



excellent tolerance of environmental harshness including temperature, pH and salinity (Al Qabany et al., 2012). The bacteria were considered ready to use once the  $OD_{600}$  reached a range of 1.00 to 1.20, as there will be sufficient bacteria to achieve appropriate levels of  $CaCO_3$  precipitation.

The mold is necessary for confining the sand sample and allowing for the injection phase of the MICP process in the presence of an AC electric field. The biocementation chamber was designed following configurations described by Terzis et al. (2020), modified to incorporate external electrical field application, as depicted in Fig. 2 (Tian et al., 2024). The cylindrical rigid chamber (inter-electrode distance = 11 cm) featured axially positioned graphite electrodes at upper and lower boundaries, with chamber walls constructed from non-conductive acrylonitrile butadiene styrene plastic.

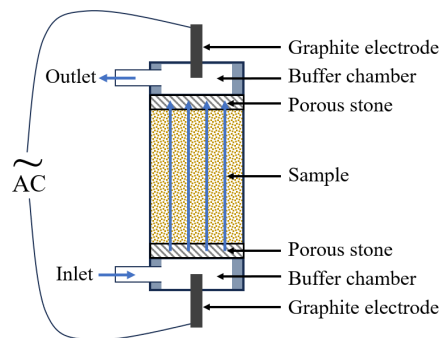


Figure 2. Mold design for the AC-assisted MICP treatment.

### 2.2 AC-assisted MICP treatment process

After completing the compaction of the specimen in layers and installing the mold, the closure of the mold was checked by injecting deionized water. Once the closure was confirmed, we injected 200 mL of bacterial solution first into the cell at the flow rate of 250 mL/h. A fixed concentration ratio between urea and calcium ions at 1: 1 was used for all the experiments, as the molar quantity of urea and that of calcium chloride are identical in the MICP reaction. A mixture of calcium chloride and urea solution was then injected into the mold at the same flow rate with a prescribed voltage applied for a duration of 20 hours at the room temperature of approximately 24 °C. The applied AC in this study was assigned with a frequency of 50 Hz in a sinusoidal waveform. For multiple successive treatments, the above process was repeated without a break. At the end of the treatment, residual chemicals were flushed with 200 mL deionized water (approximately 3.7 times the pore volume). The specimens were removed from the mold and dried at 110°C for 20 h. The final size of the bio-treated specimens was a cylinder of 5 cm in diameter and 10 cm in height.

### 2.3 Experimental procedure

UCS tests were performed to evaluate the mechanical properties of the bio-treated specimens using standard methods (British Standards Institution, 1990). The applied axial compressive stress and the axial strain were recorded throughout the loading process, and the specimens after failure were preserved in plastic bags to conduct the following calcium carbonate content tests.

For the calcium carbonate content measurements, 10 g of the bio-treated specimen was taken from every 2 cm length of the specimen, and excess hydrochloric acid was added to dissolve the calcium carbonate into the solution. After allowing the solution to stand for 10 minutes, 10 mL of supernatant was taken to determine the calcium ion concentration using the EDTA titrimetric method (ISO 6058:1984). The height at the

bottom of the specimen was defined as a 0 cm while the height at the top was defined as 10 cm. The mass of calcium carbonate precipitated along the height of the sand column was recorded, and the average mass of calcium carbonate in the 50 g of bio-cemented silica sand was then calculated.

## 3 RESULTS

### 3.1 Effect of chemical concentration

The effectiveness of MICP is governed by chemical concentration, which dictates reaction kinetics, total precipitated mass, spatial homogeneity, and crystalline morphology (Al Qabany et al., 2012). Two configurations were tested: AC application and a non-electrified control, with urea- $CaCl_2$  concentrations ranging from 0.25 to 1.00 mol/L.

Fig. 3 demonstrates the correlations between chemical concentration, UCS, and CCC. In terms of both the UCS and the calcium carbonate content, the application of AC introduces positive effects at all the specified chemical concentrations. Comparing the experimental results of the set without AC, we find the lowest chemical concentration (0.25 mol/L) results in the highest UCS of 585.99 kPa and greatest CCC of 7.11%, suggesting a more uniform distribution of calcium carbonate precipitates within the bio-treated specimen as depicted in Fig. 4. With AC applied, the fabricated specimen with chemical concentration of 0.25 mol/L fails at a UCS of 2.29 MPa, that is 2.91 times higher than the no-AC counterpart. At an intermediate chemical concentration of 0.50 mol/L, the UCS and CCC improvement by the application of AC is relatively insignificant. At the chemical concentration of 0.75 mol/L and 1.00 mol/L, the treated specimens fail with their UCS and CCC significantly elevated than the counterparts without AC applied. At 1.00 mol/L, excessive chemical concentration inhibits bacterial activity or alter reaction kinetics, resulting in suboptimal performance in the generation of calcium carbonate precipitates (Al Qabany et al., 2012). The application of AC leads to a prominent increase in the UCS and CCC of specimens at high chemical concentrations (0.75 mol/L and 1.00 mol/L), suggesting that the electric field offsets the inhibition of urease activity that limits the reaction rate by a high chemical concentration.

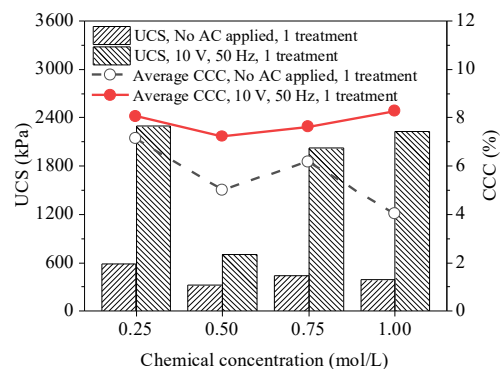


Figure 3. Correlation between UCS and CCC of the bio-samples with varying chemical concentrations under single treatment.

In terms of both the UCS and the calcium carbonate content, the application of AC introduces positive effects at all the specified chemical concentrations. It is noticed that among the specimens without AC applied, the UCS value is the lowest at an intermediate chemical concentration of 0.50 mol/L, and the improvement on UCS due to applying AC is very minimal at this concentration. This is attributed to an overall smaller amount of calcium carbonate precipitates and an uneven distribution of the precipitates in the final product; specifically,

the calcium carbonate content at the top region is significantly lower as depicted in Fig. 4. In addition, an alteration in the crystalline form of the precipitated calcium carbonate due to AC application contributes to the measured UCS values. Repeated tests have consistently shown that with the chemical concentration of 0.50 mol/L, the specimens failed featuring a portion of the top region detached from the specimen, resulting in a low UCS value.

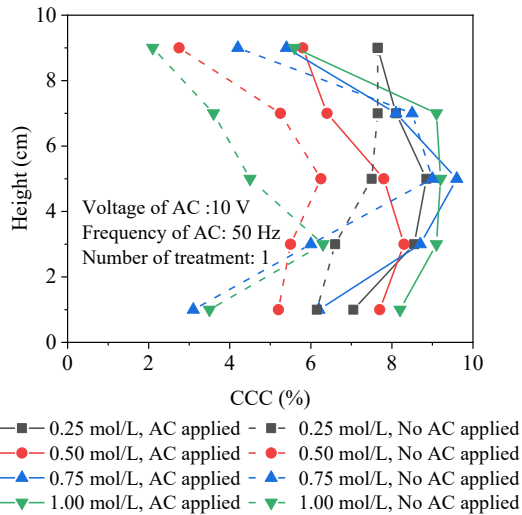


Figure 4. Calcium carbonate distribution along the height of the sand column of the bio-samples fabricated with different chemical concentrations under single treatment.

### 3.2 Effect of voltage

Voltage critically regulates AC-assisted MICP by modulating ion migration, crystallization kinetics, and urease activity. While higher voltages accelerate reaction dynamics via enhanced electric field strength and energy input, excessive voltages risk suppressing urease-driven precipitation (Kawamata et al., 2021). Experiments employing 0.50 mol/L urea-CaCl<sub>2</sub> and 50 Hz AC ranging from 0 V (No AC applied) to 30 V revealed voltage-dependent mechanical outcomes.

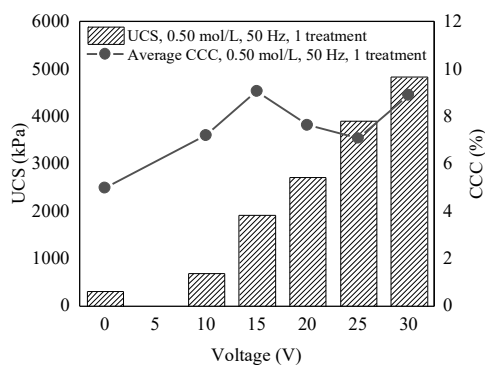


Figure 5. Correlation between UCS and CCC of the bio-samples with varying applied voltages under single treatment.

The correlation between UCS and CCC of the bio-samples with varying applied voltages under single treatment is depicted in Fig. 5. UCS scaled linearly with voltage up to 30 V, despite CCC plateauing beyond 15 V. The divergence between the continuous increase in UCS and the plateauing of CCC beyond 15 V highlighted that the voltage-driven enhancement was due to microstructural refinement. This refinement improved cementation homogeneity and effectiveness, meaning that AC-MICP enhances strength both increasing CaCO<sub>3</sub> quantity,

optimizing its distribution and changing its crystalline form. Notably, at standard 50 Hz AC, voltage elevation improved treatment efficacy through crystallization pathway modifications rather than mere CaCO<sub>3</sub> accumulation. These findings suggest that electric field optimization could overcome conventional MICP strength limits by maximizing UCS per unit volume CaCO<sub>3</sub>. It needs to be noted here that the bacteria may not necessarily be still alive at the end of the treatment period under the voltage of 30 V, as the catalyst for the MICP reaction is only the produced urease.

The calcium carbonate distribution along the height of the sand column, as shown in Fig. 6 demonstrates that by applying an AC electric field the overall amount of the generated calcium carbonate can be improved while maintaining the uniformity of the distribution. It is also indicated that an application of a relatively high voltage elevates the overall treatment effectiveness via multiple mechanisms including modification of the forms and microstructure of crystallization, rather than relying only on increasing the calcium carbonate content (Tian et al., 2024).

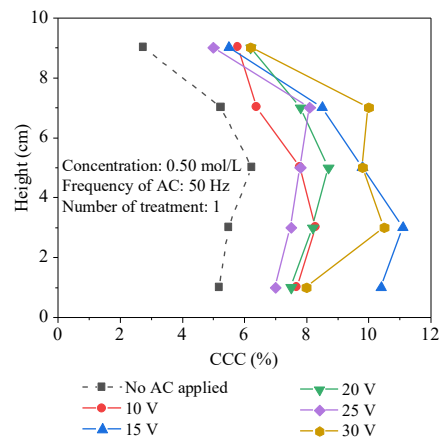


Figure 6. Calcium carbonate distribution along the height of the sand column of the bio-samples fabricated with varying applied voltages under single treatment.

### 3.3 Effect of frequency

The frequency of AC, defined by its polarity alternation rate, modulates the electrochemically coupled dynamics in AC-assisted MICP. The bio-samples were fabricated using various AC frequencies, ranging from 50 Hz to 400 Hz to evaluate the effect of frequency.

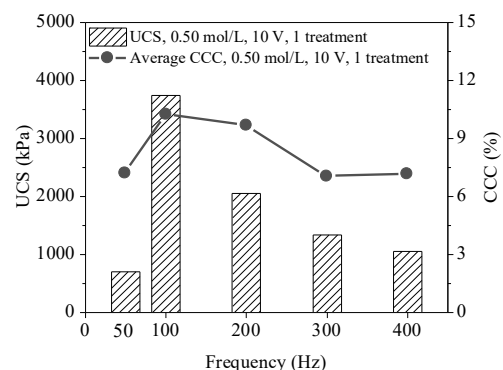


Figure 7. Correlation between UCS and CCC of the bio-samples with varying applied frequencies under single treatment.

The relationship between the UCS and CCC in each sample fabricated under different frequency is shown in Fig. 7, which corroborates that the optimum quality of the sample was achieved at 100 Hz, as the maximum value of calcium

carbonate content obtained in the current series of experiments was also obtained in the sample treated at 100 Hz, suggesting that the improvement in UCS of the bio-sample can be attributed to the increase in the total mass of calcium carbonate precipitates. We also report here that a significantly uneven distribution of calcium carbonate precipitates was observed in the specimens with the most precipitated mass concentrated in the middle portion of the sample as shown in Fig. 8, when a high frequency of 300 Hz or 400 Hz was applied.

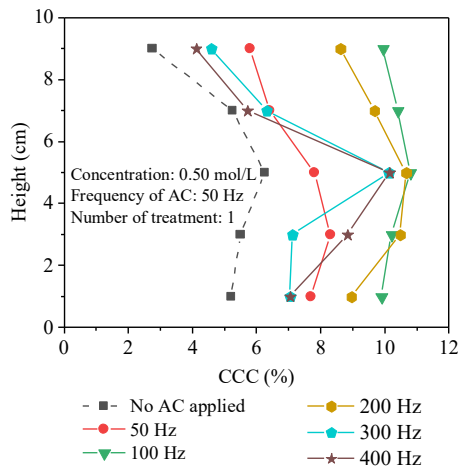


Figure 8. Calcium carbonate distribution along the height of the sand column of the bio-samples fabricated with varying applied frequencies under single treatment.

### 3.4 Effect after multiple treatments

To optimize the strength of AC-assisted MICP-treated specimens, multi-treatment experiments were conducted under varying voltages as depicted in Fig. 9. The maximum UCS of 9.4 MPa was achieved at 30 V after three treatments. Further treatment cycles yielded negligible UCS improvements due to permeability reduction and calcium carbonate-induced pore clogging, establishing three treatments as the practical limit for chemical solution reinjection. UCS enhancement correlated strongly with voltage-dependent  $\text{CaCO}_3$  accumulation.

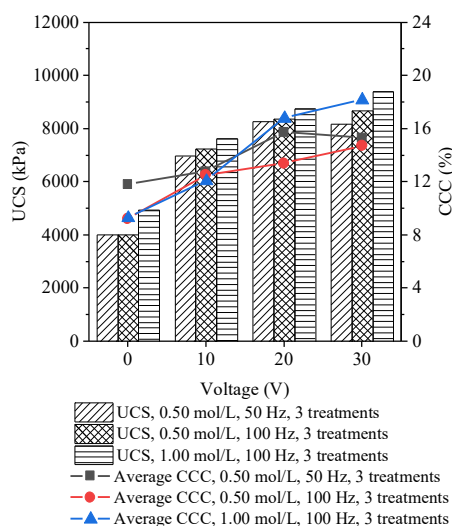


Figure 9. Correlation between UCS and CCC of the bio-samples with varying applied voltages under multiple treatments.

## 4 CONCLUSIONS

In this paper, we propose a new method for soil improvement utilising an AC electric field for assisting the MICP treatment

in sand. With an increased voltage, the UCS of the specimens increases notably, reaching up to 10 folds at 30 V without electric field applied. An optimal AC frequency of approximately 100 Hz in our experiments is determined by comparing UCS and CCC of the bio-treated specimens. By comparing the UCS values at the same calcium carbonate content, the specimens treated by the AC-assisted MICP method exhibit higher UCS values than those treated without AC applied. The maximum UCS value among all the specimens fabricated in the current study is 9.4 MPa, achieved at a chemical concentration of 1.00 mol/L, a voltage of 30 V and a frequency of 100 Hz, after 3 treatments. The experimental results demonstrate that the application of an AC electric field offsets the inhibitory effect on urease activity in high chemical concentration conditions, recovering the effective precipitation rate. In conclusion, actively utilising multiphysics processes, specifically employing an AC field, to assist with the MICP process shows great promise for sand reinforcement.

## 5 ACKNOWLEDGEMENTS

We thank the support from Prof. Tong Zhang's Environmental Microbiome Engineering and Biotechnology Lab at HKU for bacteria cultivation. The financial support by Research Grant Council of Hong Kong (ECS 27203720, GRF 17206521) is acknowledged.

## 6 REFERENCES

Al Qabany, A., Soga, K., & Santamarina, C. 2012. Factors affecting efficiency of microbially induced calcite precipitation. *Journal of Geotechnical and Geoenvironmental Engineering* 138(8), 992-1001.

British Standards Institution. Road Engineering Standards Policy Committee. 1990. *Stabilized Materials for Civil Engineering Purposes: Methods of Test for Cement-stabilized and Lime-stabilized Materials*. BSI.

Jiang, N. J., & Soga, K. 2017. The applicability of microbially induced calcite precipitation (MICP) for internal erosion control in gravel-sand mixtures. *Geotechnique* 67(1), 42-55.

Jiang, N. J., Wang, Y. J., Chu, J., Kawasaki, S., Tang, C. S., Cheng, L., ... & Wang, Y. Z. 2022. Bio-mediated soil improvement: An introspection into processes, materials, characterization and applications. *Soil Use and Management* 38(1), 68-93.

Kawamata, Y., Hayashi, K., Carlson, E., Shaji, S., Waldmann, D., Simmons, B. J., ... & Baran, P. S. 2021. Chemosynthetic electrosynthesis using rapid alternating polarity. *Journal of the American Chemical Society* 143(40), 16580-16588.

Rebata-Landa, V. 2007. *Microbial activity in sediments: effects on soil behavior*. Georgia Institute of Technology.

Shi, L., Han, Z., Feng, Y., Zhang, C., Zhang, Q., Zhu, H., & Zhu, S. 2023. Joule heating of ionic conductors using zero-phase frequency alternating current to suppress electrochemical reactions. *Engineering* 25, 138-143.

Tang, X., & Hu, M. 2024. On the resilience of bio-cemented silica sands in chemically reactive environment. *Geomechanics for Energy and the Environment* 37, 100527.

Terzis, D., & Laloui, L. 2019. A decade of progress and turning points in the understanding of bio-improved soils: A review. *Geomechanics for Energy and the Environment* 19, 100116.

Terzis, D., Hicher, P., & Laloui, L. 2020. Direct currents stimulate carbonate mineralization for soil improvement under various chemical conditions. *Scientific reports* 10(1), 17014.

Tian, A., Tang, X., Chen, J., & Hu, M. 2024. AC-assisted microbially induced carbonate precipitation for sand reinforcement: An experimental study. *Geomechanics for Energy and the Environment* 40, 100609.

Whiffin, V. S., Van Paassen, L. A., & Harkes, M. P. 2007. Microbial carbonate precipitation as a soil improvement technique. *Geomicrobiology Journal* 24(5), 417-423.