International Journal of Environmental Science and Technology
https://doi.org/10.1007/s13762-018-1788-6

ORIGINAL PAPER

Mitigation of the liquefaction potential of soil by Ca-carbonate precipitation induced by indigenous urease-producing *Staphylococcus* sp. IR-103

R. Moosazadeh[1,2] · F. Tabandeh[2] · F. Kalantary[3] · N. Ganjian[1] · H. Fallah[2] · T. Bagheri Lotfabad[2] · F. Yazdian[4]

Received: 4 October 2017 / Revised: 5 May 2018 / Accepted: 11 May 2018

Abstract
Biocementation is a microbially induced technology that increases the shear strength of soil through the production of soil particle-binding materials known as calcite (calcium carbonate). This process makes use of urease-positive microorganisms, urea and calcium ions. The main aim of this study was to introduce an indigenous soil bacterium belonging to the genus *Staphylococcus* that was capable of hydrolyzing urea and precipitating calcium carbonate ($CaCO_3$). Molecular identification of this isolate by 16S rDNA sequencing showed 98% homology to *Staphylococcus* sp. Several culture media were employed to investigate the growth, urease production and $CaCO_3$ precipitation of this strain (designated *Staphylococcus* sp. IR-103, accession number LT853888). When *Staphylococcus* sp. IR-103 was grown in YN medium containing 20 g L^{-1} of yeast extract and 10 g L^{-1} of NH_4Cl, maximal growth yield (OD_{600}), urease activity and carbonate precipitation values of 2.8 ± 0.1, 3.33 ± 0.12 IU and 47.6 ± 0.9 mg mL^{-1} were obtained, respectively. The precipitated $CaCO_3$ was characterized by FTIR, AAS, XRD and SEM analyses. In order to study the effects of the bacterium's biocementation activities on soil strength, bacterial suspension and cementation solution was injected into a column packed with uniform sandy soil under defined condition. In conclusion, *Staphylococcus* sp. IR-103 is a high urease producer, which can grow on a simple and cost-effective medium without staying viable for long following the biocementation process. Hence this newly isolated strain has the potential to be employed in soil improvement for large-scale field applications.

Keywords Biocementation · Calcite precipitation · Soil improvement · *Sporosarcina pasteurii* · *Staphylococcus* sp. · Urease

Introduction

Biocementation has been shown to enhance the mechanical properties of soil such as strength and stiffness, through microbial metabolic activities (Ivanov and Chu 2008). Microbial-induced carbonate precipitation (MICP) involves the microbial hydrolysis of urea in a calcium-rich environment (Castanier et al. 1999). This biomineralization process has been the subject of recent studies regarding several environmental applications, such as soil improvement, dust control, strengthening of concrete, treating pavement surfaces, increasing the bearing capacity of piled or non-piled foundations and mitigating the liquefaction potential of sand (Ramachandran et al. 2001; Kucharski et al. 2006; Whiffin et al. 2007; Al-Thawadi 2013). The formation of $CaCO_3$ deposits, commonly known as biocement, by the MICP process is controlled by four main factors, which include the calcium concentration, the CO_2 partial pressure that affects the carbonate

Editorial responsibility: Josef Trögl.

✉ F. Tabandeh
taban_f@nigeb.ac.ir

[1] Department of Geotechnical Engineering, Science and Research Branch, Islamic Azad University, Tehran, Iran

[2] Department of Industrial and Environmental Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), 14965161, Tehran 1497716316, Iran

[3] Department of Geotechnical Engineering, Khaje Nasir Toosi University of Technology, Tehran, Iran

[4] Department of Biomedical Engineering, Faculty of Interdisciplinary New Sciences and Technologies, Tehran University, Tehran 14395-1561, Iran

Published online: 29 May 2018

concentration, the local pH and the nucleation and subsequent crystal growth (DeJong et al. 2006). The most promising application of MICP is urea hydrolysis via urease-producing bacteria and precipitation of $CaCO_3$ in the presence of calcium chloride and urea. In order to carry out the MICP process, urease-producing bacteria are first cultivated aerobically in nutrient medium and then harvested at the late-exponential phase of growth. The bacterial suspension is then introduced to soil and supplied with a cementation solution containing urea and calcium chloride. The urease enzyme catalyzes the hydrolysis of urea to ammonium and carbonate (Eq. 1). Accordingly, the local pH increases as a result of ammonium production by urease activity. The bacterial cell surface has a negative charge which may serve as a nucleation site for the precipitation of positively charged calcium ions (DeJong et al. 2006). Under these conditions, Ca^{2+} and CO_3^{2-} precipitate as $CaCO_3$ crystals (Ramakrishnan et al. 2001), which form cementing bridges between the soil particles (Eq. 2). In this way, loose sand is converted to cemented sand or sandstone (Saxena and Lastrico 1978).

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

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

A wide range of microorganisms are able to produce urease (Mobley et al. 1995); however, the abilities of a few number of microorganisms have only been studied with regard to the biocementation process. These include bacteria *such as Sporosarcina pasteurii* and *Proteus vulgaris* (Whiffin 2004; Ivanov and Chu 2008; Stabnikov et al. 2015; Macegoniuk 2013; Porter et al. 2017). *S. pasteurii* is a well-known urease-positive bacterium that is used routinely in the MICP process. Because this is an endospore-forming soil bacterium, its spores remain dormant in the soil, until they germinate under suitable conditions. This can be considered as an advantage from a geotechnical perspective. However, adding numerous microorganisms to any environment will change the normal microbial flora of that environment, inevitably leading to negative ecological consequences. It is then evident that non-pathogenic bacteria with a fast rate of growth that only require simple nutrients and produce high amounts of urease and precipitate crystalline $CaCO_3$, will be highly valuable and applicable to the biocementation process. Therefore, the main objective of this study was to investigate the potential ability of a non-sporulating soil-isolated bacterium that does not remain viable in the soil for too long, in carrying out the MICP process. The growth and urease activity of this bacterium were investigated in different culture media, and its ability to precipitate calcium carbonate in liquid medium and in a uniform sandy soil-packed column was also studied.

Date and location of the study

This study has been carried out in Bioprocess Lab, NIGEB, Tehran, Iran, since 2016.

Materials and methods

Microorganisms

A bacterium, isolated from soil (NIGEB lawn; National Institute of Genetic Engineering and Biotechnology, Tehran, Iran) and belonging to the genus, *Staphylococcus*, was used throughout this study. The isolate was grown in Luria-Bertani medium (LB) containing (w/v) 1% peptone, 1% NaCl and 0.5% yeast extract. Semisolid medium was prepared by adding 1.5% agar to the liquid medium.

Sporosarcina pasteurii PTCC 1645 (DSM 33), a well-known bacterium in the biocementation process, was purchased from the Persian Type Culture Collection (PTCC) and activated according to the protocol mentioned in its brochure.

Both bacteria were stored as stock cultures at − 20 and − 70 °C in peptone water supplemented with 15 or 30% glycerol, respectively. For short-term storage, − 20 °C is a suitable temperature. However, long-term storage temperature of − 70 °C is recommended.

Biochemical tests and molecular identification

Soil samples were aseptically taken from a lawn located at NIGEB, Tehran, Iran. They were serially diluted and grown in LB medium at 37 °C, for 24 h. The isolated colonies were screened for their ability to produce high levels of urease following growth in urea agar medium, at 37 °C. The resulting urease-producing isolate was then grown on LB agar by the streak method to obtain single colonies in pure culture. The morphology of the colonies was investigated, and microscopic characteristics of the Gram-stained bacterial cells were also analyzed. For further characterization of the isolate, biochemical tests were carried out according to the standard flowchart protocol for identification of Gram-positive cocci (Holt et al. 1994).

Genomic DNA was extracted from the isolate using a DNA extraction kit (Roche). For the polymerase chain reaction (PCR) process, the following universal primers, 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (GGTTAC CTTGTTA CGACTT), were used to amplify approximately 1500 bp of 16S rDNA. Amplified PCR fragments were then visualized by agarose gel electrophoresis and purified using a DNA purification kit (Roche). After sequencing of

the purified fragments, sequence similarities for the complete sequence of the 16S rDNA gene were determined via BLAST analysis (Altschul et al. 1997) available in the NCBI database.

Culture media and growth conditions

Seven simple culture media were considered for evaluation of bacterial growth and urease production. All media ingredients used in this study are shown in Table 1. The media were sterilized by autoclaving at 121 °C, for 15 min at a pressure of 15 psi. Urea was sterilized by a 0.2 µm disposable membrane filter before being added to the sterile medium. All media were initially adjusted to pH 7.0 for *Staphylococcus* sp. and pH 8.0 for the moderately alkaliphilic bacterium, *S. pasteurii*.

Both bacteria were cultured under sterile aerobic batch conditions in LB medium at 30 °C, with shaking at 180 rpm. When bacterial cells reached the late-exponential phase ($OD_{600} \approx 2.5$), they were inoculated into the abovementioned media at 5% (v/v). The cultures were then incubated for 8 h, during which bacterial growth and enzyme production were measured.

Soil column preparation

In order to test the MICP process on sandy soil under laboratory conditions, a core preparation of cells was needed to be developed in which the sandy soil could be treated and then extracted. Thus, according to ASTM D 4320 specifications, a 30-cm-long PVC tube with an internal diameter of 55 mm was positioned vertically and packed with Anzali's sand (grain size characteristics: $d_{10} = 0.4$ mm (10% of the grains have a diameter of this size or lower); $d_{50} = 1$ mm; $d_{90} = 3.8$ mm) to a dry density of 1.50 g cm^{-3} and porosity of 44% (Table 2). The column was positioned vertically with downward and upward flow directions to ensure that the reaction fluid is available to all parts of the sandy soil particles. Each end of the column was fitted with filter material consisting of two layers of scouring pad (Scotch-Brite) on the outside, and approximately 3 cm of filter gravel on the inside, next to the sand (Fig. 1). Packing of the sand column was conducted under water to the required density, so as to avoid the inclusion of air pockets.

Before any experiments were conducted, water was flushed through the column from the bottom inlet, filling the sample gradually with a pressure low enough to avoid hydrofracture, and to test the pressure transducers and maintain flow regulation of the pump at a constant flow rate of about 450 mL h^{-1}. Subsequent to flushing with water, fluids were injected with a similar procedure, from bottom to

Table 1 The name and components of different culture media

Name	Components
YU medium	Yeast extract (20 g L^{-1}), Urea (10 g L^{-1})
YN medium	Yeast extract (20 g L^{-1}), NH_4Cl (10 g L^{-1})
YAS medium	Yeast extract (20 g L^{-1}), $(NH_4)_2SO_4$ (10 g L^{-1})
Y medium	Yeast extract (30 g L^{-1})
LB medium	Peptone (10 g L^{-1}), yeast extract (5 g L^{-1}), NaCl (10 g L^{-1})
UP medium	Urea (20 g L^{-1}), peptone casein (15 g L^{-1}), NaCl (5 g L^{-1}), soymeal (5 g L^{-1})

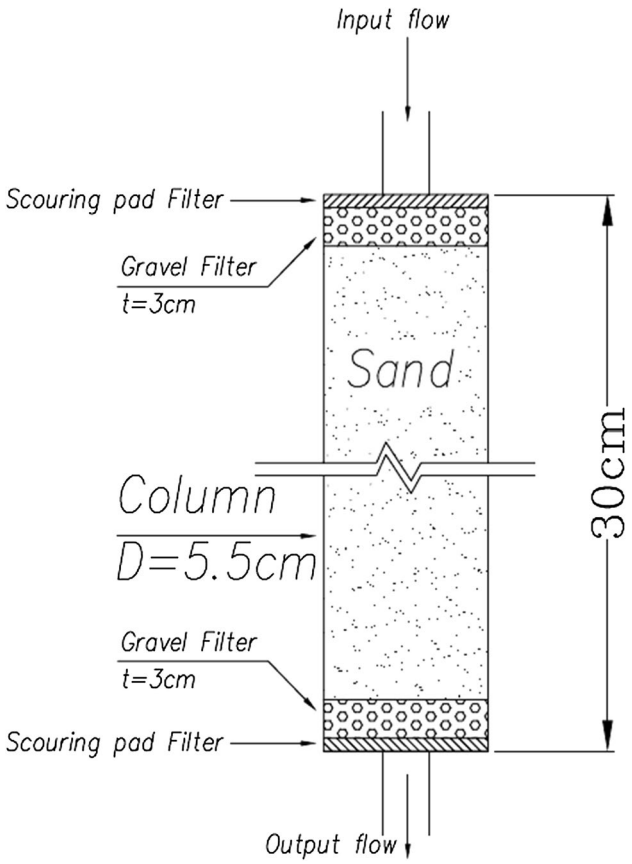

Fig. 1 Schematic details of column packed with Anzali's sandy soil

Table 2 Anzali's sandy soil properties according to laboratory tests

Soil name	USCS classification	d_{10} (mm)	d_{30} (mm)	d_{60} (mm)	d_{90} (mm)	Cc	Cu	Gs	γd_{min} (g/cm^3)	γd_{max} (g/cm^3)
Anzali's sandy soil	SP	0.4	0.5	1.5	3.8	0.50	3.75	2.70	1.31	1.73

top in order to displace the water. In the first step, 180 mL (equal to 1 V_v (volume of voids in the column)) of bacterial culture at an OD_{600} of 2.8 with a urease activity equivalent to 3300 mS was mixed with 360 ml (equal to 2 V_v) of an equimolar solution of urea and calcium chloride (1 M) and then injected into the column, which was subsequently left for 72 h at room temperature. Then, in the next step, all the aforementioned stages were repeated but the direction of flow was changed from top to bottom and vice versa for six injections alternately. After the injection phases were completed, the column was cut and the biotreated sand was divided into appropriate pieces for further analysis. One of the columns was injected with *S. pasteurii* in order to compare the resulting soil strength to that of sandy soil treated with *Staphylococcus* sp. IR-103.

Growth measurement

Optical density (OD) was used as an indication of bacterial growth in the culture medium. It was measured using a spectrophotometer at a wavelength of 600 nm (OD_{600}). One milliliter of culture broth was harvested every 1 or 2 h and immediately transferred to a glass cuvette, and its OD was measured as mentioned above. According to the Beer–Lambert law, the concentrated samples were diluted as needed. The dilution coefficient was multiplied by the OD value.

Urease assay

Urea agar culture medium containing urea 20 g L^{-1}, agar 15 g L^{-1}, peptone 1 g L^{-1} and phenol red 0.012 g L^{-1} as pH indicator was used to assay the urease-producing ability of both bacteria qualitatively.

In the absence of calcium ions, urease activity was quantitatively determined by the conductivity method. The urease reaction involves the hydrolysis of the non-ionic substrate urea to ionic products, thus generating a proportionate increase in conductivity under standard conditions. One milliliter of bacterial suspension was added to 9 mL of 1.11 M urea (reaction concentration; 1 M urea), and the relative conductivity change in mS min^{-1} was recorded during 5 min. (Whiffin 2004).

Precipitation in liquid medium

To evaluate the precipitation capability of both bacteria in liquid medium, 5 mL of bacterial suspension at the logarithmic phase of growth ($OD \approx 3.0$) was mixed with 5 mL of an equimolar solution of urea (1 M) and calcium chloride (1 M) and incubated at 37 °C for 24 h. Thereafter, the mixture was placed at room temperature to form precipitates. The supernatant was subsequently discarded, and the resulting precipitate was washed with distilled water, dried in an oven at 50 °C for 1 h and then weighed.

Analytical measurements

Fourier transform infrared (FTIR) analysis

The precipitated samples were dried, grounded and hand-pressed in potassium bromide powder in order to form homogenous clear disks, which were then subjected to FTIR spectroscopic analyses (SHIMADZU, FTIR-8900, Japan). Compliance with in-house specifications was determined by computerized comparison of the spectrum with spectra previously obtained from reference materials.

Atomic absorption spectrophotometry (AAS)

A series of standard solutions were used to construct a calibration curve of atomic absorption. They were prepared in purified water based on initial sample concentration and sensitivity of the instrument. For preparation of samples, 1.0 g of the precipitate consisting of $CaCO_3$ was diluted in nitric acid (1 M) and shaken well to dissolve, and after filtration, it was analyzed by AAS (Varian, AA240S, USA).

X-ray diffraction (XRD) analysis

X-ray diffraction (XRD) measurements were taken by a SEIFERT diffractometer (XRD 3003 PTS, Germany) using monochromatized Cu K radiation, in order to follow the $CaCO_3$ precipitation pattern.

Scanning electron microscopy (SEM)

To provide a clear image of the connection between sandy soil particles by calcium carbonate crystal precipitation during the MICP process, SEM was used. Accordingly, samples from cemented sandy soil were cut into appropriate sizes to mount on the SEM apparatus (Philips, XL30). As the specimens are brittle and non-conductive, they were coated with an ultra-thin coating of gold, which acts as an electrically conductive material.

Unconfined compression strength (UCS) test

The effect of the biocementation process on sand column strength (initial sand cohesion is assumed to be zero) was determined by the UCS test on treated sandy soil specimens. This test was performed on an unconfined tester apparatus (Pars Geo Azma, Iran), in accordance with the ASTM D2166 specifications. The load was applied to produce an axial strain at a rate of 2% min^{-1}.

Results and discussion

Gram staining and biochemical tests are classic methods for identification of bacteria. The *Staphylococcus* isolate showed up as white-yellow colonies, about 1–2 mm in diameter, which were easily observed on semisolid medium after overnight incubation on LB agar. Following Gram staining, microscopic observation of the cells showed spherical shapes with purple-colored irregular clusters of cocci. This isolate is a Gram-positive coccus which produces catalase but is not able to produce coagulase and DNase. It ferments mannitol and is sensitive to Novobiocin. According to the determinative classification of bacteria, it is highly similar to *Staphylococcus epidermidis* (Holt et al. 1994). Molecular identification of the isolate was carried out using 16S rDNA sequencing, which showed 98% homology to *Staphylococcus* sp. confirming the classic identification results. The sequence of the bacterial isolate, designated *Staphylococcus* sp. IR-103, was submitted to EMBL, under accession number LT853888. A phylogenetic tree was constructed by means of the MEGA4 software using the neighbor joining (NJ) algorithm.

When *Staphylococcus* sp. IR-103 was grown on urea agar, the color of the medium changed from yellow to pink. The color change to dark pink occurred after 8 h of cultivation which was spread throughout the petri dish. However, it took 18 h for *S. pasteurii* to finally change the color of the medium to light pink. It can be concluded that *Staphylococcus* sp. IR-103 is a high urease producer.

Bacterial growth and urease production in different culture media

It is obvious that the composition of the culture medium has a great impact on bacterial growth and metabolism. Experimental results indicating the maximum growth, conductivity and urease activity of both bacteria in different culture media are summarized in Table 3. The results showed that the minimum amount of bacterial growth and urease activity was obtained in the UP medium. Despite the presence of 20 g L^{-1} of organic nitrogen sources, such as peptone from casein and soymeal, which was equivalent to the amount of yeast extract in YU, YN and YAS media, they had no positive effects on the growth and urease production of both bacteria. It has previously been reported that the culture medium containing yeast extract is suitable for *S. pasteurii* growth and urease production (Harkes et al. 2010, Whiffin 2004). The data obtained in this study indicated that despite the presence of yeast extract as an essential component in the culture medium, higher amounts of yeast extract or substitution of inorganic nitrogen sources, such as $(NH_4)_2SO_4$ and NH_4Cl, had no remarkable impact on the urease activity of *Staphylococcus* sp. IR-103. Both bacteria grew on YU and YN media and reached maximum growth after 6 h. It is obvious that the growth media can be very strain specific and should be studied further with respect to each strain.

As shown in Fig. 2a, b, urease is a growth-associated enzyme because bacterial growth and enzyme production occurred simultaneously. Urea is not only a nitrogen source, but can also be employed for different applications by some microorganisms. For example, *Helicobacter pylori* produces urease and catalyzes urea to NH_4^+ to provide a microenvironment of neutral pH near its cell surface, so as to protect the bacterial cell from the very low pH environment of the stomach (Marshall et al. 1990). *S. pasteurii* consumes urea via the urease catalyst and produces NH_4^+ ions in order to generate the required ATP in a high pH environment (Whiffin 2004). Maximum urease production was observed in the YU culture medium, which could be due to urea acting as an inducer of urease production in the medium (urease activity of 10.1 and 5.7 IU for coccus and bacillus cells, respectively).

Although the nickel ion is located at the urease active site and has been suggested for use in culture medium to improve enzyme activity during the MICP process (Harkes et al. 2010; Whiffin 2004), but in our study, $NiCl_2$ had no significant effect on urease activity (data not shown).

Table 3 The maximum growth (OD; close to 7 h), conductivity and urease activity of *Staphylococcus* sp. IR-103 and *S. pasteurii* in different culture media

Culture medium	*Staphylococcus* sp. IR-103			*S. pasteurii*		
	OD	Conductivity (µs)	Urease activity (IU)	OD	Conductivity (µs)	Urease activity (IU)
YU medium	6.1	800	10.10	4.0	500	5.70
YN medium	2.8	340	3.33	2.4	330	3.19
YAS medium	2.2	300	2.74	0.5	10	–[a]
Y medium	6.5	410	4.37	4.6	380	3.93
LB medium	4.8	450	4.96	4.2	400	4.22
UP medium	1.7	250	2.22	1.5	150	2.24

[a]No activity was detected

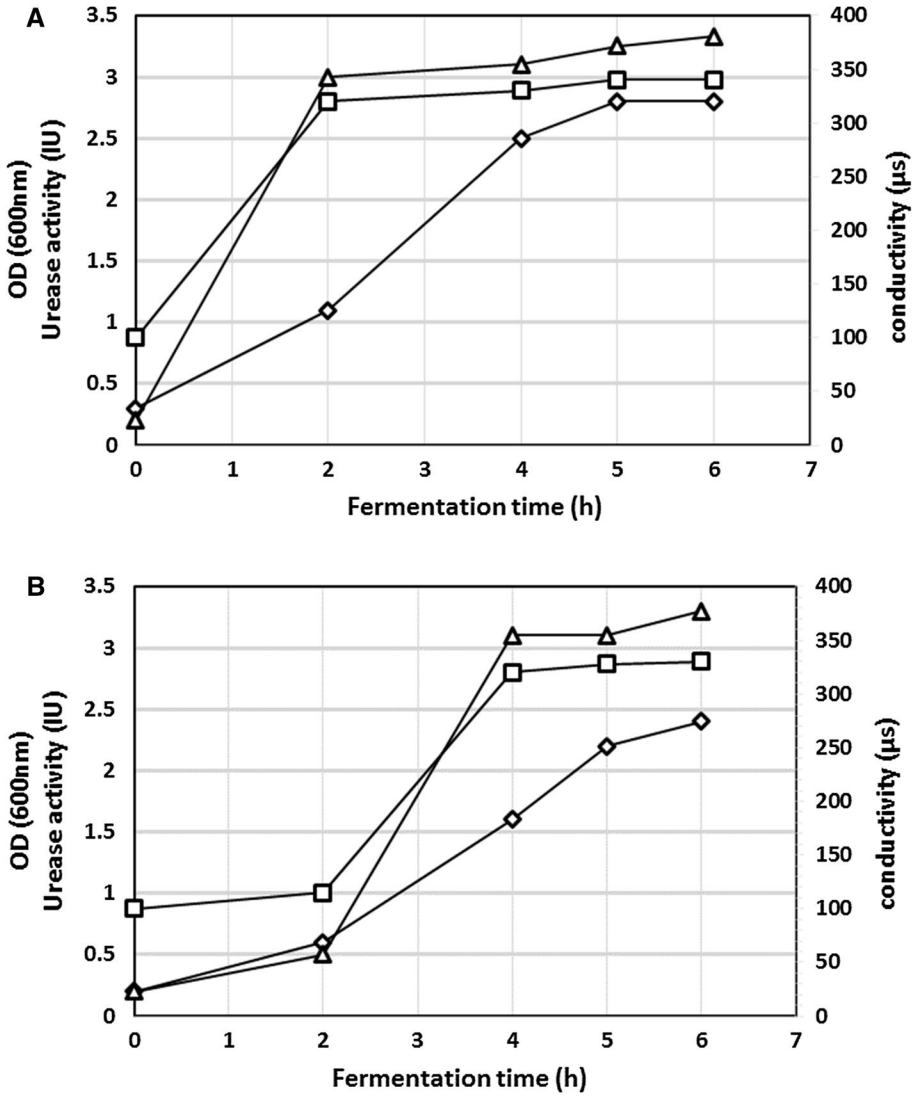

Fig. 2 **a** Optical density (OD_{600}) (diamond), medium conductivity (square) and urease activity (triangle) of *Staphylococcus* sp. IR-103 and **b** *S. pasteurii* grown in YN medium

Ca-carbonate precipitation

The various amounts of $CaCO_3$ that were precipitated by the biocementation reaction when *Staphylococcus* sp. IR-103 was grown in the YU, YN, YAS, Y, LB and UP culture media, were found to be 47.0 ± 1.6, 47.6 ± 0.9, 30.8 ± 1.3, 44.8 ± 1.1, 19.6 ± 0.5 and 33.2 ± 1.6 mg mL^{-1}, respectively. The maximum amount of calcium carbonate precipitated by S. *pasteurii* strain when grown in the YN culture medium was recorded as 43.2 ± 1.4 mg mL^{-1}.

In Fig. 2, the performance of the *Staphylococcus* sp. IR-103 strain relative to that of *S. pasteurii* grown in YN medium was studied. It can be seen that *Staphylococcus* sp. IR-103 grows faster than *S. pasteurii,* with a slightly higher growth yield. In addition, urease production reached a maximum level, which was higher than that of *S. pasteurii*.

Although higher urease activity and growth were obtained in the YU medium, $CaCO_3$ precipitation in the YN medium occurred over a 5-day time period, which is notably much earlier than that for the YU medium (15 days). This may be attributed to the instability of the urease enzyme. Urease produced during bacterial growth in the YU culture medium catalyzed the available urea to NH_4^+, thereby increasing the surrounding pH. After adding cementation solution, $CaCO_3$ aggregates were observed immediately as a cloudy mass, but the crystals attached to the wall and bottom of the tube formed slowly during a period of 15 days. However, it seems that higher amounts of urease were produced rapidly in the YN medium following the addition of cementation solution containing urea that affected the MICP process significantly during 5 days of the biocementation process. Figure 3 shows the $CaCO_3$ crystals after 15 days of adding cementation solution to the bacterial suspension. Consequently, YN was chosen as a suitable medium for maximal *Staphylococcus* sp. IR-103 growth, urease production and $CaCO_3$ precipitation.

Fig. 3 Calcium carbonate crystals precipitated after mixing *Staphylococcus* sp. IR-103 suspension grown in YN medium with cementation solution (1:1 v/v) for 15 days at room temperature

Microorganisms that are considered as suitable for biocementation, must in addition to being non-pathogenic, also have the capacity to produce high levels of urease. The urease enzyme must be highly stable and should be produced by the microorganism in a consistent and reliable manner. Moreover, the microorganism should not require preparative processes prior to its use in the biocementation process (Whiffin 2004). In this investigation, *Staphylococcus* sp. IR-103 demonstrated suitable growth, proliferation and urease enzyme production in all culture media, including those that contained ammonium. It was also capable of producing $CaCO_3$ under the 6 aforementioned conditions, thus demonstrating its stability and reliability with regard to the production of calcium carbonate precipitates. Furthermore, the mode of proliferation and enzyme production by *Staphylococcus* sp. IR-103 is not so different from that by *S. pasteurii*. In fact, when the *Staphylococcus* strain is used, precipitation time is reduced substantially. This can cut operational costs in the long run.

Characterization of the bioprecipitated $CaCO_3$

The FTIR spectrum of the precipitated disk is presented in Fig. 4. It shows a smooth peak at 3404 cm^{-1}, and because of the humidity of the disk or precipitate, it has no serious risk for analysis. The peak in the 1483–1404 cm^{-1} region is referred to as the carbonate functional group. Due to the resonance effect and increase in the bonding energy, this peak shifted from region 1500–1600 cm^{-1} to region 1483–1404 cm^{-1} (Ganendra et al. 2014; Cheng and Shahin 2016; Zhu and Dittrich 2016).

The amount of calcium in the precipitate was measured by the atomic absorption spectroscopy method (AAS) in order to assess its levels in the sandy soil following the

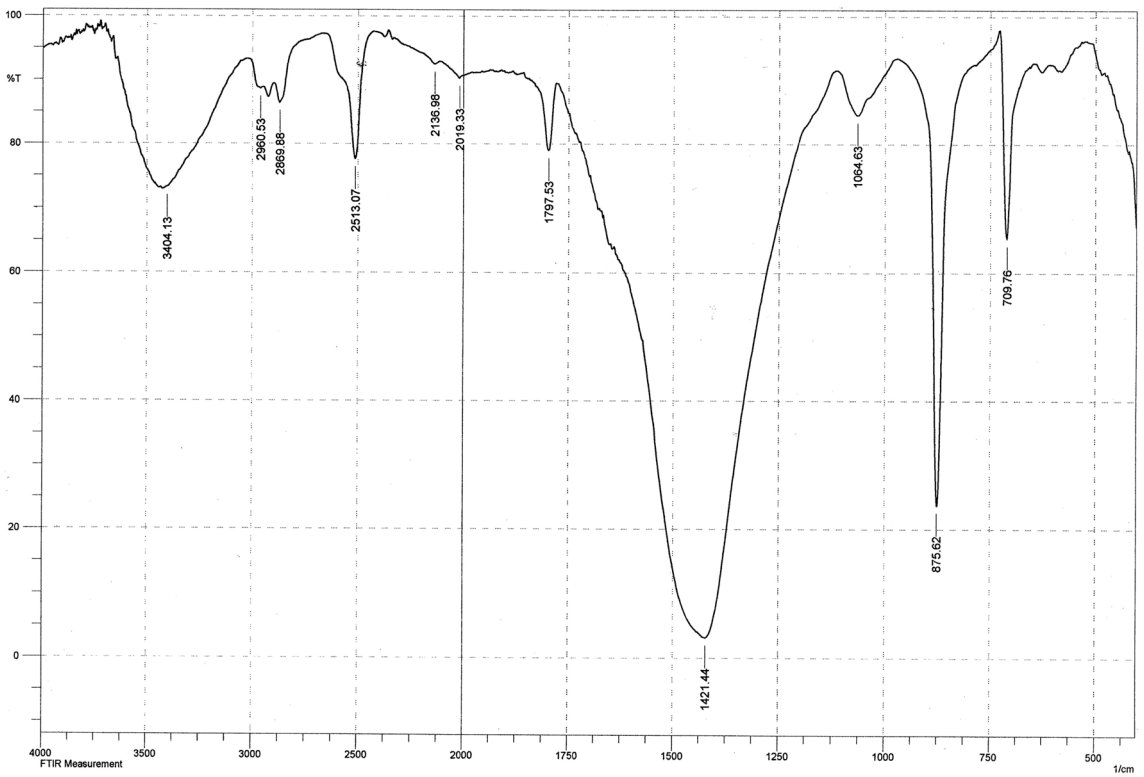

Fig. 4 FTIR spectrum of calcium carbonate precipitated by *Staphylococcus* sp. IR-103 grown on YN medium

biocementation process. Analysis was based on three standard solutions (Fig. 5). Accordingly, the concentration of $CaCO_3$ in the sample was determined as 71.76% (sample taken from a batch experiment).

X-ray diffraction was carried out to confirm the presence of calcium carbonate precipitated by *Staphylococcus* sp. IR-103 (Fig. 6). By comparison with the standard pattern for the synthesized porous $CaCO_3$, it was revealed that the products of the precipitation test were composed of the mineral, vaterite.

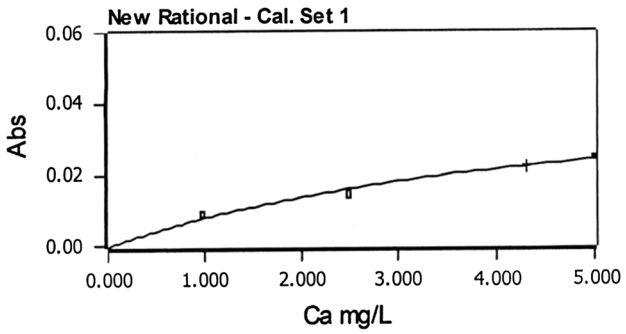

Fig. 5 AAS graph of calcium carbonate precipitated during the biocementation reaction by *Staphylococcus* sp. IR-103

Calcite, vaterite and aragonite are three polymorphs of $CaCO_3$ in aquatic environments. All of them are insoluble in water and are able to cement soil particles together, thus strengthening and hardening the soil, thereby increasing its liquefaction resistance (Burbank et al. 2011). As it has been previously reported, vaterite is the predominant crystal at high urease activity (90–180 mM urea h^{-1}) (Kralj et al. 1990).

The scanning electron micrographs of the samples obtained from the treated column showed that calcium carbonate crystals precipitated on the surfaces and pores between the sandy soil particles (Fig. 7). The bridges made up of the $CaCO_3$ crystals were formed between the sandy soil particles connecting them to each other.

From the perspective of soil improvement, placing $CaCO_3$ precipitates in close contact with soil particles enables them to bind together, thereby leading to better cementation and increased sand resistance. Scanning electron micrographs of the improved sand particles show that a notable amount of the precipitated solids is located in the immediate vicinity where sand particles bind to each other. It has been reported that needle-shaped crystals were formed both on the surface and between sand particles in MICP treated specimens (DeJong et al. 2006). As concentration of microbes is a critical factor in MICP process, more bacterial injections during

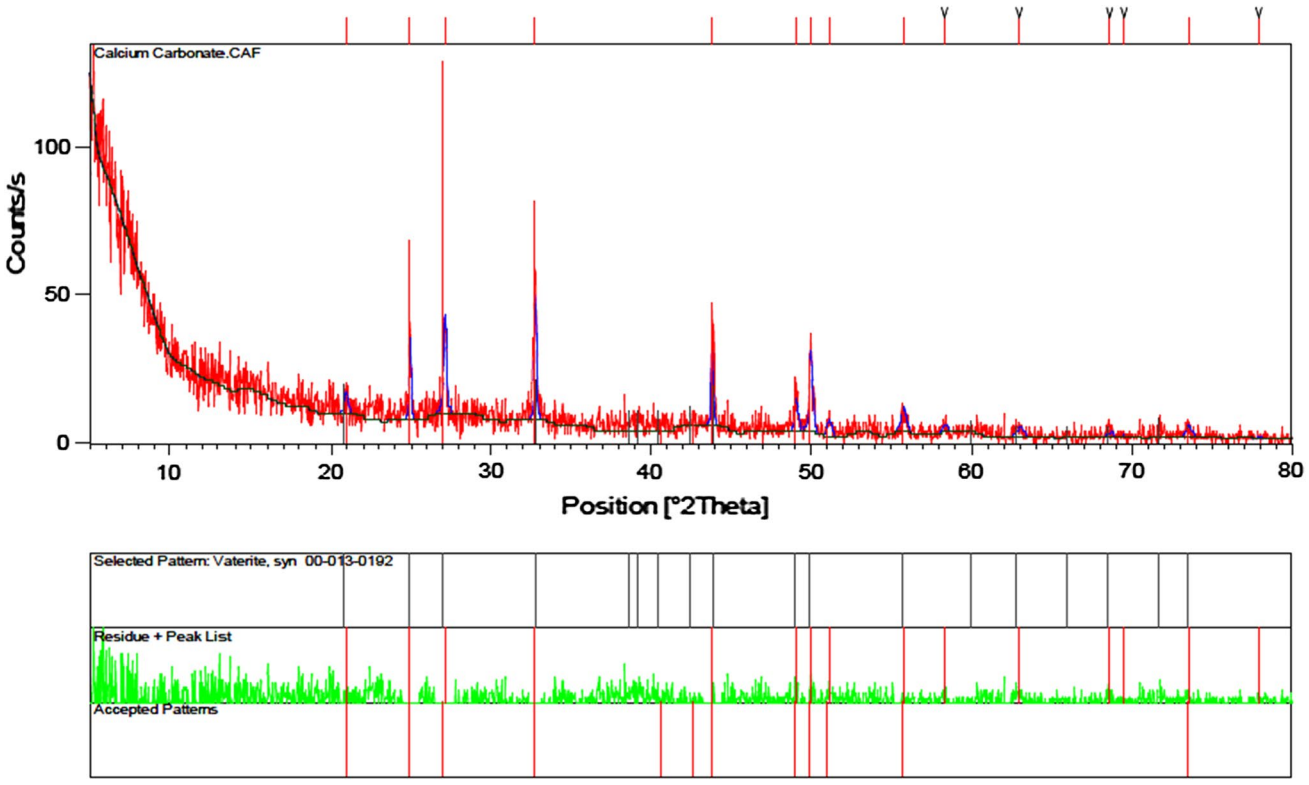

Fig. 6 The XRD pattern for calcium carbonate precipitated by the biocementation reaction demonstrates that the most dominant mineral composition is that of vaterite

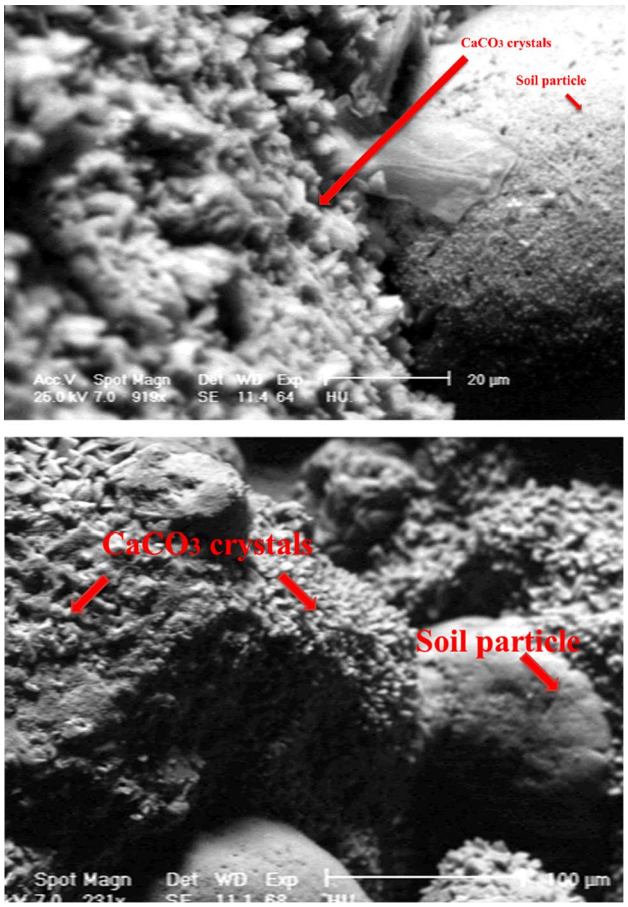

Fig. 7 SEM micrograph of biocemented sandy soil. The biotreated column samples were cut into small sections, coated with gold and then scanned. $CaCO_3$ crystals formed between the sandy soil particles and on their surfaces are clearly observed

column experiments, higher amounts of precipitated $CaCO_3$ crystals appeared on the surface of sand grains and at particle contacts.

Effect of biocementation process on sandy soil strength in the columns

The characteristics of the two columns filled with Anzali's sandy soil and bacterial suspension are shown in Table 4.

After injection was completed, the UCS test was performed on the samples obtained from the columns. The dimensions of the samples cut from the columns treated with *Staphylococcus* sp. IR-103 and *S. pasteurii* were found to be ($D = 5.47$ cm and $H = 8.84$ cm) and ($D = 5.37$ cm and $H = 8.2$ cm), respectively. Considering that the unconfined compression strength for the untreated sand is assumed to be zero and columns 1 and 2 achieved strengths of ca. 2.2 kg cm^{-2}, it can be concluded that a significant increase in UCS was observed when sandy soil was treated with *Staphylococcus* sp. IR-103, which was also similar to that observed for *S. pasteurii*.

Finally, small pieces of each column were then cut, pestled and then poured into LB medium for subsequent cultivation. The results indicated the absence of viable staphylococci in the column after 5 days following the last injection. In contrast, *S. pasteurii* remained viable in the column because of its ability to sporulate.

Biocementation is a new technology in geotechnical engineering based on the application of microbial activity to improve the mechanical properties of soils. Contrary to the environmental limitations of chemical and cement stabilization during soil improvement, MICP is an environmentally friendly method. Calcite precipitation may be achieved by many different processes, but enzymatic hydrolysis of urea is the most energy efficient of these procedures (Ivanov and Chu 2008; Burbank et al. 2011). The process of MICP depends on several factors such as calcium ion concentration, pH, and availability of nucleation sites, but it seems that the main factor influencing the process efficiency is the type of microorganism and its ability to produce urease. Therefore, finding high urease-producing microbes, which are capable of developing strength in the soil, but with no adverse effects on the environment when applied to the soil, is of great importance to the biocementation technology.

Conclusion

In order to improve soil strength, this study aimed to investigate and compare the ability of an indigenous urease-positive bacterium belonging to the *Staphylococcus* genus to that of the most commonly used bacterium, *S. pasteurii,*

Table 4 The list of column characteristics and species of bacteria injected

Column number	D (cm)	H (cm)	e*	n**	V_v^{***} (cm^3)	γd^{****} (g cm^{-3})	Bacteria	Medium	OD_{600}	Conductivity
1	5.5	22	0.66	0.33	172	1.63	*S. pasteurii*	YN	2.4	330
2	5.5	22	0.68	0.34	178	1.61	*Staphylococcus* sp. IR-103	YN	2.8	340

*Void ratio; **porosity; ***volume of voids; ****dry unit weight

in carrying out the MICP process. First, growth and urease activity of both bacteria were studied in various culture media. Staphylococcal growth was higher than that of *S. pasteurii* in all culture media. Both bacteria showed maximum urease activity in the YU medium containing urea as an enzyme inducer. But the highest amount of precipitation was found in the YN medium, which was then selected for further column studies. Finally, in order to study the performance of *Staphylococcus* sp. IR-103 in the presence of sandy soil, and its ability to cement soil particles, a sandy soil column experiment was set up and a bacterial suspension and reactant solution were mixed and injected into the sandy soil columns six times. The UCS test showed that when *Staphylococcus* sp. IR-103 was used in the MICP process, unconfined compression strength of the biocemented sand increased by the same amount as that when *S. pasteurii* was used. Furthermore, *Staphylococcal* cells did not remain alive in the sandy soil following treatment and did not change the soil microbial flora. To sum up, because of the potential of this bacterium in calcium carbonate precipitation, it can be recommended as a suitable candidate for improving the geotechnical properties of soil.

Acknowledgements This work was financially supported by a research Grant from the National Institute of Genetic Engineering and Biotechnology (NIGEB Project #103), which is greatly appreciated. We also thank Dr. Shariati for her great help.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Al-Thawadi SM (2013) Consolidation of sand particles by aggregates of calcite nanoparticles synthesized by ureolytic bacteria under non-sterile conditions. J Chem Sci Technol 2:141–146

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Burbank MB, Weaver TJ, Green TL, Williams BC, Crawford RL (2011) Precipitation of calcite by indigenous microorganisms to strengthen liquefiable soils. Geomicrobiol J 28:301–312

Castanier S, Le Métayer-Levrel G, Perthuisot JP (1999) Ca-carbonates precipitation and limestone genesis—the microbiogeologist point of view. Sediment Geol 126:9–23

Cheng L, Shahin MA (2016) Urease active bio-slurry: a novel soil improvement approach based on microbially induced carbonate precipitation. Can Geotechn Eng 53:1376–1385

DeJong JT, Fritzges MB, Nüsslein K (2006) Microbially induced cementation to control sand response to undrained shear. J Geotechn Geoenviron Eng 132:1381–1392

Ganendra G, De Muynck W, Ho A, Arvaniti EC, Hosseinkhani B, Ramos JA, Rahier H, Boon N (2014) Formation of oxidation-driven calcium carbonate precipitation by *Methylocystis parvus* OBBP. Appl Environ Microb 80:4659–4667

Harkes MP, van Paassen LA, Booster JL, Whiffin VS, van Loosdrecht MCM (2010) Fixation and distribution of bacterial activity in sand to induce carbonate precipitation for ground reinforcement. Ecol Eng 36:112–117

Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994) Bergey's manual of determinative bacteriology, 9th edn. Lippincott Williams and Wilkins Publisher, Baltimore

Ivanov V, Chu J (2008) Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ. Rev Environ Sci Biotechnol 7:139–153

Kralj D, Brečević L, Nielsen AE (1990) Vaterite growth and dissolution in aqueous solution I. Kinetics of crystal growth. J Cryst Growth 104:793–799

Kucharski ES, Cord-Ruwisch R, Whiffin V, Al-Thawadi SMJ (2006) Microbial biocementation. World Intellectual Property Organization, WO2006/066326 A1

Macegoniuk K (2013) Inhibitors of bacterial and plants urease. Folia Biol Oecol 9:9–16

Marshall BJ, Barett LJ, Prakash C, McCallun RW, Guerrant RL (1990) Urease protects *Helicobacter (Campylobacter) pylori* from the bacterial effect of acid. Gastroenterology 99:697–702

Mobley HL, Island MD, Hausinger RP (1995) Molecular biology of microbial ureases. Microbiol Rev 59:451–480

Porter H, Kaur Dhami N, Mukherjee A (2017) Synergistic chemical and microbial cementation for stabilization of aggregates. Cement Concr Compos 83:160–170

Ramachandran SK, Ramakrishnan V, Bang SS (2001) Remediation of concrete using micro-organisms. ACI Mater J Am Concr Inst 98:3–9

Ramakrishnan V, Panchalan RK, Bang SS (2001) Improvement of concrete durability by bacterial mineral precipitation. In: 11th international conference on fracture, Turin, Italy

Saxena SK, Lastrico RM (1978) Static properties of lightly cemented sand. J Geotechn Geoenviron Eng 104 (ASCE 14259)

Stabnikov V, Ivanov V, Chu J (2015) Construction biotechnology: a new area of biotechnological research and applications. World J Microbiol Biotechnol 31:1303–1314

Whiffin VS (2004) Microbial $CaCO_3$ precipitation for the production of biocement. Dissertation, Murdoch University, Australia

Whiffin VS, van Paassen LA, Harkes MP (2007) Microbial carbonate precipitation as a soil improvement technique. Geomicrobiol J 24:417–423

Zhu T, Dittrich M (2016) Carbonate precipitation through microbial activities in natural environment, and their potential in biotechnology: a review. Front Bioeng Biotechnol 4:4–10