Exploring an Environmentally Friendly Microbially Induced Calcite Precipitation (MICP) Technology for Improving Engineering Properties of Cement-Stabilized Granite Residual Soil

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ABSTRACT
This study explored Microbially Induced Calcite Precipitation (MICP) technology to improve the engineering properties (i.e., unconfined compressive strength (UCS)) of granite residual cemented-soil through calcite precipitation. The influence of age and cement mixing ratio on strength, stiffness and the stress-strain relationship of MICP induced calcite precipitation in granite residual cemented-soil was investigated. Scanning electron microscope (SEM) was used to analyse the microstructure characteristics of the cemented-soil. Based on the results, the cemented granite residual soil reinforcement mechanism was proposed. The following conclusions were obtained: (1) MICP technology can significantly enhance and improve the engineering properties such as strength, stiffness and toughness of cemented-soil. Compared with the control group, the maximum growth rate of the test group was 87.5%, and the maximum growth rate of the elastic modulus was 141.18%; (2) Soil particles were cemented through MICP technology, making the cemented-soil surface denser; (3) The MICP technology makes the cemented-soil treatment method more sustainable for its use in improving the stability of geo-structures.

INTRODUCTION
The granite reserves in the southern region, including Hong Kong and Macao, are widely distributed, mainly in low hilly areas (Chen 2007). Granite residual soil is a special type of soil with a small gap in its mineral composition in different regions. In Guangdong, the content of kaolinite in granite residual soil is between 70% and 94%. Besides, the granite residual soil also has engineering properties such as water disintegration, and strong hydrophilicity. Lin et al. (2011) performed a series of studies on the properties of granite residual soil with reduced strength and softening under different water contents (Zhang 2009, Liang et al. 2015). This kind of property makes it a great safety hazard in the extreme climate, especially in the rainstorm season. At present, it is usually adding cement to improve the shear strength and resistance to disintegration ability. Liu et al. (2018) used fly ash to improve granite residual soil. The experimental results showed that adding a certain amount of fly ash to granite residual soil can significantly improve its water stability. However, most of the chemical slurries synthesized with external admixtures are toxic, which also cause environmental pollution (Karol 2003).

Microbially Induced Calcite Precipitation (MICP) is a new technology that has evolved in recent years along with the continuous development of discipline-crossing on environmental sciences and civil engineering. It is a series of biochemical reactions in bacterial metabolism to produce a cementation process of calcium carbonate, which does not produce toxic substances, is environmentally friendly and efficient. MICP technology has been applied in many fields, such as sewage treatment, concrete material repair, anti-seepage repair, soil remediation of heavy metal pollution, soft foundation reinforcement and improvement. The solutions used in MICP technology (including bacterial solution and cement solution) have lower consistency than traditional chemical slurries and are more likely to penetrate the interior of rock and soil (Chu et al. 2012), which has obvious effects on deep cracks or deep rock soils (Ivanov & Chu 2008).
The application of *Bacillus* mixed nutrient solution and cement solution to degraded limestone can deposit calcium carbonate on the limestone surface and greatly reduce the water absorption coefficient of the limestone surface (Dick et al. 2006).

Whiffin et al. (2007) first proposed the use of microbial induced calcium carbonate to cement sand and proved that the method is effective. Van et al. (2010) used MICP technology to carry out a large-scale *in-situ* sand test of 100 m$^3$, successfully cementing nearly 1/2 of the sand together, and proved that the mechanical strength of the sand treated by MICP technology improved substantially. In addition to the research on the strength, a large number of scientific research results have been obtained in terms of stiffness (Lin et al. 2011), permeability (Fang et al. 2015, Jia et al. 2016), and durability (Huang et al. 2018, Peng et al. 2019).

At present, the use of MICP technology mainly focuses on the single use of the technology to reinforce the soil, while the MCP technology is less used in combination with other types of external additives such as cement, to strengthen the soil. Mukherjee et al. (2013) stirred bacteria and cement soil into cement bricks. The results showed that the water absorption and porosity of the cement bricks treated by MICP were significantly reduced, and the compressive strength was improved. Liang et al. (2019) investigated the effects of different calcium sources on the shear strength of granite residual soil. The results showed that the shear strength of granite residual soil treated by MICP technology was improved. When calcium acetate was used as a calcium source, the curing effect of MICP was improved better.

Based on the above results, this study researched the engineering characteristics of cement-soil treated by MICP technology. It is expected that the strength of cement-soil can meet the engineering needs in the shorter curing age and lower cement dosage.

**MATERIALS AND METHODS**

**Materials**

**Soil preparation and physical and mechanical properties:**
The soil used in the test was granite residual soil and baked at 105°C for 24 h, crushed and passed through 2 mm sieve. The basic physical and mechanical indexes were determined according to the “Standard for Geotechnical Test Methods” (GB/T50123-1999), as given in Table 1. The kind of cement was P.O 42.5.

**Preparation of bacterial solution and cement solution:**
The strain used in this experiment was *Sporosarcina pasteurii* (China General Microorganisms Collection and Management Center, No. CGMCC 1.3687). The culture medium was prepared according to the 0907 medium (Table 2) provided by CGMCC, and the pH was adjusted to 8.0. They were put then into the high-pressure steam sterilization pot, and later into the aseptic operating table and cooled. The activated bacteria were inoculated into 200 mL culture medium in a 250 mL Erlenmeyer flask in a constant temperature shaking incubator (30°C, 150 r/min), and after culturing for 24 to 36 hours, the OD600 = 0.8 was determined. The cement solution was 1 mol/L CaCl$_2$ and 1 mol/L urea.

**Methods**

We set two groups, one group was ordinary cement-soil, and the other group was microbial cement-soil. The moisture content of each group of cement soil was 25%. Table 3 gives the mixing ratio of cement-soil in the test group, and Table 4 gives the mixing ratio of cement-soil in the control group.

| Physical index       | Value   |
|----------------------|---------|
| Water content (%)    | 28.5    |
| Density (g.cm$^{-3}$)| 1.93    |
| Void ratio           | 0.78    |
| Liquid limit (%)     | 34.2    |
| Plasticity index     | 11.5    |

Table 1: Basic physical properties of granite residual soil.

| Soil mass (g)       | 1800     |
|---------------------|----------|
| Water content (%)   | 25       |
| Cement ratio (%)    | 10, 15, 20 |
| Cement mass (g)     | 180, 270, 360 |
| Total mass of solution (g) | 540, 585, 630 |
| Bacterial fluid mass (g) | 360, 390, 420 |
| Urea mass (g)       | 90, 97.5, 105 |
| CaCl$_2$ mass (g)   | 90, 97.5, 105 |

Table 2: 0907 *Sporosarcina pasteurii* medium.

| Number | 0907  |
|--------|-------|
| Peptone | 5.0 g |
| Beef extract | 3.0 g |
| Urea | 20.0 g |
| Deionized water | 1.0 L |

| Soil mass/g       | 1800     |
|-------------------|----------|
| Water content (%) | 25       |
| Cement ratio (%)  | 10, 15, 20 |
| Cement mass (g)   | 180, 270, 360 |
| Water mass (g)    | 540, 585, 630 |
Three parallel samples were set for each group. The sample size was 3.91 cm in diameter and 8.0 cm in height. Knockout time was 24 hours, then they were sealed with plastic sealed bags, and placed in a standard curing room for further maintenance. The curing conditions were: temperature (25 ± 2) °C, relative humidity ≥ 95%, and the curing age 7d, 14d, 28d.

RESULTS

Result Analysis

Analysis of the UCS test: Taking average UCS values of three parallel samples as the intensity value, the results of the UCS test and analysis are given in Table 7. It can be seen from Fig. 1 that under the same age, the UCS value of the microbial enhanced cement soil is greater than the UCS value of the ordinary cement-soil, and the growth rates were 51.85%, 87.5% and 47.37% at 7d; the growth rates were 19.77%, 50.3% and 23.26%, at 14d; and the growth rates were 22.81%, 64.46% and 41% at 28d.

It can be seen from Fig. 2 that under the same cement mixing ratio, the UCS values of the cement soils of the two groups increased with the increase of the curing age and the UCS value of the cement-soil of the experimental group is larger than that of the control group. When the cement ratio is 10%, the difference between the two groups is not large, and the difference increases with the increase of cement ratio. In the control group, when the cement ratio increased from 10% to 15%, the growth rates are significantly lower than that of the test group. From the overall view of Fig. 2, the
UCS values of the test group were significantly larger than that of the control group when the curing age was 7 days, and the UCS values of the control group are similar to that of the control group at 14 days, indicating that the MICP technology can also play a significant role in reinforcing the granite residual soil.

The general formula (1) is obtained by linear fitting the curves of Fig. 1 and Fig. 2. The values of each parameter are given in Table 5 and Table 6. As provided in Table 5 and Table 6, the strength of cement-soil treated by MICP increases. The law of strength growth is more in line with the linear relationship, and the fitting effect is more significant.

\[ f = a + b \cdot x \]

**Stress-strain curves and analysis**: Fig. 3 is the stress-strain curve at the curing age of 28d. It can be seen from Fig. 3 (a) and (b) that the stress-strain curves of the cement-soils in both groups have undergone the compaction process in the initial stage, but the stress changes of the test group are smaller than that of the control group at the same strain; after the upper concave section, a section of the approximately straight line appears, and the deformation of the cement-soil conforms to the linear elastic relationship; after this stage,

![Stress-strain curve of cement-soil corresponding to 28d age.](image)

**Table 5: Fitting results of UCS of the test group and the control group (refer to Fig. 1).**

| Group       | \( \varepsilon \) (%) | a    | b      | R²   |
|-------------|------------------------|------|--------|------|
| Test group  | 10                     | 0.635| 0.027  | 0.998|
|             | 15                     | 1.555| 0.061  | 0.993|
|             | 20                     | 2.265| 0.092  | 0.959|
| Control group | 10                    | 0.400| 0.027  | 0.897|
|             | 15                     | 0.870| 0.041  | 0.772|
|             | 20                     | 1.710| 0.065  | 0.597|

**Table 6: Fitting results of the UCS of the test group and control group.**

| Group       | T/d | a    | b      | R²   |
|-------------|-----|------|--------|------|
| Test group  | 7   | -1.113| 0.198  | 0.988|
|             | 14  | -1.623| 0.268  | 0.998|
|             | 28  | -1.93 | 0.338  | 0.995|
| Control group | 7    | -0.880| 0.136  | 0.954|
|             | 14  | -1.555| 0.229  | 0.931|
|             | 28  | -1.208| 0.225  | 0.955|

![Stress-strain curve of cement-soil corresponding to 28d age.](image)
the curve becomes curved before the stress reaches the peak stress, accompanied by plastic deformation. When the stress peak is reached, the curve begins to fall and, the sample begins to break. From the comparison of (a) and (b), when the cement mixing ratio changes from small to large, the strain corresponding to the peak stress of the cement-soil of the control group tends to become larger. When the cement mixing ratios are 10%, 15% and 20%, the strains of ordinary cement-soils are 2.89%, 3.01% and 3.64%, respectively, while the strains corresponding to the peak stress of the cement-soil in the test group show a trend of decreasing, and the corresponding strains of cement-soil are 5.85%, 4.79%, 2.80%. It shows that the addition of cement can improve the toughness of the residual soil, and the MICP technology can further improve the toughness of the cement-soil in the condition that the cement content is not high.

Fig. 4 is the stress-strain curve of the two groups when the cement content is 15%. It can be seen from Fig. 4 (a) and (b) that the values of the UCS test of the two groups are related to the age of the curing. The strain corresponding to the peak stress of ordinary cement-soil in the control group is smaller than that in the test group, and the strains corresponding to the peak stress at the age of 7d, 14d and 28d are 3.13%, 3.16% and 3.07%, respectively. The strains corresponding to the peak stress of the test group were 4.06%, 3.77% and 4.79%, respectively. It shows that at the same curing age, MICP technology can improve the toughness of cement-soil. According to the strain values corresponding to the peak stress of the two groups, it can be found that the influence of curing age on the toughness of cement soil is smaller than the cement mixing ratio.

**Deformation modulus:** The stress-strain curve shows that the initial compaction phenomenon exists in the cement-soil samples, and stress-strain relationship increases approximately linearly. The elastic modulus (E) is an important parameter to reflect an object resisting the elastic deformation. It can be calculated according to the ratio of stress and strain in the elastic-strain curve of the stress-strain curve. The slope can be obtained through linear fitting the linear elastic stage of the stress-strain curve, which can be regarded as the elastic modulus. The calculation results are provided in Table 8.

![stress-strain curve](image)

Table 8: Elasticity modulus of cemented-soil samples.

| E (%) | T/d | 7     | 14    | 28    |
|------|-----|-------|-------|-------|
| 10   | T-E/MPa | 0.27 | 0.56  | 0.61  |
|      | C-E/MPa | 0.21 | 0.48  | 0.55  |
| 15   | T-E/MPa | 1.14 | 1.63  | 1.7   |
|      | C-E/MPa | 0.69 | 0.75  | 0.99  |
| 20   | T-E/MPa | 1.9  | 2.41  | 3.28  |
|      | C-E/MPa | 0.84 | 1.1   | 1.37  |

**Note:** T-E represents elastic modulus of the test group, C-E represents elastic modulus of the control group.
It can be seen from Fig. 5 that the elastic modulus (E) of the two groups increases with the increase of the curing age and the cement mixing ratio. The elastic modulus of the test group is significantly larger than that of the control group, and the samples are more likely to exhibit brittle fracture. It also can be seen from Fig. 5(a) and (b) that the difference between the elastic modulus becomes larger and larger with the cement mixing ratio increasing, while the effect in the control group is not obvious. When the cement mixing ratio is 15%, the elastic modulus of the test group is 3.22 times, 1.91 times, 1.79 times than the cement mixing ratio 10%, respectively. When the cement mixing ratio is 20%, the elastic modulus of the test group is 0.67 times, 0.48 times, 0.93 times than the cement mixing ratio 15%, respectively. When the cement mixing ratio is 15%, the elastic modulus of the control group is 2.29 times, 0.56 times, 0.8 times than that of the cement mixing ratio 10%, respectively. When the cement mixing ratio is 20%, the elastic modulus of the control group is 0.22 times, 0.47 times, 0.37 times than the cement mixing ratio 15%, respectively. According to the results in Table 7, the elastic modulus of microbial enhanced cement soil is the largest compared with ordinary cement soil at 7d, while the overall growth rate at 14d is lower than that of the other two groups. The cement-soil with cement mixing ratio 20% has the largest elastic modulus (3.28MPa), which is 1.41 times than that of ordinary cement with the same mixing ratio.

**CURING MECHANISM**

**Cement hydration process:** The cement hydration is the coagulation and hardening of the cement. When the cement meets water, it produces gelling substances, which can cement the soil particles, thereby increasing the strength of the cement-soil. The addition of the microorganisms will affect the cement hardening process, and in general, it will develop in a direction that is conducive to increasing the strength of the cement soil.

**Pozzolanic effect:** The strong Pozzolanic effect can increase the strength of the cement soil at various ages, and the free oxides in the granite residual soil such as SiO$_2$ and Al$_2$O$_3$.

![Fig. 5: Relationship between elastic modulus, age and cement mixing ratio for cemented-soil.](image)

**Table 7: Summary of measured UCS of the cemented-soil samples.**

| $\varepsilon$ (%) | T/d | 7   | 14  | 28  |
|-------------------|-----|-----|-----|-----|
| 10                | T-UCS/MPa | 0.82 | 1.03 | 1.40 |
|                   | C-UCS/MPa | 0.54 | 0.86 | 1.14 |
| 15                | T-UCS/MPa | 1.95 | 2.45 | 3.24 |
|                   | C-UCS/MPa | 1.04 | 1.63 | 1.97 |
| 20                | T-UCS/MPa | 2.8  | 3.71 | 4.78 |
|                   | C-UCS/MPa | 1.9  | 3.01 | 3.39 |

*Note:* T-UCS represents the values of UCS of the test group, C-UCS represents the values of UCS of the control group.
also have a pozzolanic effect, thereby further amplifying the reaction (Goldman & Bentur 1993, Hou et al. 2014).

**Filling and cementation effect:** Fig. 6 is a comparison of scanning electron microscopy (SEM) results between the test group and the control group when the cement mixing ratio is 15% and the curing age is 7 days. Comparing the figures (a) and (b), we can see that the surface of the cement-soil of the control group has many pores which are also large, while the surface of the cement-soil of the test group has very few pores at this magnification, and the surface is denser than the control group. (a) There are already gelatinous substances between the soil particles, however, some of the soil particles are not cemented, and the cement-soil is generally loose; (b) the soil particles are tightly cemented, and the cement-soil is generally denser. Calcium carbonate deposited by microorganisms has a cementation effect to further bond the soil particles, thereby increasing the strength of the cement soil.

**CONCLUSION**

MICP technology can effectively reinforce granite residual soil. Compared with ordinary cement-soil, the values of UCS of cement-soil with the added bacterial solution are greatly improved, and the maximum growth rate is 87.5%. Also, the UCS at the 14-day of the test group exceeded the unconfined compressive strength at the 28-day age of the control group.

The strain corresponding to the peak stress of the cement-soil treated by MICP is generally larger than that of ordinary cement-soil. The strain corresponding to the peak stress of the cement-soil decreases with the increase of the cement mixing ratio. However, the UCS of ordinary cement-soil increases with the increase of cement mixing ratio, indicating that cement hydration will increase the toughness of residual soil, but microbes will change this direction of action. The experimental results of the two groups show that the effect of curing age on toughness is small.

The elastic modulus values of the two groups were obtained by fitting the line segments of the stress-strain curve. The results show that the MICP technology can increase the elastic modulus of the cement-soil compared with ordinary cement soil. The maximum growth rate is 141.18%.

The mechanism of MICP technology to treat cement soil is cement hardening, pozzolanic reaction and filling effect. Due to the fine soil of granite residual soil and small pores, it will limit the living space of microorganisms to a certain extent, so in the microbial-cement slurry-soil system, these effects interact and work together, so the curing mechanism is very complicated. The curing effect and mechanism of the fine-grained soil on the concentration of bacteria and water are to be further studied.

In this study, the MICP technology was successfully applied to the reinforcement of granite residual soil, which proved that the strength characteristics of cemented-soil treated by MICP technology were significantly enhanced. In the future, the effects of factors such as bacterial liquid concentration, calcium salt types and water content on the strength characteristics of cemented-soil will be studied.

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REFERENCES

Chen, Z. J. 2007. The engineering characteristics of granite residual soil and analysis of the cut slope stability in Shantou City. Geology and Mineral Resources of South China, 2.

Chu, J., Stabinikov, V. and Ivanov, V. 2012. Microbially induced calcium carbonate precipitation on surface or in the bulk of soil. Geomicrobiology Journal, 29(6): 544-549.

Dick, J., De Windt, W., De Graef, B., Saveyn, H., Van der Meeren, P., De Belie, N. and Verstraete, W. 2006. Bio-deposition of a calcium carbonate layer on degraded limestone by bacillus species. Biodegradation, 17(4): 357-367.

Fang, X.W., Shen, C.N., Chu, J., Wu, S.F. and Li, Y.S. 2015. An experimental study of coral sand enhanced through microbially-induced precipitation of calcium carbonate. Yantu Lixue/Rock and Soil Mechanics, 36(10): 2773-2779.

Goldman, A. and Bentur, A. 1993. The influence of microfillers on enhancement of concrete strength. Cement and Concrete Research, 23(4): 962-972.

Hou, P., Qian, J., Cheng, X. and Sha, S. P. 2014. Effects of the pozzolan reactivity of nano SiO₂ on cement-based materials. Cement and Concrete Composites, 55.

Huang, M., Zhang, J., Jin, G., Jiang, Y., Qiu, J., Gong, H. and Guo, S. 2018. Magnetic resonance image experiments on the damage feature of microbial induced calcite precipitated residual soil during freezing-thawing cycles. MICP. Yanshilixue Yu Gongcheng Xuebao/Chinese Journal of Rock Mechanics and Engineering, 37(12): 2846-55.

Ivanov, V. and Chu, J. 2008. Applications of microorganisms to geotechnical engineering for bio-logging and biocementation of soil in situ. Reviews in Environmental Science & Biotechnology, 7(2): 139-153.

Jia, Q., Zhang, X. and Jiang, H. 2016. Experiment research on microbial induced clogging in soil by bacterially induced calcium carbonate deposition. Sichuan Building Science, 42(1): 93-95.

Karol, R.H. 2003. Chemical Grouting and Soil Stabilization, Revised and Expand. CRC Press, Boca Raton.

Liang, S. H., Fang, C. X., Niu, J. G. and Zeng, W. H. 2019. Research on effect of microbial induced calcite precipitation adopting different calcium sources on cement granite residual soil. Industrial Construction, 49(7): 102-107.

Li, P., Chen, H. M. and Wang, Y. Q. 2011. Behavior of the unsaturated granite residual soil and its effects on earth dam project. Geotechnical Special Publications (GSP), 217: 68-75.

Liu, S., Chen, Z. B., Chen, W. W. and Wei, Y. 2018. Experience study on granite residual soil improved by fly ash. Journal of Fuzhou University (Natural Science Edition), 46(5): 115-120.

Mukherjee, A., Dhami, N. K., Reddy, B. V. V. and Sudhakara Reddy, M. 2013. Bacterial calcification for enhancing performance of low embodied energy soil-cement bricks. 3rd International Conference on Sustainable Construction Materials and Technologies, Kyoto, Japan.

Peng, J., Wen, Z.L., Liu, Z.M., Sun, Y.C., Feng, Q.P. and He, J. 2019. Experimental research on MICP-treated organic clay. Yantu Gongcheng Xuebao/Chinese Journal of Geotechnical Engineering, 41(4): 733-740.

Van, L. A., Ghose, R., Van-der-Linden, T.J.M. and Van-der-Star, W.R.L. 2010. Quantifying bio-mediated ground improvement by ureolysis:a large scale biogrout experiment. Journal of Geotechnical & Geoenvironmental Engineering, 136(12): 1721-1728.

Wang, W.J., Zhu, X.R. and Fang, P.F. 2005. Analysis on reinforcement mechanism of nanometer silica fume reinforced cemented clay. Zhejiang Daxue Xuebao (Gongxue Ban)/Journal of Zhejiang University (Engineering Science), 39(1): 148-153.

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