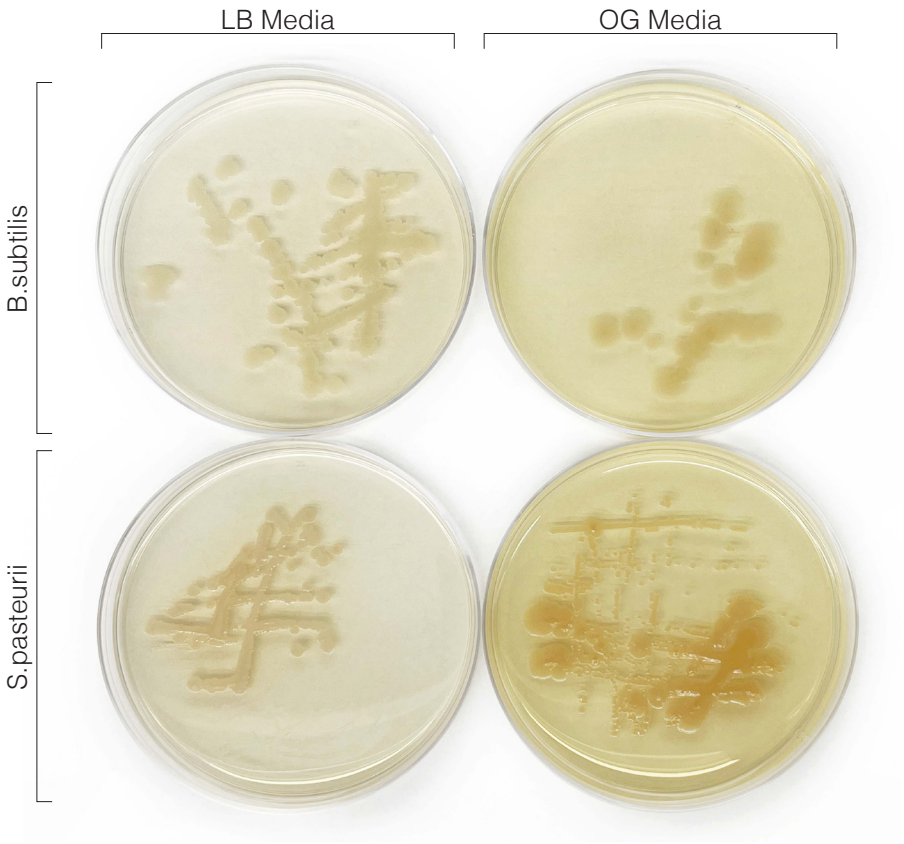

Figure 37 S.pasteurii and B.subtilis Plated on Various Media: Top row includes B.subtilis plated on it's preffered media LB (Left) and OG media (Right). Bottom row contains S.pasteurii plated on LB media (Left) and it's preffered media OG (Right).

The first study conducted was an initial investigation into the preferred media for each microbe. For example, S.pasteurii ATCC 11859 prefers a yeast extract and ammonium sulfate media adjusted to a pH of 9, referred to as OG media hereinafter, whereas B.subtilis W168 can grow on commonly used Luria-Bertani media (LB media). Microbial growth was documented with a spectrophotometer over thirteen hours to determine which media composition would be preferred by the living microbial community and understand the effects on the microbes of their preferred and non-preferred media solutions. The results are plotted in Figure 41.

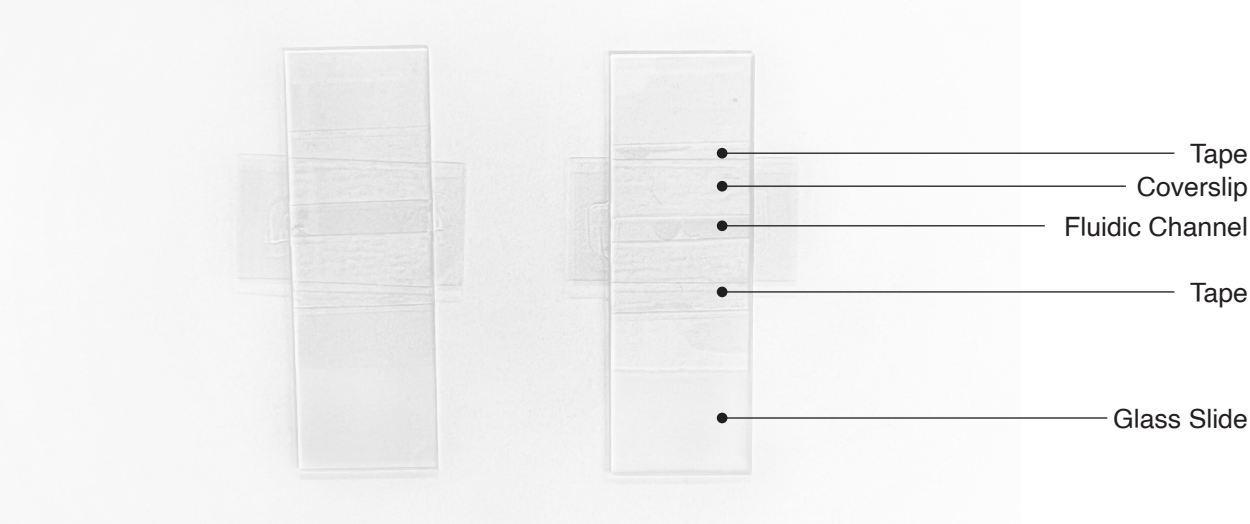

Figure 38 Prepared Slides: Two glass slides containing small fluidic channels to study the effect of crystal growth under different media solutions.

The second study examined the effects of media selection on calcium carbonate crystal formation. Switching to LB media for cementation would enable a single source of nutrition for growing, biocementing, and bioremediation with the living material. The use of the traditional media could also have implications on the ability of the system to scale in use and implementation. Cultures containing OG and LB media were examined using brightfield and fluorescent microscopy at 12 and 48 hours to investigate the effects on crystal formation. The solutions were imaged on glass slides by creating small fluidic channels with double-sided tape and a coverslip, as shown in Figure 38.

A separate study was conducted on a biocemented petri dish to understand the viability of the sensing microbe. Staining was first explored as a method for studying microbial presence. However, the color was too faint to differentiate against the natural fluorescence of the calcium carbonate crystals (Figure 39). To remediate the issue, a plasmid was constructed to transform the bacteria with a fluorescent gene: mCherry. This gene allows the bacteria to produce a bright pink color that is clearly distinguishable from other sources of fluorescence.

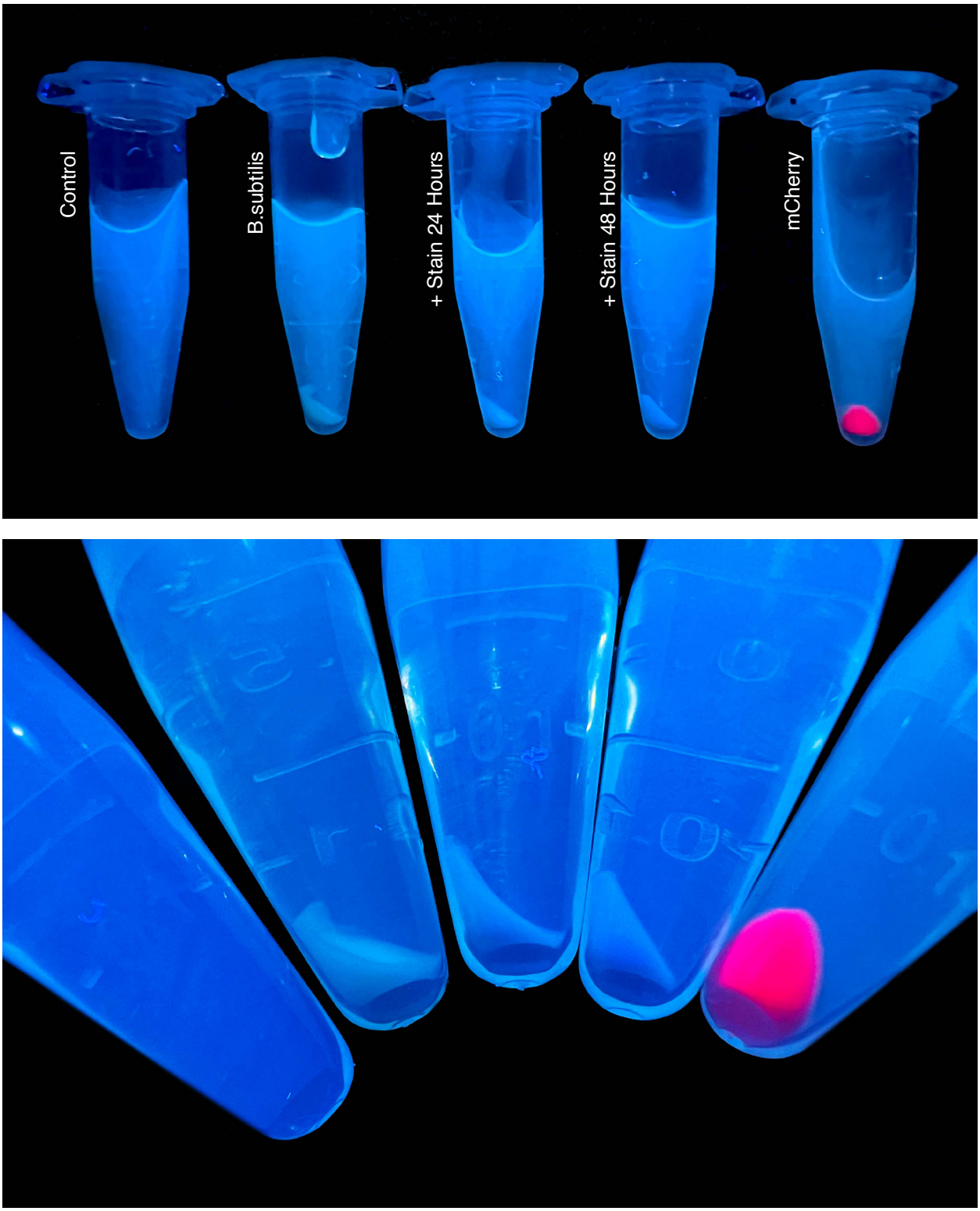

Figure 39 Various Stains and Fluorescence: Different approaches for visualizing bacteria. The various microcentrifuge tubes show pellets of bacteria with no alterations, a culture stain after 24 and 48 hours and the mCherry gene (Top). Close up of the various pellets (Bottom)

Figure 40 Genetic Systems: The genes mCherry, expressed as a flourescent pink (Left), and lacZ, expressed as a bright blue shade were selected to evaluate the biocemented living material. The mCherry shown is expressed in E.coli, the lacZ is expressed in B.subtilis.

To construct the mCherry plasmid, the empty vector backbone pBS1C was selected (Radeck, 2013). It is a backbone created for B.subtilis integration at the amyE' locus that also encodes for chloramphenicol antibiotic resistance. A gene insert was then formulated that included RFC10 standard prefix and suffix (iGEM, 2022), the constitutive promoter pVeg (BBa_K143012), B.subtilis specific RBS (BBa_K090505), and the mCherry gene which was codon-optimized for B.subtilis (BBa_K3697000). Finally, the gene was inserted into the multiple cloning site of the backbone using restriction cloning and linearized before integration into B.subtilis W168.

The last living study examined the feasibility of using an engineered microbe to sense and report the presence of a chemical via color. To test this possibility, the well-established LacZ genetic circuit was chosen. The lacZ gene is part of a larger lac operon that allows bacteria to use lactose as an energy source (Khan Academy, 2022). The lacZ encodes for β-galactosidase, an enzyme that can hydrolyze X-gal, a chemical similar to β-galactosides, to produce a bright blue color, as shown in Figure 40 (Goldbio, 2022). The plasmid pBS1CLacZ was chosen to transform into B.subtilis and subsequently tested on the biocemented Petri dishes (Radeck, 2013).

3.4.2 Living Bricks Methods

Media Composition for Transformed B.subtilis. Luria-Bertani broth was prepared for transformed B.subtilis cells with the following components: 10g/L of Tryptone, 5g/L of Yeast Extract, and 10g/L of Sodium Chloride. Selective media and plates were supplemented with 5mg/mL of Chloramphenicol, dissolved in 70% ethanol (Radeck, 2013).

Microscopy of Biocement Crystals. The Keyence All-In-One Fluorescence BZ-X microscope was used to image the calcium carbonate crystals under brightfield and fluorescent conditions. A standard glass microscope slide was used with two pieces of double-sided tape and a coverslip to create millifluidic channels for imaging. Liquid cultures were pre-prepared in culture tubes that were representative of the various conditions, including biocemented OG and LB S.pasteurii ATCC 11859 cultures, and loaded onto the glass slides for imaging. All images shown were created with Z-stack imaging capabilities of the Keyence to ensure focus throughout.

Assembly of mCherry Plasmid. To assemble the mCherry pBS1C plasmid, the backbone was first extracted using a QIAprep Spin Miniprep Kit. The backbone and gene insert were then digested using EcoRI-HF® and NotI-HF® restriction enzymes. Due to the small amount of DNA material, the gene insert was purified using the QIAquick PCR Purification Kit. The backbone was isolated with gel electrophoresis, which was then purified with the QIAquick Gel Extraction Kit. The two parts were then ligated with the T4 DNA Ligase and linearized with ScaI-HF® prior to the transformation of B.subtilis.

Preparation of PC Buffer. This media was used to create competent B.subtilis cells. The buffer consists of 107g/L K2HPO4 anhydrous, 60 g/L KH2PO4, and 10g/L Trisodium Citrate Dehydrate. The pH was adjusted to 7.5 with Potassium Hydroxide. All components were filter sterilized. This protocol was graciously provided by the Grossman lab.

Preparation of MD Media. This media was used to create competent B.subtilis cell. The components included 9.1mL of 1.1X PC Buffer, 400uL of 50% glucose, 250uL of 100mg/mL K-Aspartate, 30uL of 1M MgSO4, 25 uL of 2.2mg/mL Ferric Ammonium Citrate, and

50uL of 10mg/mL Tryptophan. All components were filter sterilized. This protocol was graciously provided by the Grossman lab.

Preparation of Competent B.subtilis Cells. To prepare competent cells a single B.subtilis W168 colony was inoculated into 3mL of LB with 3mM MgSO4 in a 10mL culture tube for two hours at 37C or until the OD600 was 1.0. 500uL of cells were then inoculated into 10mL of MD media in a 125mL flask. The cells were left to grow for three and a half hours before transformation. This competent B.subtilis protocol was graciously shared by the Grossman Lab at MIT and modified with advice from Erika Wirachman for the specific study.

Transformation of B.subtilis. 200uL of competent B.subtilis cells were transferred to a 10mL culture tube. 1ug of pre-linearized DNA was then added to each culture tube along with a control that did not include DNA. The cells were left to grow for one and a half hours at 37C and then spread on a selective plate to grow overnight. This competent B.subtilis protocol was graciously shared by the Grossman Lab at MIT and modified with advice from Erika Wirachman for the specific study.

X-Gal and Rose-Gal Experiments in Liquid Culture. X-gal and Rose-Gal aliquots were prepared to a concentration of 100mg/mL dissolved in Dimethylformamide (DMF). An IPTG solution of 100mM in dH2O was also prepared. 2uL of 100mg/mL X-gal solution was added per 1mL of media along with 10uL of 100mM IPTG solution per mL (Seaver, 2014). The solutions were then inoculated with the transformed pBS1CLacZ B.subtilis and grown overnight at 30C in a shaking incubator at 200rpm.

X-Gal and Rose-Gal Experiments on Biocemented Petri Dish. X-gal and Rose-Gal aliquots were prepared to a concentration of 100mg/mL dissolved in Dimethylformamide (DMF). An IPTG solution of 100mM in dH2O was also prepared. 2uL of 100mg/mL X-gal solution was added per 1mL of media along with 10uL of 100mM IPTG solution per mL. 10mL of each solution was then added to the corresponding biocemented petri dish and left at room temperature (25C) for two hours, after which they were inoculated with the transformed pBS1CLacZ B.subtilis and left to grow overnight at 30C.

3.4.3 Living Bricks Results

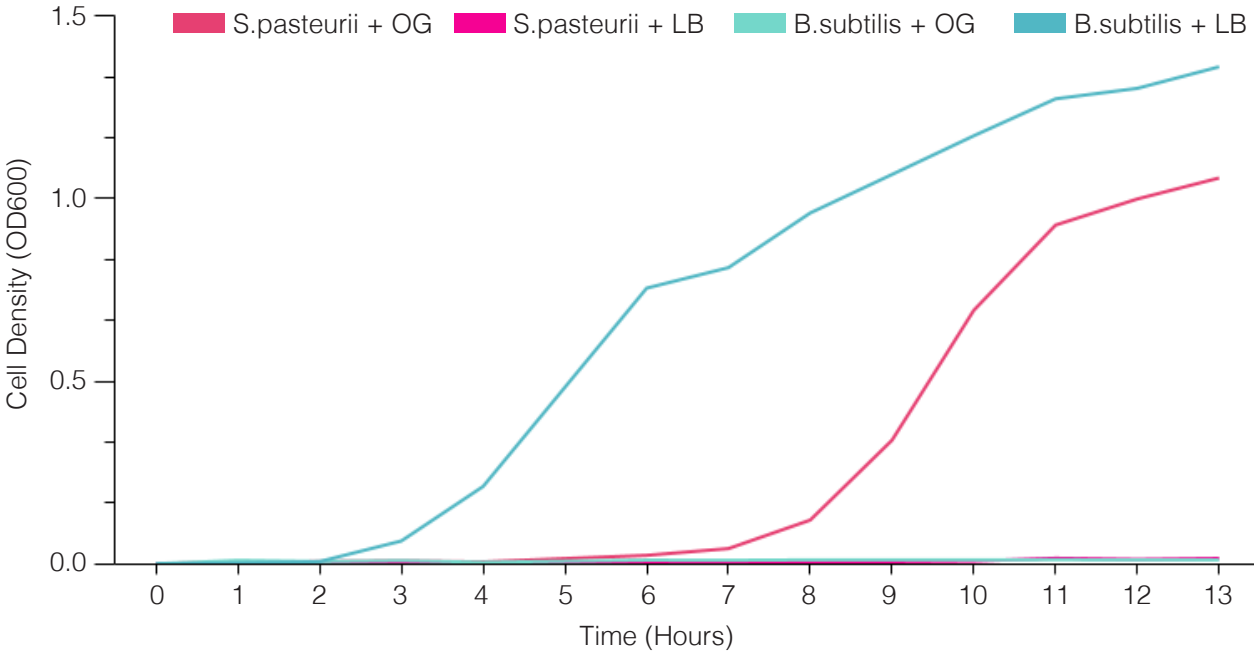

Figure 41 Doubling Time: Evaluation of doubling time of S.pasteurii and B.subtilis on different growth media including Yeast Extract + Ammonium Sulfate Media (OG) and Luria-Bertani Media (LB).

The media study revealed that the microbes do not grow well outside of their preferred media. Each microbe thrived in its preferred media but grew poorly when cultivated in each other's media (Figure 41). The results point to a separation in sensing and biocementing capabilities of the living material to appease each microbe's preferred nutritional requirements. An alternative approach could also include formulating a different media solution that meets both microbial preferences. Alternatively, a possible justification for the drastic change in growth could also be based on the sudden shift in media composition. The microbes were not previously cultivated in non-preferred media solutions resulting in a sudden change that they could not acclimate to.

The media shift did not significantly affect the formation of calcium carbonate crystals. Both solutions generated crystals after 48 hours (Figure 42). However, a noticeable difference is visible after 12 hours. The LB media biocementation shows little to no crystal

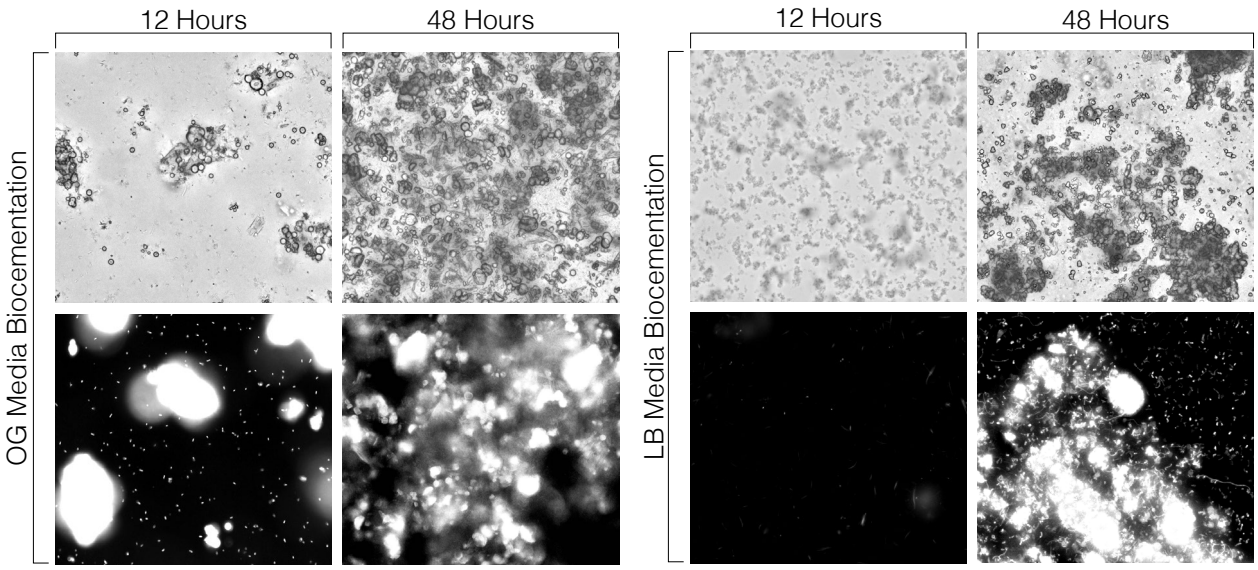

Figure 42 Microscopy of Crystalization on Original and LB Media: Biocementation using two different medias including the S.pasteurii preferred OG media and the B.subtilis preferred LB Media to understand impact on crystalization. Images were taken at 40X using Brightfield settings (Top Row) and DAPI Fluorescence (Bottom Row) on the Keyence All-In-One Microscope.

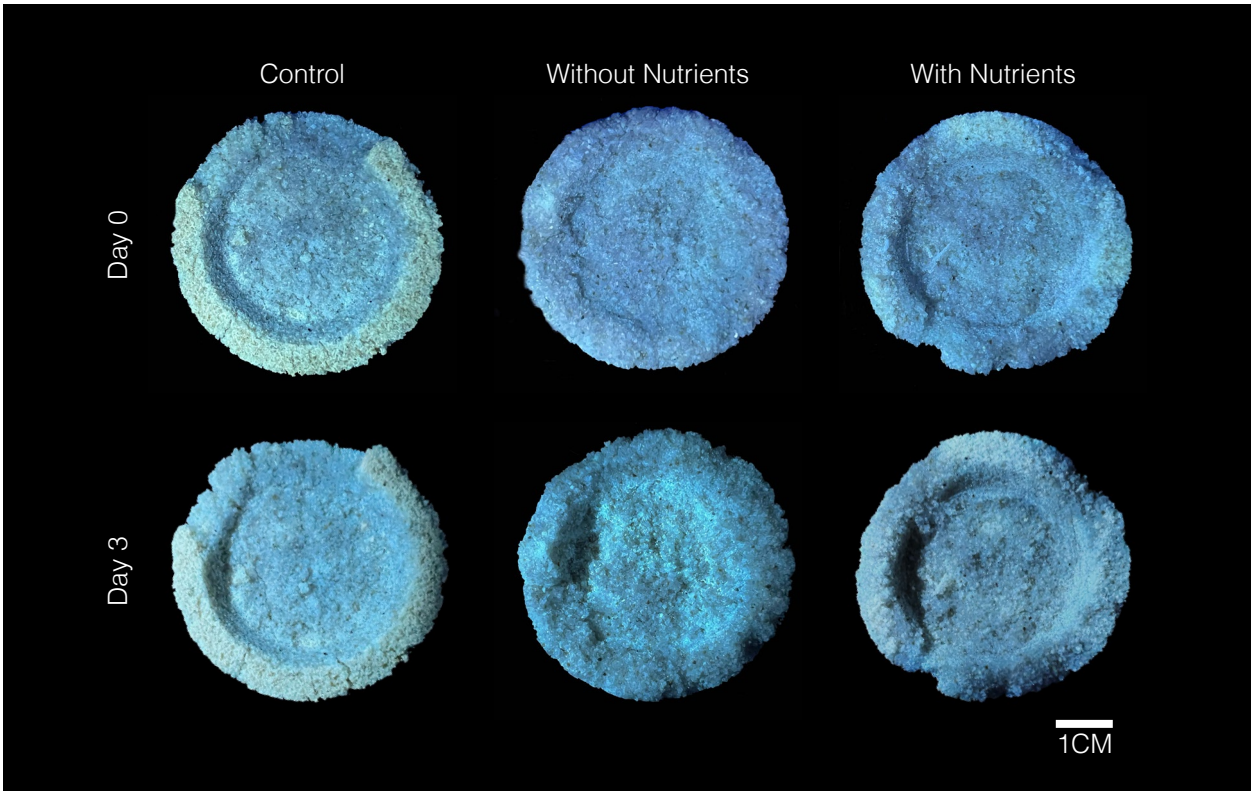

Figure 43 B.subtilis Viability: B.subtilis growing on biocemented petri dishes. The cells were transformed with mCherry to visualize their coverage of the surface over a few days.

Figure 44 B.subtilis lacZ Expression: Microcentrifuge tubes with various concentrations of Rose-Gal and X-Gal in liquid media with the transformed B.subtilis.

formation, while the OG media solution has generated visible calcium carbonate clumps. The outcome could signify a lag in the construction of calcite crystals. Furthermore, the clusters in LB media are consolidated, whereas the OG media crystals are evenly dispersed. Further studies testing the effect of sand should be conducted to understand whether these factors impact the overall viability or strength of the microbial system.

The mCherry viability study was inconclusive. While B.subtilis was effectively transformed with the plasmid, it did not emit strong fluorescence. There are a few reasons for the low expression, including a possibly weak RBS site. The transformed cells were still used in the study. However, it was challenging to discern calcium carbonate fluorescence from B.subtilis fluorescence (Figure 43).

The B.subtilis transformation with the lacZ gene was successful. It was used to test the effect of various X-Gal and Rose-Gal concentrations in liquid media (Figure 44) and on biocemented Petri dishes. The initial results shown in Figure 45 are promising. However, the surfaces have dried out, resulting in a pale color since the microbial community did not flourish.

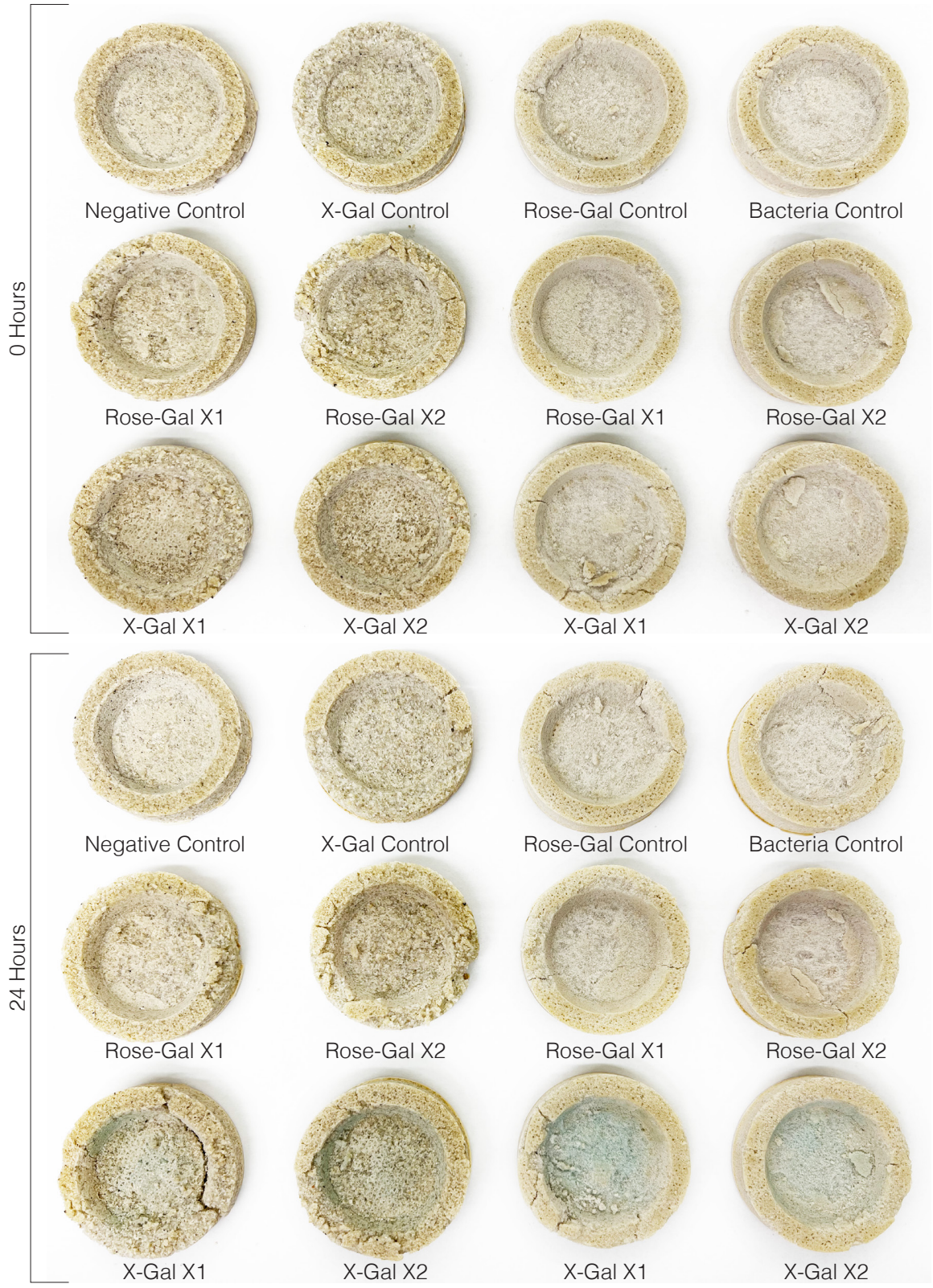

Figure 45 B.subtilis lacZ Expression on Biocemented Petri Dish: Color produced through the expression of lacZ in the presence of Rose-Gal and X-Gal on biomineralized petri dishes at 0 and 24 Hours.

Figure 46 B.subtilis Functionalized Biocemented Surface: Close up of the color produced by the engineered B.subtilis microbe on a biocemented surface.

4 Conclusion

4.1 Concluding Remarks

“We no longer are the inventors,” claims Tobias Rees (Rees, 2018). The emergence of synthetic biology has ushered in a new era that challenges our notions of technology. Technology and intelligence have separated humans from the biological world throughout history. However, synthetic biology is only possible because of microbial ingenuity. For instance, CRISPR is a genetic technology that humans did not create. Instead, it is a microbial capability that allows bacteria to fight off viral infections through a form of bacterial memory (Zomorodi, 2022). The incorporation of CRISPR as a tool in research is profound because it shifts our conceptions of technology away from a dissociative instrument to an intertwining mechanism that blurs the divide between human and non-human contributions.

This thesis developed a biocemented living material as a case study for the future proliferation of microbial collaborations in design and architecture. The work is a testament to the increasing accessibility of scientific processes. For example, I used protocols from open source websites such as iGEM and protocols.io to generate the living material. Furthermore, I designed the methods for communications with established SBOL standards and assembled gene sequences by combining several modular genetic parts into a plasmid sourced from a genetic toolbox (Radeck, 2013). The accessibility of these processes is imperative. The collaborative aspects inherent in the approach will only permeate design and architecture’s cultural realms if its accessibility to non-biologists increases.

Both synthetic biology and design processes were crucial to establishing methods for collaboration with microbes. The scientific techniques allowed me to rigorously evaluate the factors impacting the microbes, thereby providing valuable information on the needs and limitations of the living organism. At the same time, design methods defined the means of interaction that would make the invisible visible. For example, tangible forms of communication such as color were incorporated to allow humans to continuously

recognize the role these microscopic living systems have on our ecosystems. In closing, I believe that we can reach alternative futures for co-habitation through collaborative engagements with living systems. That, it will not be technology but acts of empathy for the biological world that our bodies and spaces intimately inhabit, which will create the sustainable future we seek.

4.2 Contributions

The contributions of this thesis include the following:

An overview of engineered living materials for the built environment, including specific constraints and recommendations for large-scale implementations of biocemented living materials.

A method for biocementing custom 3D printed PLA and silicone molds.

A process for biocementing units together into larger assemblies to enable the construction of architectural elements and enclosures.

An initial evaluation of the growth of an engineered microbe on a biocemented surface.

A design and assessment of an engineered microbe with sensing and reporting capabilities on a biomineralized substrate.

4.3 Future Work

The work completed in this thesis touched upon a wide range of topics and revealed a plethora of exciting future work to explore. Therefore, I have split them into the following categories for clarity:

Hardware

The key to successful biocementation is the even distribution of bacteria and biocementation solutions. In this thesis, modules were fabricated by manually flushing solutions, resulting in uneven biocementation. An automated peristaltic pump system should be developed to improve the speed and complexity of geometries that can be achieved with biocementation. Automation can also be used to increase the scale of the modules.

Software

In addition to hardware, software can also enable more complex geometries as necessitated by the microbes. Developing fluid simulation software would be essential to address this challenge. The simulation can also be supplemented with information on oxygen distribution and structural analysis to create a range of designs for biocemented modules and objects. Additionally, the software can be used to inform the design of molds, providing information on outlet locations for even fluid flow.

Biocomposite Material Approaches

Hybrid biocomposite approaches should be explored to address several challenges, including difficulties maintaining structural integrity while soaking the bricks with a nutrition solution. Moreover, biocomposites could reduce the decay of the living system under non-ideal conditions such as high heat or low temperatures. Possible scenarios could involve the integration of microfluidic channels to protect the microbes and supply necessary nutrition while removing waste.

Organism Selection

Further studies regarding the choice of organism should be conducted, particularly for selecting biosensing microbe. Possible microbes may include other strains of B.subtilis and native soil microbes. Exploring a variety of strains may result in greater robustness or compatibility with the biocementation microbe. Furthermore, microbes could be selected based on local context. Instead of introducing non-native bacteria to various ecosystems, local biocementing microbes could be cultivated and implemented in the process.

Living Biosensors

Further characterization of promoters and RBS sites for protein expression should be explored to allow for more robust visual reporting on the biomineralized substrate. Other reporting methods could also be explored, including smell or sound. Lastly, future studies should also include the development and characterization of heavy metal sensors.

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