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# Sustainable construction: Toward growing biocement with synthetic biology

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## Impact Paper

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**Abstract**

The built environment contributes to global carbon dioxide emissions with carbon-emitting building materials and construction processes. While achieving carbon-neutral construction is not feasible with conventional construction methods, microbial-based construction processes were suggested over three decades ago to reduce carbon dioxide emissions. With time, questions regarding scaling, predictability, and the applicability of microbial growth and biomass production emerged and still needed to be resolved to allow manufacturing. Within this opinion, we will discuss what can be achieved not to 'grow a building' per se but to 'grow environmentally friendly biocement'. Elaborate pathways leading to the formation of cementitious materials by genetically manipulatable microorganisms have been described so far, providing options to enhance the suitability of these pathways for construction with synthetic biology and bioconvergence. These processes can also be combined with additional beneficial properties of cement-producing organisms, such as antimicrobial properties and carbon fixation by photosynthesis. Therefore, while we cannot yet 'grow a building', we can grow and design biocement for the construction industry.

## Current challenges in construction

Bacterial biomineralization was frequently considered for bioconstruction with an emphasis on bioconcrete. The overall aim is to design self-healing construction materials, and to reduce the extensive carbon dioxide emission from the construction industry, responsible for a significant portion of carbon dioxide emissions (Myhr et al., 2019; Zamora-Castro et al., 2021).

While achieving carbon-neutral construction is not feasible with conventional construction methods, a conceptual framework to 'grow buildings' with bacteria was suggested over three decades ago (Dade-Robertson et al., 2017). Cement is primarily composed of calcium carbonate from limestones and lime. The cement manufacturing sector leads 8% of the overall greenhouse gas production (CO2), growing at uncontrollable amounts as a result of speedy industrialization and the rise of the human population (Alghamdi, 2022). Furthermore, cement production is expected to exceed 6 billion metric tons by 2050 (Sharif and Tauqir, 2021). A continuous increase in near-surface atmospheric temperature is often reported, and the additional energy stored in the climate system contributes to ocean warming. Thereof, reducing carbon dioxide emissions is one of the goals of the Agenda for Sustainable Development, highlighted in Goal 13: Climate Action (Brigitte Baptiste, 2015; Durmisevic et al., 2017; Alghamdi, 2022).

Like in anthropogenic environments, in nature, construction constantly takes place. Multicellular organisms develop from a single cell embryo into complex structured organisms, containing organelles from organic and inorganic (such as bones and teeth) building blocks. Corals produce from a single polyp into 3D complex communities held together by a mineralized scaffold, and bacteria construct architectonically complex 3D communities that frequently contain mineral and organic extracellular component (Keren-Paz and Kolodkin-Gal, 2020). Unlike human bones and teeth, designed with calcium phosphate mineral (assembled over hydroxyapatite or apatite) over a collagen template (Jeong et al., 2019), scaffolds formed by soil and marine bacteria and corals are frequently composed of calcium carbonate (Dhami et al., 2013; Phillips et al., 2013; Dardau et al., 2021), although accumulation of crystalline calcium phosphate was also reported (Hirschler et al., 1990). Crystals formed over the organic templates are either vaterite [generated by cyanobacteria (Zafar et al., 2022)], aragonite [generated by corals, and microalgae/cyanobacteria (Xu et al., 2019)] and calcite formed by *Bacillus subtilis* and the *Bacillus phylum* members (Oppenheimer-Shaanan et al., 2016; Keren-Paz et al., 2022), *Pseudomonas aeruginosa* (Li et al., 2015; Cohen-Cymberknoh et al., 2022), and cyanobacteria (Kranz et al., 2010). These systems offer a clear advantage to the construction as potential sources for the generation of environmentally friendly cementitious materials (Yang et al., 2022). The regulatory principles of bacterial systems are extensively characterized (Brown, 1992) and the genetics of biomineralization is increasingly resolved (Keren-Paz and Kolodkin-Gal, 2020), making bacterial biotechnology especially appealing for the construction industry.



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Within this opinion, we will discuss the state of the art of the field allowing to grow a functional built environment microbiome. We suggest that an achievable goal is introducing self-regenerating communities producing Cementitious materials while considering synthetic biology as a tool to improve their function. Our conclusion is that while we cannot yet 'grow a building', we can grow and design functional bacterial species for biocement optimization.

## Molecular mechanisms promoting the production of cementitious materials by microorganisms

Bacteria are well known for producing cementitious materials. For example, *Sporosarcina pasteurii, Bacillus subtilis*, and *B. megaterium* promote calcium carbonate precipitation (Oppenheimer-Shaanan et al., 2016; Ma et al., 2020). While calcium is available from the environment, bicarbonate is actively produced by $CO_2$ hydration ($CO_2 + H_2O \leftrightarrow HCO_3 + H^+$), where the source of $CO_2$ can be a byproduct of bacterial metabolism or the atmosphere (Dhami et al., 2013). The growth of calcium carbonate crystals occurs in layers while the biogenic (organic) environment and organic polymeric substances influence crystal shape and morphology (Weiner and Addadi, 2011; Zhang et al., 2016).

In structured microbial communities (mats, biofilms and aggregates) cells are embedded in self-produced organic polymers (Flemming and Wuertz, 2019) absorb $Ca^{2+}$ and promote calcium carbonate formation by providing nucleation sites. In addition to the extracellular composition, the precipitation of calcium carbonate depends on concentrations of (i) calcium ions and (ii) carbonate ions. It is also determined by two additional factors: (iii) the pH and (iv) the availability of crystal nucleation sites. The formation of the cementous minerals of calcium carbonate requires alkaline pH to promote calcium sequestration. The most widely used microbial process of $CaCO_3$ precipitation is arguably the one based on the hydrolysis of urea. The reaction is catalyzed by the enzyme urease. Urease exerts one conserved catalytic function that is the hydrolysis of urea. The products of the reaction and the resulting increase in pH of the reaction environment that can reach pH up to 9.2. The products of the reaction are carbonic acid and ammonia (Phillips et al., 2013).

Ureolytic biomineralization is carried out with ureolytic bacterial strains (expressing detectable amount of the enzyme Urease). In addition to the bulk phase, calcite/aragonite/varterite crystallization takes place on bacterial cell walls, which serve as crystal nucleation sites. The cell walls possess negatively charged functional groups and attract and bind $Ca^{2+}$ ions, resulting in their deposition and accumulation, a process that also contributes to microbial aggregation. Consequently, carbonate crystals can grow on the external surfaces of cells. The involvement of the enzymes of carbonic anhydrase (CAs), zinc-binding enzymes, that catalyze the reversible conversion of carbon dioxide and water to bicarbonate and one proton (Tripp et al., 2001). Specific CAs are involved in the carbonate biomineralization in distinct metazoan lineages, including sponges (le Roy et al., 2014), and their role in microbial mineralization of calcium carbonate was also recently reported (Lotlikar et al., 2013; Cohen-Cymberknoh et al., 2022; Keren-Paz et al., 2022).

In addition to extracellular formation of extracellular cementous materials, intracellular mineralization of amorphous calcium carbonate (ACC) (a non-crystalline material) was described in the genetically manipulatable Gram-positive bacteria *Bacillus licheniformis* (Han et al., 2018) and *Bacillus subtilis* (Keren-Paz et al., 2022). Intracellular calcium carbonate storage was also documented in photosynthetic bacteria (Xu et al., 2019; Benzerara et al., 2022), where it was considered to contribute to carbon dioxide homeostasis. The Intracellular calcium storage is expected to affect calcium carbonate deposition and therefore should be carefully considered. For example, lysed cells may release ACC to interact with the extracellular nucleators, and affect the overall net production of calcium carbonate. While intracellular CAs are the preferred targets for utilization in sustainable construction, several additional intracellular pathways associated with calcium carbonate storage were also reported in bacteria (Benzerara et al., 2022). These pathways could be manipulated to control microbial mineralization once resolved.

Alkaline pH effectively promotes calcium carbonate precipitation. Therefore, in addition to ureolysis, bacterial metabolic pathways that can increase the solution pH can promote calcium deposition. These include photosynthesis, ammonification, denitrification, sulfate reduction, and formate oxidation (Hammes and Verstraete, 2002; Ganendra et al., 2014). For most, if not all, of these pathways it remains to be determined how their synthetic/biological activation will improve the performance of the strains for biotechnological application.

The potential of microbial mineralization for bioconcrete applications has been thoroughly investigated and includes, so far, the restoration of cement mortar cubes, sand consolidation and limestone monument repair, reduction of water and chloride ion permeability in concrete, filling of pores and cracks in concrete, and enhanced strength of bricks (Dhami et al., 2013). Overall, the richness of microbial biomineralization pathways discussed here provides a comprehensive tool set for future applications.

## Enhancing the microbial performance for biocement production

An introduction artificial circuits in microorganisms with synthetic biology, now acknowledged as a useful resource to solve environmental problems, can be used to improve microbial performance as well as to generate novel microbial capacities (Heinemann and Panke, 2006; Serrano, 2007; Hanczyc, 2020). One example of the application of synthetic biology to enhance construction materials is the engineered SEEVIX polymer, which relies on a synthetic circuit that mimics the natural process of spider silk creation by inducing the fiber's spontaneous self-assembly (Stern-Tal et al., 2022). Similar synthetic circuits discussed below can augment the biotechnological applicability of bacteria for construction.

In addition to the developments in synthetic biology, integrating bioengineering and classical engineering tools to improve microbial performance is widely recognized as 'bioconvergence'. This approach calls for controlling the microenvironment of microbial organisms used in various biotechnological applications instead of their genetic modification. Below we will elaborate on methods from synthetic biology and bioconvergence to improve the performance of microorganisms in the construction industry. Below we will elaborate on such potential interventions in the microbiome of the building that can enhance the performance and applicability of the utilized bacteria.

### *Improving microbial durability to concrete*

A key issue for introducing self-regenerating properties into the assembled construction material is the long-term viability of microbial cells applied to the material. These microorganisms must resist manufacturing temperature, friction and potential

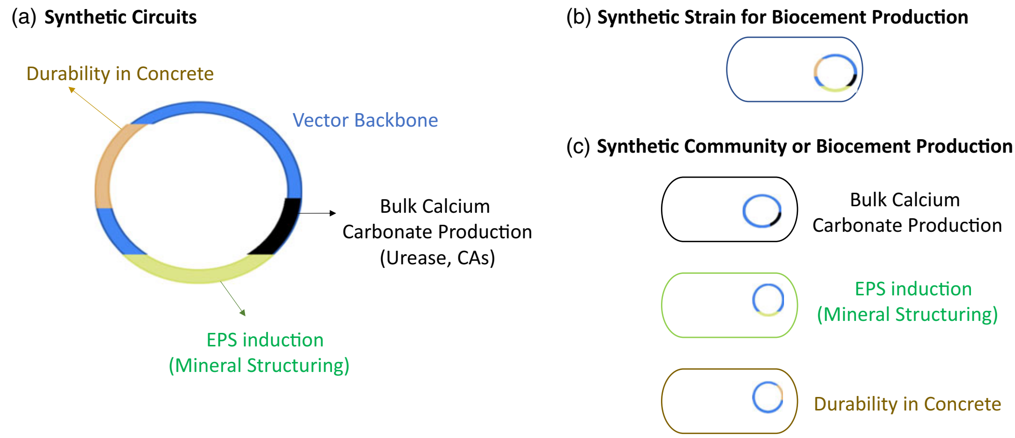


**Figure 1.** Summary of opportunities in enhancing the capacities of bacteria for construction. Left panel: The design of genetic circuits that could significantly enhance the performance of bacteria in environmentally friendly concrete: circuits that control the survival of bacteria in concrete (durability in concrete), enzymes that enhance calcium carbonate production with artificial inducers (9bulk calcium carbonate production) and genes involved in the biosynthesis of EPS (mineral structuring). Right panel: Methods to introduce the circuits: in a single strain, or with a few strains specialized for each function that will function as a consortium.

sterilization processes. Currently, an effort is made to comprehend the concrete microbiome (Kiledal et al., 2021). Among other issues, the resistance of concrete microbiome to extreme alkaline conditions is mandatory. When concrete is poured, its pH is ~12.5, higher than most known naturally alkaline environments (Kiledal et al., 2021). An emerging application of alkaliphiles is in the construction industry, where alkaliphiles are winning growing attention to treat concrete. Alkaliphiles have been trying to make concrete surface coatings, repair concrete cracks, and engineer self-healing concretes. Alternatively, to generate reparable concrete, the microbes that alter the concrete structure are introduced from outside after the structure has been poured.

One common strategy is appending spores to concrete. Spore-forming bacteria initiate the sporulation process after stressful conditions and particularly nutrient deprivation. Spores can remain dormant for extended periods (researchers have argued for up to millions of years) (Vreeland et al., 2000) and possess a remarkable resistance to environmental damage (i.e., heat, radiation, toxic chemicals, and extreme pH values) (Riley et al., 2021). Under favorable environmental conditions, the spore initiates a process called spore germination and outgrowth, where cells will begin to grow and reproduce (Bressuire-Isoard et al., 2018). In one setting, the microbial spores were mixed with mortar – a building material composed of cement, mixed with fine sands and water. In this setting lime is also added to improve durability (Jiang et al., 2020). The microcapsule broke under force representing concrete cracking, and the embedded bacteria self-healed the cracks with self-produced minerals within three weeks (Dhami et al., 2013).

The alternative strategy, offering challenges and opportunities for synthetic biology, is to utilize the adaptability of certain extremophiles to concrete microenvironments. The adaptability to alkaline environments can be considered while selecting strains and consortia of microorganisms for construction and formulation (Horikoshi, 2011). Several alkalophilic or alkali-tolerant species have already been implicated for bio concrete enhancement (Mamo and Mattiasson, 2020a), but did not always have advantage over other spore formers. For example, *B. halodurans* was tested, showing surprisingly less efficiency in calcium carbonate deposition than the lab strain of *Bacillus subtilis, B. subtilis* 168, and its poor performance was linked to reduced exopolymeric substances (for the roles of EPS in mineralization, see below) (Guéguen Minerbe et al., 2020). In addition to *Bacillus halodurans*, the halophilic *B. pseudofirmus* was efficient in improving cement performance (Sharma et al., 2017).

The genomes of alkaliphiles have also been studied in detail. Alkaline pH exerts metabolic and physical challenges to the embedded bacteria, among them a reversed proton gradient, with implications for ATP production, proteins (and thereby enzyme) inactivation, and instability of membranes and DNA (Horikoshi, 1999). In alkaline niches such as soda lakes and saline freshwater, a combination of abiotic pressures, including low $CO_2$ and metal ions concentrations, low proton gradient, and high salinity, impose a thermodynamic burden on core biological functions. These functions include but are not limited to carbon fixation, oxidative phosphorylation, and motility. Although highly diverse, alkaliphiles share two main features: the ability to maintain pH homeostasis and perform bioenergetics processes in an environment with an inverse, or 'reversed', chemical gradient.

These properties are attributed to the enrichment in genes for cation/proton antiporters and cation/substrate symporters. The enrichment of such transporters reflects the need to balance both PMF (Proton Motor Force) to achieve pH homeostasis and fuel energetically expensive processes such as substrate acquisition and chemotaxis. In addition, alkaliphile genomes encode many membrane-localized proton and electron-retaining proteins, such as cytochrome oxidases, which allow for the maintenance of a PMF across the membrane that favors efficient proton-coupled ATP synthesis (Horikoshi, 1999). Many alkaliphilic cyanobacteria express genes involved in $CO_2$-concentration mechanisms, which allow for efficient carbon fixation in environments where the levels of $CO_2$ are low and are extremely useful for carbonate biomineralization (Horikoshi, 1999; Preiss et al., 2015; Rampelotto, 2016; Mamo and Mattiasson, 2020b). All these pathways can be in theory introduced to members of the same family (e.g., genes from the alkalophilic firmicutes *B. pseudofirmus* can be easily introduced to the highly competent labs stain of *B. subtilis*, or undomesticated stains with enhanced genetic competence) (McLoon et al., 2011; Parashar et al., 2013) (Figure 1).

## *Artificial activation of enzymes promoting biomineralization*

*B. subtilis* colonies can rapidly accumulate up to 20% calcium carbonate of their dry weight (Keren-Paz et al., 2018). In addition,

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we and others have identified genes directly promoting biomineralization, for example, urease, carbonic anhydrase, and calcium channels. In this case, synthetic biology can efficiently generate environmentally friendly concrete: by placing the enzymes involved in biomineralization, for example, Urease and carbon anhydrase, under inducible promoters. The inducible promoters can be activated in the construction site with an appropriate inducer. The induction of Urease/carbonic anhydrase is expected to induce calcium carbonate precipitation (with an advantage to urease generating a significant local pH shift towards alkaline pH). The induction of carbonate precipitation and high pH is expected to yield higher efficiency of biomineralization at the required site. Still, it may need to be more promising regarding the control of the shape and function of the final cementitious product (Figure 1).

### Applications of microbial exopolymeric substances

Growth of calcite crystals occurs in layers. The relative growth rates in the various axes may alter crystal shape and morphology and may be influenced by the biogenic (organic) environment (Weiner and Addadi, 2011) and by organic polymeric substances (Dhami et al., 2013). In bacterial communities exopolymeric substances (EPS) are secreted and the genes that encode them are resolved. It is known that extracellular matrix absorbs $Ca^{2+}$. Furthermore, the EPS contribute to calcium carbonate formation by providing nucleation sites (Dupraz et al., 2009; Azulay et al., 2018). However, the exact exopolymeric substances critical for biomineralization and crystal structure remain to be determined. In *B. subtilis*, the effect of each EPS component was evaluated by comparing the morphology of calcium carbonate crystals. Calcium carbonate assembly within the wrinkles, 3D structures associated with enhanced EPS production was significantly reduced in all matrix mutants, consistent with the concept of mineral growth aided by nucleation sites provided by the matrix (Oppenheimer-Shaanan et al., 2016; Azulay et al., 2018). The analysis of crystal morphology in the different matrix mutants showed that matrix macromolecules interact with the mineral phase to affect the growth of calcium carbonate crystals. The interaction between the extracellular matrix components and mineral crystals was further assessed and confirmed by Fourier transform infrared analysis of the crystals (Azulay et al., 2022; Oppenheimer-Shaanan et al., 2016). Similarly, in *P. aeruginosa*, alginate and exopolysaccharides were shown to interact with calcium and calcium carbonate (Li et al., 2015; Cohen-Cymberknoh et al., 2022; Jacobs et al., 2022). These results strongly indicate that using artificial overexpression constructs for exopolysaccharides or amyloids, as well as mutant strains is likely to generate differential calcium carbonate-based materials. Furthermore, the current observations provide a proof of concept for controlling the final product of bacterial construction by generating or isolatingEPS mutants for each structural element, or alternatively inducing exopolymers at desired growth direction.

The effect of EPS-based nucleation on crystal assembly is probably a general feature of biofilm mats. Importantly, the shape of the calcium carbonate crystals varies between different species. For example, *Mycobacterium smegmatis* that produces fatty acids-based EPS (Ojha et al., 2005; Esteban and García-Coca, 2018) and accumulates calcium carbonate (Keren-Paz et al., 2018). Many soil bacteria are genetically manipulable, generate versatile EPS and provide a flexible toolbox for future engineers and architects (Figure 1).

In theory, the desired calcium carbonate element to be used in biocement or building foundations can be produced by designing the composition of the bacterial communities.

### Synthetic biology and bioconvergence to enhance the sustainability of buildings' microbiome

While microbial calcium carbonate for sustainable biocement production offers significant potential advancement for the construction industry, there are several additional applications that need to be considered. Synthetic genetic circuits implemented in robust bacterial hosts (Heinemann and Panke, 2006; Serrano, 2007) offer a different approach where the bacteria serve as dedicated biosensors engineered to detect the integrity of buildings. Relying on the available repertoire of mechanosensors characterized in bacteria as probes for the stability of construct (Cox et al., 2018; Gordon and Wang, 2019), engineered bacteria can be used to monitor decay processes within the foundations. Coupling the detection of the damage to the formation of cementitious materials may ensure that damage is not only reported but also prevented. The successful development of bacterial-based biosensors to detect 2,4-dinitrotoluene and 2,4,6-trinitrotoluene in buried landmines is encouraging support for this application (Belkin et al., 2017).

The formation of calcium carbonate has been studied and requires special consideration of microbial survival in soils in the context of construction foundations (Seifan and Berenjian, 2018). For this purpose, several approaches were suggested to increase the competitiveness and survival of bacteria in the complex soil microbiome and were reviewed extensively by us and others (Hou and Kolodkin-Gal, 2020; Rebello et al., 2021).

Engineered bacteria should also be considered to enhance anti-bacterial and anti-fungal activities of the construct, and thereby preventing the decay of construction materials by biodegradation. While designing dyes with anti-mold properties was frequently examined from the chemical perspective, many of the strains suggested for bioconstruction, and especially Bacillus species, are potent producers of broad-spectrum anti-fungal agents (Maan et al., 2022). While anti-mold compounds tend to have a single bioactive compound, bacteria produce multiple compounds on the same time, which can be all activated synchronically by deleting their natural repressing proteins (For example, in *B. subtilis* multiple antibiotics and anti-fungal compounds are regulating by a single response regulator Abh (Strauch et al., 2007) prior to introducing them into paints/construction materials.)

## Conclusions

The formation of biocement is emerging as a platform to harvest natural biological processes of producing alternative construction materials for construction in an environmentally friendly manner (free of or significantly reduced greenhouse gas emissions). Before, we and others highlighted the usefulness of microbial biomineralization in genetically manipulatable bacteria for this process. While the initial focus was on designing the bacterially produced cementitious materials (Kolodkin-Gal et al., 2023; Dhami et al., 2013; Alghamdi, 2022), it became clear that reducing the costs and increasing the use of bacteria for cement production is more complex: The strains should be chosen or designed to endure the harsh environment of the cement well while performing the enzymatic processes that promote biomineralization, the control in the production of exopolymers that provide additional nucleation sites for carbonate minerals should be improved. It is also desired that enzymes (e.g., Urease (Anbu et al., 2016; Keren-Paz et al., 2018; Wu et al., 2021; Nodehi et al., 2022), but also additional pathways discussed above) that promote the perception of cementitious materials could be activated when required. We here (Figure 1) suggest how this can be achieved with current technologies in synthetic biology.

We conclude that while it is still challenging to 'grow a building', for example, manufacturing the structure from biological products of living organisms, the time is already mature to consider rebuilding the construction materials with bacteria. The informed design discussed here of available strains for the scaffolds, foundations, and shells of each building can be a conceptual leap toward sustainable construction. It is the time to grow and design the buildings' microbiomes with improved biocement producers.

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