Advances in Biomineralized Building Materials

Living
Materials
Laboratory

Wil V. Srubar III, PhD, Associate Professor

Department of Civil, Environmental, and Architectural Engineering
Materials Science and Engineering Program
University of Colorado Boulder

My group engineers low-carbon, biomineralized, living materials for the built environment.

Living Materials Laboratory

Question Convention

Carbonate Mineralization

1 kg $CaCO_3$ = -0.44 kg CO_2

There are multiple mechanisms of microbial biomineralization*.

Urea Hydrolysis

$$CO(NH_2)_2 + 2H_2O \xrightarrow{\text{Urease enzyme}} H_2CO_3 + 2NH_3,$$

$$H_2CO_3 \leftrightarrow HCO_3^- + H^+,$$

$$2NH_3 + 2H_2O \leftrightarrow 2NH_4^+ + 2OH^-,$$

$$HCO_3^- + OH^- \leftrightarrow CO_3^{2-} + H_2O,$$

$$Ca^{2+} + CO_3^{2-} \rightarrow \underline{CaCO_3}.$$

Photosynthesis

$$CO_2 + H_2O \xrightarrow{\text{CA enzyme}} H_2CO_3,$$

$$H_2CO_3 \leftrightarrow HCO_3^- + H^+,$$

$$Ca^{2+} + HCO_3^- \rightarrow \underline{CaCO_3} + H^+.$$

Sulfate Reduction

$$CaSO_4 \cdot 2H_2O \rightarrow Ca^{2+} + SO_4^{2-} + 2H_2O,$$

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^- + CO_2 + H_2O,$$

$$Ca^{2+} + HCO_3^- \rightarrow \underline{CaCO_3} + H^+,$$

or

$$CaSO_4 + 2CH_2O \rightarrow CaS + 2CO_2 + 2H_2O,$$

$$CaS + 2H_2O \rightarrow Ca(OH)_2 + H_2S,$$

$$CO_2 + H_2O \rightarrow H_2CO_3,$$

$$Ca(OH)_2 + H_2CO_3 \rightarrow \underline{CaCO_3} + 2H_2O.$$

Denitrification

$$4NO_3^- + 5CH_2O \xrightarrow{\text{Denitrification}} 2N_2 + 5CO_2 + 3H_2O + 4OH^-,$$

$$Ca^{2+} + CO_2 + 2OH^- \rightarrow \underline{CaCO_3} + H_2O.$$

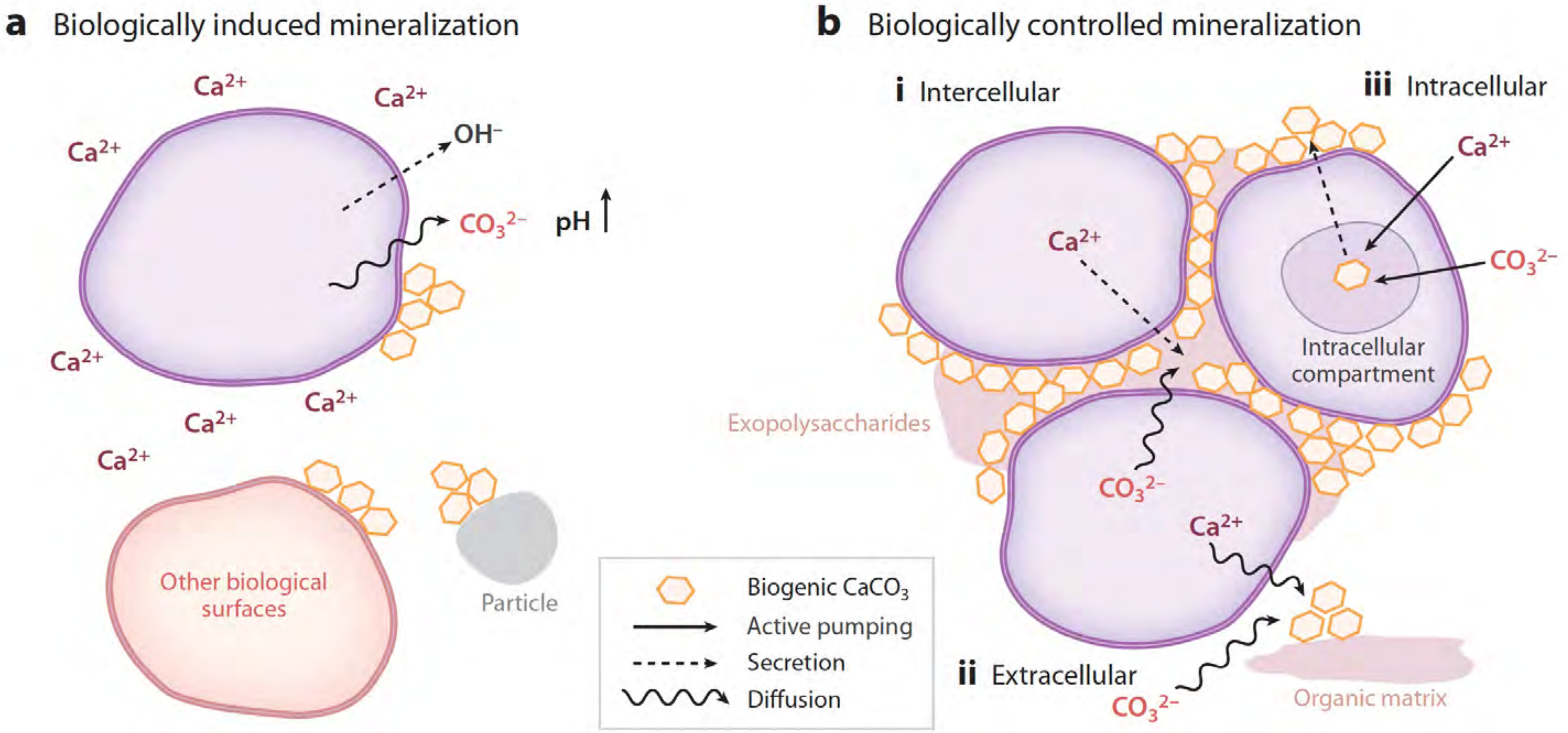
Organic Compound Conversion

$$CaC_6H_{10}O_6 + 6O_2 \rightarrow \underline{CaCO_3} + 5CO_2 + 5H_2O.$$

*Calcium carbonate precipitation; mechanisms for biomineralization of other minerals (e.g., SiO_2) also exist.

Beatty, Williams, and Srubar (2022). *In press.*

Biomineralization can be biologically induced or biologically controlled.



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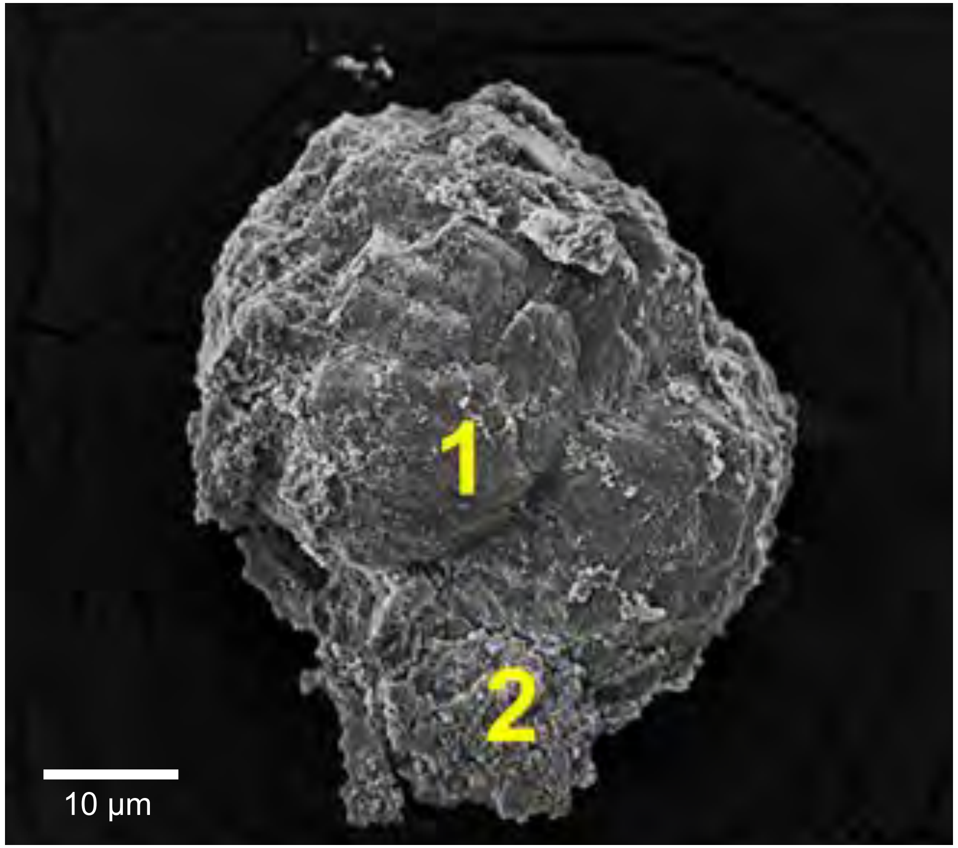
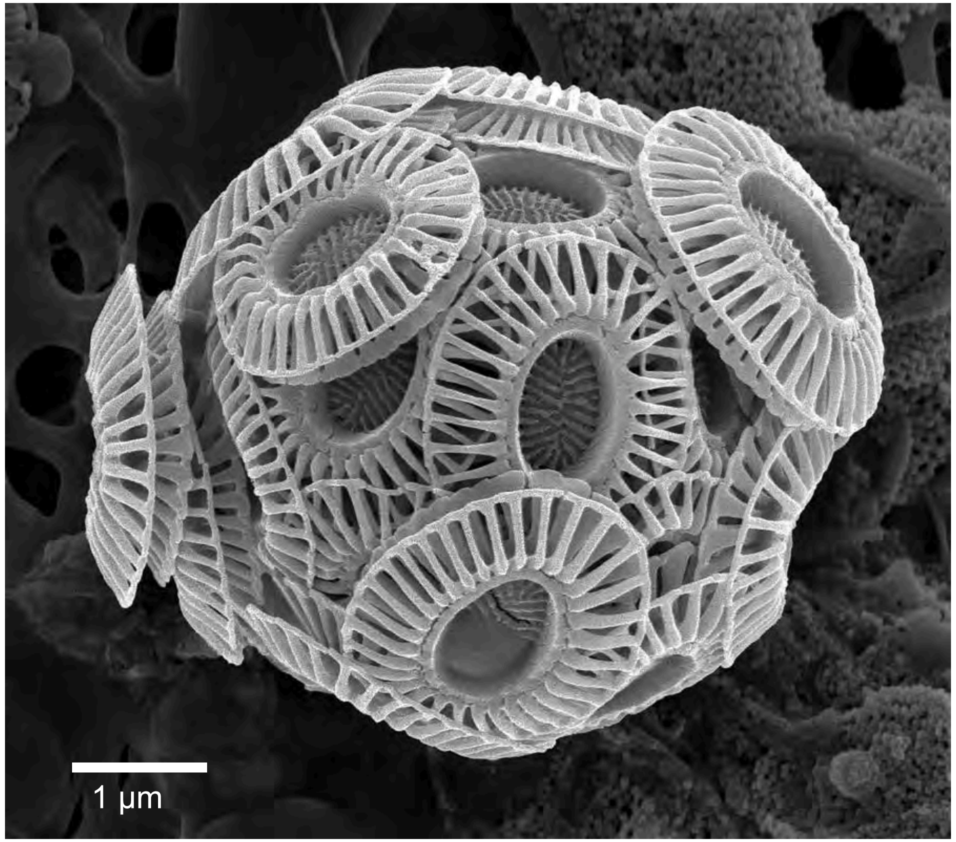
Biologically Induced $CaCO_3$ Precipitation // Urea Hydrolysis & Photosynthesis



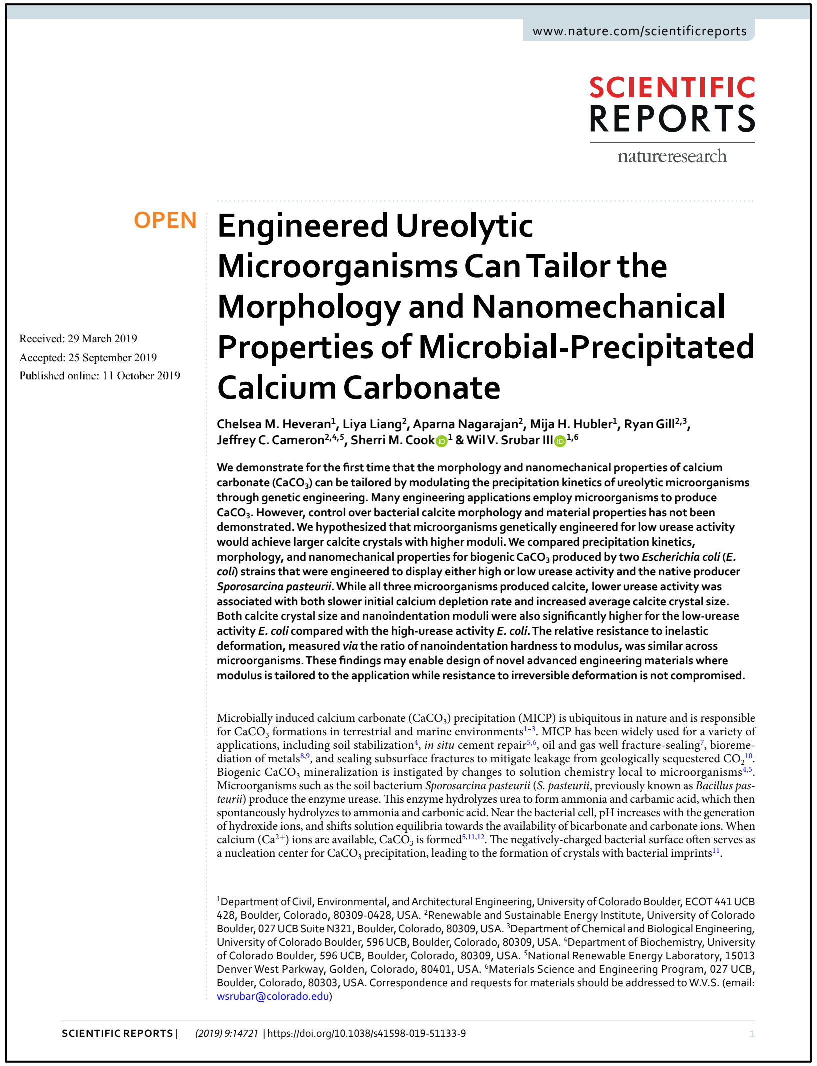
Heveran, et al. (2019).

Biologically Controlled $CaCO_3$ Precipitation // Photosynthesis



SEM Photograph by S. Gschmeissner

Microbial metabolisms can affect crystal structure and properties.



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Engineered Ureolytic Microorganisms Can Tailor the Morphology and Nanomechanical Properties of Microbial-Precipitated Calcium Carbonate

Received: 29 March 2019
Accepted: 25 September 2019

Chelsea M. Heveran[1], Liya Liang[2], Aparna Nagarajan[2], Mija H. Hubler[1], Ryan Gill[2,3], Jeffrey C. Cameron[2,4,5], Sherri M. Cook[1] & Wil V. Srubar III[1,6]

We demonstrate for the first time that the morphology and nanomechanical properties of calcium carbonate ($CaCO_3$) can be tailored by modulating the precipitation kinetics of ureolytic microorganisms through genetic engineering. Many engineering applications employ microorganisms to produce $CaCO_3$. However, control over bacterial calcite morphology and material properties has not been demonstrated. We hypothesized that microorganisms genetically engineered for low urease activity would achieve larger calcite crystals with higher moduli. We compared precipitation kinetics, morphology, and nanomechanical properties for biogenic $CaCO_3$ produced by two *Escherichia coli* (*E. coli*) strains that were engineered to display either high or low urease activity and the native producer *Sporosarcina pasteurii*. While all three microorganisms produced calcite, lower urease activity was associated with both slower initial calcium depletion rate and increased average calcite crystal size. Both calcite crystal size and nanoindentation moduli were also significantly higher for the low-urease activity *E. coli* compared with the high-urease activity *E. coli*. The relative resistance to inelastic deformation, measured *via* the ratio of nanoindentation hardness to modulus, was similar across microorganisms. These findings may enable design of novel advanced engineering materials where modulus is tailored to the application while resistance to irreversible deformation is not compromised.

Microbially induced calcium carbonate ($CaCO_3$) precipitation (MICP) is ubiquitous in nature and is responsible for $CaCO_3$ formations in terrestrial and marine environments[1–3]. MICP has been widely used for a variety of applications, including soil stabilization[4], *in situ* cement repair[5,6], oil and gas well fracture-sealing[7], bioremediation of metals[8,9], and sealing subsurface fractures to mitigate leakage from geologically sequestered CO_2[10]. Biogenic $CaCO_3$ mineralization is instigated by changes to solution chemistry local to microorganisms[4,5]. Microorganisms such as the soil bacterium *Sporosarcina pasteurii* (*S. pasteurii*, previously known as *Bacillus pasteurii*) produce the enzyme urease. This enzyme hydrolyzes urea to form ammonia and carbamic acid, which then spontaneously hydrolyzes to ammonia and carbonic acid. Near the bacterial cell, pH increases with the generation of hydroxide ions, and shifts solution equilibria towards the availability of bicarbonate and carbonate ions. When calcium (Ca^{2+}) ions are available, $CaCO_3$ is formed[5,11,12]. The negatively-charged bacterial surface often serves as a nucleation center for $CaCO_3$ precipitation, leading to the formation of crystals with bacterial imprints[11].

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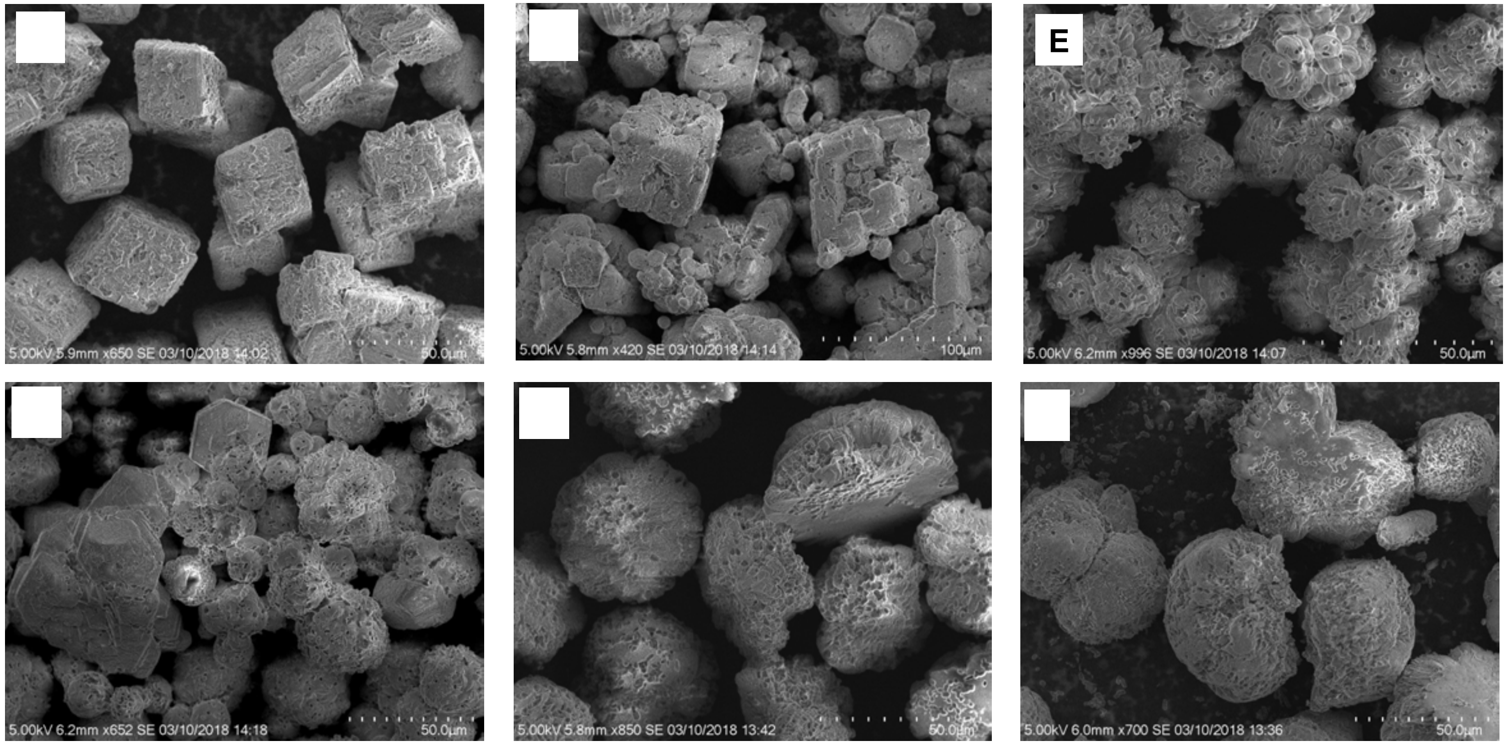
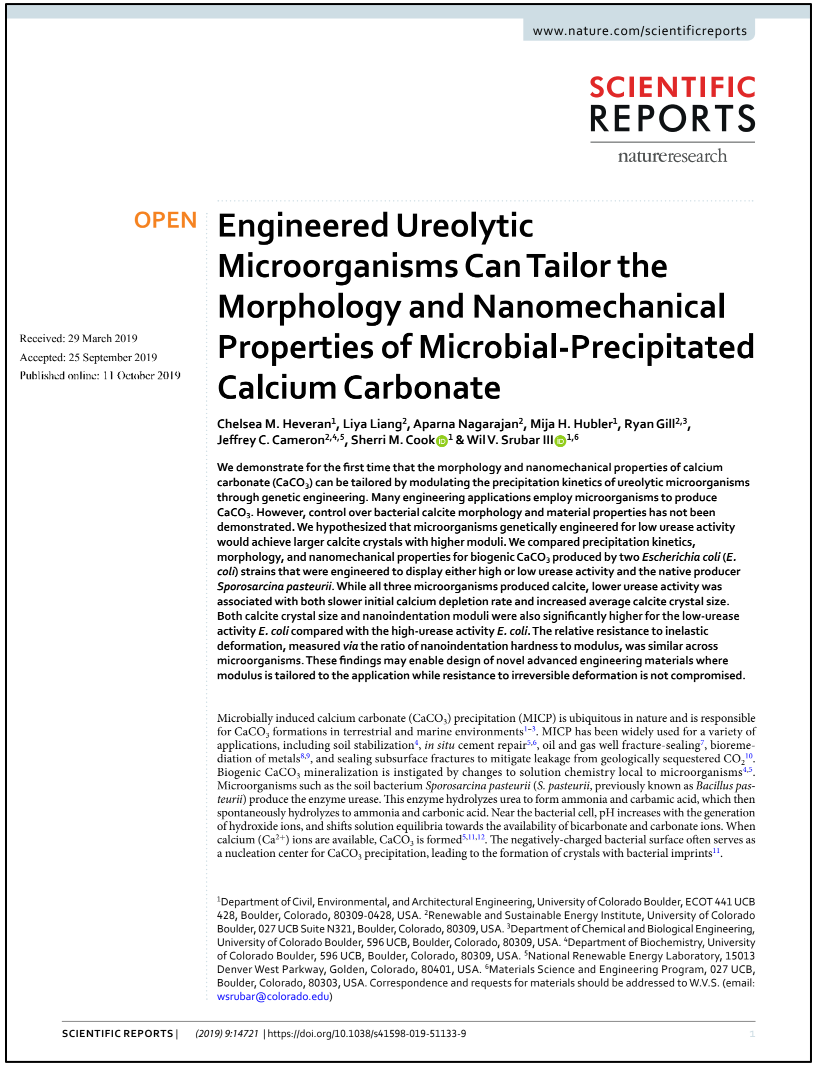
SCIENTIFIC REPORTS | *(2019) 9:14721* | https://doi.org/10.1038/s41598-019-51133-9 1

Biologically Induced $CaCO_3$ Precipitation for Biocement Applications // Urea Hydrolysis



Microbial metabolisms directly affect crystal structure and properties.



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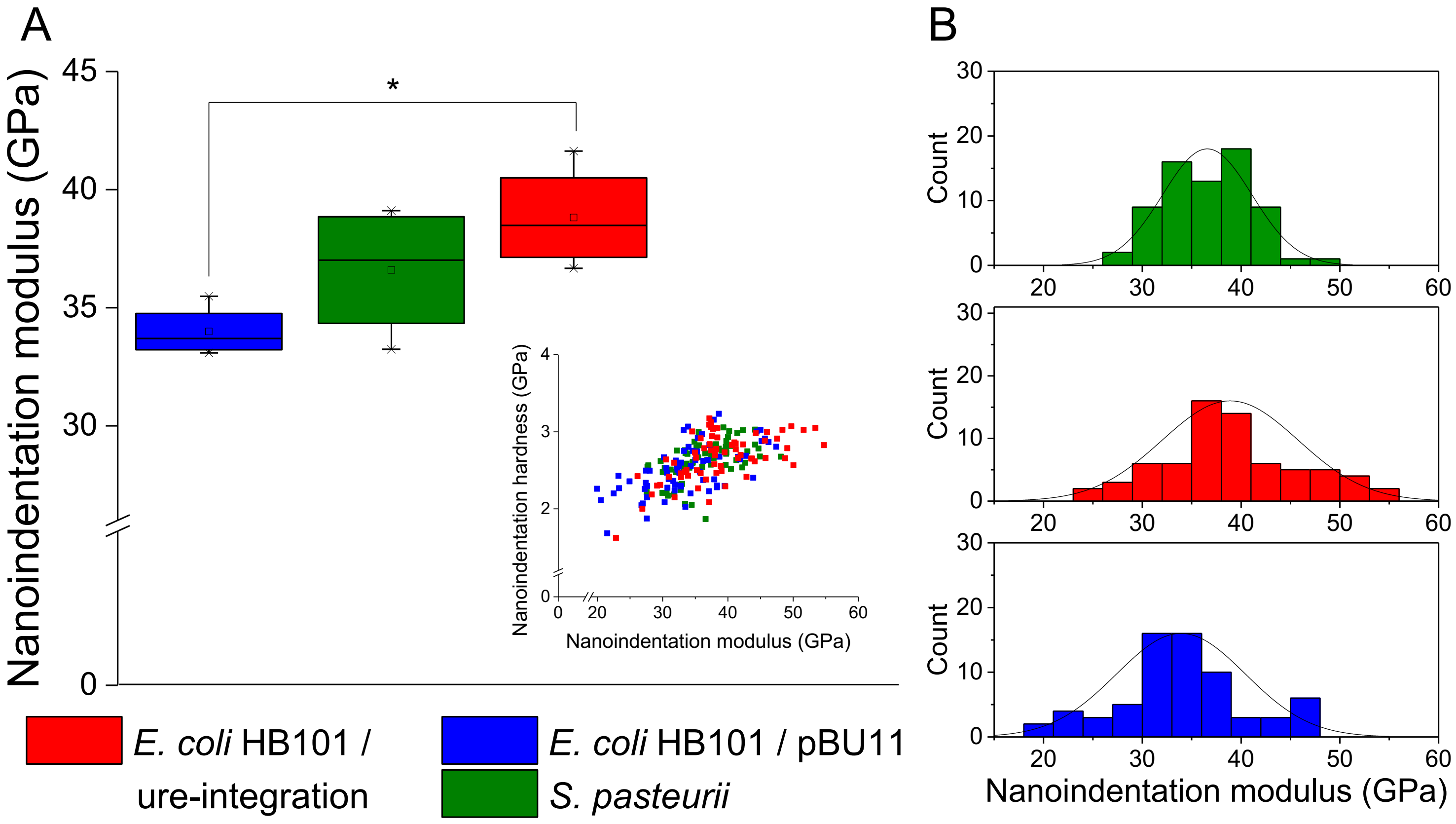


Microbial biomineralization is used widely in construction applications.

1. Biodeposition for Historic Preservation

Conservation of monumental stones by bacterial biomineralization

Brunella Perito & Giorgio Mastromei

Microbes are often responsible for the decay of stonework, but Brunello Perito and Giorgio Mastromei have found a way of using bacteria to conserve monuments through the technique of biomineralization.

Monumental stone decay is a consequence of the weathering action of physical, chemical and biological factors, which induce a progressive dissolution of the mineral matrix (Fig. 1). Attempts to slow down monument deterioration have used conservation treatments with inorganic or organic products, but their use presents several drawbacks. A new approach to conservation treatment of calcareous stones exploits bacterial biomineralization.

Calcium carbonate ($CaCO_3$) precipitation is a major biogeochemical process very common to microbes living in different environments. Several studies have pointed out the complexity of the phenomenon, which is influenced by the environmental physico-chemical conditions, and is correlated both with metabolic activity and cell-surface structures.

Application of living cultures of selected calcinogenic bacteria on limestone has been shown to induce calcite precipitation inside stone porosity, but it might generate other problems. In fact, chemical reactions due to metabolic by-products and growth of fungi, resulting from the application of organic nutrients for bacterial development, may have negative effects on the stone itself. For this reason, development of a stone treatment without viable cells seems a better biotechnological tool. To achieve this, it is necessary to understand the molecular mechanisms by which bacteria foster $CaCO_3$ precipitation, since this phenomenon is poorly understood both at molecular and genetic levels.

Work with *Bacillus subtilis*

In our laboratory we are studying calcite crystal formation in *Bacillus subtilis* in order to identify bacterial genes and cell structures involved in the biomineralization process. This work is part of the 'Bioreinforce' EC project (http://www.ub.es/rpat/bioreinforce/bioreinforce.htm) directed to develop a biomediation calcite precipitation method for conservation treatment of monumental stones.

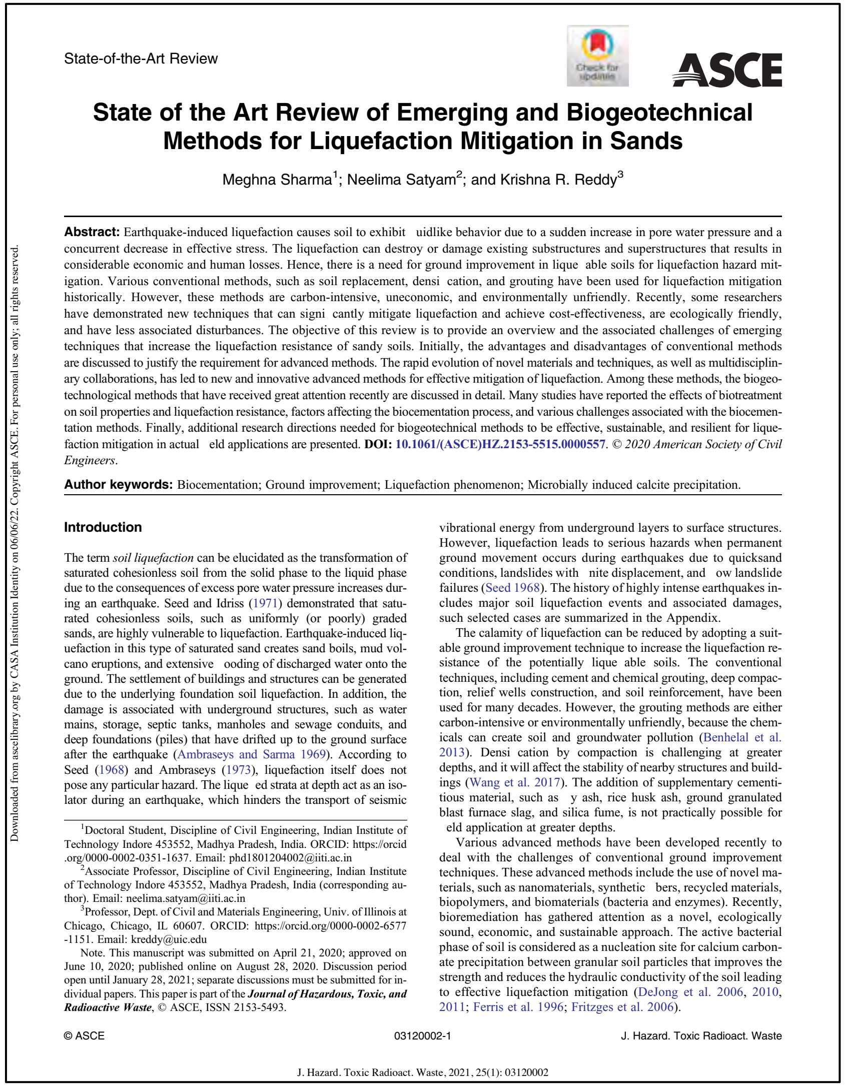
B. subtilis produces calcite crystals when grown on an appropriate medium (Fig. 2). We isolated several *B. subtilis* mutants impaired in calcite crystal

TOP LEFT:
Fig. 1. Marble Statue (1700), on the balustrade of the Villa Dosi-Dolfini, Pontremoli, Italy. The presence of old biological patina and new lichen encrustation can be seen.
COURTESY B. PERITO & G. MASTROMEI

LEFT:
Fig. 2. Calcite crystal production by *B. subtilis*. (a) Crystal formation on the surface of *B. subtilis* cells. (b) Crystals produced by *B. subtilis* observed by optical microscopy.
COURTESY B. PERITO & G. MASTROMEI

Microbial biomineralization is used widely in construction applications.

1. Biodeposition for Historic Preservation
2. **Soil Stabilization and Biogeotechnics**



State of the Art Review of Emerging and Biogeotechnical Methods for Liquefaction Mitigation in Sands

Meghna Sharma[1]; Neelima Satyam[2]; and Krishna R. Reddy[3]

Abstract: Earthquake-induced liquefaction causes soil to exhibit fluidlike behavior due to a sudden increase in pore water pressure and a concurrent decrease in effective stress. The liquefaction can destroy or damage existing substructures and superstructures that results in considerable economic and human losses. Hence, there is a need for ground improvement in liquefiable soils for liquefaction hazard mitigation. Various conventional methods, such as soil replacement, densification, and grouting have been used for liquefaction mitigation historically. However, these methods are carbon-intensive, uneconomic, and environmentally unfriendly. Recently, some researchers have demonstrated new techniques that can significantly mitigate liquefaction and achieve cost-effectiveness, are ecologically friendly, and have less associated disturbances. The objective of this review is to provide an overview and the associated challenges of emerging techniques that increase the liquefaction resistance of sandy soils. Initially, the advantages and disadvantages of conventional methods are discussed to justify the requirement for advanced methods. The rapid evolution of novel materials and techniques, as well as multidisciplinary collaborations, has led to new and innovative advanced methods for effective mitigation of liquefaction. Among these methods, the biogeotechnological methods that have received great attention recently are discussed in detail. Many studies have reported the effects of biotreatment on soil properties and liquefaction resistance, factors affecting the biocementation process, and various challenges associated with the biocementation methods. Finally, additional research directions needed for biogeotechnical methods to be effective, sustainable, and resilient for liquefaction mitigation in actual field applications are presented. **DOI:** 10.1061/(ASCE)HZ.2153-5515.0000557. *© 2020 American Society of Civil Engineers*.

Author keywords: Biocementation; Ground improvement; Liquefaction phenomenon; Microbially induced calcite precipitation.

Introduction

The term *soil liquefaction* can be elucidated as the transformation of saturated cohesionless soil from the solid phase to the liquid phase due to the consequences of excess pore water pressure increases during an earthquake. Seed and Idriss (1971) demonstrated that saturated cohesionless soils, such as uniformly (or poorly) graded sands, are highly vulnerable to liquefaction. Earthquake-induced liquefaction in this type of saturated sand creates sand boils, mud volcano eruptions, and extensive flooding of discharged water onto the ground. The settlement of buildings and structures can be generated due to the underlying foundation soil liquefaction. In addition, the damage is associated with underground structures, such as water mains, storage, septic tanks, manholes and sewage conduits, and deep foundations (piles) that have drifted up to the ground surface after the earthquake (Ambraseys and Sarma 1969). According to Seed (1968) and Ambraseys (1973), liquefaction itself does not pose any particular hazard. The liquefied strata at depth act as an isolator during an earthquake, which hinders the transport of seismic vibrational energy from underground layers to surface structures. However, liquefaction leads to serious hazards when permanent ground movement occurs during earthquakes due to quicksand conditions, landslides with finite displacement, and flow landslide failures (Seed 1968). The history of highly intense earthquakes includes major soil liquefaction events and associated damages, such selected cases are summarized in the Appendix.

The calamity of liquefaction can be reduced by adopting a suitable ground improvement technique to increase the liquefaction resistance of the potentially liquefiable soils. The conventional techniques, including cement and chemical grouting, deep compaction, relief wells construction, and soil reinforcement, have been used for many decades. However, the grouting methods are either carbon-intensive or environmentally unfriendly, because the chemicals can create soil and groundwater pollution (Benhelal et al. 2013). Densification by compaction is challenging at greater depths, and it will affect the stability of nearby structures and buildings (Wang et al. 2017). The addition of supplementary cementitious material, such as fly ash, rice husk ash, ground granulated blast furnace slag, and silica fume, is not practically possible for field application at greater depths.

Various advanced methods have been developed recently to deal with the challenges of conventional ground improvement techniques. These advanced methods include the use of novel materials, such as nanomaterials, synthetic fibers, recycled materials, biopolymers, and biomaterials (bacteria and enzymes). Recently, bioremediation has gathered attention as a novel, ecologically sound, economic, and sustainable approach. The active bacterial phase of soil is considered as a nucleation site for calcium carbonate precipitation between granular soil particles that improves the strength and reduces the hydraulic conductivity of the soil leading to effective liquefaction mitigation (DeJong et al. 2006, 2010, 2011; Ferris et al. 1996; Fritzges et al. 2006).

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J. Hazard. Toxic Radioact. Waste, 2021, 25(1): 03120002

Microbial biomineralization is used widely in construction applications.

1. Biodeposition for Historic Preservation
2. Soil Stabilization and Biogeotechnics
3. **Self-Healing Concrete**

REVIEW
Self-Healing Concrete
ADVANCED MATERIALS INTERFACES
www.advmatinterfaces.de

A Review of Self-Healing Concrete for Damage Management of Structures

Nele De Belie, Elke Gruyaert,* Abir Al-Tabbaa, Paola Antonaci, Cornelia Baera, Diana Bajare, Aveline Darquennes, Robert Davies, Liberato Ferrara, Tony Jefferson, Chrysoula Litina, Bojan Miljevic, Anna Otlewska, Jonjaua Ranogajec, Marta Roig-Flores, Kevin Paine, Pawel Lukowski, Pedro Serna, Jean-Marc Tulliani, Snezana Vucetic, Jianyun Wang, and Henk M. Jonkers**

The increasing concern for safety and sustainability of structures is calling for the development of smart self-healing materials and preventive repair methods. The appearance of small cracks (<300 µm in width) in concrete is almost unavoidable, not necessarily causing a risk of collapse for the structure, but surely impairing its functionality, accelerating its degradation, and diminishing its service life and sustainability. This review provides the state-of-the-art of recent developments of self-healing concrete, covering autogenous or intrinsic healing of traditional concrete followed by stimulated autogenous healing via use of mineral additives, crystalline admixtures or (superabsorbent) polymers, and subsequently autonomous self-healing mechanisms, i.e. via, application of micro-, macro-, or vascular encapsulated polymers, minerals, or bacteria. The (stimulated) autogenous mechanisms are generally limited to healing crack widths of about 100–150 µm. In contrast, most autonomous self-healing mechanisms can heal cracks of 300 µm, even sometimes up to more than 1 mm, and usually act faster. After explaining the basic concept for each self-healing technique, the most recent advances are collected, explaining the progress and current limitations, to provide insights toward the future developments. This review addresses the research needs required to remove hindrances that limit market penetration of self-healing concrete technologies.

1. Autogenous and Nonencapsulated Autonomous Self-Healing

Aging and degradation of concrete are connected to its porous structure and are fostered by the unavoidable proneness of concrete to cracking. The tremendous developments of concrete technology, which have enabled the design of concrete with extremely low porosity, have not altered likewise the inherent cracking hazard, with high-performance concretes being even more brittle and sensitive to early age cracking than normal strength ones. This has resulted into the development of crack-treating methodologies, which can be categorized into passive treatments that are applied manually after inspection and only heal the surface cracks, and active methods that are incorporated at the construction stage, may fill both interior and exterior cracks, and are regarded as self-healing techniques.

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1. Biodeposition for Historic Preservation
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3. Self-Healing Concrete
4. **Living Building Materials**

Matter

CellPress

Article

Biomineralization and Successive Regeneration of Engineered Living Building Materials

Chelsea M. Heveran, Sarah L. Williams, Jishen Qiu, ..., Sherri M. Cook, Jeffrey C. Cameron, Wil V. Srubar III

wsrubar@colorado.edu

HIGHLIGHTS

Living building materials (LBMs) were grown and regrown using physical switches

Cyanobacteria biomineralized hydrogel-sand scaffolds

Biomineralization increased the fracture toughness of LBMs

Three child generations of LBMs were grown from one parent generation

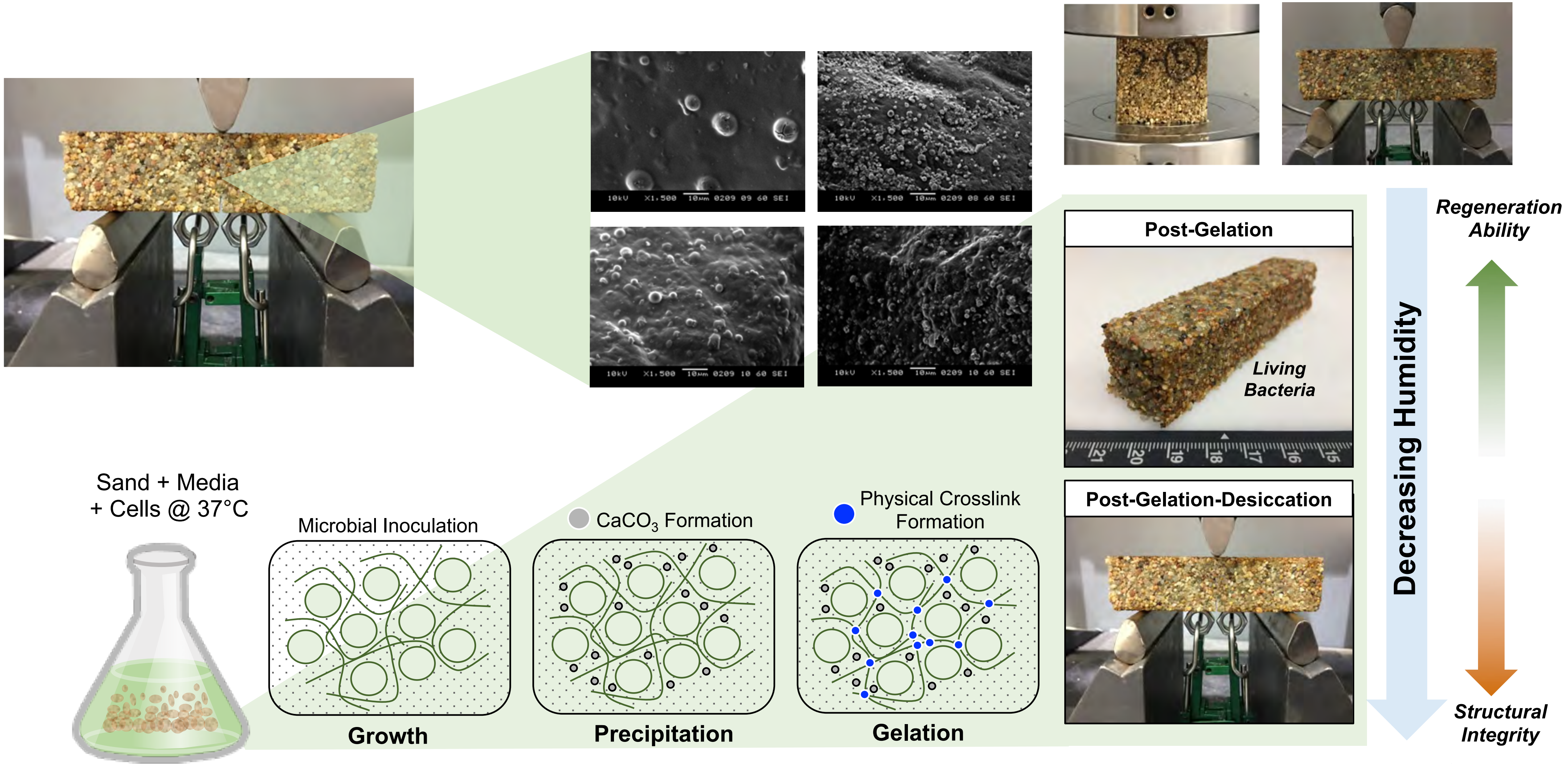
Microbial viability in the living building materials was maintained through 30 days

Living building materials (LBMs) were engineered using photosynthetic cyanobacteria and an inert sand-gelatin scaffold. Microorganisms biomineralized LBMs with calcium carbonate, which imparted higher fracture toughness compared with no-cell controls. The microorganisms maintained relatively high viability in LBMs as long as sufficient humidity conditions were provided. The microorganisms were capable of on-demand exponential regeneration in response to temperature and humidity switches. Looking forward, LBMs represent a new class of structural materials that can be engineered to exhibit multiple biological functionalities.

2 **Benchmark**
First qualification/assessment of material properties and/or performance

Heveran et al., Matter *2*, 1–14
February 5, 2020
https://doi.org/10.1016/j.matt.2019.11.016

We use biomineralizing photosynthetic microorganisms to “grow” strong, tough biological concretes.



Sand + Media
+ Cells @ 37°C

Microbial Inoculation

Growth

CaCO$_3$ Formation

Precipitation

● Physical Crosslink Formation

Gelation

Post-Gelation

Living Bacteria

Post-Gelation-Desiccation

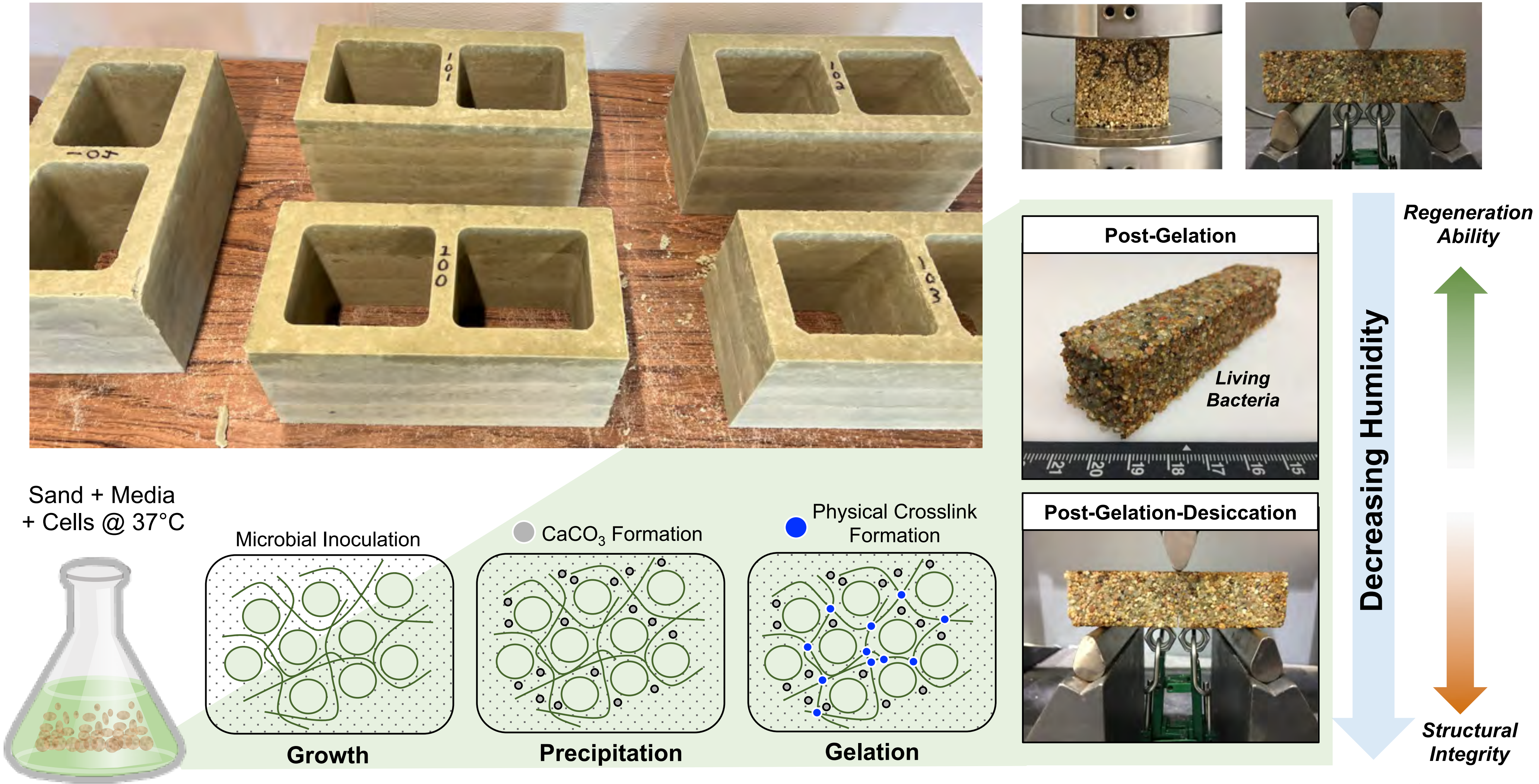
Decreasing Humidity

Regeneration Ability

Structural Integrity

Heveran, et al. 2020; Qiu, et al. 2021

We use biomineralization to “grow” strong, tough biological concretes.



Heveran, et al. 2020; Qiu, et al. 2021

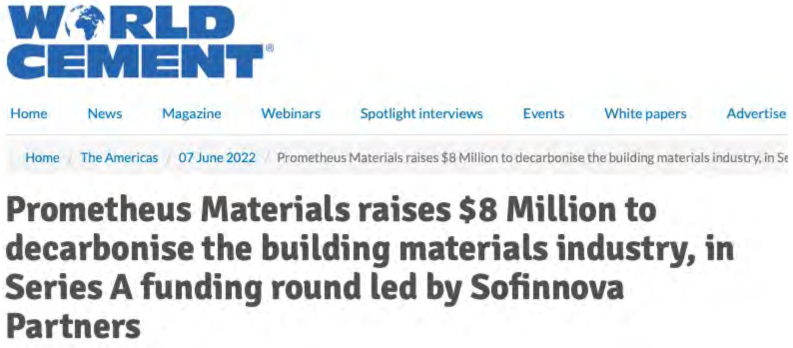
Prometheus Materials is commercializing our biomineralized concrete technology.

The New York Times

Bricks Alive! Scientists Create Living Concrete

"A Frankenstein material" is teeming with — and ultimately made by — photosynthetic microbes. And it can reproduce.

PROMETHEUS
MATERIALS



Home / The Americas / 07 June 2022 / Prometheus Materials raises $8 Million to decarbonise the building materials industry, in Se

Prometheus Materials raises $8 Million to decarbonise the building materials industry, in Series A funding round led by Sofinnova Partners

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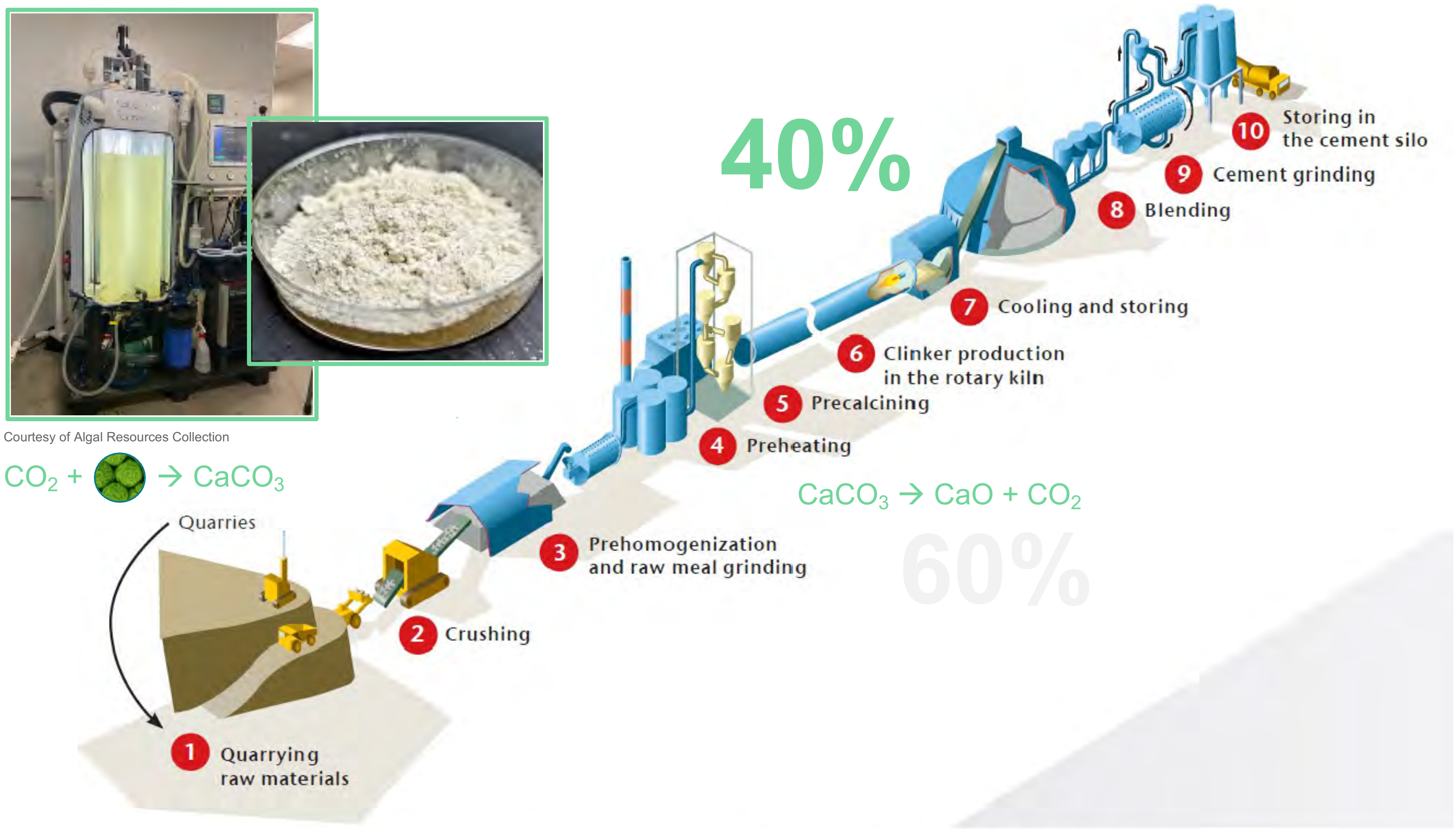
1. Biodeposition for Historic Preservation
2. Soil Stabilization and Biogeotechnics
3. Self-Healing Concrete
4. Living Building Materials
5. **Biogenic Limestone for PLC and Clinker Production**

We are growing CO_2-storing limestone quarries using photosynthesis.



Courtesy of Algal Resources Collection

CO_2 + → $CaCO_3$

40%

$CaCO_3$ → CaO + CO_2

60%

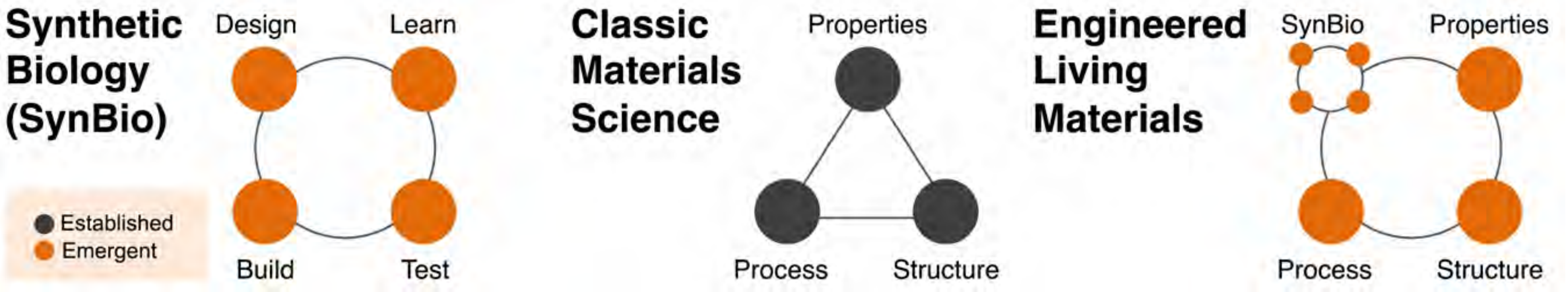
Minus Materials

Applications:
- + Carbonation Emissions-Free Clinker Production
- + CO_2-storing PLC Fillers

What are the metrology & measurement science needs for biomineralized materials?

Challenges

- *In situ* polymorph characterization (i.e., calcite, vaterite, aragonite)
- Standard viability measurements within concrete and other biomineralized building materials
- Characterization of the type and quantity of embedded *in situ* biominerals
- Standards for characterization and durability testing of biomineralized building materials
- Prototyping and standardized demonstration testbeds
- Scale-up production of biological organisms for large-scale testbed applications
- Linking genomics with process-structure-property relationships of minerals and materials



Srubar III, WV, Trends in Biotechnology, 2021.

Thank you!

Living
Materials
Laboratory

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