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Soil biocementation using the carbonic anhydrase metabolic pathway

Biocimentation des sols par la voie métabolique de l'anhydrase carbonique

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ABSTRACT: Biocementation, i.e., the soil cementation using cements produced biomimetically, has recently been introduced as a method of ground improvement, which is potentially more eco-friendly than conventional ground improvement methods. To date, the vast majority of researchers have used the urea hydrolysis metabolic pathway to biocement soils. We present instead work focusing on a less researched and potentially more interesting pathway, the carbonic anhydrase route, which produces biocement while sequestering CO_2. In this paper we present experimental results of bioprecipitate analysis and biocementation using CA producing bacteria; indicative results using the same bacteria though the ureolytic pathway are also shown; their modelling is then discussed instructed by studies on the ureolytic pathway. The results of this study based on bioprecipitate characterisation prove the precipitation of calcium carbonate using the CA pathway. The biocementation by biostimulation of the soil using the CA pathway was also proven, based on the unconfined compressive strengths of the treated soil ranging 0.5-1 MPa depending on cementing solution molarity vs the zero unconfined compressive strength of the untreated soil.

RÉSUMÉ: La biocimentation, c.a.d. la production de ciments biomimétiques, a été récemment introduite comme une méthode d' amélioration du terrain potentiellement plus éco-respectueuse que les techniques d' amélioration du terrain conventionnelles. Jusqu' à présent la plupart des chercheurs ont adopté la voie métabolique par uréolyse pour biocimenter les sols. En revanche, notre étude a pour objectif d' examiner une voie métabolique mois étudiée mais potentiellement plus intéressante, à savoir la voie métabolique de l'anhydrase carbonique, qui permet à la fois la biocimentation du sol ainsi que la séquestration du CO_2. Cette communication présente des résultats expérimentaux portant sur l' étude des minéraux bioprécipités et la résistance à la compression non confinée, confirmant la biocimentation du sol traité. Des résultats indicatifs obtenus par la voie métabolique par uréolyse (utilisant les souches isolées) sont aussi présentés. Nous traitons enfin du sujet de la modélisation de ce processus en puisant dans les exemples de la modélisation de la biocimentation par la voie uréolytique.

Keywords: Biostimulation; carbonic anhydrase; biocementation.

1 INTRODUCTION

To meet the objectives of net-zero carbon transition and environmental sustainability, geotechnical engineering practices must change radically. Whilst ground improvement will become increasingly important towards sustainable industry practices, ground improvement methods are still machinery-heavy and energy-intensive and often rely on Portland cement or lime to improve the ground; thus, they involve considerable GHG emissions for their production (Mavroulidou et al, 2022). Biomimetic cements, i.e., biocements, produced by the metabolic action of non-pathogenic microorganisms, have recently emerged as potentially more environmentally-friendly alternatives. Recent Life Cycle Assessment (LCA) research however, which considered different metabolic pathways to obtain biocements, questioned the actual sustainability of most processes and pathways (Porter et al, 2021). Porter et al's results showed that different metabolic pathways used for biocementation may have considerably varying and potentially negative environmental impact (especially considering the extent of industrial scale projects). These may be linked to the by-products of the particular metabolic pathway and also the purity of the input chemicals. However, they pointed out that the biotic metabolic pathway of interconversion between CO_2 and the bicarbonate ion HCO_3^- mediated by heterotrophic carbonic-anhydrase (CA) producing bacteria, would be the most environmentally friendly

and sustainable metabolic biocementation pathway. This pathway has the potential of consuming CO_2 (e.g. captured, industrial CO_2), while biocementing the soil, thus offering a dual way of addressing net zero carbon targets within the ground improvement industry. In this process CA utilises gaseous CO_2 to form hydrated aqueous CO_2 (aq) (Eq. 1), which reacts with water to form H_2CO_3 (Eq. 2). Once the H_2CO_3 is formed it ionises in water to generate H^+ and HCO_3^- (Eq. 2); under alkaline conditions, the HCO_3^- further ionises to form CO_3^{2-} and H_2O (Eq. 3). To form a biomineral, metal ion (e.g., Ca^{2+}, Mg^{2+} or Fe^{2+}) precipitates are formed from the reaction. For example, Ca^{2+} with CO_3^{2-} (Eq. 5) forms calcium carbonate. This reaction can occur either with CA-producing bacteria or purified CA enzyme (E) serving as nucleation sites.

$$CO_2\,(g) \leftrightarrow CO_2\,(aq) \quad (1)$$

$$CO_2\,(aq) + E.ZnH_2O \leftrightarrow H_2CO_3 \quad (2)$$

$$H_2CO_3 \leftrightarrow H^+ + E.ZnHCO_3^- \quad (3)$$

$$E.ZnHCO_3^- + E.ZnOH^- \leftrightarrow CO_3^{2-} + E.ZnH_2O \quad (4)$$

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

Recent work of our team, funded by the European Commission (project *NOBILIS*, 101025184), has focused on the study of biocementation using the CA metabolic pathway (Mwandira et al 2023a-d). The study involved isolation and screening of CA-producing bacteria from a railway embankment foundation fine-grained soil in East Anglia. Bacteria with high CA activity were applied to biocement the native soil. Some salient results of the laboratory study are shown in this paper. This is followed by a discussion on the modelling of the processes, which is the next task of our ongoing work. Although CA metabolic pathway was the main focus of this study, results based on the ureolytic pathway using the same isolated strains are also shown indicatively, as this is a pathway used in most other biocementation research.

2 MATERIALS AND METHODS

The soils studied came from two trial pits accompanying the corresponding window samples from two boreholes (labelled BH01 and BH02 respectively in Table 1) from a site in East Anglia, UK. They came from depths of 0.6-2.9m. They were sandy clay or silty sandy clay soils except for soil sample BH01_0.6-1.1m, which was clayey sandy silt.

CA-producing bacteria were isolated from the accompanying window samples. Namely, 1 g of soil was placed in 10 mL of sterile deionised water and homogenised using a vortex mixer. The soil suspension was serially diluted to 10^{-4} using sterile deionised water; 0.1 mL of the dilution was plated on CM0003 Nutrient agar (Oxoid) consisting of 1g/L meat extract, 2g/L yeast extract, 5g/L peptone, 5.0g/L sodium chloride and 15 g/L agar); 3 mM p-Nitrophenyl acetate (p-NPA), was used as an indicator for CA-positive-producing bacteria. Bacteria colonies with CA-producing ability gave an intense yellow colouration as a result of the hydrolysis of p-NPA into paranitrophenol (pNP). The plates were placed in an incubator for 3 days at 30 °C, At the end of the incubation period the soil yielded 41 CA-producing bacteria with different morphologies. A preliminary microorganism identification was performed using matrix-assisted laser desorption/ionization time-of-flight/time-of flight tandem mass spectrometry (MALDI-TOF/TOF MS) proteomic-based biotyping approach, using a Bruker Daltonics MALDI Biotyper (Mwandira et al, 2023a).

Table 1. Basic physicochemical characteristics of site soils.

ID	LL %	PL %	PI %	W %	pH	Organ. cont. %
BH01_ 0.6-1.1m	-	-	-	32.4 %	7.9	7.8%
BH01_ 1.2-2.2m	63	33	30	47.6 %	7.7	3.9%
BH02_ 0.6-1.1m	35	22	13	34.4 %	7.8	5.5%
BH02_ 1.2-2.2m	39	23	16	36.1 %	7.8	5.8%
BH02_ 2.2-2.9m	40	26	15	34.3 %	7.7	4.0%

The CA enzyme activity was determined colorimetrically as follows: the activity of p-nitrophenyl acetate hydrolysis was determined at room temperature in a reaction mixture (1.35 ml) containing freshly prepared 3 mM p-nitrophenyl acetate in phosphate buffer (0.13M and pH 7.2). The reaction was allowed to proceed for 5 minutes, and the change to A348 per min recorded. Then the CA activity was characterised by the amount of p-nitrophenol produced per unit of time, and enzyme activity expressed as shown in Eq 6.

$$\text{A activity}\left(\frac{\text{U}}{\text{mL}}\right) = \frac{(\Delta A_{348}T - \Delta A_{348}B)\times 1000}{5\times V} \quad (6)$$

where $\Delta A_{348}B$ is the initial uncatalysed reaction (Blank) at a wavelength of 348 nm; $\Delta A_{348}T$ is the final reading of absorbance; 1 U (μmol/min) is the

amount of the enzyme that catalyses the conversion of 1 micromole of substrate per minute; and V is the volume of bacterial suspension added to the cell.

The urease activity of the isolated strains was also determined using the electrical conductivity (EC) method. It was expressed in mM of urea hydrolysed per minute, calculated as shown in Eq. 7, where EC_1 and EC_5 are respectively EC measured at 1 and 5 min.

$$\text{Urease activity}\left(\text{mM}\frac{\text{Urea}}{\text{min}}\right) = \frac{EC_1 - EC_5}{5} 10 \cdot 11 \quad (7)$$

Bioprecipitate analyses were conducted using precultured bacteria in peptone agar p-NPA and B4 (0.4% yeast extract,0.5% dextrose, 0.25% calcium acetate, 1.4% agar) agar media respectively for CA and ureolytic bacteria) at a concentration of 9×10^8 cells/L (i.e., suspension of OD_{600} of 0.5 -MacFarland Standard). This cell concentration was diluted in the applied bioaugmentation solutions ($Ca(CH_3COO)_2$ and $NaHCO_3$ and $CaCl_2$ and $CO(NH_2)_2$ respectively for the CA and ureolytic pathways). The mixtures were incubated for 6 hours at 37°C with shaking (160 rpm) and then further centrifuged (15,000 rpm for 5 minutes) to collect the bioprecipitates, which were then characterised.

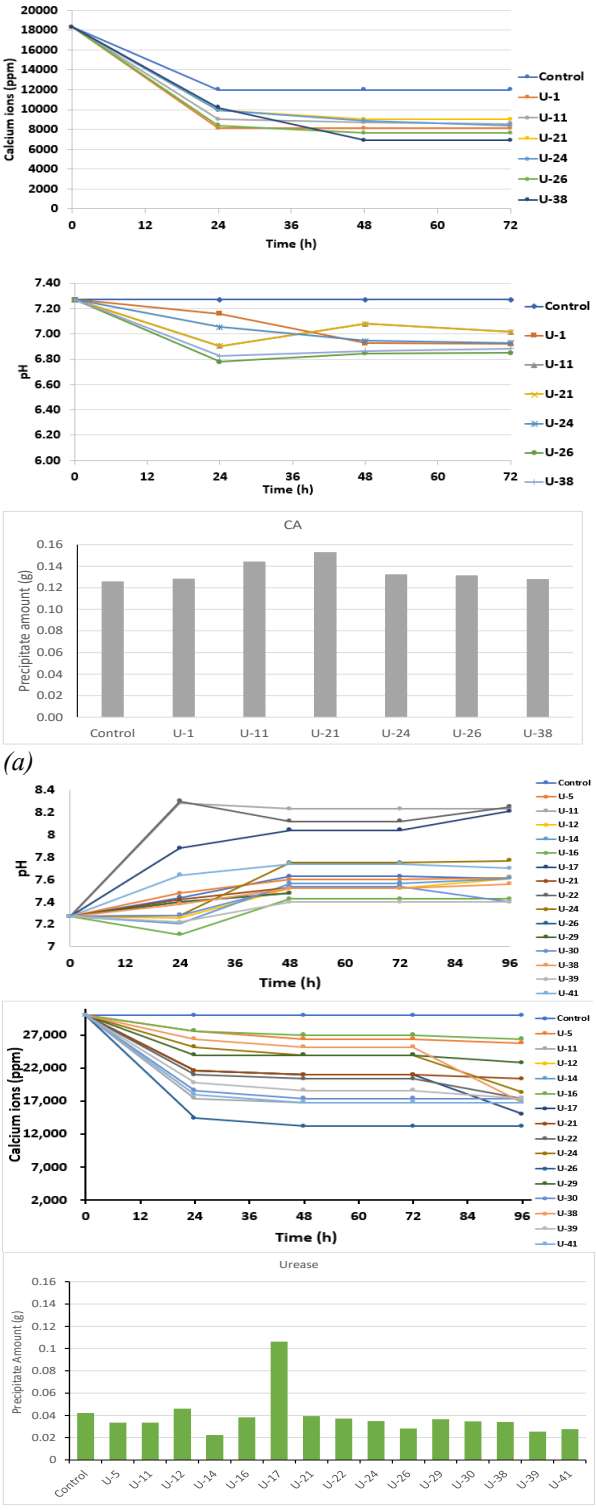
To induce biocementation, biostimulation was used. The treatments used are shown in Table 2. This study refers to the treatment of the clayey sandy silt soil BH01_0.6-1.1 (see Table 1), which was just about coarse enough to allow treatment by injection. Soil layers which were more fine-grained were treated electrokinetically (see Mwandira et al, 2023a). The biostimulation process was conducted as in Mwandira et al 2023(a), according to optimised conditions. Biostimulation media were injected first; then after 3 days (i.e., the optimal growth time of the isolated bacteria, see Mwandira et al, 2023a,d), the optimised cementation reagent solutions respectively for CA and UA pathways (see Table 2) were injected.

Table 2. Biostimulation treatments.

ID	Biostimulation	Cementation
CA pathway		
Yeast Extract	10 g/L	-
Sodium Bicarbonate	100 mM	0.25M/0.5M
Zinc Sulphate	1 µM	-
Calcium Acetate	-	0.25M/0.5M
Ureolytic pathway		
Yeast Extract	20 g/L	-
Urea	330mM	-
Urea	-	1M
Calcium Chloride	-	1M

3. RESULTS

Maldi-TOF results on the 41 CA-producing strains that returned matches of secure or probable genus identification were identified as of the *Bacillus* genus. Indicative results of bioprecipitate study on selected CA-producing strains which also showed ureolytic activity are presented in Figure 1.

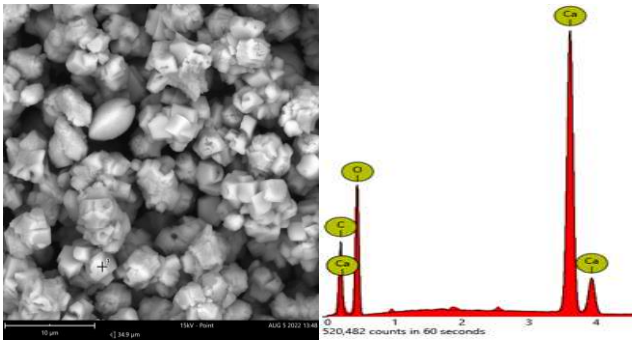


(a)

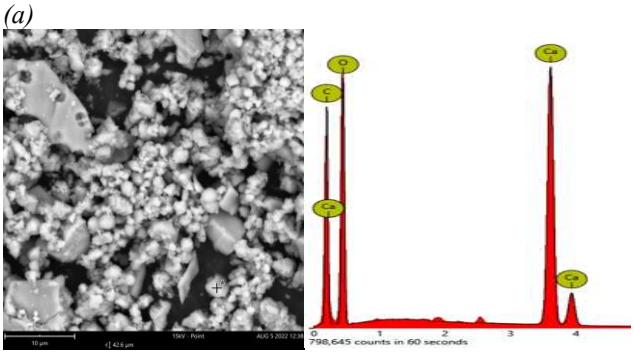
(b)

Figure 1. Bioprecipitates (a) CA route; (b) ureolytic route.

Figure 2 shows indicative SEM-EDS results of bioprecipitates obtained from the CA and ureolytic pathways. Carbonate minerals were confirmed, in most cases showing the typical calcite morphologies but rounder vaterite crystals were also detected depending on cementing solution molarity (not shown, for brevity). Figure 3 shows indicative photos of biocemented samples (after biostimulation) using the CA pathway, against untreated soil samples (control) and Figure 4 their unconfined compression strengths. The biocement can be clearly seen on the surface of biostimulated samples, concurring with the unconfined strength increase of the treated samples. Note that the ureolytic results were produced using a higher cementing solution molarity and this could be a factor leading to their higher strength increase.



(a)



(b)

Figure 2. SEM-EDS (a) CA route; (b) ureolytic route.

(a) (b)

Figure 3. Soil samples (a)control; (b)biostimulated (CA).

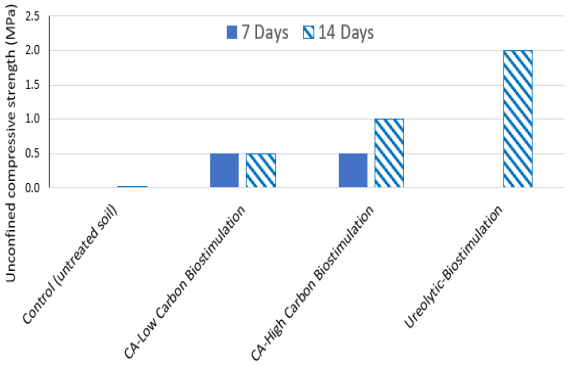


Figure 4. Unconfined compression test results.

4. MODELLING

Biocementation modelling comes under the general heading of reactive transport, whose origins go back to the 1980s with concerns about various environmental issues generally involving the flow and/or removal of contaminating chemicals in the ground (e.g. Lichtner et al., 1996). Traditionally three coupled processes are modelled: advection, dispersion, and chemical reactions taking place between solutes in the groundwater. Applying mass continuity, we obtain the basic differential equation for 1D advection-dispersion:

$$D^* \frac{\partial^2 c}{\partial x^2} - v_x \frac{\partial c}{\partial x} = \frac{\partial c}{\partial t} \qquad (8)$$

where v_x is the Darcy velocity in x direction, t is time

Generalisation of (8) to 3-D is straightforward and solutions are normally obtained numerically. In the general case there will be several different solutes (say N with different initial distributions of concentration) and this will lead to all these concentrations varying with time. The problem of solving a set of equations for a single set of concentrations which vary with time and space has been transformed by multiplying the number of equations to be solved by N. The final part of the picture is that these different solutes may react together (possibly forming new chemical substances) in a process which continues with time until overall chemical equilibrium is achieved. Techniques for the computer-based modelling of simultaneous chemical reactions have been in use since the 1990s and are now well established (Bethke, 2022). The most basic approach of coupling chemical reactions with the transport processes is to carry out the chemical reaction calculations following each transport time step (known as the Sequential non-iterative approach or SNIA -it is important here to consider the relative time rates between the chemical and transport processes to determine the appropriate approach). There are some additional processes which are significant in modelling biocementation as bacteria play an important role in biocementation by producing

enzymes which catalyse up important chemical reactions in the precipitation of calcite; the bacterial growth and enzyme activity needs therefore to be included in the modelling. Bacteria are commonly 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. Biofilm creation (coating the soil particles help promote sites for the calcite to precipitate) has been discussed and modelled for ureolytic bacteria (e.g. Faesli et al, 2023). The rate of the forward reaction (r_e) of CO_2 hydration catalysed by generic carbonic anhydrase may be described e.g. according to the Michaelis–Menten model. The reaction mechanism of CA has been extensively studied in other scientific fields or applications but we found no work so far modelling CA biocementation. Kinetics of the recombinant CA from bacteria have been reported in some CO_2 sequestration studies (e.g., Russo et al, 2016) and can be instructive. The final step for CA biocementation would be the precipitation of $CaCO_3$ as an immobile mass. This can be incorporated in the CA-pathway models in a similar way as in existing ureolytic models (e.g., Cunningham et al., 2019; Martinez et al., 2014; van Wijngaarden et al., 2011).

5. CONCLUSIONS

The work has focused on the little researched CA biocementation pathway, studying the biocemntation potential of bacteria isolated from soil under a railway embankment. The results proved the formation of calcium carbonate precipitates with calcite morphology, following the CA pathway, and the biocementation potential of these strains, corroborated by the increase in the unconfined compressive strength of the soil. Future work is addressing the modelling of the CA biocementation process, for which no information has been found in the literature. However existing studies on biocementation via the ureolytic pathway can be instructive, whereas further laboratory testing can serve in providing modelling parameters.

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