Biotechnology approaches and improvement strategies in biocement production

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Abstract

Introduction. This review summarizes existing research focused on overcoming the key limitations of the biocementation process.

Materials and methods. Biocement was used as a main key term in combination with terms related to its production and applications.

Results and discussion. Among environmentally friendly building materials, biocement is of special importance as an alternative to traditional cement, since it possesses valuable properties, its production does not require significant energy consumption, and it helps reduce the environmental burden by lowering carbon dioxide emissions. The precipitation of calcite due to the activity of urease producing bacteria (UPB) can be used to improve the geotechnical properties of soils and to restore concrete structures.

Obstacles to the large-scale use of biocement include its relatively high cost and the release of ammonia and ammonium during the biocementation process, the levels of which in the environment are strictly regulated.

Biocement production requires a calcium source, urea, and the enzyme urease to enable calcite precipitation. Bacteria adhere to soil particles, adsorb Ca^{2+}, and act as nucleation sites, improving soil strength and concrete repair. Safety can be enhanced by using biosafe or inactivated cels of microbial urease producers, applying plant urease, or alternative bioagents such as microalgae or cyanobacteria. Costs may be reduced by applying industrial wastes and replacing expensive chemicals.

Several methods help reduce ammonium and ammonia emissions during biocementation, such as replacing $CaCl_2$ with calcium acetate, adding zeolite to adsorb ammonium, or using electrobiocementation with graphite electrodes. Other approaches include cation-enriched injections, ammonium recovery as struvite, and modified nutrient solutions that both strengthen soil and cut ammonia release. Additionally, captured ammonia can be adsorbed with sulfuric acid. A promising eco-friendly alternative is calcium phosphate-based biocementation.

Conclusions. Using industrial waste, plant-based urease, and safer or inactivated microorganisms can reduce costs and enhance the biosafety of biocementation.

Introduction

Concrete is the most widely used man-made material on Earth, but it is also among the most energy-intensive. In 2022, carbon dioxide emissions from cement manufacturing totaled 1.6 billion metric tons (Statista, 2023), and global emissions are projected to reach 2.34 billion metric tons of CO_2 by 2050. Meanwhile, the release of greenhouse gases into the atmosphere contributes to global warming, and climate change is recognized as one of the most urgent global challenges.

In recent years, the use of microorganisms for the production of building materials, a new direction in biotechnology, has been developing rapidly (Achal and Chin, 2021; Ivanov and Stabnikov, 2023). Among these innovations, biocement production holds a special place. The advantages of biocementation include not only the low viscosity and high permeability of biocement into concrete or soil, but also it's relatively low cost and energy consumption, along with a significant reduction in greenhouse gas emissions compared to conventional cement production.

To produce biocement, at least three components are required: (1) a source of soluble calcium, (2) a compound such as urea that alters the pH to facilitate calcium carbonate precipitation and supplies CO_2 for carbonation, and (3) microbial cells with urease activity or the urease enzyme that catalyze urea hydrolysis. The most widely used biocementation process is known as microbially induced calcite precipitation (MICP). The enzymes urease (EC 3.5.1.5) and carbonic anhydrase (EC 4.2.1.1) play crucial roles in this process:

$$(NH_2)_2CO + 2\ H_2O\ (+\ \text{urease}) \rightarrow CO_2 + 2\ NH_4^+ + 2\ OH^-$$

$$CO_2 + H_2O\ (+\text{carbonic anhydrase}) \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2\ H^+ + CO_3^{2-}$$

$$CaCl_2 + (NH_2)_2CO + 2\ H_2O + (+\text{urease} + \text{carbonic anhydrase}) \rightarrow CaCO_3\downarrow + 2\ NH_4Cl$$

Bacterial cells adhere to soil particles, and since bacterial cells have a negative surface charge, they can easily adsorb Ca^{2+} cations and serve as nucleation sites for crystallization. The precipitation of calcite due to the activity of urease producing bacteria (UPB) can be used to improve the geotechnical properties of soils and to restore concrete structures (DeJong et al., 2013; De Muynck et al., 2010; Ivanov and Chu, 2008; Van Paassen et al., 2010; Wang Z. et al., 2017).

However, the production of biocement obtained by MICP has certain drawbacks that limit its widespread practical application. These include economic challenges, such as high production costs, and ecological concerns, mainly the introduction of a large number of live bacterial cells into the environmen and, the release of toxic ammonia into the atmosphere, as well as ammonium ions into ground and surface waters when MICP is used for soil stabilization (Ivanov et al., 2019a, b) (Figure 1).

Figure 1. Main obstacles for large-scale biocement application: biosafety concerns, high cost, and ammonia emissions

To improve biocement production technology and make its manufacturing and application safer and more cost-effective, two main directions are being pursued, focusing on its key components: (a) the source of urease and (b) the source of calcium ions. This review is devoted to analyzing the research conducted in these areas.

Materials and methods

The literature search was performed using the Scopus, Web of Science and Google Scholar databases. Biocement was used as a main key term in combination with terms related to its production and applications, such as microbial urease producers, biological agents, biosafety, replacement of chemicals, intensification, cost, ecology, ammonia release, and others. Full texts of relevant articles were obtained for further evaluation. Additional publications were identified by examining the reference lists of relevant journal publications and review articles.

Results and discussion

Ways to increase the safety and reduce the cost of the microbial component of biocement

Selection of microbial urease producer

Microbial urease is a high-molecular-weight, nickel-containing multi-subunit enzyme that hydrolyzes urea to ammonia and carbon dioxide, resulting in an increase in pH. Urease-producing bacteria used for biocementation must be active in geotechnical environments characterized by high salt concentrations and alkaline pH levels. Therefore, halophilic or halotolerant and alkaliphilic UPB are preferred for biomement production. For effective biocementation, microbial urease must function at inorganic salt ($CaCl_2$) concentrations of 1.0–1.5 M and at pH values above 8.5.

Constitutive synthesis of urease is a common property of many strains of soil bacteria. Urease-producing bacteria are widely distributed in soils, especially in areas where urea is constantly present either as a product of nitrogen metabolism in mammals or because of the use of ammonium-containing fertilizers in agriculture. The possibility of strengthening soil by activating indigenous urease-producing bacteria through the addition of nutrients and forced aeration has been demonstrated (Burbank et al., 2012). The addition of molasses or yeast extract to coastal sand from a semi-arid environment to activate native URB, resulting in a change in the composition of the natural microbial population, its enrichment with representatives of the genus *Bacillus*, and a significant increase in urease activity (Gat et al., 2016). Strengthening the soil by activating of URB present in it was also used in the works (Gomes et al., 2018; Rajasekar et al., 2017). However, the use of artificially activated natural microflora with urease activity may be limited by several factors: (1) a low concentration of UPB in deep soil layers and their absence in mountains breeds; (2) negligible urease activity at high salt concentrations or at pH levels above 8.5; (3) potential pathogenicity; and (4) unpredictable and unstable bioactivity during the MICP process.

To carry out MICP, the use of mixed or enrichment bacterial cultures has been proposed (Al-Thawadi, 2012; Hammes et al., 2003). For example, the use of a mixed culture of ureolytic and non-ureolytic bacteria increased the efficiency of $CaCO_3$ precipitation, possibly due to the formation of additional crystallization centers facilitated by the adhesion of non-ureolytic bacteria (Gat et al., 2014). In addition, the cost of biocement production can be reduced by using an enrichment microbial culture with urease activity instead of a pure culture of URB. The fact that bacterial strains used for biocementation or soil strengthening are alkaliphilic and halotolerant makes them highly competitive, enabling their cultivation in batch or even continuous processes under non-sterile conditions (Cheng and Cord-Ruwisch, 2013). However, along with UPB, undesirable microorganisms, including opportunistic pathogens, may also develop. Some studies propose using activated sludge from wastewater treatment plants (WWTP) for biocementation. This sludge can be artificially enriched with UPB through non-sterile cultivation at an initial pH of 10.0, with the addition of ammonium chloride (5 g/l) and urea (10 g/l) to the nutrient medium (Yang et al., 2020). Using this method, the cost of UPB biomass can be reduced by up to 30%; however, biosafety concerns are not addressed. Therefore, the use of pure cultures of UPB with well-characterized properties may offer a safer alternative for implementing MICP than the use of activated indigenous urease-producing bacteria or enrichment cultures.

To ensure safety in biotechnologies involving the release of microorganisms into the environment, it is preferable to work with bioagents classified as Risk Group 1 (RG1) under the European Union Directive 2000/54/EC, which includes microorganisms that do not potentially cause diseases in humans and animals. Strains of *Sporosarcina pasteurii*, which belong to Risk Group 1 microorganisms, are among the most widely used URB recommended for biocementation, especially the strain *S. pasteurii* ATCC 11859, which has been commonly employed in MICP research (Eryürük et al., 2015; Mortensen and DeJong, 2011; Whiffin and van Paassen, 2007). In addition, many biocementation studies utilize the strain *Bacillus* sp.VS1 (Chu et al., 2012; Stabnikov et al., 2016), whose 16S rRNA gene sequence shares 99% identity with *Bacillus pasteurii*, a species also classified within Risk Group 1 (Figure 2c1).

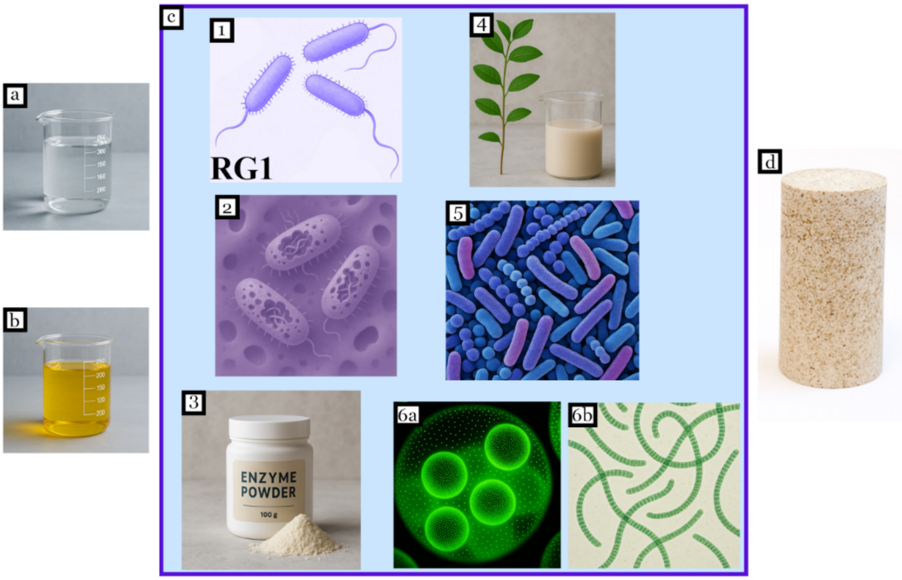

Figure 2. Biosafety of biocementation:
(a) calcium ion solution; (b) urea solution; (c) safe sources of urease:
(1) bacteria belonging to Risk group1; (2) inactivated bacterial cells; (3) pure enzyme;
(4) extract of plant material with urease activity; (5) lactic acid bacteria;
(6) photosynthetic microorganisms – microalgae (6a) and cyanobacteria (6b); (d) biocement

Currently, many studies on biocementation have used strains from the genus *Lysinibacillus,* Gram-positive, rod-shaped, motile, endospore-forming bacilli belonging to RG1 group and isolated from soil (Chen M., 2021; Ekprasert et al., 2020; Gowthaman et al., 2019; Leeprasert et al., 2022; Sreekala et al., 2024; Vashisht et al., 2018), or industrial sources (Han et al., 2014; Jeong et al., 2017). Additionally, strains *Bacillus megaterium* (Andalib et al., 2016; Sun et al., 2019; Wani et al., 2024) and *Cytobacillus horneckiae* (Moqsud and Gochi, 2024), both classified within RG1, have also been successfully applied in biocementation research.

However, many halotolerant and alkaliphilic bacterial strains having significant urease activity belong to opportunistic and even pathogenic species. Numerous studies have proposed the use of urease-producing bacteria classified as RG2 group, which may cause disease in humans, for biocementation. Examples include opportunistic pathogens such as *Bacillus cereus* (Brasileiro et al., 2020), *Bacillus mycoides* (Elmanama, and Alhour, 2013), *Proteus* sp. (Khanafari et al., 2011), *Staphylococcus aureus, Klebsiella pneumoniae* (Varalakshmi and Devi, 2014), and even the pathogen *Helicobacter pylori* (Dosier, 2014) as potential biological agents for biocementation.

Non-pathogenic microbial urease producers can be isolated from extreme environments considered unfavorable for microbial growth, such as speleothems from limestone caves (Garcia et al., 2016; Omoregie et al., 2018), calcareous soil (Dhami et al., 2013) or highly alkaline cement samples (Achal et al., 2010). Meanwhile, active UPB isolated from activated sludge from WWTP have also been proposed for biocementation (Al-Thawadi, 2012; Varalakshmi and Devi, 2014; Xu G. et al., 2017), although the risk of isolating opportunistic bacteria remains high. Recently, research has focused on using mutant strains of UPB for biocementation, including mutants of *Sporosarcina pasteurii* ATCC 11859 (Han et al., 2021) and strains from the genus *Bacillus* (Li H. et al., 2014; Tayyem et al., 2021).

There is an opinion that all bacteria on Earth are natural, so transferring them to new habitats will not cause environmental changes or risks (Fritzges, 2005). However, it has been shown that it is impossible to predict the behavior of bacterial populations introduced into soil, so generalizations are unwarranted. Instead, each case requires a specific analysis of the introduction pattern and potential risks (Almajed et al., 2019). Given these biorisk concerns, the selection of bacteria for use in MICP must be grounded in a thorough biosafety analysis.

To ensure biological safety when introducing large quantities of foreign microbial cultures into soil, inactivation of bacterial cells can be employed, if the cells retain their functional activity (Fig. 2c2). For example, the use of UPB *Yaniella* sp. cells, inactivated by soaking in a 0.5% (w/v) sodium dodecyl sulfate solution, was demonstrated for constructing a model aquaculture pond through biocementation (Stabnikov et al., 2016). Some studies have explored the direct use of urease enzyme (Fig. 2c3) for repairing cracks in building materials (De Muynck et al., 2010) or for biogrouting (Almajed et al., 2019; Neupane et al., 2015). However, the high cost of urease remains a major obstacle to its practical application. Therefore, reducing the cost of urease is crucial for its practical application in biocementation (Khodadadi Tirkolaei et al., 2017).

Summarizing the above, it can be concluded that urease-producing bacteria are the basis of the biocementation process. These bacteria should be harmless to humans and the environment; capable of forming aggregates and adhering effectively to treated surfaces; tolerant to high salt concentrations, as they need to remain active in saline environments; and able to synthesize urease that functions efficiently in alkaline conditions (pH 8.5–9.2) (Stabnikov et al., 2013).

Reducing the cost for microbial urease producer

Obtaining biomass of URB includes the preparation of an expensive nutrient medium, sterile cultivation, biomass separation, and, in the case of storage, freeze-drying to preserve bacterial cell viability. To reduce the cost of the nutrient medium for growing bacteria, it is proposed to use various wastes, including activated sludge hydrolysates (Whiffin 2004; Stabnikov, 2016), liquid dairy waste, lactose-containing mother syrup (Achal et al., 2009), whey from cheese production (Chaparro et al., 2021), whey powder (Kahani et al., 2020), enzymatically hydrolyzed kitchen waste (Meng et al., 2021), effluent from biogas production using chicken manure (Yoosathaporn et al., 2016), and cheap soybean flour instead of yeast extract. The use of urine sterilized with ultraviolet light as a component of the medium for URB cultivation has been reported (Yang et al., 2022).

Plant urease instead of microbial enzyme for biocementation

Plants for urease production

In biocementation, bacterial urease can be replaced by plant urease (Fig. 2c4) (Dilrukshi et al., 2015; Ivanov et al., 2019a). Urease is found in the leaves, stems, and roots of many higher plants, allowing them to use urea as a nitrogen source. It is present in relatively high amounts in the seeds of beans, melons, and pumpkins (Das et al., 2002). The production of urease from jack beans (*Canavalia ensiformis* and *Canavalia adans*) was patented by Kakimoto and coauthors (1992). Each plant-derived urease has its own optimal conditions for catalytic activity. For example, urease from chickpea seeds shows maximum activity at a temperature of 48°C and pH 7.2 (Pervin et al., 2013), while urease from pea seeds is most active at 40°C and pH 7.5 (EL-Hefnawy et al., 2014), urease from mulberry leaves at 30°C and pH around 9.0 (Hirayama, 2000), and urease from dehusked seeds of pigeonpea had an optimum pH of 7.3, with activity over a pH range of 5.0 to 8.5 (Das et al., 2002).

It is preferable to use local plant materials or food processing waste to obtain extracts for subsequent applications in biocementation. Thus, the application of extracts from watermelon seeds (Al Imran et al., 2021; Dilrukshi et al., 2018), cabbage, wheat, or soybean leaves, and soybean pulp (Baiq et al., 2020; Hogan et al., 1983) has been shown. The technique for extracting urease from plant raw materials is extremely simple and allows one to easily obtain a solution with urease activity for its later use in the biocementation process. The plant raw material is cleaned, washed, and crushed. An extractant, which is most often distilled, deionized or tap water (Al Imran et al., 2021; Cui et al., 2023; Dilrukshi et al., 2018; Stabnikov et al., 2022), but sometimes it is 20 mM phosphate buffer (Park et al., 2014), 20 mM phosphate buffer containing 2 mM ethylenediaminetetraacetic acid (Khodadadi Tirkolaei et al., 2020), 0.025 M Tris–acetate containing 50 µg/ml leupeptin (Das et al., 2022) or chilled 50 mM phosphate buffer containing 10 mM ß-mercaptoethanol (Hamzah, 2014), is added to the crushed raw material. Chemicals such as 20-30% (v/v) ethanol solution (Lai et al., 2023; Weng et al., 2024) or 20% chilled acetone (EL-Hefnawy et al., 2014; Pervin et al., 2013) are also used as extractants to obtain crude urease extracts. The ratio of raw material to extractant varies across different studies, more commonly being 1:3, but sometimes 1:4, 1:10 or even 1:20 (Table 1).

Table 1

Urease activity of crude extracts from various plant sources

Extract from	Plant, g, to extractant, ml	Urease activity	Units	Reference
Germinated chickpea (*Cicer arietinum*) seeds	1:4	0.011	mM NH_3/min·mg protein	Pervin et al., 2013
Watermelon (*Citrullus lanatus*) seeds	1:20	0.01	mM NH_3/min·ml	Al Imran et al., 2021
Watermelon (*C. lanatus*) seeds	1:20	0.08	mM NH_3/min·g	Dilrukshi et al., 2018
Dehusked watermelon (*C. lanatus*) seeds	1:4	0.01	mM NH_3/min·mg of protein	Khodadadi Tirkolaei et al., 2020
Dehusked jack bean (*Canavalia enformis*)	1:4	0.03		
Jack bean (*Canavalia enformis*) meal	1:4	0.024		
Soybeans (*Glycine max*)	1:4	0.004		
Germinated soybean (*Glycine max*)	1:20	0.70	mM urea/min·g	Stabnikov et al., 2022
Soybean	1:10	3.90	mM urea/min·l	Weng et al., 2024
Soybean	n/a	0.14	mM urea /min·g	Gao et al., 2020
Mulberry (*Morus alba*) leaves	1:3	0.076	nkat/mg protein	Hirayama et al., 2000
Sword bean (*Canavalia gladiata*)	n/a	0.70	mM urea/min·g	Liu L. et al., 2024
Germinated pea (*Pisum sativum*) seeds	1:4	0.46	mM NH_3/min·mg protein	EL-Hefnawy et al., 2014
Broad beans (*Vicia faba*)	1:3	0.03	mM NH_3/min·ml	Bedan 2020
Dehusked pigeonpea (*Cajanus cajan*) seeds	n/a	0.032	mM NH_3/ min·mg protein	Das et al., 2002
Syrian mesquite (*Prosopis farcta*) seeds	1:2	0.125 0.037	mM NH_3/min mM NH_3/min·mg protein	Hamzah 2014
Indian sweet-clover (*Melilotus indicus*) seeds	1:2	0.015 0.007	mM NH_3/min mM NH_3/min·mg protein	Hamzah 2014

Note: n.a. – not applicable.

The extraction time and temperature vary across different studies. The resulting suspension is filtrated, and the filtrate is then centrifuged to remove suspended pulp. The solution with urease activity is referred to as the crude extract, which is often used for biocementation. Sometimes, the crude urease is further purified by the precipitation using acetone, ethanol, or ammonium sulphate (Bedan, 2020; EL-Hefnawy et al., 2014; Hirayama et al., 2000). For example, 50 g of dehusked watermelon seeds were soaked in 200 ml of an extractant at pH 7.5 for 12 h at 4°C. The mixture was then homogenized to disrupt plant cells for urease extraction, followed by filtration and centrifugation. The crude extract obtained was used to isolate purified enzyme via a two-step acetone precipitation. However, the highest strength was observed for sand treated with the crude urease rather than with urease with a higher degree of purification (Khodadadi Tirkolaei et al., 2020). Urease activity of some crude extracts from different plants is shown in Table 1.

The efficiency of extraction is influenced by the particle size of the plant material. It was shown that extract from soybean powder with particle sizes of 0.075-0.25 mm exhibited urease activity of 4.92 mM urea/min, whereas larger particles (1-2 mm) showed significantly lower activity, 2.95 mM urea/min (Shu et al., 2022). The enzymatic activity of the crude extract also depends on the concentration of plant powder used for extraction. Thus, extracts prepared from soybean powder at concentrations of 20, 40, 60, and 80 g/l exhibited urease activities of 2.15, 4.86, 6.62, and 8.17 mM urea/min, respectively (Shu et al., 2022). It is well known that during seed germination, metabolic processes are intensified due to the activation of enzymes (Guzman-Ortiz et al., 2019; Nkhata et al., 2018; Stabnikova et al., 2019; Wunthunyarat et al., 2020). In some studies, the authors carry out preliminary seed germination to enhance enzymatic activity prior to obtaining urease-containing extracts (EL-Hefnawy et al., 2014). The specific urease activity of soybean seeds reached a peak at 24 hour of germination, 0.24 mM/g·min, and then gradually declined, decreasing by 45% at 96 hours of germination (Stabnikov et al., 2022). The highest urease activity of the extract from germinated peas was observed at 192 hours of germination, 0.03 mM/ml, followed by a subsequent decrease (Pervin et al., 2013). Notably, the sprouts of germinated seeds from beans, soy, watermelon, and melon exhibited 23.3–34.5% lower urease activity compared to the seeds themselves (Stabnikov et al., 2022).

Application of plant extracts with urease activity for biocementation

The use of plant extracts with urease activity offers a sustainable and environmentally friendly alternative for various biogeotechnical applications, including soil improvement, mitigation of earthquake-induced liquefaction, and coastal erosion protection. Until recently, most studies have relied on commercially available urease with high enzymatic activity derived from jack beans. However, the successful use of ureases extracted from various plant sources for biocementation and soil biogrouting has been demonstrated. These plant-derived enzymes have proven their effectiveness, but their cost was significantly lower. Some examples of plant urease application in biocementation are given in Table 2.

Although the urease activity of plant extracts is generally lower than that of urease-producing bacteria commonly used in biocementation processes, their use is still advisanle for soil bio-strengthening and the mitigation of wind and dust erosion, as surface treatment of soil with small doses of biocement is effective in controlling wind and water erosion and significantly reduces the release of dust, bacterial contaminants, and chemical pollutants into the environment (Hao et al., 2021; Ivanov and Stabnikov, 2020; Namdar-Khojasteh et al., 2022; Stabnikov et al., 2013b). Moreover, it is believed that high urease activity is not necessary during biocementation, since the process of calcium carbonate crystal formation should proceed slowly, consistently filling the pores and cavities in the cemented material.

Table 2

Application of plant urease for biocementation

Source of urease and its activity	Characteristics of		Reference
	biocementation process	biocemented oil	
Crude soybean urease solution, 3.9 mM/l·min	Silica sand with a grain size of 0.425-0.6 mm; calcium chloride and urea 1M:1M; initial pH 7.5; 8 treatments	Permeability decreased from 10^{-3} m/s to 10^{-5} m/s, UCS 1680 kPa	Weng et al., 2024
Crude soybean extract, 0.7 mM urea/g·min	Screened river sand with a grain size of 0.0-0.6 mm (93%); calcium chloride and urea 1M:1M; 8 treatments	Permeability decreased from $6 \cdot 10^{-4}$ m/s to $1 \cdot 10^{-6}$ m/s	Stabnikov et al., 2022
Crude soybean extract, 6.5 mM/min	Soil prepared containing 10% clay particles (<0.005 mm), 50% silt particles (0.005–0.075 mm), and 40% sand particles (>0.075 mm); solution (calcium chloride and urea 0.5M:0.5M); 15 treatments	After 15 treatments, the soil at 100 kPa effective confining pressure had peak deviatoric stresses in the triaxial tests 250.0 kPa; for untreated soil it was 120.8 kPa	Gao et al., 2020
Jack bean extract	Sieved river sand with a grain size of 0.25- 0.075 mm, solution ($CaCl_2$ and urea), 3 days of treatment	UCS of the treated sand was 317 kPa; content of precipitated calcite 6.58%	Park et al., 2014
Watermelon seed extract, 3.9 µmol urea hydrolyzed/ min	Mikawa sand with mean diameter 0.87 mm, 0.7 M calcium chloride and 0.7 M urea, 14 treatments	UCS of the treated sand was 3.0 MPa	Dilrukshi et al., 2018
Soybean extract, 13 mM/min	Sand with average particle size 0.76 mm, 1.0 M calcium chloride and 1.0 urea, 30 °C for 7 days	UCS of the treated sand was 18.5 kPa	Cui et al., 2023
Crude soybean extract, 6.62 mM urea/min	Ottawa sand with a mean size of 0.36 mm, 0.5 M urea and 0.5 M $CaCl_2$, 8 treatments at 25°C	UCS of the treated sand was 4 MPa, $CaCO_3$ content 8%	Shu et al., 2022
Watermelon seed extract	Mikawa sand with a mean diameter 0.87 mm, solution ($CaCl_2$ and urea)	UCS about 1.2 MPa	Al Imran et al., 2021

Note: UCS, unconfined compressive strength.

However, it has been shown that the efficiency of plant urease in producing $CaCO_3$ is comparable to that of bacterial urease (Cui et al., 2024). Comparison of properties of sand treated with UPB and plant urease demonstrated that, for the same amount of precipitated calcium carbonate, samples treated with plant urease exhibited higher unconfined compressive strength (Ahenkorah et al., 2020; Almajed et al., 2018). Additionally, sand treated via enzyme-induced calcium precipitation (EICP) displayed also significantly greater splitting tensile strength compared to sand with similar average $CaCO_3$ content treated by MICP (Ahenkorah et al., 2020).

For example, treatment using plant-derived urease and solutions of urea and calcium chloride with molar ratio of 1.5:1 was proposed to be used for mitigating fugitive dust emissions in semi-arid and arid environments (Hamdan and Kavazanjian, 2016). The successful application of jack bean and soybean ureases for surface stabilization of desert sands has also been demonstrated (Liu L. et al., 2024). Notebly, the cost of the proposed plant-based enzyme sources for biocementation was reported to be 380-1400 times lower than that of commercially available ureases.

Numerous studies have shown the effectiveness of plant-derived urease in the biocementation of desert sand through $CaCO_3$ precipitation to protect it against wind erosion, enhance surface strength, stabilize sloped soils prone to landslides, and combat desertification. In these studies, soybean urease was the most commonly used enzyme source (Gao et al., 2020; He et al., 2023; Liu Y. et al., 2023, 2024).

The application of mix–induced carbonate precipitation, which combines microbial and plant-derived ureases to carry out biocementation, has been investigated. Biocementation of sand foreshore slopes with a mixture of *Sporosarcina pasteurii* cell suspension and soybean powder extract proved to be more effective than using microbial and plant ureases separately (Sun et al., 2022).

The advantages of enzyme-induced calcium precipitation are undeniable: (a) there is no need to cultivate microbial producer, significantly simplifying the overall process; (b) crude extract can be prepared immediately prior to use; (c) the cost of biocementation is considerably reduced, as there is no requirement for nutrient media, electricity for aeration, or highly qualified personnel to produce microbial biomass with urease activity; (d) one of the important problems of biocementation is completely solved – no microbial cells are introduced into the environment, and the aqueous solution of homogenized plant biomass generally does not require additional biosafety assessments.

Thus, biocementation using plant-derived urease represents an environmentally friendly and sustainable approach for addressing a range of geotechnical challenges, including soil stabilization, mitigation of earthquake-induced soil liquefaction, erosion control, and dust suppression (Liu L. et al., 2022; Stabnikov et al., 2022). Additionally, EICP has been proposed for applications such as crack repair and enhancing the mechanical strength of construction materials (Nam et al., 2014; Roksana et al., 2023).

Increase of urease activity in plant materials

Several studies have investigated the influence of plant growth conditions on the urease activity in plant material. Given that urease is a nickel-containing enzyme, the effect of nickel supplementtion in soil on soybean urease activity has been examined (Zhou et al., 2023). The addition of nickel to the soil contributed to an increase in urease activity in both the roots and shoots of soybeans (*Glycine max*). The introduction of NiO nanoparticles, nickel oxide (NiO), and nickel sulfate ($NiSO_4$), in the amount of 50 mg/kg of soil increased urease activity in both the shoots and roots of plants. It was also demonstrated that nickel supplementation is essential when rice is cultivated in soil amended with urea-containing fertilizer, as it ensures sufficient urease activity in the plants (Gerenda et al., 1998).

The presence of urea in the soil helps to increase the activity of the plant ureolytic enzymes, which provide them with nitrogen through the decomposition of urea and the release of ammonia. This ammonia is then utilized in the synthesis of amino acids and, subsequently proteins (Ono et al., 2021). In a field experiment on soybean cultivation, addition of nitrogen-containing fertilizer to clay loam soil resulted in a significant increase in the urease activity of the harvested seeds (Achakzai et al., 2003).

Some approaches to improve biocementation process

Calcium sources and their effectiveness for biocementation

A comparative study on the application of calcium chloride, calcium acetate, and calcium nitrate in silica sand biocementation using crude soybean extract with urease activity of 3.9 mmol/l/min and the cementation solution containing 1 mol/l of calcium salt and urea revealed that $CaCl_2$ produced the best results, followed by calcium acetate $CH_3(COO)_2Ca$, while calcium nitrate was the least effective calcium source (Weng et al., 2024). For sand samples with the same calcium carbonate content of 15%, the unconfined compressive strength (UCS) was 1680 kPa for those treated with $CaCl_2$, while samples treated with calcium acetate exhibited a significantly lower UCS of approximately 1000 kPa. The authors attributed these differences to variations in the morphology of $CaCO_3$ crystals formed during precipitation, which depend on the type of calcium salt used.

However, Xiang et al. (2022), testing $CaCl_2$, $(CH_3COO)_2Ca$, and $Ca(NO_3)_2$ in MICP using *S. pasteurii,* concluded that the best source for biocementation is calcium acetate followed by calcium chloride and calcium nitrate. The application of calcium acetate made it possible to produce more $CaCO_3$, which contributed to an increase in the biocement compactness.

Application of additives to enhance biocementation

There are some studies aimed to enhance the biocementation process by incorporating various additives into the cementation solution. While this strategy benefits both microbial and plant-based urease systems, it is particularly critical for plant urease treatments, as the absence of microbial cells on sand grain surfaces reduces available nucleation sites for $CaCO_3$ precipitation and can diminish overall treatment efficacy (Ahenkorah et al., 2021; Miyake et al., 2022).

A biocementing solution containing $CaCl_2$, urea, plant urease was added with non-fat milk powder (Almajed et al., 2019). Silica sand was mixed with this solution, placed in an acrylic column, and left to cure at room temperature (20°C) for three days. The resulting samples then were washed, oven-dried at 40 °C to a constant weight, and subjected to unconfined compressive strength testing. The authors supposed that milk powder could stabilize the urease, and enhance the biocementation process by reducing the rate of calcium carbonate precipitation and providing additional nucleation sites for carbonate deposition. The addition of milk powder resulted in an increase of the maximum axial compressive stress (MACS) of sand from 138±18 kPa to 1745±83 kPa (Table 3).

Table 3

Application of additives to enhance biocementation

Source of urease and its activity	**Characteristics of**		**Reference**
	Biocementation process	**Biocemented soil**	
Commercial urease from jack bean, ≈3.5 mM of NH_3 from urea/min·g	Ottawa silica sand, a mean grain size of 0.6 mm, non-fat powdered milk, 4 g/l, 1.0 M urea and 0.67 M $CaCl_2$ (1:1), 2.5 g/L of urease enzyme	Increased sand strenght: MACS of sand increased from 138±18 kPa to 1745±83 kPa	Almajed et al., 2019
Urease from jack bean (CAS 9002-13-5, Japan)	Toyoura silica sand, a mean grain size of 0.17 mm, casein, 64.28 g/l, 0.893 M urea and 0.581 M $CaCl_2$, 2.6 g/l urease enzyme	UCS increased to 3.58 MPa	Miyake et al., 2022
Bacillus sp. MTCC 4445	Poorly graded silty sand, rice husk ash, 10%, 1.0 M urea and 1.0 M $CaCl_2$	Reduced permiability by 22.5%	Sorum and Kalita, 2023
Pararhodobacter sp. SO1	Mikawa sand, mean diameter of 0.6 mm, particle density 2.66 g/cm^3, 0.03% chitosan, 0.3 M urea and 0.3 M $CaCl_2$ (1:1)	Increased sand strength up to 40%.	Nawarathna et al., 2020
Crude extract of soybean, ≈11 U/ml	Mikawa sand mean diameter of 0.87 mm, 0.2 – 8.0% eggshell powder, 0.5 mol/L $CaCl_2$ and 0.5 mol/l urea (1:1)	Increased sand strength	Yan et al., 2024
Sporosarcina pasteurii ATCC 11859	Ottawa silica sand, the average particle diameter 0.54 mm, synthetic fibers 0.2-0.3% by weight of dry sand, 0.187 M $CaCl_2$ and urea 0.187 M (1:1)	Increased the shear strength, ductility, and failure strain	Li M. et al., 2016
Bacillus sp., 3.7 mM/min	Ottawa silica sand, mean grain size of 0.73 mm, PVA fiber 0.8% by weight of sand, 0.3 M urea and 0.3 M $CaCl_2$ (1:1)	Increased UCS by 138%, STS by 186%, decreased permeability by 126%, reduction in brittleness	Choi et al., 2016a
S. pasteurii ATCC 11859	Glass beads (diameter 0.05–3 mm), bentanite (5% w/w of beads), 0.5 M $CaCl_2$ and 0.5 M urea (1:1)	Decreased hydraulic conductivity by 25%	Eryürük et al., 2015

Note: STS, splitting tensile strength; UCS, unconfined compressive strength.

It was shown that using casein instead of skim-milk in EICP allowed to receive biocemented sand with much higher compressive strength (Miyake et al., 2022). Under identical EICP conditions, the addition of 38.87 g/l of either milk powder or casein resulted in obtaining biocemented sand with a compressive strength of 1251 and 2439 kPa, respectively. This improvement may be attributed to the binding of calcium ions to the phosphate and carboxyl groups of casein, leading to the formation of casein micelles, which play the role of centers for calcium carbonate nucleation (Li X. et al., 2019). The improved cohesion of sand has also been observed when casein micelles were incorporated in the MICP process (Nakano, 2024).

Positive effect of chitosan addition on the MICP initiated by *Pararhodobacter* sp. SO1was shown by (Nawarathna et al., 2020). Chitosan powder was first dissolved in 1% acetic acid, then the resulting 1% chitosan solution was neutralized to pH 6.8 using 0.1 M NaOH. Prepared solution was then added to the MICP process at a final concentration of 0.03% during the biocementation of sand. The presence of chitosan led to a higher amount of precipitate compared to the control, with the precipitate consisting of both $CaCO_3$ and chitosan hydrogel. As a result, the compressive strengths of cemented sand increased by up to 40% relative to sand treated without chitosan.

The addition of eggshell powder to sand treated with crude soybean extract increased the number of nucleation sites, resulting in enhanced strength of the biocemented sand compared to samples processed without the additive (Yan et al., 2024). Similarly, the incorporation of 0.25 wt.% of chitosan, a natural biodegradable linear polysaccharide derived from shrimp shells, into silica sand led to a slight improvement in the mechanical properties of the biocement after 28 days of curing (Baykara et al., 2024). It was found that the addition of fibers of different origin such as polypropylene monofilament fibers (Li M. et al., 2016), poly(vinyl alcohol) (PVA) fibers (Choi et al., 2016a), and basalt fibers (Xiao et al., 2019) increased the mechanical properties of biocemented sand (Table 3).

Positive effects on the properties of biocemented sand have been reported with the addition of various organic and biodegradable materials. These include wastepaper fibers addition to sand treated with *Lysinibacillus xylanilyticus* (Chen et al., 2021); glutinous rice powder, brown sugar, and skim milk powder in systems utilizing soybean urease (Yuan et al., 2020); and guar gum, xanthan gum, and polyol-cellulose hydrogel in treatments with jack bean urease (Hamdan et al., 2016).

Magnesium chloride has been shown to be one of the most effective additives for enhancing soil strength during EICP. The addition of 0.5 M Mg^{2+} to a solution of calcium chloride and urea resulted in biocemented sand with a maximum UCS of 6.2 MPa (Lv et al., 2022). Similar results were reported by Xu X. et al. (2020), who demonstrated that the presence of 0.01 M Mg^{2+} ions in a 0.5 M calcium acetate/urea solution enhanced the UCS of biocemented sand by 40%, while a higher Mg^{2+} concentration of 0.5 M led to a twofold improvement in strength. It was found that the presence of Mg^{2+} changes the crystal morphology of precipitates from calcite to Mg-calcite, vaterite, rosette and nesquehonite (Wang W. et al., 2024).

Cost reduction strategies for biocementation using industrial waste

One of the main chemical components used in traditional MICP biocementation is a soluble calcium salt, typically calcium chloride $CaCl_2$. However, its use as a chemical reagent contributes to the overall cost of the biocementation process. Moreover, while $CaCl_2$ is generally considered harmless to plants and soil, possible adverse effects have been noted as chloride ions increase the likelihood of corrosion of steel reinforcement (Wang Q. et al., 2024).

Thus, one approach to improving the biocementation process involves replacing calcium chloride with different calcium containing compounds. Currently, extensive research is focused on isdentifying suitable substitutes for calcium ion sources in biocementation (Yan et al., 2025). Calcium acetate has attracted particular attention as a possible source of calcium in biocementation processes due to its ability to produce biocement with higher mechanical strength compared to that ontained using $CaCl_2$, while also reducing NH_3 emissions by 54.2% (Xiang et al., 2022; Zhang et al., 2014).

As possible replacer for $CaCl_2$, solution containing Ca^{2+} obtained by dissolving a limestone powder in acetic acid solution, a byproduct from the fast pyrolysis of lignocellulosic biomass, was proposed (Choi et al., 2017). The calcium ion was produced:

$$CaCO_3 + 2\ CH_3COOH \rightarrow Ca^{2+} + 2\ (CH_3COO^-) + CO_2 + H_2O$$

Solution used in biocementation of sand had calcium ion concentration 0.3 M, and pH, adjusted by solidum hydroxide, 7.0–7.5. The permeability of Ottawa sand treated with *S. pasteurii* reduced from 1×10^{-4} m/s to 8.2×10^{-6} m/s (Table 5).

Table 5

Replacement of calcium chloride in biocementation process

Source of urease and its activity	**Characteristics of**		**Reference**
	substitute of $CaCl_2$	**biocementation process**	
Sporosarcina pasteurii ATCC 11859, 8 - 15 mM/min	Dissolved limestone powder containg 50.7% CaO in 7% (w/v) acetic acid	Ottawa sand was treated with solution of urea, 0.3 M, + Ca^{2+}, 0.3M, for 7 days. The permeability was reduced from 1×10^{-4} m/s to 8.2×10^{-6} m/s	Choi et al., 2017
Extract of soya bean seeds (seed powder, 50 g, in distilled water, 1 l)	Eggshells treated with acetic acid to obtain solution of calcium acetate, 0.39 mol/l	Poorly graded natural soil was treated with solution of calcium acetate and urea in molar ratio 1:1 for 7 days to obtain sand with UCS of 371 kPa	Hoque and Islam, 2023
Sporosarcina pasteurii ATCC 11859	A limestone powder treated with acetic acid	Desert eolian sand with particle sizes 0.106-0.178 mm, ratio of Ca^{2+} : urea= 1:2.6, UCS increased by 10.6%	Feng et al., 2023
Extract of soybean, 6.66 mM/min	Waste concrete treated with 31.0% HCl	Compressive strength did not differ from sand treated with $CaCl_2$ reagent	He et al., 2022

A number of works are devoted to the use of eggshells in biocementation (Choi et al., 2016b; Kulanthaivel et al., 2022; Raheem and Jabbar, 2024). Eggshell powder was treated with an aqueous acetic acid solution for 7 days, to obtain concentration of calcium acetate, 0.39 mol/l (Hoque and Islam, 2023). Treatment using urease soybean extract and solution containing calcium acetate and urea in molar ratio 1:1 resulted in producing the biocemented sand with UCS of 371 kPa, which was by 8.5% higher than that of sand with the same calcium content, but treated with $CaCl_2$.

Waste concrete was treated with 31% hydrochloric acid to obtain dissolved calcium ions for further use in sand biocementation (He et al., 2022):

$$CaCO_3 + 2\ HCl \rightarrow Ca^{2+} + 2\ Cl^- + H_2O + CO_2\uparrow$$

A comparative study with sand treated with equimolar $CaCl_2$ and urea of the same concentration as a control showed no significant difference in the compressive strength of both biocemented samples (Table 4).

To obtain dissolved calcium ions, limestone powder with a particle size of about 0.282 mm was treated with acetic acid at 55°C, using a solid-to-liquid ratio of 1:14 and an acetic acid dosage of 163% (Feng et al., 2023). The UCS of sand samples treated with calcium ions derived from limestone was 10.6% higher compared to the control sand treated with $CaCl_2$, while the cost of MICP using $Ca(CH_3COO)_2$ (prepared from limestone) was reduced by 31.9%.

To replace $CaCl_2$ in the EICP process for treating sandy soil, it was proposed to use industrial waste calcium carbide slag powder dissolved in an acetic acid solution (Qi et al., 2022). No significant differences were observed between the properties of sand treated with reagent-grade $CaCl_2$ and those treated with the substitute. Therefore, utilizing waste or recycled materials in biocementation can significantly reduce the cost of soil improvement while also mitigating environmental pollution from unused industrial by-products. A replacement for both chemical reagents commonly used in the biocementation process, calcium chloride and urea, has been proposed. For example, eggshells have been used as a source of Ca^{2+} and cow urine as a source of urea (Sreekala et al., 2024). Similarly, waste carbide sludge dissolved in 0.8 M waste acid has served as a Ca^{2+} source, while urine has been used instead of urea for the cultivation of urease-producing bacteria (Yang et al., 2022).

Some studies have proposed using cheaper sources of urea to reduce the cost of the biocementation process by replacing the laboratory-grade materials with commercially available alternatives, for example, substituting urea with agricultural fertilizers and using commercially available $CaCl_2$ sold as a snow-melting agent (Moqsud and Gochi 2024). This approach has been shown to reduce the cost of materials for the biocementation process by up to 90%.

Methods for prevention of ammonium release in the environment

One of the obstacles to the large-scale application of biocementation is the release of ammonia gas into the atmosphere, along with the leaching of ammonium and hydroxide ions into groundwater and surface water (Gowthaman et al., 2021a; Ivanov et al., 2019b). During conventional MICP, the pH of the environment typically rises to 8.5 - 9.5 due to the release of ammonium ions and ammonia. It is known that at a pH ≤ 7.3, approximately 99% of the released nitrogen remains in the form of ammonium ions (NH_4^+). However, when the pH exceeds 7.5, a sharp shift occurs toward gaseous the formation of gaseous ammonia (NH_3) (Gowthaman et al., 2021a; Whiffin, 2004).

Ammonium concentrations in groundwater are typically below 0.2 mg/l. According to the European Commission (2012), the threshold value for NH_4^+ in groundwater is 5 mg/l. Ammonia is considered as one of the five main air pollutants in the European Union, and its permissible concentration in surface waters in the United States ranges from 0.25 to 32.5 mg/l, depending on local conditions and regulations (Ammonia, 2020).

It has been estimated that biocementation or biogrouting of sandy soils requires the precipitation of at least 62 kg of calcium carbonate per one m^3 of sand. This process results in the emission of approximately 10.5 kg of ammonia into the air and 11.2 kg of ammonium into ground or surface waters (Ivanov et al., 2019a). Therefore, it becomes clear that a critical challenge for the widespread adoption of biocementation is the effective limitation of ammonium and ammonia emissions into the environment.

To prevent environment contamination with ammonium ions, several methods involving additional treatments steps have been proposed including: (1) the use of calcium acetate instead of calcium chloride as calcium source. For example, replacing $CaCl_2$ with $Ca(CH_3COO)_2$ during the biocementation of sand using *Sporosarcina pasteurii* reduced NH_3 emission by 54.2% while also improving the strength of biocemented samples (Xiang et al., 2022); (2) the addition of zeolite, a negatively charged aluminosilicate mineral, to adsorb the resulting ammonium and produce an environmentally friendly soil stabilizer altogether with increasing of biocemented soil strength and reducing its permeability (Erdmann and Strieth, 2022; Keykha et al., 2019; Su et al., 2022); (3) the maintenance of ammonium by the graphite cathode electrode during electrobiocementation of soil to prevent the leakage of NH_4^+ ions into the bulk of the soil (Keykha and Asadi, 2017); (4) removal of ammonium formed during soil biocementation by injection of solutions enriched with cations (Lee and Gomez, 2024; Lee et al., 2019); (5) rinsing the ammonium from the sand with subsequent its recovery by addition of Na_2HPO_4 and $MgCl_2$ at pH 8.5 with Mg^{2+}:NH_4^+: PO_4^{3-} molar ratio of 1.2:1:1 as struvite ($NH_4MgPO_4 \cdot 6H_2O$) (Mohsenzadeh et al., 2022); (6) biocementation of sand with *Sporosarcina pasteurii* using solutions containing $K_2HPO_4 \cdot 3H_2O$, $MgCl_2$, and urea led to the formation of struvite in the treated sand. UCS of the treated sand was 1.47 MPa, while ammonia gas emission was reduced by 75% compared with traditional process using $CaCl_2$ (Yu et al., 2021). To protect the atmosphere from ammonia release, adsorption of removed gas ammonia with sulfuric acid could be used (Ivanov and Stabnikov, 2017).

A promising and environmentally friendly alternative to reduce toxic ammonia emissions is calcium phosphate-based biocementation (Avramenko et al., 2022; Ivanov et al., 2019a). Calcium phosphate is a promising structural material with sufficient strength, while its biosafety is known (Toshima et al., 2014). Its use for soil improvement offers several advantages: (a) calcium phosphates are non-toxic and environmentally friendly materials (Kohn et al., 2002); (b) calcium phosphate present in the soil can act as a fertilizer, promoting plant growth. The formation of strong calcium phosphate crystals can be carried out from both inorganic and organic phosphate sources. Precipitation via this method can be achieved through enzymatic hydrolysis of urea, during which the pH increases from 4.4 to 7.0.

The economic advantage of hydroxyapatite biosolution lies in the possibility of using bone meal, an abundant by-product of meat processing, as a starting material. It has been found that calcium and phosphate in bone meal are present in the form of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$ (Gowthaman et al., 2021b). Acid hydrolysis of bone meal converts calcium into a soluble form and solution for biocementation with pH 3.4 and containing 8800 mg of Ca^{2+} per 1 l was received:

$$Ca_{10}(PO_4)_6(OH)_2 + 8\ HCl \rightarrow 10\ Ca^{2+} + 6\ HPO_4^{2-} + 8\ Cl^- + 2\ H_2O$$

To provide biocementation process, commercial acid urease Nagapshin, 4 g/l, was dissolved in the solution for biocementation to obtain urease activity of 2.5 mM/min at pH 4.0. Ratio of calcium: urea was 1.5, while in traditional biocementation it typicall ranges from 0.66 to 1.0. The biocemened poorly graded fine sand achieved a uniaxial compressive strength of 1.5 MPa. It was estimated that the release of ammonium ions was reduced by approximately 50%, and toxic ammonia emissions were about 90% lower compared to traditional biocementation.

An alternative biocementation process that combines the precipitation of struvite (NH_4MgPO_4) and calcium carbonate using triple superphosphate and a magnesium salt has also shown to be more environmentally friendly. This approach prevents the formation of free ammonium and the release of ammonia into the atmosphere during the biocementation process (Ivanov and Stabnikov, 2020):

$$Ca(H_2PO_4)_2 + 2\ Mg^{2+} + CO(NH_2)_2 + H_2O + \text{acid urease} \rightarrow 2\ NH_4MgPO_4\downarrow + CaCO_3\downarrow$$

To perform these types of biocementation, urease active at acidic pH is required. Commercial acid urease synthesized by *Lactobacillus fermentum* was used in the study (Gowthaman et al., 2021a), while the strain *Staphylococcus saprophyticus* AU1, isolated from acid soil, was employed in biocementation based on the calcium phosphate formation (Stabnikov et al., 2024).

Potential new bioagents for biocementation

Carbonate rocks such as calcite, aragonite, and dolomite contain large amounts of carbon in the form of calcium carbonate and represent the largest natural carbon storage sites (Zhu and Dittrich, 2016). Biodeposition of minerals is a common phenomenon in the biological world, mediated by bacteria, fungi, protozoa, and plants. Biomineralization in nature leads to the formation of more than sixty different biological minerals that exist as either extracellular or intracellular inorganic crystals. Calcium carbonate is one such mineral that naturally precipitates as a byproduct of microbial metabolic activity (Seifan and Berenjian, 2019). Carbonate precipitation can be initiated by chemotrophic and phototrophic microorganisms that produce carbonate ions through an increase in pH (Cacchio et al., 2003; Castanier et al., 1999; Wright and Oren, 2005).

There are three main groups of microorganisms responsible for carbonate precipitation in nature. These are photosynthetic microorganisms such as cyanobacteria and microalgae (Ariyanti et al., 2012); sulfate-reducing bacteria (Warthman et al., 2000), and some types of microorganisms involved in the nitrogen cycle, namely nitrogen-fixing bacteria, nitrifying bacteria, denitrifying bacteria or ureolytic bacteria (Castanier et al., 1999; Hammes and Verstraete, 2002; Stocks-Fischer et al., 1999). The formation of calcium carbonate, $CaCO_3$, is one of the most studied and understood biomineralization processes (Bang et al., 2010; Fernandez et al., 2018; Ronholm et al., 2014). Thus, biocement is considered an ecological material, the creation of which was inspired by nature itself (Achal et al., 2015) (Figure 3a) in contrast to artificial biocementation using urease-producing bacteria (Figure 3b).

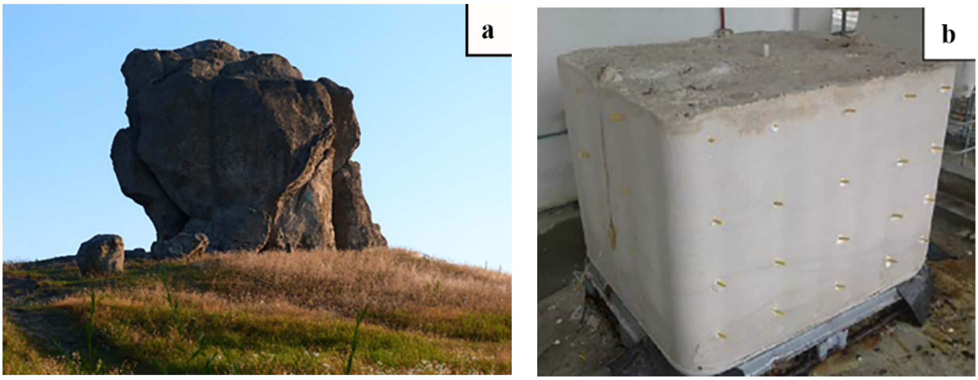

Figure 3. A rock in the village of Pidkamin, Ukraine, an object of natural biocementation, approximately 11 million years old (Stabnikov et al., 2022) (a); 1 m^3 of stone and sand mixture after biocementation (Stabnikov et al., 2015) (b)

Lactic acid bacteria

Ureases currently used in biocementation processes have optimal activity at pH values in the neutral to alkaline range, and are not only labile and prone to deactivation in an acidic environment, but also do not even catalyze the urea hydrolysis. More often, biocement is produced by precipitation of calcium carbonate at alkaline pH. However, the use of acid urease may be advantageous for biocementation in environments with a low initial pH, such as when calcium bicarbonate is used as the calcium source or during the production of calcium phosphate (Gowthaman et al., 2021a).

Lactic acid bacteria (Fig. 2c5), which are generally recognized as safe by both the US Food and Drug Administration and the European Food Safety Authority (Gowthaman et al.„ 2021a; Stabnikova et al., 2024), can serve as producers of acid urease (Stabnikov et al., 2025). Lactic acid bacteria can produce urease as a response to stress caused by low pH for neutralizing the acids formed. Among lactic acid bacteria producing urease there are strains of *Streptococcus thermophilus* used for production of yogurts and cheeses (Arioli et al., 2017; Scala et al., 2019), *Bifidobacterium longum* subsp. *infantis* (LoCascio et al., 2010), probiotic bacteria *Lactobacillus reuteri, Streptococcus thermophilus*, *S. salivarius* (Power et al., 2008; Udymovych, 2021; Wilson et al., 2014). Urease active at a pH range of 2.0–4.0 was first discovered in *Lactobacillus* sp. by Japanese researchers in 1988 (Fidaleo et al., 2006; Ough and Trioli, 1988). Commercial acid urease, Nagapshin, produced using *Lactobacillus fermentum*, was applied in the study of Gowthaman et al. (2021a) for the biocementation of sand, employing acid hydrolysate of bone meal as a source of hydroxyapatite.

Fungi

The potential use of fungal strains such as *Penicillium chrysogenum* (Fang et al., 2018; Martuscelli et al., 2020), *Aspergillus* sp. and *Fusarium oxysporum* (Dhami et al., 2017), *Pestalotiopsis* sp. and *Myrothecium gramineum* (Li et al., 2015) for calcium carbonate precipitation in soil biocementation has been shown. It was reported that the fungi *Aspergillus* sp. UF3 and *Fusarium oxysporum* UF8 synthesized carbonic anhydrase in addition to urease and produced calcite, vaterite, and aragonite during biomineralization using calcium oxalate as the calcium source (Dhami et al., 2017). Beside to the fact that the use of urease-producing fungi leads to the precipitation of calcium carbonate, the introduction of mycelial biomass into biocemented material can serve as a biological fiber additive, contributing to increased strength (Martuscelli et al., 2020). The fungal strains used for biocementation were isolated from alkaline soils (Devgon et al., 2024; Fang et al., 2018), calcareous soil (Li Q. et al., 2015) or karstic caves (Dhami et al., 2013). However, in the studies conducted, the potential biosafety risks associated with the use of fungi, including plant pathogens such as *Fusarium oxysporum*, *Myrothecium gramineum*, *Pestalotiopsis* sp., and others, in biocementation processes remain unexplored.

Microalgae

Photosynthetic organisms are considered to have played a major role in the formation of the world's carbonate deposits over the last 3 million years of Earth’s history (Altermann et al., 2006). Photosynthetic microorganisms utilize carbon dioxide, which is in equilibrium with HCO_3^- and CO_3^{2-}. When carbon dioxide is consumed by photosynthetic microorganisms, the pH increases and, in the presence of calcium ions, calcium carbonate is formed (Ariyanti et al., 2011, 2012):

$$CO_2 + H_2O \rightarrow (CH_2O) + O_2$$
$$2\ HCO_3^- \leftrightarrow CO_2 + CO_3^{2-} + H_2O$$
$$CO_3^{2-} + H_2O \leftrightarrow HCO_3^- + OH^-$$
$$Ca^{2+} + HCO_3^- + OH^- \leftrightarrow CaCO_3 + 2\ H_2O.$$

Microalgae can promote the formation of calcium carbonate ($CaCO_3$) by thriving in alkaline environments saturated with Ca^{2+} ions. For instance, the formation of calcite and aragonite was observed in solutions with an initial pH 8.5, saturated with Ca^{2+} and containing the microalgae *Scenedesmus obliquus* (Santomauro et al., 2012). Currently, the application of microalgae (Fig. 2c6a) is proposed for biocement production and the self-healing of concrete cracks (Nur and Dewi, 2024).

Coccoliths, calcified haptophytes, are unicellular free-living microalgae whose cells are covered with calcified scales. They play an important role in the global carbonate cycle (Young and Henriksen, 2003). The potential use of these microalgae for biocementation is being explored as a promising approach (Al-Mardeai et al., 2024), alongside green microalgae such as *Chlorella* sp. (Ariyanti et al., 2012). Notably, the ability of microalgae *Chlorella kessleri* to produce calcium carbonate precipitates using waste cement kiln dust was demonstrated in a study (Irfan et al., 2019).

Cyanobacteria

Several studies have investigated the use of cyanobacteria (Fig. 2c6b) for biocementation (Chandra et al., 2023; Jang et al., 2023; Sidhu et al., 2022; Son et al., 2024). Cyanobacteria, commonly known as blue-green algae, are prokaryotic autotrophic algae-like bacteria that contain chlorophyll and are capable of photosynthesis, releasing molecular oxygen, which enriches the environment. Cyanobacteria inhabit diverse environments, including fresh, brackish, and saltwater ecosystems, and are active members of the soil microbiota.

A schematic illustrating the use of cyanobacteria in the manufacturing of living building materials (LBMs) is shown in Figure 4 (adopted from Son et al., 2024). The production of calcium carbonate ($CaCO_3$) by cyanobacteria offers the advantage that there is no CO_2 gas emissions during precipitate formation.

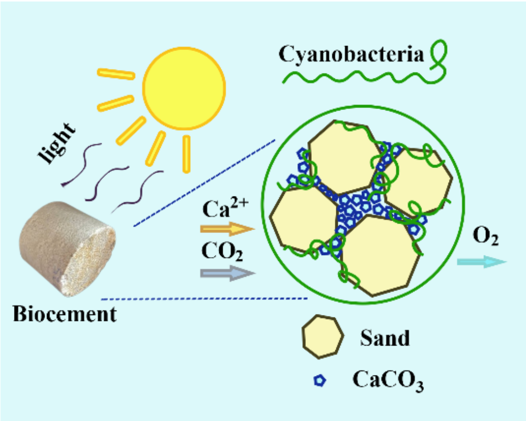

Figure 4. Schematic use of cyanobacteria for MICP formation (adopted from Son et al., 2024)

It has been suggested that cyanobacteria could enhance the mechanical properties of concrete by precipitating $CaCO_3$ crystals within its pores and microcracks. For example, the compressive strength of sand treated with cyanobacteria increased after 28 days of curing by 25.5% using *Synechocystis pevalekii* (Sidhu et al., 2022) and by 13% using *Leptolyngbya boryana* (Son et al., 2024) compared to control specimens without microorganisms.

Assesment of the carbon footprint for the production 1 kg of $CaCO_3$ by different methods showed that using cyanobacteria results in a lower carbon footprint of 0.815 kg CO_2/kg $CaCO_3$, compared to ureolytic bacteria at 1.51 kg CO_2/kg $CaCO_3$, and the convential carobonation process at 2.37 CO_2/kg $CaCO_3$ (Porter et al., 2021).

Conclusions

Biocementation, as a microbial-initiated calcite precipitation (MICP), presents a promising alternative to traditional cement production. It enables the creation of building materials with valuable properties while reducing energy consumption and environmental impact through lower greenhouse gas emissions. However, large scale application of biocementation faces key challenges: economically, due to relatively high production costs, and the ecologically, due to the introduction of live bacterial cells and the associated release of ammonium and ammonia into the environment.

To overcome these obstacles it is possible: (i) to reduce the cost of the biocementation process (1) by using various industrial wastes both to obtain the microbial component and to replace the chemical components of the process, namely calcium chloride and urea; (2) to use plant urease instead of urease of microbial origin; (ii) to improve the biosafety of biocementation (1) by using safe microbial urease producers or inactivated bacterial cells of urease-producing bacteria with preserved urease activity; (2) by using plant urease; (3) by using technologies that reduce the release of toxic ammonia and ammonium ions into the environment.

To improve the efficiency of the biocementation process, methods such as the introduction of additives, increasing the urease activity of plants, and the development of biocementation technologies using new bioagents are promising areas for further research.

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