Biocementation through the carbonic anhydrase (CA) activity of microorganisms -A review

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Abstract

Biocementation is the process of producing cement naturally through the metabolic activity of microorganisms. This technique has emerged as a sustainable alternative to conventional cement for various applications in the civil engineering construction industry, such as crack repairs in concrete or heritage buildings or soil stabilization, among others. While a few metabolic pathways have the potential to induce biocementation by mineral precipitation, most of the research works and applications of biocementation are based on urease enzyme mediated biocementation. Despite being the most straightforward process, the ureolysis route suffers from the disadvantage of ammonia production. The carbonic anhydrase pathway for biocementation is thus of interest due to its potential of producing biocement while also sequestering atmospheric CO_2. This paper discusses the possibility of microorganisms sequestering CO_2 and fixing it into different carbonate minerals using carbonic anhydrase activity. This contribution reviews this metabolic pathway and the few works that have studied it for applications such as CO_2 sequestration and biocementation for civil engineering and environmental applications.

Keywords: biocementation, carbonic anhydrase, carbon sequestration, soil, concrete

1. INTRODUCTION

Biocementation is the process of producing cement naturally through the metabolic activity of microorganisms via microbial calcium carbonate precipitation (MICP). This technique has emerged as a sustainable alternative to the use of conventional cement for various applications in the civil engineering construction industry, such as crack repairs in concrete [1], biobricks [2], repair of heritage buildings [3]; soil stabilization [4], [5], bioremediation [6], and wastewater treatment [7]. The wide application of this technique is due to reduced CO_2 emissions [8], increased resistance to both wind and water erosion [9], [10], potential for self-healing [8], and durability in terms of the dry-wet cycle and freeze-thaw cycle [11]. The formation of calcium carbonate as a binding agent in the biocementation process is facilitated by various microbial metabolic pathways that include ureolysis [12]–[14], carbonic anhydrase producing microorganisms (CA) [15], photosynthesis [16], denitrification [17], [18], and methane oxidation [19]. A summary of the relative advantages and limitations of the biocementation processes is shown in Table 1.

The vast majority of research works are based on urease enzyme-mediated biocementation through ureolytic bacteria [24], [25], [34], [35]. The disadvantage of this route is the production of ammonia as a by-product. Ammonia has adverse effects on the soil environment [36], biodiversity [37], and groundwater resources [38]; hence it is not desirable. Thus, this paper discusses the potential of microorganisms that can assist in sequestering CO_2 and fixing it into different carbonate minerals used for various civil engineering applications using the CA activity as an alternative to the ureolytic pathway. According to the authors' knowledge, CA has been suggested for CO_2 sequestration [20], [39], [40], and very few works have studied it for biocementation for civil engineering and environmental engineering applications [41]. The following sections review the advantages of carbonic anhydrase pathway in the biocementation process and make suggestions.

Table 1: A summary of the literature showing advantages and limitations of various biocementation pathway

Biocementation routes	Advantages	Limitations
Carbonic anhydrase	• Less carbon footprint via CO_2 sequestration [20], [21] • Energy-efficient process [8] • No toxic by-products [22]	• Poor stability and suboptimal use of CA [23]
Ureolysis	• Straightforward and easily controlled [24], [25] • Fast process [26]	• Large carbon footprint [8] • Energy inefficient process [27] • Toxic by-product (NH_4) [28]
Photosynthesis	• No toxic by-products [29],[30]	• Application limited to structures exposed to CO_2 and sunlight [31] • More carbon footprint [8]
Denitrifying	• Applicable under aerobic and anoxic conditions [32]	• Slow reaction rate [33] • Possibility of gas generation [33] • More carbon footprint [8]
Methane oxidation	• Less aggressive by-products to building materials [19]	• Large carbon footprint [8]

2. Mechanism of MICP via Carbonic Anhydrase pathway

Carbonic anhydrase is an enzyme with an active site that contains a Zinc ion (Zn^{2+}) that can convert CO_2 and water into carbonic acid, protons, and bicarbonate ions. The role of the Zn^{2+} in CO_2 conversion is to facilitate the water to create a proton H+ and a nucleophilic hydroxide ion with the mechanism well investigated [42]. The enzyme CA has been of interest and a subject of study since its first discovery in 1933 by Meldrum and Roughton in mammalian red blood cells of cattle [43]. Since then, the CA has been subsequently found in plants [44], algae [45], and microorganisms [46], [47]. The past few decades have proposed the usage of CA for industrial applications such as carbon sequestration[48]–[50]and biofuel production [51]–[53]because of the distinctive CO_2-catalyzing properties. CA has been proposed more recently for biocementation as CA can hydrolyse 600 000 molecules of CO_2 per CA per second [54]. For the application of CA in construction, both bacterial-CA and purified enzymes induce calcium carbonate precipitation. The biochemical process involves gaseous CO_2 dissolving in water to form hydrated aqueous CO_2 (aq) (Eq. 1), which reacts with water to form H_2CO_3 (Eq. 2), whose ionisation in water generates H^+ and HCO_3^- (Eq. 2). Under alkaline conditions, the HCO_3^- further ionizes to form CO_3^{2-} and H_2O (Eq. 3). In the presence of a calcium source (Ca^{2+}), $CaCO_3$ precipitates are formed by reacting with CO_3^{2-} (Eq. 4) with the bacterial cells serving as nucleation sites [55]. The rate at which the CA biocementation occurs depends on several factors such as temperature and pH value [21], calcium source and concentration [56], and the enzyme activity of CA bacteria [57].

$CO_2\ (g) \leftrightarrow CO_2\ (aq)$ Eq. 1
$CO_2\ (aq) + H_2O \leftrightarrow H_2CO_3$ Eq. 2

$H_2CO_3 \leftrightarrow H^+ + HCO_3^-$ Eq. 3

$HCO_3^- + OH^- \leftrightarrow CO_3^{2-} + H_2O$ Eq. 4

$Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO(s) \downarrow$ Eq. 5

The above indicates that the CA-producing bacteria or enzyme application to civil engineering applications is environmentally friendly as it has less carbon footprint than the other pathways [58]. The potential applications in the subsequent subsections will discuss how this can be applied in civil and environmental engineering and suggest areas for further investigations.

3. CURRENT APPLICATIONS OF CA-MICP

Carbonic Anhydrase-MICP (here referred to as CA-MICP) has been suggested for concrete and self-healing, ground improvement, and bioremediation. The biocementation technology via the MICP to develop biocement has gained attention as an alternative to conventional building materials as the latter forms at ambient temperature [43]–[46]. This technology paves the way for low temperature and low energy biocement compared to the cement production process produced at high temperatures ranging from 1300-1450 °C [47]. A typical setup for CA biocement production would involve combining CA-producing bacteria, CO_2, media, and a calcium source mixed with sand [29].

3.1. Concrete and self-healing of cracks in concrete

The CA-MICP method was proposed for concrete and self-healing applications for concrete-based cracks repair [59], [60]. The formation of cracks on concrete surfaces is normal as concrete ages or due to stresses caused by various factors [61], [62]. The conventional way of addressing the cracks involves the injection of either cement grout or epoxy into the concrete. However, environmental and health hazards such as allergies, asthma, and irritations of the eyes, nose, and throat have been cited for using these chemicals [63], [64]. CA-MICP crack repair occurs by two methods; cracks are repaired by sealing the microcrack and incorporating the CA enzyme in the cement paste. When a gap appears, it is restored by activation of the components in the concrete. The two methods used are illustrated in Figure 2.

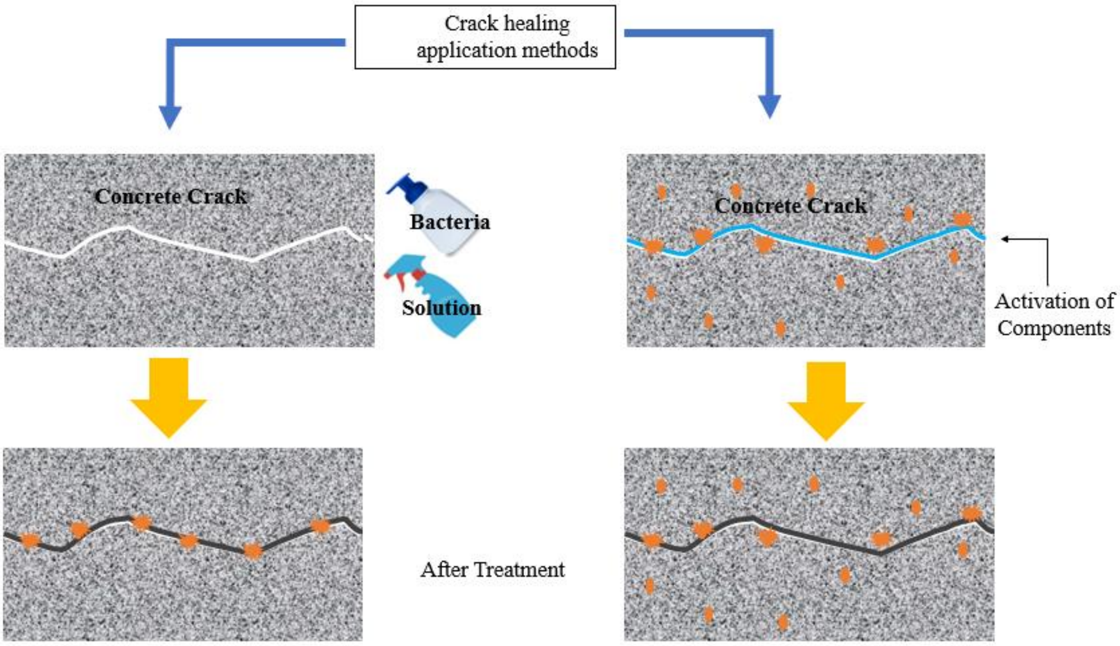

Figure 1: A schematic presentation of methods for crack self-healing CA-MICP

A recent study illustrates the first methodology of crack repair, which utilised *Bacillus mucilaginous L3*, a CA-producing bacteria that showed crack repairing ability by healing small cracks within seven days [59]. This study was able to repair tiny cracks and predict the depth of the

$CaCO_3$ precipitated layer based on the expected and experiment results. Cracks in the concrete below 0.4mm were almost fully closed using this method. Further, in 2016, investigators reported bio-restoration and self-healing of cement-based damages by immobilizing bacteria and nutrients [65]. This study showed that *Bacillus sphaericus* could use CO_2 to precipitate $CaCO_3$ in cracks when reacting with soluble Ca^{2+} providing a self-healing effect of the cement-based materials.

The second methodology is where the CA-enzyme is mixed in the cement during the concrete preparation. This method relies on the fact that any seepage of water and air through cracks leads to the release of incorporated components in the concrete premix to initiate the CA-MICP process leading to sealing of the gaps. A recent study suggested using CA into a cement paste mix to develop a self-activated healing cement paste [66]. The results showed that hydration of samples containing CA after fracture promoted the formation of calcium carbonate crystals at ambient temperature. These results further prove that using the CA enzyme to repair cracks is practical as it can percolate into the small cracks and seal microcracks in concrete. Similar results have been reported using other pathways [67], [68]; however, CA-MICP has been deemed superior because of the stability of the CA enzyme, the possibility of low production cost of CA, and reusability [27].

3.2. Soil improvement

The CA-MICP can be used for soil improvement either by using CA enzyme or CA-producing bacteria that can induce calcium carbonate precipitates between the soil particles and improve the mechanical properties. To date very few works have studied CA-MICP for soil improvement [69]. A recent study used different calcium sources to examine the sand biocementation by a CA-carbonic anhydrase-producing bacteria [56]. The study showed that CA-producing bacteria could precipitate calcite in a sand column using various calcium sources calcium nitrate ($Ca(NO_3)_2$), calcium acetate ($Ca(CH_3COO)$, and calcium chloride ($CaCl_2$), indicating improved soil mechanical properties. The results showed that the sand column using the $Ca(NO_3)_2$ had the highest shear strength of 62.33 kPa, followed by the $CaCl_2$, whereas $Ca(CH_3COO)_2$ had the least (11.19 kPa). Another study explored the CA-MICP pathway by biostimulation. Biostimulation is the addition of nutrients to a population of microorganisms to stimulate growth and activity during soil improvement or bioremediation, or biotreatment of soils. This study showed how the microbial community of calcareous soils was improved to precipitate calcium carbonate by ureolytic and CA-producing bacteria [69]. The synergy between ureolytic and CA-producing bacteria was observed and could be promoted by different pathways simultaneously,, as in the natural environment no single process exists in isolation. The advantage was an increased biocementation efficiency as high nutrient conditions as calcite precipitation ranging from 50 - 72% was observed in the treated soils.

3.3. Bioremediation

Apart from the reported effectiveness in improving and enhancing the mechanical properties of soils, CA-MICP can be applied to bioremediation by either fixation or leaching, as suggested by a recent study on fly ash [70]. The results showed that *Bacillus mucilaginosus* in the carbonation of steel slag increased the compressive strength ranging from 7.4 to 11.2 MPa. The mechanical property improvement in bioremediation is desirable as it reduces permeability, eliminating the contact of water and oxygen to leach heavy metals from biocemented material. A recent study revealed similar results for MICP induced by ureolytic bacteria on kiln slag [71] and chromium slag [72]. This shows promise that CA-MICP could work to the same effect, but further research is required as there are few studies.

4. CA-MICP FUTURE SCOPE AND PERSPECTIVES

The carbonic anhydrase pathway is a promising pathway to investigate due to its many advantages for biocementation applications in civil and environmental engineering. As argued above, the CA-MICP has excellent potential for application in concrete, concrete crack self-healing, liquefaction, bioremediation of contaminated construction sites, problematic soil improvement and restoration of heritage buildings (Figure 2) as the CA-MICP pathway was proven capable of inducing the precipitation of calcium carbonate, which acts as a binding agent. Despite the proof of concept, several limitations still exist for the commercialization of CA-MICP. Different sources of CA could be investigated to improve the transition process from bench scale to field application to expand and enhance advantaged cited previously in subsections above that include: less carbon footprint via CO_2 sequestration; energy efficient process; and no toxic by-products in the process. Finally, approaches learned from the CO_2 sequestration studies can be incorporated into the biocementation process to enhance the process [73]. These strategies include the CO_2-converting enzymes at a near molecular separation distance, which can efficiently transfer CO_2 captured by CA [23]; the development of CA as a catalyst and its ability to function on an industrial scale [50]; and modification methods of CA for improved CO_2 capture and conversion by protein engineering [74]. The immobilization of CA enzyme using nanostructured materials can also contribute to an increased biocementation efficiency.

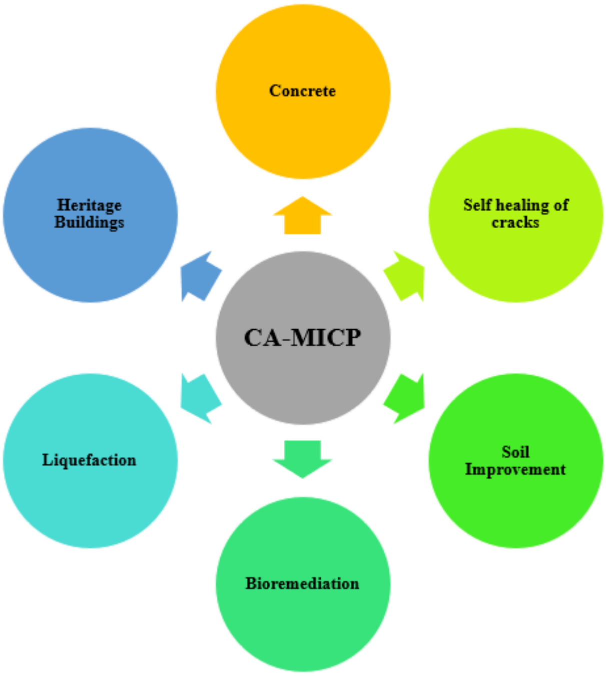

Figure 2: Future scope for application of CA-MICP techniques in the construction industry

5. CONCLUSION

CA-MICP has the potential to be an attractive alternative to ground improvement techniques in geotechnical and environmental engineering using CA-producing bacteria for both biocement and soil stabilization. The idea of utilizing microbes to this effect for construction applications is novel and exciting. It can combine CO_2 capture with the production of novel, greener and sustainable construction materials and assist in combating climate change. Despite the various advantages and prospects of CA-MICP, further studies are required to fill the knowledge gaps and address a number of issues common to other MICP pathways (e.g. the high cost of raw materials, culture media or the possible inhomogeneity of biocement treatments) thus overcoming barriers to industrial scale applications.

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