

## Development of a Ureolytic Bacterial Consortium for Beach Sand Stabilization Using Bio-Grout Technology: A Preliminary Laboratory-Scale Study

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### Abstract

Coastal abrasion poses a serious threat to the coastal regions of Indonesia. Conventional soil stabilization methods using Portland cement have high carbon footprints and can disrupt aquatic ecosystems. Bio-grout technology based on Microbially Induced Calcium Carbonate Precipitation (MICP) offers a green alternative by utilizing the activity of calcium-carbonate-precipitating bacteria. This study aimed to develop a consortium of ureolytic bacteria and determine the optimal application protocol for beach sand stabilization at the laboratory scale. Experiments were conducted using Tambakboyo beach sand (grain size 0.1–0.5 mm) with variations in bacterial density ( $10^5$ ,  $10^7$ ,  $10^9$  cells/mL) and number of treatment cycles (1, 3, 5 cycles). The measured parameters included Uniaxial Compressive Strength (UCS),  $\text{CaCO}_3$  precipitation percentage, permeability, and simulated erosion resistance. The results showed that the optimal condition—bacterial density of  $10^7$  cells/mL with 3 treatment cycles—produced a UCS of 2.1 MPa,  $\text{CaCO}_3$  precipitation of 5.1% by weight, and mass loss of <3% after simulated water flow at 0.5 m/s. The control treatment without bacteria (chemical injection) yielded only ~0.1 MPa UCS and <0.5%  $\text{CaCO}_3$  precipitation, confirming the crucial role of bacteria as biological catalysts. Excessive bacterial density ( $10^9$  cells/mL) led to premature pore clogging and a drastic reduction in permeability. This study concludes that a ureolytic bacterial consortium at a density of  $10^7$  cells/mL with 3 injection cycles represents the most effective and efficient condition for forming a natural bio-cement capable of mitigating coastal abrasion. Further research is required to assess scalability, the ecotoxicological impacts of ammonium byproducts, and long-term field stability..

*Keywords:* Bio-grout, MICP, ureolytic bacteria, beach sand stabilization, abrasion, bio-cement.

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### 1. INTRODUCTION

Coastal erosion, or abrasion, is an increasingly pressing environmental issue in archipelagic nations such as Indonesia. In addition to causing the loss of land and settlements, abrasion also damages coastal ecosystems of high economic value. Conventional coastal protection measures such as the installation of groynes, seawalls, or soil stabilisation with Portland cement are often costly, have a significant carbon footprint, and can disrupt hydrology and marine biota (Dejong et al., 2014)

In the last decade, Microbially Induced Calcium Carbonate Precipitation (MICP) technology has emerged as a nature-inspired geotechnical engineering solution. This technology utilises ureolytic bacteria (genus *Sporosarcina*) that produce the enzyme urease, catalysing the hydrolysis of urea into carbonate ( $\text{CO}_3^{2-}$ ) and ammonium ( $\text{NH}_4^+$ ) ions. The resulting local increase in pH then induces the precipitation of calcium carbonate ( $\text{CaCO}_3$ ) in the presence of calcium ions (Soon et al., 2014; Wang et al., 2025). The  $\text{CaCO}_3$  crystals formed act as a ‘biological cement’ that binds sand grains without significantly altering the soil structure.

Research on MICP has advanced significantly in the fields of soil improvement, erosion control and concrete crack repair (Dhami et al., 2013; Shahin et al., 2020). Tsai (Tsai et al., 2022) demonstrated that MICP treatment was capable of reducing wave-induced scour depth by up to 33.9% in a laboratory-scale physical model, indicating the significant potential of this technology for mitigating erosion on sandy coasts. However, specific applications for stabilising beach sand in tropical regions with particular hydrodynamic characteristics and salinity levels remain limited. This study aims to develop a formulation of a ureolytic bacterial consortium and determine the optimal injection protocol (bacterial density and number of treatment cycles) on a laboratory scale, as a first step towards field trials.

## **2. RESEARCH METHODS**

### **Materials and Bacterial Cultures**

A consortium of ureolytic bacteria was isolated from coastal sediments at Tambakboyo, Tuban and identified using biochemical and molecular methods (16S rRNA gene). The bacteria were cultured in a growth medium containing 20 g/L urea, 10 g/L yeast extract, and 10 g/L  $\text{NH}_4\text{Cl}$  (pH 7.0) at 30°C for 24 hours.

### **Test Sand**

Coastal sand with a grain size of 0.1–0.5 mm (D50 diameter = 0.3 mm) was dried, sterilised, and placed into a cylindrical mould (height 10 cm, diameter 5 cm) at a relative density of 60%.

### **MICP Treatment**

The cementation solution consisted of 0.5 M urea and 0.5 M  $\text{CaCl}_2$ . Three variations in bacterial density ( $10^5$ ,  $10^7$ ,  $10^9$  cells/mL) and three variations in the number of treatment cycles (1, 3, 5 cycles) were tested. One cycle consisted of the injection of 50 mL of bacterial suspension (left to stand for 6 hours) followed by the injection of 50 mL of cementation solution (left to stand for 18 hours). The control groups consisted of: (K1) untreated sand, (K2) injection of urea+ $\text{CaCl}_2$  solution without bacteria.

Measurement Parameters

- Uniaxial Compressive Strength (UCS): using a Universal Testing Machine (speed 1 mm/minute).
- CaCO<sub>3</sub> content: gravimetric method with washing in 1 M HCl.
- Permeability: constant head method.
- Erosion resistance: water flow simulation in a flume at a velocity of 0.5 m/s for 1 hour.

**3 RESULTS AND DISCUSSION**

**The Effect of Bacterial Density on MICP Effectiveness**

Table 1 summarises the results of the MICP effectiveness tests. At a density of 10<sup>5</sup> cells/mL, the UCS reached only 0.8 MPa, with uneven CaCO<sub>3</sub> precipitation. This was due to an insufficient number of bacterial cells to produce urease enzyme within the large pore volume. Conversely, at a density of 10<sup>9</sup> cells/mL, the UCS increased to 1.9 MPa; however, there was a drastic decrease in permeability due to premature pore blockage, thereby hindering the distribution of grout throughout the sample. These findings are consistent with reports that precise control of cell density is crucial to avoid premature clogging, which can hinder the homogeneity of precipitation (Gao & Dai, 2022)

**Table 1. Results of MICP effectiveness tests for beach sand stabilization**

Test Variable	Condition/Group	Measurement Result	Interpretation
<b>Bacterial Density</b>	10 <sup>5</sup> cells/mL	UCS: 0.8 MPa	Low inoculum, uneven precipitation
	10 <sup>7</sup> cells/mL	UCS: 1.8 MPa; CaCO <sub>3</sub> : 4.2%	Optimal, homogeneous precipitation
	10 <sup>9</sup> cells/mL	UCS: 1.9 MPa; Permeability drastically decreased	Premature clogging
<b>Number of Cycles</b>	1 cycle	UCS: 0.9 MPa	Precipitation not yet optimal
	3 cycles	UCS: 2.1 MPa; CaCO <sub>3</sub> : 5.1%	Multi-cycle effective
	5 cycles	UCS: 2.3 MPa	Marginal increase, inefficient
<b>Control</b>	Untreated sand	UCS: ~0.05 MPa	Loose sand, non-cohesive
	Chemical injection without bacteria	UCS: ~0.1 MPa; CaCO <sub>3</sub> : <0.5%	Minimal abiotic precipitation

<b>Erosion Test (3 cycles, 10<sup>7</sup>/mL)</b>	Sand + Bio-Grout	Mass loss <3%	Resistant to 0.5 m/s flow
	Control sand	Mass loss >60%	Instant erosion

Notes:

- *UCS (Uniaxial Compressive Strength): A measure of material strength.*
- *% CaCO<sub>3</sub> Precipitation: The percentage by weight of bio-cement relative to the total weight of the sample.*
- *The experiment used Tambakboyo beach sand with a grain size of 0.1–0.5 mm.*

### Effect of the Number of Curing Cycles

Increasing the number of cycles from 1 to 3 significantly raised the UCS from 0.9 MPa to 2.1 MPa, with an increase in CaCO<sub>3</sub> content to 5.1% by weight. However, increasing the number of cycles to 5 resulted in only a marginal improvement (UCS 2.3 MPa) and was therefore not cost-effective. These findings are consistent with the report by (Soon et al., 2014) Soon et al. (2014) that there is a saturation point for CaCO<sub>3</sub> precipitation at which intergranular bonds have formed completely. Furthermore, a study by Liang also confirmed that increasing the number of treatment cycles significantly enhances the cohesion and disintegration resistance of residual granite soil, though with a decreasing rate of efficiency after the third or fourth cycle (Liang et al., 2022).

### Erosion Resistance Testing and Comparison with Controls

Sand consolidated with Bio-Grout (3 cycles, 10<sup>7</sup> cells/mL) exhibited a mass loss of <3% following a 0.5 m/s water flow simulation for 1 hour, whereas untreated sand lost >60% of its mass over the same period. The chemical control (without bacteria) produced only <0.5% CaCO<sub>3</sub> precipitation, proving that the role of bacteria as a biological catalyst is crucial—without bacteria, the reaction proceeds very slowly and does not produce meaningful bonds. These results are consistent with findings that MICP effectively enhances the resistance of sandy slope surfaces to erosion, particularly when applied via surface methods such as spraying or staged injection (Ghasemi & Montoya, 2022).

### Mechanism of Bio-Cement Formation

The MICP process involves the following reactions:

1. Urea hydrolysis:  $\text{CO}(\text{NH}_2)_2 + 2\text{H}_2\text{O} \rightarrow 2\text{NH}_4^+ + \text{CO}_3^{2-}$  (catalysed by bacterial urease)
2. CaCO<sub>3</sub> precipitation:  $\text{Ca}^{2+} + \text{CO}_3^{2-} \rightarrow \text{CaCO}_3 \downarrow$

The CaCO<sub>3</sub> crystals formed (identified as calcite via XRD) precipitate at the contact points between sand grains, forming biological cement bridges. This structure enhances internal cohesion without completely sealing the pores, thereby maintaining partial permeability, which is vital for coastal ecosystems.

#### Implications for Sustainable Coastal Engineering

The results of this study indicate that Bio-Grout has the potential to be applied for:

- Stabilisation of sand dune bases and slopes prone to landslides (Shahin et al., 2020).
- Forming a semi-rigid protective layer resistant to light to moderate waves.
- Habitat rehabilitation: by selecting non-pathogenic bacteria, precipitation can be tailored to bind the substrate of transplanted coral reefs or seagrass beds.

The main advantages of Bio-Grout over conventional cement are its low carbon footprint, its natural room-temperature process, and its environmental compatibility (Dhami et al., 2013). However, challenges that need to be addressed include: ammonium by-products that are toxic at high concentrations (requiring remediation strategies or nutrient capture), the cost of large-scale culture production, and the long-term stability of bio-cement under tidal cycles and changes in salinity. Further research is required to test scalability, the ecotoxicological impact of ammonium by-products, and long-term stability in the field.

## **4. CONCLUSIONS AND RECOMMENDATIONS**

### **Conclusions**

This study successfully developed a Bio-Grout application protocol based on a ureolytic bacterial consortium for the stabilisation of beach sand. Optimal conditions were achieved at a bacterial density of  $10^7$  cells/mL with three treatment cycles, resulting in an unconfined compressive strength (UCS) of 2.1 MPa,  $\text{CaCO}_3$  precipitation of 5.1% by weight, and mass loss due to erosion of <3% under a 0.5 m/s current simulation. In the absence of bacteria, calcium carbonate precipitation is abiotic and very weak. This technology offers a potential green solution for naturally slowing coastal abrasion.

### **Recommendations for Further Research**

1. Mesocosm and field tests with variations in area size, injection methods (gravity vs. pressure), and real-world hydrodynamic conditions.
2. Long-term ecotoxicological studies, particularly regarding the fate of ammonium by-products and their impact on benthic biota.
3. Optimisation of production costs through the utilisation of urea-rich wastewater (e.g. from the fertiliser industry) as a growth medium.
4. Development of a consortium of high-salinity-tolerant bacteria for application on coastlines with extreme seawater intrusion.

## **REFERENCE**

Dejong, J. T., Soga, K., Kavazanjian, E., Burns, S., Van Paassen, L. A., Al Qabany, A., Aydilek, A., Bang, S. S., Burbank, M., & Caslake, L. F. (2014). Biogeochemical processes and geotechnical applications: progress, opportunities and challenges. *Bio-and Chemo-Mechanical Processes in Geotechnical Engineering: Géotechnique*

*Symposium in Print 2013*, 143–157.

- Dhami, N. K., Reddy, M. S., & Mukherjee, A. (2013). Biomineralization of calcium carbonates and their engineered applications: a review. *Frontiers in Microbiology*, *4*, 314.
- Gao, H., & Dai, S. (2022). Influence of Culture Medium on Cementation of Coarse Grains Based on Microbially Induced Carbonate Precipitation. *Crystals*, *12*(2). <https://doi.org/10.3390/cryst12020188>
- Ghasemi, P., & Montoya, B. M. (2022). Field Implementation of Microbially Induced Calcium Carbonate Precipitation for Surface Erosion Reduction of a Coastal Plain Sandy Slope. *Journal of Geotechnical and Geoenvironmental Engineering*, *148*(9). [https://doi.org/10.1061/\(ASCE\)GT.1943-5606.0002836](https://doi.org/10.1061/(ASCE)GT.1943-5606.0002836)
- Liang, S., Xiao, X., Fang, C., Feng, D., & Wang, Y. (2022). Experimental Study on the Mechanical Properties and Disintegration Resistance of Microbially Solidified Granite Residual Soil. *Crystals*, *12*(2). <https://doi.org/10.3390/cryst12020132>
- Shahin, M. A., Jamieson, K., & Cheng, L. (2020). Microbial-induced carbonate precipitation for coastal erosion mitigation of sandy slopes. *Géotechnique Letters*, *10*(2), 211–215.
- Soon, N. W., Lee, L. M., Khun, T. C., & Ling, H. S. (2014). Factors affecting improvement in engineering properties of residual soil through microbial-induced calcite precipitation. *Journal of Geotechnical and Geoenvironmental Engineering*, *140*(5), 4014006.
- Tsai, C.-P., Ye, J.-H., Ko, C.-H., & Lin, Y.-R. (2022). An Experimental Investigation of Microbial-Induced Carbonate Precipitation on Mitigating Beach Erosion. *Sustainability (Switzerland)*, *14*(5). <https://doi.org/10.3390/su14052513>
- Wang, R., Peng, C., Zhao, H., Liu, H., Wei, T., Wang, L., & Li, H. (2025). Strengthening mechanisms of indigenous bacteria in granite residual soil improvement via microbial induced calcite precipitation. *Scientific Reports*.