Living Concrete

Mummy: how can concrete be alive?

Figure 1: Combining microbial cells and cement to generate "living concrete". Microbial communities are placed into the concrete to enhance its properties

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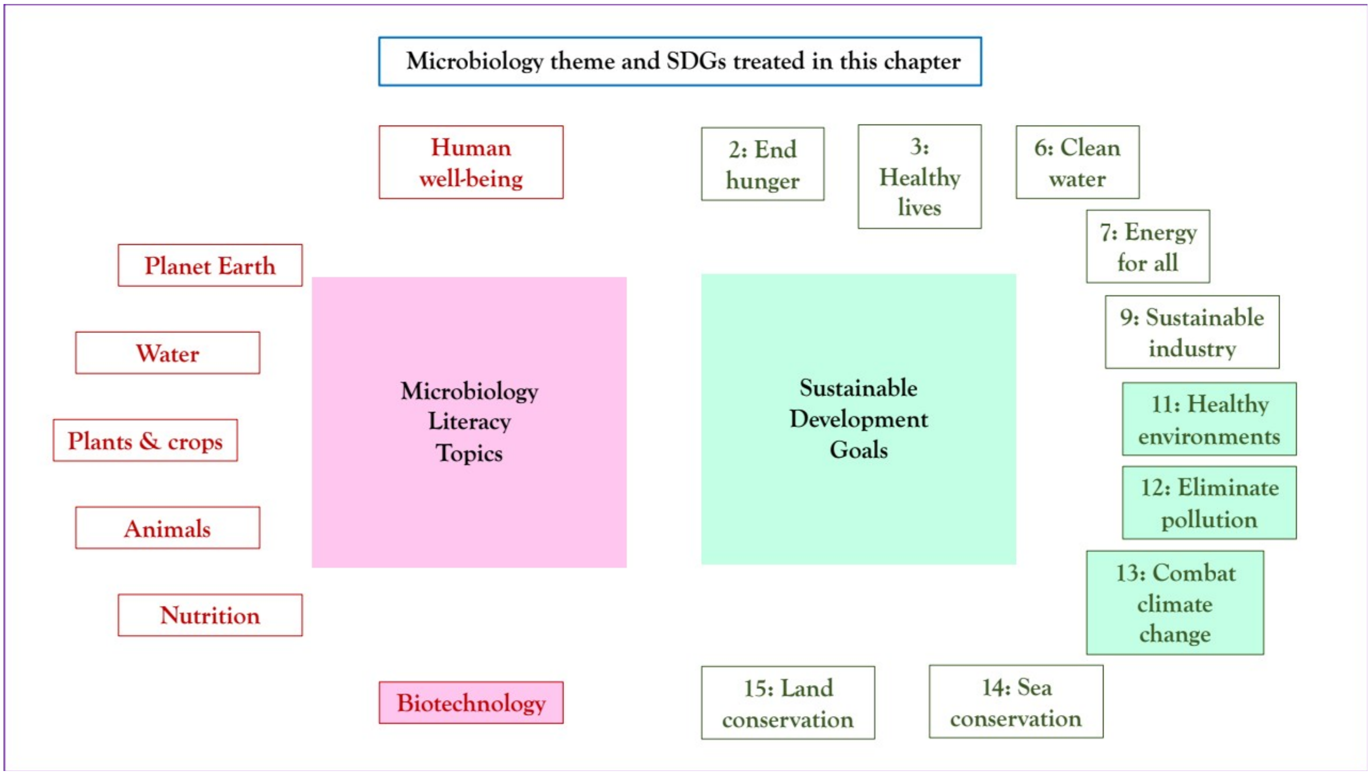
Storyline

Concrete is essential for the construction of the human built environment and is used for most if not all civil buildings for health care, industry, education and transport. Modern life is not feasible without concrete. However, as with many man-made materials, the production of concrete comes with a high environmental cost. The production of concrete is responsible for a substantial 4-8% of all **carbon dioxide** (CO_2) released in the world, increasing the greenhouse effect and subsequent climate change. Modern architecture and microbiology can join forces to develop sustainable construction approaches. Here, we discuss the potential use of bacteria in designing "self-healing" materials for construction with a versatility of morphology and function. We also discuss how these recent discoveries may change current strategies of construction, and speculate that biofilm communities and stone microbiome may be used to construct environmentally friendly buildings with "self-healing" capacities.

The Microbiology and Societal context

The microbiology: biocement; biomineralization; spores and sporulation; extracellular matrix; biofilm; bioprotection of buildings and stone works of art; pollution; smart materials; carbon dioxide emission and greenhouse gas production from concrete; microbially-based substitutes. And *peripherally for completeness of the storyline*: stone microbiome, novel materials design, microbial spores. *Sustainability issues:* health; economy and employment; environmental pollution; global warming.



Living Concrete: the Microbiology

1. Concrete and cement are essential for modern construction. Concrete is an essential material for human civilization. Concrete is the bulk material used in virtually all of our critical building and infrastructure and, literally, allows us to put a roof over our heads. Therefore, modern life is not possible without concrete. Concrete is a complex material composed of fine and coarse aggregate – sand, gravel, crushed stone— bonded together with a cement paste that hardens over time. In general, concrete is composed of calcium carbonate. This mineral is a salt of calcium, an alkaline earth reactive metal, and carbonate. Crystalline forms of calcium carbonate are also often used as a structural material in complex organisms. For example, mammalian bones and teeth are composed of crystalline calcium phosphate, while corals and star-fish structures are based on crystalline calcium carbonate exoskeletons.

Cement reacts with the water and other ingredients to form a hard matrix that binds aggregate materials together into a durable stone-like material that has many uses. Cement is favored due to its high endurance, lower cost, and ease of production, durability and flexibility to be cast into to various geometric shapes. Concrete usually has high compressive strength and, when combined with steel, the composite material also has high tensile strength and can be used to build megastructures from skyscrapers to dams and bridges.

2. Concrete and cement are damaging for the environment and cause significant emission of greenhouse gasses. However, as is the case with most manufacturing technologies, production of concrete comes with an environmental cost. Concrete is responsible for 4-8% of all **carbon dioxide** release in the world, taking into account all stages of production, while the construction industry as a whole may account for 18%.

Carbon dioxide (CO_2) is an important trace gas in the earth's atmosphere, currently constituting about 0.04% (400 parts per million) of the atmosphere. CO_2 is a potent player in the greenhouse effect, which causes global warming. The present concentration of atmospheric CO_2 is likely to be the highest of the past 20 million years. Because of the increased accumulation of CO_2, there has been a rapid growth of near-surface temperatures. Most of the additional energy stored in the climate system since 1970 has gone into ocean warming which has, in turn, melted ice, and warmed the continents and atmosphere. Many of the observed changes since the 1950s are unprecedented. As CO_2 accumulates in the atmosphere, it also dissolves in oceans, rivers and lakes, which contributes to ocean acidification.

3. Concrete deteriorates over time. Once it has been constructed, concrete is considered a resilient material. However, known problems such as cracking or flaking can occur. Reinforced concrete damage causes the steel reinforcement to become exposed and leads to water corroding the reinforcements and causing permanent and unrepairable damage.

4. Microorganisms that can improve the performance of concrete. Bacteria are single-celled microorganisms, often capable of fast reproduction. Diverse species inhabit a vast array of different environments. The capacity of microbes to generate fast-growing biomass is exploited in a range of industrial and environmental settings.

Bacteria can be used as bio-friendly additives to improve cement and concrete performances, and a cement enhanced with microorganisms or their products is often referred to as **biocement**. The concept of using bacteria to improve the performance of concrete is well established. Concrete is composed of $CaCO_3$, and bacteria can naturally produce this mineral in response to environmental stimuli. The application of bacteria to concrete to create biocements or self-healing materials has a number of advantages over more traditional approaches. For example, concrete with spores (bacterial seeds) mixed into the aggregate has been shown to significantly increase the life span of concrete materials by triggering a process of biomineralization when the spores are exposed to water. The resulting bacterial growth triggers the formation of calcium carbonate and reseals the cracks stopping further water penetration, protecting steel reinforcements and, therefore, improving the structural performance and longevity of the material. In addition, the process of biomineralization consumes carbon dioxide and requires no energy input.

5. Microbial production of carbonate for construction. Bacterial biomineralization of carbonate is a well-established phenomenon. While calcium is available from the environment, bicarbonate is actively produced by CO_2 hydration ($CO_2 + H_2O$ $\boxed{\leftrightarrow}$ $HCO_3 + H^+$$CO2 + H2O \leftrightarrow HCO3^+ + H^+$), where the source of CO_2 can be a byproduct of bacterial metabolism or is available in the immediate environment. Bacterially-induced calcium carbonate precipitation has been proposed as an environmentally-friendly method of material synthesis for various applications in construction (Figure 2).

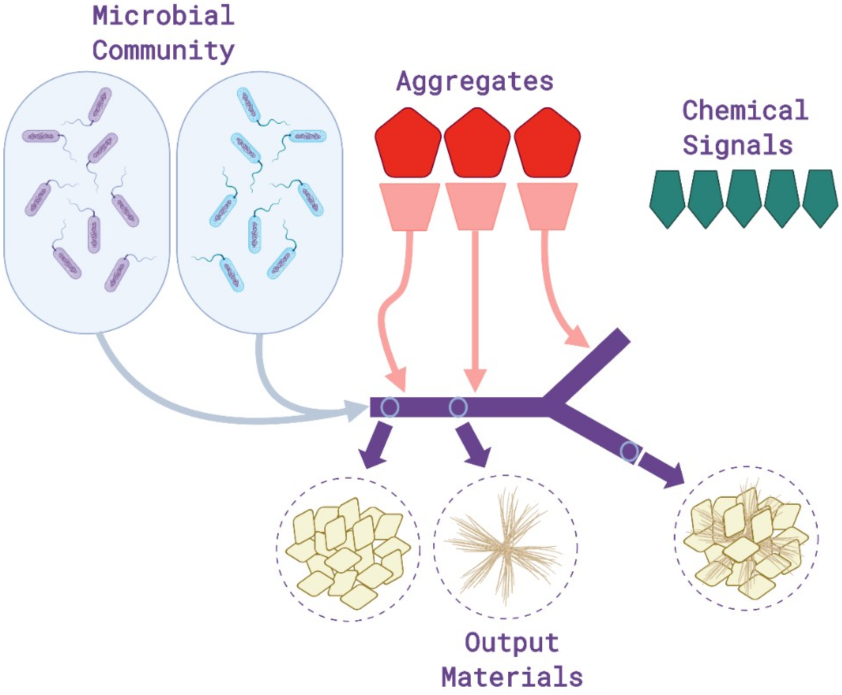


Figure 2: A framework for using microbial communities for construction. The microbial community is mixed with different aggregated substances that will affect the final shape of the microbial minerals. These communities can be enhanced with chemical stimuli to improve their communal performance and calcium carbonate production. The final cementous material is environmentally friendly, and can be designed in multiple shapes and with versatile properties. The graphic was made with BioRender software.

Applications of bacteria in construction include:

a. *Strengthening soil and sand materials for ground improvements*. Ground improvement techniques are used when the underlying soil does not meet required criteria. Such criteria include adequate bearing capacity for building foundations, sufficient stability of slopes, and adequate drainage capacity/ soil permeability for buildings, roads and other settlement foundations.

b. *Rescuing of buildings of historical interest.* Increasing environmental pollution in many areas can endanger the survival of carbonate stone of historic importance through, for example, acid rain that tends to dissolve the surface layers of the stone material. Microbial cultures can be applied to these surfaces in order to protect the outer layers and give them self-healing capabilities.

c. *Improvement of the durability of cementitious materials.* Microbial products and activities were shown to improve the resilience of cement to environmental stress.

6. Using microbes for "self-healing" concrete. Microbial cultures can be used for generation of "self-healing concrete" to repair cracks in concrete. In a recent application, *Bacillus megaterium* spores and suitable dried nutrients were mixed and applied to steel-reinforced concrete. When the concrete cracks, water ingress dissolves the nutrients and the bacteria germinate, triggering calcium carbonate precipitation, resealing the crack and protecting the steel reinforcement from corrosion.

Microbial cells are often used for technological innovation that seeks to minimize the problem of cracks in concrete structures. The incorporation of bacteria in the concrete matrix has been used with the ultimate goal of closing pores and cracks. Thus, the bacteria are considered a self-healing agent of concrete cracks, because they have the ability to precipitate minerals that close the cracks autonomously. In recent times, a basic principle of applying microbial $CaCO_3$-production to create self-healing concrete is that bacteria and other relevant agents are added into the concrete matrix during casting has become widely accepted.

Figure 2 illustrates this process. Microbial spores, a dormant cell type that can survive extreme conditions, or vegetative cells, are placed in microcapsules (represented in light blue). The microcapsules are then mixed with mortar – a building material composed of cement mixed with fine sand, water, and lime to improve the durability of the product. The desired material shape and performance can be controlled by adding aggregated substances that affect the growth of microbial $CaCO_3$. Microbial activities can be enhanced by adding chemical stimuli that induce microbial mineralization (Figure 2). The microcapsules break under a force representing concrete cracking, and the embedded bacteria self-heal the cracks with versatile self-produced minerals.

7. Bacteria can survive in cement by generating spores. The pH of the concrete is highly alkaline, has no nutrients, and oxygen is only available in limited amounts through open pores and on the surface. These factors will restrict bacterial growth in the core of the material. The use of spore-forming bacteria can overcome this challenge and generate "self-healing" smart material. Spore-forming bacteria have distinct elements of their lifecycle that includes three different phases: vegetative growth, sporulation and germination (Figure 3).

Vegetative growth occurs when environmental conditions are favorable, and nutrients are available. Stressful conditions, particularly nutrient deprivation, induce spore-forming bacteria to initiate the sporulation process. Spores can remain dormant for extended periods (researchers have argued for up to millions of years) and possess a remarkable resistance to damage by extreme environmental conditions, such as heat, radiation, toxic chemicals and pH extremes. Under favorable environmental conditions, the spore initiates a process called spore germination and outgrowth, creating a normal cell that will begin to grow and reproduce.

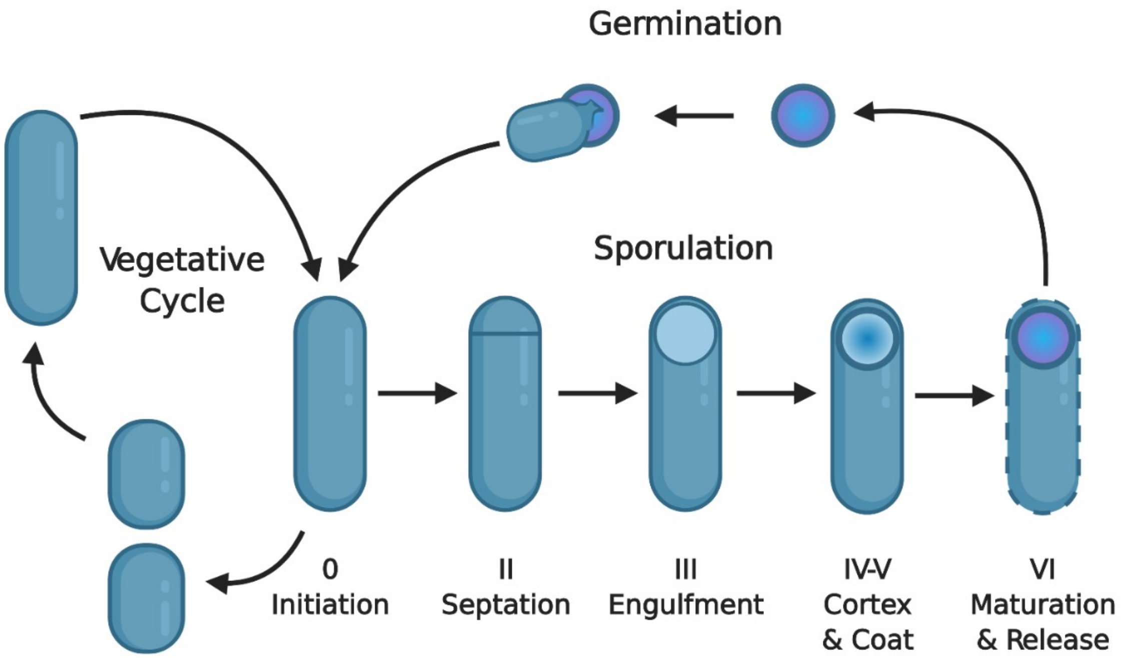


Figure 3: The formation and revival of dormant seed cells (spores). The graphic was made with BioRender software.

*8. **Enriching concrete with bacteria.*** In general, two approaches can induce microbial mineralization: biostimulation and bioaugmentation. In the case of biostimulation, the natural indigenous microbes (e.g. stone or cement microbiome) are stimulated or induced for growth by the addition of specific nutrients and carbon sources. In the case of bioaugmentation, the system is supplemented with exogenous bacteria. The potential of foreign cultures to survive and work effectively in a new environment is challenging due to competition from native communities residing in the habitat. However, the use of spore forming bacteria can overcome harsh conditions. The challenges of introducing beneficial bacteria into concrete are in fact very similar to the challenges of introducing beneficial bacteria (probiotic) into our microbiomes.

*9. **Designing new cementitious materials.*** In recent years, man-made technologies are going beyond simply improving concrete-like materials. In addition to improving the properties of traditional concrete, microbial cells may help to create entirely new materials: a new generation of intelligently designed microbial minerals.

Briefly, microbial minerals can be controlled by manipulating the environment or genetics of bacteria affecting: (a) microbial community composition, dynamics and metabolism, (b) calcium carbonate precipitation efficacy, (c) the shape of crystals and related functional properties of the final biomineralization product. Frequently, the sophistication required to control microbial activities relies on a life-style choice of residing in biofilms or, when associated with a host, microbiomes (multicellular communities composed of different cell types of the same specie or multi-species). Individual species of bacteria do not usually live alone but in complex microbial communities surrounded by materials known as extracellular matrix (ECM), which helps them attach to surfaces of inert (e.g., rocks, glass, plastic) or organic (e.g., skin, cuticle, mucosa) materials. ECMs are composed of different organic substances, such as extracellular proteins, exopolysaccharides, and nucleic acids.

In the context of ECMs, the growth of calcium carbonate crystals occurs in layers. The interaction of crystal growth with the environment can affect crystal shape and morphology, and this in turn may be affected by the biogenic (organic) environment, and by organic polymeric substances. For many biofilm-producing bacteria, the identity of the exopolymeric substances and the genes that encode them are well understood. It is known, for example, that ECM absorbs Ca^{2+} and promotes calcium carbonate formation by providing nucleation sites for new crystals.

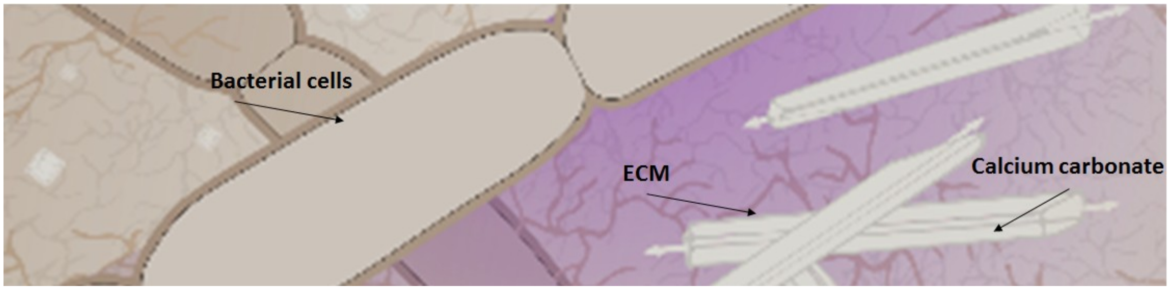


Figure 4: The growth of calcium carbonate on microbially-produced ECM template

For example, in *Bacillus subtilis*, a known promoter of biomineralization, the different crystal morphologies of the final product were found to be dependent on secreted fibers, secreted protein adhesins, and secreted exopolysaccharides in the ECM (Figure 4).

As the capacity of self-produced polymers to shape the minerals has been established, research is now targeted to identify the influence of the species composition of microbial communities on crystal growth. In principle, the understanding these natural mechanisms of crystal growth and control could lead to new techniques for mineral material production, using engineered microbial communities. For example, different soil *microbiome members* all have putative carbonic anhydrases and urease in their genomes, enzymes that were shown to participate in the formation of carbonate for mineralization, but vary in their ECM genes. The presence of calcium, carbon, and nitrogen sources in the environment can also affect biomineralization efficiency. In addition, using synthetic biology (a process where the genetics of the bacteria is altered to create new functions), microbes that can sense changes in their environment (such as mechanical load) and make materials where necessary (Figure 2).

10. Correlating the microbial activities from biocement with CO_2 atmospheric levels. In microbial communities, different subpopulations of specialized cells coexist and show spatial and temporal organization within biofilms. Monitoring performance of bacteria on concrete in a controlled atmosphere and directly testing the impact of atmospheric CO_2 on biomineralization, together with monitoring the structure and function of the cement-associated community, may allow us to design new and useful microbial communities for construction that absorb carbon dioxide.

Relevance for Sustainable Development Goals and Grand Challenges

- **Goal 11: Sustainable Cities and Communities** As the world population increases, there is an increased need for living space. Increasing the number of houses can result in the destruction of forests and other habitats, impact natural resources, such as soil, and disrupt soil, water and air ecosystems. Constructing with ecologically friendly biocement embedded with bacteria can reduce the damage to the soil ecosystems by introducing beneficial soil and stone microbiota in the foundations of buildings, reduce the damages of buildings decay, and minimize the production of greenhouse effects.

- **Goal 12. Ensure sustainable consumption and production patterns.** Concrete production creates carbon dioxide emissions, and therefore modern construction is in conflict with sustainable consumption and production. The use of biocement can modernize the construction industry by making our materials resilient and longer lasting or creating materials in a carbon neutral or carbon negative way.

- **Goal 13. Take urgent action to combat climate change and its impacts.** Applying bacteria to the development of eco-friendly building materials and bio-cements will result in carbon dioxide sequestration in the form of a functional mineral. Thus, incorporating bacterial biofilms that form calcium carbonate into our construction materials may provide a sustainable solution for the critical issue of global warming.

Potential Implications for Decisions

National policies relating to bioconstruction:

a. Environmental pollution

b. Greenhouse gas production and global warming. Constructors should be financially supported to construct with eco-friendly biomaterials.

c. Non-microbial parameters: policies relating to construction: reducing construction costs for sustainable housing

Pupil Participation

1. Class discussion of the issues associated with construction, concrete and bacteria

2. Pupil stakeholder awareness

a. Using microbial cultures in construction has positive consequences for the SDGs. Which of these are most important to you personally/as a class?

b. Can you think of anything that might be done to reduce the negative consequences of modern construction on the earths' climate and soil?

c. Can you think of anything you might personally do to reduce the environmental footprint of constructing building?

3. Exercises (could be made at any level, but these are probably secondary education level)

a. Construction with concrete is a sign of modern cities. What sustainable options are for construction?

b. What ideal concrete you would design for construction?

c. Looking at the SDGs, how can we change our approach to construction strategies to generate environmentally friendly cities?

4. Class experiments (select appropriate experiment from the Class Experiment list)

a. Inducing the formation of "microbial stones" e.g: calcium minerals.

- Prepare biomineralization plates from simple commercial materials: (Agar 1.5%, Glucose 0.5%, Yeast Extract 0.4%). Sterilize the materials by autoclaving. Pour the sterilized solution into Petri dishes. Dry on room temperature overnight.
- Inoculate non-hazardous *Bacillus subtilis* in rich growth media in 15 ml Eppendorf tubes (http://2014.igem.org/wiki/images/6/63/WPI_LB_Liquid_and_Agar.pdf) overnight until the medium is turbid.
- Place a drop of bacterial culture on the plates using Pasteur pippete.
- Incubate the plates for 10 days in 37^{o}C incubator
- Observe microbial "stones" emerge (Figure 5).
- If safety regulations are compatible, the "stones" can be washed with commercial bleach by inoculating the microbial culture into bleach for 10 minutes, removing the bleach using a pipette, and washing the remaining material with acetone, to demonstrate that they are as resilient as "regular" stones.

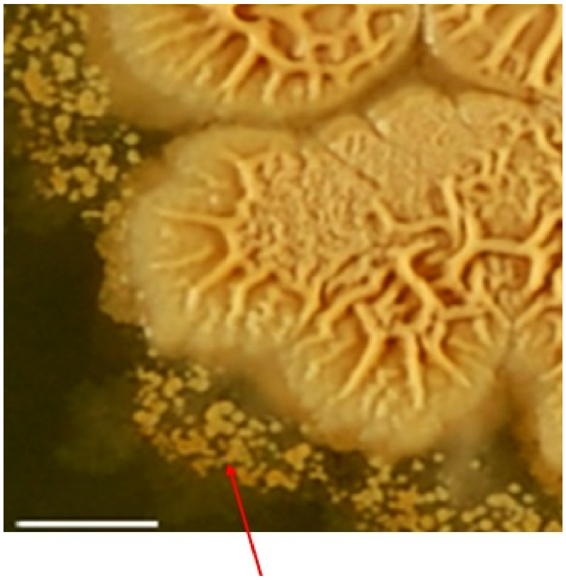


Figure 5: The growth of calcium carbonate stones (red arrow) induced by microbial colonies on biomineralization medium. Scale bar corresponds to 1 mm.

b. Isolating microorganisms from sand, stone and concrete. What is the microbiome of these construction materials?

- Prepare laboratory plates of LB (http://2014.igem.org/wiki/images/6/63/WPI_LB_Liquid_and_Agar.pdf)
- Use a sterile toothpick. Gently touch the surface in mind: concrete, stone, or sand.
- Spread the (invisible) content of the toothpick on the plate
- Incubate the plates for 2-5 days in 37^{o}C incubator
- Examine the plates

c. Can inoculated microbes survive in soils? Bacteria will be grown in the lab, added to sterile stone/sand and extracted for plating.

- Grow nonhazardous bacteria as in A. Mix them with previously sterilized sand/soil.
- Incubate for 24 hours

- Use the toothpick method (b) to test whether the bacteria are still viable.

The Evidence Base, Further Reading and Teaching Aids

https://www.thespruce.com/difference-between-cement-concrete-and-mortar-2130884

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Worland, J. (2016). Why Your Office Is the Cause Of—and the Solution to—Climate Change https://time.com/4311258/climate-change-energy-efficient-buildings/. Time Magazine.

Glossary

Cement: The binding material used in building and civil engineering construction. Cements of this kind are finely ground powders that, when mixed with water, set to a hard mass. When formed by living organisms, it is referred to as **Biocement.**

Concrete: The structural component of buildings in civil construction: A composite material composed of fine and coarse aggregate bonded together with a fluid cement (cement paste) that hardens over time.

Greenhouse gas: A gas that absorbs and emit infrared radiation in the wavelength range emitted by Earth. Carbon dioxide (0.04%), nitrous oxide, methane and ozone are gases that account for almost one tenth of 1% of Earth's atmosphere and have an appreciable greenhouse effect (the process by which radiation from a planet's atmosphere warms the planet's surface)

Biofilms: Microbial communities formed by different species, or a single specie, that are held together with self-produced polymers

Microbiomes: A community of microorganisms (such as bacteria, fungi, and viruses) that inhabit a particular environment (in here, stone). Often refers to the collection of microorganisms living in or on the human body

Spore: A unit of sexual or asexual reproduction that may be adapted for dispersal and for survival, often for extended periods of time, in unfavourable conditions. Bacterial spores are not part of a sexual cycle but are resistant structures used for survival under unfavourable conditions.

pH: A measure of acidity or alkalinity. Alkalinity is considred to promote biomineralization

Extracellular matrix (ECM): A three-dimensional network of extracellular macromolecules, such as polysaccharides and proteins, that provide structural and biochemical support to surrounding cells. Self-produced polymers which are secreted by single cell bacteria to generate functional multicellular communites.

Exopolymeric substance: molecules released by microorganisms in response to the physiological stress encountered in the natural environment. Exopolymeric substances are structural components of the extracellular matrix in which cells are embedded during biofilm development.

Calcium carbonate: Calcium carbonate is a chemical compound with the formula $CaCO_3$. When it forms crystals, they are designated calcite, varterite or argnoite. It is a common substance found in rocks (most notably as limestone, which is a type of sedimentary rock consisting mainly of calcite). Calcium carbonate is the main component of pearls and the shells of marine organisms (corals and starfishes), snails, and eggs.

Crystal/Crystalline: A solid material whose constituents (such as atoms, molecules, or ions) are arranged in a highly ordered microscopic structure.When a chemical is generating a crystal, it is referred to as crystalline.

Gene: A segment of DNA that codes for production of a given protein. DNA is a collection of chemical information that carries the instructions for making all the proteins a cell will ever need.