Bio-Cementation: Material Innovation in Architecture

A Thesis Submitted in Partial Fulfillment of the Requirements
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Abstract

"Bio-Cementation: Material Innovation in Architecture" explores and develops a new biomaterial for architectural use sourced from existing material waste systems. Initial research was conducted to examine the current state of biomaterial development throughout a range of disciplines and to identify a potential waste stream to be diverted. It was concluded that crustacean shells discarded by the seafood industry had the greatest potential for new material development while also being a highly abundant waste product. This project utilizes crustacean shells in the form of a fine powder. A process called microbially-induced calcium carbonate precipitation ("MICP") was identified as a biological alternative to cement that would bind the grains of crustacean shell powder together to form a hardened material. Following this research, a series of material tests was conducted to determine A) the feasibility of the material concept and B) the material composition that would produce the most advantageous material properties.

The project is concluded by a set of speculative designs that aim to present possible applications for the material with careful consideration of the properties it currently has at this early stage in its development.

Executive Summary

"Bio-Cementation: Material Innovation in Architecture" is a research and design project whose goal is to create a sustainable, biological alternative to existing material practices while diverting a material waste stream in the process. The project then aims to demonstrate the properties and abilities of the resulting biomaterial through speculative designs. The project is part of a larger body of research and design projects produced by a studio of ten fifth-year architecture students, all exploring various biomaterials.

In the field of architecture, a "biomaterial" can be defined as a material derived from or produced by living organisms. Biomaterials have become an increasingly-important topic of conversation throughout many disciplines as our society shifts away from unsustainable practices. In architecture, biomaterials are being used for a plethora of reasons: to reduce the carbon footprint of the building industry, to further and enhance design concepts such as biophilia (a term for our innate love of nature), and to produce new and interesting material effects that might lend themselves well to various aspects of building design and construction. Biomaterial research is part of the larger discourse surrounding humanity's faltering relationship with nature: "We can no longer see ourselves as separate from the natural world or our technology, but as a part of them, integrated, co-dependent, and entangled" (Dade-Robertson, p. 13).

Bio-cementation, the process at the heart of this research project, refers to a biological phenomenon that closely mirrors the hardening and binding effects of typical construction cement. Generally, the goal of bio-cementation is "to bind soil particles together with minerals and other substances to increase the compressive strength of soil" (Chu et al., 2014, p. 2). The term takes the prefix "bio" because the process is typically performed by bacteria (Chu et al., 2014, p. 2). The chemical process undergone by the bacteria to achieve the desired cementing effect is called microbially-induced calcium carbonate precipitation, or MICP (Chu et al., 2014, p. 2). The bacteria (specifically urease-producing bacteria, or UPBs) produce an enzyme that is capable of breaking down (through hydrolysis) the compound urea into its consituent parts:

free carbonate (CO_2^{3-}) and ammonium (NH_4^+) (Gowthaman et al., 2023, p. 2). The carbonate ions are then able to mineralize (bind) with free calcium ions (Ca^{2+}) to form calcium carbonate ($CaCO_3$) (Gowthaman et al., 2023, p. 2). In this study, the bacteria *Sporosarcina pasteurii* was used as it is one of the most widely studied UPBs due to its ability to produce large amounts of calcium carbonate under certain conditions (Wong, 2015, p. 8).

Previous applications of MICP have typically focused on the cementation of sands and soils to prevent erosion. One study successfully explored the application of MICP in stabilizing dunes in desert environments, focusing primarily on reducing erosion and movement due to wind (Naemi et al.). Another used the process to prevent coastal dune erosion resulting from shear forces from crashing waves (Montoya et al.).

Instead of typical soil and sand applications, this study aimed to reproduce the successful efforts of Naemi's and Montoya's teams using crustacean shells. This material was selected for experimentation due to the shells' innate material properties as well as the ability to source large quantities of the material from seafood waste. Global fisheries production exceeds 100 million tons annually, resulting in upwards of 6-8 million tons of shell waste (Vidal et al.). As crustacean fisheries can be found around the world (Scheld et al.), this is clearly a global resource that is typically discarded either in land fills or directly back into the ocean.

As with MICP, many projects and studies exist that explore various possible applications of crustacean shells. The primary compounds of interest found in crustacean shells are calcium carbonate (at approximately 90% of a shell's mass (Suzuki and Nagasawa, p. 349)) and chitin, the biopolymer that, when combined with calcium carbonate, affords crustacean shells their lightweight strength (Ifuku et al., 2009, p. 1584). Most previous studies explore the shells' chitin, undergoing costly and energy intensive processes to isolate the biopolymer from the other compounds within the shells (Rinaudo, 2006, p. 611).

Generally, this pure form of chitin has medical and pharmaceutical applications (Rinaudo, 2006, p. 611), however some design teams have experimented with the material for architectural application as well. Neri Oxman and her team utilized chitin to 3D print fan-like structures ("Aguahoja"). The structures eventually disintegrated over time due to drying out, however the temporal aspect of the project was welcomed by Oxman as she and her team aimed to "utilize decay as a design feature" ("Aguahoja").

For this research project, it was decided that pure chitin was not advantageous to the end goal due to its high cost and seemingly limited applicability in the field of architectural design. Instead, the study utilized crustacean shells in the form of crab shell powder, a material typically used for fertilizer. The study explored the potential of creating a material through the calcification of the crab shell powder via MICP. It was theorized that the high concentration of calcium carbonate already present in crustacean shells would meld well with the calcium carbonate precipitated by MICP. Conceptually, the study aimed to create a moldable material that preserves the lightweight strength and chemical makeup of crab shells.

As the material stands at the conclusion of this study, such an effect has yet to be achieved. Utilizing purely bacteria and crab shell powder, the material was unable to progress beyond a rather weak and brittle state. Thus, the speculative applications presented at the conclusion of the study embrace these properties by exploring uses revolving the construction and preservation of landscapes. The first proposed application thus becomes large-scale landscape art composed of large, geometric cuts in the ground created by the bio-cementation of large amounts of crustacean shells over a large area of land. Initially, the cite would be used an art park where visitors are invited to enjoy the architecture effects of the landscape art. Over time, the cemented crab shells would leach into the soil beneath them, creating fertile soil that would eventually come to be used as farmland.

The second application proposes a seawall design composed of a series of cemented crustacean shell mounds to create a break water that would prevent the erosion of deteriorating beach environments,

especially at times of high tide and storm surges. As with the landscape art, over time, this seawall would itself erode, sacrificing itself for the longevity of the beach on which it rests. Another layer of cemented crustacean shells would simply need to be added on top of the existing, eroded mounds. The advantage of this system compared to typical seawall construction is the elimination of possible sources of environmental pollution. Concrete is currently the most widely used material in seawall construction (Blankendaal et al., 2014, p. 30). However, concrete (especially reinforced concrete) can have adverse effects on the environment as the high salinity of seawater eventually releases chemical compounds from the rebar reinforcements within the concrete (Blankendaal et al., 2014, p. 30).

Next steps in the development of this research would be to determine possible aggregates that could be added to the bio-cemented crustacean shells in order to achieve more desireabe and useful material properties, such as an increase in the shells' ability to resist compressive and bending forces.

Acknowledgements

I would like to express my deepest gratitude to my friend and partner Isabella Bai, with whom I've worked very closely over the past several months to develop this project. We have supported each other throughout this process in ways that have only deepened our connection as friends and colleagues. She is the other half of this project; forgive the cliché, but suffice it to say that the material would not be what it is today without her work.

Isabella and I would also like to express immense thanks to the professors who have guided us along the way. Kiana Memaran Dadgar in the School of Architecture has provided extensive research materials and support, for which we are very grateful. Davoud Mozhdehi at the Center for Science and Technology has patiently taught us two architecture students the ways of the lab and has been gracious enough to allow us to use his lab spaces. Without his support, our research would not have been nearly as successful as it was.

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Chapter I: Research

Section 1.1: Crustacean Shells

This study's interest in crustacean shells stems from its abundant supply in the form of waste from the seafood industry. Annually, more than 100 millions tons of catch are harvested from the world's fisheries (Vidal et al.). As only 40% of a crab's biomass is edible and consumed (Su et al.), this can produce 6-8 million tons of crustacean shell waste, the vast majority of which is either sent to landfills or simply dumped back into the ocean (Vidal et al.). As crustacean fisheries can be found along the coasts of every continent, aside from Antarctica (Scheld et al.), their waste streams can be sourced and utilized locally by the vast majority of the world's population. In the United States, for example, perhaps the most iconic seafood crustaceans sourced from adjacent waters include the American lobster, sourced primarily along the coast of northern New England (of which 119 million pounds were harvested in 2022 alone) (Fisheries), and the blue crab, one of the most valuable resources in the Chesapeake Bay (Fisheries). The gazami crab, sourced primarily off the coast of China, are the most abundantly-harvested crustaceans in the world by weight, at 558,000 tons in 2016 (Penn et al., 2019). In Africa, Namibia ranks as the largest fishery for crabs of the *chaceon* genus (notably red crabs) in the world (Penn et al., 2001). These examples name but a select few of the countless crustacean fisheries to be found worldwide.

Crustacean shells are a remarkable material due to their molecular composition as well as the intricate geometric structure of that composition. The two compounds that afford crustacean shells their unique and advantageous properties are chitin and calcium carbonate. In nature, chitin is often modified as a component of composite materials. Combined with calcium carbonate, chitin produces a much stronger composite. This composite material is much harder and stiffer than pure chitin and is tougher and less brittle than pure calcium carbonate, with increased hydrogen bonding between adjacent polymers giving the chitin-polymer matrix increased strength (Ifuku). As Dr. Shinsuke Ifuku and his team surmise in their study of chitin nanofibers, chitin is becoming a much more prevalent biomaterial due to its environmentally benign, biodegradable, biocompatible, renewable, and sustainable qualities (Ifuku). Other advantages of using chitin as a biomaterial include its low toxicity, being inert in the gastrointestinal tract of mammals (making it safe should it happen to contaminate a surrounding area), its antibacterial qualities that can accelerate wound healing, its hydrophilic properties, and its ability to form gels (Rinaudo, 2006, p. 6011). As one of the most common biopolymers–second only, in fact, to cellulose–it is a widely available resource, primarily found in the exoskeletons of shellfish and insects and even in the cell walls of mushrooms (Ifuku). Combined, these sources produce an estimated 1 billion tons of chitin annually ("Chitin"). In a world dealing with a waste crisis, the adoption of chitin into widely-used biomaterials would provide an opportunity to divert a waste stream from the seafood industry (Ifuku et al., 2009, p. 1584).

The structure of chitin microfibrils within the shells is what gives them their strength. "In general, the exoskeletons of crustacea have a strictly hierarchical organization which reveals various structural levels" (Ifuku et al., 2009, p. 1585). Dr. Ifuku and his team describe the shells' hierarchical structure as follows:

> "These chitin molecules are aligned in an antiparallel manner that gives rise to R-chitin crystals in the form of thinner nanofibers of about 2-5 nm diameter. These nanofibers are wrapped in protein layers, which can

be regarded as the next level. The next level in the scale consists of the clustering of some of these nanofibers into chitin/ protein fibers of about 50-300 nm thicker diameter. The next step is the formation of a planar woven and branched network of such chitin-protein fibers with a variety of thickness. These strands are embedded in a variety of proteins and minerals. The minerals mainly consist of crystalline calcium carbonate. Thickness of strands vary widely among crustaceans. Furthermore, these woven and network planes form twisted plywood pattern. This structure is formed by the helicoidal stacking sequences of the fibrous chitin-protein layers. The thickness of the twisted plywood layer corresponds to a certain stacking density of planes, which are gradually rotated about their normal axis" (Ifuku et al., 2009, p. 1585). See *Figure 1.1.1*, below.

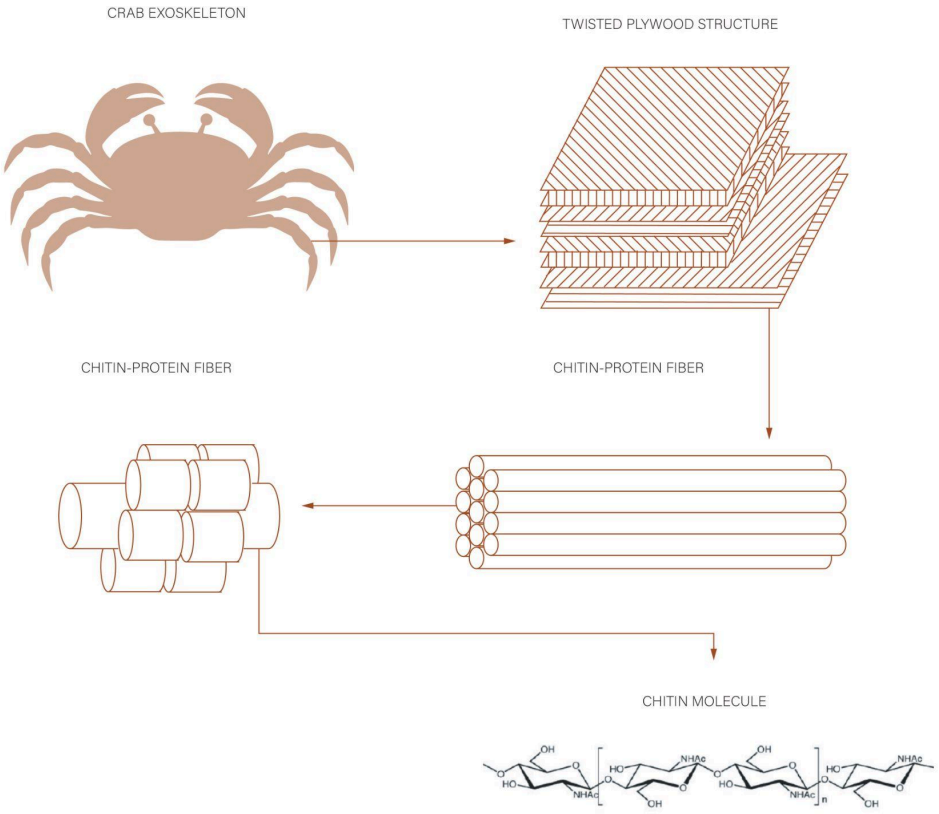


Figure 1.1.1 *Crustacean shell microstructure.*

Current applications of chitin can be found throughout various disciplines, a versatility attributable to the many forms it can take: "Chitin can be transformed and used as fiber, film, sponge or powder" (Rinaudo, 2006, p. 624). Chitin is already extensively used in the pharmaceuticals, cosmetics, environment, and energy industries (Su et al.). To name a few applications, chitin has been used to create biodegradable plastics (Zhou), as nanofiber coatings (Kaku), and even bone regrowth: "Another interesting application is in a hydroxyapatite–chitin–chitosan composite bone-filling material, which forms a self-hardening paste for guided tissue regeneration in treatment of periodontal bony defects [92]." (Rinaudo, 2006, p. 611).

To develop these technologies, processes have been explored to isolate chitin (Rinaudo) and to extract chitin nanofibers from crab shells (Ifuku). However, this study will focus on experimenting with chitin in its unpurified form: as a component of crustacean shell powder. The extraction and isolation of chitin are processes principally explored by the medical and pharmaceutical industries due to their high cost (Rinaudo, 2006, p. 624). This study, conversely, aims to explore the uses of chitin in architecture in a manner that could become widely and inexpensively available.

Along with chitin, crustacean shells also contain large amounts of calcium carbonate (more than 90% by mass) and a small amount of other organic matrices (Suzuki). The abundance of calcium carbonate in crustacean shells prompts speculation into its properties and possible applications. Already, in fact, calcium carbonate has found many applications in the pharmaceutical, agricultural, construction, chemical, and polymer industries (Su et al.).

Calcium carbonate is not a compound unique to crustacean shells. Its primary sources in today's material industries are geological, such as it is derived from marble or limestone (Yan and Chen). Though these sources are advantageous in their abundance, the downside to sourcing calcium carbonate geologically is that the compound may then also contain unwanted and even dangerous substances, such as heavy metals, that are difficult to remove completely (Yan and Chen). Thus, sourcing calcium carbonate from crustacean shells provides a much less toxic alternative (Yan and Chen) with the added benefit of renewability (crustacean shells can be grown and harvested continuously; marble and limestone cannot). It is important to note the various crystal polymorphs of calcium carbonate: calcite, aragonite, and caterite; of which most crustacean shells contain aragonite and/or calcite (Suzuki).

Section 1.2: Microbially-Induced Calcium Carbonate Precipitation (MICP)

Calcium carbonate (detailed above) is the compound produced by a process called microbially-induced carbonate precipitation ("MICP"), or sometimes as microbially-induced cementation (Chu et al., 2014, p. 3). This process is described as follows:

> "During [MICP], the enriched bacteria culture and cementation media (mainly consisting of urea and calcium slat) are introduced through the soil surface and allowed to percolate onto the stratum, either one after another or simultaneously [4,5]. The bacteria cells containing urease enzyme facilitate the hydrolysis of urea, resulting in the production of carbonate ions (CO_3^{2-}) and ammonium ions (NH_4^+). Thereupon, the supplied calcium ions (Ca^{2+}) react with carbonates, and mineralize as calcium carbonate ($CaCO_3$) within the surrounded soil matrix, i.e., on soil surfaces and at particle contacts [6]." (Gowthaman et al., 2023, p. 2) See *Figure 1.2.1*, below.

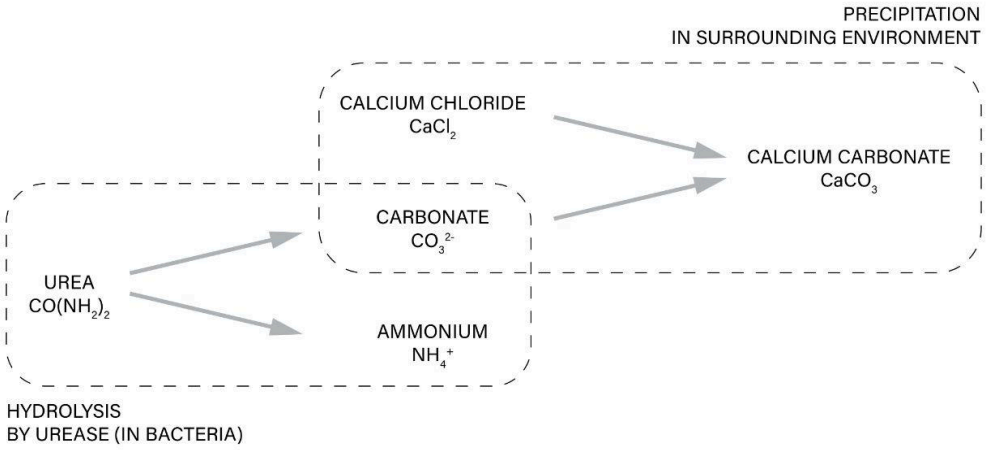


Figure 1.2.1 *Chemical reactions of microbially-induced calcium carbonate precipitation.*

The calcium carbonate molecules produced are known to act as the microbiological binders of cement-based biomaterials (Wong, 2015, p. 6). In this research project, MICP is used to cement crustacean shell powder. See *Figure 1.2.2*, below.

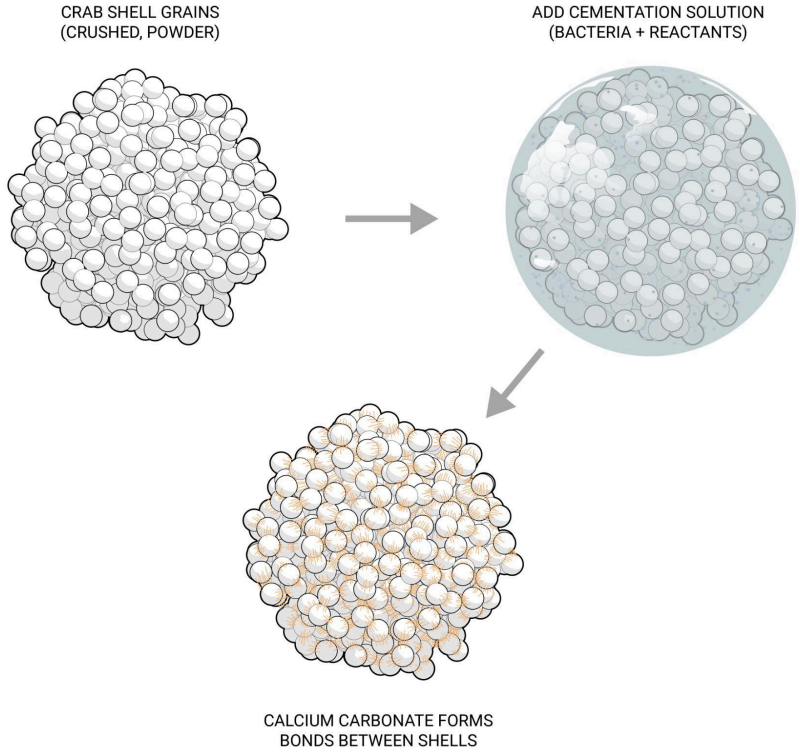


Figure 1.2.2 *Bio-cementation of crustacean shell powder via MICP diagrammatic visualization*

Applications of MICP are limited as of yet. While many future applications are being explored, one primary application showing significant promise is often referred to as bioclogging or biocementation. Chu and team, in their study of the two processes, describe bioclogging as "a process of filling the pores in soil with minerals and other substances that are generated microbially to reduce the soil permeability," and biocementation as "a process to bind soil particles together with minerals and other substances to increase the compressive strength of soil" (Chu et al., 2014, p. 2). Both bioclogging and biocementation are currently being used to combat and solidify shifting sand dunes in populated desert areas, where dunes often block roadways (Chu). Studies conducted to determine the feasibility of applying bio-cementation to the issue of wind erosion and shifting sand dunes in deserts found that the process of MICP produced evident layers of cementation in the sand, the depth of which depended on the quantities of cementation and bacterial solutions used (Naeimi et al.). The cemented "crust" produced by MICP was shown to successfully protect the loose sand present in the layers below (Naeimi et al.). Similarly to Naeimi's study, another has explored using MICP to combat water erosion on coastal dunes (Montoya et al.). This body of research found that sand treated with MICP were largely more capable of resisting shear forces from incident waves as compared to untreated sand (Montoya et al.). As for other applications, there have even been numerous studies attempting to create self-healing concrete using MICP (Myer).

Section 1.3: Sporosarcina Pasteurii

The "active ingredient" of MICP are the microbes for which the process is named. The particular microbes of interest for MICP are those that produce urease (urease-producing bacteria, or UPB), an enzyme capable of separating calcium from calcium chloride (which then binds with carbonate molecules found in urea) (Chu et al., 2014, p. 3). Two strains of bacteria are primarily used in MICP technologies: *Sporosarcina pasteurii* and *Lysinibacillus sphaericus*, both of the genus *Bacillus* (Gowthaman et al., 2023, p. 3). *Sporosarcina pasteurii* has become more prevalently used for its high urease-producing ability (Wong, 2015, p. 8). This bacteria is also able to survive much more alkaline environments than other UPB (Bhaduri et al., 2016, p. 1), making it an attractive additive for self-healing concrete formulas (Wong, 2015, p. 9).

To understand the best practices and methodologies of using *Sporosarcina pasteurii* in lab experiments, many studies utilizing the bacteria were consulted. The bacteria specifically used in this experiment was determined to be sourced from the American Type Culture Collection (*Sporosarcina Pasteurii (Miquel) Yoon et al. - 11859 | ATCC*). Examining the protocols of several research papers, it was found that the ideal growth medium for culturing this specific strain of bacteria consists of yeast extract at 20.0 grams/liter, ammonium sulfate ($(NH_4)_2SO_4$) at 10.0 grams/liter, and 0.13 M (or 15.75 grams/liter) of Tris buffer at pH 9.0 (Gowthaman et al., 2023, p. 3) (Williams et al., 2016, p. 569). To grow the bacteria in petri dishes, the same formula was used with the addition of Agar at 40.0 grams/liter concentration.

Chapter II: Lab Protocols

Section 2.1: Hypothesis

By utilizing bacteria to precipitate calcite we hope to replicate the strength and lightweight properties of crustacean shells while obtaining control of the form.

Section 2.2: Architectural Research Question

Can we artificially manipulate the form of a crab shell while maintaining the properties through utilization of bacteria to precipitate calcium carbonate between crab shell granules?

Section 2.3: Scientific Research Question

Can we utilize Sporosarcina pasteurii to precipitate calcium carbonate between crab shell granules to create a strong composite material?

Section 2.4: Materials Used

Sporosarcina pasteurii - "SP."
(*Sporosarcina Pasteurii (Miquel) Yoon et al. - 11859 | ATCC*)

1. Product arrives freeze-dried and is to be stored between 2℃ and 8℃.

Urease U1500 - UU; from canavalia ensiformis (jack bean).
(*Urease Type III, Powder, Main 15,000-50,000units/g Solid 9002-13-5*)

1. The enzyme is soluble in 0.2 M sodium phosphate buffer, pH 7.0, (10 mg/ml) yielding a solution with a possible haze. The following buffers have been shown not to inhibit urease activity: MES, HEPES, and CHES.
2. Product arrives as powder and is to be stored between 2℃ and 8℃.

Urease 94280 - U9; from canavalia ensiformis (jack bean).
(*Urease Powder, 1units/Mg 9002-13-5*)

1. Soluble in 1 MG/ML in Enzyme dilution buffer.
2. Product arrives as a powder.

Crab Shell Fertilizer - "CF."

Section 2.5: Material Handling

Sporosarcina pasteurii

1. Open vial according to enclosed instructions.
2. Using a single tube of #1376 broth (5 to 6 mL), withdraw approximately 0.5 to 1.0 mL with a Pasteur or 1.0 mL pipette. Rehydrate the entire pellet.

   ATCC medium: 1376 Bacillus pasteurii NH4-YE medium (per liter)

   i. Yeast extract.....20.0 g
   ii. (NH4)2SO4.....10.0 g
   iii. 0.13 M Tris buffer (pH 9.0).....1.0 L
   iv. Agar (if needed).....40.0 g

   Autoclave ingredients separately. No growth occurs when ingredients are sterilized together.
3. Transfer this aliquot back into the broth tube. Mix well.
4. Transfer 1.0 mL of the suspension to a second tube of broth. From the second tube, use several drops to inoculate a slant and/or plate if desired. The cryoprotectant used in the freeze-drying procedure may inhibit growth in the primary tube, hence the necessity for immediate transfer.
5. Incubate all tubes and plates at 30°C for 48 to 72 hours. If growth is not heavy on agar medium, make additional transfers from the incubated broth at this time.

Section 2.6: Bacteria Culturing

The following protocol details the methods used to culture the bacteria *Sporosarcina pasteurii* and maintain a healthy population of the bacteria throughout the duration of the study.

1. Culture Bacteria - Agar Plate Medium Preparation
   - 1.1. Assemble equipment and ingredients such as Petri dishes, flasks, Tris-base, HCI, agar, Millipore water, pH-meter, etc. Sterilize all containers by autoclaving at 121◦C.
   - 1.2. Prepare 1 L of 0.13 M aqueous solution of Tris-buffer by mixing 15.75 g Tris-base with 1 L of Millipore water. To lower the pH level of the original solution (pH 10.4) add 2,800 µl of HCI (50% concentration). Check continuously using a pH-meter to set pH = 9.
   - 1.3. Divide the 1 L buffer solution into two parts as follows:
     - 1.3.1. Take 800 ml of this solution. Divide it equally into two parts of 400 ml each. Dissolve 8 g $(NH_4)_2SO_4$ to one solution and 16 g yeast extract to the other solution.
     - 1.3.2. Take the remaining 200 ml of solution and divide it again into two parts of 100 ml each. Mix 2 g $(NH_4)_2SO_4$ to one. Add 4 g yeast extract and 4 g agar to the other.
   - 1.4. Autoclave the 4 solutions separately after wrapping the respective flasks in Al foil and sticking autoclave tapes.

     NOTE: If a benchtop autoclave unit is used, the volume should be set to 500 ml (temperature and pressure automatically specified as a function of volume).
   - 1.5. After taking them out of the autoclave, set the two 400 ml solutions aside for step 3.1 (below). Mix the two 100 ml solutions to have a 200 ml solution. Pour the mixture into 10-12 Petri dishes.
2. Culture Bacteria - Agar Plate Sample Preparation

2.1. Remove the bacterial stock from freezer (-80◦C) and allow it to thaw. After thawing properly, place the bacterial stock and the agar plate inside the biosafety hood.

2.2. Select the micropipette of smallest available dimension (0.5-10 µl is a good choice) to infest the tip with the *Sporosarcina pasteurii* stock. Streak an agar plate with the micropipette tip. Place the streaked agar plate inside a non-shaking incubator at 31◦C for 48 hours.

2.3. After 48 hours, remove the plate from the incubator and visually examine for the existence of single colonies. If there are no single colonies, then place it in the incubator for another 24 hours.

2.4. Repeat the process until single colonies are detected. Do not exceeds 7 days of trial.
NOTE: If single colonies do not appear even after a week, then it is concluded that the entire process must be repeated from step 1.

3. Culture Bacteria - Final Sample Preparation

3.1. Mix the two 400 ml solutions (Tris buffer + $(NH_4)_2SO_4$ and Tris buffer + yeast extract (step 1.5) together to obtain an 800 ml solution. Transfer 125 ml of this solution into a flask.

3.2. Perform a visual examination of the surface of the agar plate to identify regions with a high concentration of single colonies. Gently nudge and break one of the colonies with a micropipette tip.

3.3. Dip the same micropipette tip into the 125 ml flask and stir it thoroughly to ensure that a sufficient number of cells for robust multiplication get transferred. Place the flask in a shaking incubator at 150 rpm, 30◦C for 2-3 days. After 2-3 days, remove the flask from the incubator.

4. Culture Bacteria - Final Cell Count

4.1. Perform serial dilution of the non-diluted culture solution using Phosphate Buffered Saline (PBS) to attain a dilution of at least ten million (10^{-7}) to ensure countable single colonies appear. Draw seven parallel equidistant lines on one of the agar-plates.

4.1.1. Do this by drawing bold lines on the bottom surface of the Petri dish, prominent enough to be visible from the top. Drop 3 little drops of non-diluted solution into one segment. Add 1 ml of non-diluted solution to 9 ml of PBS to obtain a 1:10 dilution.

4.2. Take a small aliquot (~0.1 ml) of this newly diluted solution with a pipette and drop 3 more small drops on the next segment. Transfer the newly diluted solution to a new flask and further dilute it ten times (10x) by adding PBS. This brings down the dilution to 10^{-2} or 1:100.

4.2.1. Use this 1:100 solution in the next segment. Repeat this process with small volumes of the freshly diluted solutions by successively transferring them to new flasks and continuously diluting ten-fold (10x) in tandem with PBS to obtain more and more dilute samples from 10^{-3} or 1:1,000 all the way down to 10^{-7} or 1:10 million into the last segment.

4.3. Perform Colony-Forming Unit (CFU) plate count to count the number of cells present in the agar plate after incubating the plate for 1-2 days at 31◦C. This gives a quantitative measure of the bacterial count in the undiluted sample.
NOTE: The CFU value is measured based on the ability of the system to give rise to

colonies under the specific conditions of nutrient medium, temperature, and time assuming that every colony is separate and founded by a single bible microbial cell.

4.4. Seal the Petri dishes with self-sealing film and store remaining items in a refrigerator for future use.

(Bhaduri et al., 2016)

Section 2.7: Experimental Protocol 1 – Enzyme-Induced Calcium Carbonate Precipitation (EICP) Control Test

This initial experiment was undergone to verify the ability of the urease enzyme alone to undergo the process of EICP. Success was measured by the visual appearance of white calcium carbonate chalk forming in the experimental test tubes.

1. Plant-derived urease enzyme
    1.1. The urease enzyme used in this experiment will be in the form of a powder sourced from jack beans. They are supplied by Sigma-Aldrich and are specified as follows: Urease U1500 - UU and Urease 94280 - U9.
    1.2. These will be added to the cementation mixture (described below) at a concentration of 60 g / L
2. Chemical reagents
    2.1. The chemical reagents that will be combined with the urease powder are urea and calcium chloride. The urea provides the carbonate molecules that will be separated by the urease enzyme to then react with calcium ions from the calcium chloride to precipitate calcium carbonate.
    2.2. Previous studies have found that maximal calcium carbonate precipitation was achieved when an equimolar mixture of 2.5 mol/L of each reagent was used (Cuccurullo et al., 2022, p. 7). This experiment will use those specified concentrations.
    2.3. Another study found that the highest precipitation ratio was at a urease concentration of 60 g/L and a urea and calcium carbonate concentration of 1.0 mol/L each (Liu et al., 2023, p. 6). The below experimentation process will be completed with one round of tests at 2.5 mol/L concentration of urea and calcium chloride followed by a second round of tests using 1.0 mol/L each of urea and calcium chloride should initial results be unsatisfactory.
3. Experimentation - test tubes (Nemati and Voordouw)
    3.1. Prepare a reaction mixture containing, per liter: 12 g urea, 30 g calcium chloride ($CaCl_2 \bullet H_2O$), 4 g stabilizer (skim milk powder), and urease (U1500 - UU).
    3.2. Transfer the reaction mixture to a set of tubes maintained at 30°C.
    3.3. Monitor tubes for calcium carbonate precipitation. Terminate the experiment when the quantity of calcium carbonate precipitated does not change from 48-72 hrs.
    3.4. Assess amounts of calcium carbonate precipitated via the method described below:
        3.4.1. Centrifuge the reaction tubes at 3000 rpm for 10 min at 4°C.
        3.4.2. Remove the supernatant. Dry the tube at 70°C for 24 hrs.

3.4.3. Weigh the dried tube before and after the removal of the calcium carbonate via acid wash.

4. Experimentation - crab shells in molds
   4.1. Separate crab shell sources by grain size. The grain sizes tested will be, in order from finest to largest: powder, <0.5mm, >0.5mm, >1.0mm.
   4.2. Prepare four molds of each grain size. Two molds will be circular to represent the single inoculation cycle of tests while the two others will be square to represent the double inoculation cycle of tests.
   4.3. Mix the recommended amounts of the reagents identified above. Information on recommended amounts of solution is below.
   NOTE: The referenced experiment used 80 mL of the cementation / urease solution on a mold 50 mm in diameter. Our molds are 3 inches in diameter. To maintain the proportional relationship between the mold's circular surface area and the amount of solution used, our study would need to create 93 mL of solution for each test.
   4.4. Inoculate the first round of molds by pouring the cementation mixture (of the above amount) over the surface of the crab shell powder in the mold. Ensure an equal distribution of solution across the entire surface of the mold. Repeat this process for the second round inoculation in the square molds only.

Section 2.8: Experimental Protocol 2 – Microbially-Induced Calcium Carbonate Precipitation (MICP) Control Test

The following protocol outlines a second initial experiment to verify the ability of the bacteria *Sporosarcina pasteurii* to precipitate calcium carbonate in a controlled environment. Success was measured based on the visual appearance of white calcium carbonate chalk forming in the experimental test tubes.

1. Transfer 9 ml of the prepared culture liquid (cells + medium) into several sterilized centrifuge tubes, each of 10 ml volume.
2. Prepare a 100 ml stock solution of the external enrichment consisting of four components in the following concentrations in fresh medium: Urea: 2 g/L, Ammonium chloride: 1 g/L, Sodium bicarbonate: 212 mg/L, Calcium chloride: 280 mg/L. Carefully measure all the ingredients using an analytical balance and mix all but urea with fresh medium in a beaker and place in the autoclave (121 °C, 15 psi, 15 min).
3. Following autoclave, mix the requisite amount of urea (2 mg) with 1 ml of fresh medium and pass through a syringe fitted with 0.22 µm syringe filter to complete the process of enrichment.
4. Add 1 ml of this enrichment medium with additives to the sterilized centrifuge tubes containing 9 ml of the prepared culture liquid (cells + medium) (see step 2.1). NOTE: Of all these components, urea being degradable at elevated temperatures, cannot be sterilized in an autoclave. Hence, after the other components have been autoclaved (121 °C, 15 psi, 15 min), urea is added last through a 0.22 µm syringe filter.
5. Vortex each tube thoroughly using a mechanical vortexer. Place the enriched liquids (cells + medium + additives) in a non-shaking incubator at 30 °C. Monitor all the units regularly for

initiation of precipitation. Using a light microscope, begin microscopic observations once onset of precipitation is detected with the naked eye. This is usually between 30 - 36 hr after start of experiments.

Section 2.9: Experimental Protocol 3 – Bio-Cementation of Crustacean Shell Powder via MICP

The following protocol outlines the primary experiment of the study: creating a solid material through the bio-cementation of crustacean shell powder using MICP.

1. Bacterial biomass of Bacillus sp. VS1 was separated by centrifugation at 4 °C and 10,000 rpm for 15 min using Micro Cooling Centrifuge 5922 (Kubota, Japan)
    1.1. Prior to centrifugation, eight test tubes of bacteria-rich culturing solution were balanced to the same weight in order to prevent failure of the centrifuge due to unbalanced samples.
2. Separate the hardened pellet of bacteria cells from the supernatant liquid left over by discarding the supernatant altogether.
3. Resuspend the pellet of bacteria cells to a concentration about 1 gram of dry biomass per 10 ml of rehydration solution.
4. Store at 4 °C before the experiments.
5. Produce a series of material test samples by varying different variables of each sample's composition. Variables tested and their protocols are outlined as follows:
    5.1. Varying chemical concentrations within the MICP reaction.
        5.1.1. Mix crustacean shell powder with varying concentrations of cementation solution. Increase/decrease concentrations of calcium chloride and urea within the solutions, beginning with 29 grams/liter and 30 grams/liter respectively (these densities were previously found to lead to the precipitation of large amounts of calcium carbonate).
        5.1.2. Pour mixture into silicone molds.
        5.1.3. Mix equal amounts of rehydrated bacteria solution (at a concentration of 1 gram bacteria/ml).
        5.1.4. Place silicone molds in an incubator at 30 °C.
        5.1.5. Remove molds once favorable hardness is achieved.
    5.2. Varying bacteria amounts within the MICP reaction.
        5.2.1. Mix crustacean shell powder with the cementation solution, consisting of calcium chloride and urea at concentrations of 29 grams/liter and 30 grams/liter respectively.
        5.2.2. Mix varying amounts of rehydrated bacteria solution (at a concentration of 1 gram bacteria/ml).
        5.2.3. Place silicone molds in an incubator at 30 °C.
        5.2.4. Remove molds once favorable hardness is achieved.
    5.3. Varying application method of cementation and bacteria solutions to crustacean shell powder.

5.3.1. Application method 1: Mix crustacean shell powder with the cementation solution, consisting of calcium chloride and urea at concentrations of 29 grams/liter and 30 grams/liter respectively. Mix equal amounts of rehydrated bacteria solution (at a concentration of 1 gram bacteria/ml). Place silicone molds in an incubator at 30 °C. Remove molds once favorable hardness is achieved.

5.3.2. Application method 2: Mix crustacean shell powder with the cementation solution, consisting of calcium chloride and urea at concentrations of 29 grams/liter and 30 grams/liter respectively. Pipette equal amounts of rehydrated bacteria solution (at a concentration of 1 gram bacteria/ml) onto the surface of the crustacean shell powder and cementation solution mixture. Allow the bacteria solution to percolate into the crustacean shell powder without the aid of physically mixing the substances together. Place silicone molds in an incubator at 30 °C. Remove molds once favorable hardness is achieved.

5.3.3. Application method 3: Mix crustacean shell powder with the cementation solution, consisting of calcium chloride and urea at concentrations of 29 grams/liter and 30 grams/liter respectively. Spray equal amounts of rehydrated bacteria solution (at a concentration of 1 gram bacteria/ml) onto the surface of the crustacean shell powder and cementation solution mixture. Place silicone molds in an incubator at 30 °C. Remove molds once favorable hardness is achieved.

Chapter III: Experimentation

Section 3.1: Preparing the Experimental Samples

Please refer to Chapter II, Section 2.9: Experimental Protocol 3 – Bio-Cementation of Crustacean Shell Powder via MICP for a protocol describing the process used for the experimental phase of the research project.

The following tables detail the three rounds of experimentation conducted. Outlined are the compositions of the samples prepared.

TEST DATE	VARIABLES	ROUND 1	
03/02/24		[1]	[2]
	Shell Source (CLF or CF)	CLF	CLF
	Shell Grain Size (mm)	n/a	n/a
	Urease Source (pure urease or SP	SP	SP
	Urea Concentration	30 g/L	30 g/L
	Ca2+ Concentration	29 g/L	29 g/L
	Amt. Crab Shell (g/l)	2000 g/L	640 g/L
	Amt. Bacteria (g/l)	4.3 g/L	4.3 g/L
	Crab : Bacteria Ratio	465:1	149:1
	Application Method (injection / spray / blend / etc)	pipette	mix
	Notes	1 chip	3 chips
	Setting Temperature	30 c	30 c
	Setting Time	3 days	3 days
	Additional Setting	airdry at room temp	airdry at room temp

TEST DATE	VARIABLES	ROUND 2			
03/04/24		[1]	[2]	[3]	[4]
	Shell Source (CLF or CF)	CLF	CLF	CF	CF
	Shell Grain Size (mm)	n/a	n/a	<0.5mm	<0.5mm
	Urease Source (pure urease or *SP*)	SP	SP	SP	SP
	Urea Concentration	30 g/L	30 g/L	30 g/L	30 g/L
	Ca2+ Concentration	29 g/L	29 g/L	29 g/L	29 g/L
	Amt. Crab Shell (g/l)	1333 g/L	1333 g/L	800 g/L	800 g/L
	Amt. Bacteria (g/l)	6.8 g/L	6.8 g/L	6.8 g/L	6.8 g/L
	Crab : Bacteria Ratio	196:1	196:1	118:1	118:1
	Application Method (injection / spray / blend / etc)	mix	mix	mix	mix
	Notes	150 ml sol 200 g crab 3 chips	150 ml sol 200 g crab 3 chips	250 ml sol 200 g crab 3 chips	250 ml sol 200 g crab 3 chips
	Setting Temperature	30 c	30 c	30 c	30 c
	Setting Time	incubated for 3 days	incubated for 3 days	incubated for 3 days	incubated for 3 days
	Additional Setting	none	none	none	none

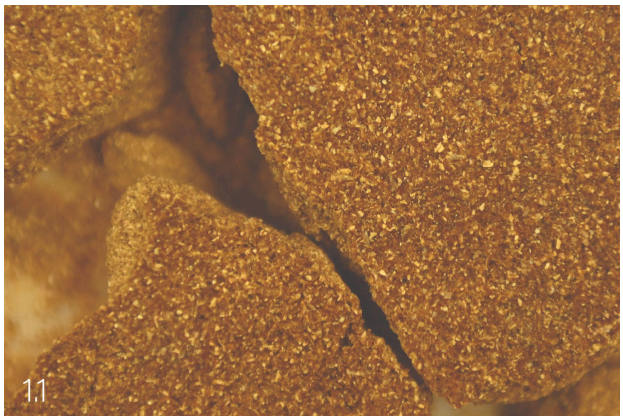
TEST DATE	VARIABLES	ROUND 3							
03/07/24		[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]
	Shell Source (CLF or CF)	CLF	CLF	CLF	CLF	CLF	CLF	CLF	CLF
	Shell Grain Size (mm)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	Urease Source (pure urease or	SP	SP	SP	SP	SP	SP	n/a	n/a
	Urea Concentration	30 g/L	30 g/L	30 g/L	30 g/L	30 g/L	30 g/L	30 g/L	30 g/L
	Ca2+ Concentration	29 g/L	29 g/L	29 g/L	29 g/L	29 g/L	29 g/L	29 g/L	29 g/L
	Amt. Crab Shell (g)	1000 g/L	1000 g/L	833 g/L	833 g/L	1333 g/L	1333 g/L	1333 g/L	1333 g/L
	Amt. Bacteria (g)	38.4 g/L	38.4 g/L	32.6 g/L	32.6 g/L	49.4 g/L	49.4 g/L	0	0
	Crab : Bacteria Ratio	26:1	26:1	26:1	26:1	27:1	27:1	0	0
	Bacteria Application Method (injection / spray / blend / etc)	mix u+Ca pip sp	mix u+Ca pip sp	mix u+Ca mix sp	mix u+Ca mix sp	pip u+Ca pip sp	pip u+Ca pip sp	control (no bacteria)	control (no bacteria)
	Notes	75 ml sol 100 g crab 3 chips	75 ml sol 100 g crab 3 chips	75 ml sol 100 g crab 3 chips	75 ml sol 100 g crab 3 chips	75 ml sol 100 g crab 3 chips	75 ml sol 100 g crab 3 chips	75 ml sol 100 g crab 3 chips	75 ml sol 100 g crab 3 chips
	Setting Temperature	30 c	30 c			30 c	30 c	30 c	30 c
	Setting Time	incubated 1wk	incubated 1wk	incubated 1wk	incubated 1wk	incubated 1wk	incubated 1wk	incubated 1wk	incubated 1wk
	Additional Setting	none	baked 200 c 24 h	none	baked 200 c 24 h	none	baked 200 c 24 h	none	baked 200 c 24 h

Section 3.2: Resulting Samples

The material samples produced during the experimentation phase all exhibited similar characteristics, differing slightly between samples due to differences in variable values. For example, the samples prepared using the fine crab shell powder were much more successfully cemented than were the samples prepared using the larger grain size of crushed crab shells (<0.5mm; samples 2.3 and 2.4). This is suspected to be due to the microscopic nature of the MICP process; with the larger grain sizes, calcium carbonate is less able to connect the pieces together.

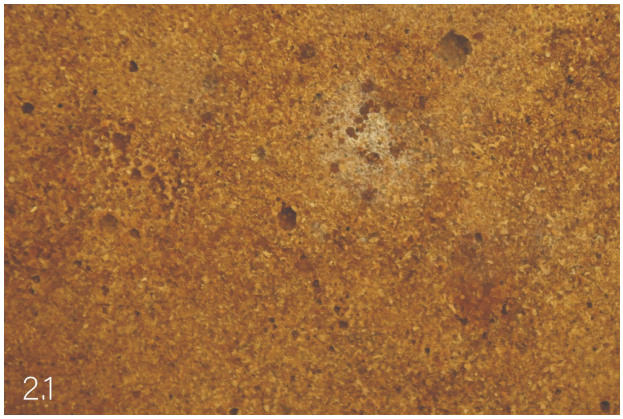
Below are close-up images depicting the textures of the 14 samples produced throughout the duration of the research project.

Sample 1.1

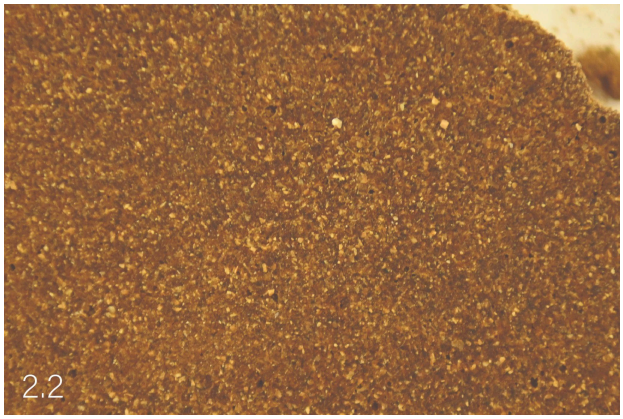


Sample 1.2

Sample 2.1



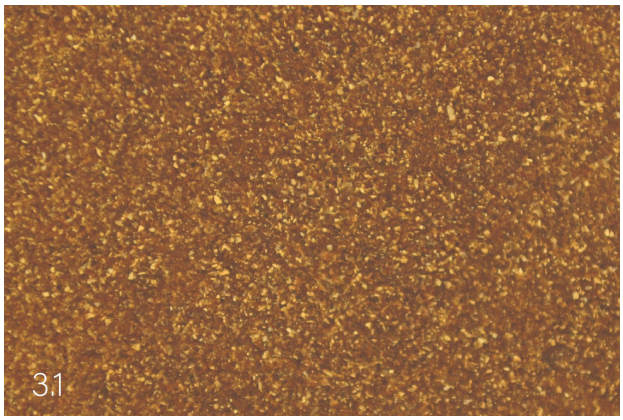
Sample 2.2



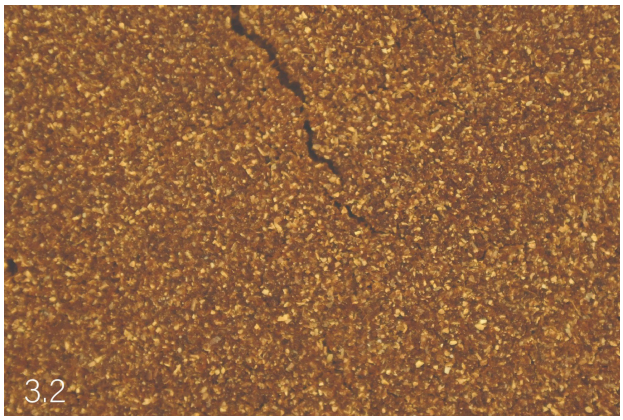
Sample 2.3

Sample 2.4

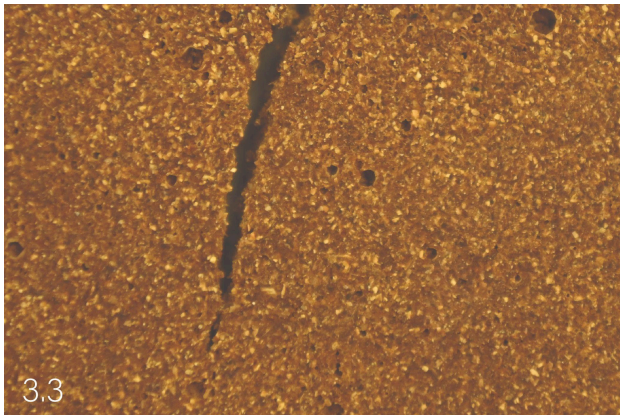
Sample 3.1



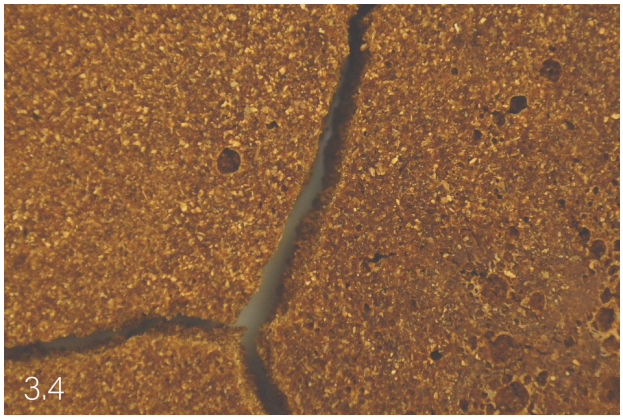
Sample 3.2



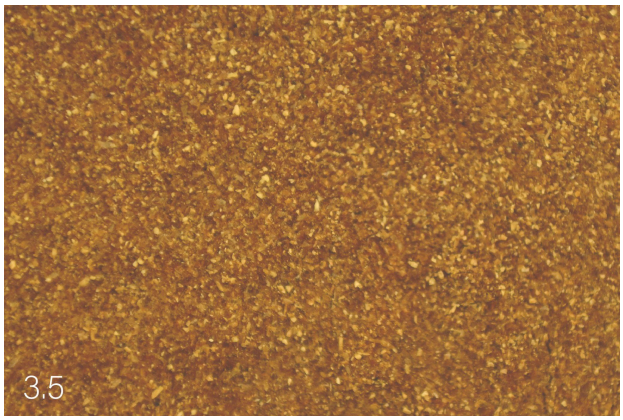
Sample 3.3



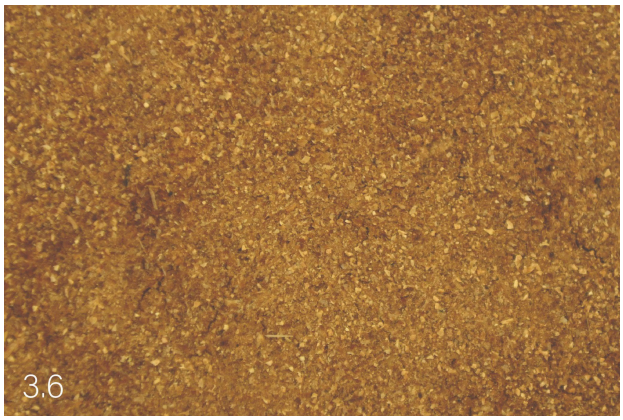
Sample 3.4



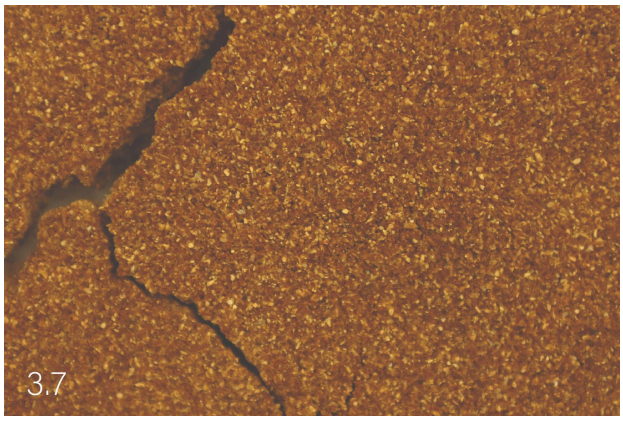
Sample 3.5



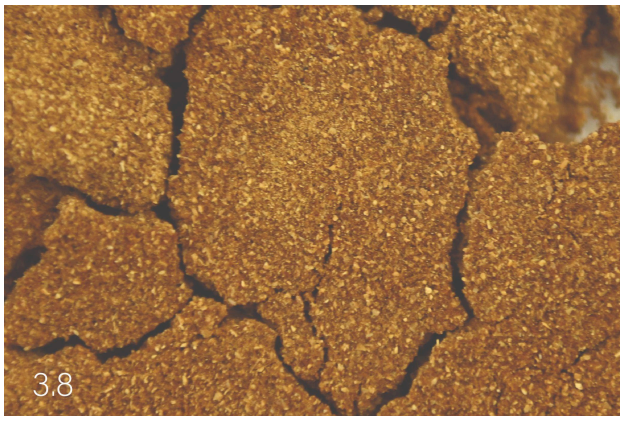
Sample 3.6



Sample 3.7



Sample 3.8



Section 3.3: Properties

The samples produced all exhibit extremely brittle and crumbly textures. The samples show that the MICP process creates layers of cementation within the crab shell flour, just as it had in desert sands

(Naeimi et al.). Additionally, the samples were all extremely lightweight compared to their relative sizes, and all samples maintained the hydrophobic qualities of the original crab shell flour. Many compositions exhibited a high propensity for cracking during incubating and drying periods.

Chapter IV: Speculation

The speculation section of the study presents two potential applications of the material and its technology as it exists in this current, initial stage of its development. The goal of the speculative applications is to take advantage of the inherent properties of the material itself while solving an issue that shows signs of worsening over the coming decades.

Section 4.1: Initial Speculations

As a first set of design speculations, the study had proposed an application in which the bio-cemented crab shell flour was molded into a facade system, either as shingles or as infill for a frame structure. Though this application had taken advantage of the hydrophilic property of the material, it had ignored its brittle nature and had attempted to impose on the material a functionality that it could not achieve.

After re-analyzing the resultant material properties, it was decided that a leap from material invention immediately to direct architectural application would not be a productive step in the material's development. Instead, the application needed to be more abstract; less literal to the built environment. Considering the samples' ability to form strong outer crusts, it was theorized that large-scale landscape interventions would make a logical first step in the direction of architectural application.

Section 4.2: Landscape Art Speculative Application

The first of the two speculative applications presented as the conclusion of this study is one of large-scale landscape art. Referencing studies exploring the ability of MICP-treated sand to withstand wind erosion (Naeimi et al.), this application proposes bio-cementing large areas of land in arid and degraded regions with the goal of 1) shaping the landscape into enjoyable art; and 2) restoring the region over a period of time.

In the face of climate change, issues of land degradation and desertification are intensifying; every year, 12 million hectares of land is lost to degradation (Ajai and Bhatnagar, p. 12). Bio-cemented crustacean shell flour has the potential to mediate the effects of this changing climate condition by enriching the soil with plant-supporting nutrients (such as potassium, magnesium, sodium, iron, and especially calcium) (Alim), and by forming a solid crust of crustacean shells that protect the soil underneath from wind erosion.

The formal composition of the proposed landscape art scheme is inspired largely by the work of Michael Heizer, an American landscape artist who often evokes the aesthetic principles of the sublime in his work (*Michael Heizer | Gagosian*). The sublime aims to evoke senses of fear, awe, and wonder in its observers. For this application, this study proposes employing those same principles in its design as a means of underscoring the scale of the waste industry from which the material of the art piece is sourced. See *Figure 4.2.1*, next page, for a rendering of the proposed design.

Figure 4.2.1 *Landscape art application rendering*

This application takes full advantage of the non-toxic, bio-enriching makeup of crustacean shells as well as the crust-forming properties of MICP. It also highlights the temporality of the material; due to its brittle nature, this study theorizes that bio-cemented crustacean shells would become subject to erosion after a long period of time. Beginning as an enriching site of art and culture and eventually becoming fertile farmland, this design has several stages of life that mimic the constant shifts and changes of the environment. See *Figure 4.2.2*, next page, for a diagram detailing this lifecycle.

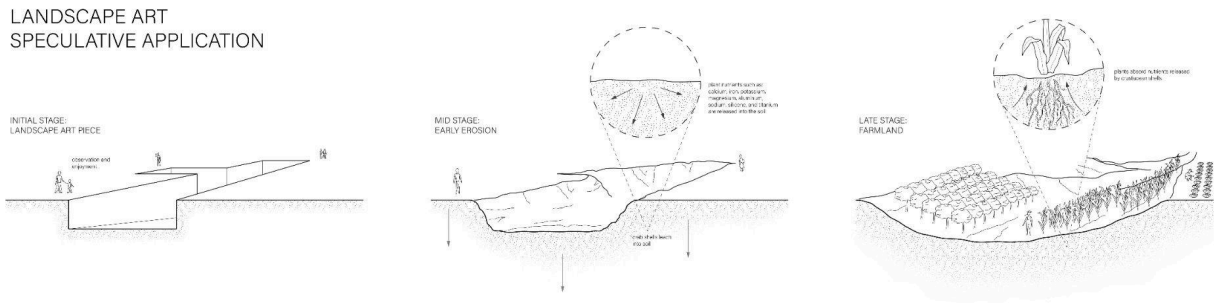


Figure 4.2.2 *Landscape art application diagram*

Section 4.3: Coastal Dune Speculative Application

The second application to which the material has been speculatively applied has been called "Coastal Dune" as it aims to mitigate erosion along coastlines caused by the shear forces of incident waves. This application draws heavily upon previous research into the ability of bio-cemented sand to resist erosion from waves (Montoya et al.). Due to climate change causing sea level rise, issues of shoreline erosion are worsening in many regions and becoming a new issue altogether in others (Chiu). Protecting these areas from erosion is vital to maintaining coastal habitats and protecting seaside communities from damage (Chiu) ("Beach Width Provides Greatest Protection Against Flooding, Erosion During Long Storms").

Currently, strategies to prevent coastline erosion are expensive and largely ineffective. Many states, such as Massachusetts, Florida, and New York spend millions to replenish beaches from coastline erosion ("Beach Width Provides Greatest Protection Against Flooding, Erosion During Long Storms"). Specific efforts have included a $500,000 project to build a protective sand dune that simply washed away days later in the first storm it had to face (Chiu). The issue is that natural sand dunes take years to progress to the point of withstanding storm surges; they require plant roots that have matured enough to give strength to the entire dune (Chiu).

This study proposes an erosion protection design that uses bio-cemented crustacean shells as the dunes' first line of defense against erosion. The design features an undulating series of mounds formed by the shells, a formal strategy that reduces wave speed and height before impact with the dune. See *Figure 4.3.1*, next page, for a rendering of the proposed design.

Figure 4.3.1 *Coastal dune application rendering*

The goal of the design is for the crustacean shells to erode before the sand beneath them. The shells would also erode far more slowly, having been treated with MICP. This method is far less intrusive than attempting to build new dunes altogether as it does not require the excavation and misplacement of large quantities of sand. Instead, the crustacean shells are a renewable resource sourced from seafood waste. Utilizing the temporality of the brittle material to its advantage, the design is able to change with its surroundings, allowing it to better meld with the environment it aims to protect. The erosion of the shells has the added advantage of releasing non-toxic compounds as opposed to alternative plans involving concrete. See *Figure 4.3.2*, next page, for a diagram describing the design.

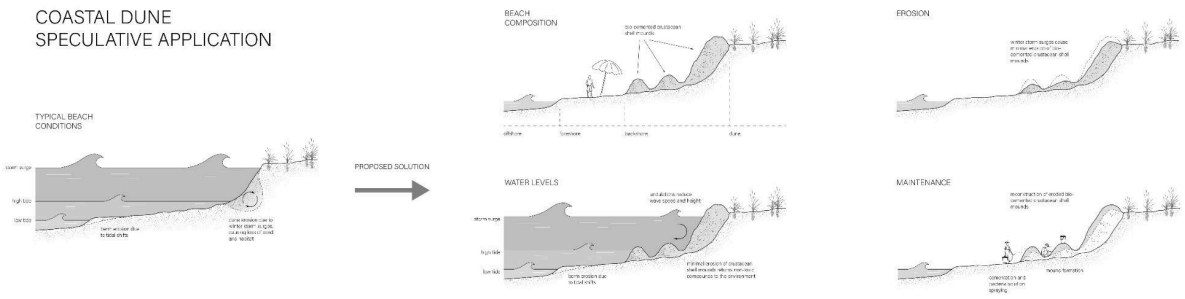


Figure 4.3.2 *Coastal dune application diagram*

Section 4.4: Next Steps

Further development of this research will include the investigation of adding aggregate materials to the composites. Potential additives could include purified chitin, as this material is already found in abundance in crustacean shells and would thus retain the original concept driving this research: to create an architecturally-useful biomaterial utilizing only compounds found in crustacean shells in conjunction with MICP. The addition of chitin to the test samples would theoretically mirror the composite structure of chitin and calcium carbonate that lend crustacean shells their lightweight strength. The samples would likely become more plastic and less brittle, broadening its speculative applications.

Continued research will also be conducted to further develop the speculative applications of the material as it exists at the conclusion of this study. The material could, for example, be used as a 3D printing medium at a multitude of scales. Research into the environmental conditions along the world's various coasts will also be conducted to further inform the design of the coastal dune application, developing it into a much more region-specific solution.

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