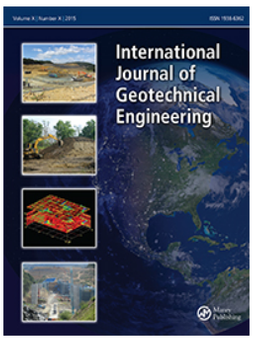

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Investigation of various gram-positive bacteria for MICP in Narmada Sand, India

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ABSTRACT

In lieu of the conventional carbon-intensive and expensive cement-based ground improvement methods, microbially induced calcite precipitation (MICP) method has gained prominence recently as a sustainable approach to improve the engineering properties of soil. MICP involves calcification by microbial processes, inducing cementation effect in granular soil particles. Several studies have demonstrated the use of *Sporosarcina pasteurii* bacteria to improve density, stiffness, shear strength, and liquefaction resistance of sand. However, the effects of different bacteria on calcite formation have not been systematically examined in the Indian context. This study investigates MICP in poorly graded, liquefiable Narmada sand, using gram-positive bacteria (i.e. *Sporosarcina pasteurii*, *Bacillus subtilis*, and *Bacillussphaericus)*. Several controlled microbial and cementation media concentrations were tested in sand-filled tubes. Calcite precipitation was analyzed using SEM, FTIR, XRD, and calcimeter. *Bacillus subtilis* and *Bacillus sphaericus* resulted in calcite precipitation comparable to that of *Sporosarcina pasteurii* and can be utilized for sand stabilization.

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KEYWORDS
Biomineralsation; ground improvement; liquefaction resistance; MICP; micro characterization

Introduction

Soils are heterogeneous, disperse, multiphase, and porous system (Fredlund and Rahardjo 2011). Various challenges which are faced during and after the construction of a structure are associated with soil due to its poor bearing capacity, settlement, drainage, erosion, liquefaction, etc. (Terzis and Laloui 2019). The conventional ground improvement methods involve enhancing the physical, chemical, mechanical and mineral composition of the soil. These techniques involve addition of cement, lime, chemical, and supplementary cementitious material such as fly ash, rice husk ash, and blast furnace slag for soil stabilization. Cement, lime, and chemicals can lead to air pollution by emitting carbon dioxide during the manufacturing phase or usage and permanent contamination of soil and groundwater (Benhelal et al. 2013). The use of synthetic materials in chemical grouting release toxins in the natural environment and endanger human health (DeJong et al. 2010). The other conventional methods comprise of mechanical compaction, which involves vibroflotation, dynamic compaction, blasting, construction of compaction piles, etc. These compaction techniques require heavy equipment, energy resources, and create soil disturbances in surrounding areas (Wang et al. 2017). The conventional compaction techniques suffer from some limitations such as they are adequate and economical up to a depth of 10 m and require more energy efforts for larger depths. Dynamic compaction methods are suitable for sandy soils only (Indraratna, Chu, and Rujikiatkamjorn 2015).

Various advanced methods have been developed in the last two decades to overcome the challenges and limitations of conventional ground improvement techniques. Multidisciplinary research and rapid development in materials technology led to the use of nanomaterials such as colloidal silica, bentonite, and laponite, short synthetic fibres, recycled material, micro-fine cement, and biological materials to enhance the engineering properties of soil (Bao et al. 2019). A comparative study of conventional methods and advanced methods gives a clear vision that there is a need for less invasive, sustainable, environment-friendly, and energy-saving technology.

Microbially induced calcite precipitation (MICP) is an alternative approach to ground improvement which strengthens the soil and reduces seepage by bio plugging and cementation induced by calcite precipitation (Ferris et al. 1996). The bacteria present in soil are either ureolitic microbes (intracellular) or produce urease enzyme from disrupted plants and microorganisms (extracellular) (Lloyd and Sheaffe 1973). The most commonly used urease-producing bacteria *S. pasteurii* for soil improvement uses urea as a source of energy and create ammonium ions which increases the pH of adjacent environment (Al Qabany, Soga, and Santamarina 2012). The increase in pH is attributed to urea hydrolysis. The added calcium chloride to soil generates calcium ions in high pH environment, and later these calcium ions are attached with carbonate ions during urea hydrolysis resulting in precipitation of calcium carbonate (Stocks-Fischer, Galinat, and Bang 1999). MICP applications are really important for various engineering areas, i.e. geological and petroleum sites; to fill the cracks and fissures in rocks and surrounding areas of oil reservoirs (Finnerty and Singer 1983), biogeochemical landfill covers in MSW landfill to attain zero emissions of CH_4, CO_2, and H_2S (Reddy, Gopakumar, and Chetri 2019), transformation in water resources and reclamation of ancient buildings, and ground improvement (DeJong, Fritzges, and Nüsslein 2006).

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In recent studies, researchers emphasized the biological phase of soil, which includes living microorganisms (Mitchell and Santamarina 2005). There are around 2×10^9 bacteria and archaea present in a gram of soil sampled from the ground surface (up to 1 m depth). These prokaryotes (bacteria and archaea) count decreases to 1×10^8 at 1–8 m depth below ground surface. In subsurface sediment layer from 10 m to 300 m and 300 m to 500 m, the prokaryotes are present in abundance around, 2.3×10^7 cells/cm^3 and 6×10^6 cells/cm^3, respectively (Mountassir et al. 2018). These bacteria allow MICP through bacterial metabolic activities (Dawoud, Chen, and Soga 2014; DeJong, Fritzges, and Nüsslein 2006; DeJong et al. 2010; Stocks-Fischer, Galinat, and Bang 1999; Van Paassen 2009; Whiffin 2004). The consequence of such metabolic action leads to an increase in pH because of production of ammonia via urea hydrolysis, also known as urease activity. A wide range of plants and microbes produce urease enzyme in large quantity (Bachmeier et al. 2002; DeJong, Fritzges, and Nüsslein 2006). Bacillus pasteurii, also known as Sporosarcina pasteurii (*S. pasteurii*), is widely known urease producing bacteria which is economically sound bio construction material (Ferris et al. 1996). The bacteria produce urease enzyme which hydrolyzes the urea into ammonium ion and carbonate ion and induce calcite precipitation in the presence of calcium ions (Chou et al. 2011; Dejong et al. 2013; Zhao et al. 2014).

MICP includes biogeochemical activity through porous soil media, which can be implemented either by biostimulation or bioaugmentation (DeJong et al. 2010). Biostimulation encourages the growth of indigenous urease producing bacteria by providing sufficient nutrient and cementation media. Bioaugmentation includes culture solutions of urease producing bacteria to introduce in soil with enrichment and cementation media (Tsesarsky, Gat, and Ronen 2016). Some lab and field-scale investigations have been conducted by researchers such as sand column method (Al Qabany, Soga, and Santamarina 2012; Choi et al. 2017), fully immersed flexible mould method (Li et al. 2015), large tank specimens (Bing 2014; Gomez et al. 2016), and field testing (Van Paassen et al., 2010a; Gomez et al. 2014). Van Paassen et al. (2010b) carried out the up-scaling of bio-grout process from laboratory scale to 100 m^3 field-scale experiment which resulted in increase of unconfined compressive strength and stiffness. Van Paassen (2011) conducted field tests to cementing gravel for borehole stability in collaboration with Deltares and Volker Wessels Companies. However, the large scale application didn't result in uniform precipitation and the cost was relatively high because of the need for ammonium chloride removal and ex-situ cultivation of bacteria. Whiffin (2004) analysed the up-scaling and economic issues of the industrial application of biocementation process in collaboration with Dutch Geotechnical Company. A cost-effective cementation method was developed considering the urease activity of bacteria and medium of growth i.e. diary waste. Gomez et al. (2016) investigated the large scale implementation of MICP via biostimulation and bioaugmentation in 1.7 m diameter identical tanks for comparison. The results indicated that the biostimulation may provide comparable calcite precipitation at the metre scale. Bing (2014) conducted the up-scaled model test in 1 m^3 tank under controlled environmental conditions and achieved 93% of cementation efficiency.

The advancement of the research with time has emerged in the diversification of bacterial examination for MICP competence. Various bacterial strains, especially *S. pasteurii* (Feng and Montoya 2017; Stocks-Fischer, Galinat, and Bang 1999; Terzis and Laloui 2019; Whiffin 2004), *B. subtilis* (Gat et al. 2014; Tsesarsky, Gat, and Ronen 2016), Proteus vulgaris (Whiffin 2004), Bacillus licheniformis (B. licheniformis) (Helmi et al. 2016; Saricicek et al. 2019), *B. sphaericus* (De Muynck et al. 2010; Moravej et al. 2018) and Bacillus megaterium (Jiang et al. 2016) have been investigated for MICP for comparison or control and capability.

The present article includes a comparative study of three bacteria on Narmada sand sample, with an aim to assess the possibility of bioaugmentation and calcite precipitation. The study involves the use of three bacteria: (i) *S. pasteurii* (ATCC 11859), a master bacterium to produce urease enzyme and known for inducing calcite precipitation, (ii) *B. subtilis* (ATCC NCIB 8533), a gram-positive, catalase-positive, rod-shaped, spore-forming bacteria (Gat, Ronen, and Tsesarsky 2016), and (iii) *B. spahericus* (ATCC 14577), a gram-positive, rod-shaped, non-pathogenic, and mesophilic bacterium. *B. sphaericus* is commonly found in soil in abundance (Berry 2012). *B. subtilis* and *B. sphaericus* are not well-recognized in improving the strength of sand. This study aims to investigate the ability of *B. subtilis* and *B. sphaericus* for MICP, and to evaluate its potential to be an alternative to *S. pasteurii*. In this regard, the amount of calcite precipitation was analysed after 14 and 28 days using calcimeter and the presence of calcite was determined through microstructural investigations using SEM, XRD, and FTIR.

Material and methods

Narmada sand

The sand used for the study is Narmada river sand which can be classified as poorly graded sand according to Indian standard classification system (IS:1498–1970, IS:1498-1970 2002). Figure 1 shows the particle size distribution of the Narmada sand. The mean particle size of Narmada sand was 0.32. The physical properties of Narmada sand are summarized in Table 1.

Microbes and growth medium

The bacterial strains i.e. *S. pasteurii, B. subtilis*, and *B. sphaericus* were stored at −20°C before use. The bacterial culture was prepared using nutrient broth medium of Himedia (Peptone 10 g/l, HM peptone B 10 g/l, Sodium chloride 5 g/l and pH after sterilization was 7.3 ± 0.1). The nutrient broth was autoclaved at 121°C for 20 minutes at 15 psi pressure followed by inoculation of each strain in laminar airflow cabinet under sterile conditions as the chances of contamination are more for bacteria. Later, the inoculated media was kept in an orbital shaking incubator for 24 hours at 30°C, rotating at a speed of 200 rpm under aerobic conditions to initiate the bacterial growth. The optical density (OD) of the bacteria was measured using

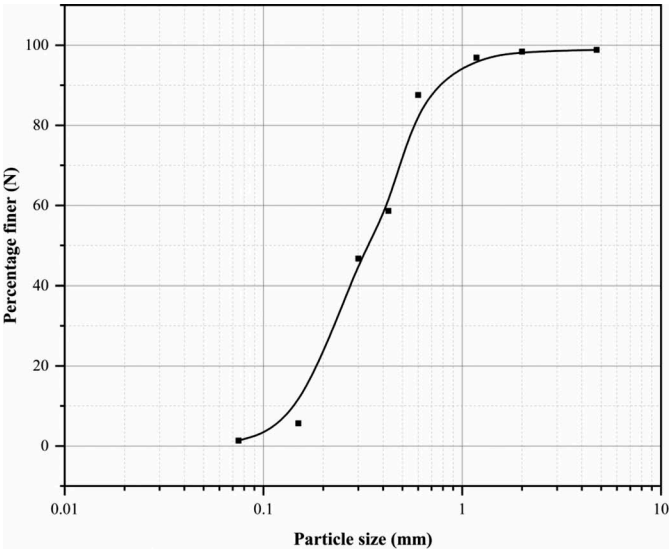

Figure 1. Grain size distribution curve of Narmada sand.

Table 1. Properties of Narmada sand.

Property	Value
Cu	2.60
Cc	0.72
D_{50} (mm)	0.32
Silt Content (%)	4.72%
Specific Gravity	2.65
e_{max}	0.72
e_{min}	0.38
OMC (%)	8.64
MDD (gm/cm^3)	1.75
Permeability (m/s)	2.74* 10^{-6}

a spectrophotometer at 600 nm wavelength. The recorded values were 1.17 for *S. pasteurii*, 1.27 for *B. subtilis*, and 2.48 for *B. sphaericus*. It was observed that the OD of *B. sphaericus* is more than *S. pasteurii* and *B. subtilis*. The OD of all species was adjusted to approximately 1 in the present study by diluting with distilled water. The bacterial solution was centrifuged at 5000 rpm for 20 minutes and the supernatant was replaced with fresh nutrient broth media (Bu et al. 2018).

Cementation solution

Cementation solution provides chemicals for urea hydrolysis, which includes urea, calcium chloride dihydrate, ammonium chloride, sodium bicarbonate, and nutrient broth among which ammonium chloride and sodium bicarbonate act as a buffer in cementation media (Bu et al. 2018). The cementation solution includes 10 g/l ammonium chloride, 2.12 g/l sodium bicarbonate, 3 g/l nutrient broth and various combinations of concentrations of urea, and calcium chloride dihydrate. In the cementation media, the nutrient broth solution was autoclaved to prevent bacterial contamination (Mortensen et al. 2011). The other components of cementation media were not autoclaved to represent more practical and field conditions. In general, autoclaving of urea will lead to its decomposition due to changes in chemical structure at high temperature (Saricicek et al. 2019).

Experimental details and treatment procedure

40 grams of Narmada river sand and 15 ml of bacterial solution was filled in each plastic tube of 50 ml capacity with the help of funnel to achieve the relative density of around 40%. Initially, one-third of the bacterial solution was poured into the plastic tube, then one-third amount of sand was filled in circular motion with the help of a funnel. The other two layers were placed following similar procedure. The Narmada river sand was oven-dried at 105°C for 24 hours before starting the treatment without any sterilization process. A total of 16 samples with three bacterial strains and various combinations of urea – calcium chloride dihydrate, were prepared in duplicate along with the controls. The details of the experimental samples are provided in Table 2. Various experimental groups have investigated the calcite precipitation using combinations of bacteria and cementation media concentrations. The concentration of cementation media used in this study was similar to Wen et al. (2019). The final results will be used for further research on shear strength improvement and liquefaction mitigation.

The following procedure was adopted throughout the treatment: (1) 15 ml of bacterial solution and 40 grams of sand were mixed and filled in three layers in the plastic tube and left for 16 hours so that the bacteria can attach with the sand; (2) The supernatant was decanted; and (3) The cementation media was provided twice a day in the same quantity as bacteria solution for further treatment up to 14 and 28 days. The stock solution of cementation media was prepared every 2nd day. Half of the samples were removed after 14 days of treatment and analysed for the amount of precipitation. The remaining samples (duplicate samples) were removed after 28 days and the degree of precipitation was compared with 14 days treated sample.

Table 2. Details of experimentation of three bacteria and cementation media concentrations used.

S. No.	Sample designation*	Concentration of Urea	Concentration of $CaCl_2$. $2H_2O$
1	14/28 B_1 0.25	0.25 M	0.25 M
2	14/28 B_1 0.50	0.50 M	0.50 M
3	14/28 B_1 2:1	0.50 M	0.25 M
4	14/28 B_1 3:1	0.75 M	0.25 M
5	14/28 B_2 0.25	0.25 M	0.25 M
6	14/28 B_2 0.50	0.50 M	0.50 M
7	14/28 B_2 2:1	0.50 M	0.25 M
8	14/28 B_2 3:1	0.75 M	0.25 M
9	14/28 B_3 0.25	0.25 M	0.25 M
10	14/28 B_3 0.50	0.50 M	0.50 M
11	14/28 B_3 2:1	0.50 M	0.25 M
12	14/28 B_3 3:1	0.75 M	0.25 M
13	14/28 C 0.25	0.25 M	0.25 M
14	14/28 C 0.50	0.50 M	0.50 M
15	14/28 C 2:1	0.50 M	0.25 M
16	14/28 C 3:1	0.75 M	0.25 M

Sample designation*: 14/28 denotes that a sample is prepared in duplicates to treat up to 14 and 28 days. B_1, B_2, and B_3 are bacteria strains S. pasteurii, B. subtilis, and B. sphaericus, respectively. 0.25 and 0.50 show equimolar concentrations of urea and $CaCl_2$. $2H_2O$, while 2:1 and 3:1 stand for the concentration ratio of urea and $CaCl_2$. $2H_2O$. Letter C refers to control samples.

Micro-characterization of biotreated samples

After biotreating the soil samples of Narmada sand; SEM, FTIR and XRD tests were carried out to calculate the amount of calcite precipitation. The samples were taken out from the tubes; the top 5 mm layer was removed from all the samples as it was injection point and the amount of precipitation was more at the top surface. Collected samples were oven-dried at 105°C up to 24 hours, then crushed into powder form to make the subsamples for micro-characterization. The SEM analysis was performed on the gold sputter-coated subsamples and the SEM images were obtained at various magnifications at 15 kV, to identify the presence of calcite crystals and microbial beds. The presence of minerals and type of crystal structure of biotreated sand samples was resolved by XRD analysis, which was done within the scanning range of 2 – 90°. Fourier transform infrared spectroscopy was used to identify the chemical bonds in biotreated sand molecules in the wavenumber range of 4000–400 cm^{-1} by KBr pellet method.

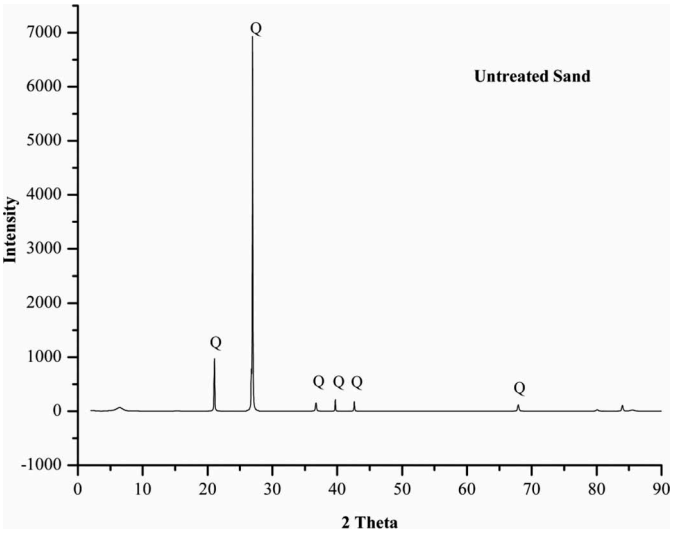

Figure 2. XRD analysis untreated sand.

Calcite content

Fann calcimeter was used to determine the percentage of calcite precipitation, formed due to MICP. When the HCl is added to calcite precipitated sand in calcimeter, CO_2 is generated, which increases the pressure in calcimeter cylinder. The increased pressure measured by a pressure gauge is used to calculate the amount of calcite precipitation (Kalantary, Abbasi Govanjik, and Safdari Seh Gonbad 2019).

Results and discussion

XRD analysis

The XRD analysis was performed on 0.50 M concentration cementation media treated samples with all three bacteria. XRD data of 14 and 28 days treated sample showed the presence of rhombohedral calcite crystals in all type of bacteria used. XRD analysis of untreated sand showed that the calcite was not present in the sand before treatment (Figure 2). It could be interpreted that the Narmada sand was free from calcite initially, and only quartz or silica was present in it. The comparative graphical analysis of treated samples with three bacteria and 0.50 M cementation media, till 14 and 28 days are shown Figure 3. The peaks of silica (quartz) and calcite were analysed.

SEM analysis

SEM images of all the bacteria and cementation media concentrations showing microbe bed and calcite crystal

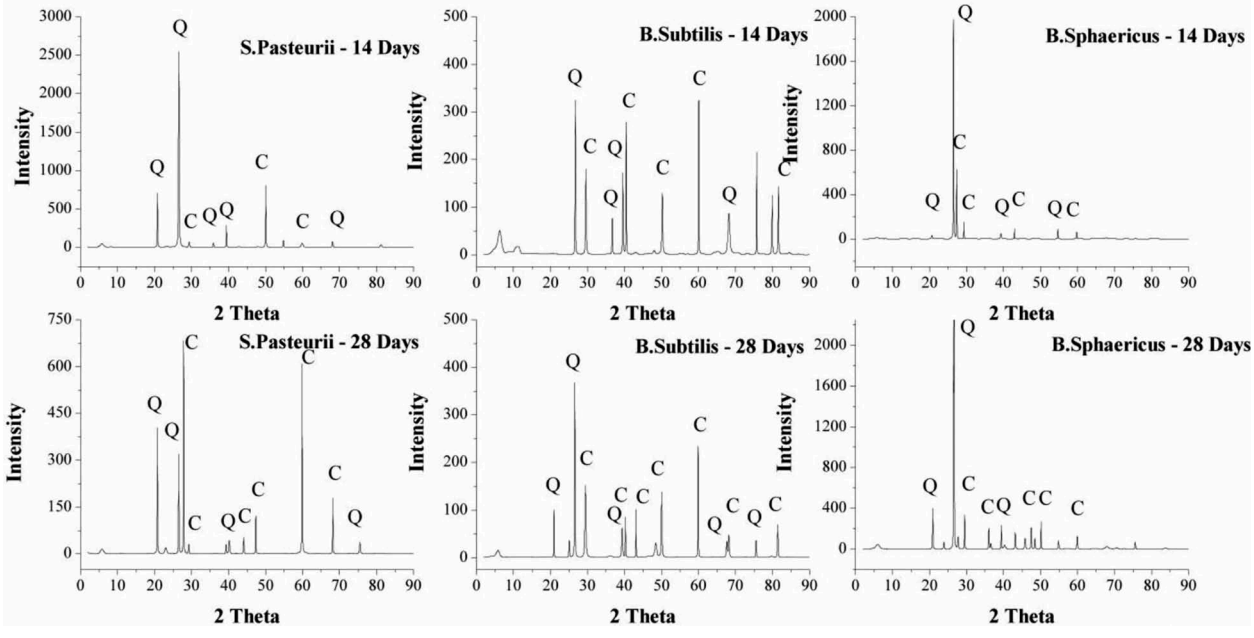

Figure 3. XRD analysis of 14–28 days biotreated sand with three bacteria and 0.50 M cementation media concentration.

formations are shown in Figures 4–9. The crystals of calcite are more in 28 days treated samples, which can be seen in SEM images at different magnifications (Figures 9–11). Microbe beds were observed in 1–5 μm images, which appeared as hollow cylindrical spaces on the surface of calcite crystals, which confirms the functioning of MICP.

The bacteria and various cementation media combinations formed distinct sizes of calcite crystals. The diversity in sizes of crystals was associated with the clashes between the growth of crystals and the formation of new crystals, as a result of interaction between the bacteria and cementation media. The clashing occurs because of the formation of new crystals which suppresses the growth rate of previously existing crystals (Gandhi, Kumar, and Ramkrishna 1995). The reaction rate of urea hydrolysis is high due to high cementation media concentration, which promotes the formation of new calcite crystals more than the growth of already existing crystals. According to the principle, the high bacteria concentration

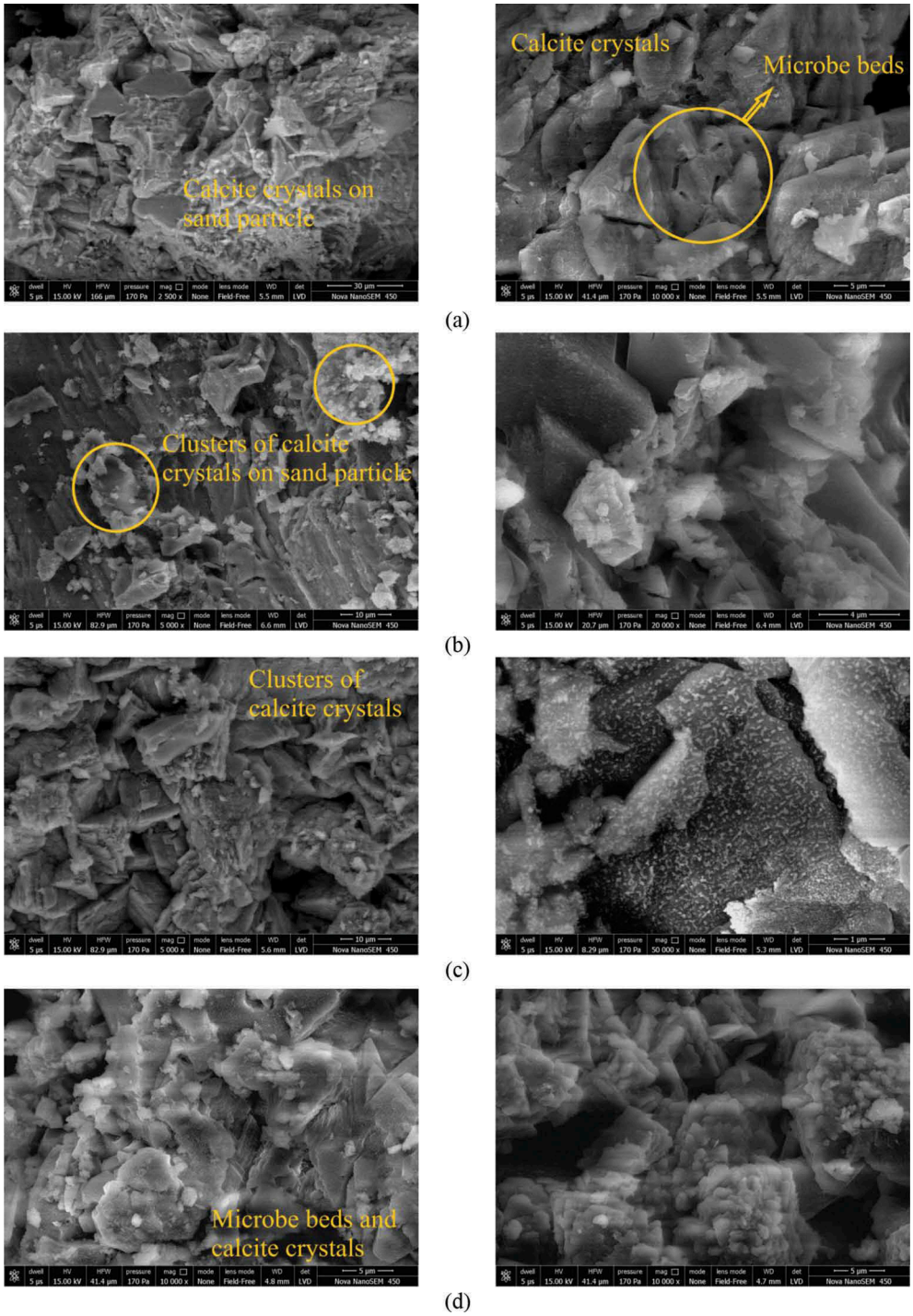

Figure 4. SEM analysis of 14 days treated samples with S.pasteurii and different concentrations of cementation media i.e. (a) 0.25 M, (b) 0.50 M, (c) 2:1, (d) 3:1, at various magnifications.

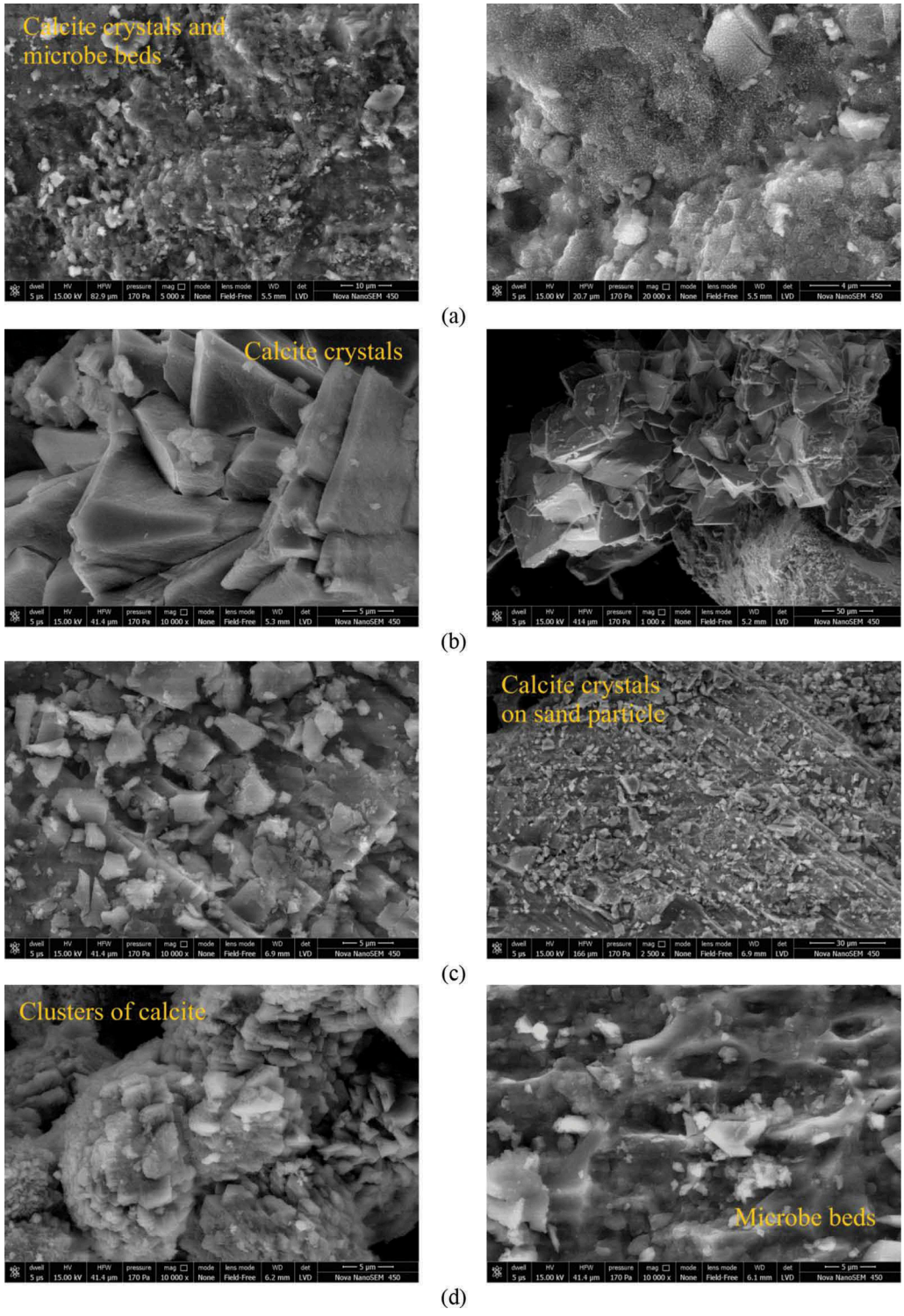

Figure 5. SEM analysis of 14 days treated samples with B. subtilis and different concentrations of cementation media i.e. (a) 0.25 M, (b) 0.50 M, (c) 2:1, (d) 3:1, at various magnifications.

provides a large number of nucleation sites in the sand matrix and forms spherulitic crystals of calcite (Mujah, Shahin, and Cheng 2017).

The reaction rate is higher in 2:1 and 3:1 concentration, as there was an increase in pH due to the release of ammonia gas. The increased pH creates a positive environment for bacterial growth (Mahawish, Bouazza, and Gates 2018). The clusters of small size crystals can be seen in Figures 4–9(c & d). The rate of urea hydrolysis is low in 0.25 M and 0.50 M concentrations, which leads to the formation of large-size crystals, as shown in Figures 4–9 (a & b). Figures 6 and 9 show the spherulitic crystals of calcite, which can be

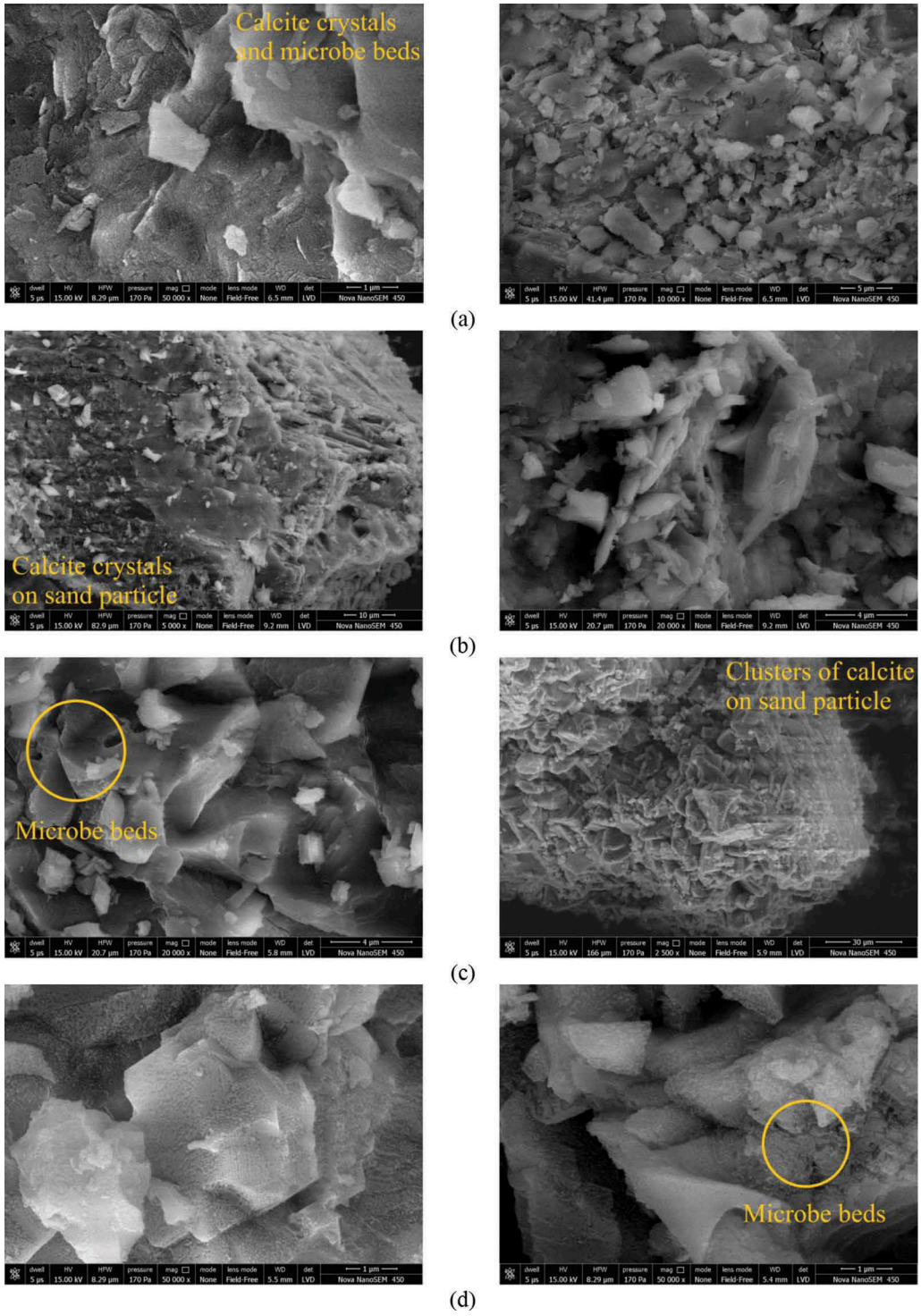

Figure 6. SEM analysis of 14 days treated samples with B. sphaericus and different concentrations of cementation media i.e. (a) 0.25 M, (b) 0.50 M, (c) 2:1, (d) 3:1, at various magnifications.

associated with the high growth rate of *B. sphaericus* cells as compared to S.pasteurii and B.subtilis. Experimentally, it was also interpreted that OD of *B. sphaericus* was almost twice the OD of *S. pasteurii* and *B. subtilis* in 24 hours. Typically, the rhombohedral calcite crystal clusters can be seen on sand particles surface, which creates an interparticle bond between calcite crystals and sand grain. According to the SEM analysis, the three bacteria and distinct cementation media treated samples showed the calcite precipitations in all the combinations. The number of calcite crystals was more in 28 days treated samples than 14 days treated samples. Uniformity and higher amount of precipitation were found in all three types of bacteria treated with 0.50 M concentration solution up to 28 days.

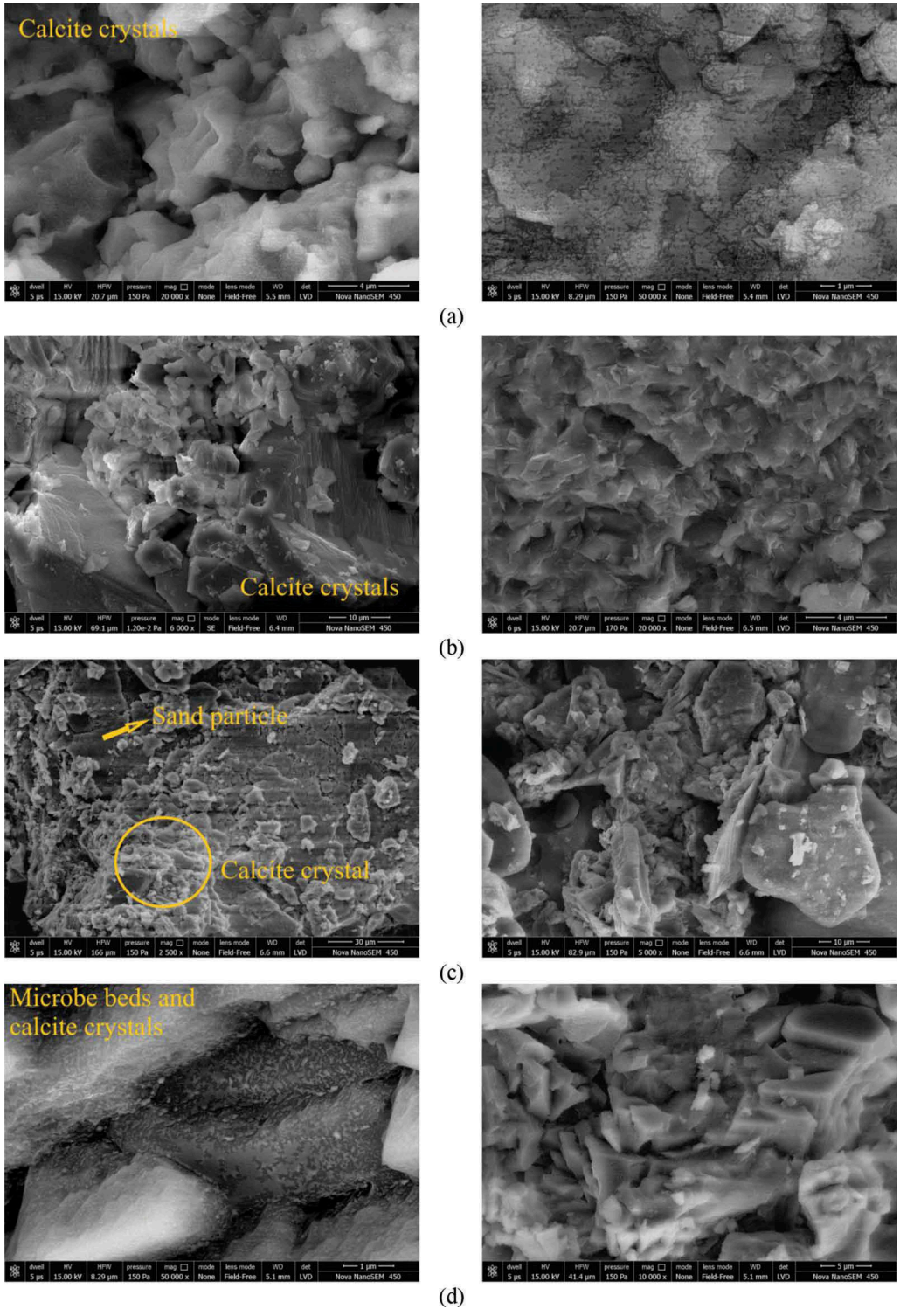

Figure 7. SEM analysis of 28 days treated samples with B. pasteurii and different concentrations of cementation media i.e. (a) 0.25 M, (b) 0.50 M, (c) 2:1, (d) 3:1, at various magnifications.

FTIR analysis

Pellet of biotreated sand sample and KBr was used to perform FTIR analysis in the spectrometer at the frequency range of 4000–400 cm^{-1}. Figures 10 and 11 show the graphical analysis of the FTIR spectrum of biotreated sand with all three types of bacteria at 0.50 M cementation media concentration at 14 and 28 days, respectively. FTIR spectrum substantiates that the used bacterial strains produce NH_4^+ and CO_3^{-2}. *B. subtilis* and *B. sphaericus* used samples showed similar bands and stretch as present in *S. pasteurii* treated samples. The peak wavenumber analysis was carried out according to the data available in the literature (Almajed et al. 2019; Cardoso et al. 2018), which is concluded in Table 3.

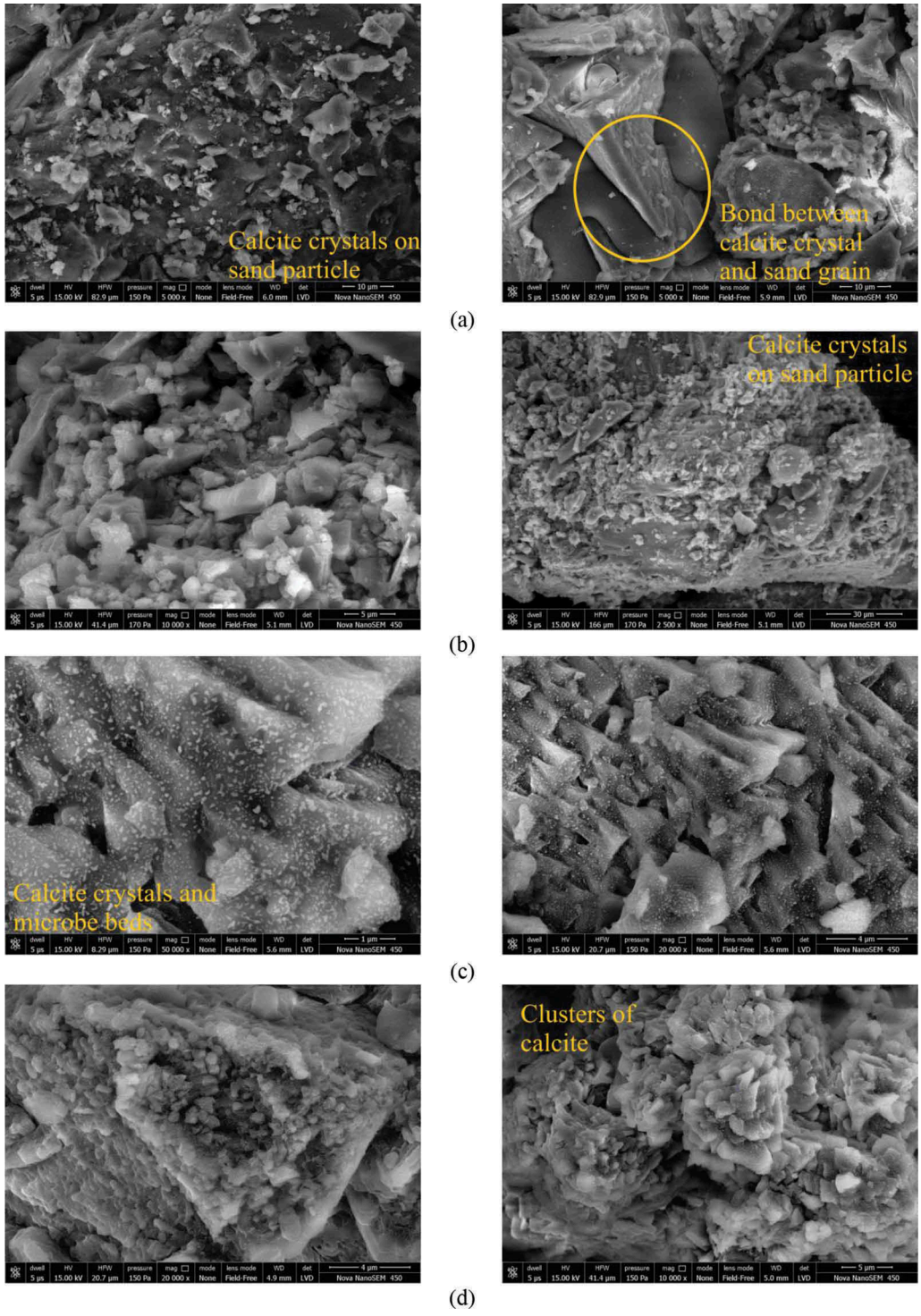

Figure 8. SEM analysis of 28 days treated samples with B. subtilis and different concentrations of cementation media i.e. (a) 0.25 M, (b) 0.50 M, (c) 2:1, (d) 3:1, at various magnifications.

Calcite precipitation analysis

The Bacterial treatment of Narmada sand was performed for 14 and 28 days. The samples were treated in tubes using bacteria and distinct cementation media concentrations. The bacteria inoculated samples demonstrated the microbe induced calcite precipitation with time. After removing the top 0.5 cm layer of calcite, the specimen from the upper part of the tube was taken for calcite content determination and the bottom part was analysed for uniformity in precipitation. The results showed that the rate of precipitation was very high in 2:1 and 3:1 samples from the second day of treatment. It was attributed to the higher molarity of urea than $CaCl_2.2H_2O$, which increase the pH and reaction rate, releasing ammonium as ammonia gas in the air. Bacteria act as a nucleation site at higher pH,

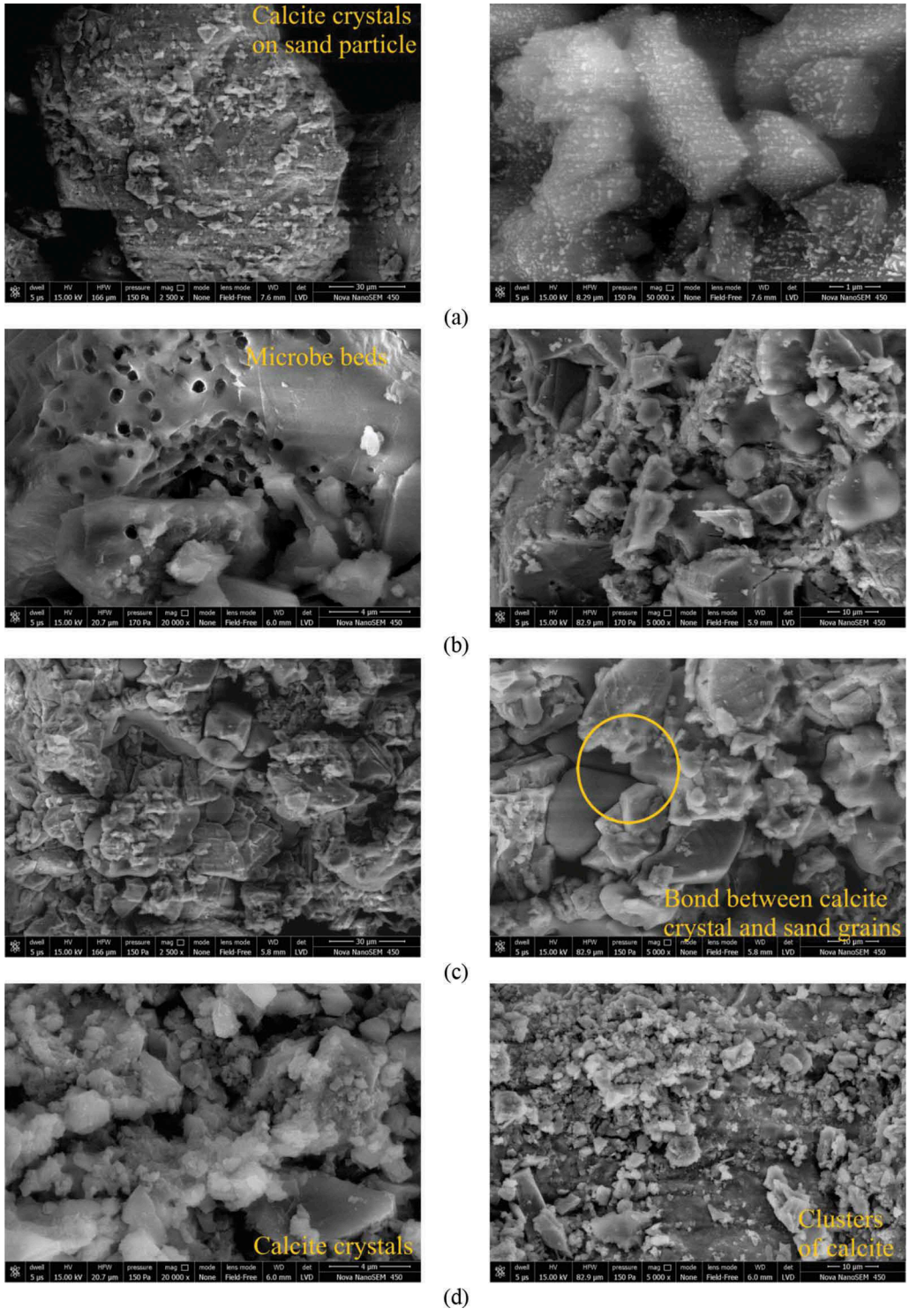

Figure 9. SEM analysis of 28 days treated samples with B. sphaericus and different concentrations of cementation c media i.e. (a) 0.25 M, (b) 0.50 M, (c) 2:1, (d) 3:1, at various magnifications.

so the rate of precipitation was very high comparative to 0.25 M and 0.50 M concentration samples. The higher reaction and precipitation rate lead to bioclogging near the injection point or at the surface. It has been observed that due to bioclogging initially in top 1–1.5 cm layer, the sand sample from the lower part of the tube showed very less amount of calcite precipitation. Even the amount of precipitation did not increase with the increase in the number of treatment days.

The samples treated with 2:1 and 3:1 cementation media concentration had a similar amount of precipitation in 14 and 28 days in the top layer, and bottom layer samples had very less precipitation which was between 1.5–3% only. In spite of this, 0.25 M and 0.50 M concentration samples showed uniform

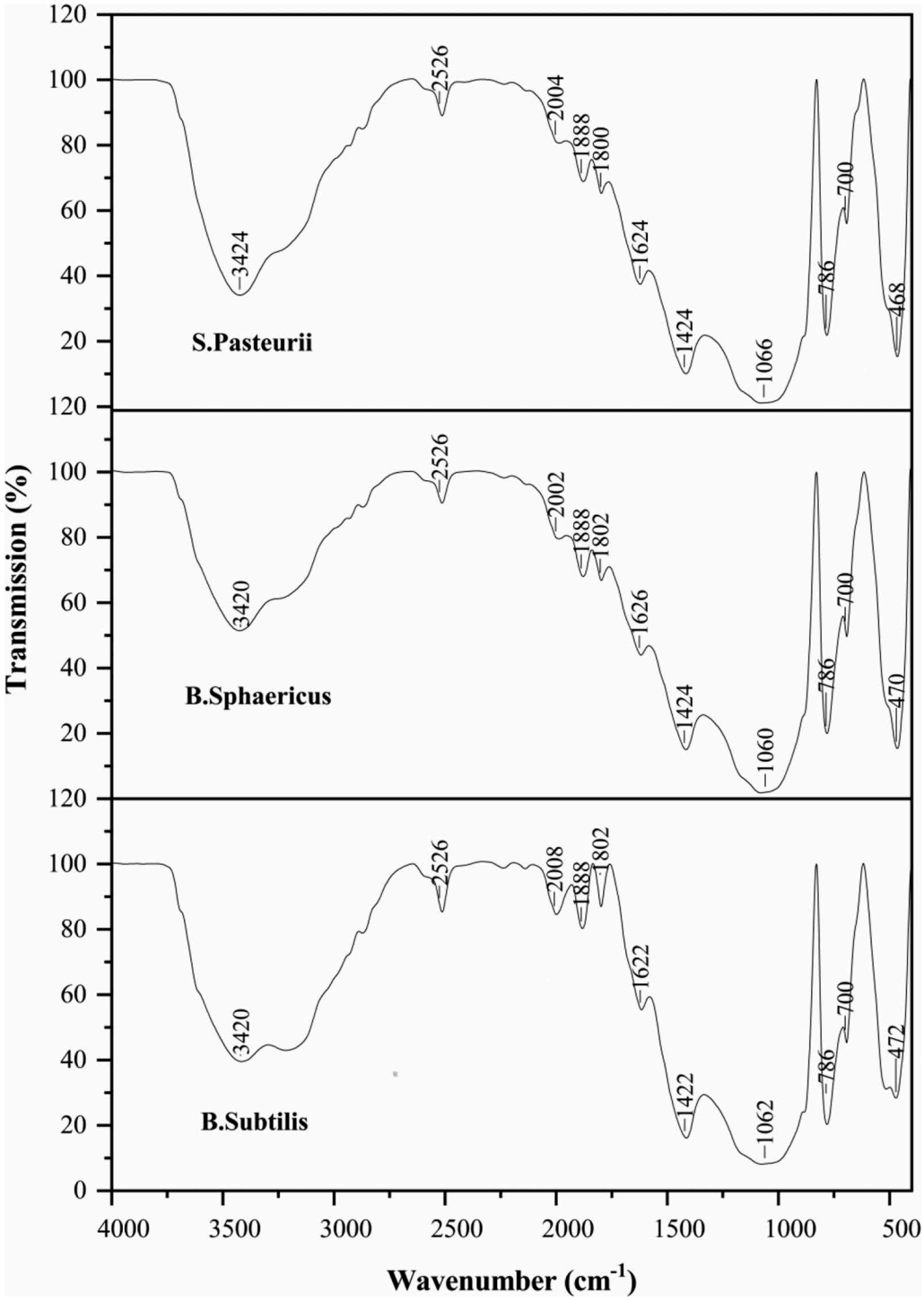

Figure 10. FTIR spectrum of 14 days biotreated sand with three bacteria and 0.50 M cementation media concentration.

precipitation. However, the 0.25 M samples had less precipitation and uniformity than 0.50 M. 14 days treated samples with 0.50 M concentration showed similar uniformity in precipitation as 28 days treated samples with 0.25 M concentration. 0.50 M concentration samples of all three bacteria showed good results after 14 and 28 days of treatment, though the uniformity and calcite bond strength was much higher in 28 days treated samples. Extrusion of 0.50 M cementation media treated samples required extra efforts due to high cementation and uniformity. The 14 days treated samples was less uniform and relatively easier to remove in comparison to 28 days treated samples. The bacteria *B. sphaericus* and *B. subtilis* resulted in a nearly similar amount of calcite precipitation and uniformity after treatment with 0.50 M

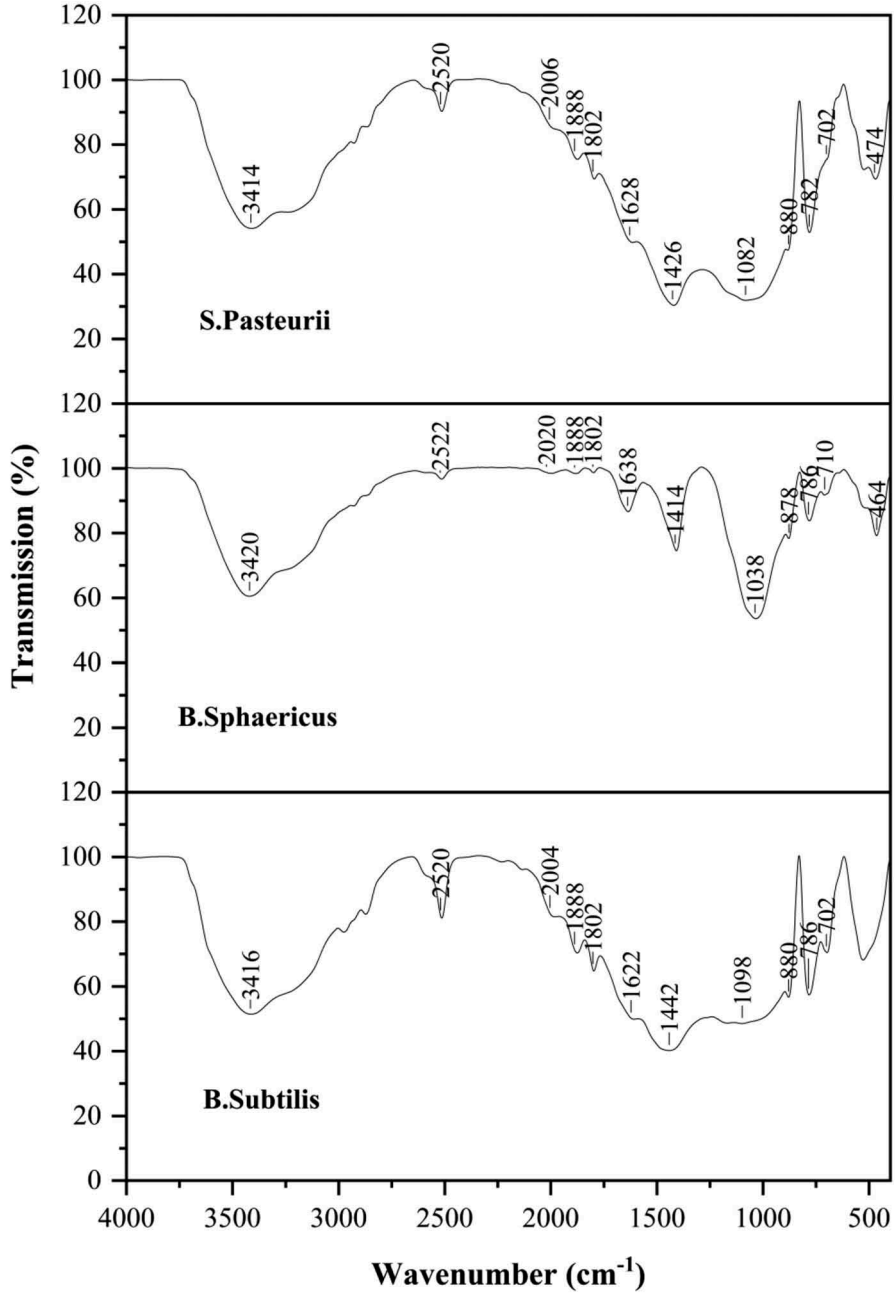

Figure 11. FTIR spectrum of 28 days biotreated sand with three bacteria and 0.50 M cementation media concentration.

cementation media concentration in comparison to *S. pasteurii*. The amount of calcite precipitation in different samples is shown in Table 4.

Conclusions

This study emphasizes on the efficiency of *B. subtilis* and *B. sphaericus* in MICP, as *S. pasteurii* is well recognized and widely used bacterium in soil stabilization to deal with geotechnical challenges. Small scale bacterial treatment of Narmada sand was performed in tubes under a sterile environment, to interpret the behaviour of different bacteria and cementation media concentrations. The findings of this study via SEM, XRD, FTIR, and determination of calcite content, confirm the formation of MICP. This study drives us to elicit the following conclusions:

(1) All three bacteria show calcite precipitation with various concentrations of cementation media used.
(2) *B. sphaericus* and *B.subtilis* can be used as a substitute for *S. pasteurii*, but the importance of the selection of a concentration of cementation media is equally important.

Table 3. Characterization of the biotreated sample using FTIR analysis.

Peak Wavenumber (cm^{-1})	Analysis
3420, 3424, 3414, 3416, 1622, 1626, 1624, 1628,1638	Corresponding to the band of N-H stretch which denotes the presence of NH_4^+
2526,2522,2520, 2002, 2004, 2006,2008, 2020	NH^+ stretching
1888	Aromatic compound CH bending
1422,1424, 1442, 1414, 1426	Attributed to the asymmetric band of C-O stretch
1800, 1802,1062,1060,1066, 1098, 1038, 1082	Strong C-O stretching
880,878	Inadequate plane deformation of CO_3
786, 782	Strong CH Bending
700,702, 710	In-plane deformation due to OCO bending
468, 470, 472, 464, 474	Bending vibrations of silicates in a plane

Table 4. Amount of calcite precipitation in biotreated samples.

		Calcite Precipitation (%)	
S. No.	Sample Designation	Top Layer	Bottom Layer
1	Untreated sand	0	N.A.
2	14 B_1 0.25	7.1	6.8
3	14 B_2 0.25	6.4	5.2
4	14 B_3 0.25	7.9	7
5	14 B_1 0.50	13.6	12.7
6	14 B_2 0.50	12.8	11
7	14 B_3 0.50	13.4	13.1
8	14 B_1 2:1	17.3	3.5
9	14 B_2 2:1	16.2	2.1
10	14 B_3 2:1	17.5	2.8
11	14 B_1 3:1	21.6	2
12	14 B_2 3:1	19.8	1.5
13	14 B_3 3:1	20.4	2.4
14	28 B_1 0.25	14.4	14.1
15	28 B_2 0.25	13.6	13.3
16	28 B_3 0.25	14.2	13.9
17	28 B_1 0.50	26.7	26.2
18	28 B_2 0.50	24.9	24.4
19	28 B_3 0.50	25.4	25
20	28 B_1 2:1	18.2	3
21	28 B_2 2:1	17.1	2.4
22	28 B_3 2:1	18	3.2
23	28 B_1 3:1	21.8	2.2
24	28 B_2 3:1	20.1	1.8
25	28 B_3 3:1	21	2.7

(3) The reaction rate of urea hydrolysis should not be very less or very high, as 0.25 M cementation media treated samples had precipitation in the range of 5.2–7.9% and 13.3–14.4% in 14 and 28 days, respectively, though the precipitation was uniform. Meanwhile, biotreated samples of 0.50 M cementation media showed the almost double amount of precipitation in 14 days as compared to 28 days treated samples of 0.25 M cementation media. The precipitation was high and uniform in 0.50 M cementation media treated samples when compared to other concentrations.
(4) The rate of ureolysis was high in 2:1 and 3:1 concentration treated samples, which had a high amount of precipitation in 14 days only but was limited in the top few cms of the treated soil. The concentrations can be applied to control seepage and wind erosion. These concentrations can create an impermeable and tough layer of calcite. There was only 2–3% variation in the amount of calcite when treated with 2:1 and 3:1 concentration, so 2:1 concentration cementation media can be economical to use.

Overall, the study showed the feasibility of MICP on loose Narmada sand. Furthermore, additional field studies are needed to confirm the results under real field conditions.

Disclosure statement

No potential conflict of interest was reported by the authors.

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