

# Biocementation of an organic soil using indigenous ureolytic bacteria

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## Abstract

This paper describes research aiming at assessing the potential for biocementation of an unsuitable organic foundation soil encountered in the UK railway network. As opposed to the majority of previous studies it focuses on isolation and use of non-pathogenic, indigenous ureolytic bacterial strains from the *in situ* soil, which are capable of inducing calcite precipitation. The paper describes the procedures for indigenous bacteria isolation and screening, their growth and urease activity and shows results from soil strength and calcite precipitation testing proving biocementation for this type of soil using indigenous bacteria.

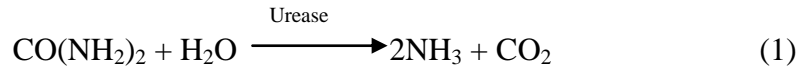
*Keywords: Biocementation; organic soil; urea hydrolysis*

## 1. INTRODUCTION

Growing urbanisation worldwide leads increasingly to construction on inferior ground in urbanised areas; at the same time the growing population in urban centres will require new infrastructure based on complex engineering with little tolerance for error (e.g. high rise buildings, deep basements in urban areas, high-speed trains). Existing infrastructure facilities will also need to be upgraded to meet future needs and changing environmental loads due to climate change. These include ageing transport earthworks in many European countries suffering from serviceability problems and requiring costly maintenance/remediation. Common ground improvement methods for foundation soils and earthworks may be successful in minimising severe damage but they commonly suffer from high costs, environmental side effects, limited lifetime, and interruption to services. Therefore, the development of innovative, superior and cost-effective soil improvement techniques to mitigate natural and man-made hazards while minimising waste and other environmental impact is necessary a field of ongoing intensive research effort.

In this context, biocementation of soils has been proposed as a potentially more sustainable and superior soil stabilisation technique (DeJong et al., 2013), as it is a nature-based solution, using the metabolic action of non-pathogenic and renewable microorganisms to cement soils, thus improving their engineering properties. Namely, the technique is inspired by the natural process of biomineralisation, i.e., the biological production of minerals through the metabolic processes of living organisms. Whereas a number of possible metabolic pathways can produce biocements, the most commonly investigated mechanism has been the precipitation of the calcium carbonate using ureolytic bacteria and predominantly *Sporosarcina pasteurii* which was proven to be effective by several a number of researchers (e.g. Whiffin, 2004; Al Thawadi, 2008; Al Qabany et al, 2012; Montoya et al, 2013; Montoya and De Jong, 2015; Gao et al., 2018, amongst many others).

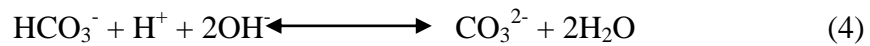
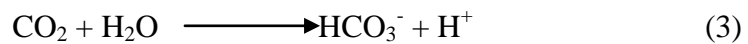
The precipitation of  $\text{CaCO}_3$  by urea hydrolysis is a multi-step chemical reaction, which can be described as follows: first, the initial urea [ $\text{CO}(\text{NH}_2)_2$ ] hydrolysis generates ammonia ( $\text{NH}_3$ ) and carbon dioxide ( $\text{CO}_2$ ) (see Eq 1).



The local increase in pH occurs due to the hydroxyl ions (OH<sup>-</sup>) generated by the conversion of ammonia to ammonium, which leads to the breakdown of bicarbonate to carbonate ions (Eq. 2).



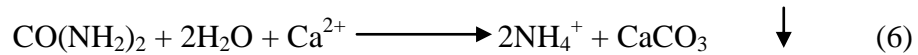
The carbon dioxide quickly reacts with the water and produces bicarbonate (HCO<sub>3</sub><sup>-</sup>) (Eq 3), which further reacts with hydroxyl ions (OH<sup>-</sup>) to generate carbonate ions (Eq 4).



Hence, the precipitation of CaCO<sub>3</sub> occurs in the presence of calcium ions (Ca<sup>2+</sup>).



The overall process of urea hydrolysis and CaCO<sub>3</sub> precipitation is thus given as:



The urea hydrolysis process was used successfully predominantly for sands but has been less investigated for other soil types. In particular the use of biocementation for problematic soils such as organic soil/peat is very little explored in the literature. In addition, the vast majority of studies used exogenous bacteria which could present issues of competition with indigenous species, and adaptability in the new environment.

This paper describes research carried out at London South Bank University in collaboration with Middlesex University, UK, aiming at assessing the potential for biocementation of an unsuitable organic/peat foundation soil encountered in the UK railway network and causing severe engineering problems to the railway infrastructure owners and operators, Network Rail. As opposed to the majority of previous studies the paper focuses on isolation and use of non-pathogenic, indigenous ureolytic bacterial strains from the *in situ* soil, which are capable of inducing calcite precipitation and summarises a number of factors affecting the biocementation outcome.

## 2. MATERIALS AND METHODS

### 2.1 Soil sample

The soil used in this study came from two boreholes at an East Anglian railway site. The properties of the sample retained for testing in its as received state were determined as shown in Table 1.

**Table 1.** Properties of organic soil sample

Property	Value
Natural gravimetric moisture content (%)	55.5
Organic matter content (%)	50.8
Loss on Ignition (%)	52.7
Liquid Limit (%)	101
Plastic Limit (%)	63
Plasticity Index (%)	38
Specific Gravity ( $G_s$ )	2.060
Bulk Density ( $\text{kg/m}^3$ )	1316
Dry Density ( $\text{kg/m}^3$ )	919
pH	7.15
Zeta potential ( $\zeta$ ) (mV)	-38.4
Colour Description (Munsel chart)	10YR 3/2
Undrained shear strength (pocket penetrometer) (kPa)	76

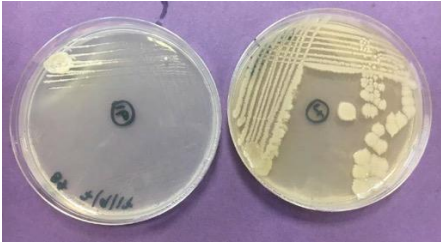
Based on its organic content (>20%), the soil was identified as sandy (sand>50%) amorphous peat (i.e. “of no visible plant structure and mushy consistency”, BS EN ISO 14688-1:2018, (BSI, 2018). The samples had a low natural moisture content which is consistent with a humified /decomposed organic soil. Based on its ash content by dry weight (< 25%) the soil is equally classified as peat (basic sapric peat) according to ASTM D4427-92 (1997).

## 2.2 Isolation and screening of bacteria

Eighteen soil samples (nine from each borehole) were selected for bacteria isolation, depending on consistent pH, moisture contents, organic contents and soil type to reduce the extensive microbiological laboratory work. Isolation of bacteria was done by adding and thoroughly mixing 1 g of soil from each soil sample to the conical flasks where it was diluted in sterile distilled water; 1mL of the diluted culture solution was then plated out on 15mL of molten Tryptic soya agar (TSA) (Oxoid, UK). The plates were inverted and incubated at 25°C for 3-7 days.

The screening of the isolated bacteria was done on the basis of several parameters, primarily rate of growth at different temperatures, ability to form crystals on the solid high carbon media, and most importantly the ability to produce calcite in the soil. After one week of incubation, 98 samples showed considerable growth in all dilutions. These were transferred to the individual B4 Agar plates (0.4% yeast extract, 0.5% dextrose, 0.25% calcium acetate and 1.4% agar in solid preparations) and incubated at 37°C for one week to form mineral crystals. 49 out of 98 samples showed good production of crystals (confirmed microscopically) and were selected and passaged twice on B4 plate to obtain purified single colonies. The 49 purified individual colonies were then transferred to Nutrient Agar (NA) (Oxoid, UK) to be identified further (bacteria cannot form crystals on NA agar). The NA is a high nutrient medium having composition (0.5% peptone, 0.3% beef or yeast extract, 1.5% agar as solidifying agent and 0.5% NaCl).

To further test the viability of the selected bacterial strain, the purified samples were incubated for 7 days but at considerably low temperature from 4°C to 7°C. All the 49 samples shown considerable growth at the lower temperature but the rate of growth was slower in the first 2-3 days, increasing gradually by the end of the 7 days period. However, the overall growth at lower temperature was one-fourth, and in some cases, was one-fifth of that of the same culture when grown at 25°C. (see Fig 1).



**Figure 1.** Comparative rate of growth of same strain incubated at 4°C (left) or at 37°C (right) after 3 days

The strains were grown to an early stationary phase i.e., Optical Density (OD): OD<sub>600</sub> ranging from 0.5-0.7; they were then harvested by centrifuging at 8000 g for 10 minutes to achieve the final concentration of approximately  $1 \times 10^8$  cfu/mL (optical density 3.3).

Microbial identification and diagnosis of the final 49 samples was performed using Matrix-Assisted Laser Desorption-Ionization Time-of-Flight tandem mass spectrometry (MALDI-TOF/TOF MS) proteomic-based biotyping approach. The sample preparation and extraction of proteins and peptides of the bacteria were performed according to the Bruker bacterial sample preparation protocol. Each extracted sample was analysed using a MALDI ground steel target plate. In order to ensure homogeneity and reliability in the results, six different sample spots (replicates) for each sample were laid to generate six combined mass spectra (MSP) per bacterial isolate. After acquisition and analysis of mass spectra, the identification of the isolated bacteria strain was performed with MALDI Biotyper software 3.0 (Bruker Daltonik) through comparison with reference strains.

### **2.3 Urease activity measurement**

The urease activity and the resulting ammonia concentration in the treated soil was directly measured by a Urease Activity Assay kit (Colorimetric; Abcam, US). Clear supernatant containing urease was obtained by centrifugation for 5 min at 14,000 x g for each bacterial species. The enzyme reaction was performed at pH 7 at 30°C for 4 hours using the following steps: 0.1 mL supernatant was collected and added in a micro vial. For the test sample, 0.1 mL of Urea was added into 0.9 mL solution tube and incubated at 37°C for 2 hours. The solution was then centrifuged at 8000 x g for 1 minute; 0.1 mL supernatant was collected and placed in a micro-vial in which reagents were added and vortexed with a mechanical mixer. The solution was again incubated at 37°C for 30 minutes. The output was measured on an ultraviolet-visible spectrophotometer at OD<sub>670</sub>.

### **2.4 Sample preparation for geotechnical property testing**

For the geotechnical analyses, all the test strains were cultivated at pH 7 under aerobic batch conditions in a sterile culture medium of Nutrient Broth (Oxoid, UK) in a shaking incubator at 200 rpm and 37 °C. The strains were grown to an early stationary phase i.e., Optical Density (OD): OD<sub>600</sub> ranging from 0.5-0.7 (measured using a Pharmacia LKB Novaspec II spectrophotometer of 325-900 nm Wave length Range); they were then harvested by centrifuging at 8000g for 10 minutes to achieve the final concentration of approximately  $1 \times 10^8$  cfu/mL (optical density 3.3); a second concentration of  $1 \times 10^7$  cfu/mL was obtained by dilution with sterile sodium chloride solution (9-g/L NaCl). Different bacterial strains took different time period to reach the final required optical density of  $1 \times 10^8$  cfu/mL. For this purpose, rate of growth for each bacterial strain was recorded against time (see Fig. 2 in the results section), and then growth controlling factors such as temperature of incubation were adjusted accordingly to quickly and effectively obtain the required growth OD.

The samples were mixed with 15 % added water or aqueous solutions by mass of the soil sample and left for 48-72 hours in air tight seal for homogeneity of treatment. The solutions contained

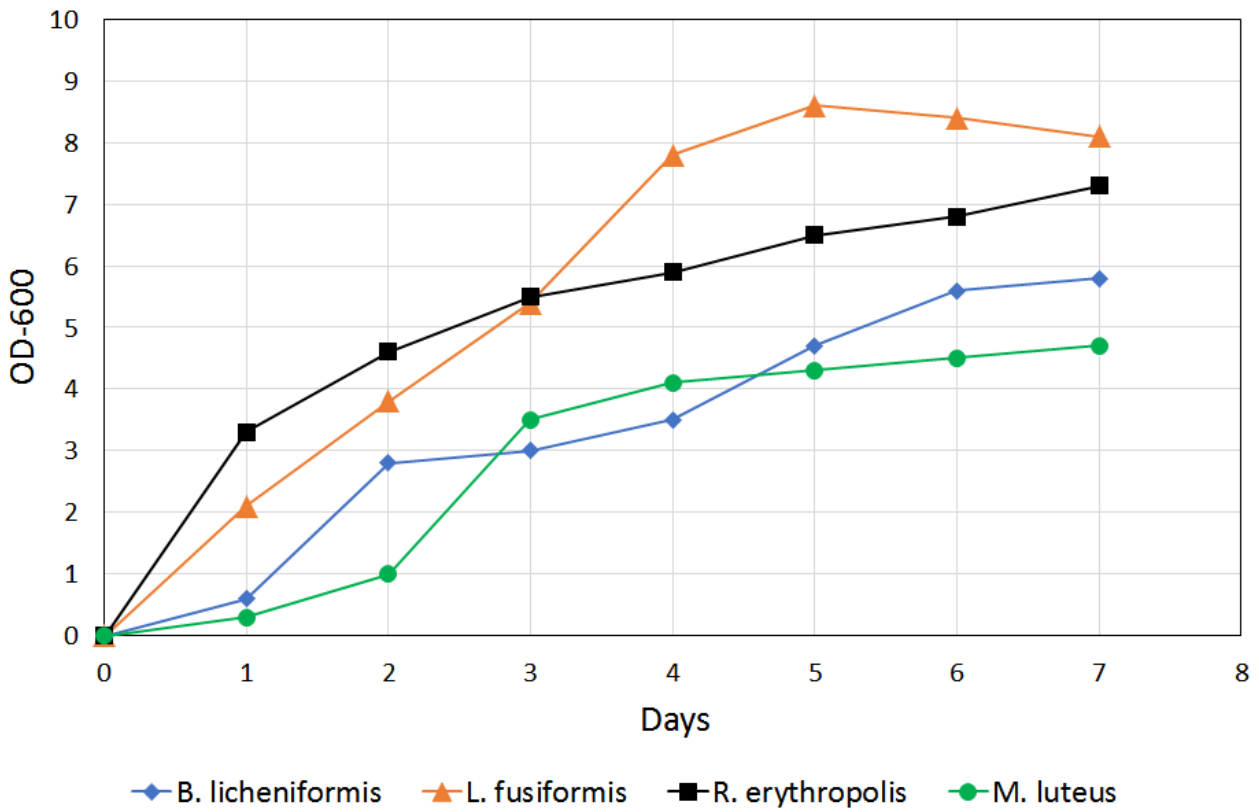
nutrients, bacteria, and cementing agents i.e. urea and calcium chloride. Statically compacted (1mm/min rate) specimens in layers of 10mm were prepared at a dry density corresponding to the field density (see Table 1). The dimensions of the samples varied according to the equipment used for the treatment implementation, i.e. hand mixing of all treatment solutions versus implementation through light pressure into a flow column as well as electrokinetic injection of the treatments. The experimental setups of the latter two methods are described in detail in Safdar et al (2020). Samples prepared as a minimum in duplicate or triplicate included: the natural soil sample at its *in situ* water content (control sample); samples injected with water only; samples injected with nutrients only; samples with nutrients and bacteria but no cementing agents; samples injected with nutrients and cementing agents but no bacteria (this method could still potentially trigger biocementation by biostimulation of the existing bacteria in the soil) and finally, full treatment (nutrients, cementing agents and bacteria). In total we prepared: 4 sets of hand mixing implementation samples (these series of tests were discontinued due to low strength results obtained); 27 pressure flow column experiment sets of samples (note that when bacteria were used these were premixed in the soil and then the cementing agents were implemented through light pressure into the column); in addition to studying the individual performance of the selected bacteria other parameters studied in the pressure flow column test were bacteria population and cementing agent concentration; finally 14 sets of electrokinetic injection samples were prepared for the best performing type of bacteria only, where the main factors tested were premixing of the bacteria in the soil compared to full treatments injected electrokinetically (i.e. also bacteria), and the effect of degree of saturation.

The samples were then subjected to unconfined compression testing (UCS) whereas monitoring of ammonia of the effluent, calcite content measurements and pH measurements were used to support and explain the shear strength values obtained from the UCS tests.

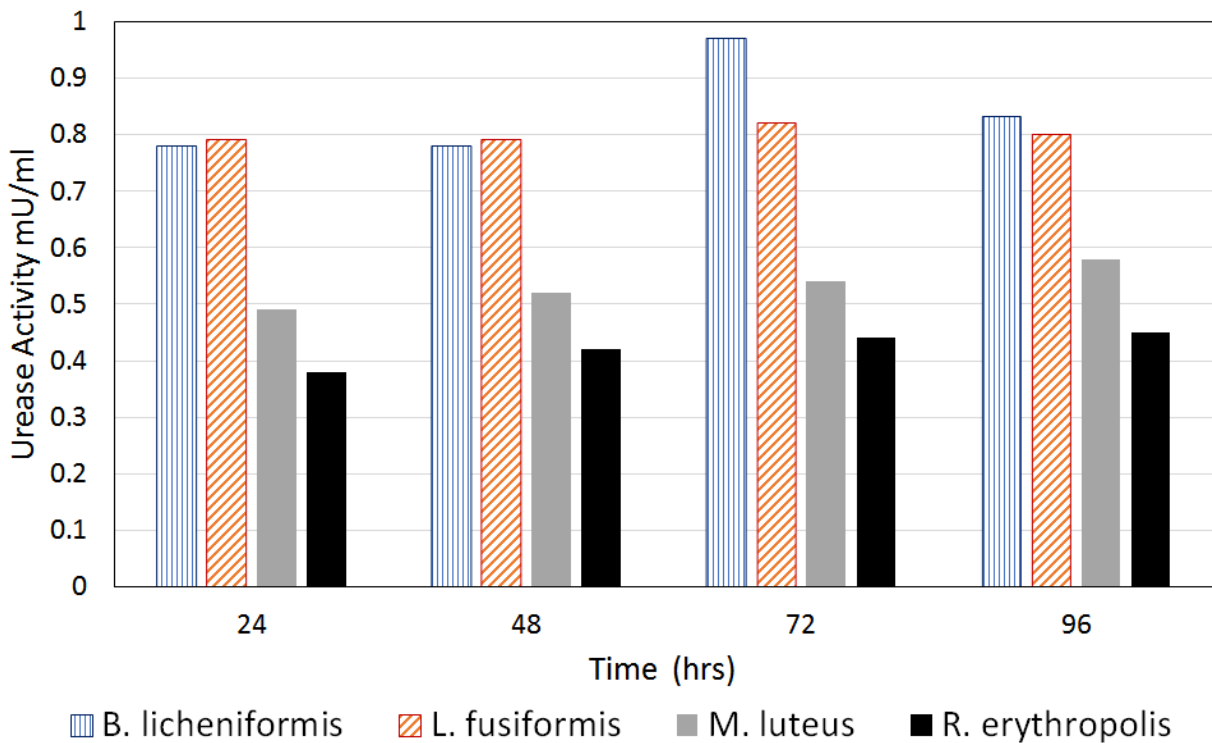
### 3. RESULTS AND DISCUSSION

#### 3.1 Bacterial Growth Rate and Urease Activity

The four best indigenous ureolytic bacterial strain candidates for biocementation were *Bacillus licheniformis*, *Rhodococcus erythropolis*, *Micrococcus luteus*, and *Lysinibacillus fusiformis*, based on their ability to grow and survive at low to medium temperatures and pH values of 4.5-10, and their urease enzyme production ability. The rate of growth of these bacteria against time is shown in Figure 2. Their urease activity during the incubation period in crude enzyme solution is shown in Figure 3. It was assumed that the rate of urea hydrolysis would be proportional to the urease activity. For the *Bacillus licheniformis* and *Lysinibacillus fusiformis* the maximum Urease activity was recorded at 72h despite the fact that cell population for both these strains continue to increase well after first 72 hours (see Fig 3). However, the urease activity for these strains did not reduce after the first 72 hours, but was maintained around the maximum value with some fluctuations. In the case of *Micrococcus luteus* and *Rhodococcus erythropolis* the urease activity kept on increasing with incubation duration. *Rhodococcus erythropolis* showed the lowest enzyme activity which also reflects in the lower urea hydrolysis and resultant lower CaCO<sub>3</sub> production and strength increase.



**Figure 2.** Rate of Growth of bacteria cells against time

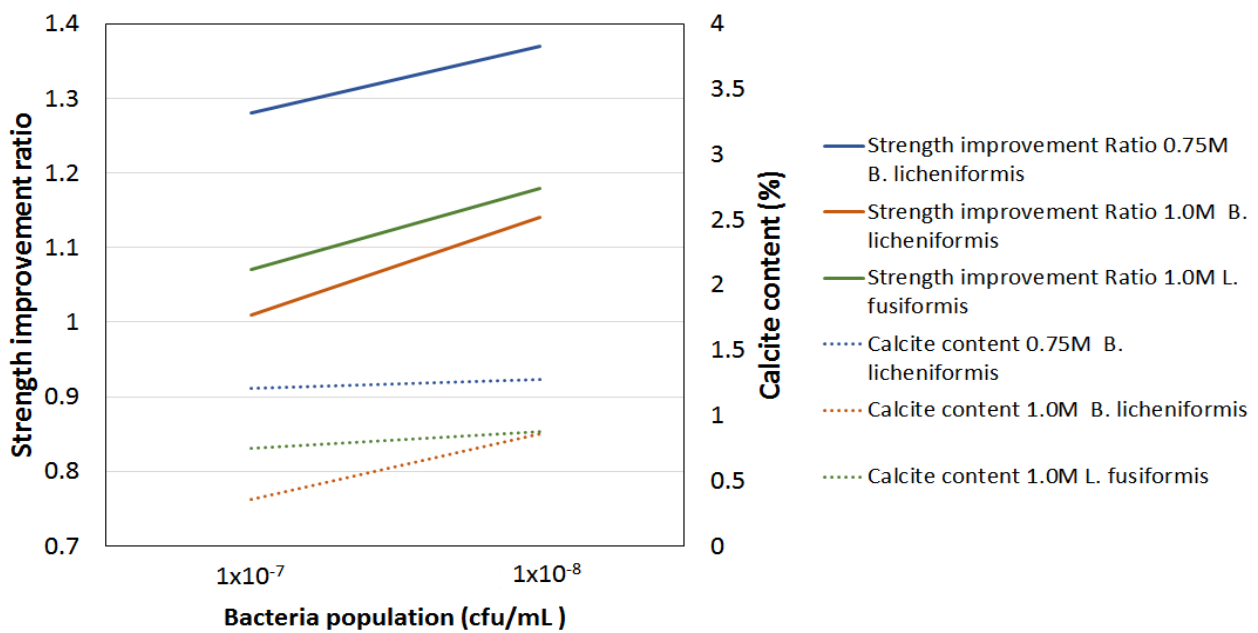


**Figure 3.** Urease activity of microorganisms at different times

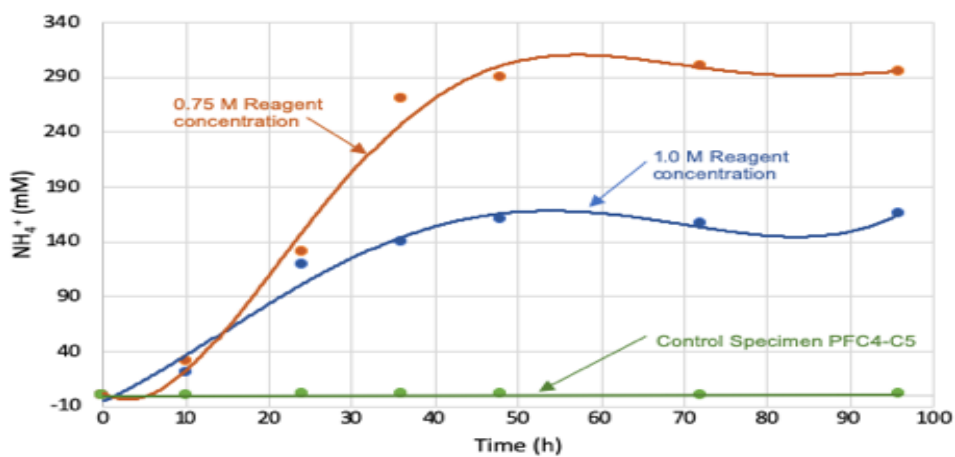
### 3.2 Unconfined compression testing results

Figure 4 shows indicative results of pressure flow column tests in terms of biocemented unconfined compressive strength (UCS) increase compared to the respective control samples with nutrients only. It illustrates the effect of bacterial populations ( $1 \times 10^7$  cfu/mL and  $1 \times 10^8$  cfu/mL respectively)

as well as cementing reagent concentration for some of the best results of the pressure flow column tests i.e. those from *Bacillus licheniformis* and *Lysinibacillus fusiformis*. From the figure it can be seen that the increase in bacterial population led to higher strength improvement ratio compared to the control mix, and higher calcite precipitation, which can be attributed to higher urease activity with the increase in the amount of bacteria. When keeping the other variable fixed, it is noted that the higher bacterial population produced higher UCS strength and  $\text{CaCO}_3$  content for both strains. It is also noted that 0.75M led to higher strength than 1M cementitious reagent solution for *Bacillus licheniformis*, implying that increasing the cementing reagent concentration does not necessarily lead to better results in terms of strength and that there is some optimal concentration for the treatment. This higher strength increase for 0.75M is consistent with the measurement of  $\text{NH}_4^+$  concentration in the effluent from the treatment (see Fig 5), which is higher for the 0.75M compared to 1M cementing solution, showing higher urease activity; the lower  $\text{NH}_4^+$  concentrations and strength can be attributed to urease activity inhibition at high  $\text{CaCl}_2$  concentrations (Whiffin, 2004).



**Figure 4.** Effect of the concentration of Bacterial population and cementing reagent (0.75 and 1.0M) on the UCS strength  $q_u$  and calcite content % of the biocemented soils.



**Figure 5.** Variation of  $\text{NH}_4^+$  concentration in the effluent in time for *Bacillus licheniformis*.

Concerning the effect of degree of saturation on the treatment, electrokinetic tests (whose results are not shown here for brevity) showed that best results were consistently achieved for the highest degree of saturation tested, i.e. 95% compared to 85% and 75%. In general electrokinetic injection led to better results than pressure flow column and this was the case whether bacteria were premixed in the soil (as in the flow column tests) or injected electrokinetically into the soil; the former implementation method produced the highest strengths and calcite content in the soil, but there when bacteria were injected electrokinetically there have been strength increases of 20-35% in parts of the sample. Whilst the non-uniformity of the treatment needs to be addressed and further investigated, the observed increase in strength and calcite content in parts of the sample shows promise that electrokinetics could be a viable technique for treating foundation soil under existing infrastructure, which is a major challenge for engineers.

#### 4. CONCLUSIONS

The study proved the feasibility of using indigenous ureolytic bacteria for the biocementation of peat Fens foundation soil of UK railway embankments. This was based on UCS testing supported by  $\text{CaCO}_3$  measurements, which proved that biocementation did occur after implementing a number of soil treatments. The use of indigenous non-pathogenic bacteria is environmentally beneficial, as the interference on the local microbial ecology is reduced compared to solutions using exogenous to the location bacteria. Ongoing work is currently investigating the effect of biocementation on a number of other soil properties and the durability of the treatment, after which upscaling of the techniques towards in situ implementation is planned in the next stage of the research.

#### ACKNOWLEDGEMENTS

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