Microbially induced calcite precipitation performance of multiple landfill indigenous bacteria compared to a commercially available bacteria in porous media

Adharsh Rajasekar, Charles K. S. Moy*, Stephen Wilkinson, Raju Sekar

1 Jiangsu Key Laboratory of Atmospheric Environment Monitoring and Pollution Control (AEMPC), Collaborative Innovation Center of Atmospheric Environment and Equipment Technology (CiC-AEET), Nanjing University of Information Science &Technology, Nanjing, China, 2 Department of Civil Engineering, Xi’an Jiaotong-Liverpool University, Suzhou, Jiangsu, China, 3 Faculty of Engineering and Information Sciences, University of Wollongong in Dubai, Dubai, UAE, 4 Department of Biological Sciences, Xi’an Jiaotong-Liverpool University, Suzhou, Jiangsu, China

* cloo8000@uni.sydney.edu.au

Abstract

Microbially Induced Carbonate Precipitation (MICP) is currently viewed as one of the potential prominent processes for field applications towards the prevention of soil erosion, healing cracks in bricks, and groundwater contamination. Typically, the bacteria involved in MICP manipulate their environment leading to calcite precipitation with an enzyme such as urease, causing calcite crystals to form on the surface of grains forming cementation bonds between particles that help in reducing soil permeability and increase overall compressive strength. In this paper, the main focus is to study the MICP performance of three indigenous landfill bacteria against a well-known commercially bought MICP bacteria (Bacillus megaterium) using sand columns. In order to check the viability of the method for potential field conditions, the tests were carried out at slightly less favourable environmental conditions, i.e., at temperatures between 15-17˚C and without the addition of urease enzymes. Furthermore, the sand was loose without any compaction to imitate real ground conditions. The results showed that the indigenous bacteria yielded similar permeability reduction (4.79 E-05 to 5.65 E-05) and calcium carbonate formation (14.4–14.7%) to the control bacteria (Bacillus megaterium), which had permeability reduction of 4.56 E-5 and CaCO₃ of 13.6%. Also, reasonably good unconfined compressive strengths (160–258 kPa) were noted for the indigenous bacteria samples (160 kPa). SEM and XRD showed the variation of biocrystals formation mainly detected as Calcite and Vaterite. Overall, all of the indigenous bacteria performed slightly better than the control bacteria in strength, permeability, and CaCO₃ precipitation. In retrospect, this study provides clear evidence that the indigenous bacteria in such environments can provide similar calcite precipitation potential as well-documented bacteria from cell culture banks. Hence, the idea of MICP field application through biostimulation of indigenous bacteria rather than bioaugmentation can become a reality in the near future.
Introduction

Applied geomicrobial engineering is an expanding field of studies, including the utilisation of microorganisms to modify the soil chemistry and physical properties for geotechnical purposes [1–12]. One promising form of biominalisation for engineering applications is Microbially Induced Carbonate Precipitation (MICP) [13,14], the MICP process involves the formation and precipitation of CaCO$_3$ polymorphs (e.g. calcite, vaterite, etc) as a result of changes in the soil environment (mainly pH and increasing the concentration of aqueous CO$_2$) induced by microorganisms’ activities [15]. Microbial metabolic activities associated with MICP include ureolysis, denitrification, ammonification, sulphate reduction, and methane oxidation [4]. Different microorganisms have been found capable of MICP and the most studied urease bacteria are *Sporosarcina pasteurii* [6,16–21] and *Bacillus* [22–25]. A recent review paper compared the performances of those microorganism towards MICP [26]. This technique has the potential for several geoengineering applications since microbes are small, pervasive, and they can enter into the interstices of geological materials, such as masonry or fine-grained soils, thus allowing CaCO$_3$ precipitation within the materials’ granular matrix.

MICP can lead to biocementation which is the generation of bonds (cements) between soil particles caused by Ca$^{2+}$-based mineral polymorphs [27–29]. Such bonds act as bridges between particles resulting in an increase in the shear strength [30–34] and a reduction of the void space between particles (and therefore a reduction in the material permeability) [2,35,36]. Numerous studies shown above have indicated the potential and possibility of using MICP to improve the sand strength with a minor problem being the movement of treatment depth since treating potential wastelands with larger depth might be a constraint [37]. Also, depth is often associated with a major reduction in permeability [38]. The bacteria and the cementation reagent are mixed before injected into sand columns under atmospheric or high pressure (depending on depth) to avoid clogging of the system near the injection point. For the purposes of soil improvement, the reduction of permeability is often viewed as undesirable because this may affect natural groundwater flow paths by increasing pore pressure in the soil. Hence, retaining permeability while providing strengthening treatments is ideal. MICP has proven to be a suitable technique for such situations allowing strengthening with minimum disturbances to the ground/soil conditions [39,40].

Experiments that have assessed the performance of MICP microorganisms are often conducted at temperatures ranging from 20˚C to 50˚C [22], elevated pressures up to 30kPa [40], and under high initial pH [21]. Limited assessments have been made to test the potential of MICP/biocementation under less favourable conditions such as lower temperatures. Similarly, study of indigenous microorganisms and their potential application for MICP/biocementation are rather limited to certain environments [2,3,6,29,41]. For example, Kang et al. (2015) [42] investigated the bioremediation potential of lead from ureolytic bacteria from abandoned metal mines in Korea. Similarly, a study by Zamarreño et al. (2009) [43] looked at the biocalcification performance of non-sporing freshwater bacteria. There are no known references focussing on the performance of indigenous bacteria in landfills. Therefore, the approach shown in this paper is based towards assessing the biocementation potential of microorganisms isolated from landfill groundwater and leachate under realistic soil conditions. Our objective is to evaluate the MICP/biocementation potential of bacteria strains indigenous to environments where MICP might be applied including polluted habitats and areas of rapid urbanization (conversion from an agricultural use). This would reduce the costs associated to the technique and mitigate environmental risks of introducing non-indigenous species into the environment.
Identification of bacteria and their precipitation potential

The three bacteria mentioned in this paper were isolated from landfill leachate (L3 and L5) and groundwater (W1) located in Suzhou, Jiangsu, China. The pH of the leachate and groundwater samples was alkaline in nature. The description of the landfill is mentioned in detail by [44].

A culture-dependent technique was used for bacteria isolation from the landfill leachate and groundwater samples. The isolation protocol and the sequencing methodology for these microorganisms were published in our previous paper [45]. Seven bacteria were found to precipitate calcium carbonate. The detailed isolation protocol and identification of their calcium carbonate precipitation ability have been reported in the previous study [45].

Application of bacteria towards precipitation

The three bacteria which produced the highest carbonate precipitation were selected to be used in the sand column experiments described below. The bacteria used were *Pseudomonas nitroreducens szh_asesj15* (Bacteria isolated from landfill groundwater), *Bacillus sp. xjlu_xherec15* (Bacteria isolated from leachate), and *Bacillus licheniformis adseedstjo15* (Bacteria isolated from leachate). More information about these bacteria can be found in our previous paper [45]. *Bacillus megaterium* was used as a control in this study, since they outperformed *Sporosarcina pasteurii* at temperatures around 15˚C [24,46].

Biocementation solution and procedure

The cementation solution consisted of 500mM calcium chloride, 500mM urea and bacterial solution (cell density at 2 x 10⁷ cells/ml). Bacterial solution consists of Nutrient broth and bacteria. Nutrient broth consists of Peptone, Yeast extract and sodium chloride. The transport of media through the column occurred due to gravity and diffusion. The experiment was conducted at a temperature of 17˚C and atmospheric pressure. Most experiments conducted using porous media have been performed at a temperature >25˚C [2,3,7,31,47]. It is well documented that the performance of bacteria to secrete enzymes (e.g. urease, carbonic anhydrase, etc.) is relatively low when the temperature gets lower than 20˚C [22]. This study aimed at achieving a more realistic environmental condition for the application of MICP. The cementation solution was replaced 6 times, once every 2 days to allow the cementation to occur. The media was replaced slowly to avoid disturbing the sample content. This became easier as the columns solidified and the permeability decreased. The experiments were carried out without agitation. The indigenous bacteria and control bacteria were tested alongside a blank column. A control specimen containing commercially bought *Bacillus megaterium* was used to compare the MICP efficiency of the indigenous bacteria. A blank specimen (without any bacteria) was subjected to the same temperature, pH and the cementation solutions.

Bacteria optical density

A qualitative calculation of viable biomass optical density (OD) was determined spectrophotometrically at 600 nm (i.e. OD600) using visible light spectrophotometry.

Column preparation

The soil columns used to evaluate biocementation were modified from a design described in [48]. Pure silica sand (ISO 9000:2001) sieved to between 0.04 and 1.18mm was used for the experiments (Fig 1). Columns were made of Polyvinyl Chloride (PVC) tubing with an internal diameter of 5.5 cm and 20 cm length (Fig 2). The columns were packed with loose dry silica.
sand. The top part of the column being open to the atmosphere and the bottom part being con-
nected to the inner tube. The media (bacterial suspension and cementation solution) was
introduced from the top of the columns. The bottom of the column was covered with a scour-
ing pad layer, to avoid sand loss. A flexible plastic tube of 20mm inner diameter was connected
to the outlet of the soil column. In order to maintain cementation solution saturation in the
sand column at all times, the outlet pipe was fitted in a u-shape parallel to the column as
shown in (Fig 2) [27]. The biocementation media including calcium chloride and urea were fil-
ter sterilized. The nutrient broth was autoclaved before adding the bacteria in them. The PVC
column, tubes and scouring pad were soaked in 75% ethanol and oven-dried at 70˚C.

Permeability measurement

Estimates of permeability were also made using a falling head procedure [49] between two
marks on the inside of the column above the sand. All specimens were also flushed with DI
water after finishing the biochemical treatments to remove residual chemicals from the pores
throat of coarse sand and then saturated before commencing the permeability test by percolat-
ing water through the coarse sand to de-air residual air in the pore matrix. The coefficient of
permeability (K) was calculated using the equation:

$$K = 2.303 \times \left[ \frac{aL}{A(t_f - t_i)} \right] \times \log_{10} \frac{h_1}{h_2}$$

where $a$ is the area of the inlet, $L$ is the distance between the two measuring points, $A$ is the
area of the sample, $(t_f - t_i)$ is the increment of time between two readings, $h_1$ is head of water
above outlet elevation at time $t_i$ and $h_2$ head of water above outlet elevation at time $t_f$.

Calcium carbonate precipitation

Finally, the extent of precipitation of calcium carbonate was assessed via titration with HCl
[50] using approximately 10 grams of randomly subsampled cemented sand from the column.
The sand taken for titration was not air-dried as described in Rajasekar et al. (2018). Before this procedure, the sand was washed with DI water to remove excess or unused calcium chloride, urea and any other by-products such as hydrochloric acid that may be retained in sand. The procedure is as follow: weigh the soil sample of 1 to 10 g (±0.001 g) into a 250-mL Erlenmeyer flask; use a volumetric pipette, add 20 mL of standardized 1N HCl to the flask; cover the Erlenmeyer flask with a watch glass and boil the soil-acid mixture for 5 minutes; and add 50–100 mL deionized water using a graduated cylinder. After it has cooled down; add 2 or 3 drops phenolphthalein indicator. Titrate the solution with 1N NaOH solution while swirling the flask and finally record the reading when a faint pink colour develops.

\[ \text{CaCO}_3 \text{equiv., } \% = \left( \frac{V_{\text{HCl}}N_{\text{HCl}} - V_{\text{NaOH}}N_{\text{NaOH}}}{\text{grams of soil}} \right) \times 0.05 \times 100 \]

where \( V_{\text{HCl}} \) and \( V_{\text{NaOH}} \) are the volume and normality of HCl and NaOH, respectively.

Fig 2. Schematic of the experimental setup of the sand columns.
https://doi.org/10.1371/journal.pone.0254676.g002
Strength measurement

A hand-held penetrometer (PP) was used to measure the penetration resistance of the samples and the readings were converted to unconfined compressive strength (UCS) values to allow comparison with values published elsewhere. This method was mainly selected to ensure continuous monitoring of the strength development. However, only the final strength measurements recorded at 14 days are reported for clarity.

Qualitative measurement

Scanning electron microscopy (SEM) was used to analyse the presence of calcium carbonate precipitation. Following the physical properties testing, small subsamples of sand agglomerations extracted from the columns, were placed onto pin stubs using double-sided sticky carbon tape and analysed with a scanning electron microscopy (SEM-Hitachi TM3000). The samples were placed uncoated into the microscope to allow a qualitative assessment of the extent of mineral precipitation that occurred after the experiment. The samples were crushed into fine powder and later mounted on a glass slide for the X-ray Diffraction analysis. The samples were tested for their calcium carbonate presence using (Advanced D8, Bruker, Germany).

Results and discussion

In our study, the permeability of the sand columns containing indigenous and commercial bacteria was lower than the control (Fig 3). The mean permeability values produced by the MICP process range from 4.79x10^{-5} ms^{-1} to 5.65x10^{-5} ms^{-1}, varying by microbe in comparison to 2.60x10^{-4} ms^{-1} for the control. All the samples had relatively reasonable standard deviations indicative of consistent measurements. The control samples had a more variable flow rate than the other samples varying from 4.83x10^{-4} ms^{-1} to 7.66x10^{-4} ms^{-1}. This is thought to be due to variations in cementation (Fig 5).

Fig 4 shows the range of unconfined compressive strength measured for the indigenous bacteria and the control bacteria. Similar performance can be observed between the samples with Sample B having slightly higher strength than the other samples and the control samples. Those values are similar to those produced by others. For example, for MICP utilising the
bacteria *Sporosarcina pasteurii*, compressive strengths ranging between 200–240 kPa have been reported where a slow flow treatment approach was used [51], compressive strengths of 290–870 kPa are reported with 42 treatment cycles in a sand column in comparison to the 10 treatment cycles in this study [52]. UCS ranging between 215–932 kPa have been reported where a different organism *Bacillus* sp. VS1 was used however, this was for a 20mm height 10mm width test sample, extracted from a larger structure [2]. A study published by [3] was performed at room temperature (20–25˚C) and used twice the concentration of biocementation reagents with a bacteria isolated from a non-contaminated environment. They were able to report higher strength values, in the 0.8 to 1.6 MPa range this could be due to the face that their samples were dried at 60˚C before strength measurement. The lack of sand consolidation studies performed at 15˚C makes it hard to compare our data with other papers. A study published by [46,53] observed that at 15˚C the strength value is higher with *Bacillus megaterium* when compared with *Sporosarcina pasteurii*. In our study, the indigenous bacteria showed higher strength, permeability and CaCO$_3$ precipitation than the control bacteria. We believe that the higher performance at lower temperature could be due to the longer retention time of bacteria combined with slow and steady enzyme performance leading to higher urea degradation into carbonate.

The growth of crystals caused a reduction in the void ratio of the soils alongside the creation of agglomerated/cemented particles (Fig 5). The reduction in the void ratio resulted in increased strength and decreased permeability in the bacterial columns. When comparing strength and permeability among the bacterial columns, the permeability change was not significantly different compared to strength. This could be attributed to the varied amount of calcium carbonate precipitated among the bacterial columns. The observed morphology of biologically-induced mineral crystals formed varied among organisms (Fig 5). It is generally accepted that the microbe has an impact on the morphology of the crystals, such as those described in Branson, Bonnin [54]. The extracellular precipitation can be associated with the morphologies of the minerals [55]. Hence, the variation in morphology can result from either the interaction of the organism with the crystals, or it can be a secondary effect caused by the extent of the chemical changes in the sand medium generated by the organism [56–58]. An assessment of this can be made by observing the microstructure of the resulting sand columns.

![Fig 4. Unconfined compressive strength among indigenous bacteria found in the sand columns. A = Pseudomonas nitroreducens szh_aesj15; B = Bacillus sp. xjlu_herc15; C = Bacillus licheniformis adseedstjo15.](https://doi.org/10.1371/journal.pone.0254676.g004)
Differences (outlined below) in crystal extent are observed between the biotic and abiotic samples indicating that the rate of growth and extent of the crystals is enhanced by the presence of the microorganisms (Fig 5A–5C). This inferred difference in crystal growth rate is also indicated by the lower pH observed in the control [43,45,59].

As predicted by the permeability tests the observable pore crystal density is approximately equivalent for all the sand samples from the columns containing bacteria. For the control
sample (Fig 5D), the crystals appear to be more widely spaced and sparse. There are clusters of crystals in some voids, while others remain entirely free from crystals. The calcite crystals are believed to be affected by factors such as CO$_2$ concentration, pH, particle surface charge, rate of carbonation, and reactants [60]. For example, Al Qabany, Soga [61] showed that the precipitation pattern at the pore scale is affected by the injection concentration, and lower chemical concentrations result in better distribution of calcite precipitation. Studies performed by Soon, Lee [62] showed effective calcite crystals formation and bonding employing $B$.megaterium concentration of $1 \times 10^8$ cfu/mL, cementation reagent concentration of 0.5M, and flow pressure of 1.1 bar for a treatment duration of 48 h for residual soil. Higher flow rate (2 bar) resulted in pore-water pressure and disturbance in the media. In addition, the growth of crystals on some grains and not others may be a function of surface roughness/mineralogy.

XRD analysis provided information of the composition, and crystalline structure of the biominerals. Calcite and vaterite were precipitated by all the bacterial isolates in this study (Fig 6A–6C). Calcite was found to be dominant in the reference sample when compared to bacterial samples (Fig 6D). This is clearly different from the full spectra for calcite developed in the samples with bacteria. Both vaterite and calcite precipitation are kinetically favoured by the high pH of the fluid phase. Vaterite (which is a metastable phase) can be dissolved and reprecipitated as calcite at low solution supersaturations [63]. They also showed that the rate of dissolution decreases at supersaturation ratios of 1.5 and above. Hence, the presence of urease enzymes may potentially favor calcite formation in the samples and since it is absent in the control, vaterite was found to be dominant. The production of both calcite and vaterite also occurred in other studies; this is particularly the case when calcium chloride is used as a calcium source [6,31,51,64]. Our data also showed vaterite in bacterial samples but at smaller levels which was also reported by several authors [3,6,29,47].

The measured percentage of CaCO$_3$ in the sand columns containing bacteria measured by titration was higher by approximately 3% compared to that of the control sand column (Fig 7). The mean CaCO$_3$% produced by the MICP process ranged from 14.42% to 14.63% varying by microbe, compared to 11.75% for the control sample. The standard deviations were below 1% for all the samples. Given the measured difference in permeability and strength, a higher difference in CaCO$_3$ was expected. It is interesting to note that the difference in CaCO$_3$ content between the samples and the control is the result of ~7% void space within the sand media. In an engineering perspective, this difference suggests that the distribution of CaCO$_3$ produced by the organism is significant for the modification of permeability and strength.

It is promising that bacteria existing in and thus, resistant to toxic environments have the potential for biomineralization. This may help addressing environmental concerns over the introduction of non-native bacterial species into the environment. Zamarreño et al. [43] reported that heavy metals concentrations were shown to have an impact on the bacterial diversity within the area from which the bacteria used in this study were isolated. The isolation of MICP capable bacteria from landfill leachate containing high concentrations of heavy metals is indicative of the potential application of this technique in such hazardous conditions. The major challenge to this approach is that it is likely that the presence of MICP capable bacteria may vary and may potentially be absent depending on the location, making it a site-specific technique. In addition, the variation in the strengths that are produced by different micro-organisms may vary creating inconsistencies.

**Conclusions**

MICP is currently observed as a promising technique for applications in construction materials and ground improvement. However, there is a need to understand if indigenous bacteria
available in contaminated environments such as landfills can precipitate calcium as well as commonly known bacteria. This can open up new possibilities, other than ground improvement, such as heavy metals entrapment avoiding environmental degradation. In that perspective, the indigenous bacteria identified from the landfill and investigated has shown potential to perform better than *Bacillus megaterium*. The permeability reduction of the indigenous bacteria ranged between 4.79 E-05 to 5.6534 E-05 as compared to the control at only 4.56 E-05. This was in line with the amount of CaCO\(_3\) precipitated, 14.4–14.7% for the indigenous and 13.6% for the control bacteria. In terms of UC strengths, the indigenous bacteria values ranged between 160–258 kPa in comparison to 160 kPa for the control.

In summary, the utilisation of *in situ* organisms within this work was an attempt to assess the existence of potentially MICP microorganisms within the natural engineering environment. Using organisms that are already on site generates much less environmental concern than injecting bacteria which could potentially change the microbial dynamics of the environment. Also, utilizing *in-situ* organisms can potentially reduce the application cost. However, it should also be acknowledged that field application poses several challenges, for e.g., the level of ground improvement produced will be highly dependent on the bacteria present on site and may not be as extensive as that produced using an organism purchased from a culture collection. In addition, the hazardous chemicals present in the leachate may pose too many variables to fully understand the toxicity limits of these organisms.

**Author Contributions**

**Conceptualization:** Stephen Wilkinson.

**Formal analysis:** Charles K. S. Moy.

**Investigation:** Adharsh Rajasekar, Charles K. S. Moy.

**Methodology:** Raju Sekar.

**Supervision:** Charles K. S. Moy.
References

1. Chu J., Stabnikov V., and Ivanov V., Microbially Induced Calcium Carbonate Precipitation on Surface or in the Bulk of Soil. Geomicrobiology Journal, 2012. 29(6): p. 544–549.

2. Chu J., et al., Microbial method for construction of an aquaculture pond in sand. Géotechnique, 2013. 63(10): p. 871–875.

3. Chu J., et al., Optimization of calcium-based bioclogging and biocementation of sand. Acta Geotechnica, 2014. 9(2): p. 277–285.

4. Zhu T. and Dittrich M., Carbonate Precipitation through Microbial Activities in Natural Environment, and Their Potential in Biotechnology: A Review. Frontiers in Microbiology, 2016. 4(4): p. 1–21.

5. Krajewska B., Urease-aided calcium carbonate mineralization for engineering applications: A review. Journal of Advanced Research, 2018. 13: p. 59–67. https://doi.org/10.1016/j.jare.2017.10.009 PMID: 30094083

6. Hoang T., et al., Sand and silty-sand soil stabilization using bacterial enzyme induced calcite precipitation (beicp). Canadian Geotechnical Journal, 2019. 56(6): p. 808–822.

7. Neupane D., et al., Distribution of mineralized carbonate and its quantification method in enzyme mediated calcite precipitation technique. Soils and Foundations, 2015. 55(2): p. 447–457.

8. Canakci H., Sidik W., and Hail Kilic I., Effect of bacterial calcium carbonate precipitation on compressibility and shear strength of organic soil. Soils and Foundations, 2015. 55(5): p. 1211–1221.

9. Montoya B., DeJong J.T., and Boulanger R., Dynamic response of liquefiable sand improved by microbial-induced calcite precipitation. Géotechnique, 2013. 63.

10. Paassen L.A.v., et al., Quantifying Biomediated Ground Improvement by Ureolysis: Large-Scale Bioout Experiment. Journal of Geotechnical and Geoenvironmental Engineering, 2010. 136: p. 1721–1728.

11. Sun X., Miao L., and Wang C., Glucose addition improves the bio-remediation efficiency for crack repair. Materials and Structures, 2019. 52: p. 111–129.

12. Sun X., et al., Theoretical quantification for cracks repair based on microbially induced carbonate precipitation (MICP) method. Cement and Concrete Composites, 2021. 118: p. 103950.

13. DeJong J.T., Fritzges M.B., and Nüsslein K., Microbially Induced Cementation to Control Sand Response to Undrained Shear. Journal of Geotechnical and Geoenvironmental Engineering, 2006. 132: p. 1381–1392.

14. Castro-Alonso M.J., et al., Microbially Induced Calcium Carbonate Precipitation (MICP) and Its Potential in Bioconcrete: Microbiological and Molecular Concepts. Frontiers in Materials, 2019. 6(126): p. 1–15.

15. Azadi M., et al., Physical and mechanical properties of reconstructed bio-cemented sand. Soils and Foundations, 2017. 57(5): p. 698–706.

16. DeJong J.T., Soga K., and Kavazanjian E., Biogeochemical processes and geotechnical applications: progress, opportunities and challenges. Géotechnique, 2013. 63.

17. Okwadha G.D. and Li J., Optimum conditions for microbial carbonate precipitation. Chemosphere, 2010. 81(9): p. 1143–1148. https://doi.org/10.1016/j.chemosphere.2010.09.066 PMID: 20947128

18. Tobler D.J., et al., Comparison of rates of ureolysis between Sporosarcina pasteurii and an indigenous groundwater community under conditions required to precipitate large volumes of calcite. Geochimica et Cosmochimica Acta, 2011. 75(11): p. 3290–3301.

19. Mahanty B., Kim S., and Kim C.G., Assessment of a biostimulated or bioaugmented calcification system with Bacillus pasteurii in a simulated soil environment. Microbial Ecology, 2013. 65(3): p. 679–688. https://doi.org/10.1007/s00248-012-0137-4 PMID: 23229414

20. Cheng L. and Cord-Ruwisch R., Selective enrichment and production of highly urease active bacteria by non-sterile (open) chemostat culture. Journal of Industrial Microbiology and Biotechnology, 2013. 40(10): p. 1095–1104. https://doi.org/10.1007/s10295-013-1310-6 PMID: 23892419

21. Kang C.H., et al., Microbially induced calcite precipitation-based sequestration of strontium by Sporosarcina pasteurii WJ-2. Applied Biochemistry and Biotechnology, 2014. 174(7): p. 2482–2491. https://doi.org/10.1007/s12010-014-1196-4 PMID: 25190302

22. Helmi F.M., et al., Calcium carbonate precipitation induced by ureolytic bacteria Bacillus licheniformis. Ecological Engineering, 2016. 90: p. 367–371.

23. Chen Z., et al., Biominalization of Pb(II) into Pb-hydroxyapatite induced by Bacillus cereus 12–2 isolated from Lead-Zinc mine tailings. J Hazard Mater, 2016. 301: p. 531–7. https://doi.org/10.1016/j. jhazmat.2015.09.023 PMID: 26468754
24. Sun X., et al., Improvement of bio-cementation at low temperature based on Bacillus megaterium. Applied Microbiology and Biotechnology, 2019. 103(17): p. 7191–7202. https://doi.org/10.1007/s00253-019-09986-7 PMID: 31250062

25. Oualha M., et al., Microbially induced calcite precipitation in calcareous soils by endogenous Bacillus cereus, at high pH and harsh weather. Journal of Environmental Management, 2020. 257: p. 109965. https://doi.org/10.1016/j.jenvman.2019.109965 PMID: 31868651

26. Rahman M.M., et al., State-of-the-Art Review of Microbial-Induced Calcite Precipitation and Its Sustainability in Engineering Applications. Sustainability, 2020. 12: p. 6281.

27. Cheng L. and Cord-Ruwisch R., In situ soil cementation with ureolytic bacteria by surface percolation. Ecological Engineering, 2012. 42: p. 64–72.

28. Rajasekar A., Moy C.K.S., and Wilkinson S., MICP and Advances towards Eco-Friendly and Economical Applications, in 8th International Conference on Environmental Science and Technology (ICAST 2017). 2017, IOP Conference Series: Earth and Environmental Science: Spain.

29. Mahawish A., Bouazza A., and Gates W.P., Improvement of Coarse Sand Engineering Properties by Microbially Induced Calcite Precipitation. Geomicrobiology Journal, 2018. 35(10): p. 887–897.

30. Stabnikov V., et al., Halotolerant, alkaliphilic urease-producing bacteria from different climate zones and their application for biocementation of sand. World Journal of Microbiology and Biotechnology, 2013. 29: p. 1453–1460. https://doi.org/10.1007/s11274-013-1309-1 PMID: 23529354

31. Sun X., et al., Improvement of Microbial-Induced Calcium Carbonate Precipitation Technology for Sand Solidification. Journal of Materials in Civil Engineering, 2018. 30(11): p. 04018301.

32. Gowthaman S., Nakashima K., and Kawasaki S., Freeze-thaw durability and shear responses of cemented slope soil treated by microbial induced carbonate precipitation. Soils and Foundations, 2020. 60: p. 840–855.

33. Liu P., Shao G.-h., and Huang R.-p., Study of the interactions between S. pasteurii and indigenous bacteria and the effect of these interactions on the MICP. Arabian Journal of Geosciences, 2019. 12: p. 724.

34. Burbank M.B., et al., Precipitation Of Calcite By Indigenous Microorganisms To Strengthen Liquefiable Soils. Geomicrobiology Journal, 2011. 28: p. 301–312.

35. Arbu P., et al., Formations of calcium carbonate minerals by bacteria and its multiple applications. Springer plus. 2016. 5(250): p. 1–26. https://doi.org/10.1186/s40064-016-1869-2 PMID: 27026942

36. Nayanthara P.G.N., et al., Native Inland Bacterium for Beach Sand Stabilization in Nearshore Areas. Applied Sciences, 2019. 9: p. 3201.

37. Dhami N.K., Reddy M.S., and Mukherjee A., Biomineralisation of calcium carbonates and their engineered applications: a review. Frontiers in Microbiology, 2013. 4(314): p. 1–13. https://doi.org/10.3389/fmicb.2013.00314 PMID: 24194735

38. Tobler D.J., Maclachlan E., and Phoenix V.R., Microbially mediated plugging of porous media and the impact of differing injection strategies. Ecological Engineering, 2012. 42: p. 270–278.

39. Yasuhara H., et al., Experiments and predictions of physical properties of sand cemented by enzymatically-induced carbonate precipitation. Soils and Foundations, 2012. 52(3): p. 539–549.

40. Neupane D., et al., Applicability of Enzymatic Calcium Carbonate Precipitation as a Soil-Strengthening Technique. Journal of Geotechnical and Geoenvironmental Engineering, 2013. 139(12): p. 2201–2211.

41. Omorogie A.I., et al., Experimental optimisation of various cultural conditions on urease activity for isolated Sporosarcina pasteurii strains and evaluation of their biocement potentials. Ecological Engineering, 2017. 109: p. 65–75.

42. Kang C.-H., et al., Bioremediation of lead by ureolytic bacteria isolated from soil at abandoned metal mines in South Korea. Ecological Engineering, 2015. 74: p. 402–407.

43. Zamarreño D.V., May E., and Inkpen R., Influence of Environmental Temperature on Biocalcification by Non-sporing Freshwater Bacteria. Geomicrobiology Journal, 2009. 26(4): p. 298–309.

44. Rajasekar A., et al., Next-generation sequencing showing potential leachate influence on bacterial communities around a landfill in China. Canadian Journal of Microbiology, 2018. 64(8): p. 537–549. https://doi.org/10.1139/cjm-2017-0543 PMID: 29633622

45. Rajasekar A., et al., Biominerallisation performance of bacteria isolated from a landfill in China. Canadian Journal of Microbiology, 2018. 64(12): p. 945–953. https://doi.org/10.1139/cjm-2018-0254 PMID: 30148972

46. Sun X., et al., Study of the effect of temperature on microbially induced carbonate precipitation. Acta Geotechnica, 2019. 14: p. 627–638.
47. Cheng L., Cord-Ruwisch R., and Shahin M.A., Cementation of sand soil by microbially induced calcite precipitation at various degrees of saturation. Canadian Geotechnical Journal, 2013. 50(1): p. 81–90.
48. Harkes M.P., et al., Fixation and distribution of bacterial activity in sand to induce carbonate precipitation for ground reinforcement. Ecological Engineering, 2010. 36(2): p. 112–117.
49. BSI, Geotechnical investigation and testing—Laboratory testing of soil Part 11: Permeability tests, BS EN ISO 17892-11:2019, British Standards Institution. p. 20.
50. Maulood P.M., et al., Comparison between Calcimetric and Titrimetric Methods for Calcium Carbonate Determination. Open Journal of Soil Science, 2012. 2: p. 263–268.
51. Shahrokhi-Shahraki R., et al., Improving sand with microbial-induced carbonate precipitation. Ground improvement, 2015. 168(3): p. 217–230.
52. Henze J. and Randall D.G., Microbial induced calcium carbonate precipitation at elevated pH values (>11) using Sporosarcina pasteurii. Journal of Environmental Chemical Engineering, 2018. 6(4): p. 5008–5013.
53. Peng J. and Liu Z., Influence of temperature on microbially induced calcium carbonate precipitation for soil treatment. PLoS One, 2019. 14(6): p. e0218996. https://doi.org/10.1371/journal.pone.0218996 PMID: 31211807
54. Branson O., et al., Nanometer-Scale Chemistry of a Calcite Biomineralization Template: Implications for Skeletal Composition and Nucleation. Proceedings of the National Academy of Sciences of the United States of America, 2016. 113(46): p. 12934–12939. https://doi.org/10.1073/pnas.1522864113 PMID: 27794119
55. Meier A., et al., Microbial communities in carbonate rocks—from soil via groundwater to rocks. Journal of Basic Microbiology, 2017. 57(9): p. 752–761. https://doi.org/10.1002/jobm.201600643 PMID: 28681946
56. Sanchez-Roman M., et al., Bimineralization of carbonate and phosphate by moderately halophilic bacteria. FEMS Microbiology Ecology, 2007. 61(2): p. 273–284. https://doi.org/10.1111/j.1574-6941.2007.00336.x PMID: 17535298
57. Zamarren o D.V., Inkpen R., and May E., Carbonate crystals precipitated by freshwater bacteria and their use as a limestone consolidant. Applied and Environmental Microbiology, 2009. 75(18): p. 5981–5990. https://doi.org/10.1128/AEM.02079-08 PMID: 19617383
58. Silva-Castro G.A., et al., Carbonate Precipitation of Bacterial Strains Isolated from Sediments and Seawater: Formation Mechanisms. Geomicrobiology Journal, 2013. 30(9): p. 840–850.
59. Achal V. and Pan X., Influence of calcium sources on microbially induced calcium carbonate precipitation by Bacillus sp. CR2. Applied Biochemistry and Biotechnology, 2014. 173(1): p. 307–317. https://doi.org/10.1007/s12010-014-0842-1 PMID: 24643454
60. Chang R., et al., Calcium Carbonate Precipitation for CO2 Storage and Utilization: A Review of the Carbonate Crystallization and Polymorphism. Frontiers in Energy Research, 2017. 5(17).
61. Al Qabany A., Soga K., and Santamarina C., Factors affecting efficiency of microbially induced calcite precipitation. J Geotech Geoenviron Eng, 2012. 138.
62. Soon N.W., et al., Factors Affecting Improvement in Engineering Properties of Residual Soil through Microbial-Induced Calcite Precipitation. Journal of Geotechnical and Geoenvironmental Engineering, 2013. 140(5): p. 1–11.
63. Spanos N. and Koutsoukos P.G., The transformation of vaterite to calcite: effect of the conditions of the solutions in contact with the mineral phase. Journal of Crystal growth, 1998. 191: p. 783–790.
64. Dilrukshi R.A.N., Nakashima K., and Kawasaki S., Soil improvement using plant-derived urease-induced calcium carbonate precipitation. Soils and Foundations, 2018. 58(4): p. 894–910.