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Formation of water-impermeable crust on sand surface using biocement

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ABSTRACT

This paper examines the feasibility of using calcium-based biocement to form an impermeable crust on top of a sand layer. The biocement used was a mixture of calcium salt, urea, and bacterial suspension, which hydrolyzed urea with production of carbonate and an increase of the pH level. Applying 0.6 g of Ca per cm^2 of sand surface, the permeability of the biocemented sand can be reduced from 10^{-4} m/s to $1.6 \cdot 10^{-7}$ m/s (or 14 mm/day) due to formation of the crust on sand surface. The rupture modulus (maximum bending stress) of the crust was 35.9 MPa, which is comparable with that of limestone. The formation of a water-impermeable and high strength crust layer on sand surface could be useful for the construction of aquaculture ponds in sand, stabilization of the sand dunes, dust fixation in the desert areas, and sealing of the channels and reservoirs in sandy soil.

1. Introduction

Biocementation is an innovative technology based mainly on application of urease-producing microorganisms together with urea and calcium ions in a permeable soil [1–4]. Hydrolysis of urea by enzyme urease causes calcium carbonate precipitation and formation of a cemented product according to the following equations [5]:

$$(NH_2)_2CO + 3H_2O + \text{urease} \rightarrow 2NH_4^+ + HCO_3^- + OH^- + \text{urease} \quad (1)$$

$$CaCl_2 + HCO_3^- + OH^- \rightarrow CaCO_3\downarrow + H_2O + 2Cl^- \quad (2)$$

It has been shown that urease-producing bacteria or enzyme urease can be used to bind sand particles through calcite formation, a process known as biocementation [4,6–8].

There are many potential applications of biocementation in civil engineering such as enhancing stability of slopes and dams, reducing the liquefaction potential of soil, road construction, prevention of soil erosion [1–5], and reparation of the cracks in concrete [7,9–11]. The formation of aquaculture ponds or reservoirs in sandy soil is a new application of biocementation, which has not been studied. These ponds could be used for outdoor commercial aquaculture, such as fish, shrimp and mollusk production [12], for large-scale cultivation of algae [13], for biofuel production in desert coastal area, or as water collecting reservoirs.

It is known that excessive seepage from aquaculture pond or reservoir is a major problem in areas with highly permeable soils. Seepage from stable ponds causes 45% to 87% total water losses [14,15] and the seepage rate (or water permeability) could rise up to 182 mm/day [16]. Seepage from the aquaculture pond causes not only the loss of water but also leakage of nutrients needed for aquaculture [17–19]. It may also cause pollution of groundwater with nutrients, organic aquacultural wastes, and pathogens from aquaculture pond. For example, when the total average of 1021.2 kg/ha potassium was applied to the newly constructed shrimp ponds, the estimated loss of potassium due to seepage was 101.2 kg/ha [20]. Therefore, cutting off seepage and decreasing permeability of soil are an important design consideration for the applications listed above. The feasibility of using calcium-based biocement to seal or construct the water pond in sand is discussed in this paper.

2. Materials and methods

2.1. Urease-producing bacteria

Halotolerant and alkalophilic strain of urease-producing bacteria *Bacillus sp.* VS1 has been isolated from sand of tropical beach. It was spore-forming, Gram-positive bacteria with rod-shaped cells. The nearly full-length 16S rRNA gene was amplified conventionally by Polymerase Chain Reaction (PCR) with the primers 27F, 530F, 926F, 519R, 907R and 1492R. Purified PCR products were sequenced using the ABI PRISM 3730xl DNA sequencer and the ABI PRISM BigDye Terminator Cycle according to the manual of the manufacturer (Life Technologies Corporation, California, US). The partial nucleotide sequences were assembled to produce the full-length nucleotide sequence of 16S rRNA gene deposited in NCBI GenBank under accession number JF896459. To identify microbial strain, the full-length nucleotide sequence of its 16S rRNA gene was compared with

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the related sequences available in the NCBI GenBank database (http://www.ncbi.nlm.nih.gov/).

2.2. Method for the measurement of urease activity

Urease activity was defined as the amount of ammonium produced per minute from 0.2 M solution of urea. Concentration of ammonium was determined using an electric conductometer by linear correlation ($R^2 = 0.9986$) between molar concentration of NH_4^+ (Y) and electric conductivity (X) in mS/m:

$$Y = 49 \cdot 10^{-6} \quad (3)$$

2.3. Microscopic observations

The structure of sand sample was observed using a scanning electron microscopy (SEM) Zeiss EV050, UK. The sand sample was fixed in 2% glutaraldehyde for 2 h, washed 3 times with 0.1 M sodium cacodylate buffer for 20 min, dehydrated step-wise in 50, 70, 85, and 95% (v/v) solutions of ethanol for 10 min, kept in 100% ethanol until dried with the critical point dryer Polavon E3100, Quorum Technologies, UK, and then sputter coated with Au–Pd using Emitech SC7620, Quorum Technologies, UK.

2.4. Size distribution of cellular aggregates

Size distribution of cellular aggregates before and after treatment of cells with solution of $CaCl_2$ was measured using Mastersizer 2000 (Malvern Instruments Ltd, UK).

2.5. Cultivation of urease-producing bacteria

Urease-producing bacteria were grown in a liquid medium of the following composition: Tryptic Soy Broth DIFCO™, 30 g/L; urea, 20 g/L; $MnSO_4 \cdot H_2O$, 12 mg/L; $NiCl_2 \cdot 6H_20$, 24 mg/L, phenol red, 10 mg/L. NaCl, 100 g/L, was used only in the medium for isolation of strain, but not for the cultivation. Selection of halotolerant strain was needed in this study to perform biocementation at high concentrations of salts and urea. Phenol red was used as a pH indicator: the color is yellow at pH 6.8, but gradually changes to a red/bright pink color at pH above pH 8.2. Conductivity of the prepared medium was 1400 mS/m. All components of this medium, for exemption of urea, were sterilized at 121 °C for 15 min. Stock solution of urea, 100 g/L, was prepared and sterilized by filtration through Whatman™ nitrocellulose membrane with 0.2 μm pores to prevent urea lost during autoclaving. Cultivation was conducted in 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. Then, the culture liquid with suspended bacterial cells was mixed with 82.5 g/L (0.75 M) $CaCl_2$ solution to obtain a final concentration of 11 g/L (0.1 M) of $CaCl_2$. This mixing was done to enhance aggregation of bacterial cells as well as their attachment to the sand grains after being added into sand. Due to the adjustment to pH 7, there was no precipitation of calcium carbonate upon mixing of the bacterial suspension with the calcium chloride solution.

2.6. Liquid biocement

Two components of liquid biocement, applied sequentially, were as follows: (1) cultural liquid (bacterial suspension) with addition of $CaCl_2$ to its concentration 0.1 M; (2) calcium chloride and urea solution contained 82.5 g/L (0.75 M) of $CaCl_2$ and 90 g/L (1.5 M) of urea. The pH was adjusted to pH 7 by 1 N HCl.

2.7. Biocementation

The glass tank at a size of 36×22×25 cm with an outlet in the bottom was filled in with 7 L of beach sand with porosity 40% (v/v). Model pond (22×14×5 cm) with volume about 1.5 L was made in this sand. There were six sequential treatments for biocementation of sand. Every treatment included the following operations: 1) cultural liquid with added solution of $CaCl_2$ was poured into the model pond to cover sand surface; 2) recirculation of effluent was with the rate of 200 mL/min for first, second and third treatments, but there was no recirculation for the fourth, fifth and sixth treatments because the seepage decreased significantly during the experiment. Recirculation was done using the peristaltic pump Masterflex® easy load® 7518–10 with silicone tubing Masterflex® 9640–25 for 1.5 h; 3) liquid was drained off the tank; 4) calcium-urea solution was poured into the model pond to cover the surface of the sand; 5) recirculation of effluent was at the rate of 200 mL/min for first, second and third treatments, but there was no recirculation for fourth, fifth and sixth treatments because significant decrease of the seepage during the experiment. Recirculation was done using the peristaltic pump for 12 h; 6) liquid was drained off the tank. Quantity of solutions was changed during the treatments: 2 L of cultural liquid with added $CaCl_2$ and 1.5 L of calcium chloride and urea solution were used for the first and second treatments; 1 L of cultural liquid with added $CaCl_2$ and 1 L of calcium chloride and urea solution were used for the third treatment; 1 L of cultural liquid with added $CaCl_2$ and 0.6 L calcium chloride and urea solution were used for the fourth treatment; 0.4 L of cultural liquid with added $CaCl_2$ and 0.4 L calcium chloride and urea solution were used for the fifth and sixth treatments.

Biggest volumes of the solutions for the first and second treatments were used to keep highest level of solutions (see Fig. 1) for the biocementation of the bottom, slopes, and bank surface of the pond. Consecutively lower volumes of the solutions for the third to sixth treatments were used to keep lower level of solutions (see Fig. 1) for the biocementation of mainly the bottom surface of the pond.

2.8. Seepage rate determination

The seepage rate (soil permeability) was determined by measuring the time taken for 1 L of tap water to flow through the model pond. It was used to assess the improvement in the reduction of permeability of the sand through the biocementation process.

2.9. Measurement of calcium concentrations and contents

Calcium concentration was determined using standard method 2340 C with ethylene diamine tetraacetate dihydrate (EDTA) titration [21]. Liquid sample of 50 mL was placed in a dry conical flask where 1–2 mL of buffer solution was added to ensure the liquid sample reach pH of 10.0. Then, a few drops Eriochrome Black T indicator were added into the flask and the sample was titrated with 0.01 M solution of ethylene diamine tetraacetate dihydrate (EDTA) until the color was changed from purple to blue. To determine calcium content in the sand, about 5.0 g of air-dried sample was placed in a 100 ml volumetric flask, and then 25 ml of 2.5% (v/v) acetic acid was added into the flask to dissolve calcium while keeping the contents well shaken for 1 h. The mixture was centrifuged and the supernatant was collected in another 100 ml volumetric flask to fill in to 100 ml with 2.5% solution of acetic acid.

Filling of the sand pores with $CaCO_3$ after treatment of sand with biocement was calculated from the determined content of $CaCO_3$ in sand. It was considered in these calculations that porosity of sand is 40% and density of $CaCO_3$ is 2.7 g/cm^3.

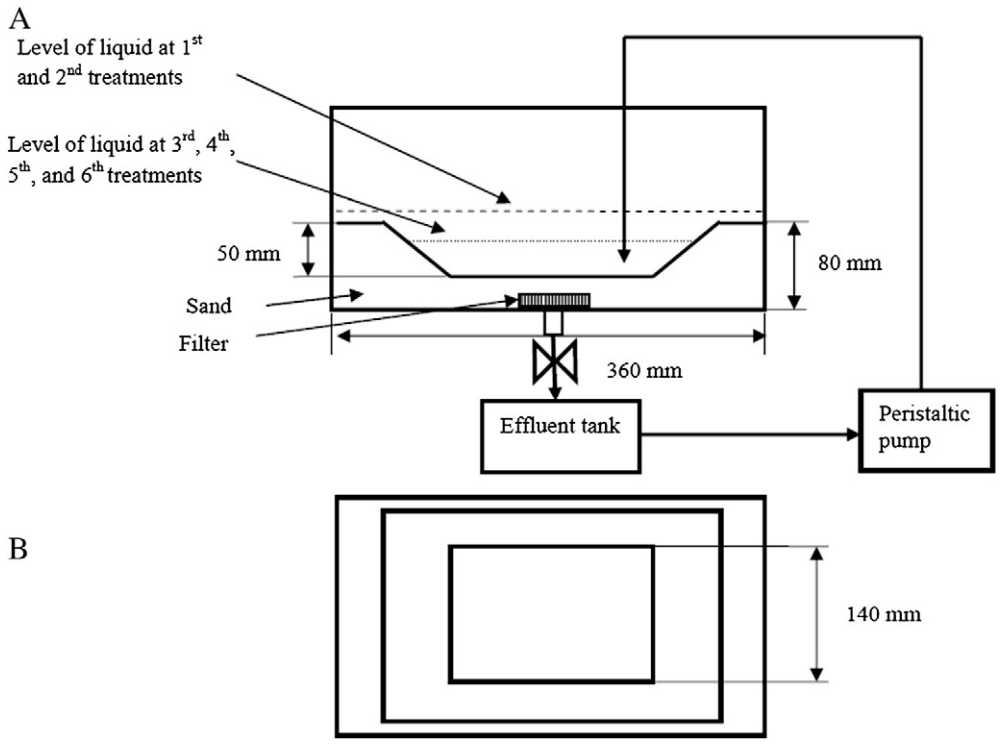

Fig. 1. Schematics of the model biocementing pond.

2.10. Measurement of flexural strength of calcite crust

The construction materials should be tested for flexural strength using standard methods. However, standard methods were not applicable for determination of the flexural strength of the crust formed in the experimental pond model, because of small and non-standard size of the crust. Therefore, the modulus of rupture of the biocemented crust was determined using the non-standard method — the three points bending flexural test. The results of this test have been used for the evaluation of biocementation efficiency but not for the production of the engineering design data.

The flexural strength of the biocemented crust was determined using a beam test in which a simply supported beam specimen was loaded by a point load in the middle of the beam. The test specimen was 0.1 cm thick, 2 cm wide and 4 cm long. The modulus of rupture (R) of the biocemented crust was calculated using following equation:

$$R = 3P\,l / 2bh^2 \quad (4)$$

where: R = modulus of rupture in Pa; P = load in N; l = span length between supports in m; b = width of beam at point of fracture in m; and h = height of beam at point of fracture, m.

3. Results and discussion

3.1. Urease activity of bacteria

Addition of 6 mL of the culture liquid to 70 ml of 1 M urea solution increased the conductivity of solution by 2.5 mS/cm in 30 min. It corresponds to urease activity of the culture liquid 2.7 mM urea/min (9.7 g urea/L·h). This activity was close to the levels used in some experiments on biocementation [22] but was lower than the urease activity used in other experiments [23]. Urease activity of bacterial strain *Bacillus sp.* VS1 was sufficient to hydrolyze 90 g of urea/L of biocement solution for approximately 9.3 h. If the time consumed by biocementation for some real geotechnical engineering application needs to be shorten due to engineering consideration, either a higher amount of starting volume of the culture liquid can be used or the urease of the culture liquid could be concentrated using nanofiltration technique.

The strain *Bacillis sp.* VS1 showed maximum specific growth rate of 0.11 h^{-1} for the first day of submerged batch cultivation. The pH increased from 8.8 at the beginning of cultivation to 9.4 after 4 days of cultivation. Maximum of total urease activity was 2.3 mM/min after 4 days of cultivation. Test of cultural liquid showed that precipitation of calcium from the mixture of 90 mL of 1 M urea solution and 90 mL of 0.5 M $CaCl_2$ solution after an addition of 20 mL of cultural liquid and incubation for 21 h was 84% of initial quantity of calcium.

3.2. Formation of crust using biocementation

Thin and dense crust with thickness approximately 1 mm was formed on the surface of the treated sand (Fig. 2A, B). The scanning electron microscopy showed that formation of $CaCO_3$ crystals was initiated not in the liquid inside the pores of sand, but directly onto the surface of the sand grain (Fig. 3). Probably, bacterial cells or urease molecules, which were attached to the sand grain surface, served as the crystallization centers.

Formation of biocemented crust on the top layer of sand could be due to the treatment of the cultural liquid with 100 mM $CaCl_2$ solution, which stimulated formation of cell aggregates (Fig. 4). Average size of cell aggregates was about 1 μm (Fig. 5A) and more than 10 μm (Fig. 5B) for aggregates formed in the absence and presence of calcium chloride, respectively. In the presence of calcium chloride, aggregates with size up to 400 μm could be observed (Fig. 5B). These big cell aggregates were immobilized in the top layer of sand since they could not penetrate the pore system. The immobilization of the cells in the top layer is responsible for formation of the calcite crust on the surface.

Concentration of calcium ions in the effluent after the treatment of the sand was low. The quantities of calcium in the supplied calcium chloride solution and in the discharged solution were 186 g and 9.3 g,

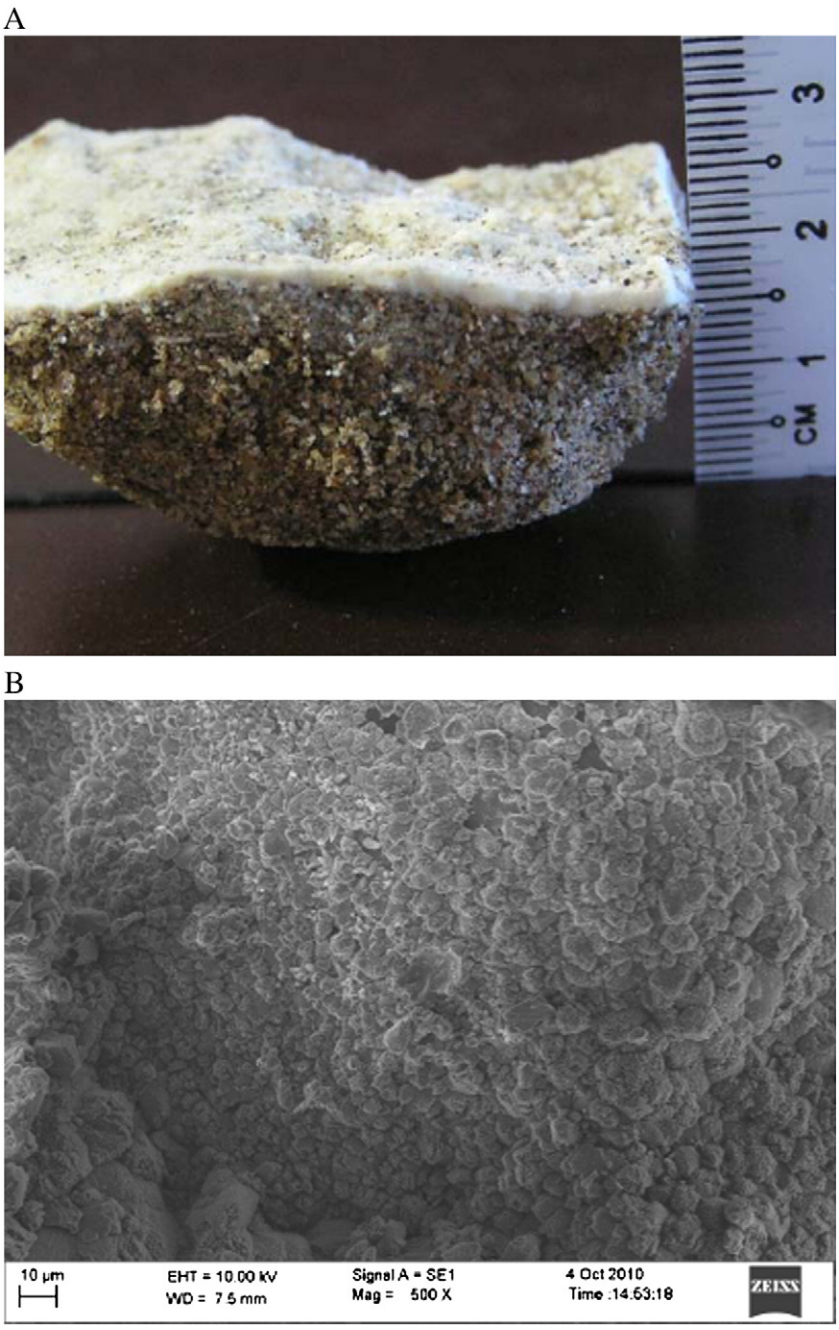

Fig. 2. (A) Crust on the surface of the treated sand and (B) SEM image of the crust.

respectively. So, almost 95% of supplied calcium was precipitated in the treated sand, mainly in the crust on the sand surface. Distribution of calcium content in the sand by depth showed that only 17–22% of the volume of the sand pores beneath the crust was filled with $CaCO_3$ (Fig. 6). The quantity of calcium for the formation of crust was 0.6 g of Ca/cm^2 or 6 kg of Ca/m^2 of sand surface.

Usually, the sealing of the pond is made using the fine sand bed with a thickness of about 10–25 cm, which is covered with the plastic liner with the thickness of about 0.5–12 mm and the upper soil layer with a thickness of about 10–25 cm [24]. Calcite crust has a lower weight than this type of the pond sealing.

The weight of the biocemented crust is significantly lower than the weight of clay, which is also used for the pond sealing [24]. For example, the thickness of the bentonite blanket should be at least 10 cm, so about 60 kg of bentonite is required to seal 1 m^2 of pond area [24].

The sealing could be made by the biocementation using sequential injections procedure that includes homogenous distribution of bacterial cells in sand followed by fixation with 50 mM solution of $CaCl_2$ [22]. However, waterproof biocementation in the bulk of sand required a big quantity of calcium salt; for example: 7.6 kg of Ca/m^2 of surface (calculated from [22]) or even 113 kg of Ca/m^2 of surface (calculated from [23]), while the formation of the waterproof crust of biocement, described in this paper, is significantly less material-consuming biocementation process.

3.3. Effect of the sand biocementation on the permeability

The permeability (daily seepage rate) decreased gradually with the number of treatments. It was $1.6 \cdot 10^{-7}$ m/s (or 14 mm/day) after the sixth treatment (Fig. 7). This seepage rate is similar to the values of permeability for the well compacted clay. It is lower than the seepage

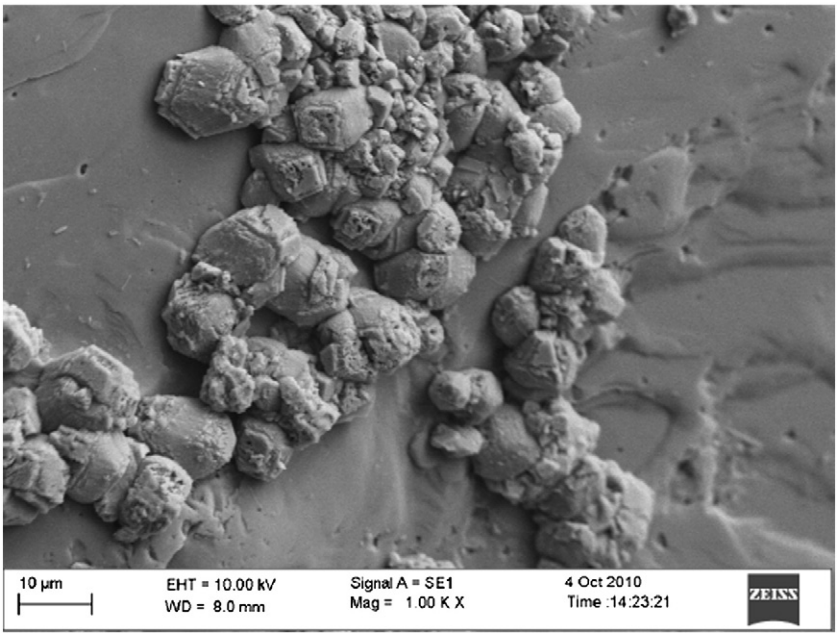

Fig. 3. Crystals of $CaCO_3$ on the surface of the treated sand.

rates of 27–37 mm/day, 19–58 mm/day for the tropical fish ponds [17,18] and 136–182 mm/day for the shrimp mariculture ponds [16].

3.4. The flexural strength of the biocemented crust

The mechanical breakdown of the bottom biocemented crust or the liner of the aquaculture pond could be due to its bending on the bed because of hydrostatic pressure. The modulus of rupture (maximum bending stress) of biocemented crust was 7.5 MPa and 35.9 MPa after 1 day and 10 days drying on air. Last value is comparable with the maximum bending stress for limestone and some types of granite, which is well known from the references on the construction material properties. The maximum bending stress of the biocemented crust was significantly higher than hydrostatic resistance of the PVC plastic liners that are used for the pond sealing. This hydrostatic resistance is typically from 0.4 to 1.0 MPa. So, mechanical strength of the biocemented crust could be sufficient for the pond or reservoir.

4. Conclusions

The study presented in this paper has demonstrated that it is feasible to use biocement for the construction of aquaculture pond or reservoir in sand. Due to precipitation of 0.6 g of Ca/cm^2 of sand surface through a biocementation process, the permeability of sand can be reduced from 10^{-4} m/s to $1.6 \cdot 10^{-7}$ m/s (or 14 mm/day). The biocementation took place mainly on the surface of a sand layer and led to the formation of a hard and impermeable crust of about 1 mm

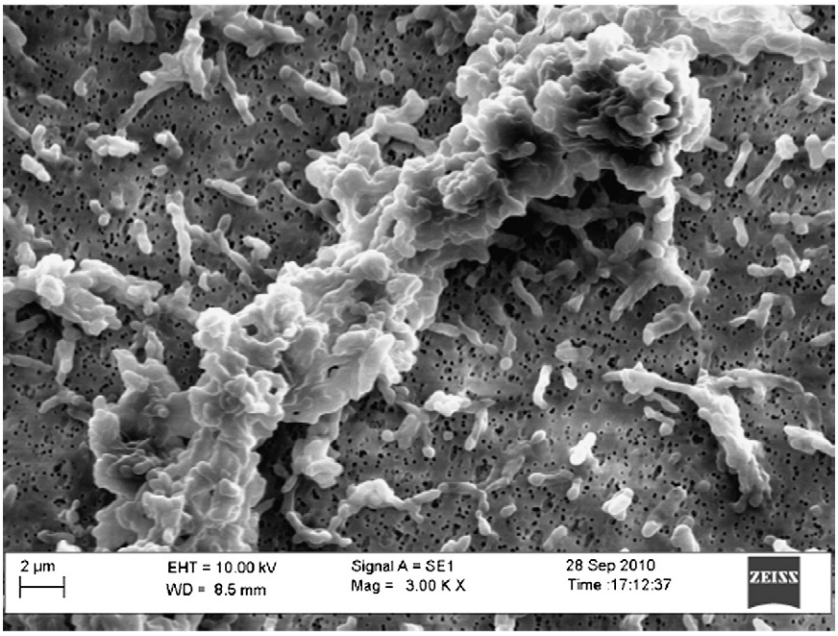

Fig. 4. Free and aggregated cells of *Bacillus* sp. VS1.

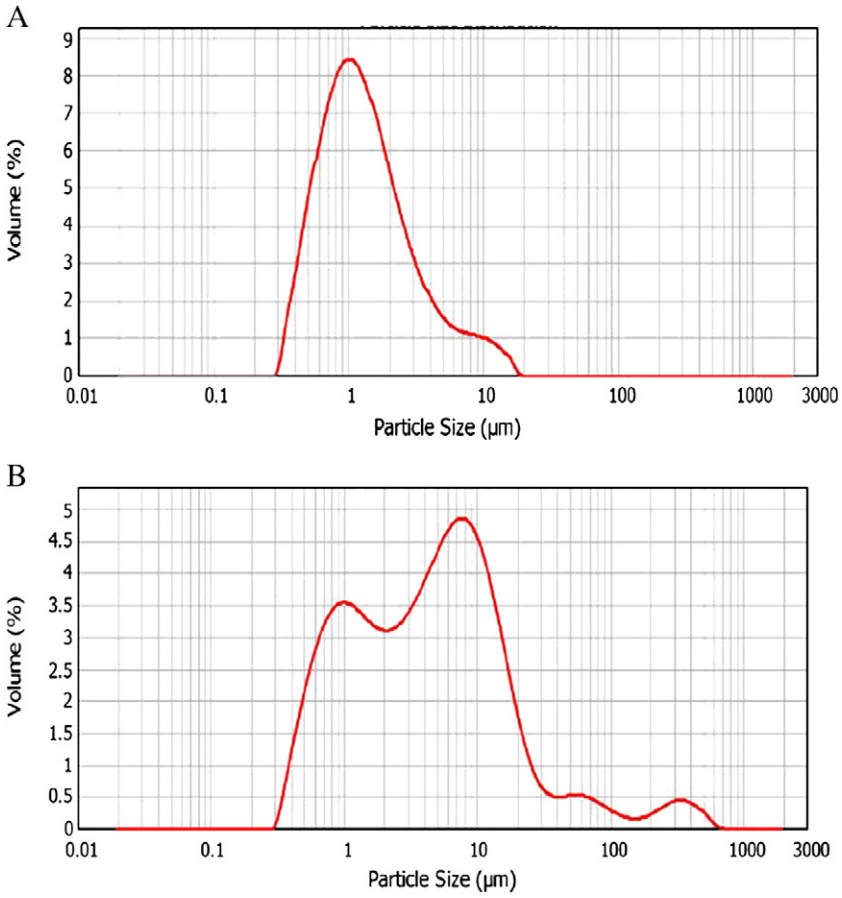

Fig. 5. Formation of cell aggregates after treatment of cultural liquid with calcium solution: A, distribution of cell and cell aggregate sizes without treatment of cultural liquid with calcium solution; B, distribution of cell and cell aggregate sizes after treatment of cultural liquid with calcium solution.

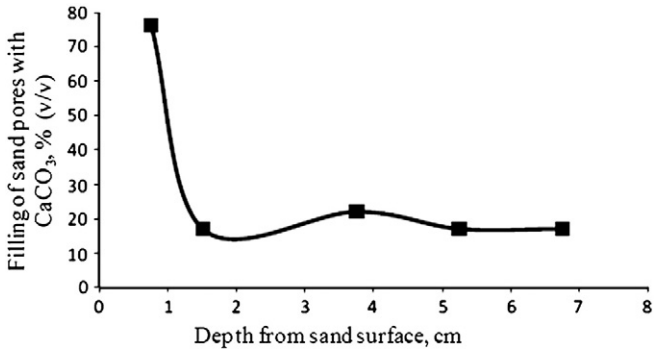

Fig. 6. Distribution of calcium in the treated sand.

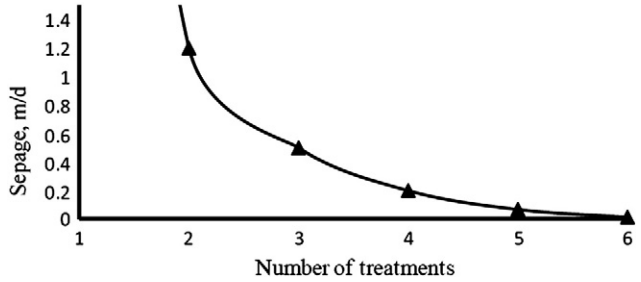

Fig. 7. Effect of treatment numbers on the seepage rate from the model biocemented pond.

thickness on the sand surface. The modulus of rupture of biocemented crust was 35.9 MPa, which is comparable with that of limestone. The formation of the water-impermeable and mechanically strong biocemented crust on the sand surface could provide a method for the construction of aquacultural ponds, stabilization of sandy soil slopes, sand dunes, dust fixation in desert areas, and sealing of the channels and reservoirs in sandy soil.

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