TECHNICAL NOTE

Bio-cementation of sandy soil using microbially induced carbonate precipitation for marine environments

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This study proposes and describes a novel approach for cementing sandy soils in marine environments by modifying the promising technique of microbially induced carbonate precipitation (MICP). In contrast to the usual MICP technique described in the literature, the method proposed herein relies on the calcium ions dissolved in seawater as the sole source of calcium for calcite formation. This proposed method involves flushing high-salinity-tolerant, urease-active bacteria followed by a mixture of urea and seawater through a porous sandy soil, leading to bacterial carbonate release from the urease reaction and precipitation of insoluble and semi-soluble carbonate salts including calcium carbonate and magnesium carbonate trihydrate. This precipitation method resulted in a physical stabilisation of sand that reached an unconfined compressive strength of up to 300 kPa, which is about two-fold higher (with same amount of crystals produced) than that of the MICP treatment in which highly concentrated calcium and urea solutions are used. Permeability was retained at about 30% for all MICP-treated samples, suggesting good drainage ability. This new exploration of MICP technology provides a high potential for using bio-cementation in marine environments, for applications such as mitigation of submarine sediment liquefaction and prevention of beach sand erosion and cliffs scouring.

KEYWORDS: coastal engineering; ground improvement; microscopy; permeability; soil stabilisation

INTRODUCTION

The rapid growth of onshore and offshore human activities has increased the demand for ground improvement of soft and unconsolidated marine sediment/seabed. Although several ground improvement techniques exist, they suffer from a number of problems. For example, the effective treatment distance of chemical grouting is only 1–2 m from the injection point due to the limitation of the mixing equipment (DeJong *et al.*, 2010). Soil mixing is usually not suitable to treat large ground volumes underneath existing constructions (Karol, 2003). Furthermore, the chemical grouts such as cement, epoxy, acrylamide, silicates and polyurethane raise the issues of cost, health and safety, and many such grouts are now banned in several countries (DeJong *et al.*, 2010).

In the last few years, the use of microbially induced carbonate precipitation (MICP) by urea hydrolysis has emerged as an attractive alternative for soil stabilisation and has recently undergone rapid developments. MICP is achieved by transporting (e.g. injecting, flushing or percolating) chemical reagents (urea and calcium chloride ($CaCl_2$)) and ureolytic bacteria, typically *Sporosarcina pasteurii* (formerly known as *Bacillus pasteurii*), to the location where soil treatment is required. Ureolytic bacteria catalyse the hydrolysis of urea to produce ammonium and carbonate ions, which react with calcium ions (e.g. calcium chloride) to form calcite that precipitates throughout the soil matrix. The microbially induced calcite crystals bridge the adjacent soil

particles, and hence increase the shear strength and stiffness of soil while retaining high permeability (Van Paassen *et al.*, 2010; Cheng & Cord-Ruwisch, 2014).

Bio-mediated ground improvement by MICP technology has been demonstrated to be applicable at a large scale of up to 100 m^3 (Van Paassen *et al.*, 2010). Although the MICP under high-salinity conditions has been previously investigated by Mortensen *et al.* (2011), the application of MICP technology using seawater as cementation solution (Ca^{2+} source) has not yet been investigated. Seawater contains free calcium ions, although at a low concentration of about 10 mM, which can be considered as a source of calcium for MICP. Based on previously established MICP methodology developed by Whiffin *et al.* (2007), the presented study aims at applying the MICP technology to a marine environment using seawater as a calcium resource. The main purpose of this study is to improve understanding of bio-cementation for marine conditions through a series of tests that will investigate several parameters, including the urease activity, crystal content, permeability and strength of bio-cemented samples.

MATERIALS AND METHODS
Preparation of soil specimens

Pure silica sand (Cook Industrial, Minerals Pty Ltd Western Australia) was selected for the current study (> 0·425 mm: 20·2%; 0·3–0·425 mm: 52·2%; 0·15–0·3 mm: 26·3; and < 0·15 mm: 1·3%). The sand column was prepared by packing the dry sand to a density of 16·0~16·1 kN/m^3 and porosity of 39·5% (pore void volume was about 150 ml) into a polyvinyl chloride (PVC) column 180 mm high, with an inner diameter of 50 mm. The coefficient of permeability of the untreated silica sand was about 1·74 × 10^{-4} m/s.

Ureolytic bacteria and cementation solution for MICP

The urease active strain used in the current study was MCP-11 (*Bacillus sp.* DSM 23526) (Al-Thawadi *et al.*, 2012). The MCP-11 was cultivated under sterile aerobic batch conditions in a yeast extract medium, as described by Cheng & Cord-Ruwisch (2012). The optical density (OD_{600}) of the bacterial culture was varied between 1·5 to 2·0, and the urease activity was approximately 10 U/ml (1 U = 1 μmol urea hydrolysed per min). The seawater cementation solution was prepared by adding 10 mM urea (0·6 g/l) into the artificial seawater, which consisted of different concentrations of salts (g/l): NaCl (23·9), Na_2SO_4 (4·0), $CaCl_2 \cdot 2H_2O$ (1·5), $MgCl_2 \cdot 6H_2O$ (10·8), KCl (0·7), $NaHCO_3$ (0·2), KBr (0·1) and H_3BO_3 (0·03). By comparison, bio-cementation was also conducted using highly concentrated cementation solution consisting of equal moles of urea (1 M, 60 g/l) and anhydrous calcium chloride (1 M, 111 g/l), as conducted by Van Paassen *et al.* (2010), Al-Thawadi *et al.* (2012) and Cheng *et al.* (2013).

MICP procedure

Microbially induced carbonate precipitation bio-cementation was conducted using a vertical upward-flushing method at a flow rate of 1 l/h. Initially, the seawater saturated sand column was flushed with bacterial culture (85 ml) and cementation solution (85 ml) in sequence, followed by 24 h of reaction time at 25 ± 1°C for the bacteria to be immobilised onto the sand particle surface. Then, repeated flushes with cementation solution (190 ml) were conducted to obtain different strength. The bio-cementation reaction time was about 6 h with the seawater-based cementation solution, and 24 h with the highly concentrated cementation solution.

Urease activity and content of precipitates

The bacterial urease activity was monitored by measuring the concentration of the end products (ammonia/ammonium) over time. Ammonia/ammonium concentration was determined by the Nessler method (Greenberg *et al.*, 1992). The mass of carbonate crystals produced was determined by measuring the carbon dioxide gas volume produced (reacted with 1 M hydrochloric acid (HCl) solution), and converting it into an equivalent amount of carbonate crystals (Whiffin *et al.*, 2007).

X-ray diffraction analysis

The crystals formed (collected from crushed samples) were dried at 50°C for 24 h prior to X-ray diffraction (XRD) (GBE, enhanced multi-materials analyser, Australia) analysis. Phase identification was made by searching the Inorganic Crystal Structure Database (ICSD) powder diffraction file database and published literature.

Permeability, unconfined compression strength and microscopy analysis

Permeability tests on the untreated and bio-cemented sand columns were conducted using the constant head permeability test in accordance with the Australian Standards AS 1289.6.7.1 (SA, 2001). Unconfined compression strength (UCS) tests were conducted according to the procedure reported in ASTM D2166 (ASTM, 2013) on bio-cemented soil specimens with selected diameter-to-height ratios of 1:1·5 to 1:2. The axial load was applied at a constant rate of 1·0 mm/min. The bio-cemented samples were flushed with 2 l of tap water (about 15 pore volumes) and dried at 50°C for 24 h prior to UCS measurements. Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX) (Philips XL20, Eindhoven, The Netherlands) analysis were conducted on the crushed, cemented, sand samples after the UCS measurements.

PRESENTATION OF RESULTS AND DISCUSSIONS

Effect of seawater on urease activity

In order to obtain an effective and efficient soil stabilisation, a high urease activity is required, which is normally influenced by the characteristics of the environment such as the temperature, salt concentration, pH and heavy metal ions (Sahrawat, 1984; Stocks-Fischer *et al.*, 1999; Nemati *et al.*, 2005). The short- and long-term effects of the dissolved salts in seawater on the urease activity of the MCP-11 strain were tested by suspending a defined amount of bacterial cells in the artificial seawater, followed by adding 30 g/l of urea immediately or after 24 h at room temperature (25 ± 1°C).

The production rate of ammonium ions, NH_4^+, was not markedly affected by the artificial seawater, apart from a slight decrease after 24 h of mixing of the cells and seawater (Fig. 1). The seawater has high salinity and osmotic pressure, which usually causes osmotic stress to the injected exogenous freshwater ureolytic bacteria, resulting in bacterial cell lysis (Takamatsu *et al.*, 2005) and loss of both the intracellular enzymes (such as urease) and enzyme activity (Frankenberger & Bingham, 1992). The ureolytic bacteria used here were adapted to high-salinity conditions because high concentrations of ammonium sulfate (($NH_4)_2SO_4$) (25 g/l) and yeast extract (YE) (20 g/l) were used in the growth medium, preventing strong osmotic effects when exposed to seawater. This suggests that the MCP-11 urease active strain (*Bacillus sp.*) could be directly used in marine environments.

X-ray diffraction characterisation

According to the literature, calcium carbonate exists in three different crystal structures: vaterite, calcite and aragonite (Pişkin & Dere Özdemir, 2012). The XRD pattern of the obtained crystal samples (see Fig. 2) indicates a typical XRD pattern of calcite. Aside from the typical calcite crystals found in previous MICP processes (Cheng *et al.*, 2013), clear diffraction peaks of magnesium carbonate trihydrate ($MgCO_3 \cdot 3H_2O$) were also observed. This is expected because the seawater magnesium ion concentration is about 50 mM (10·8 g/l magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$)), which is five times higher than the calcium ion concentration (10 mM).
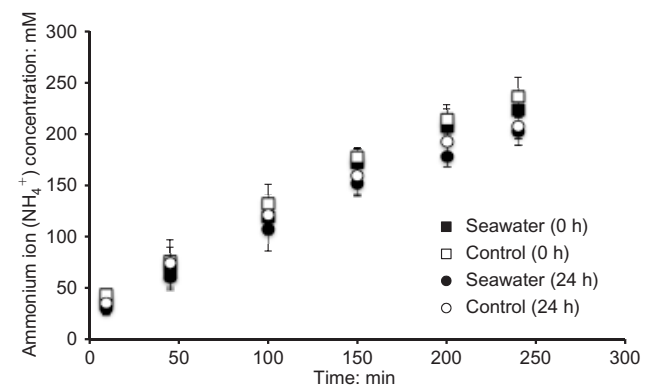

Fig. 1. Effect of the dissolved salts in seawater on the rate of urea hydrolysis by MCP-11, the control solution contained 1·5 g/l of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$)
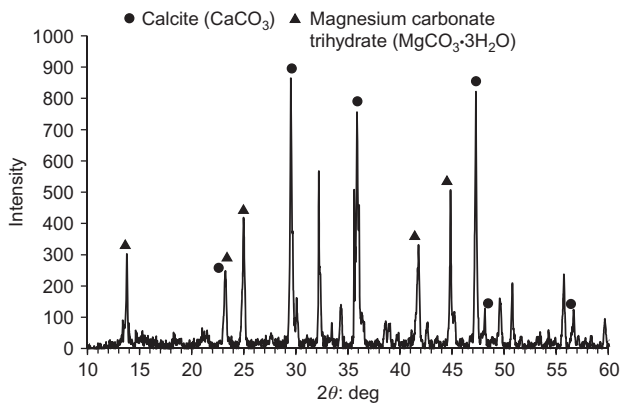
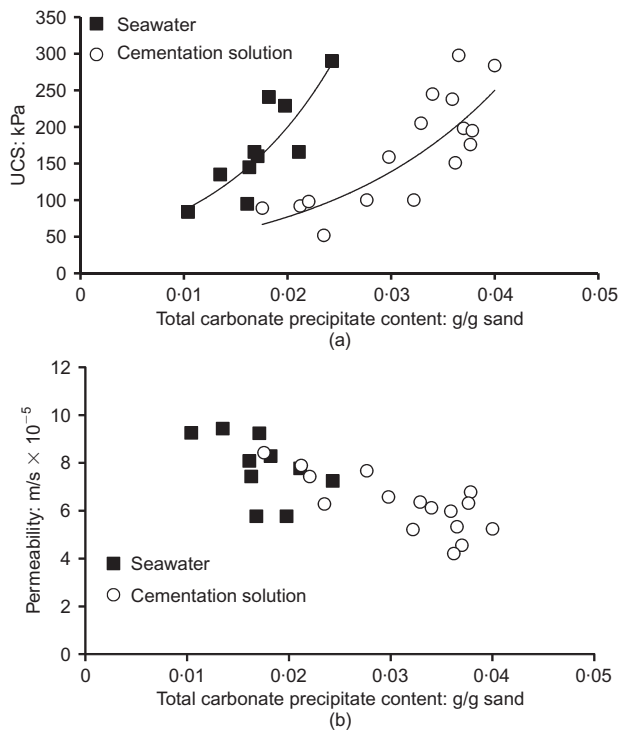**Fig. 2. X-ray diffraction pattern of precipitated crystal sample**

Fig. 3. Biocemented sand samples treated with seawater as the sole calcium source and highly concentrated cementation solution of 60 g/l urea and 111 g/l of anhydrous calcium chloride (CaCl$_2$): (a) UCS strength; (b) permeability

UCS and permeability

The compressive strength was exponentially related to the precipitated carbonate content (Fig. 3(a)), which is in line with the previous published results (Van Paassen *et al.*, 2010; Cheng *et al.*, 2013). It shows that for the same amount of precipitated carbonate, the compressive strength of samples treated with seawater was higher than that of the control samples treated with the high concentration of cementation solution. This is in line with the reported previous observations where higher concentrations (0·5–1·0 M) of urea and calcium chloride generated a significant amount of calcite; however, the efficiency (strength per mass of calcite) was lower compared to the formation of calcite at lower concentration (Nemati *et al.*, 2005; De Muynck *et al.*, 2010; Okwadha & Li, 2010).

The use of seawater supplemented with urea instead of concentrated cementation solution did not significantly affect the permeability per carbonate formed (Fig. 3(b)). However, because the same carbonate resulted in more strength (Fig. 3(a)), the seawater cementation trials enabled more permeability for a given strength (Fig. 3(b)). The final per-

meability of all samples is about 40 times higher than 1×10^{-6} m/s, which according to Fell *et al.* (1992), provides good drainage. Overall, continued gain of compressive strength up to 300 kPa was possible for the seawater-treated sand with about 200 flushes. This still retains sufficient permeability for further treatment if needed.
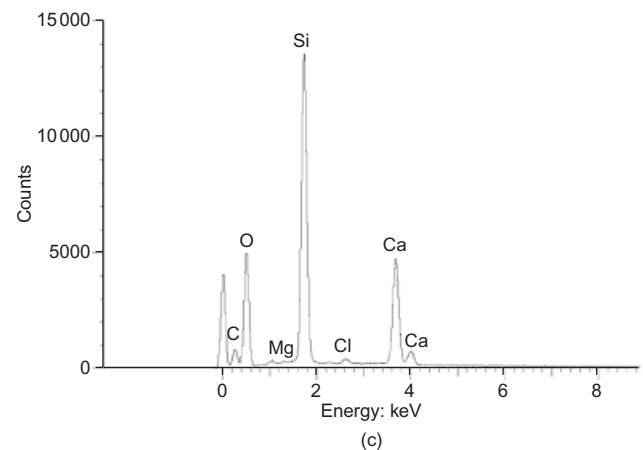
SEM and EDX analyses

The MICP treatment with seawater showed abundant crystals formed on the surface of the sand particles and at the contact points between them (Figs 4(a) and 4(b)). The
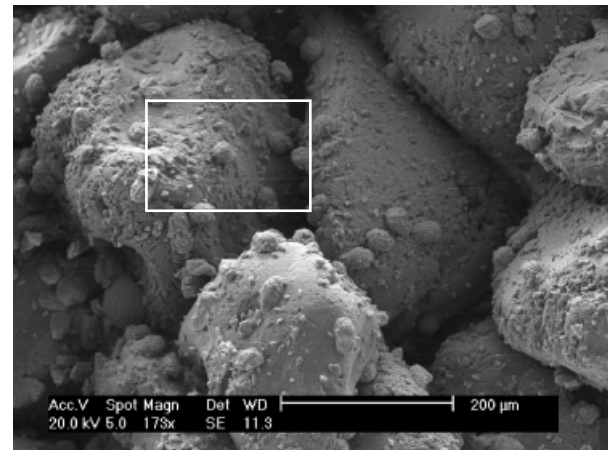
(a)

(b)

(c)

Fig. 4. (a), (b) SEM and (c) EDX analyses of the MICP treated sand sample with seawater (UCS = 150 kPa). (The EDX detection area was the rectangle area indicated in Fig. 4(a))

precipitated crystals were generally small and dispersed, or as large clusters (about 50 μm) of ovoid shape.

Both magnesium and calcium can be detected by EDX analysis (Fig. 4(c)), which indicates the formation of calcium carbonate and magnesium carbonate precipitates. However, the amount of magnesium is much lower than the calcium in the formed crystals, which may be explained by the partial dissolution of magnesium carbonate hydrate upon flushing of the samples with about 15 pore volumes of tap water. In marine environments, the contribution of the magnesium carbonate hydrate to the strength development is of potential interest and will be investigated in a following paper. In particular, it would be interesting to study to what extent a re-crystallisation of carbonate from magnesium to calcium carbonate occurs under the seawater conditions (Mavis & Checkovich, 1975) and how it effects the duration and stability of bio-cemented sand.

Limitation and significance of current work

In the current study, artificial seawater was used as a source of calcium ions to establish proof of concept of seawater bio-cementation and to evaluate the MICP for ground improvement. Although the calcification rate (crystals formed per time) using seawater is lower than that of the concentrated cementation solution, it allows savings in cost of chemicals. This suggests that, instead of relying on industrial chemicals, naturally occurring calcium and urea (urine) enables bio-cementation in marine environments, which opens up novel ways for soil stabilisation in marine and coastal applications, where continued gain of strength would be desirable.

In the presented experiments, the amount of urea supplied as a carbonate source for calcite precipitation was limited to stoichiometrically match the low calcium concentration in seawater. This limitation slowed the overall reaction rate and required 200 flushes to reach a strength gain of about 300 kPa. If sufficient urea was added to enable the precipitation of magnesium carbonate, the reaction rate could be faster and only about 40 to 50 flushes would be needed. The magnesium carbonate hydrate produced is known to re-crystallise as calcium carbonate in seawater over time. In fact magnesium carbonate has been used to remove calcium from seawater (Mavis & Checkovich, 1975).

CONCLUSIONS

The current study has demonstrated the feasibility of using the calcium ions from seawater as the sole calcium source in the application of MICP-based ground improvement under marine conditions. The strength of sand columns can be significantly increased by the MICP from repeated treatment of seawater. The application of MICP under marine conditions using seawater can possibly offer a cost-effective and sustainable way to strengthen the ground in marine and coastal environments.

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