Microstructural and Geomechanical Study on Microbial-Carbonized Sand Using Streptomyces Microflavus for Dust Control

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Microbial carbonization based on carbon-capturing bacteria induced calcium carbonate precipitation has become a new construction material for soil improvement. This paper presents a new technique for dust control in sand using Streptomyces microflavus induced calcium carbonate precipitation. This kind of carbon-capturing bacteria can capture and transform CO2 into carbonate then precipitate calcium carbonate crystals through the microbial carbonization process, which then acts as cementitious materials to bind sand particles. In this way, loose sand particles can be bridged and increase their hardness, consequently forming a consolidated layer for dust control. The Shore hardness of the consolidated layer using Streptomyces microflavus could be improved to 25 HD with a calcium carbonate content of 5.5% after 7 times of carbonization treatment. The microstructure, Shore hardness, and obtained calcium carbonate content were also compared with sand samples treated by Paenibacillus mucilaginosus. These results reveal that the consolidated sand layer using Streptomyces microflavus is sufficient to against wind erosion or rainfall erosion and has better geomechanical performance than Paenibacillus mucilaginosus.

Keywords: calcium carbonate precipitation, microbial carbonization, carbon-capturing bacteria, dust control, shore hardness

INTRODUCTION

Fugitive dust is a common issue in desert areas that leads to sandstorms (Raymond et al., 2019). Planting is the most adopted method for dust control in the desert area (Grantz et al., 1998). However, the growth cycle of planting is too long to be effective for dust control. Apart from the planting, using Portland Cement (PC) is another conventional method for dust control. Considering the complex operation, unexpected energy consumption, environmental concerns, and considerable CO2 emission associated with PC production, investigation on alternative cement has been raised (Benhelal et al., 2013; Ruan and Unluer, 2017).

Microbial cement has been proposed in various applications such as erosion control, mitigation of liquefaction, and slope reinforcement (Yu et al., 2019; Yu and Rong, 2022). Unlike cement paste, the small size of microorganisms (2–5 μm) can easily penetrate the desert sand (0.1–0.25 mm) particles without injection, bridging the loose sand particles together and forming a consolidated layer with certain hardness (Hamdan and Kavazanjian, 2016; Zhan et al., 2016). More importantly, the
NH_{3} and CO_{2}, further precipitating calcium carbonate in a microbial or plant seeds can decompose urea to form \( \text{HCO}_3^- \). The urease enzyme from either sand particles as a dust suppressant (Hamdan and Kavazanjian, 2014). This leads to microbial cement becoming an innovative method for dust control. Other than the microbial method, enzyme induced calcite precipitation (EICP) is another widely used method to produce calcium carbonate crystals that can bind sand particles as a dust suppressant (Cheng, 2012; Cheng and Cord-Ruwisch, 2014). This leads to microbial cement becoming an innovative method for dust control. Other than the microbial method, enzyme induced calcite precipitation (EICP) is another widely used method to produce calcium carbonate crystals that can bind sand particles as a dust suppressant (Hamdan and Kavazanjian, 2016; Sun et al., 2021). The precipitates act as a binding material to connect soil particles, consequently providing strength but keeping certain permeability (Cheng, 2012; Cheng and Cord-Ruwisch, 2014). The feasibility of using Streptomyces microflavus for dust control is investigated in this technical note, which can accelerate the conversion of CO_{2} into \( \text{HCO}_3^- \) in an aqueous environment. The method for dust control and several parameters related to the consolidation performance using Streptomyces microflavus are discussed. These include the Shore hardness of the consolidated layer, the content of precipitates, and the microstructure of consolidated sand.

**MATERIALS AND METHODS**

**Carbon-Capturing Bacteria Preparation**

The carbon-capturing bacteria of *Streptomyces microflavus* (DSM 40070) adopted in this study was purchased from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH. The growth culture of *Streptomyces microflavus* comprises 15 g/L of glucose, 10 g/L of yeast extract, and 5 g/L of peptone with an initial pH of 9 adjusted by 1 M of NaOH. The growth culture was put into a sterilization pot at 121°C for 25 min and then cooled to room temperature. Inoculation of the *Streptomyces microflavus* strain solution (4%) was carried out on a sterile operation platform, followed by shaking in an orbital shaker at 260 rpm for 72 h. The harvested bacteria were stored in a refrigerator at 4°C prior to use. The average concentration of CA was measured by the enzyme linked immunosorbent assay (ELISA) kit method (Xu et al., 2021) and determined as 0.0082 mg/L. The OD values of *Streptomyces microflavus* were 2.4 ± 0.1. The pH of the *Streptomyces microflavus* was about 5–6.

**Consolidation Test of the Sand Layer**

Petri dish (90 mm in diameter) was filled with 50 g of Ottawa sand with a mean grain size of D_{50} = 0.4 mm. The sand was evenly spread out to form a sand layer with a thickness of 0.5 cm. This sand layer had a void volume of 12 cm³. During treatment, a mixture of 15 ml of *Streptomyces microflavus* and 0.01 M of Ca(NO_{3})_{2} (volume ratio 1 : 1) was evenly sprayed on sand surface, followed by curing in the carbonation box with a 10% of carbon dioxide concentration. Treatment was repeated 1, 3, 5, and 7 times and each treatment was 24 h. After carbonization, all the sand samples were rinsed with tap water and oven-dried at

\[
\text{CA} + \text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{HCO}_3^- + \text{H}^+ \quad (1)
\]
\[
\text{HCO}_3^- + \text{OH}^- \leftrightarrow \text{CO}_3^{2-} + \text{H}_2\text{O} \quad (2)
\]
\[
\text{Ca}^{2+} + \text{CO}_3^{2-} \leftrightarrow \text{CaCO}_3 \quad (3)
\]
70°C for 48 h prior to hardness testing and calcium carbonate content measurement. A comparative study using *Paenibacillus mucilaginosus* (DSM 24461) was also conducted with the same cultivation and treatment process. All tests were conducted in duplicate.

**Hardness of Consolidated Sand Layer and Calcium Carbonate Content Measurement**

A digital Shore hardness tester was used to determine the hardness of the consolidated sand layer. The calcium carbonate content was determined by the acid-washing and rinsing method (Cheng et al., 2013). For each test, hardness and calcium carbonate content were measured at least three times to obtain an average value.

**Microstructural Analyses**

The microstructure of all microbial carbonized sand samples was analyzed by Field Emission Scanning Electron Microscope (FESEM) (Zeiss EV050, United Kingdom). All samples were rinsed with tap water and oven-dried at 105°C for 24 h prior to the SEM analysis. Chemical composition of all samples was determined by powder X-ray diffraction analysis (5–80°) (XRD, Bruker D8-Advance, λ = 1.5406Å, Bruker Company, Karlsruhe, Germany).

**RESULTS AND DISCUSSION**

**Shore Hardness of Consolidated Sand Layer**

The correlation between the hardness and the treatment times using different carbon-capturing bacteria species is presented in Figure 2. It can be seen that the hardness of all sand samples increased after the consolidation test using *Streptomyces microflavus* and *Paenibacillus mucilaginosus*. This was because the CO₂ was adsorbed, transformed, and generated carbonate ions under the enzymatic action of both carbon-capturing bacteria. Consequently, the carbonate ions could react with soluble calcium and form calcium carbonate precipitation, bridging the loose sand particles together and forming a consolidated sand layer that has mechanical strength. A similar observation was made by Zhan et al. (2016).

More importantly, the hardness increased with the increase of treatment times, which was attributed to the higher calcium carbonate precipitation that was associated with the greater hardness as previous literature reported (DeJong et al., 2013; Mousavi et al., 2021). As shown in Figure 3, the average shore hardness of consolidated layer using *Streptomyces microflavus* and Ca(NO₃)₂ were 0.95, 10.70, 15.00, and 24.70 HD after 1, 3, 5, and 7 times of treatment, separately. In comparison, the samples treated by *Paenibacillus mucilaginosus* and Ca(NO₃)₂ had average shore hardness of 0.25, 4.42, 11.42, and 15.25 HD, respectively. The results suggested that the carbonization efficiency of *Streptomyces microflavus* improved 62% after 7 treatments compared to *Paenibacillus mucilaginosus*. This was probably due to the *Paenibacillus mucilaginosus* being characterized as a facultatively anaerobic bacterium (Liu and Lian, 2019). The 10% of carbon dioxide concentration inhibited the performance of CA from *Paenibacillus mucilaginosus*. Thus, the absorption rate of CO₂ catalyzed by CA from *Paenibacillus mucilaginosus* was lower than *Streptomyces microflavus* in the carbonation box. The result suggests the consolidation layer strengthened by *Streptomyces microflavus* has prospective application like dust control that require certain mechanical properties. For example, the calcite-consolidation layer could cement fugitive dust and against wind-erosion with the hardness of 24.6 HD (Zhan et al., 2016).

**Calcium Carbonate Content Measurement**

The effect of treatment times on the calcium carbonate content using different carbon-capturing bacteria is shown in Figure 3.
Increasing the number of sprays led to the increase in calcium carbonate content. The calcium carbonate content of sand samples treated by *Streptomyces microflavus* and Ca(NO₃)₂ system were varied from 1.06, 3.11, 4.67, and 5.58% corresponding to 1, 3, 5, and 7 times of treatments. In the *Paenibacillus mucilaginosus* and Ca(NO₃)₂ system, the calcium carbonate content were 1.19, 2.59, 3.40, and 4.26% after 1, 3, 5, and 7 times of treatment, which were lower than those obtained by *Streptomyces microflavus* and Ca(NO₃)₂ system. This also can be explained by the characteristic of anaerobic *Paenibacillus mucilaginosus*, which shows weaker carbonization efficiency than aerobic bacterium of *Streptomyces microflavus* at high CO₂ level. The calcium carbonate contents results were in line with the previous hardness results, where the calcium carbonate content increased as the increase of the number of sprays, consequently contributed to the greater hardness. Therefore, the *Streptomyces microflavus* could better capture and transform CO₂ than the *Paenibacillus mucilaginosus* to precipitate calcium carbonate crystals in the carbonization box.

**Microstructure of the Microbial Carbonized Samples**

The microstructure of precipitates induced from two types of carbon-capturing bacteria was analyzed by FESEM, as shown in Figures 4A,B, respectively. FESEM showed that the morphology of the mineralized sediments by *Streptomyces microflavus* was typical rhombohedral-shaped crystals with a particle size varied from 10–30 μm (Figure 4A), known as calcite (Reddy, 2013). This is the most stable polymorph of CaCO₃ due to its lowest solubility (Van Paassen, 2009). Unlike the regular CaCO₃ crystals precipitated by *Streptomyces microflavus*, the morphology of the *Paenibacillus mucilaginosus* precipitated CaCO₃ was mainly spherical crystals with a single crystal size of 1–10 μm, (Figure 4B). The spherical shape of CaCO₃ is consistent with the shape of vaterite (Chu et al., 2013), which can be transformed into a more stable polymorph of CaCO₃, such as calcite, via the dissolution and reprecipitation process (Van Paassen, 2009). Although both shapes of CaCO₃ crystals were able to connect

![Figure 4](image-url)
sand particles and provide mechanical strength during the carbonization process, the Shore hardness of the consolidated layer using *Streptomyces microflavus* was higher than *Paenibacillus mucilaginosus* from previous results. This is because the shapes, sizes, and stabilities of CaCO₃ precipitates are critical factors that control its engineering properties, similar observations were made by Al Qabany et al. (2012). For example, the crystal transformed by *Streptomyces microflavus* was stable calcite, while *Paenibacillus mucilaginosus* generated unstable vaterite. However, the final formation of crystals could undergo phase transformation under the influence of other parameters, such as temperature, treatment times, and concentration of cementation solution (Wang et al., 2019).

**CONCLUSION**

This paper presented a method to consolidate sand using *Streptomyces microflavus* for dust control. The proposed microbial carbonization method provided an effective, low-cost, environmentally friendly, and publicity acceptable method for dust control in the desert area, which could effectively accelerate the conversion of CO₂ into HCO₃⁻ and drive biomimeralization through microbial carbonization pathway. The following conclusions can be drawn from this study:

1) Under the enzymatic action of *Streptomyces microflavus*, CO₂ was absorbed and transformed, consequently producing carbonate ions to mineralize calcium ions into calcium carbonate precipitation. In this process, sand was cemented and formed a consolidated sand layer, which had adequate mechanical strength for dust control.

2) The Shore hardness and calcium carbonate content were measured on the consolidated layer using *Streptomyces microflavus*, which had higher Shore hardness and calcium carbonate content than the samples treated by *Paenibacillus mucilaginosus* under the same treatment conditions. The comparison indicates that using *Streptomyces microflavus* is a more efficient and sustainable method for dust control.

3) The SEM images show effective cementation among sand grains by both carbon-capturing bacteria. The *Streptomyces microflavus* mainly precipitated rhombohedral-shaped calcite, which is the most stable polymorph than the *Paenibacillus mucilaginosus* generated unstable vaterite. This resulted in the variance of the engineering properties of the consolidated sand layer through the microbial carbonization process.

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

**AUTHOR CONTRIBUTIONS**

XY: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, and Writing—original draft. QM: Methodology, Investigation, and Data curation. YY: Conceptualization, Methodology, Validation, Resources, Writing—review and editing, and Funding acquisition. WZ: Validation and Writing—review and editing. LC: Conceptualization, Validation, and Writing—review and editing.

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**REFERENCES**

Al Qabany, A., Soga, K., and Santamaria, C. (2012). Factors Affecting Efficiency of Microbially Induced Calcite Precipitation. *J. Geotech. Geoenviron. Eng.* 138 (8), 992–1001. doi:10.1061/(asce)gt.1943-5606.0000666

Benhelal, E., Zahedi, G., Shamsaei, E., and Bahadori, A. (2013). Global Strategies and Potentials to Curb CO₂ Emissions in Cement Industry. *J. Clean. Prod.* 51, 142–161. doi:10.1016/j.jclepro.2012.10.049

Cheng, L., Cord-Ruwisch, R., and Shahin, M. A. (2013). Cementation of Sand Soil by Microbially Induced Calcite Precipitation at Various Degrees of Saturation. *Can. Geotech. J.* 50 (1), 81–90. doi:10.1139/cgj-2012-0023

Cheng, L., and Cord-Ruwisch, R. (2014). Upscaling Effects of Soil Improvement by Microbially Induced Calcite Precipitation by Surface Percolation. *Geomicrobiology J.* 31 (5), 396–406. doi:10.1080/01490451.2013.836579

Cheng, L. (2012). Innovative Ground Enhancement by Improved Microbially Induced CaCO₃ Precipitation Technology. Murdoch University.

Chu, D. H., Vinoba, M., Bhagyalakshmi, M., Hyun Baek, I., Nam, S. C., Yoon, Y., et al. (2013). CO₂ Mineralization into Different Polymorphs of CaCO₃ Using an aqueous-CO₂ System. *RSC Adv.* 3 (44), 21722–21729. doi:10.1039/c3ra44007a

De Jong, J. T., Mortensen, B. M., Martinez, B. C., and Nelson, D. C. (2010). Biomediated Soil Improvement. *Ecol. Eng.* 36 (2), 197–210. doi:10.1016/j.ecoleng.2008.12.029

De Jong, J. T., Soga, K., Kavazanjian, E., Burns, S., Van Paassen, L. A., Al Qabany, A., et al. (2013). Biogeochemical Processes and Geotechnical Applications: Progress, Opportunities and Challenges. *Géotechnique* 63 (4), 287–301. doi:10.1680/geot.13.p.017

Grantz, D. A., Vaughn, D. L., Farber, R., Kim, B., Zeldin, M., VanCuren, T., et al. (1998). Seeding Native Plants to Restore Desert farmland and Mitigate Fugitive Dust and PM10. Wiley Online Library.

Hamdan, N., and Kavazanjian, E., Jr (2016). Enzyme-induced Carbonate mineral Precipitation for Fugitive Dust Control. *Géotechnique* 66 (7), 546–555. doi:10.1680/geot.15.p.168

Harkes, M. P., Van Paassen, L. A., Booster, J. L., Whiffin, V. S., and van Loosdrecht, M. C. M. (2010). Fixation and Distribution of Bacterial Activity in Sand to Induce Carbonate Precipitation for Ground Reinforcement. *Ecol. Eng.* 36 (2), 112–117. doi:10.1016/j.ecoleng.2009.01.004

Liu, H., and Lian, B. (2019). Quantitative Evaluation of Different Fractions of Extracellular Polymeric Substances Derived from Paenibacillus Mucilaginosus against the Toxicity of Gold Ions. *Colloids Surf. B: Biointerfaces* 175, 195–201. doi:10.1016/j.colsurfb.2018.11.075
Lo, C.-Y., Tirkolaei, H. K., Hua, M., De Rosa, I. M., Carlson, L., Kavazanjian, E., Jr, et al. (2020). Durable and Dustcile Double-Network Material for Dust Control. Geoderma 361, 114090. doi:10.1016/j.geoderma.2019.114090

Mousavi, F., Abdi, E., Ghalandarayeshi, S., and Page-Dumroese, D. S. (2021). Modeling Unconfined Compressive Strength of fine-grained Soils: Application of Pocket Penetrometer for Predicting Soil Strength. Catena 196, 104890. doi:10.1016/j.catena.2020.104890

Mujah, D., Shahin, M. A., and Cheng, L. (2017). State-of-the-art Review of Bioconfinement by Microbially Induced Calcite Precipitation (MICP) for Soil Stabilization. Geomicrobiology J. 34 (6), 524–537. doi:10.1080/01490451.2016.1225866

Raymond, A., Purdy, C., Fox, T., Kendall, A., DeJong, J., Kavazanjian, E., Jr, et al. (2019). Life Cycle Sustainability Assessment of Enzyme-Induced Carbonate Precipitation (EICP) for Fugitive Dust Control. Adv. Civil Eng. 37 (2), 600–607.

Redu, M. S. (2013). Biominalization of Calcium Carbonates and Their Engineered Applications: a Review. Front. Microbiol. 4, 314.

Ruan, S., and Unluer, C. (2017). Influence of Mix Design on the Carbonation, Mechanical Properties and Microstructure of Reactive MgO Cement-Based concrete. Cement and Concrete Composites 80, 104–114. doi:10.1016/j.cemconcomp.2017.03.004

Shi, G., Qi, J., Teng, G., Wang, Y., and Shen, H. (2021). Influence of Coal Properties on Dust Suppression Effect of Biological Dust Supressant. Advanced Powder Technology.

Shi, G., Qi, J., Wang, Y., and Liu, S. (2021). Experimental Study on the Prevention of Coal Mine Dust with Biological Dust Supressant. Powder Technology.

Sun, X., Miao, L., Wang, H., Yuan, J., and Fan, G. (2021). Enhanced Rainfall Erosion Durability of Enzymatically Induced Carbonate Precipitation for Dust Control, 791. Science of The Total Environment, 148369. doi:10.1016/j.scitotenv.2021.148369

Van Paassen, L. (2009). Biogroat, Ground Improvement by Microbial Induced Carbonate Precipitation.

Wang, Y., Soga, K., DeJong, J. T., and Kabla, A. J. (2019). Microscale Visualization of Microbial-Induced Calcium Carbonate Precipitation Processes. J. Geotech. Geoenviron. Eng. 145 (9), 04019045. doi:10.1061/(asce)gt.1943-5606.0002079

Whiffen, V. S. (2004). Microbial CaCO3 Precipitation for the Production of Bio cement. Murdoch University.

Xu, Y., Yu, C., and Yu, X. (2021). Microbial Mineralization and Carbonation Consolidation of Drdger Fill and its Mechanical Properties. J. Mater. Civil Eng. 33 (7), 04021144. doi:10.1061/(asce)mt.1943-5533.0003769

Yu, X., Qian, C., and Jiang, J. (2019). Desert Sand Cemented by Bio-Magnesium Ammonium Phosphate Cement and its Microscopic Properties. Construction Building Mater. 200, 116–123. doi:10.1016/j.conbuildmat.2018.12.046

Yu, X., and Rong, H. (2022). Seawater Based MICP Cements Two/one-phase Cemented Sand Blocks. Appl. Ocean Res. 118, 102972. doi:10.1016/j.apor.2021.102972

Zhan, Q., Qian, C., and Yi, H. (2016). Microbial-induced Mineralization and Cementation of Fugitive Dust and Engineering Application. Construction Building Mater. 121, 437–444. doi:10.1016/j.conbuildmat.2016.06.016

Zhang, D., Shahin, M. A., Yang, Y., Liu, H., and Cheng, L. (2022). Effect of Microbially Induced Calcite Precipitation Treatment on the Bonding Properties of Steel Fiber in Ultra-high Performance concrete. J. Building Eng. 50, 104132. doi:10.1016/j.jobe.2022.104132

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