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# Sealing of sand using spraying and percolating biogrouts for the construction of model aquaculture pond in arid desert

**Viktor Stabnikov · Volodymyr Ivanov · Jian Chu**

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**Abstract** The topic of the paper is the biotechnological sealing of sand using calcium- or iron-based biogrouts. These processes are modeling the sealing of sand during construction of aquaculture pond in the arid desert. The experiments showed that it is possible to conduct biosealing of sand using microbially induced calcium carbonate precipitation performed by the spraying of dead but urease-active bacteria. The sealing was also effective due to ferric hydroxide precipitation in sand after percolation of ferrous-containing solution produced from iron ore and cellulose by the community of acidogenic and iron-reducing bacteria. These treatments of sand can decrease its hydraulic conductivity from the level of $10^{-4}$ m/s to the level of $10^{-8}$ m/s, which is an acceptable level for the aquaculture ponds. The cost of this sealing, especially when the local sources of calcium chloride brain or low grade iron (hydr)oxides of iron ore are applied, could be several times lower than any other known methods of the sand sealing, and could be used in aquaculture practice for the construction of fish, prawns, or algae ponds in sand of the arid deserts.

**Keywords** Aquaculture pond · Biosealing of sand · Microbially induced calcium carbonate precipitation · Microbially induced ferric hydroxide precipitation

## Introduction

Non-arable land in an arid desert can be used for algae, fish, and prawns pond cultures (Winckelmann et al. 2015) but the problem is the high cost of the sealing a pond constructed in sandy soil. Potential solution of this problem can be a biosealing of sand as shown in this paper. A new scientific and engineering discipline, Construction Biotechnology, is the development of the microbially mediated construction processes and construction biomaterials (Ivanov et al. 2015; Stabnikov et al. 2015). The new type of construction materials, biocement and biogrouts, are developing extensively as an alternative to cement and chemical grouts (Bachmeier et al. 2002; Ferris et al. 1996; Whiffin et al. 2007; Ivanov and Chu 2008; DeJong et al. 2010; De Muynck et al. 2010; Burbank et al. 2011, 2012; Stabnikov et al. 2011; Al-Thawadi and Cord-Ruwisch 2012; Chu et al. 2012; Rong et al. 2012) because they have low viscosity, can penetrate deeper into porous soil, and usually are less harmful for environment than conventional grouts.

V. Stabnikov · V. Ivanov (✉) · J. Chu
School of Civil and Environmental Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore
e-mail: cvivanov@ntu.edu.sg

Biocementation and biosealing of sand could be based on microbially induced calcium carbonate or ferric hydroxide precipitation due to the activity of urease-producing bacteria (UPB) in the presence of urea and ions of calcium or ferrous:

$$Ca^{2+} + (NH_2)_2 + 2\,H_2O \rightarrow CaCO_3 \downarrow + 2\,NH_4^+ \quad (1)$$

$$Fe^{2+} + 0.25\,O_2 + 4H^+ + 3\,(NH_2)_2CO + 5.5\,H_2O \rightarrow Fe(OH)_3 \downarrow + 6\,NH_4^+ + 3\,CO_2 \quad (2)$$

To produce solution of ferrous ions, organic acids must be produced by acidogenic bacteria fermenting cellulose at the first step as shown below (molar ratios of volatile fatty acids were taken from Madigan et al. 2014):

$$C_6H_{12}O_6 + 0.82\,H_2O \rightarrow 1.13\,CH_3COOH + 0.35\,C_2H_5COOH + 0.26\,C_3H_7COOH + 1.67\,CO_2 + 2.47\,H_2 \quad (3)$$

where $C_6H_{12}O_6$ is a monomer of cellulose, and $CH_3COOH$, $C_2H_5COOH$, and $C_3H_7COOH$ are acetic, propionic, and butyric acids, respectively. Then, iron ore should be reduced by iron-reducing bacteria using organic acids, mainly acetate:

$$8\,Fe^{3+} + CH_3COOH + 2\,H_2O \rightarrow 8\,Fe^{2+} + 4\,CO_2 + 8H^+ \quad (4)$$

Urease-producing bacteria for biocementation/biosealing of sand should be able to synthesize active urease under alkaline environment with high concentration of salts (Stabnikov et al. 2013), should show low aggregation ability at high concentration of calcium ions (DeJong et al. 2006; Stabnikov et al. 2011), and should be biological safe, e.g., non-pathogenic ones. Usually, the strains of *Sporosarcina pasteurii*, formerly *Bacillus pasteurii,* are commonly used for biocementation (Bang et al. 2001; Bachmeier et al. 2002; Whiffin 2004; Whiffin et al. 2007; Dupraz et al. 2006; Mortensen and DeJong 2011; Tobler et al. 2014).

These bacteria belong to Risk group 1 with low individual and community risk. However, there are a lot of studies when such opportunistic pathogens as *Bacillus cereus* (Maheswaran et al. 2014), *Bacillus mycoides* (Elmanama and Alhour 2013), *Proteus* sp., *Proteus vulgaris* or *Proteus mirabilis* (Dosier 2014; Khanafari et al. 2011; Whiffin 2004; Varalakshmi 2014), *Staphylococcus aureus* and *Klebsiella pnemoniae* (Varalakshmi 2014), and even pathogenic bacteria *Helicobacter pylori* (Dosier 2014) were proposed to be used for biocementation. So, the critical point of biocementation is an introduction of live bacterial cells in environment, which is a risk.

In our previous research on the sand biosealing, we used live urease-producing bacteria for the construction of the model aquaculture pond using sealing of sand by microbially induced calcium carbonate precipitation (Stabnikov et al. 2011; Chu et al. 2013). The aim of the present study was to check possibility to seal the pond in sand using calcium or iron salts and dead but urease-active cells of urease-producing bacteria to ensure biosafety of the process for human and environment.

## Materials and methods

The main idea of the safe biosealing using spaying was to kill cells of UPB in such way that their urease activity will remain intact. However, experiments with existing strains of *Sporosarcina pasteurii*/*Bacillus pasteurii* did not produce such results probably because of ability of these bacteria to produce stable spores and due to thick cell wall. Therefore, another strain of UPB was isolated and tested. The strain of non-sporogenic Y*aniella* sp. VS8 (GenBank accession number of nucleotide sequence of 16S rRNA is KT182991), belongs to *Actinobacteria*, the phyla Bacteria and represents Gram-positive bacteria with a high G+C content in their DNA, the order *Micrococcales*, the family *Micrococcaceae*, was isolated from enrichment culture of UPB that was used in practice for several years. The bacteria of the genus Y*aniella* belong to Risk group 1.

The strain Y*aniella* sp. VS8 demonstrated a high enough urease activity, so application of its inactivated cells for biocementation was studied.

Batch cultivation of Y*aniella* sp. VS8 was provided under shaking at 200 rpm at temperature 25 °C. Medium for microbial cultivation had the following composition: Tryptic Soya Broth DIFCO$^{TM}$, 20 g; NaCl, 20 g, $NiCl_2 \bullet 6H_2O$, 24 mg, phenol red, 10 mg, distilled water 1 L, pH 8.2. This medium was sterilized at 121 °C for 15 min. Stock solution of urea, 100 g/L, was sterilized by filtration through Millipore filter with diameter 0.2 μm to avoid urea loss due to thermal treatment. 200 mL of urea stock solution was added to 800 mL of described above medium, and this urea-containing medium was used for bacteria cultivation (TSB medium).

Treatment of bacterial cells (culture liquid of strain VS8) was done by addition of 0.5 % (w/v) sodium dodecyl sulfate (SDS) for 960 min. Bacterial biomass was separated by centrifugation at 4 °C and 10,000 rpm for 10 min using Micro Cooling Centrifuge 5922 (Kubota, Japan), resuspended in NaCl, 2 %, and used for biocementation of sand.

To determine the concentration of cells in bacterial suspensions, the enumeration of colony forming units (CFU) was done after spread-plate inoculation of the Petri plates aseptically filled with Tryptic Soy Agar (TSA) (DIFCO, Lawrence, USA) by 0.1 mL of the serial tenfold dilutions of the bacterial suspension. To check the content of live bacterial cells in biocemented sand, 0.1 g of sand was mixed with 10 mL of solution NaCl, 2 %, and after vortexing, this suspension was used for spreading onto solid medium in the Petri dishes. The enumeration of CFU was made after incubation of inoculated Petri plates at 30 °C for 5 days.

Urease activity was defined as the amount of ammonium produced from 1 M solution of urea per minute. Amount of ammonium produced from urea was determined using electric conductometer showing linear correlation ($R^2 = 0.9997$) between the molar concentrations of $NH_4^+$ ($Y$) and the changes of electric conductivity of solutions ($\Delta X$) in mS/cm.

The hydraulic conductivity of sand, $k$, in the model pond was measured by falling head method at water head falling from 5 to 2 cm according to the equation:

$$k = V/t \cdot S(m/s),$$

where $V$ is the volume of water passed through the sand, $t$ is a time of the liquid was passed through the sand, and $S$ is a cross-sectional area of water flow through sand.

A standard sand ASTM C778 was used for the experiments. This rounded grain silica sand had a mean grain size of 0.42 mm. The specific gravity was 2.65. Sand, total 6 kg, was placed into the plastic box with the sizes: 260 mm in width, 360 mm in length, and 55 mm in height.

Construction of the model pond using sand biocementation comprised with the steps of the formation of the bottom and the walls in sand: sand, 4.5 kg, was placed in the box, and then 4 plastic sheets were fixed into sand for the formation of walls. Sand, 1.5 kg, was spread between the walls of plastic box and plastic sheets to form the walls of pond.

Biosealing with $CaCO_3$ was done by spaying of the bacterial suspension and the solution of $CaCl_2$ and urea. Bacterial suspension was spread onto the surface of the model pond and left for 30 min. Then, mixture of the


**Fig. 1** Iron ore particles of different sizes

solutions of calcium chloride and urea was spread onto the sand surface and left for 16 h. Spray of the mixture was done some times for each treatment, thus the sand was not submerged into liquid. The treatments were repeated 11 times. After 8th, 9th, and 10th treatments, the model pond was dried by placing in oven at 60 °C: after 8 and 9 treatments for night; after 10th treatment or 2 h. The study period included: (1) biotreatment—15 days, (2) drying on air—60 days, (3) additional biotreatment—4 days; (4) washing with water—3 days.

The production of dissolved ferrous ions was done by consortium of cellulose-fermenting and iron-reducing bacteria producing sealing solution in the 3-L plastic bottle filled with 400 mL of tap water, 50 mL of anaerobic sludge from local municipal wastewater treatment plant, 1 kg of beach sand, 100 g of the particles of hematite iron ore (ferric oxide) with average size of 2.4 ± 0.4 mm (Fig. 1), 30 g of cellulose (shredded paper tissue), and 20 g of $CaCO_3$ for pH buffering.

Cultivation was done for 20–45 days at 25 °C and was stopped when concentration of $Fe^{2+}$ was above 0.5 g/L. Then, 300 mL of ferrous-containing solution and 50 mL of 0.05 M solution of urea altogether with 50 mL of UPB bacterial suspension were added to the 5-cm diameter plastic column filled with 650 g of sand for 3 days incubation. Then, solution was drained and the same treatment was repeated. There were 11 such treatments for the sand clogging.

## Results and discussion

### Biosealing of sand with calcium carbonate

The best way to ensure biosafety of the aquaculture pond biosealing could be killing of bacterial cells and application of dead bacterial cells, because application of enzyme urease for large-scale biocementation could be too expensive and probably the presence of bacterial cells is needed to create crystallization centers for calcium carbonate precipitation on sand surface (Stocks-Fischer et al. 1999; Gat et al. 2014).

Killing of bacterial cells *Yaniella* sp. VS8 was done with sodium dodecyl sulfate (SDS) added to culture liquid to concentration 0.5 % (w/v) for 960 min. SDS, $C_{12}H_{25}NaO_4S$, is an anionic surfactant inhibiting growth of many Gram-positive (Flahaut et al. 1996) and Gram-negative bacteria (Woldringh and Van Iterson 1972; Adamowicz et al. 1991) via dissolution of the cell membrane (Kramer et al. 1984; Hansen et al. 2011). Concentration of live bacterial cells and urease activity (UA) of bacterial suspension were determined in the samples after treatment and were compared with control (Table 1).

Bacterial cells were killed, but urease activity of culture liquid after treatment remained at the level of 4.9 mM hydrolyzed urea/min, while this level before treatment was 5.6 mM hydrolyzed urea/min.

Totally, 2 L of treated bacterial suspension containing 5.8-g dry biomass, 108 g of calcium, and 225 g of urea was used for biosealing of the model pond by spraying onto the sand surface with area of 722.5 $cm^2$. Finally, after eleven biotreatments the model pond with sizes 180 × 275 × 25 mm was constructed (Fig. 2).

Calcium in effluent was absent after every treatment. Due to this treatment, the hydraulic conductivity of sand was decreased from $5.2 \times 10^{-4}$ to $7.7 \times 10^{-9}$ m/s. Quantity of water percolated through pond bottom sand layer for the testing pond and control per day vs duration of study period is shown in Fig. 3. It was changed from 7000 to 96 mm/day.

Effect of water depth on the hydraulic conductivity was measured by the water seepage from 8-mm diameter plastic tube connected with the bottom. Average values of the seepage for the layers of biocemented sand and not cemented sand at water head 500–1000 mm were 96 and about 7000 mm/day. The hydraulic conductivity of the biosealing layer in tap water did not changed for at least 90 days.

**Table 1** Concentration of live bacterial cells and UA of bacterial suspensions

| Object | UA, mM hydrolyzed urea/min for contact time with urea solution | | CFU/mL |
|---|---|---|---|
| | 5 min | 30 min | |
| Control | 4.95 | 2.28 | $5.2 \times 10^8$ |
| Cells treated with SDS | 3.20 | 6.49 | 0 |

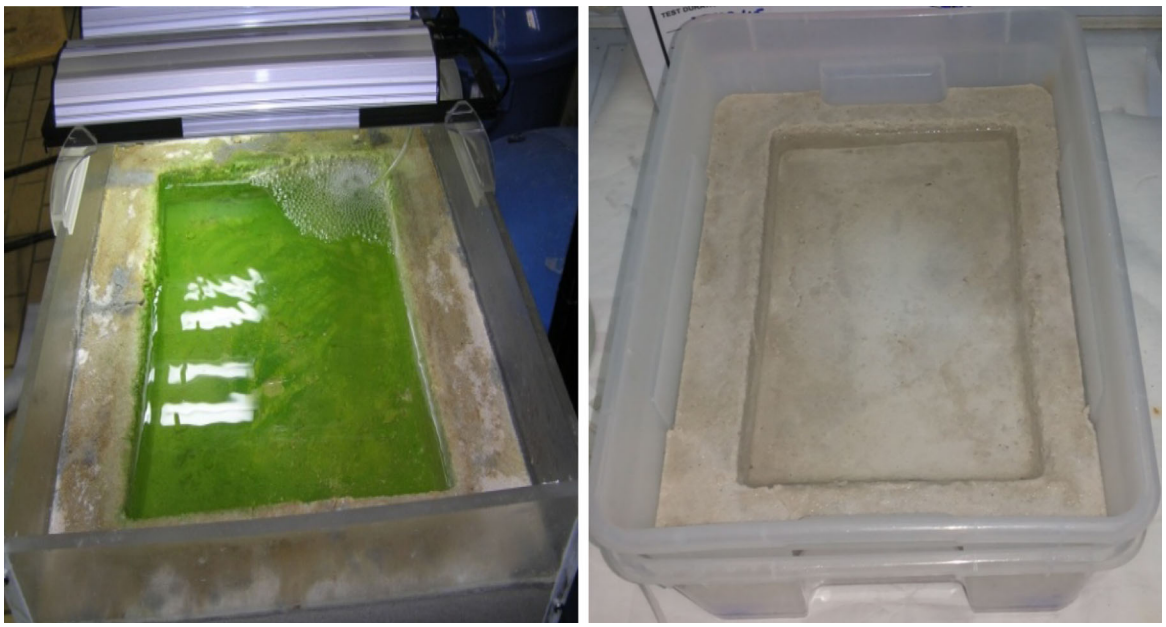

**Fig. 2** Images of model ponds constructed in sand using live cells of urease-producing bacteria *Bacillus* sp. VS1 (*left image*, bottom is coated with the grown biofilm of algae) and dead but urease active cells of *Yaniella* sp. VS8 (*right image*)

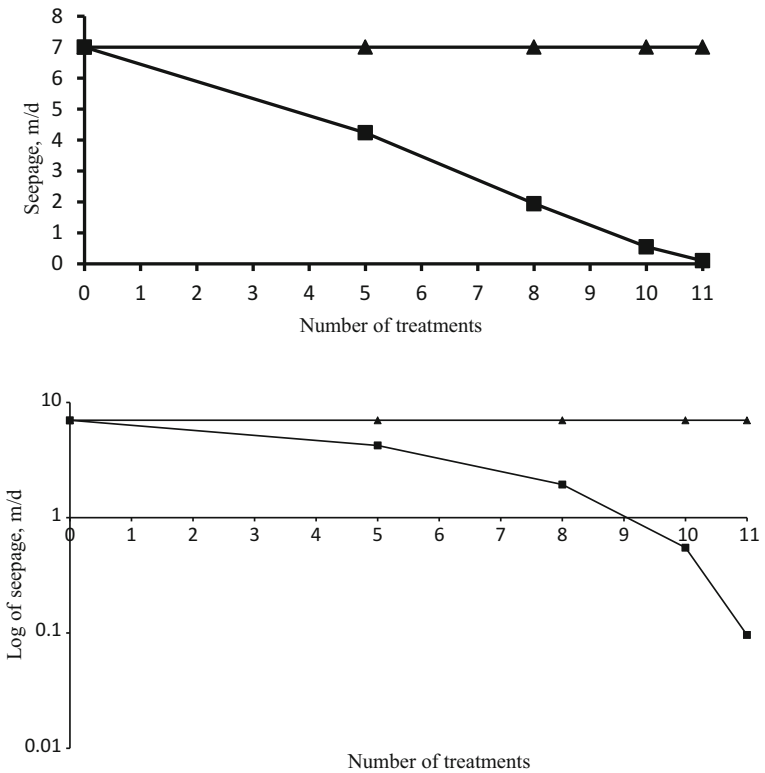


**Fig. 3** Dynamics of bioclogging with $CaCO_3$ (in *linear* and logarithm *scales*). *Rectangles* show experimental treatment with dead but urease-active bacteria, *triangles* show control treatment without bacteria

The content of live microbial cells in the sample taken from the pond surface was $4 \times 10^2$ CFU/g of the biocemented sand, which is almost the same as initial bacterial contamination of sand, $1.1 \times 10^2$ CFU/g.

## Quality of water after biotreatment

Initial water was tap water with electric conductivity 0.35 mS. The model pond produced after bioclogging and drying was washed with tap water three times for about 12 h of each washing. After that, the electrical conductivity and concentrations of dissolved calcium and magnesium were studied (Table 2).

Meanwhile, effluent accumulated after 16 h of seepage had the same pH 7.8, but significantly higher electric conductivity of 2.54 mS, and concentrations of $Ca^{2+}$ and $Mg^{2+}$ were 2.65 and 0.12 mM, respectively. Ammonium was not determined in water of the model pond but accumulated in effluent up to 2.7 mM. Probably, calcium, magnesium, and ammonium ions were extracted from a layer of not biocemented sand, which was under a layer of sand that was cemented with calcium carbonate.

### Biosealing of sand with iron hydroxide

Solution of ferrous ions can be produced from iron ore and cellulose for about 4–10 days in batch anaerobic cultivation (Fig. 4).

To create anaerobic conditions, the diffusion of oxygen from air was prevented by an addition of sand into the reactor with iron ore and cellulose. Strong anaerobic conditions with oxidation–reduction potential −320 mV were self-developed at 5–6 cm depth below sand surface by bacterial community. pH of effluent was 6.5 with concentration of $Fe^{2+}$ above 0.5 g/L.

Produced solution of soluble ferrous salts was supplied to the sand column for bioclogging by dropping through air, so percolation of sand was by aerated ferrous-containing grout. pH of liquid increased after the treatment of sand with a mixture of this grout, 0.05 M urea solution and suspension of UPB to 8.5 with almost complete precipitation of iron when final concentration of $Fe^{2+}$ was below 5 mg/L. After ten treatments, the hydraulic conductivity of sand decreased from $5 \times 10^{-4}$ to $1 \times 10^{-6}$ m/s. The pores of sand were visibly filled with brown ferric hydroxide (Fig. 5).

**Table 2** The electric conductivity and concentrations of dissolved calcium and magnesium in water after treatment and washing of the model pond

| Time after filling of the model pool with water (h) | pH | Electric conductivity (mS) | $Ca^{2+}$ (mM) | $Mg^{2+}$ (mM) |
|---|---|---|---|---|
| 0 | 7.9 | 0.35 | 0.30 | 0.02 |
| 4 | 7.8 | 0.49 | 0.45 | 0.02 |
| 24 | 7.9 | 0.50 | 0.65 | 0.02 |
| 28 | 8.0 | 0.57 | 1.35 | 0.04 |
| 100 | 8.1 | 0.74 | 1.58 | 0.05 |

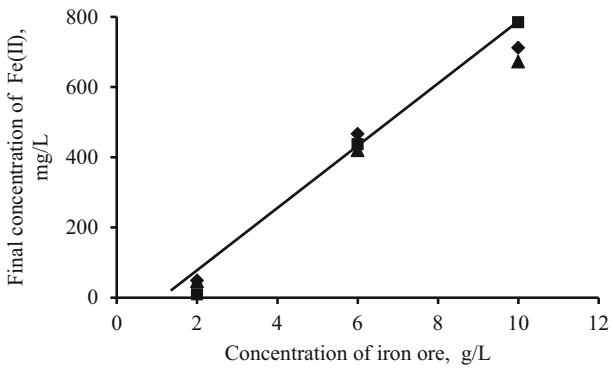


**Fig. 4** Production of Fe(II) in experiments with different concentrations of iron ore at the following concentrations of cellulose: 2 g/L (*filled rhombus*); 6 g/L (*filled square*); 10 g/L (*filled triangle*)

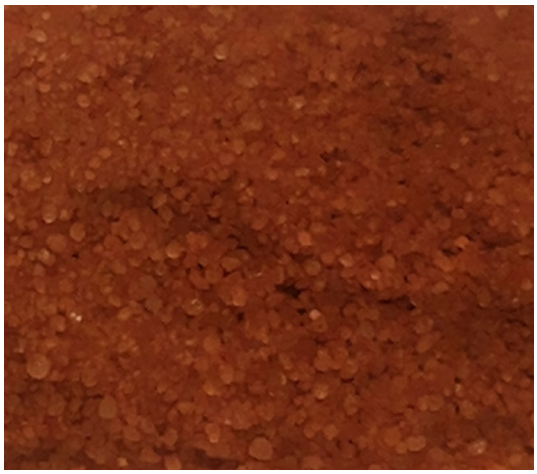

**Fig. 5** Sand after bioclogging with iron-based biogrout

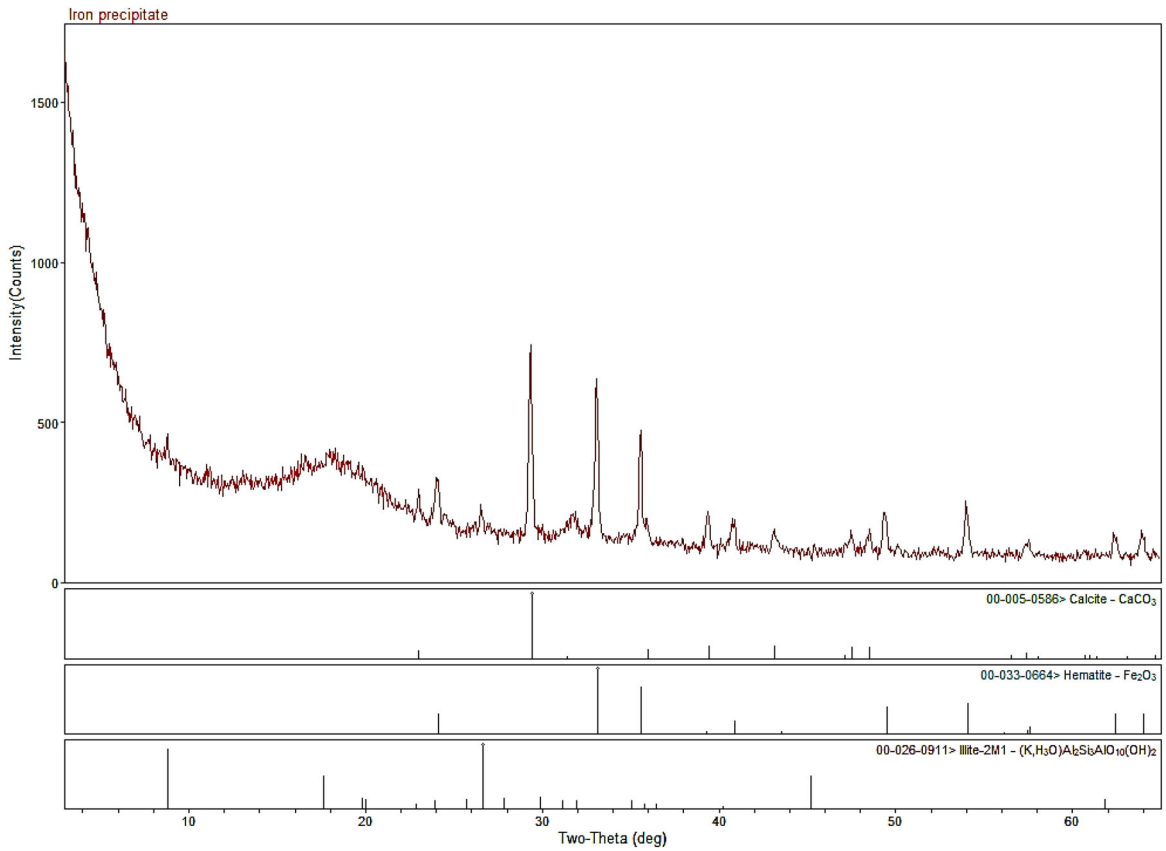


**Fig. 6** XRD of the material precipitated in sand

XRD of the material precipitated in sand shows that this precipitate was a mixture of hematite and calcite (Fig. 6).

Treatment of sand with iron-based biogrout did not increase the unconfined compressive strengths of the treated sand significantly. Therefore, major application of iron-based biogrout produced from iron ore and cellulose could be bioclogging of the porous soils for the sealing of aquaculture ponds or the fractured sedimentary rocks during the dam or tunnel construction.

### Comparison of the sand biosealing with other methods

The calculated consumptions of biomass, calcium, and urea per 1 $m^2$ of cemented sand surface were as follows: 0.08 kg of dry bacterial biomass; 1.5 kg of calcium (4.1 kg of $CaCl_2$); 3.1 kg of urea. Considering that the costs of dry calcium chloride, urea, and bacterial suspension are about US$250, US$360, and US$500 per ton, respectively, the evaluated total cost of the calcium chloride-based biosealing materials is about US$18,000. It is lower than the cost of the sealing with bentonite, US$48,000/ha, and is significantly lower than sealing cost with geosynthetic liners, which is about US$100,000/ha.

The sealing of sand (i.e., formation of 10-cm layer of sand with the content of iron oxide 5 % w/w) with iron-based biogrout produced from iron ore and waste cellulose from municipal solid waste, agricultural waste, and food-processing wastes will require per 1 $m^2$ of cemented sand surface 0.08 kg of dry bacterial biomass; 8 kg of iron oxide (hematite iron ore), 3 kg of urea, and 1 kg of waste cellulose. Considering that the costs of iron ore dry iron ore, urea, bacterial biomass, and waste cellulose are about US$100, US$360, US$500, and US$100 per ton, respectively, the evaluated total cost of the iron ore-based biosealing materials is about US$23,800. It is also lower than the cost of known sealing materials. The cost of this sealing could be especially low when the local sources of calcium chloride brain can be used.

### Scale-up factors

Theoretical comparison of the supplies of urea, which is a major component of the biosealing composition, using Eqs. 1 and 2 for calcium chloride-based biosealing (density of calcite is 2.71 g/$cm^3$) and iron ore-based biosealing (density of ferric hydroxide is 4.25 g/$cm^3$) are 0.221 g urea/$cm^3$ of porous space in sand and 0.396 g urea/$cm^3$ of porous space in sand, respectively. So, calcium chloride-based biosealing requires almost twice bigger quantity of urea per volume of the porous space than iron ore-based biosealing method needs. Respectively, twice bigger quantity of ammonium will be released to environment in case of iron ore-based biosealing. Therefore, selection of the most suitable scaled-up application must be based on both economy and environmental considerations, as well as on the local availability of either sources of calcium salts, for example, underground calcium chloride brain, or iron ore, especially its fine powder, which is most suitable for bioreduction of iron (III).

Important scale-up factor is the thickness of the biosealing layer which has to be sufficiently mechanically strong to prevent cracking because of the bending from hydraulic head and/or thermal expansion. This thickness must be determined in the field tests at the defined area at the different water heads and solar radiation. In every case, it will not be bigger than several centimeters.

The hydraulic conductivity and strength after biotreatment is determined by the third, and probably the major scale-up factor, which is the content of precipitated $CaCO_3$. In our experiments, the contents of $CaCO_3$ in sand were 45, 6, 8, and 8 % (w/w) on the distance 1, 2, 3, and 4 cm from surface of the bottom, while there were 13 and 19 % of $CaCO_3$ on the distance of 1–2 cm from the top and side surfaces of the wall.

The seepage rate from the model pond was 96 mm/day at the content of calcium in 1 cm layer on the bottom 45 and 18 % (w/w) in 1 cm layer of the side surface of the wall. This seepage was almost the same as the seepage rate from the model pond constructed in sand using un-inactivated cells of UPB *Bacillus* sp. VS1, when an average 2.1 kg of calcium per $m^2$ of sand surface was precipitated (Stabnikov et al. 2011; Chu et al. 2013). These seepage rates are comparable with the seepage rates for the aquaculture ponds (Teichert-Coddington et al. 1989; Weisburd and Laws 1998).

The technology with sprayed calcium-based biogrout simplifies considerably the procedure, because there is no use of live microorganisms and thus there is no need for the numerous and complicated biosafety approvals needed for the real construction projects. The technology with percolated iron-based biogrout simplifies considerably the production of the sealing materials, because raw materials, low-grade iron ore and cellulose-containing waste, need for construction are abundant everywhere. However, essential part of the sand biogrouting is drying of the treated sand under sun light. Our data showed that this drying removes ammonia and ammonium from the treated sand, which is essential for fish and prawns aquaculture, but not favorable for algae cultivation. Sand sealed with iron-based biogrout is more mechanically stable for drying under sun light than sand treated with calcium-based biogrout. In the last case, the treated sand is more brittle

and there is possible the formation of fine cracks due to thermal expansion of the treated sand. Repair of these cracks requires additional treatment of sand after sun light drying.

Major limitation of the proposed biosealing method of the sand pond is the release of ammonia to atmosphere and ammonium to water, which can produce toxic effects for human being, animals, fishes and prawns. So, the best solution is the use of the constructed pond for the cultivation of oil-producing algae in the coastal desert area. Then, when ammonium will be washed out from the cemented sand and consumed by the oil-producing algae during one or several cycles of their cultivation, the pond could be used for the fish aquaculture. There must be a source of carbon dioxide for algae growth, so additional layer of calcium carbonate on the bottom of the pond can be a source of carbon dioxide as well as a source of calcium ions for further strengthen and biosealing of the pond bottom.

In conclusion, because the cost of biosealing of sand using calcium chloride- or iron ore-based biogrouts could be several times lower than any other known methods of the sand sealing, the biosealing has to be test in aquaculture practice for the construction of fish, prawns, or algae ponds in sand of the arid deserts.

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