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TECHNICAL NOTE

Microbial method for construction of an aquaculture pond in sand

J. CHU^{*†}, V. IVANOV^{*†}, V. STABNIKOV^{*‡} and B. LI^{*}

A method to construct an aquaculture pond in sand using microbial biocementation is presented in this paper. The microbially induced calcium carbonate precipitation process was used to form a low-permeability layer in sand for the construction of a water pond model in the laboratory. The test results indicated that the permeability of sand was reduced from the order of 10^{-4} m/s to 10^{-7} m/s when an average 2.1 kg of calcium (Ca) per m^2 of sand surface was precipitated. The bending strengths of the walls and the base of the model pond were in the range of 90–256 kPa. The unconfined compressive strengths for the samples taken from the walls and the base of the pond were in the range of 215–932 kPa.

KEYWORDS: ground improvement; grouting; permeability; seepage

INTRODUCTION

Conventionally, compacted bentonite, geosynthetic clay liner or plastic liners are used to seal ponds, channels and reservoirs in sand. However, these methods are costly. For example, about 60 kg of bentonite is required to seal 1 m^2 of pond area according to Pillay & Kutty (2005). The technology with plastic liner is more expensive depending on the design situations (Pillay & Kutty, 2005).

Studies have shown that the microbially induced carbonate precipitation (MICP) process can be used to increase the shear strength and decrease the permeability of sand, owing to the filling of the pores with the minerals precipitated (Gollapudi *et al.*, 1995; Mitchell & Santamarina, 2005; Whiffin *et al.*, 2007; Chu *et al.*, 2009; DeJong *et al.*, 2010; Tobler *et al.*, 2011). The MICP process is catalysed by urease-producing bacteria (UPB) owing to an increase in pH to produce carbonate in the presence of calcium ions (Ferris *et al.*, 1996; Stocks-Fischer *et al.*, 1999) as shown in the following

However, practical applications of the MICP process for soil treatment are far more complicated (Ivanov & Chu, 2008; Ivanov, 2010) because the quantity of reagents required by conventional biocementation through percolation of soil with bacterial suspension and solutions of calcium salt and urea is large. Recently, a new approach has been developed to form a low-permeability layer several centimetres thick on top of a sand surface through the MICP process (Chu *et al.*, 2012). This method has been adopted to

build a laboratory-scale water pond model in sand. The method for the construction of this pond and the properties of the microbial mediated sand are discussed in this paper. Potential applications of the method presented could include the construction of runoff collection ponds in deserts' sand (Boers, 1994), aquaculture ponds (Kitto & Reginald, 2011; Lucas & Southgate, 2012) or algal biofuel production ponds (Campbell, 2008).

CONSTRUCTION OF MODEL POND

Sand and reagents

Ottawa sand with a mean grain size of 0.42 mm was used for the construction of the water pond model. The sand was deposited dry in a box and the density achieved was 1600 kg/m^3 .

The reagents used for creating a low-permeability layer in sand included

- a suspension of bacterial cells of *Bacillus* sp. VS1 with urease activity 2.7 mM hydrolysed urea/min (9.7 g urea/l·h)
- a solution containing 82.5 g/l (0.75 M) calcium chloride and 90 g/l (1.5 M) urea. The strain of UPB *Bacillus* sp. VS1 was used as a bioagent inducing formation of calcite from calcium chloride and urea (Stabnikov *et al.*, 2011; Chu *et al.*, 2012).

Urease-producing bacteria were grown in a liquid medium of the following composition: Tryptic soy broth DIFCOTM, 30 g/l; urea, 20 g/l; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 12 mg/l; $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, 24 mg/l; conductivity of the prepared medium was 1400 mS/m. All components of this medium, with the exemption of urea, were sterilised at 121°C for 15 min. The stock solution of urea, 100 g/l, was prepared and sterilised by filtration through WhatmanTM nitrocellulose membrane with 0.2 μm pores to prevent urea being lost during autoclaving. Cultivation was conducted in a B. Braun biostat fermentor (International Equipment Trading Ltd., Vernon Hills, Illinois, USA) with working volume 1.5 l at temperature 30°C under aeration rate 3 l/min. Palm oil was used as an antifoaming agent in quantity 0.2% (v/v). Duration of cultivation was 4 days. The pH value of the culture liquid, with a concentration of bacterial cells of about 8 g of dry biomass/l, was adjusted to pH 7 by 1 N HCl.

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* School of Civil and Environmental Engineering, Nanyang Technological University, Singapore.

† Department of Civil, Construction & Environmental Engineering, Iowa State University, USA.

‡ Department of Microbiology and Biotechnology, Ukrainian National University of Food Technologies, Kiev, Ukraine.

Formation of low-permeability layer in sand

The model pond was built within a rectangle glass tank of 48 cm × 38 cm × 20 cm (length × width × height) filled with 28 kg of sand, in which a rectangular hole of 34 cm × 26 cm × 5 cm (length × width × height) was excavated manually as shown in Fig. 1(a). A plastic board was used to support the stability of the walls of the excavated hole.

The following five steps were used to treat the surface of the model pond

- (a) spray with bacterial suspension
- (b) spray with calcium chloride and urea solution

(a)

(b)

(c)

Fig. 1. Formation of a water pond model in sand: (a) before treatment; (b) after treatment; (c) cross-sectional view of the bottom of the pond after cultivation of algae

- (c) spray with bacterial suspension for a second time
- (d) cover the sand surface with calcium chloride and urea solution
- (e) dry the model pond outdoors under sunlight for 2 days.

The first spray (first treatment) of the bacterial suspension on the bottom and four walls of the pond supplied a dosage of about 1.35 ml/cm² (or 0.11 kg/m²) of dry biomass and the average supply rate was 0.7 ml/cm² per h (or 7 l/m² per h). The spray of the solution containing calcium chloride and urea on the bottom and four walls provided a dosage of approximately 2.3 ml/cm² (or 23 l/m²) of calcium (Ca) and the average supply rate was 0.7 ml/cm² per h (or 7 l/m² per h). The supply rate and time of the treatment were adjusted so that there was no liquid above the sand surface. The second spray (second treatment) of the bacterial suspension on the bottom and the four walls provided a dosage of 0.7 ml/cm² (0.05 kg/m²) of dry biomass and the average supply rate was approximately 0.7 ml/cm² per h (7 l/m² per h). The supply rate and time of the treatment were adjusted so that there was no liquid above the sand surface. Then, the model pond was filled with calcium chloride and urea solution so that the level of liquid was 5 mm above the sand surface for 2 days. This third treatment enhanced the biocementation of the surface layer (Chu *et al.*, 2012) but is not mandatory for real construction technology. Subsequently, the liquid was drained off from the tank and the model pond was left to dry outdoors for 2 days. The water pond formed is shown in Fig. 1. A cross-sectional view of the model pond after it had been used for cultivation of algae is shown in Fig. 1(c). It can be seen that the water pond is formed by a low-permeability layer surrounding the boundary of the pond. The sand below the low-permeability layer was not treated. In this way, the biotreatment was localised and thus the cost of treatment was reduced.

Design of the biocemented layer of sand

The formation of the biocemented layer of sand during the construction of the pond can be calculated using the following model. The maximum deposition of calcium (Q , kg) in the biocemented layer of sand is

$$Q = CV_p + CV_s \quad (2)$$

or

$$Q = CA(H_p n + H_s) \quad (3)$$

where C is calcium concentration (kg/m³); V_p is volume of pores in the soil (m³); V_s is volume of calcium salt solution (m³); A is treated area (m²); H_p is height of the treated sand (m); n is sand porosity; H_s is height of the calcium salt solution above soil surface (m). The calculated ratio of the quantities of precipitated calcium in the biocemented crust and in the bulk of sand for the model pond was 0.83, which was almost the same as the experimental value of 0.80. This ratio could be diminished by the formation of a low-permeability layer on top of the sand by sequential spraying of biocementing solution performed before major precipitation of calcium from solution above the surface of the sand.

The thickness of the biocemented layer, H_p , of sand can be calculated as follows

$$H_p = (V_b/A)n \quad (4)$$

where V_b is volume of bacterial suspension and solution of calcium chloride and urea, which was used for spraying the sand surface. The thickness of the biocemented layer was from 20 to 30 mm for the bottom and from 15 to 20 mm for the walls (Fig. 1(c)).

A field emission scanning electron microscope (FE-SEM)

JSM-7600 (Jeol, Japan) was used to study the dry, platinum (Pt)-coated samples of bio-treated sand. From the SEM image, it can be seen that the pores between sand grains were filled by the minerals precipitated. According to the energy dispersive X-ray spectroscopy (EDS) spectrum, the fill material between the sand grains had the following contents, in atomic %: C, 19.9; O, 31.1; Ca, 49.0, whereas the sand grains had: C, 8.7; O, 60.8; Al, 1.3; Si, 23.9; Ca, 0.5; Zr, 4.8. The higher contents of calcium and carbon in the fill material could be an indication of the formation of calcium carbonate (CaCO_3).

GEOTECHNICAL PARAMETERS OF MODEL POND

Water leakage tests

The average leakage rate from the bio-treated model pond was determined roughly by the change of water table over a given time interval. The water level change was monitored after the pond was filled with 5 l of tap water. This was done at the end of every treatment. For every measurement, the time taken for 25 mm of water drop to occur was recorded. The leakage rate calculated after each treatment is shown in Fig. 2. It can be seen that the leakage rate reduced with each additional treatment. The leakage rate after the fourth treatment was 3×10^{-7} m/s. It is possible to reduce the leakage rate further by using one or two additional treatments. However, a leakage rate in the order of 10^{-7} m/s was comparable with the leakage rate required for tropical fish ponds, aquaculture ponds (Teichert-Coddington *et al.*, 1989) or for shrimp mariculture ponds (Weisburd & Laws, 1990). Therefore, the leakage rate was considered adequate for aquaculture or fish ponds.

Permeability tests

The engineering properties of the soil in the bio-treated low-permeability layer were measured using different laboratory tests. Samples taken from the walls and the bottom of the pond were tested separately. Permeability tests were conducted using a triaxial cell by following the constant head method (Head, 1986). As the bio-treated sand was hard and brittle, it was difficult to trim a sample into a perfect cylinder. Small cracks could also occur on the surface of the sample. To overcome these problems, model clay was used to seal the cylindrical surface of a 38 mm dia. specimen, as shown in Fig. 3. The specimen was then installed into a triaxial cell and sealed using a membrane. A confining pressure of 50 kPa was used. Two GDS pressure volume controllers that could apply a pressure and measure the volume at the same time (Chu *et al.*, 1992) were used to apply pressures of 45 and 40 kPa to the bottom and top of the specimen respectively. Once steady-state flow conditions

Fig. 3. Specimen for permeability test

were obtained, the quantity of flow over a time interval was measured to calculate the permeability. Two permeability tests each were conducted on samples taken from the walls and the bottom of the pond. The results are presented in Fig. 4. It can be seen that the permeability of the bio-treated sand taken from both the walls and the bottom of the pond had been reduced from the initial 4.0×10^{-4} to 3.5×10^{-7} m/s, on average. This permeability value was still not low enough to make a leakage-free water pond. However, it was adequate for building aquaculture ponds.

Four-point bending tests

The MICP process not only reduced the permeability of sand substantially, but also increased the stiffness and shear strength of the soil. By observation, it can be seen that the bio-treated soil cemented together like sandstone. The cementation effect was likely to have been created by the minerals produced through the MICP process. The cementation effect not only increased the shear strength, but also created cohesion or tensile strength in the soil. To assess the tensile strength of the bio-treated soil, some four-point bending tests were carried out by following a procedure detailed in ASTM standard D6272-10 (ASTM, 2010). One

Fig. 2. The leakage rate measured after each treatment

Fig. 4. Results of permeability tests

reason for doing the four-point bending tests was that the samples were only suitable for this type of test. The test set-up is shown schematically in Fig. 5. The support span was 80 mm. The dimensions of the specimen were 5 mm thick, 100 mm long and 20 mm wide, which did not exceed one-quarter of the support span. The loading span was 26.7 mm, which was one-third of the length of the supported span. The diameter of the round loading noses was 6 mm. The maximum bending stress S for a load span of one-third of the support span is calculated as follows (ASTM D6272-10 (ASTM, 2010))

$$S = P \frac{L}{t} d^2 \quad (5)$$

where S is the bending strength in the outer fibre throughout the load span (MPa); P is the load at a given point on the load–deflection curve (N); L is the support span (mm); t is the thickness of the beam (mm); and d is the depth of the beam (mm).

The bending strengths measured by the four-point bending tests for samples taken from the walls and base are shown in Fig. 6. It can be seen that the bending strengths measured are in the range of 90–256 kPa. These bending strengths were still much smaller compared with those of rocks. However, as the untreated sand had no cohesion or tensile strength at all, the measured bending strength is a good indication of the cementation effect due to bio-treatment. This bending strength of the soil will enhance the stability of the pond and all steeper slopes or even walls to be used for the pond. This will help to cut down further the construction cost for the pond.

Fig. 5. Set-up for four-point bending tests

Fig. 6. Results of four-point bending tests on samples taken from the wall and the base

Unconfined compression tests

Unconfined compression (UC) tests were also conducted on specimens 10 mm in diameter and 20 mm high. Small-diameter samples had to be used as the cementation zone was only 15–30 mm thick. Nevertheless, the UC test provided a way to assess the degree of improvement of the sand given the fact that clean sand would not even stand as a column. A loading rate of 0.4 mm/min (or 2% strain) was used in the tests. The UC strengths obtained for samples taken from both the walls and the base were in the range 215–932 kPa (Fig. 7). There was a large variation in the UC strength even for samples taken from the same zone. This could be partially attributed to the non-uniformity in the treatment of the soil and the disturbance to the samples during sample preparation. Similar to the bending strength obtained from the four-point tests, the strength of the sample at the bottom of the pond is slightly lower than that of the sample at the wall. This could be because the walls had a larger surface area than the base for the application of biocementation solution. Additionally, sample size may have a significant effect on the absolute value and variations of the unconfined compressive strength.

CONCLUSIONS

A method to construct a water pond in sand based on an MICP approach is presented in this paper. The calcite formed through the MICP process filled the pores in sand and resulted in a substantial reduction in the permeability as well as an increase in the stiffness and shear strength of sand. A water pond model was built in the laboratory using this method. The test results indicated that the permeability of sand was reduced from the order of 10^{-4} m/s to 10^{-7} m/s when an average 2.1 kg/m^2 of calcium was precipitated in sand. Although clean sand has no tensile or unconfined compressive strength, the bio-treated soil had a bending strength of up to 256 kPa, as measured by four-point bending tests, and an unconfined compressive strength of up to 932 kPa.

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Fig. 7. Unconfined compressive strength measured on samples taken from the wall and the base

NOTATION

A	treated area (m^2)
C	calcium concentration (kg/m^3)
d	depth of beam (mm)
H_p	height of the treated sand (m)
H_s	height of the calcium salt solution above soil surface (m)
L	support span (mm)
n	sand porosity
P	load at a given point on the load–deflection curve (N)
Q	maximum deposition of calcium in biocemented layer of sand (kg)
S	bending strength in outer fibre throughout load span (MPa)
t	thickness of beam (mm)
V_b	volume of bacterial suspension and solution of calcium chloride and urea, used for spraying sand surface (m^3)
V_p	volume of pores in the soil (m^3)
V_s	volume of calcium salt solution (m^3)

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