# Microbial induced calcite precipitation as a viable ground improvement technique

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#### Abstract

Traditional ground improvement techniques, such as grouting or compaction, can be invasive, energy demanding and expensive. Microbially induced calcite precipitation (MICP) offers a sustainable alternative by utilizing a natural process, and has therefore been the focus of extensive interest and laboratory research over the past decade. Most of that research has been at laboratory-scale on the factors that affect process efficiency. The use of MICP in the field have been discussed in numerous research papers but remains largely theoretical and examples of field-scale trials are rare.

MICP uses ureolytic bacteria, such as the common soil bacteria Sporosarcina pasteurii, which are given access to an ample supply of urea and calcium chloride. The bacteria hydrolyse the urea into ammonium and carbonate, raising the pH and in the presence of calcium in solution, facilitating the precipitation of calcite crystals (CaCO3). It is particularly effective when used with fine grained sands as those calcite crystals form a bridge between the individual sand grains, cementing them together and creating a weak bio-sandstone.

This project, through bench-scale column experiments on MICP treated sands, has investigated optimization of the influencing factors of the bacteria concentration, the treatment strategy employed and the number of treatment cycles administered. The influence these parameters have on the ultimate core strength, from unconfined compressive strength (UCS) tests, and the homogeneity of the calcite distribution, have been determined. These results have then been used to design an efficient treatment process to underpin large-scale trials of MICP for ground improvement and erosion protection.

## Introduction

Soil as a construction material has typically been viewed by engineers as a passive substance, with poor engineering properties, that often requires intervention to make fit for purpose [1]. One commonly used soil improvement technique is chemical grouting, involving the injection of cement or chemical grouts into the ground to bind the soil particles together, creating strength and stiffness within the soil [2]. Unfortunately, such treatments "increase the pH of groundwater to highly alkaline levels", which can alter the local ecosystem and generate a number of environmental concerns [3]. In response to this, Wang *et al.* [1] state that "it is necessary to develop a new type of environmental friendly and cost-effective material which can be used for ground improvement for sustainable development".

Microbial induced calcite precipitation (MICP) has the potential to offer that sustainable alternative to traditional ground improvement techniques. Bao *et al.*, [4] note that, "Soil is a living ecosystem in which biogeochemical processes such as mineral precipitation, gas generation, biofilm formation, and biopolymer generation are ubiquitous". MICP is a biogeochemical process that involves the artificial inducement of calcium carbonate (calcite) precipitation by "microbial metabolic activity" [4]. It is sustainable as it can be applied in-situ and can utilize common bacteria such as the ureolytic soil bacteria *Sporosarcina pasteurii* (*S. pasteurii*). By giving the bacteria access to a supply of urea and calcium chloride, the urease positive bacteria hydrolyse the urea into ammonium and carbonate (Equation 1), raising the pH and facilitating the precipitation of calcite crystals [5].

The bacteria also play a critical role as nucleation sites for mineral growth within the soil matrix. This encourages calcite development between the grains, effectively cementing them together and increasing the mechanical properties of the soil such as the shear strength, compressibility and stiffness [4]. It is particularly effective when used with fine grained sands as those calcite crystals form bridges between the individual sand grains and create a weak bio-sandstone [6]. Figure 1 illustrates the calcite crystals bridging the gaps between the larger quartz sand grains, cementing them together.

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-}$$
 (1)  
 $Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3$ 

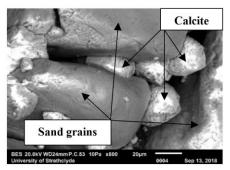


Figure 1 - S.E.M. image

In practice, the MICP process must be both economical and efficient if it is to be used as a sustainable and eco-friendly alternative to traditional methods [7]. To become a viable ground improvement technique there are a number of influential factors that first need to be optimised at the bench-scale. These factors include, but are not limited to, the concentration of the bacteria suspension, the treatment strategy employed and the number of completed treatment cycles [7]. This project, through bench-scale column experiments on MICP treated sands, has investigated optimization of some influencing factors. The impact these parameters have on the ultimate core strength, from unconfined compressive strength (UCS) tests, and the homogeneity of the calcite distribution, have been determined. These results have then been used to design an efficient treatment process to underpin large-scale trials of MICP for ground improvement and erosion protection.

#### Materials and methods

# Bacteria and cementing fluid

The species of bacteria used in all experiments were *Sporosarcina pasteurii* (*S. pasteurii*), taken from cold-stored plated colonies and inoculated into autoclaved flasks of yeast extract broth media, with 20 g/l urea. Those flasks were then placed in a floor shaker at 115 rpm and 30 °C for up to 24-hr until adequate growth and an optical density ( $OD_{600}$ ) greater than 1 was achieved. Fresh batches of broth media were prepared and inoculated with bacteria daily, to grow and be ready for use the following day.

Two main approximate concentrations of bacteria were used for the experiments; *in water* and *in broth*. For the *in water* concentration, each batch of bacteria were first separated from the supernatant by centrifuge before being re-suspended with tap water and restored to the original volume and a concentration which was measured as just above 1 OD<sub>600</sub>. As the name suggests, *in broth* refers to the bacteria being kept in the original growth media with a measured optical density also just above 1.

To facilitate the MICP process, the bacteria must have a supply of urea to hydrolyse and a source of calcium to precipitate into calcite. These were supplied through the addition of a

cementing solution made from 0.5 equimolar of urea and calcium chloride dissolved in deionised water.

# <u>Soil</u>

To create a fine soil only three sizes of sand were used; 28% at 0.15 mm, 33% at 0.212 mm and 39% at 0.3 mm. After initial fraction separation, the sand was washed and dried before the exact composition for each sample was individually weighed and mixed to ensure all contained the correct proportion. The initial average flow rate through the samples was 11ml/min, with a discharge velocity of 58 cm/hr, however it was possible to reduce that to roughly 6 ml/min, and a discharge velocity of 32 cm/hr, by including 3% of silica flour in the mix. Finally, the samples were vibrated to increase the bulk density to 1.7 Mg/m³, with a sample porosity between 35 and 37%.

### Set-up

Clear plastic tubing, of 38mm inner diameter, was cut into lengths to create experiment moulds. As shown in Figure 2, a fine net material was secured over the bottom to prevent any loss of sand when the fluids were added, and to allow the samples to free drain under gravity. Prior to initial treatment, the samples were flushed with 2 pore volume (PV) of tap water to create a partially saturated state. At the end of the treatment, the samples were flushed with 10PV of tap water to stop all reactions and expel any unused fluids. Finally, the samples were allowed to air dry for approx. 1 week before being removed from their moulds and prepared for testing.

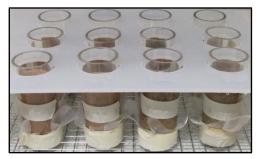


Figure 2 - Prepared samples for treatment

#### Treatments

Each treatment cycle began with 2PV of the bacteria, at each concentration, simply poured into the top of the sample and creating a temporary head. A static period of approximately 1-hr was allowed so the fluid would percolate through the sample and the bacteria would attach themselves. 2PV of cementing fluid was then added in the same manner as the bacteria and the samples were then left static until the next day (approx. 22-hr). A further 2PV of cementing fluid was then added to each sample before a further static period of approx. 24-hr to complete the cycle. The MICP treatment process was continued for 12 days until a total of 6 treatment cycles were completed on all samples.

# **Testing**

Samples extracted intact from the moulds were adjusted to a ratio of 2:1 (76mm x 38mm) and tested by unconfined compression (UCS) to ascertain the strength properties. Each test was continued until failure was observed by a sudden drop in peak strength displayed on the real-time results graph.

Using a Jeol JSM-IT100 machine, scanning electron microscope (SEM) images were taken of sample fragments to ascertain the crystal growth with the sand grains. Using the same machine, it was also possible to conduct further analysis on the samples with energy-dispersive X-ray spectroscopy (EDS), giving an overview of the main chemical elements present.

# Supplemental investigation

Further investigation was conducted to ascertain any difference in precipitation from administering the bacteria in different media. Simple bench experiments using bacteria in broth media, bacteria re-suspended in tap water, and supernatant that the re-suspended bacteria had been removed from, were performed in triplicate. 40ml of cementing fluid was added to beakers that already contained 40ml of the fore mentioned three varieties of fluid, then left static for 2-hr to allow for precipitation. Figure 3 shows an example of the supernatant during the 2-hr static period. The fluid was then vacuumed off through filter paper, which was dried and weighed to give the quantity of precipitate produced.



Figure 3 – Supernatant and CaCl

#### Results and discussion

It can be seen from Figure 4, that the samples treated with the bacteria still in broth produced higher strengths and total calcite contents than the samples treated with re-suspended bacteria. Despite the small number of results attained, the *in broth* results appear linear, with increasing calcite contents relating to increasing UCS, which corresponds with observations from other authors such as Cheng et al. [8] and Whiffin et al. [9].

Closer examination from the SEM images, Figure 5 and 6, show that there is a distinct difference in the formation/growth of the calcite crystals between the *in water* and *in broth* samples. Most notable is that the re-suspended bacteria has effectively formed a uniform blanket of calcite that is coating or encapsulating the sand grains. In contrast, the crystals created with *in broth* bacteria are more irregular in shape and size, while also appearing to be amassed between the sand grains rather than coating them. Crystal growth at the contact points between the sand grains is desired in MICP treatment and is associated with an increase in mechanical properties, such as strength and stiffness, of treated sands [2].

Apart from the bacteria suspension fluid, both samples were the same, from composition to post treatment, so any difference in results must be due to that fluid. The results of the simple experiments, shown in Figure 7, reveal that the supernatant alone is able to produce a significant amount of calcite. As the bacteria had been removed and there was no ureolytic activity present, there must be carbonate ions (from the bacteria growth) present in the supernatant fluid [10]. Those ions react instantaneously with the calcium chloride in the cementing fluid to precipitate calcite.

This means that the *in broth* samples effectively have an initial burst of precipitation forming calcite crystals before the bacteria start the hydrolysis of the urea. The impact of this phenomenon has both positive and negative implications and associations.

Although that initial precipitation from the supernatant may produce a boost for the MICP process, it may also significantly reduce the pore space and flow paths at the inlet, preventing fluids freely migrating through the entire sample [11]. Such an occurrence would effectively prevent homogeneous calcite distribution, instead creating samples with a strong top/inlet and a high calcite content, while the bottom/outlet was weak with a low calcite content.

#### **Total Calcite Content vs UCS** 2.5 Ж 2 Ж UCS (MPa) 1.5 1 +'in water' **≭** 'in broth' 0.5 0 10 10.5 11 11.5 12 12.5 Calcite Content (%)

Figure 4 - Experiment results by bacteria concentration

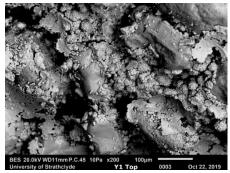


Figure 5 - SEM image of 'in water' sample

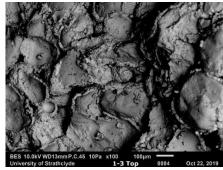


Figure 6 - SEM image of 'in broth' sample

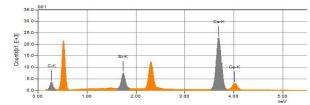


Figure 7 - EDS analysis of 'in broth' sample

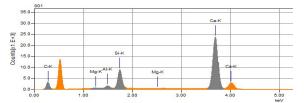


Figure 8 - EDS analysis of 'in water' sample

# Precipitate Produced in 2 hrs

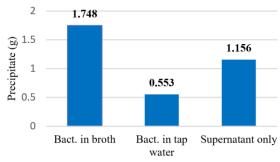


Figure 9- Precipitate results

EDS analysis of the samples reveals that the *in broth* sample, shown in Figure 8, has more impurities than the *in water* sample, shown in Figure 9, which has the basic chemical elements of only carbon, silicone and calcium. According to the analysis the *in broth* samples also have a small percentage of magnesium and aluminium present, although only at trace levels within these samples.

#### Conclusion

MICP offers an environmentally friendly and sustainable alternative to traditional ground improvement techniques by generating calcite within the soil matrix and increasing the strength properties of the soil. Although treatment with bacteria kept in their growth media increases calcite content, that initial precipitation could prove a hindrance to continuing a treatment programme. Ideally, for ground improvement, MICP treatment should result in homogeneous distribution of calcite to ensure consistent improvement of the engineering properties throughout the treated area. In contrast, coastal erosion prevention or dust suppression improvement may be able to utilise that fast precipitation to create a strong protective crust on the surface, effectively protecting the underlying soil layers.

Sufficient knowledge and understanding of these factors, and others, will ensure that any large-scale trials will be appropriately designed and targeted for that application, ensuring an efficient process.

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