Environmental Processes (2023) 10:56
https://doi.org/10.1007/s40710-023-00667-2

REVIEW

Concurrent Carbon Capture and Biocementation through the Carbonic Anhydrase (CA) Activity of Microorganisms -a Review and Outlook

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Received: 3 April 2023 / Accepted: 30 August 2023

Abstract

Biocementation, i.e., the production of biomimetic cement through the metabolic activity of microorganisms, offers exciting new prospects for various civil and environmental engineering applications. This paper presents a systematic literature review on a biocementation pathway, which uses the carbonic anhydrase (CA) activity of microorganisms that sequester CO_2 to produce biocement. The aim is the future development of this technique for civil and (geo-)environmental engineering applications towards CO_2-neutral or negative processes. After screening 248 potentially relevant peer-reviewed journal papers published between 2002 and 2023, 38 publications studying CA-biocementation were considered in the review. Some of these studies used pure CA enzyme rather than bacteria-produced CA. Of these studies, 7 used biocementation for self-healing concrete, 6 for CO_2 sequestration, 10 for geotechnical applications, and 15 for (geo-)environmental applications. A total of 34 bacterial strains were studied, and optimal conditions for their growth and enzymatic activity were identified. The review concluded that the topic is little researched; more studies are required both in the laboratory and field (particularly long-term field experiments, which are totally lacking). No studies on the numerical modelling of CA-biocementation and the required kinetic parameters were found. The paper thus consulted the more widely researched field of CO_2 sequestration using the CA-pathway, to identify other microorganisms recommended for further research and reaction kinetic parameters for numerical modelling. Finally, challenges to be addressed and future research needs were discussed.

Highlights

- A review of biocementation via carbonic anhydrase (CA) was conducted.
- The role of CA in the biocementation mechanism was discussed.

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- The application of CA in civil and geo-environmental engineering was reviewed and discussed.
- Challenges, research gaps and prospects of CA application were identified.

Keywords Bacteria · Biocementation · Carbonic anhydrase · Carbon sequestration

1 Introduction

According to recent estimates (United Nations Environment Programme 2022) 15% of global carbon emissions are due to construction operations and the manufacture of construction materials. Historically, the most significant of these construction materials has been cement which has been reckoned to contribute 7% or 8% of global carbon dioxide emissions (Mavroulidou et al. 2015). While cement is primarily used in making concrete it has also been used for other purposes in civil construction, e.g., ground improvement. Together with the other main chemical substance used in ground improvement (lime), cement is an aggressive chemical which has the potential for causing environmental pollution.

Natural soils such as peat play a significant role in the natural carbon cycle but are unsuitable as foundation soils and need improvement (Safdar et al. 2021a, 2022). With increasing environmental awareness and the mandate to improve sustainability in the industry, engineers are now striving to find ways of providing economical and environmentally responsible ways of improving the properties of such soils. Thus, it is unsurprising that intensive research worldwide focuses on producing low-carbon cement via microbially induced calcium carbonate precipitation (MICP). Biocements are emerging as the most promising and transformative nature-based material in the construction industry. Biocements are binding agents produced through the natural biological process of biomineralisation, i.e., the biological production of minerals due to metabolic processes of different types of microorganisms/plants. Worldwide research efforts have focused on the potential application of biocements in the civil and environmental engineering industry, and a few commercial products have started entering the market. Applications include surface treatment or crack repairs in concrete (De Muynck et al. 2008; Zheng and Qian 2020b, Li and Qu 2015; Van Tittelboom et al. 2010; Sharma et al. 2017; Wiktor and Jonkers 2011; Achal et al. 2013; Joshi et al. 2021), biobricks (Bu et al. 2018; Bernardi et al. 2014; Lambert and Randall 2019; Kumar et al. 2019), restoration of heritage buildings (Castanier et al. 2000; Jroundi et al. 2012; Ortega-Villamagua et al. 2020; Tiano et al. 1999), and soil stabilisation (Al Qabany and Soga 2013; Al-Thawadi 2011; DeJong et al. 2010; Jiang et al. 2020; Gomez et al. 2017; Keykha et al. 2019; Martinez et al. 2014; Moravej et al. 2018; Oliveira et al. 2017; Punnoi et al. 2021; Safdar et al. 2021a, b).

Other applications include wind and water erosion control and suppression of dust generated from natural processes or construction activities (Cheng et al. 2021; Clarà Saracho et al. 2021; Dubey et al. 2021; Jiang et al. 2019; Salifu et al. 2016), bioremediation (Achal et al. 2012; de Oliveira et al. 2021; Li et al. 2016; Mwandira et al. 2017; Song et al. 2022; Zhang et al. 2022), and water treatment (Duarte-Nass et al. 2020; Mitchell and Ferris 2005; Torres-Aravena et al. 2018). The introduction of biocement as a potentially environmentally friendly and sustainable material is a nature-based solution. Microorganisms offer the potential of natural self-healing of biocement (Botusharova et al. 2020; da Silva et al. 2015;

Tziviloglou et al. 2016). Very importantly, several microorganisms offer the potential to capture CO_2 to use it to produce biominerals via carbonic anhydrase enzyme (Yadav et al. 2014). Moreover, the diversity of microorganisms can offer vital enzymes, withstanding the extreme operating conditions of the process. Consequently, upon further development, bio-based CO_2 mineralisation to produce biocement by capturing CO_2 can offer economic and environmental benefits over other chemicals/physical-based mineralisation processes (Jansson and Northen 2010).

Biobased alternatives to conventional cements have surged in the past decades, with the use of MICP getting particular attention. However, no comprehensive literature review has examined the carbonic anhydrase pathway. This pathway has been proposed to produce novel CO_2-absorbing biocements towards civil/(geo-)environmental engineering industry activities that are potentially neutral or carbon negative, and can play an important role in the net carbon zero strategies globally.

Therefore, the specific objectives of the current review were to: identify the evolution and trends in biocementation via carbonic anhydrase and discuss its merits and demerits; examine the different civil and geo-environmental engineering applications of the CA pathway and factors affecting its development; and finally, provide an outlook on applications, potential advantages, and limitations. The results of this study will significantly improve the understanding of the present status of biocementation via carbonic anhydrase and identify gaps for future research leading to new perspectives and options for future studies.

1.1 Biocementation Pathways and their Merits and Demerits

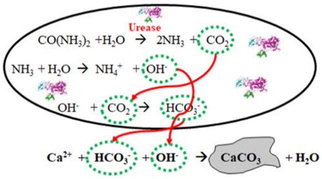
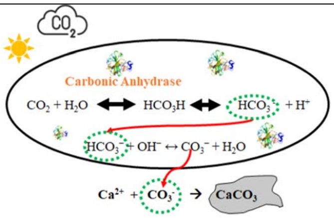
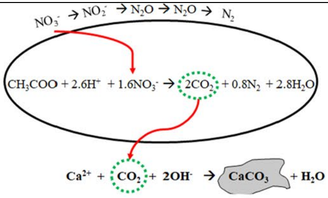
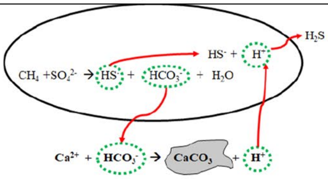
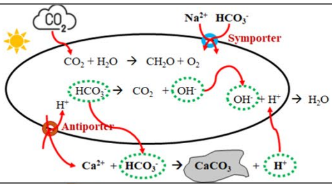
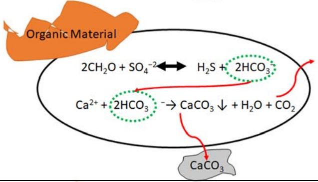
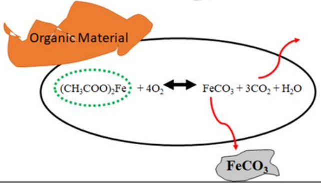
Biocementation can be achieved via several pathways, including ureolysis, carbonic anhydrase, denitrification, methane oxidation, photosynthesis, iron reduction, and sulphate reduction. These pathways have been discussed in a number of recent reviews (e.g., Castro-Alonso et al. 2019; He et al. 2020; Ivanov and Stabnikov 2020; Jain et al. 2021; Khodadadi et al. 2017; Lee and Park 2018). Therefore, with the exception of the carbonic anhydrase pathway (the focus of this review), the other pathways are only briefly presented in the subsections below and summarised in Table 1. Each figure in the table illustrates the chemical reaction for producing biominerals via MICP.

1.1.1 Ureolysis-driven Biocementation

Ureolytic-driven biocementation utilises urease enzyme which catalyses the hydrolysis of urea into ammonia and carbamate, subsequently leading to the formation of bicarbonate, ammonium, and hydroxyl ions. The local increase in pH due to the hydroxyl ions shifts the bicarbonate equilibrium, leading to the formation of carbonate ions. In the presence of soluble calcium ions, $CaCO_3$ precipitation can thus occur (Safdar et al. 2022). The most commonly used bacteria for this pathway are *Sporosarcina pasteurii* because they produce high concentrations of urease enzyme and are non-pathogenic (Nasser et al. 2022; Hadi et al. 2022). Additionally, indigenous ureolytic strains have been isolated worldwide from: a) mine tailings (Mwandira et al. 2019); b) caves (Li et al. 2019); c) beach rock (Imran et al. 2019); and d) indigenous soils (Burbank et al. 2012; Safdar et al. 2022).

The ureolytic pathway is the one most commonly applied and developed. The process is fast and easy to control (Stabnikov et al. 2022; Dilrukshi et al. 2018). However, it produces ammonium byproducts which are undesirable from an environmental point of view

Table 1 Summary of various biocementation pathways and respective merits and demerits

Biocementation routes	Biochemical reactions of different pathways of MICP	Merits	Demerits
Ureolysis		• Straightforward and easily controlled (Bibi et al. 2018) • Fast process (Al-Thawadi 2011)	• Large carbon footprint and energy-inefficient process (Deng et al. 2021) • Toxic byproduct (NH_4) (Su et al. 2022)
Carbonic anhydrase		• Less carbon footprint via CO_2 sequestration (Zhen and Qian 2020a) • Energy-efficient process (Deng et al. 2021) • No toxic byproducts (Pan et al. 2019)	• Poor stability and suboptimal use of CA
Denitrification		• Applicable under anoxic and anaerobic conditions (Erşan et al. 2015)	• Slow reaction rate (DeJong et al. 2010; Jiang et al. 2020) • Possibility of gas generation (DeJong et al. 2010) • Possiblity of high carbon footprint (Deng et al. 2021)
Methane oxidation		• Less aggressive byproducts of building materials (Ganendra et al. 2015)	• A large carbon footprint (Deng et al. 2021).
Photosynthesis		• No toxic byproducts (Ludwig et al. 2005)	• Application limited to structures exposed to CO_2 and sunlight (Seifan et al. 2016)
Sulphate Reducing Bacteria		• Can use anoxic and extreme environments (Baumgartner et al. 2006)	• Application limited
Iron Reducing Bacteria		• No toxic byproducts (Guo et al. 2010).	• Application limited (Castro-Alonso et al. 2019)

(Parvathy et al. 2023; Insausti et al. 2020). Moreover, if ammonium is oxidised, it may create acidic conditions dissolving the $CaCO_3$ precipitate over time (Khodadadi et al. 2017).

1.1.2 Denitrification Pathway

The denitrification pathway uses denitrifying microorganisms (i.e., denitrifiers), which transform nitrogen into different nitrogen-based oxides (Jain et al. 2021; O'Donnell et al. 2017). For geotechnical applications the process has been commonly proposed to desaturate soil for soil liquefaction mitigation (Azeiteiro et al. 2017). This is because of nitrogen gas formed in the subsurface when denitrifying microorganisms are provided with a solution containing nitrate and dissolved organic matter. Organic matter is oxidized to inorganic carbon and nitrate is reduced to nitrogen gas. MICP can thus also occur using this process, as dissolved inorganic carbon produced during denitrification, can lead to calcium carbonate precipitation in the presence of soluble calcium ions. To this effect, most studies used calcium salts such as calcium acetate and calcium nitrate as substrates, so that the inorganic carbon produced by the microbial metabolism can readily precipitate with the dissolved calcium to form calcium carbonate minerals (van Paassen et al. 2018; Pham et al. 2018). However, the required amount of substrates to biocement the soil is much higher than that required to desaturate the soil. Significant advantages of this pathway for civil and (geo-)environmental engineering applications, are that denitrifiers are ubiquitous in the subsurface, so that denitrification can be induced in most soils by stimulation of indigenous microorganisms (van Paassen et al. 2018), and that the process can occur under anoxic or anaerobic conditions, so that it can be applied at depth/low oxygen level (Martienssen and Schöps 1999). However, biocementation by denitrification is a slow process, considerably slower compared to biocementation by ureolysis. Other disadvantages are that incomplete reaction may lead to accumulation of nitrous oxide (N_2O), a greenhouse gas, as well as nitrite (NO_2^-) and nitric oxide (NO) byproducts that are potentially toxic and harmful to the environment and additionally inhibit the MICP process (van Paassen et al. 2018; Castro-Alonso et al. 2019). It is also possible that the production of nitrogen gas may have undesirable effects for geotechnical applications if soil matrix stability is compromised before biocementation has been achieved. The pathway can also be associated with a high eutrophication potential due to the nitrogen gas production (Porter et al. 2021).

1.1.3 Methane Oxidation Pathway

This autotrophic pathway involves microorganisms of the methane cycle that produce enzymes that release and consume methane (Murrell and Jetten 2009; Ganena et al. 2014, 2015). In this process, methane is oxidised with molecular oxygen to carbon dioxide, then converted to carbonates that can be used to form biominerals. The advantage of this process is that it has the potential to capture methane, a well-known greenhouse gas, and store it in biocements/building materials. This way methane becomes benign and the process can contribute to climate change mitigation. Also the process can occur under aerobic, anoxic and anaerobic conditions (Jain et al. 2021). Still, it suffers from the emission of hydrogen sulphide as a byproduct (Murrell and Jetten

2009). Furthermore, studies on this metabolic pathway were carried out under laboratory conditions but it is yet to be studied how the poccess can be implemented in situ under different environmental conditions (Jain et al. 2021).

1.1.4 Photosynthetic Pathway

The photosynthetic pathway uses the photolithoautotrophic nature of (micro-)algae and cyanobacteria to facilitate colonisation and biomineral formation (Irfan et al. 2019). As stated in Jain et al. (2021), during this process, the alkalinity across the microbial cell increases during the exchange of HCO_3^- /OH^- ions. The microorganisms use CO_2 (gaseous or dissolved) to form organic matter via photosynthesis, while bicarbonate is converted into CO_2 and OH^-, eventually forming carbonate minerals. Photosynthesis can be used to realise MICP cheaply and straightforwardly. However, the main limitations are the need for sunlight, restricting its applicability, especially for geotechnical applications at depth. Moreover, it can potentially have a considerable carbon footprint due to the production of CO_2 (Vecchi et al. 2020).

1.1.5 Iron-reduction Pathway

Under anaerobic conditions, anaerobic heterotrophic iron-reducing bacteria produce dissolved salts or chelates of Fe(II) that can be transformed to ferrous carbonate (Guo et al. 2010). A significant advantage for geo-environmental and geotechnical applications is the attainment of biocementation at depth under anoxic conditions as well as at low pH conditions. However, due to the aggressive nature of the environment in which the process occurs, its application is limited and potentially undesirable (Castro-Alonso et al. 2019). Only a few studies have used the process and have reported that the iron based biocement is not as strong as calcium based biocement but that the clogging effect is high, hence the process would be most suitable for bioclogging (Ivanov et al. 2015).

1.1.6 Sulphate-reduction Pathway

In the sulphur cycle, sulfate-reducing bacteria can oxidise organic carbon to bicarbonate (Alshalif et al. 2016; Baumgartner et al. 2006; Tambunan et al. 2019); at the same time, hydrogen sulphide production increases the pH, favouring the precipitation of calcium carbonate. The potential advantages of this pathway are the ability of sulfate-reducing bacteria to produce biocement under anaerobic conditions and the use of organic matter, which can be readily available from waste sources such as food waste (Chetty et al. 2023). However, sulfate-reduction is a slow process and hydrogen sulfide gases are odorous and toxic, which potentially limits the use of this process for large-scale (geo-) environmental and civil engineering applications. Another challenge is that anaerobic conditions must be maintained throughout the process, which is quite difficult to ensure under real-field conditions (Jain et al. 2021).

1.2 Carbonic Anhydrase Pathway

The carbonic anhydrase is an enzyme with an active site that contains a Zinc ion (Zn^{2+}) (Kim et al. 2020). It 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 (Meldrum and Roughton 1933). Since then, CA has been found in plants (Bradfield 1947), algae (Moroney et al. 2001), and bacteria (Veitch and Blankenship 1963; Supuran and Capasso 2017). The past few decades have proposed the usage of CA for industrial applications such as carbon sequestration (Molina-Fernández and Luis 2021; Yadav et al. 2014; Zhan et al. 2021), and biofuel production (Boone et al. 2013; Thakur et al. 2018). In the medical field, CA has found application in dental health (Abou Neel et al. 2016), as a component of artificial blood (Bian et al. 2012), for the prevention of kidney stones (Ghorai et al. 2020) and eye therapy (Jansook et al. 2021).

Its numerous applications are due to its distinctive CO_2-catalyzing properties. CA has been proposed for biocementation, as CA can hydrolyse 600,000 molecules of CO_2 per CA per second (Trachtenberg et al. 1999). For applications of CA-biocementation in civil or (geo-)environmental engineering, bacterial-CA (Zheng and Qian 2020a, b), as well as purified enzymes (Sharma et al. 2022) were used to 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 ionises to form CO_3^{2-} and H_2O (Eq. 3):

$$CO_2(g) \leftrightarrow CO_2(aq) \tag{1}$$

$$CO_2(aq) + H_2O(aq) \leftrightarrow H_2CO_3(aq) \tag{2}$$

$$H_2CO_3(aq) \leftrightarrow H^+ + HC_3^-(aq) \tag{3}$$

In the presence of a calcium ion source, $CaCO_3$ precipitates are formed from the reaction of (Ca^{2+}) with CO_3^{2-} (Eq. 5). Whilst other divalent ions can be used (e.g., Mg^{2+} or Fe^{2+}), Ca^{2+} remains the most widely ions used; therefore, $CaCO_3$ is precipitated in most biocementation studies. If bacteria are used rather than the free enzyme, bacteria cells serve as nucleation sites (Kahani et al. 2020):

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

$$Ca^{2+}(aq) + CO_3^{2-}(aq) \leftrightarrow CaCO_3(s) \downarrow \tag{5}$$

A typical setup for CA-biocement production for possible civil or (geo-)environmental engineering applications would thus involve combining CA-producing bacteria, CO_2, nutrient media, and a calcium (or another metal ion) source mixed with soil or soil-like waste (e.g., mine tailings), as illustrated in Fig. 1. Typically, bacteria cells are negatively charged, and thus attract and bind to the provided metal ion (Mg^{2+}, Ca^{2+}, or Fe^{2+}), forming crystals (Power et al. 2013). The potential applications take advantage of the precipitated carbonate (in most cases, $CaCO_3$) that acts as a binding agent between soil or soil-like waste particles (Charpe et al. 2019). The biomineral formed creates a cement that can also fill the void spaces. As a result, soil engineering properties such as strength and stiffness are enhanced (Chen et al. 2021).

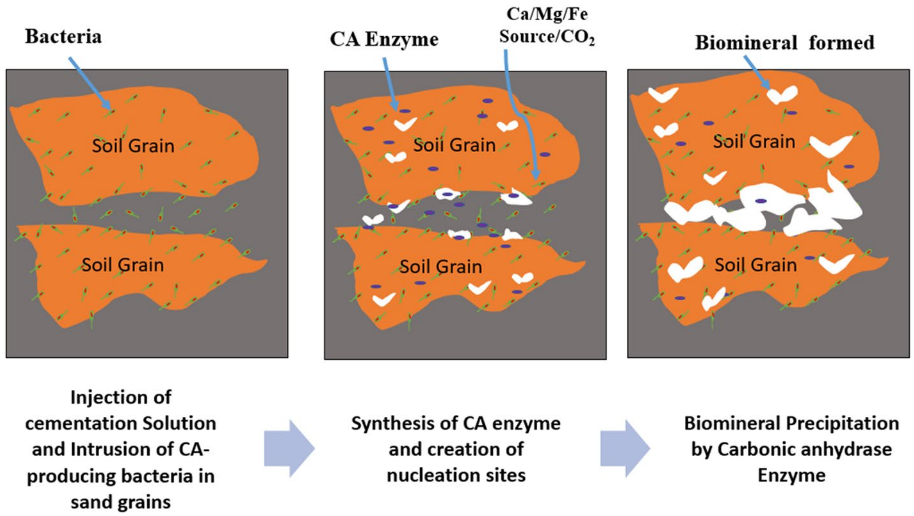


Fig. 1 Typical presentation of CA-biocementation

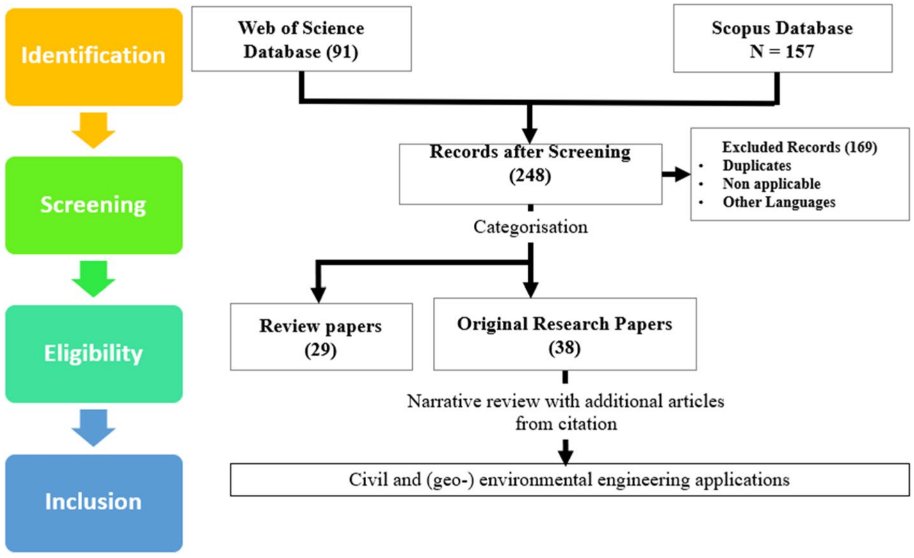


Fig. 2 Flow chart of the systematic review framework used in this study

2 Literature Review Methodology

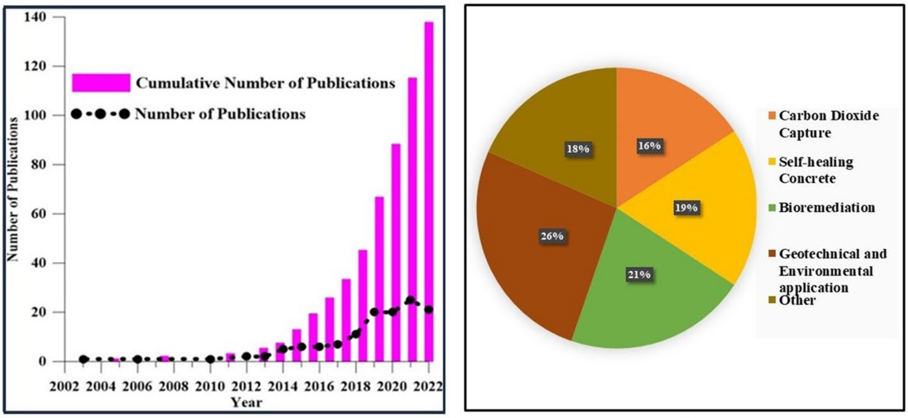
This paper adopted a systematic literature review (Xiao and Watson 2019), as illustrated in Fig. 2. The first step was to search for literature from the Web of Science (WOS), Scopus database, and other databases until 20th January 2023. An appropriate keyword combination described as (bacteria) AND (microbial calcium carbonate precipitation

OR biocementation AND (carbonic anhydrase*) was employed in the search command of respective databases to identify the relevant publications (Swartz 2011). Step 2 involved screening the literature search results, excluding all languages except English and removing duplicates, and excluding magazines, conference proceedings, book chapters, series, and newsletters to have only full and reviewed papers. The categorisation performed in steps 3 and 4 involved reading through the title and abstract of documents to check whether the study is related to civil and (geo-)environmental engineering. Both "primary articles" and "review" papers were considered. "Primary articles" include those papers publishing original experimental data from laboratory or field studies. "Review" are papers that summarise and report on the past or recent progress in the study area. Only papers that had adopted the carbonic anhydrase route for microbial calcium carbonate precipitation (MICP) or biocementation were reviewed and included in the narration of the review paper (Step 5).

3 Results and Discussion

3.1 Number and Categorisation of Research Articles

The Web of Science and Scopus search results generated 91 and 157 studies, respectively, excluding identical studies, not applicable, and languages other than English. One hundred sixty-nine (169) studies were excluded based on the article's abstract-level screening. The remaining studies, 38 in total, were considered in the systematic review. The trend in the number of research publications from 2002 to 2022 is represented in Fig. 3a and b, showing the thematic application areas. A steady increase can be observed in the number of publications on the topic. The literature shows that CA has been applied for carbon capture, self-healing concrete, bioremediation, geo-environmental applications and other. The increased number is due to the recent interest in the MICP technique as a sustainable technology (Fukue et al. 2011; Bhattacharya et al.



(a) (b)

Fig. 3 (a)Trend in the number of research publications from 2000 to 2023; (b) Schematic areas of research publications

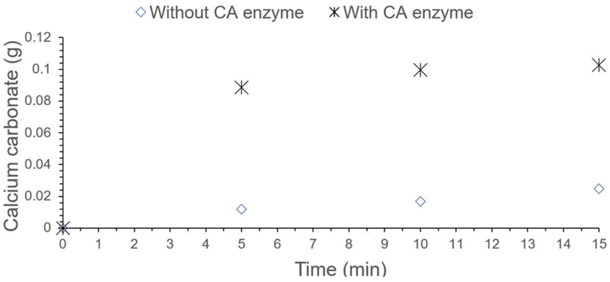


Fig. 4 Comparison of precipitation with and without carbonic anhydrase enzyme (Reproduced based on data from Mirjafari et al. 2007)

2018). This increased interest has yielded more excellent knowledge of the mechanism and factors affecting MICP (Soon et al. 2014; Chek et al. 2021; Soon et al. 2014). However, despite the advances in MICP, it is estimated that less than 1% of publications on biocementation studied the CA pathway.

3.2 Factors Affecting CA-biocementation

Many factors influence the process of biocementation, such as microorganism type and environmental factors (e.g., temperature and pH affecting microorganism viability, microbial growth, and enzymatic activity), the concentration of CO_2, and metal cation source and concentration. These factors are described in the subsections below, explicitly referring to the CA pathway.

3.2.1 Bacteria Type

Bacteria are the source of enzymes and provide nucleation sites for mineral precipitation (Zhao et al. 2014). Many researchers have confirmed that the CA-producing bacteria enzyme plays a vital role in promoting the conversion of CO_2 to bicarbonate and calcite (Steger et al. 2022). The hydration of CO_2 triggers the proton transfer initiated by the CA, as it attacks the carbon atom of CO_2 by zinc-bound OH^- to produce bicarbonate, as described earlier (Fu et al. 2021). Thus, the CA enzyme (whether from bacteria or purified enzyme) affects the biocementation process (Giri and Pant 2019; Mirjafari et al. 2007). Results have shown that purified CA enzymes could significantly enhance the carbonate/bicarbonate formation and deposition in solution, compared to control samples without CA enzyme (Fig. 4). Therefore, selecting a CA-producing bacterial strain is critical to the biocementation process using the CA metabolic pathway. Additionally, the bacteria serve as the nucleation site for the $CaCO_3$ precipitation process during the CA-biocementation process. A small number of bacterial cells will yield a small number of nucleation sites; consequently, a small amount of biocement will form between soil particles. In a recent study by Jin et al. (2021) using *Bacillus mucilaginosus*, it was confirmed that under the action of CA secreted by microbes, carbon dioxide was captured, enriched, and converted into carbonate ions, which reacted with calcium ions to form calcite. Similar results were reported by Zhan et al. (2021) using *Paenibacillus mucilaginosus* to produce CA and provide nucleation sites for the bio-mineralisation reaction. Selected CA strains screened by this study are shown in Table 2.

Generally, biocementation applications can harness the enzymatic activity of bacteria through bioaugmentation or biostimulation (Gomez et al. 2017). Bioaugmentation involves

Table 2 Summary of recent works using Carbonic Anhydrase applications detailing the study findings, bacteria used, and optimal temperature and pH conditions

No	Application type	Paper highlights/Conclusions	Microbial strain	Temp (°C)	pH	Reference
1.	Self-healing concrete and soil biocementation	Mixed culture of ureolytic and CA bacteria can be used for MICP at high temperatures	*Neobacillus drentensis* and *Priestia aryabhattai*	40	12	(Harnpicharnchai et al. 2022)
2.	Self-healing concrete	MICP was used to repair concrete cracks of widths ranging 0.3–0.5 mm	*Bacillus mucilaginous* L3	20	-	(Qian et al. 2021)
3.	Self-healing concrete	Self-healing of early-age cracks in cement-based materials by mineralisation of CA microorganisms	*Bacillus mucilaginous* L3	20	-	(Qian et al. 2015)
4.	Self-healing concrete	With *B. subtilis* M9 as the healing agent, the self-healing concrete beam specimens were successfully prepared by incorporating polyvinyl alcohol fibres	*Bacillus subtilis*	25	10.0	(Feng et al. 2021)
5.	Self-healing cementitious mortar	Calcium lactate was used as the calcium source to improve the calcification rate healing depth for microbial self-healing agents	*Bacillus alcalophilus*	25	11	(Zheng et al. 2021)
6.	Self-healing Concrete	Morphologies of spores with and without encapsulation immersed in the simulated pore solution of cement-based materials at different times were studied to characterise the protective effect of the carrier on spores	*Bacillus mucilaginous L3*	25	11	(Zheng et al. 2020)
7.	Self-healing Concrete	Microbial spores are beneficial when encapsulated with low alkaline potassium magnesium phosphate (MKPC) and sulphoaluminate cement (SC) and increase the survival time of spores in cement-based materials for the self-healing of cracks	*Bacillus alcalophilus*	25	12.1	(Zheng and Qian 2020b)

Table 2 (continued)

No	Application type	Paper highlights/Conclusions	Microbial strain	Temp (°C)	pH	Reference
8.	Construction industry	As an eco-friendly method, streptomyces-induced calcite precipitation (SICP) was used to bind loose dredger fill into soil columns	*Streptomyces microflavus*	25	11	(Xu et al. 2021)
9.	Construction industry	CA microorganisms are an environmentally friendly method with a fast calcium carbonate deposition rate, which can provide a theoretical basis for promoting and applying MICP technology	*Bacillus mucilaginosus*	25	10	(Zheng 2021)
10.	Construction industry	CA-producing bacteria can successfully promote the reinforcement and repair of underground projects including mines and tunnels	*Bacillus sphaericus* and *Bacillus mucilaginosus*	28	7—8	(Hu et al. 2020)
11.	Construction industry	Calcium carbonate induced by CA can be used as filler in the papermaking industry and in construction materials	*Corynebacterium flavescens*	35	7.5	(Sharma et al. 2022)
12.	Construction industry	Construction using CA-producing bacteria to make Steel slag pavement bricks	*Bacillus mucilaginous*	30	8.0	(Yi et al. 2022)
13.	Construction industry	Depositional calcium carbonate induced by microorganisms can fill the pores of the cement or mortar and compact the matrix	*Bacillus mucilaginous*	25	7.0	(Qian et al. 2022)
14.	Construction industry	Study of different calcium sources to improve the application of microbial-induced carbonate precipitation for MICP	*Bacillus cereus*	30	7.4–7.6	(Pan et al. 2019)

Table 2 (continued)

No	Application type	Paper highlights/Conclusions	Microbial strain	Temp (°C)	pH	Reference
15.	Ground Improvement	Biostimulation can be favoured at sites with high organic carbon content, whereas augmentation with repeated injections of nutrients can be applied on poor nutrient soils via different enrichment routes of microbial metabolism	*Bacillus cereus* C1 (CB)	37	8.0	(Dhami et al. 2017)
16.	Construction industry	Upon CO_2 influx compressive strength increased up to 117% compared to control and 47% with urea-treated specimens	*Bacillus megaterium* SS3	20	10.0	(Kaur et al. 2016)
17.	Construction industry	Construction materials can be engineered to improve mechanical properties of the carbonates by controlling the biochemical processes	*Bacillus pumilis* (BPm)	30	8.0	(Dhami et al. 2016)
18.	CO_2 Capture and storage	MICP via CA-producing bacteria can sequester CO_2 and calcium in aquatic environments	*Bacillus pumilus, Bacillus marisflavi, Virgibacillus pantothenticus* S3	25	7.2	(Silva-Castro et al. 2015)
19.	CO_2 Capture and storage	Carbonic anhydrase can accelerate the hydration of CO_2 to form $CaCO_3$ precipitation in an alkaline environment, and the presence of a calcium source for CO_2 Capture and storage	*Bacillus mucilaginosus (*GLRT202Ca*)*	30	9.0	(Zheng and Qian 2020b)
20.	CO_2 Capture and storage	The increase in pH and CO_2 hydration by CA play synergetic roles in providing supersaturated alkaline conditions in the system with bacteria for MICP	*Curvibacter lanceolatus* strain HJ-*1*	30	-	(Yang et al. 2021)
21.	CO_2 Capture and storage	Improved CA production and enzyme stability for CO_2 capture and storage	*Corynebacterium flavescens*	30	7.0	(Sharma and Kumar 2021)

Table 2 (continued)

No	Application type	Paper highlights/Conclusions	Microbial strain	Temp (°C)	pH	Reference
22.	CO_2 Capture and storage	CA-producing bacteria are used to mitigate CO_2 emissions and hence the greenhouse effect	*B. cereus* strain LV-1	30	7.60	(Huang et al. 2022)
23.	CO_2 Capture and storage	CA-producing bacteria are used to mitigate CO_2 emissions using bacterial strains from extreme environments	*Virgibacillus* sp and *Bacillus licheniformis*	30	7.0	(Abdelsamad et al. 2022)
24.	CO_2 Capture and storage	CA-mediated MICP by B. *altitudinis* M8 strain reduced CO_2 by 75% in a microcosm of sterilised soil with CA-producing bacteria and 97% in a microcosm with sterile soil with free enzyme	*Bacillus. altitudinis* M8	35	8.3	(Nathan and Ammini 2019)
25.	Heritage building Restoration	Microbial urease and CA can be used effectively for calcification to remediate defects in building structures	*Bacillus* sp., *Bacillus. megaterium*, and *B. simplex*	37	8.4	(Bozbeyoglu et al. 2020)
26.	Heritage building Restoration	Application of *E. mexicanum* on concrete specimens significantly increased the compressive strength (23.5%)	*Exiguobacterium mexicanum*	37	8.0	(Bansal et al. 2016)
27.	Coastal erosion protection	Microbial urease and CA are used in building eco-materials to protect coastal areas against erosion	Various urease and CA-producing bacteria	25	7.3	(Vincent et al. 2020)
28.	Bioremediation	MICP using *B. linens* bioremediated Cd^{2+} and Pb^{2+} contamination by effectively sequestering Cd^{2+} and Pb^{2+} with precipitation of calcite	*Actinobacterium Brevibacterium linens* BS258	28	7.4–7.6	(Zhu et al. 2017)

Table 2 (continued)

No	Application type	Paper highlights/Conclusions	Microbial strain	Temp (°C)	pH	Reference
29.	Bioremediation	*B. mucilaginosus* achieved remediation by the carbonation of steel slag and improved the CO_2 sequestration capacity	*Bacillus mucilaginosus*	30	7.4	(Jin et al. 2021)
30.	Bioremediation	CA bacteria could be economical and environmentally friendly to remove Ca^{2+} ions from industrial wastewater	*Bacillus amyloliquefaciens* DMS6	37	7.0	(Li et al. 2022)
31.	Bioremediation	Submerged fixed-film bioreactor was used for the treatment of urban wastewater in both natural and artificial media for wastewater treatment	Various CA-producing bacteria	-	-	(Uad et al. 2014)
32.	Bioremediation	Using CA bacteria could be an economical and environmentally friendly way of removing Mg^{2+} and Ca^{2+} ions from industrial wastewater	*Bacillus licheniformis* SRB2	37	7.2	(Zhao et al. 2019)
33.	Bioremediation	*Paracoccus denitrificans* AC-3 possessed an efficient ammonia removal ability	*Paracoccus denitrificans* AC-3	35	7.0	(Zheng et al. 2022)
34.	Various MICP applications	Demonstrated the role of microbial organic matter degradation and that carbonic anhydrase may play a substantial role in calcium carbonate precipitation in paleo- and recent shallow marine environments, findings that could apply to MICP	*Alcanivorax borkumensis*	20	7.7–8.3	(Krause et al. 2018)
35.	Various MICP applications	It was suggested to use Mg^{2+} for biomineralisation using the CA pathway	*Arthrobacter* sp. strain MF-2	30	8.0	(Zhang et al. 2018)
36.	Various MICP applications	The potential relationship between EPS, CPS and calcium carbonate precipitation was investigated by calcifying bacterial isolate *Bacillus megaterium*	*Bacillus megaterium* (SS3)	37	8.0	(Bains et al. 2015)

Table 2 (continued)

No	Application type	Paper highlights/Conclusions	Microbial strain	Temp (°C)	pH	Reference
37.	Various MICP applications	The study explained the kinetics and mechanisms of calcite precipitation induced by microbial CA	*Bacillus* strain	30	7.5	(Li et al. 2013)
38.	Various MICP applications	The paleoenvironment provides useful evidence for further recognising biotic and abiotic calcite in the geological record in nature and the laboratory	*Bacillus cereus* MRR2	30	7.0	(Zhuang et al. 2018)

the introduction of microorganisms to soils. These can be imported non-native microorganisms or native microorganisms that have been isolated from the native soil and screened for the intended usage; they are then cultivated and introduced back into the soil. For the bioaugmentation process, various strains of CA-producing bacteria have been isolated from soils. These include but are not limited to *Bacillus mucilaginous* L3 (Qian et al. 2015), *Bacillus cereus* C1 (Dhami et al. 2017), and *Bacillus altitudinis* M8 strain (Nathan and Ammini 2019). However, many researchers argue that bioaugmentation is not sustainable and eco-friendly (Raveh-Amit and Tsesarsky 2020). So far, the source of reliable high CA-producing bacteria is achieved by cultivating pure cultures which is a major cost factor of MICP application. Borchert and Saunders (2011) have successfully demonstrated the production of CA from a recombinant *E. coli* bacterial strain. Still, a high capital cost is associated with the large-scale production of enzymes from native and recombinant *E. coli* cell lines.

An alternative process is the stimulation of existing microorganisms in the soils by providing them with suitable nutrients (Pan et al. 2019). Biocementation by biostimulation can be of interest for practical geotechnical and geo-environmental engineering applications as native bacteria are well-distributed spatially within the subsurface and this can lead to an improved biocementation treatment with spatial uniformity (Gomez et al. 2017; Behzadipour and Sadrekarimi 2021; Dubey et al. 2021). Moreover, native microorganisms are well-suited and adapted to their native subsurface environment. Although little studied, biocementation by biostimulation of native CA-producing bacteria is possible and would merit further research. Consequently, before any full-scale CA-biocementation application in the field, an economically viable production of CA or CA-producing bacteria is necessary.

3.2.2 pH

The pH plays a vital role in biocementation, affecting microbial growth, metabolism/enzymatic activity, calcite solubility and crystallization and nature of biomineral formed. Moreover, the pH changes throughout the mineral precipitation process. Generally, the enzymatic activity is consistent with the growth and reproduction of bacteria: the better the bacteria grow, the higher the observed enzymatic activity. The optimal pH for most studied microbial CA-producing bacteria ranges from 7.0 to 9.0 (see Table 2). A high or low pH value generally results in the denaturation of enzyme activity due to the structural alteration of the amino acids of the CA enzymes. The optimal pH values for various CA-producing enzymes reported in recent studies were as follows: pH 6.5 for *Bacillus mucilaginosus* K02; pH 6.0 for *Lactobacillus delbrueckii* (Li et al. 2015); pH 7.0 for *Corynebacterium flavescens* (Sharma and Kumar 2021), and pH 8 for *Bacillus sp* (Sundaram and Thakur 2018). A study by Zheng and Qian (2020a) showed that as pH increased due to CO_3^{2-} and OH^- from the hydration of CO_2, a pH value of 9.5 inhibited the growth of bacteria and eventually resulted in no bacterial growth at a pH value above 11.0. The study postulated that higher pH values lead to increased permeability of microbial cell membranes, reducing the absorption of nutrients by microorganisms and leading to bacterial death. Lai et al. (2023) performed a microscopic analysis of EICP-treated sand specimens. This showed only a few small crystals forming for $pH \leq 4.5$. Conversely, at a higher pH, large calcium carbonate crystals were observed on the sand particle surface and/or at the interparticle contacts. It was thus postulated that lowering pH would lead to a reduction in enzyme activity affecting biocementation.

The pH value of concrete can reach 13 which is extremely unfavourable to the growth of most microorganisms. Thus, for such applications researchers adopted spore forming techniques and ways of shielding the bacteria during the hydration process. This could be achieved for example by using gels (Wang et al. 2012). Of all CA-producing microbes studied, *Bacillus sphaericus* alkali-resistant and spore-forming bacteria are used widely for self-healing concrete purposes (Zhu et al. 2021; Jonkers and Schlangen 2008). The concrete's matrix capillary water is reported to have pH values between 11 and 13, and *Bacillus sphaericus* still survives and can thrive at these pH ranges (Jonkers et al. 2010; Jonkers and Schlangen 2008; Zhu et al. 2021). The fundamental strategy for implementing bacteria in concrete is to protect these, for example, in a hydrogel, enabling them to last from several to hundreds of years under extreme environments (Setlow 1994; Wang et al. 2018). Indeed, although alkaliphilic bacteria would normally tolerate the highly alkaline concrete environment, their metabolic activity could still be affected by the high pH and the dry environment of hardened concrete. This was evidenced by Jonkers et al. (2010) who observed only 10% of the directly incorporated *Bacillus cohnii* spores in concrete after 42 days. Therefore, encapsulation rather than direct application is recommended, using carriers such as capillaries, porous materials, microcapsules, and hydrogel, as well as graphite nanoplatelets (GNPs) and lightweight aggregates (LWAs) (He et al. 2020; Lee and Park 2018). Further research would be required to study the viability of CA-producing bacteria in extreme environments and the performance of spore-forming CA bacteria encapsulated using different carriers for possible application in the construction sector.

In addition to and also as a result of its effect on microbial growth, pH plays a significant role in calcium carbonate crystallisation. Different initial pH lead to different crystalline morphology of precipitates, as evidenced by microstructural analysis of CA-treated materials shown by Zheng and Qian 2020b) for initial pH ranging from 7.0 to 10.0. Namely, the SEM micrographs showed that the morphologies of precipitates evolved progressively from agglomerations of tiny spheres in lower pH of 7.0 to larger compact crystals when pH increased to 9.0, and bacteria acted as nucleation sites to induce more regular spherical morphology. The different morphologies can be attributed to the fact that at low pH value, excess H^+ produced by CO_2 dissolving in water, would further reduce the pH of the solution, gradually dissolving $CaCO_3$ products; hence, smaller particle sizes were observed. Conversely, at $pH = 9.0$, which was found to be the optimum pH for mineralisation for the CA bacteria used in this study, precipitation was enhanced. The bacteria acted as the nucleation sites to induce a more regular spherical morphology, which resulted in increased sizes of precipitates. Finally, an initial pH of 10.0, would restrict bacterial growth leading to a decrease in enzymatic activity and to the inhibition of the hydration reaction of CO_2. This would result in a limited precipitation, mainly caused by the dissolution of CO_2 in the alkaline solution. Further increases in initial pH values could eventually lead to the death and dissolution of CA bacteria, thus impacting on the quantity and stability of enzyme produced by microorganisms activity (Zheng and Qian 2020a). It should be noted that the large variation in the size of the biominerals has an impact on the contact points forming between soil grains, and consequently, on the strength of biocemented geomaterial. Longer retention times of enzymatic activity, will yield larger crystal sizes, enhancing the bonding between the biomineral and the geomaterial particles, hence leading to higher strengths.

3.2.3 Temperature

In addition to pH changes, temperature changes also have a significant impact on biocementation as they affect the enzymatic activity of the microorganisms, as well as the chemical stability of the biominerals. Increased or decreased temperature affects microbial metabolism and growth, hence affecting enzymatic activity. Therefore temperature is a key factor for the success of CA-biocementation. Some literature has pointed out that the optimal temperature for microbially induced calcium carbonate precipitation is around 35 °C, which correlates well with the optimal growth temperature of most CA-producing bacteria. A recent soil microcosm study by Jaya et al. (2019) showed that marine *Bacillus safenis* had optimal CA activity at 40 °C. Another study found that *Citrobacter freundii* was active and optimal at 37 °C (Giri et al. 2018). Unlike the other CA-producing bacterial strains studied, *Methanobacterium thermoautotrophicum* had an optimal high temperature of 75 °C (Smith and Ferry 1999). Therefore, the temperature should be optimised in CA-biocementation to cement different geomaterials efficiently for different applications in natural environments (Justo-Reinoso et al. 2021). However, few studies have optimised this process for application in the field as most of the experiments were conducted at room temperature. Future investigations should mimic the ground conditions, as low temperature conditions significantly affect microbial activity. For example, according to Sun et al. (2019), microbial activity is low at temperatures of 10 °C, affecting the success of biocementation. On the other hand the study by Zhang et al. (2011) shows that the CA activity of *Bacillus mucilaginosus* K02 at temperatures of 10 °C is reduced by approximately 30% of the activity achieved at optimal temperatures (Fig. 5). Therefore, studies focusing on how to achieve sufficient for biocementation microbial activity at low temperatures are of most relevance. In particular for geotechnical and geo-envirronmental applications, it will be impractical to control or maintain a constant temperature in the field. The soil temperature in the field would vary with altitude, latitude, soil type and depth, water content, proximity to industrial or agricultural site (Jain 2021). Though the native CA bacteria will be acclimated and able to survive in a wide range of temperatures in their natural environment, the effect of different temperatures on bacteria growth and activity will need to be thoroughly studied before field application, as it will be a major factor affecting biocementation success.

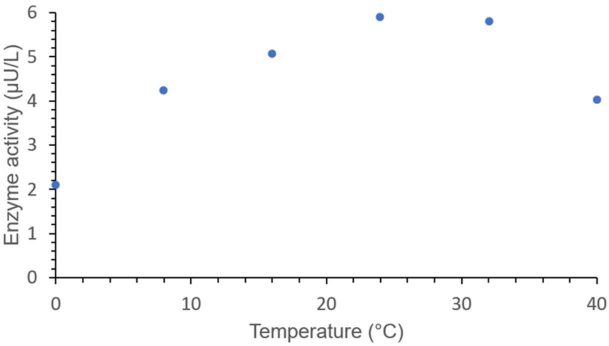


Fig. 5 Effect of different temperatures on carbonic anhydrase produced by *Bacillus mucilaginosus* K02 (Reproduced using data from Zhang et al. 2011)

3.2.4 Cementation Solution

The review of existing experimental studies shows that the cementation solution is vital in the CA-biocementation process. Previous biocementation studies showed that the composition and concentration of the cementation solution affect the crystal type, appearance, size, composition of pore fluid, and pH value. Typically, the cementation solutions in CA-biocementation are a mixture of metal ion salt, a CO_2 source, and trace nutrients (Bozbeyoglu et al. 2020; Dhami et al. 2014; Pan et al. 2019). In calcite precipitation studies, three calcium sources have been used widely, namely calcium nitrate ($Ca(NO_3)_2$), calcium acetate ($Ca(CH_3COO)$), and calcium chloride ($CaCl_2$). Pan et al. (2019) used three calcium sources that demonstrated that CA-producing bacteria could precipitate calcite in a sand column and showed that the three sources of calcium systems produced different crystal types (see Fig. 6). The morphologies of the crystals were mapped against the strengths achieved. Namely, the sand column with $Ca(NO_3)_2$ had the highest shear strength of 62.33 kPa, followed by the $CaCl_2$ group, while the $Ca(CH_3COO)_2$ group had the worst strength of only 11.19 kPa. The authors attributed the higher strengths of the $CaCl_2$ and $CaNO_3$ systems to the fact that $CaCO_3$ crystals were closely packed, attached to the sand particles, and well distributed, as opposed to $CaCO_3$ crystals from the $Ca(CH_3COO)_2$ systems, where precipitates were much more scattered and not attached to the sand particles. Still, in the context of biocementation for ground improvement these strengths are too low: when using the ureolytic route for sand biocementation strengths as high as 30 MPa have been reported (Whiffin 2004). An interesting observation made by the authors is that holes were formed on the surface of their specimens, which could justify the low strength. The authors attributed these holes to the continuous production of CO_2 during the process, so that gas bubbles disrupted the soil matrix. It is also notable that XRD identified the $CaCO_3$ crystals as vaterite, i.e., the least stable $CaCO_3$ form. Vaterite formation instead of calcite impacts the mechanical properties of the soil and can explain the lower strengths. This was particularly the case for the $Ca(CH_3COO)_2$ system, which had the lowest strengths, consistently with the formation of vaterite. However there was generally a limited evidence of calcite forming (the most stable polymorph) even in the other two systems, which overall concurs with the low strengths achieved.

Zheng and Qian (2020a), investigated the effect of cementing solution concentration. Using cementing solutions of different molarities (0 to 160 mM) of calcium ions, they found that molarity also affects the growth and enzyme activity of CA bacteria, hence biocement production. Bacteria grew best, and the enzyme activity reached its maximum, when the Ca^{2+} concentration was 60 mM. An increased Ca^{2+} concentration inhibited the

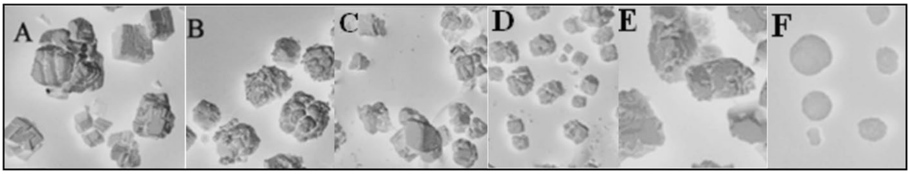


Fig. 6 The morphology of $CaCO_3$ formed with the three different calcium sources in the presence of *Bacillus* cereus (+) or not (−): (A) $CaCl_2$ system (−); (B) $CaCl_2$ system (+); (C) $Ca(NO_3)_2$ system (−); (D) $Ca(NO_3)_2$ system (+); (E) $Ca(CH_3COO)_2$ system (−); (F) $Ca(CH_3COO)_2$ system (+) (Figure reproduced from Pan et al. (2019)

growth of CA-producing bacteria, and the enzyme activity of CA-producing bacteria decreased gradually. Once the Ca^{2+} concentration reached 160 mM, the bacteria hardly grew, and the activity of CA-producing bacteria almost disappeared. A possible explanation for this is that increased Ca^{2+} concentration affects the osmotic pressure around the membrane of bacteria cell. This triggers flow from the low Ca^{2+} concentration zone to the high Ca^{2+} concentration zone through the cell membrane, which leads to the dehydration of cells and the separation of plasma wall. This can cause bacteria to stop growing or even die (Zheng and Qian 2020a).

Finally, the source of CO_2 is essential. CO_2 used could be from the atmosphere or externally supplied; this could include captured industrial CO_2. Most CO_2 sequestration studies suggest using CO_2 directly from point sources such as industrial sources (Leimbrink et al. 2017; Russo et al. 2013, 2016). However, this source of CO_2 has impurities and, as such, needs to be purified before usage as other pollutants exist in the flue gas (Wattanaphan et al. 2013; Weber et al. 2000). This is plausible for CO_2-capturing application purposes but could be less suitable for biocementation for soil improvement or bioremediation. For this purpose, the usage of $NaHCO_3$ has been suggested by many researchers as a source of CO_2 (Hanifa et al. 2023) although it is less attractive in terms of climate mitigation than the use of captured CO_2. Alternatively, using CO_2 directly from the atmosphere is plausible in the same fashion as when CO_2 from the atmosphere is used for curing cement-based materials (Liu et al. 2021) but this has been little investigated. It should, however, be noted that CO_2 addition can lead to a disruption of the soil matrix as observed in the experiments by Pan et al. (2019) and this requires further investigation especially for ground improvement applications.

To conclude, cementation solution composition/concentration variations can affect biocementation success. Investigators must carefully select the appropriate cementation solution type and concentration ratio before implementing CA-biocementation in various engineering applications. The limited evidence for the use of CA enzyme for ground improvement shows that strengths may be considerably lower compared to those achieved by the ureolytic route (see, e.g., Pan et al. 2019). To precipitate more calcium carbonate between sand particles, a higher cementation solution may be required; however, the MICP process may be retarded or even terminated with an increased solution concentration (Mahawish et al. 2018; Lai et al. 2021). Alternatively, multiple applications of cementing solutions of lower concentations may have to be adopted as a way of increasing precipitates and strength. However, this would reduce the efficiency of the process in large scale applications, increasing treatment duration and costs. This would be a major limitation for the CA based MICP soil treatment and needs to be studied thoroughly.

3.3 Applications of CA-biocementation in Civil and Environmental Engineering

3.3.1 Concrete Repair

As discussed earlier, biocementation can be applied for concrete crack repair and self-healing, soil/geomaterial stabilisation/ground improvement, carbon capture, and bioremediation. The CA-biocementation method has been used primarily for concrete crack repair or self-healing concrete (Ali et al. 2023). The conventional way of repairing concrete cracks involves injecting cement grout or epoxy into the concrete. However, environmental and health hazards such as allergies, asthma, and irritation of the eyes, nose, and throat have been reported when using these chemicals (Kapustka et al. 2020). CA-biocementation can be used instead for the self-healing of microcracks. In the latter method, restoration occurs by activating the biocementing components incorporated in the concrete when a gap appears. Namely, any seepage of water and air through cracks leads to the release of incorporated components in the concrete to initiate the CA-biocementation process which seals the microcracks. A recent study used *Bacillus mucilaginous* L3, a CA-producing strain that was shown to seal small cracks within seven days (Qian et al. 2015). This study predicted the depth of the $CaCO_3$ precipitated layer based on the expected experimental results. Cracks in the concrete below 0.4 mm were almost entirely closed using this method. Chen et al. (2016) also repaired cement-based material damage by immobilising *Bacillus sphaericus* CA-bacteria and nutrients.

Free CA-enzyme was also introduced in the cement paste mix during the concrete preparation to develop a self-activated healing cement paste (Rosewitz et al. 2021). The results showed that after fracture, the hydration of samples containing CA promoted the formation of calcium carbonate crystals at ambient temperature. These results prove that using the CA enzyme to repair small cracks in concrete is practical, as reported using other pathways (Chuo et al. 2020).

3.3.2 Ground Improvement

To date, very few works have studied CA-biocementation for soil stabilisation. These include the recent study by Pan et al. (2019), who used different calcium sources to investigate sand biocementation by a CA-carbonic anhydrase-producing bacteria, as mentioned earlier. The study showed that CA-producing bacteria could precipitate calcite in a sand column of a diameter of 60 mm and a height of 50 mm using various calcium sources. Another study by Dhami et al. (2017) explored the CA-biocementation pathway by biostimulation. It showed that calcium carbonate could be precipitated by the synergy between ureolytic and CA-producing bacteria, as in the natural environment, no single process exists in isolation. The outcome was an improved biocementation efficiency as calcite precipitation increased by 50–72%.

3.3.3 Bioremediation

CA can also be applied for the bioremediation by either fixation or leaching of contaminants, as suggested by a recent study of steel slag carbonation using *Bacillus mucilaginosus* (Jin et al. 2021). The resulting inert material had a compressive strength of 11.2 MPa and could be used for contaminant encapsulation by adhering to the waste materials and resisting biodegradation. Further investigations of CA-biocementation for contaminant encapsulation are required as there is paucity of information regarding this application.

3.3.4 Future CA-biocementation Application

The CA-biocementation process could be used for other applications in the future, such as soil liquefaction mitigation, erosion protection, or heritage building restoration, in the same way as other pathways (e.g., denitrification or ureolytic pathways). However no studies were found using CA for these applications. This is a research gap that future studies can address (Fig. 7).

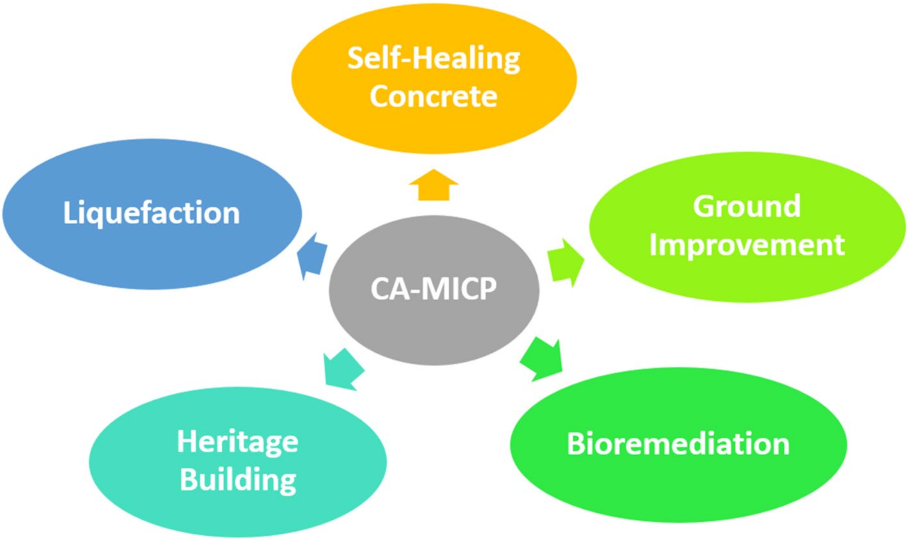


Fig. 7 Possible future CA-biocementation applications in civil and (geo)environmental engineering

3.4 Modelling of CA-biocementation Processes

Although the biocementation technique has been extensively investigated in the recent past, to date, no predictive model has been formulated for the CA-biocementation pathway despite its various advantages. The vast majority of biocementation models are for the ureolytic route and are calibrated at laboratory scale (Dupraz et al. 2009; Fauriel and Laloui 2012; Kashizadeh et al. 2021; Martinez et al. 2014; Nassar et al. 2018; Matsubara and Yamada 2020; Sharma et al. 2021; van Wijngaarden et al. 2011; Wang and Nackenhorst 2020). Very few models are calibrated with field scale data (Minto et al. 2019; Cunningham et al. 2019). Such models are helpful for upscaled or field applications. It is costly and takes many years to carry out field experiments before the technology can be transferred from a laboratory-scale process to a practical field-scale deployable technology. Thus, it is necessary to have CA-biocementation models developed. Forward-looking, for CA-biocementation predictive modelling, the CO_2 sequestration literature could be consulted as no modelling studies on CA-biocementation were found. This process could provide the required information (parameter values) for the CA enzyme kinetics. Figure 8 shows the step-by-step process of predictive model development.

The first step would be to understand and implement the general form of mass balance for chemical species transport by advection–dispersion-reaction relationship. CA-biocementation involves multi-component systems that require solving transport for many species (CO_2, H_2O, Ca^{2+}, CO_3^{2-}). Learning from previous models in the ureolytic pathway, all these species in the system are generally simulated using the advection–dispersion equation (Eq. 6) (Abbas et al. 2020; Cunningham et al. 2019; Fauriel and Laloui 2012; Martinez et al. 2014). The terms on the left-hand side express the advective transport, the first term on the right-hand side considers dispersion, and the second term considers reaction; in its simplest 1-D form, it is written as:

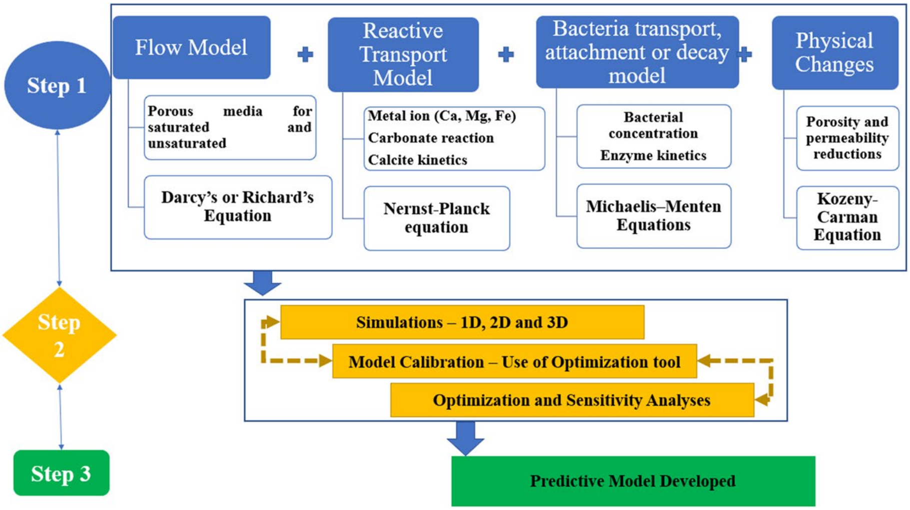


Fig. 8 Schematic diagram of the predictive modelling of CA-biocementation techniques

$$\frac{\partial C}{\partial t} + u\frac{\partial C}{\partial x} = D\frac{\partial^2 C}{dx^2} - KC \tag{6}$$

where

C	Constituent concentration (mol/m^3),
t	time (s)
u	cross-sectional average flow velocity (m/s),
x	distance along the longitudinal axis (m),
D	Dispersion coefficient (m^2/s), and
K	Reaction rate (1/s).

Secondly, during typical batch culture, the bacterial growth curve shows distinct stages of growth that describe the lag phase, exponential growth, and death phase, when conditions become unfavourable for growth and bacteria stop replicating (Balakrishnan et al. 2021). The microbial growth and enzyme activity can be expressed using the Monod equation. The Monod equation considers the number of microorganisms and the substrate concentration (Nežerka et al. 2022). This phenomenon has been modelled as chemical species, where the bacterium in suspension is considered irreversibly attached to solid surfaces in the soil profile and independent of velocity and bacteria growth; the Monod equation then simplifies into Eq. (7) (Minto et al. 2019). The rate of the forward reaction (r_{CA}) for CO_2 hydration rate (mol/(m^3 s)) catalysed by generic carbonic anhydrase (CA) may be described according to the Michaelis–Menten model (Eq. 7) as follows:

$$r_{CA} = \frac{k_{cat}}{K_M}[CA]\left(\left[CO_2\right] - \left[CO_2\right]_{eq}\right) \tag{7}$$

where k_{cat} is the turnover number (1/s), and K_M is the Michaelis–Menten kinetic constant (mol/m^3) and $\left[CO_2\right]$ and $[CO_2]_{eq}$ the total CO_2 concentration and the CO_2 concentration in equilibrium respectively (mol/m^3).

The reaction mechanism of CA has been extensively studied, and the reaction scheme is reported in the literature. Generally, the CA turnover numbers range between 104 and 106 s^{-1} for free CA and immobilised enzymes (Di Fiore et al. 2015). Russo et al. (2013, 2016) reported the kinetics of the recombinant CA from the thermophilic bacterium *Sulfurihydrogenibium yellowstonens,* namely that $k_{cat}/K_M = 9.16 \times 10^6\ M^{-1}\ s^{-1}$. In another study, the k_{cat}/K_M assessed for the α-class human carbonic anhydrase HCAII was reported to be about $10^8\ M^{-1}\ s^{-1}$ (Gaspar et al. 2017). Such parameters could be helpful for the models. The final step for CA-biocementation would be the precipitation of biominerals where $CaCO_3$ is precipitated as an immobile mass. This can be incorporated into the CA-pathway models in the same way as in existing ureolytic models (e.g., Abbas et al. 2020; Cunningham et al. 2019; Martinez et al. 2011; Matsubara and Yamada 2020; van Wijngaarden et al. 2011).

Numerical models for the ureolytic pathway have been produced using software such as COMSOL-MULTIPHYSICS (Faeli et al. 2023), TOUGHREACT (Martinez et al. 2013), PHT3-D (Nassar et al. 2018), OpenFOAM (Minto et al. 2019) and others. These and other platforms could be useful for simulating the CA-biocementation with parameters obtained from CO_2 sequestration studies. Once a model has been produced, the next step would be to determine the main influential parameters and calibrate the model, considering both aqueous and solid phase data from the experiments (Abbas et al. 2020). The stoichiometric constraints of individual reactions and the interconnectivity between the responses would be utilised in the calibration process (Martinez et al. 2011; Minto et al. 2019). However, most of the current mathematical models are limited to CO_2 absorption into aqueous solutions along a biomimetic route. There are no models for CA-biocementation, which requires further study by researchers.

4 Discussion: Challenges and Future Directions

The CA pathway is an attractive nature-based method of producing environmentally friendly cement for civil and (geo-)environmental engineering applications. Combining CO_2 capture with biocement production for different applications, is novel and exciting. It assists in climate change mitigation and adaptation. The carbonic anhydrase biocementation pathway is also free of undesirable byproducs. Due to its many advantages the pathway has excellent potential for large scale civil and environmental engineering applications, including concrete crack repair or self-healing, restoration of heritage buildings, bioremediation of contaminated construction sites, as well as problematic soil improvement, including preventing soil liquefaction and erosion problems. However, despite the proof of concept, several limitations still exist for commercialising CA-biocementation. Many research gaps were identified in this study and suggestions for future research to address these are listed below:

i. Firstly, the different sources of CA (CA from bacteria or free/purified enzyme) need further investigation to improve the transition process from bench scale to field application. For this, it is also necessary to identify robust and site-specific microorganisms that can operate under a broad range of environmental parameters such as pH or temperature. The ability of the CA-biocementation route to achieve design requirements also needs to be addressed. For example, the soil strength gains reported currently, are generally lower than those achieved when the ureolytic route with *Sporosarcina pasteurii* was used. Moreover, to realise CA-bocementaton at field scale it is essential to find ways of producing biominerals cheaply. Overall sustainability assessments of the proposed processes through Life Cycle Analysis will also be required and these are currently lacking.

ii. Secondly, immobilising CA enzymes using nanostructured materials could be investigated. This technique was proven to be helpful in the carbon capture process. Future studies can thus adapt the technique to increase biocementation efficiency (Al-Maqdi et al. 2021; Shende et al. 2018). A critical anticipated advantage of CA enzyme immobilisation is an improved stability.

iii. Thirdly, CA-biocementation studies are currently limited to a few applications. Further research could broaden the use of the technique to other civil and (geo-)environmental engineering applications such as liquefaction and heritage building restoration.

iv. Another aspect that has not been discussed in the literature but it is of most relevance for industrial scale implementation is finding practical ways of implementing CO_2 for large scale projects, especially for geo-environmental and geotechnical applications. It should be considered for example how the CO_2 can be implemented in the ground using currently available equipment (e.g., air sparging or electrokinetic setups), without disrupting the soil matrix, especially under existing infrastructure. Moreover, the literature review has shown that the type of the cementing solution and its purity may affect the biocementation success. Whilst the effect of impurities on the success of biocementation should be the focus of future laboratory work, the logistics of supplying CO_2, especially captured industry waste CO_2, free of potentially deleterious impurities would need to be considered for large scale projects. The possibility and practicality (in terms, for example, of rates of calcite precitation, and whether these would be practical in terms of timeframes required) of using atmospheric CO_2 should be considered, so that CO_2 biocementation becomes a carbon sink in the natural environment, but no such studies were found.

v. Finally, the formulation of predictive models of the CA-biocementation pathway, which are currently lacking, needs to be addressed in future work, to support uplscaling towards field applications.

5 Conclusions

This study provided a comprehensive literature review on the use of the carbonic anhydrase biocementation pathway for environmental and geo-environmental engineering applications. The review covered the period from 1st January 2002 to 20th January 2023. The study of this pathway in comparison to other biocementation pathways revealed that the carbonic anhydrase biocementation pathway could be the net-zero biocementation solution for the construction sector. However this study also showed that although very promising, this pathway remains little researched. It was, therefore, concluded that more studies are required both in the laboratory and in the field for the different possible applications of the process. In particular long-term field experiments, which are lacking, are essential to assess the feasibility of this exciting route and to overcome barriers towards uptake by industry.

The study also showed that studies on the numerical modelling of the processes and information on the required kinetic parameters are also lacking; yet, these are important for industrial implementation. To complement the information and as an outlook for future research in this direction, it is relevant to consult the field of CO_2 sequestration using CA-producing bacteria, to fill the knowledge gaps.

Finally, several issues common to other biocementation pathways need to be further investigated to overcome barriers to industrial-scale applications. These include the high cost of raw materials and culture media. Sustainability analyses of the proposed processes are of primary importance for the application of the technique at an industrial scale. Practical considerations regarding capturing CO_2 for field scale applications must also be addressed towards industry uptake of the technique.

Author Contributions All authors contributed to the study's conception. Maria Mavroulidou and Michael J. Gunn are the LSBU supervisors of Dr Mwandira, a Fellow of MSCA-IF NOBILIS. Diane Purchase, Hemda Garelick and Jonathan Garelick are the secondment supervisors/mentors of Dr Mwandira from Middlesex University and Network Rail, UK, respectively, partnering with project NOBILIS. Wilson Mwandira performed literature collection and analysis. Wilson Mwandira wrote the first draft of the manuscript, and Maria Mavroulidou produced the second draft. All authors commented on the latter version of the manuscript, suggested amendments and approved the submission of the final manuscript.

Funding The European Commission has funded this work under the Horizon 2020 Marie Skłodowska-Curie Individual Fellowships, project NOBILIS, Grant Number 101025184 (Grant holder: London South Bank University). Additional support for the research has been received from Network Rail, UK, which partner with project NOBILIS.

Data Availability The research did not generate primary data. All collected literature sources can be found in the list of references.

Declarations

Competing Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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