

Investigating ammonia-free urea hydrolysis biocementation processes

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Abstract. Biocementation (i.e., the production of biomimetic cement through the metabolic activity of microorganisms) has attracted the vivid interest of researchers worldwide in the last decade. To date most research works and commercial products proposed biocementation using the urea hydrolysis metabolic route, as it is a fast and easy to control process. However, its major limitation is ammonia production, with adverse environmental impacts. Consequently, research effort has focused on how to alleviate or mitigate ammonia by-products, while using this metabolic route.

The paper presents results of soft organic soil biocementation using an *ex-situ* urea hydrolysis process, developed so that the produced ammonia does not reach the soil. Indigenous ureolytic bacteria extracted from the soil of a site in East Anglia, UK were used to produce the urease enzyme, which catalyses the urea hydrolysis reaction. Following the proposed *ex situ* process, measured ammonia contents were found to be within acceptable limits. Soil strength increased and calcite precipitated in the soil although biocementation by bioaugmentation with the same indigenous bacteria gave higher soil strengths and CaCO₃ precipitation than the ammonia-free process. After the presentation of results, further advantages and disadvantages of the respective biocementation methods (*ex situ* vs. *in situ*) are then discussed.

Keywords: low-carbon cements, biocementation, urea hydrolysis, ground improvement, soft soils

1. Introduction

Soil biocementation (i.e., the production of biomimetic cement through the metabolic activity of microorganisms) is an emerging soil stabilisation method that has attracted the vivid interest of researchers worldwide. This is because it gives promise for the development of nature-based low-carbon and overall, more sustainable solutions for ground improvement, towards the net zero carbon transition. It has been proposed as an alternative chemical ground improvement method to stabilization with Portland cement or lime, both emitting considerable CO₂ amount during their production. However, to-date research works studied mostly biocementation using the urease hydrolysis

metabolic route. The main advantage of this metabolic pathway is that it is fast and easy to control. However, its major limitation is the production of ammonia, which dissolves easily in water and can be toxic to various organisms. Additionally, when converted into nitrate, another concern is that it can harm human health and promote eutrophication. Research effort has therefore focused on how to mitigate mitigate the negative side-effects of ammonia by-products, while using this metabolic route, which is the best researched hence the most promising to-date for industrial scale application, with a few products starting to be commercially available (e.g. Biocalcis; MeduSol).

A number of mitigating measures for the ammonia by-products have been proposed if the urea-hydrolysis route is used for biocementation. These include: a) flushing /rinsing until ammonia concentration levels are within acceptable limits (Lee et al, 2019); b) removal of ammonia by-products from the effluent through struvite precipitation (Gowthaman et al, 2022); c) use of nitrifying bacteria; d) electrokinetic removal of ammonia (Keykha and Asadi, 2017); e) production of the carboante ions *ex situ* through the action of bacteria, then, after filtering out ammonia (using for example, zeolite, as in Keykha et al, 2018) CO₃²⁻ are then implemented into the soil together with calcium ions to precipitate CaCO₃; f) collection of ammonia using a biofiltration system to produce soil fertilizer e.g. for nearby farmlands.

This paper follows on previous biocementation work carried out at London South Bank University, to treat a soft organic soil of a railway embankment site in East Anglia, UK, a novelty of the authors' research (Safdar et al, 2020, 2021a-b, 2022). The work used indigenous ureolytic bacteria isolated from the *in situ* soil to perform biocementation by bioaugmentation. Unlike previous work which bioaugmented the soil with the indigenous microorganisms to promote *in situ* urea hydrolysis, in the process presented in this paper, urea hydrolysis occurs outside of the soil, so this part of the process can be done *ex situ*. A solution rich in CO₃²⁻ is produced outside the soil using ureolytic bacteria to produce urease enzyme, which catalyses urea hydrolysis; a zeolite filter system is the used

to filter out the ammonia. Carbonate ions are then sent into the soil and then, a calcium source is provided so that CaCO_3 is precipitated in situ.

The following sections show the experimental methods and *ex situ* hydrolysis and injection system setup and provide results.

2. Materials and methods

2.1 Soil

The soil used in this study was the same as in Safdar et al (2021a,b, and 2022) for the purposes of comparison. It came from bulk samples from East Anglia, UK. In its as-received state the natural soil was a mixture of mineral and organic fractions of very dark greyish brown colour (10YR 3/2 according to Munsell chart); based on pocket penetrometer measurements its undrained shear strength indicated was 76 kPa. Based on its organic content (>20%), the soil was identified as sandy (sand>50%) amorphous peat (i.e., “of no visible plant structure and mushy consistency”, BS EN ISO 14688-1:2018, BSI, 2018). The sample had a low natural moisture content of 55.5% consistent with a humified /decomposed organic soil. Based on its ash content by dry weight (< 25%) the soil was equally classified as peat (basic sapric peat) according to ASTM D4427-92 (1997). For the tests the sample was pulverised and sieved to remove inorganic debris; the portion passing the 1.18mm sieve was retained for testing, as in Safdar et al (2021a,b, and 2022).

2.2 Microbial strains

The study used screened and isolated non-pathogenic indigenous ureolytic microbial strains from the soil sample which showed potential for biocementation, as described in Safdar et al 2020 and 2021a,b. Of the four strains studied *Bacillus licheniformis* was selected as the most promising for this soil treatment and was used for further study. The optimised treatments according to Safdar et al (2020, 2021a, 2022) were used; namely bacteria concentrations of 1×10^8 (cfu/mL) supplemented in nutrient broth of 3g/L nutrient broth (Oxoid, UK, consisting of 5-g/L peptone, 5-g/L sodium chloride, 2-g/L yeast extract, and 1-g/L beef extract), and equimolar solutions of 0.75M urea and 0.75 M CaCl_2 (cementing reagents).

2.3 Injection system and process for *ex situ* urea hydrolysis

To implement treatments through a soil column a pressure driven flow was used as described in Safdar et al, (2021a, 2022). The apparatus consisted of a Plexiglas cylindrical mould, a hydraulic pump, a compression frame and an effluent collector. A modification of the process in Safdar et al, (2021a, 2022) was then used for *ex situ* microbially induced calcite precipitation (MICP). For this purpose, the experimental procedure was divided into three phases.

In the first phase, the aqueous solution of bacterial strain (*Bacillus licheniformis*) (see Safdar et al., 2021a and 2022) was mixed with 0.75M urea solution to produce CO_3^{2-} ions and NH_4^+ ions in solution. Note that the initial population of the bacterial solution was kept 1×10^8 (cfu/mL) and 24 hours of mixing time to correlate to previous experiments in Safdar et al (2021a,2022). Justifications of the selected bacteria populations and

solution molarities can be found in Safdar et al (2021a,2022). After 24 hours the NH_4^+ concentration of the bacteria urea solution was measured.

Phase 2 then involves the extraction of bacteria from the solution and filtration to remove NH_4^+ ions. The bacterial extraction was done by centrifuging the solution at 10,000 g and supernatant was pipetted out and filtered through 0.20- μm Sterile PES Syringe Filter (Fisher scientific). In the next step the filtered solution was passed through natural purified granular (0.75-1.25mm) zeolite. The zeolite was filled in a circular tube of 50mm diameter and filled to achieve a thickness of 250mm (Fig 1). The NH_4^+ concentration of the effluent passing through the zeolite was then measured. The zeolite filtration was conducted twice to bring NH_4^+ concentration below 0.5 mg/L.

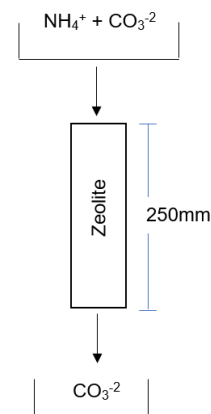


Figure 1. Schematic representation of phase 2

Phase 3 includes the injection of ammonia-free effluent containing CO_3^{2-} ions and 0.75M calcium chloride (CaCl_2) solution into the soil. In this study, the filtration through zeolite was conducted under gravity. However, the injection of CO_3^{2-} and CaCl_2 was done under 150 kPa pressure, as in Safdar et al (2021a, 2022). The bacterial-nutrient broth solution and CaCl_2 were supplied in quantities of a total of 15% by mass of the soil sample.

2.4 Testing procedures

At the end of the treatment period (after 1 day of curing), duplicate cylindrical specimens of 50mm diameter and 100 mm height were extracted from the soil column. They were subjected to Unconfined Compressive Strength (UCS) testing at a constant rate of strain of 1mm/min. Calcium carbonate (CaCO_3) content of the UCS samples (after testing) was determined by acid digestion test using 20 g of oven-dried (at 105 °C) soil samples soaked with 2 M hydrochloric acid (HCl) (Ng et al, 2014). The residue was collected on filter paper and oven dried at 105 °C and the mass loss measured to estimate the CaCO_3 content in the soil, expressed as a percentage of the dry soil sample mass. The effluent from the column was sampled for pH and ammonium (NH_4^+) measurements.

3. Results and discussion

Table 1 summarises the main results and compares them against the *in situ* method, using the same bacteria at the same concentrations and the same cementing reagent

solution molarities and quantities per soil mass. It can be seen that both in the *in situ* method as well as in the *ex situ* method before filtration and after first filtration the NH_4^+ levels exceeded the allowable limits of total ammonia for drinking water according to UK legislation, set to 0.5 mg/L (The Water Supply (Water Quality) (Amendment) Regulations, 2018). In fact, the NH_4^+ concentration for the *ex situ* tests before zeolite filtration was found to be higher compared to the *in situ* procedure, possibly because bacteria may have hydrolysed more urea in liquid state compared to when mixed directly in the soil. This requires further investigation, and for this purpose the concentration of CO_3^{2-} in the cementation solution will be measured in future tests.

However, after the second filtration stage NH_4^+ levels fell well below the maximum allowable concentration limit. It is believed that a finer grade of zeolite could potentially remove ammonia effectively in one filtration. This will be investigated in future work.

Unconfined compressive strengths (q_u) and calcite contents were higher in the *in situ* rather than the *ex situ* method. Still, the strength increase with the *ex situ* method was 28% higher than the control mix with nutrient-broth only (the nutrient broth was used as control mix because it contains salts that could contribute to some strength increase). Also, the treatment clearly improved the undrained shear strength of the untreated soil which was found to be 76 kPa.

Table 1. Results summary

	Ex situ urea hydrolysis	In situ urea hydrolysis (with bacteria in soil)
Average q_u (kPa)	401.5	428
Average calcite (%)	0.86	1.28
Average Ammonium (NH_4^+) (mg/L) before filtration:	1.55	0.98
Average Ammonium (NH_4^+) (mg/L) after 1st filtration:	0.69	N/A
Average Ammonium (NH_4^+) (mg/L) after 2nd filtration:	0.21	N/A

The *ex-situ* method has the main advantage discussed earlier: it is likely more sustainable than flushing the soil with water to reduce the ammonia concentrations. Additionally, it circumvents complexities of controlling

bacteria in the soil (sensitive to numerous environmental conditions) to produce the desired biocementation outcome and also addresses any concerns about potentially encouraging the growth of pathogens in addition to the targeted bacteria as nutrients come into the soil.

However, it negates most arguments put forward in favour of the biocementation concept as it loses the advantage of using the action of bacteria in the soil: first of all, bacteria in the soil act as further nucleation sites for the calcite precipitation, thus enhancing the process; this can explain the higher strengths achieved using the *in situ* process (see Table 1). Secondly, by using bacteria in the soil the process is potentially renewable and self-healing mechanisms can be explored as one could ‘revive’ the bacteria/spores in the soil to continue the process as required. Conversely, the products of the bacteria from *ex situ* urea hydrolysis are finite. Once the enzyme and CO_3^{2-} have been depleted the process stops, as opposed to when using the bacteria *in situ*. In essence, one is reducing the process to just a chemical treatment of the soil (using the bacteria as a way to produce enzyme and CO_3^{2-}) rather than a biochemical process in the soil.

Furthermore, fermenting bacteria to just centrifuge them and filter them out appears to be a rather onerous and probably not the most efficient way of producing CO_3^{2-} . Instead, one could potentially induce calcite precipitation purely using a chemical route of a reaction between Na_2CO_3 and CaCl_2 . Na_2CO_3 sources are widely found in nature, they can be sourced by brines and are cheap (although arguably not renewable). The relative efficiency and overall sustainability of the *ex situ* biocementation vs. the purely chemical method can be assessed further through detailed sustainability analyses.

Therefore, although the *ex-situ* process is possible, other recommended options to investigate comparatively are to either: a) use bacteria that are not ureolytic so that the ammonia problem is bypassed; b) use the electrokinetic process to remove NH_4^+ by electromigration; c) use nitrifying bacteria to consume the ammonia; in soil there are many organisms, which can oxidise ammonia during the *in situ* process. Also, efficient ways of collecting and using ammonia for example as a N source for plants or for hydrogen production could be developed in future works.

4. Conclusion

The aim of this work was to assess a biocementation method of *ex situ* urea hydrolysis through the action of ureolytic bacteria, versus an *in situ* biocementation method by bioaugmentation of native non-pathogenic ureolytic bacteria. The method is meant to mitigate ammonia by-products of the urea hydrolysis process during biocementation with ureolytic bacteria. The treatments were applied to a soft organic soil from East Anglia which is a weak foundation soil of railway embankments, prone to large settlements. The assessment of the technique was done through measurements of the ammonium content (whose reduction was the specific aim of the method) but also in terms of biocementation treatment of the soil, i.e. in terms of the improvement of the unconfined compressive

strength (UCS) of the soil and accompanying CaCO₃ precipitation measurements. It was found that after a two-step filtration process through a zeolite filter the method was effective in reducing ammonium content well below acceptable levels (as opposed levels measured before filtration during the ex situ process or during the in situ process). Strengths and calcium carbonate contents increased, proving biocementation has occurred, but

remained lower to those of the respective in situ process, which also has some further advantages. It is recommended to study comparatively different ways of circumvent ammonia by-product production during soil biocementation or ways of removing and using ammonia in order to identify the most efficient and sustainable overall process.

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