Ecological Engineering 42 (2012) 64–72

Contents lists available at SciVerse ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

In situ soil cementation with ureolytic bacteria by surface percolation

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ARTICLE INFO

Article history:
Received 20 September 2011
Received in revised form
19 December 2011
Accepted 30 January 2012
Available online 1 March 2012

Keywords:
Biocementation
Microbially induced carbonate precipitation (MICP)
Calcium carbonate
Soil improvement
Unsaturated soil
Soil liquefaction

ABSTRACT

The possibility of using microbiological processes to improve the mechanical properties of soil by undisturbed in situ application has gained attention over recent years. This paper describes a new variation of in situ soil reinforcement technology based on microbially induced carbonate precipitation (MICP), which involves both the hydrolysis of urea by soil bacteria enzyme and calcium carbonate precipitation in the presence of dissolved calcium ions. In contrast to other previously published approaches, the current work uses surface percolation for in situ placement of bacteria and cementation solution. Bacteria could be immobilised over the full length of a 1 m column by surface percolation. To accomplish this it was necessary to percolate alternate solutions containing either bacteria or fixation solution containing calcium ions.

The biologically triggered cementation resulted in homogeneous cementation over the entire length of the 1-m sand column. The efficiency of calcite crystals to form strength was found to be related to the pore water content of the continuously drained column with less water content enabling more efficient strength formation. Scanning electron microscopy supported the idea that lower water contents lead to selective positioning of crystals at the bridging points between sand grains. These findings imply that the cost of MICP technology can be reduced by optimising the conditions for effective crystals precipitation. This is expected to make this technology more readily acceptable for large scale applications.

1. Introduction

Microbial induced calcium carbonate precipitation (MICP) by urea hydrolysis has been a topical subject of research in recent years (Ivanov and Chu, 2008; De Muynck et al., 2010). Enabled by interdisciplinary research at the confluence of microbiology, geochemistry, and civil engineering (DeJong et al., 2010), MICP technology has been applied to relevant applications, such as wastewater treatment (Hammes et al., 2003), and calcareous stone restoration (Stocks-Fischer et al., 1999; Castanier et al., 2000). In addition, this process has been explored for the improvement of the strength and stability of soft and poorly consolidated sand soil (Whiffin et al., 2007; van Paassen et al., 2009). Compared to chemical or cement grouting techniques, which are usually harmful to the environment, MICP has been proposed as an environmentally friendly method (Le Metayer-Levrel et al., 1999).

The microbial method of soil improvement generally involves three steps:

1. Urea is hydrolyzed by microbial urease to form ammonium and carbonate ions (Eq. (1)).

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

2. The produced carbonate ions react with calcium ions and precipitate as calcium carbonate crystals (Eq. (2)).

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3(s) \quad (2)$$

3. Sand grains are bound together by the calcium carbonate crystals.

As the bacterial cells are distributed throughout the solution, or sand core, the reaction of generating an oversaturation of dissolved calcium carbonate happens uniformly throughout the sample and at a controlled rate. This is in contrast to reactions resulting from mixing calcium solutions with carbonate solutions.

Current exploitations of the bacterial capacity to release carbonate in situ in the presence of calcium ions has been limited to using water logged soils (DeJong et al., 2006; Whiffin et al., 2007; van Paassen et al., 2009), requiring heavy machinery and hydraulic injection of the cementation solutions. In order to induce MICP in the water saturated soil subsurface, a method of two-phase injection of bacterial suspension and cementation solution for

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doi:10.1016/j.ecoleng.2012.01.013

bacterial immobilisation has been developed (Whiffin et al., 2007; van Paassen et al., 2010). This method makes use of the effect of ionic strength, on microbial fixation to sand particles. Increased ionic strength, in particular calcium ion concentration, encourages bacterial adsorption onto the surface of sand particles (Scholl et al., 1990; Torkzaban et al., 2008). Initially, a solution of suspended bacteria is injected into a soil matrix. This is followed by the addition of a fixation solution consisting of 50 mM $CaCl_2$.

For sufficient bacterial adsorption, a slow flow rate of fixation solution is required which will enable adequate intermixing between the bacterial suspension and fixation solution (Harkes et al., 2010).

The cementation of non-water saturated sandy soils, as they are encountered above the groundwater table, and in areas such as sand dykes, road or train embankments, and sand dunes, has not been studied and described extensively. This is most likely due to the less control of flow that can be exercised in the non-saturated soil environment.

The aims of this paper are to:

- develop a bacterial immobilisation process that works for unsaturated sand,
- allow the in situ soil cementation of non saturated soils by using a simple surface percolation application, and
- understand calcite crystal formation in a soil matrix under a non-water saturated condition.

2. Materials and methods

2.1. Bacterial culture and cementation solution

The urease active bacteria named as MCP-11 (*Bacillus sphaericus*, available now from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Germany) have been isolated from local soil samples in the Laboratory of Biological and Biotech Science of Murdoch University, WA (Al-Thawadi, 2008). For the current study, samples of the isolated strain (MCP-11) were cultivated under sterile aerobic batch conditions in a medium consisting of 20 g/L yeast extract, 0.17 M ammonia sulphate and 0.1 mM $NiCl_2$, at pH of 9.25. After 24 h incubation at 28 °C, the culture was collected and stored at 4 °C prior to use. The optical density (OD_{600}) of harvested culture varied between 1.5 and 2 and the urease activity was approximately 10 U/mL (1 U = 1 μmol urea hydrolyzed per minute). Cementation solution consisted of 1 M $CaCl_2$ and 1 M urea.

2.2. Sand column setup and sampling

Pure silica sand (Cook Industrial, Minerals Pty. Ltd., Western Australia) was used (>0.3 mm: 1.13%, 0.212–0.3 mm: 63.39%, 0.15–0.212 mm: 29.59%, and <0.15 mm: 5.89%) for all experiments. All columns used in this study were made of Poly Vinyl Chloride (PVC) tubing (internal diameter of 4.5 cm, length of 30 cm and 1 m). Sand columns were packed with dry silica sand under continuous vibration to give an even density of 1.61–1.63 g/cm^3 (porosity 37.3–38.5%). The top and bottom of column were covered with a layer of scouring pads (porous plastic pad) as filters.

Under surface percolation and fully drained conditions, the water retention capacity of 30 cm and 1 m sand columns was determined to be about 180 mL and 360 mL respectively. The retained water was characterised as adsorbed water, that is the water had been adsorbed onto the sand grain surface, and capillary water, the water found between the grains (Baker and Frydman, 2009).

In this study, the sand columns were treated under two different water saturation conditions. (1) Water fully saturated condition: sand columns were treated by the submersed flow method. The setup and treatment process were based on the method that was described by Whiffin et al. (2007) (Fig. 1A), except for the flow rate, which was kept constant at 220 mL/h. (2) Water unsaturated condition: sand columns were free drained and treated by surface percolation, as described below.

2.3. Percolation method of cementation

For biocementation under unsaturated conditions the percolation method was used. Sand columns were positioned vertically with top and bottom fully open. Reagents (bacterial suspension and cementation solution) were introduced from the top of the columns (Fig. 1B). The transport of liquid was the result of gravity and capillary forces.

Unless otherwise stated for specific experiments, the percolation method consisted of the following 4 steps:

(1) Percolation of bacterial suspension (50% of the water retention capacity of the sand columns (90 mL for 30 cm columns, 180 mL for 1 m columns)).
(2) Percolation of fixation solution (same amount as aforementioned bacterial suspension), which was identical to the cementation unless otherwise specified.
(3) Incubation for 12 h at 25 ± 1 °C, allowing the added layers to diffuse into each other.
(4) Percolation of cementation solution (100% of the water retention capacity of the sand columns) and allowing the reaction process (urease hydrolysis and calcite precipitation) to occur for 12 h.

The separate addition of bacterial suspension (BS) and fixation solution (FS) could also be in more than two layers, for example by adding 25% of the water retention capacity of BS + FS + BS + FS to accomplish 4 layers (Fig. 2). By alternately injecting smaller volumes of bacterial suspension and cementation solution multiple layers (2–12 layers) were created in each of four sand columns.

During the course of all experiments, samples were taken from the outlet and immediately tested for urease activity and ammonium concentration. By recording the urease activity in the effluent, the bacterial activity lost from the columns was determined. The bacterial urease activity retained in the column was calculated by subtraction from introduced activity.

2.4. Monitoring methods

2.4.1. Total ammonia nitrogen concentration

Total ammonia nitrogen concentration was determined by the Nessler method (Greenburg et al., 1992).

2.4.2. Biomass measurement (OD_{600})

Biomass concentration was determined by measuring optical density of bacterial suspension with a spectrophotometer (Pharmacia Biotech NovaspecII, Cambridge, England) at a wavelength of 600 nm (Harkes et al., 2010). If required, samples were diluted to stay in the absorbance range of 0.2–1.

2.4.3. Urease activity

In the absence of calcium ions, urease activity was determined via solution conductivity (Whiffin, 2004). 1 mL of bacterial suspension was added to 5 mL of 3 M urea and 4 mL of DI water (reaction concentration 1.5 M urea) and the relative conductivity change was recorded over 5 min at 25 ± 1 °C. The urease activity was then calculated taking the dilution into account. In the presence of calcium ions, urease activity was determined from the ammonia production