Influence of Resting Periods on the Efficiency of Microbially Induced Calcite Precipitation (MICP) in Non-saturated Conditions

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Abstract. Microbially Induced Calcite Precipitation (MICP) is a relatively new soil improvement technique that has been extensively studied during the last two decades. Most of these studies have been performed in saturated conditions and only few papers deal with non-saturated conditions. In this study, we investigate two injection protocols with or without resting periods between treatment steps. The results of unconfined compression tests performed on treated samples, as well as the measurement of the calcite contents, lead to the conclusion that the resting periods improve the stiffness of the samples and the calcite content.

1 Introduction

Microbially Induced Calcite Precipitation (MICP), also known as biocalcification, is a relatively new process relying on the formation of precipitated calcium carbonate. This technique is mostly used for granular soils and consists in injecting aerobic bacteria in the soil that can hydrolyze urea to produce carbonate ions. This injection of bacteria is usually followed by an injection of urea, nutrients and a calcium-rich solution. The calcium ions combine with carbonate ions issued from the bacterial activity and lead to the precipitation of calcium carbonate around the bacteria. Ultimately, the precipitation results in the formation of bridges between the grains of soil, such as the natural cementing process of sandstone and other carbonated rocks (Martinez and DeJong 2009; Harkes et al. 2010; Montoya et al. 2012).

A wide variety of parameters must be considered to have a successful reaction, such as the pH, nucleation spots and the availability of calcium and carbonate ions (Stocks-Fischer et al. 1999, Hammes and Verstraete 2002; Girinski 2009). According to the *in-situ* conditions, two different methods can be implemented. The first one, and the most used, consists in injecting directly into the soil all the nutriments in saturated conditions and simultaneously pumping the reactants or biproducts in excess (Montoya et al. 2012; Cheng et al. 2013; Dejong et al. 2013; Martinez et al. 2013; Montoya et al. 2013; Cheng and Cord-Ruwisch 2014). This technique allows below-the-surface and wide spread (until 5 m wide) treatments (van Paassen et al. 2010). The other technique is the injection of all the required reactants by gravity percolation. This technique is not suitable for deep treatment but seems promising for surface level problems as, unlike the previous one, it does not require two sets of wells. Finally, the saturation of the soil is a

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critical factor in the efficiency of the methodology. Using a saturation lower than 100% means that the treatment is more focused on the grains of soil (Cheng et al. 2013).

The objective of this paper is to present two protocols of MICP through gravity percolation and to investigate the influence of resting periods on the efficiency of MICP treatments.

2 Materials and Methods

The general approach of the experiments relies on the treatment of non-saturated soils according to two different protocols, with and without resting periods. The experiments were performed in duplicate to ensure the accuracy of the results.

2.1 Soil Specimens

The treated soil was pure silica sand (Ottawa) with a homogeneous particle-size distribution. This sand is considered poorly graded according to the Unified Soil Classification System (USCS) and its relative density has been evaluated to 2.644 g/cm^3 according to the Canadian National Standard CAN/BNQ 2501-070/2014. The minimum and maximum densities were respectively evaluated to 1.453 and 1.700 g/cm^3 according to the Canadian National Standard CAN/BNQ 2501-062/2005.

2.2 Treatment Solutions

To fulfill the treatment, three kind of solutions must be used: (1) a **bacterial solution**, with a specific concentration of bacteria, (2) a **fixation solution** to facilitate the attachment of bacteria onto the soil's grain and finally, (3) a **cementation solution** to grow the carbonate crystals.

The bacterial strain of Sporosarcina pasteurii (ATCC 11859) was grown on a solid sterile aerobic yeast-extract medium (Tris buffer 15.75 g/L, yeast extract 20 g/L, ammonium sulfate 10 g/L, agar 10 g, pH = 9.0) for 72 h at 30 °C, then put in a fridge at 4 °C for conservation.

For the preparation of the bacterial suspension, a liquid yeast-extract medium was prepared. The composition of this liquid medium was identical to the previous solid medium, less the agar. About 10 colonies were extracted from the yeast-extract medium and placed in 100 mL of the liquid medium. Then, a 800 rpm agitation was performed with a magnetic bar at 20 °C ± 1 °C during 60 h. After this period, the absorbance at 600 nm was a bit more than 1 and samples of 50 ml were centrifuged at 5 000 rpm for 15 min to produce, after removing the supernatant, a single pellet of bacteria.

The bacterial solution consisted of 2 pellets in a urea medium to reach an absorbance of about 2.5. The urea medium was composed of 6 g/L Bacto nutrient powder, 40 g/L of Urea, 10 g/L ammonium chloride, 4.24 g/L sodium carbonate at a pH 6 before autoclave.

The fixation solution uses the same urea medium with the addition of equimolar content of calcium chloride. Dehydrated calcium chloride was used, which brings the concentration to 98 g/L.

Finally, the cementation solution was identical to the fixation solution, but the name was different to help in identifying the stage of the protocol.

2.3 Treatment Protocols

Two protocols were successfully elaborated, their difference lying in the curing time. The first step of each protocol was the packing of the dry sand in an Acrylonitrile Butadiene Styrene (ABS) mold of 2 in. (50.8 mm) in diameter and 4 in. (101.6 mm) in height with the methodology to reach the minimum density allowed by the soil, the density is then verified with the known volume of the mold and the weight of the sand. To support the sand, a filter and a perforated cap were placed at the bottom of the mold. The perforated cap was dedicated to the evacuation of the solution in excess during the injection.

The sand was then extracted and mixed by hand with the bacterial solution in a quantity equal to 35% of the void volume. The sand and bacteria were then reintroduced in the mold and a percolation of an identical volume of the fixation solution was started at a flow rate of 120 ml/h from the top of the sample. A curing time of 24 h was allowed to fix the bacteria on the grain particles.

Finally, the cementation phase started. In the first protocol, there were 5 cementation phases separated by 24-h curing periods. For each cementation phase, a volume corresponding to about 110% of the void volume was injected. In the second protocol, only one cementation phase of a volume equivalent of the total volume of the first protocol was performed. In other words, the second protocol was identical to the first one, without the 24-h curing periods.

2.4 Microscopy Observation and X-Ray Analysis

To visualize how the bridges were formed, some sub-samples were taken from the sample treated with the second protocol and analyzed through Scanning Electronic Microscope (SEM) with a JEOL JSM840 apparatus. The samples used to understand the composition thanks to X-ray tests were the same as for the microscopy. They were analyzed with a Philips X'PERT apparatus.

2.5 Calcite Content

The easiest and fastest way to measure the calcite content is to weight the mold and the dry sand before treatment and to compare it with the weight of dry samples after treatment. This procedure was performed on samples dried at 50 °C after treatment. The main advantage of this procedure is that it is easy to implement and reproductible.

2.6 UCS Tests

Unconfined compressive strength tests were performed according to the D-2166 ASTM Standard. After treatment, the samples were flattened using a file and the height and diameter were measured. The speed of the press was adjusted between 0.5% and 2% of the height of the samples.

3 Results and Discussion

3.1 Strength and Calcium Carbonate Content

The results of the compressive tests were put in perspective with the calcium carbonate content in Fig. 1. In this figure, P1 correspond to the first protocol and the P2 to the second one. The duplicates demonstrated the good reproducibility of both protocols. The results show that the Young modulus is largely influenced by the presence of a rest period: mean value equals to 39.70 MPa for P1 and only 11.28 MPa for P2. This observation is mainly due to the low concentration in calcite obtained for P2 specimens, which illustrates the lowest efficiency when there is no rest period during injection steps. As a consequence, the rest periods in protocol P1 produce two times more of calcite and increases the Young modulus by a factor of 3.5 compared to P2.

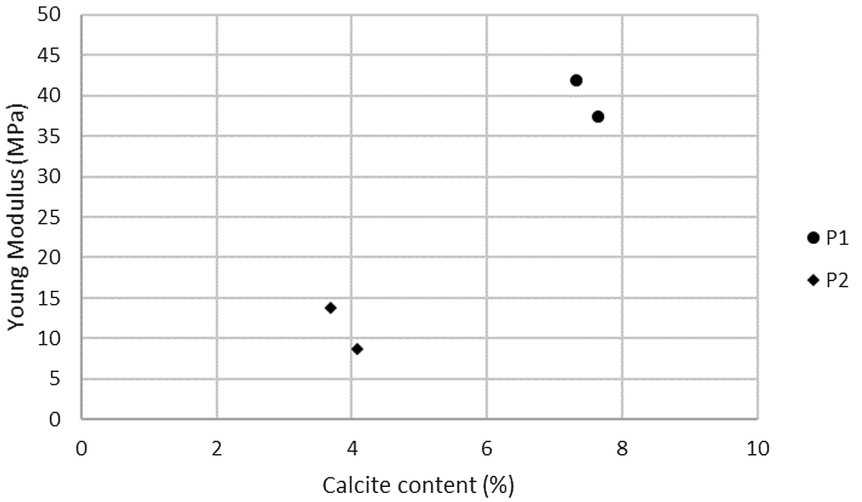

Fig. 1. Young modulus as a function of calcite content

3.2 X-Ray Analysis

An X-ray analysis was performed on a specimen of each protocol and the results are provided in Figs. 2 and 3, respectively for protocols P1 and P2. For protocol P1, it is possible to observe that the measurement is very close to the calcite reference. On the contrary, the comparison to vaterite and aragonite references showed the absence of aragonite and some traces of vaterite. The observation of the P2 specimen offers similar results, the difference being that there is no vaterite in this second sample. Moreover, the presence of silica is self-explanatory as soil grains are 100% pure silica sand. Finally, the investigation presents ammonium chloride as a byproduct of the reaction. As the sample needs to be dried before testing, some residue might still be imprisoned in the water and slowly crystalizing as the water evaporates. Nevertheless, their solubility index is about 414 g/L at 30 °C whereas the calcite is 8.6 10^{-2} g/L at the same temperature (Stchouzkoy-Muxart 1971). The calcite being less soluble than the ammonium chloride, the hypothesis that the augmentation of mass during the process is mainly due to the calcite is plausible. This analysis leaded to the conclusion that the precipitated crystal, cause of the improvement of strength, is calcite, and not vaterite or aragonite.

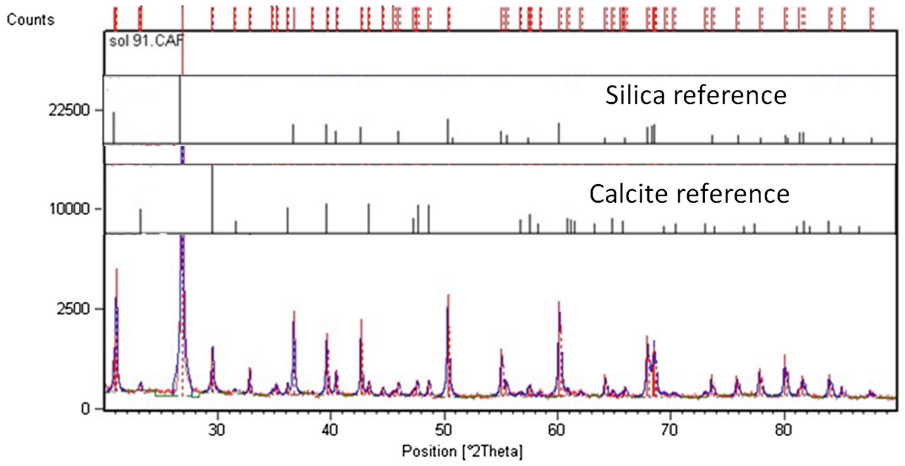

Fig. 2. X-ray analysis (Protocol P1)

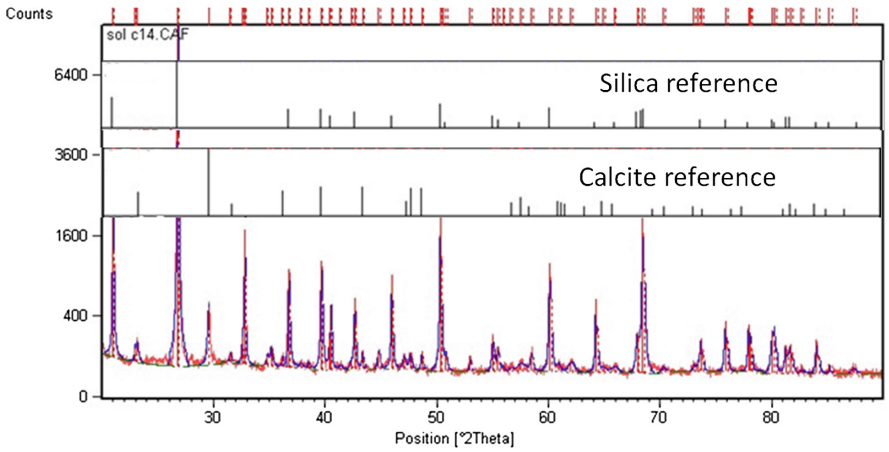

Fig. 3. X-ray analysis (Protocol P2)

3.3 Microscopy Imagery

An example of image taken on P2 specimen is presented in Fig. 4. This sample had a local percentage of calcite of about 3%. The bridges of calcite are visible, and it is

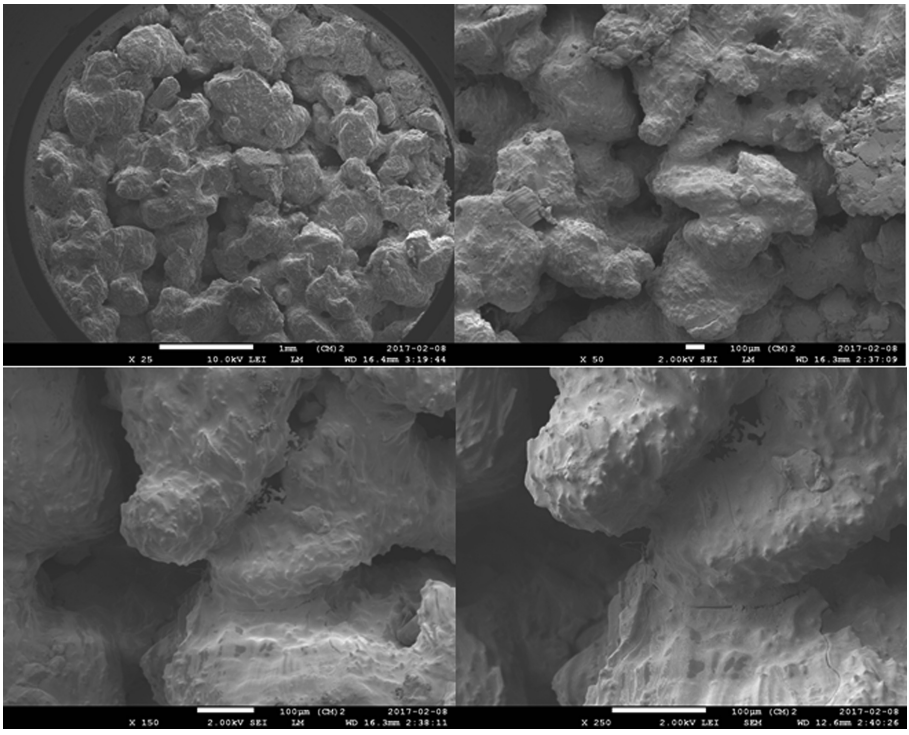

Fig. 4. Microscopic imagery (Protocol P2)

possible to observe a breach in one of them (white arrow in Fig. 4). After the breach of the most fragile zone, the calcite precipitated around the grains is supposed to improve the internal angle friction of the sample, compared to non-treated soils.

4 Conclusions

The goal of this paper was to establish an easy to use protocol to implement MICP in non-saturated conditions. Two protocols were created, and they allowed the samples to reach calcium carbonate content between 3 and 6%. The unconfined compression tests performed on the samples showed that rests periods during injection steps lead to stiffer specimens as well as higher calcite contents. These results are particularly promising for the treatment of loose sand in superficial layers.

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