

# MBCMG2024

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**ABSTRACT BOOK**

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# Session 1

**Title:**

Inorganic Flocculant-Based Soybean Urease Extraction and its Effect on Biomineralization of Soil

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**Abstract:**

Enzyme induced carbonate precipitation (EICP), based on crude soybean urease solution (CSUS) self-extracted from soybean powder, has the potential to become an efficient, cost-effective, and environmentally friendly ground improvement method. However, the deionized water-extracted CSUS contains a large amount of impurities, which easily lead to bioclogging during the biogrouting process, resulting in a nonuniform biomineralization effect. In this study, a purification method using inorganic flocculants is proposed to extract CSUS with relatively high purity and urease activity for EICP treatment. Seven commonly used inorganic flocculants were tested in this study, including  $KAl(SO_4)_2 \cdot 12H_2O$ ,  $AlCl_3 \cdot 6H_2O$ ,  $Al_2(SO_4)_3 \cdot 18H_2O$ ,  $Fe_2(SO_4)_3$ , poly aluminum chloride, poly ferric sulfate, and poly aluminum ferric chloride. Three sets of tests, including CSUS extraction tests, solution tests, and sand column treatment tests, were conducted in this study to investigate the feasibility of this purification method. The test results show that the inorganic flocculants can effectively remove impurities from the soybean powder mixture, but cause a reduction in the urease activity (UA) of the extracted CSUS. CSUS with relatively high UA and low turbidity can be extracted by a solvent containing a certain amount of inorganic flocculants. Compared with the deionized water-based CSUS, the use of inorganic flocculant-based CSUS has little effect on the EICP process, and can effectively improve or even prevent the bioclogging during the biogrouting process, thus improving the strength enhancement of the biocemented sand column. Considering the UA, turbidity, and biomineralization effect of the extracted CSUS, the optimal inorganic flocculants and their contents are 3.0 g/L for  $KAl(SO_4)_2 \cdot 12H_2O$ , 2.0 g/L for  $AlCl_3 \cdot 6H_2O$ , 2.5 g/L for  $Al_2(SO_4)_3 \cdot 18H_2O$ , respectively.

**Title:**

Development and application of microbial-induced restoration and conservation techniques for cultural relics

**Author:**

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**Abstract:**

China has a long history and abundant cultural relics. However, due to historical factors, inadequate preservation, and other reasons, a large number of stone cultural relics suffer from diseases such as flaking and incompleteness. Nevertheless, traditional restoration materials have certain disadvantages, so there is an urgent need to develop and promote the compatibility of excellent compatibility, strong weather-resistance, non-toxic cultural relics protection, and restoration materials. The emerging microbial geotechnical engineering is a new cross-disciplinary subject that applies the microbial mineralization process to geotechnical engineering, and the technology has significant effects on improving the strength, permeability, and other basic properties of the soil body. With the development of microbial geotechnical reinforcement technology, this technology has also received the key attention of researchers related to geotechnical relics. Many scholars are currently conducting research on microbial technology for repairing and reinforcing soil sites and cave relics. Therefore, based on microbial mineralization consolidation technology, this study proposes the patch repair method for stone relics and the bonding repair method for movable relics (e.g. pottery and bone and keratin relics). Through a large number of macroscopic and microscopic experiments, it verifies the validity and practicability of the microbial mineralization restoration method in the restoration of stone relics and movable relics, which provides a novel approach to thinking for the restoration of stone relics and movable relics.

**Title:**

Multiple heavy metals immobilization in contaminated water and soil by Enzyme-induced carbonate precipitation method

**Author:**

Yi Bian, Yanbo Chen & Liangtong Zhan

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**Abstract:**

Water and soil in the ecosystem have been seriously contaminated by heavy metals from the electronic industry, mine tailings, waste disposal, and fuel consumption. Usually, heavy metal contamination in wastewater was regarded as a significant issue. The toxic heavy metals (e.g., Zn, Ni, Cr, Pb, and Cd) can lead to plant pollution, soil erosion, and even potential risks to human life due to inappropriate treatment methods. The rapid generation of wastewater or contaminated soil in the expanding industry becomes a tough problem and necessitates effective remediation treatment. Conventional methods to remediate wastewater like chemical precipitation, and adsorption, are not only highly-cost but also environmentally unfriendly. Recently, Enzyme Induced Carbonate Precipitation (EICP) has been extensively investigated and shows great potential for the immobilization of heavy metal ions in contaminated water and soil.

In this study, a crude urease extracted from sword bean was used to immobilize heavy metals in contaminated water and soil with individual or multiple heavy metals. The liquid batch tests and soil column experiments were conducted to verify the effectiveness of the EICP method. In liquid batch tests, about 109 mg/L of individual and multiple heavy metals solutions including Zn, Ni, and Cr(VI) were treated with the EICP method. Calcium ions were also introduced to evaluate the immobilization efficacy. The results show that, in groups without calcium ions, the immobilization efficiency of Zn, Ni, and Cr(VI) in solutions is about 39.9-49.9 %, 13.8-18.3 %, and 7.9-10.5 %, respectively. In groups with the addition of calcium ions, the immobilization efficiency of Zn, Ni, and Cr(VI) increased to 99 %, 40-50 %, and 35-40 %, respectively. In contaminated soil, the immobilization percentage increased with the addition of calcium ions and treatment cycles. The final immobilization percentage of Zn, Ni, and Cr(VI) was up to 99.87~99.99%, 83.43~86.38%, and 73.43~75.18%, respectively. The results indicate that the addition of calcium ions is beneficial for heavy metal immobilization, which can be attributed to the adsorption effect. Conclusions can be drawn that EICP is a promising method to immobilize (Zn, Ni, and Cr(VI)) multiple heavy metals in contaminated water and soil with crude extracted sword bean urease.



**Title:**

Investigation on the bioremediation of heavy metal-contaminated solution utilizing the two-step MICP method based on the urea-medium

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**Abstract:**

The application of *Bacillus pasteurii*-based microbial-induced carbonate precipitation (MICP) method in the remediation of heavy metals (HM) has attracted quite a bit of interest recently. Nevertheless, researches have demonstrated that the toxic Cd, Pb, Zn, and Cu ions in the contaminated solution will obstruct microbial and urease activity, thereby limiting the bioremediation efficiency. In this study, the impact of heavy metal ions on the urease activity and bioremediation efficiency are first investigated with the traditional MICP method (in which the bacterial solution, nutrient solution, and heavy metals are mixed at the same moment) in the heavy metal concentration range from 1 to 10 mM. Then, SEM-EDS and XRD are employed to examine the bioremediation mechanism for HMs by *B. pasteurii*. Additionally, a two-step MICP method, which separates urea hydrolysis and heavy metal precipitation, is introduced to demolish the inhibitory effect of heavy metal. The following results are obtained. Cu is highly poisonous to *B. pasteurii*. The synthesis of ammonium significantly decreases and the bioremediation efficacy drops when the Cd and Zn concentrations are over 5 mM and 2 mM, respectively. Pb can inhibit urease expression to some extent, but the carbonate ions produced are sufficient to completely remove the soluble Pb. *B. pasteurii* primarily induces Cd, Zn, and Pb to generate carbonate precipitation, and the weakly alkaline solution pH (from 7 to 8) aids in the precipitation of soluble Cu by abiotic effect. Increasing the initial urea hydrolysis time can significantly increase the removal rates of Cd and Zn when employing the two-step MICP method. When the Cu concentration is greater than 5 mM, the optimal first-step reaction time is 0.5 h, which benefits the removal of Cu. The two-step MICP approach has the potential to restore heavy metal-contaminated solutions as it promotes heavy metal removal efficiency while decreasing the urea addition. For Cu-contaminated solutions, however, further investigation is needed to improve removal effects.

**Title:**

Synergistic solidification of heavy metal tailings by polyethylene glycol (PEG) and microorganisms

**Author:**

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**Affiliation:**

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**Abstract:**

A large amount of tailings rich in heavy metals is left behind after mining, causing environmental pollution due to long-term storage. In recent years, microbial-induced carbonate precipitation (MICP) has shown potential to solidify and stabilize heavy metal-contaminated soils. However, high concentrations and complex heavy metals have toxic effects on microorganisms, resulting in a decrease in carbonate yield. Therefore, a low pH treatment method using PEG-MICP was proposed. The unconfined compressive strength (UCS) of the tailings sand treated by this method increased by times, and the soil uniformity was greatly improved, with a substantial reduction in exchangeable heavy metal ions in the soil. Microscopic results showed that the introduction of PEG could modify the morphology of calcium carbonate. The transformation of calcite from a mineral to sheet-like and faceted calcite improves solidification efficiency. In this study, PEG-MICP shows broad application prospects for solidifying heavy metal tailings sand.

**Title:**

Experimental study on the seepage proof and leak plugging of vertical cracks in concrete by microbial grouting

**Author:**

Qiang Jia

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**Abstract:**

The technology of microbial grouting to seal concrete cracks is an effective means to solve the leakage of underground concrete structures. However, in engineering practice, it is found that due to the low viscosity of grouting components such as bacterial fluid, it is not easy to remain in vertical cracks under the action of its own weight. In order to optimize the grouting process and improve the repair efficiency, an experimental study on microbial grouting to repair vertical concrete cracks was carried out. The experiment set parameters such as crack width, grouting port spacing, whether to inject fixed fluid in advance, whether the crack is embedded with a medium, the type and dosage of the medium, etc. Through multiple rounds of microbial grouting, the appearance changes of the crack were observed, and indicators such as the seepage speed of the crack, the wave speed of the ultrasonic wave across the crack, the amount of calcium carbonate generated in the backfill soil outside the crack, and the deposition and distribution of calcium carbonate in the crack were tested. The test results show that as the number of grouting cycles increases, the seepage speed of the crack gradually decreases; the wave speed of the ultrasonic waves at each measuring point has significantly increased, indicating that with the deposition of calcium carbonate in the crack, the compactness has significantly increased. The distribution of calcium carbonate in the backfill and in the crack generally shows the characteristic of more at the bottom and less at the top. Taking measures such as reducing the crack width, injecting the fixed fluid in advance, embedding the medium in the crack, and adding activated carbon in the medium can all reduce the number of grouting cycles and accelerate the sealing process.



**Title:**

Impact of different water-reducing agents on the properties of Limonite Self-compacting conductive concrete

**Author:**

Zhenhua Ren

**Affiliation:**

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**Abstract:**

The application environment for concrete is becoming increasingly complex, accompanied by an intensification of its functional requirements. This paper presents a method for developing self-compacting concrete with conductive properties using limonite and graphite as the concrete conductive phases. In the process of concrete preparation, the limonite is initially treated by a pre-wetting method to prevent the surface depression caused by the addition of limonite during the concrete curing process. The second stage of the process involved optimising different proportions of limonite and graphite and different dosages of water-reducing agent, defoamer and dispersant to prepare concrete. The influence of different dosages of limonite and graphite and different dosages of water-reducing agent on the mechanics and electrical conductivity of concrete was studied in order to obtain self-compacting conductive concrete with performance indicators meeting the requirements of self-compacting and electrical conductivity. The results demonstrate that the mechanical and electrical properties of self-compacting conductive concrete prepared with polycarboxylic acid superplasticizer and retarding superplasticizer combined with superplasticizer are satisfactory, and the composite superplasticizer can function in conjunction with dispersant. The self-compaction index, slump expansion, expansion time T50 and J-ring expansion of fluid concrete meet the requisite standards. Once the concrete has reached the designated curing age, its compressive strength and flexural strength align with the anticipated design expectations, while its resistivity meets the stipulated conductivity index requirements.

**Title:**

Assessment of Surface Treatment Systems for Protecting Concrete Structure

**Author:**

Jin Ping Lu; Sam Kim Leng Choon

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**Abstract:**

Surface treatments are grouped into three types according to the BS EN 1504-2 : 2004 (i) Impregnations reduce the surface porosity by filling totally or partially the concrete pores; (ii) Hydrophobic Impregnations produce a water repellent surface that generally has no pore filling effect; and (iii) Surface Applied Corrosion Inhibitors form a protective film on the rebar surface. In the first type of impregnations, the reaction product can block the pores, strengthening the concrete surface. The underlying basis of the impregnation mechanism is to utilise pore blockers to act as a physical barrier that reduces the ingress of aggressive agents (chloride ions and water/moisture). Molecules of the employed surface treatment chemicals are able to penetrate the surface into the concrete pores and react with the hydration products of concrete, the product of which will fill up the existing voids in the concrete's pore system, thus reducing the surface porosity. Hydrophobic impregnation mechanism relies on the production of a hydrophobic and water-repellent surface that seeks to minimise the ingress of water. With such a surface, water molecules that find their way onto the surface will exhibit the characteristic large contact angle it makes with the surface. Silanes, siloxanes and similar substances function by penetrating concrete pores, forming a hydrophobic layer, thus inhibiting penetration by water in liquid form (which may be contaminated with chloride), but allowing water vapour to enter and exit, allowing concrete to "breathe" freely. Migrating corrosion inhibitors can repair the corroded steel when applied on the concrete surface without damaging the concrete. The application of corrosion inhibitors onto the concrete surface involves the transport of the corrosion inhibitors to the rebar where it has to reach a sufficiently high concentration to protect steel against corrosion or reduce the rate of the ongoing corrosion. When corrosion inhibitor migrates to the steel surface, a mono-molecular film is formed on the steel surface. This additional layer of protection, prevents the steel from further corrosion. In addition to this, the salting-forming component of the corrosion inhibitor can react with calcium hydroxide resulting in a gel layer blocking the pores of the concrete surface. This paper presents series of testing results for the performance of these 3 types of surface treatment systems. The performance characteristic tests were performed using light weight concrete (LWC), grade G30, G40 and G60 concretes. Light weight concrete was used as substrates for the Permeability to Water Vapour – Dry Cup Method Test (BS EN ISO 12572: 2016) and Capillary Absorption & Permeability to Water

Test (BS EN 1062-3:2008). Grade G30, G40, G60 concretes were used for the remaining tests: Depth of Penetration (BS EN 14630:2006, BS EN 1504-2:2004); Water Penetration Test (DIN 1048 Pt5: 1991); Rapid Chloride Penetration Test (ASTM C1202-17A); Drying Rate Coefficient Test (BS EN 13579:2002) and Water Absorption & Resistance to Alkali Test (ASTM C1202-17A). From the testing results, it can be concluded that hydrophobic treatment as well as the impregnation of concrete does achieve a reduction in water absorption and chloride penetration. However, the degree to which they achieve that reduction is different. Overall, the hydrophobic agents are comparatively more effective than the traditional impregnates.

## Session 2

**Title:**

Experimental study on corrosion resistance of granite residual soil strengthened by biostimulation-induced calcium carbonate precipitation

**Author:**

Shiyu Liu<sup>1</sup>, Yujia Sui<sup>1</sup>, Jin Yu<sup>1</sup>, Cai Yanyan<sup>1</sup>, Tu Bingxiong<sup>1</sup>, Yang Liu<sup>2</sup>

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**Abstract:**

An extensive experimental study underpinned our robust research endeavors to enhance the erosion resistance of granite residual soils in South China. We rigorously explored the stimulation of indigenous microbial reinforcement of soils through PB-CCD optimization. Our comprehensive approach, featuring systematic indoor tests, effectively unveiled the mineralization mechanism and its influencing factors, establishing a solid foundation for our findings. The results of our research demonstrate that factors such as the type of carbon and nitrogen source, growth factors, and environmental conditions directly influence the effectiveness of biostimulation. We identified the best stimulation solution as sodium acetate (63.96mM), ammonium chloride (140.40mM), YE (0.20g/L), nickel chloride (0.01mM), urea (333mM), pH adjusted to 9.07. Among the parameters set for the mineralization scheme, the concentration of cementation solution and the number of treatments significantly affect the effectiveness of biomineralization. The concentration of cementation solution at 1.0 mol/L and 10 times treatments achieved the best enhancement results with a surface crust thickness of 17.36 mm, penetration resistance values of up to 480 kPa, calcium carbonate production of around 4.2%, and a reduction in the permeability coefficient of up to two orders of magnitude. SEM and XRD analyses show that calcite from bio-induced mineralization combines the soil particles into a single structure and significantly improves the mechanical properties of the granite residual soil. The wind erosion rate of the mineralized samples was negligible. In the rainfall splash test, the splash volume and the mean weight diameter of agglomerates (MWD) of the mineralized specimens were significantly reduced. The better the reinforcement of the specimens in the rainfall and thin runoff tests, the higher the effluent's initial pH, electrical conductivity (EC), and NH<sub>4</sub><sup>+</sup> concentrations, gradually converging to stable values as the tests progressed. This also confirms that biostimulation-induced mineralization has good resistance to water erosion and provides a new strategy for mitigating erosion caused by rainfall on soil erosion, offering practical insights for soil conservation and engineering.

**Title:**

Saturated permeability and water retention capacity in biochar-methanotrophs-clay for new landfill cover system

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**Abstract:**

A new landfill cover system, biochar-methanotrophs-clay (BMC) cover is recommended for reducing methane emissions at landfills. It also contributes to decreasing soil permeability and improving soil water retention in a long time, due to highly porous structure of biochar and the growth metabolism of methanotrophs. To determine the effects of biochar content, oxidation aging times and methane-filled days on hydraulic properties, a total of 60 groups of experiments were conducted. The saturated hydraulic conductivity ( $k_{\text{sat}}$ ) was obtained by flexible wall permeameter with controllable hydraulic head pressure. The results showed that the  $k_{\text{sat}}$  of BMC increased with increasing biochar content and oxidation aging times, while decreased with adding methane-filled days. The soil-water characteristic curves (SWCCs) were obtained with soil suction measured by the filter paper method. The results indicated the water retention capacity of BMC reduced with increasing oxidation aging times but increased with adding methane-filled days. Detected by mercury intrusion porosimetry (MIP), fourier transform infrared spectroscopy (FTIR) and scanning electron microscope (SEM), the differences displayed the changes of pore structures and extracellular polymeric substances (EPS). The oxidation aging of biochar increased the volume of pores, resulting the increased  $k_{\text{sat}}$  and the decreased water retention capacity. However, the growing of methanotrophs decreased the volume of pores, resulting the  $k_{\text{sat}}$  decreased and the water retention capacity increased due to EPS. No matter how many times the oxidation aging process was experienced, the BMC with longer methane-filled days exhibited relatively lower  $k_{\text{sat}}$  and better water retention capacity. This implied a more stable barrier capacity to reduce water infiltration in the long term. By combing a series of macro and micro experiments, this paper provides theoretical guidance for the application of biochar-methanotrophs-clay mixture to landfill covers.



**Title:**

Biochar-vegetation interactions on slope stabilization

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**Abstract:**

Biochar is the pyrolysed biomass which has been used as a soil amendment to improve plant performance. Biochar and plant roots can affect soil hydraulic properties and hence slope stability. This presentation includes (i) the effects of biochar on plant growth and the evolution of soil hydraulic properties (i.e., soil water retention curve (SWRC) and  $k_s$ ) during a two-year period; (ii) the coupled effects of biochar and vegetation on gas permeability and emission in unsaturated landfill cover through an integrated theoretical modelling and laboratory investigation; (iii) plant reinforcement to the stability of coarse-grained soil slopes, by exploring the relative contribution of mechanical root reinforcement and hydrological effects of plant-induced matric suction; (iv) a new three-dimensional (3D) theoretical model describing the effects of hydro-mechanical reinforcement of root systems on the stability of initially unsaturated soil slopes; (v) the effects of soil–plant–biochar interactions on soil PWP response and slope stability under various rainfall patterns. The above studies clearly demonstrate the effectiveness of soil–plant–biochar interactions in reducing water infiltration and enhancing slope stability.

**Title:**

Bio-cementation technique for lateral injection in a test pit: evaluation of treatment uniformity

**Author:**

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**Abstract:**

Microbial-induced carbonate precipitation (MICP) is an environmentally friendly ground improvement technique. Implementation in the field is the ultimate goal of the MICP technique. This study attempted to establish the procedures for lateral injection in the field with a 65 cm (L) × 50 cm (W) × 100 cm (H) test pit and evaluate the uniformity of treatment. The test pit was filled with 50% of the relative density of Da Nang sand. A simple configuration with a designed lateral injection system was used to simulate double-packer grouting. Four injection inlets were set up at depths 33 and 66 cm respectively. Four designed samplers were embedded in the sand at the depth between two injection inlet sections for sampling triaxial specimens. One curing cycle was conducted on the sand using a single-phase treatment solution with injection pressure graduated to 2 kgf/cm<sup>2</sup>. After curing for 7 days, we examined the water content and calcium carbonate distributions and performed a triaxial consolidated drained test to evaluate the uniformity of the treatment and the mechanical behavior of treated sand. The results show that the water content was slightly higher at the injection point, and increased with depth. This indicates that the treatment solution injected into the sand was downward seepage by gravity. Calcium carbonate content also increased with depth, illustrating the gravity effect. The peak strength of MICP-treated specimens is lower than that of untreated specimen because of poor remolding quality. Despite this, specimens that have received more treatment solution have a greater peak strength than specimens that have received less treatment solution. Although the results are not ideal, it is still possible to use them as a guide for MICP field implementation.

**Title:**

4D printing construction of living materials based on microbially-induced carbonate precipitation

**Author:**

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**Abstract:**

The construction sector substantially impacts global CO<sub>2</sub> emissions, with cement manufacturing being a leading contributor. It is imperative to explore sustainable alternatives to traditional cement with excellent cementitious properties, thus promoting carbon neutrality. Inspired by the natural biological precipitation of calcium carbonate which forms structures such as nacre and sea urchin spikes, hydrogel-based structures are 3D printed containing bacterial spores. After curing in pre-determined conditions, in-situ carbonate precipitation can be facilitated along the hydrogel networks and gradually fill in the pores, thus reinforcing the structure. Results show that the methacrylate gelatin/alginate (GelMA/Alg)-based pre-gel has good printability and can provide a great microenvironment for the survival, germination, and metabolic activity of microbes. The compressive strength of the hydrogel after bio-carbonate precipitation can be increased up to 20 times. The 3D-printed living structure is able to be utilized in many scenarios, such as nonbearing walls and artificial reefs, which could be a sustainable and eco-friendly candidate to replace conventional cement-based structures.

**Title:**

Experimental study on the dynamic fracture properties of granite exposed to cyclic freeze-thaw treatment and different mixed modes dynamic loading

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b College of Architecture and Environment, Sichuan University, Chengdu, 610065, China

**Abstract:**

Rock masses are susceptible to the combined action of freeze-thaw (F-T) weathering and dynamic mixed mode loading in cold or high-altitude regions, which is adverse to the safety and stability of geotechnical engineering. It is of great significance to reveal the effect of the cyclic F-T treatment on fracture properties of brittle rocks with different dynamic mixed modes. In this study, different mixed-mode dynamic fracture tests are carried out on the notched semi-circular bend (NSCB) granite specimens subjected to different cyclic F-T treatments. By virtue of the digital image correlation (DIC) technique combined with high-speed photography and three-dimensional (3D) scanning, the progressive fracture process and macroscopic morphology of fracture surface of the F-T treated specimens are observed, and the roughness of fracture surface is determined. The experimental results verify that the dynamic effective fracture toughness of NSCB specimens has a significant rate dependence, which increases with dynamic loading rate. At a fixed loading rate, the cyclic F-T treatment leads to the degradation of dynamic fracture toughness values, and the 30 F-T cycles are the turning points for changes in descent speed. Under higher F-T cycles, the crack propagation speed accelerated and the number of fine cracks is increased. The fractal dimension of fracture surface rises due to cyclic F-T treatment, which means F-T cycles lead to a rougher fracture surface, and this effect is more significant with the decrease of mixed mode degree. In addition, the maximum tangential stress criterion (MTS) and the generalized maximum tangential stress-based semi-analytical theoretical criterion (SA-GMTS) are used to analyze the fracture resistance contrastively. The theoretical solution predicted based on the SA-GMTS criterion is more consistent with the experimental results, and the critical distance of fracture process zone (FPZ) oscillatorily diminishes with the rise of F-T cycle numbers.

**Title:**

Research on Energy Evolution and Strain Localization of Freeze-thawed Sandstone under Uniaxial Loading-unloading

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**Abstract:**

In cold regions, combined cyclic freeze-thaw (F-T) and loading-unloading can cause rock slope instability and sliding. Hence, understanding rock damage mechanisms and fracture behavior is important in the management of engineering hazards. In essence, the macro-failure of rock masses originates from the expansion of strain localization areas driven by energy. Therefore, studying the energy evolution and strain distribution can reveal the instability process. We subjected intact and intermittently jointed sandstone specimens to a cyclic F-T environment with uniaxial graded loading-unloading to observe their mechanical parameters and obtain CT images. Investigation of energy density found that the input, elastic, dissipated, and damage energies all increased with loading according to a quadratic polynomial function, with the elastic and dissipated energies obeying a linear allocation law. Meanwhile, F-T changed the energy storage capacity and energy dissipation coefficient of rock by causing meso-damage. To measure the meso-scale strain localization characteristics, the advanced digital volume correlation technique was used to obtain three-dimensional strain fields. In terms of local strain, the failure behavior of intact specimens under low and high numbers of F-T cycles were controlled by potential fracture bands and frost-heave cracks, respectively, while fractured specimens were mainly dependent on the macro-fracture arrangement, so that the strain distribution was similar before and after F-T. However, both frost heaving and thawing transformed sandstone from brittle to ductile. In terms of statistical strain, the equivalent strain ( $\epsilon_{eq}$ ) showed a log-normal distribution and continued to deviate to the right due to loading-unloading. Meanwhile, the normal strain ( $\epsilon_{xx}$ ,  $\epsilon_{yy}$ ,  $\epsilon_{zz}$ ) presented a normal distribution, where fractured sandstone mainly developed along the slip direction, and the standard deviation of  $\epsilon_{xx}$  was about 1.6 times that of  $\epsilon_{yy}$  after failure, while the anisotropy of intact sandstone was not obvious. Finally, the intrinsic correlations among energy parameters, meso-damage, and macro-failure area discussed.

**Title:**

Study on the mechanism of biomass ash in carbonation of magnesium slag and its main mineral phases

**Author:**

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**Abstract:**

Magnesium slag is the main solid waste produced by industrial smelting of raw magnesium, and its main mineral phases are calcium silicates (27%  $\beta$ -C<sub>2</sub>S and 53%  $\gamma$ -C<sub>2</sub>S). Biomass ash is the remaining waste after extracting biomass energy in plants. This paper focused on the influence of biomass ash on carbonation of magnesium slag,  $\beta$ -C<sub>2</sub>S and  $\gamma$ -C<sub>2</sub>S, the microstructure of the carbonated products was also studied. It could be found that the addition of biomass ash has increased the compressive strength by 100% in magnesium slag and by more than 200% in  $\gamma$ -C<sub>2</sub>S after CO<sub>2</sub> curing, which was from 12.0 MPa to 24.8 MPa and from 8.1 MPa to 21.5 MPa, respectively. However, the enhancement effect of biomass ash on  $\beta$ -C<sub>2</sub>S was not significant, whose compressive strength increased only by 6.8%. It indicated that the effect of biomass ash is more obvious in low-calcium silicate phases with high carbonation activity. The structural characteristics and functional components of biomass ash are the main factors. Ca<sup>2+</sup> dissolved in the system was absorbed by functional groups such as SiO<sub>2</sub>, -OH and -CH in biomass ash, and micro-nano sized vaterite was induced to generate on the surface of biomass ash during carbonation, which could form reticular vaterite layers instead of the dense calcite layers on the surface of magnesium slag paste, so that CO<sub>2</sub> could be transmitted inside system for deep carbonation. Therefore, the products could be enriched inside the paste, and thus increasing the compressive strength of magnesium slag paste. To sum up, biomass ash has great potential and research value in the field of solid waste that may be carbonated.



**Title:**

Effects of a new synthetic Fe-PAM flocculants on filtration and consolidation of bentonite slurry

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**Abstract:**

To improve the dewatering performances of the waste slurry, a new Fe-Polyacrylamide (Fe-PAM) flocculant was synthesized. Comparative analysis with various ionic forms of polyacrylamide (PAM) reveals that the Fe-PAM flocculant has the shortest flocculation time, the lowest optimal shear gradient for flocculation, and the best filtration performance. The capillary suction time (CST) of Fe-PAM treated slurry was improved from 262.3 s (raw sample) to 6.0 s. Moreover, sludge treated with Fe-PAM exhibits the lowest porosity under the same consolidation pressure and the highest permeability at the same void ratio. In addition, mercury intrusion porosimetry (MIP) results indicated that the Fe-PAM-treated sample features the highest number of largesized inter-aggregate pores (average value of 184.2 nm and median value of 23.2 nm) and the greatest pore connectivity (60.55%), contributing to its superior permeability. The effectiveness of Fe-PAM is further elaborated through a synergistic flocculation mechanism, encompassing the electrical neutralization effect of the inorganic metal Fe<sup>3+</sup> and the adsorption bridging effect of PAM's long-chains. This study demonstrates the potential of the synthesized Fe-PAM flocculant to enhance the dewatering efficiency of high-water content slurry and offers valuable insights for synthesizing advanced flocculants in slurry treatment engineering.

## Session 3

**Title:**

Synergistic solidification of Calcareous sand by Magnesium oxide and microorganisms

**Author:**

Hailei Kou

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**Abstract:**

The synergistic solidification of activated magnesium oxide and microbial organisms is a new type of sand solidification technology. A series of biological carbonation experiments were conducted on calcareous sand specimens with different MgO contents to verify the feasibility of the method. The experimental results indicate that the synergistic solidification technology of MgO and microorganisms has the ability to improve the engineering characteristics of calcareous sand. When the MgO content is 25%, the unconfined compressive strength (UCS) of the specimen reaches 2.46 GPa. The UCS of a specimen with a MgO content of 25% is six times that of a specimen with a MgO content of 5%. The synergistic solidification of MgO and microorganisms enhances the compressive deformation ability of calcareous sand. In the one-dimensional compression test, the deformation of the specimen with a MgO content of 5% is 1.77 times that of the specimen with a MgO content of 25%. The high MgO content also changes the pore structure and distribution inside the specimen, transitioning from air pores to gel pores. Based on nuclear magnetic resonance (NMR) testing, the mechanism of the synergistic solidification of MgO and microorganisms on the internal pores of the visual specimen was discussed through T2 spectrum, GR sequences, nuclear magnetic resonance imaging, and grayscale values. The mechanism of synergistic solidification between MgO and microorganisms was analyzed from a microscopic perspective by SEM and XRD testing. The hydration of MgO together with the carbonation of brucite produces hydrated magnesium carbonate (HMCs), which improves the mechanical properties of calcareous sand. The large amount of HMCs forms a stable spatial network structure that facilitates stabilization performance and improves the mechanical properties.

**Title:**

Effective heavy metal bioremediation through enhanced biomineralization from microbiological and crystallographic perspectives

**Author:**

Liang Cheng<sup>1,2</sup>, Hui Jia<sup>1</sup>

**Affiliation:**

<sup>1</sup>School of Environment and safety engineering, Jiangsu University

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**Abstract:**

Microbial-induced carbonate precipitation (MICP) has been widely employed in the biomineralisation of heavy metals. However, the limited heavy metal tolerance of ureolytic bacteria restricts its application to environments with low heavy metal concentrations. In this study, we present a straightforward approach to significantly enhance the Cd<sup>2+</sup> resistance of ureolytic bacteria through the immediate supplementation of Ca<sup>2+</sup> ions. The presence of Ca<sup>2+</sup> ions protects the cells by reducing extracellular and intracellular Cd<sup>2+</sup> concentrations by approximately 50%. Consequently, the Cd<sup>2+</sup> removal efficiency improved by nearly 100%. Additionally, Ca<sup>2+</sup> ions also confer protection to ureolytic bacteria against the toxicity of a broad range of heavy metals. The introduction of Ca<sup>2+</sup> ions also facilitates the formation of a novel vaterite-type calcium carbonate adsorbent (biogenic CaCO<sub>3</sub>), which demonstrates a maximum adsorption capacity for Cd<sup>2+</sup> of 1074.04 mg Cd<sup>2+</sup>/g CaCO<sub>3</sub> in Cd<sup>2+</sup>-contaminated water. X-ray diffraction (XRD) and scanning electron microscopy (SEM) analyses reveal that the semi-stable vaterite form of biogenic CaCO<sub>3</sub> spontaneously undergoes dissolution and recrystallization to form thermodynamically stable heavy metal carbonate minerals in aquatic environments. Moreover, biogenic CaCO<sub>3</sub> has proven to be an efficient and viable method for removing Pb<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, and Mn<sup>2+</sup> from water samples, outperforming previously reported adsorbents. The combination of biogenic CaCO<sub>3</sub> and MICP enables secondary biomineralization, resulting in the formation of a CaCO<sub>3</sub> layer on the surface of heavy metal carbonate minerals. This process enhances the stability of the minerals in harsh environments.

**Title:**

Influence of particle and root bark morphology on the the root pull-out behavior and direct shear behavior of the root-soil matrix

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**Abstract:**

The interfacial friction behavior between the root and soils plays an essential role in the root pull-out and shear behavior of root-soil composite. This paper presents an experimental investigation of the root pull-out and shear behavior of root-soil composite, by concentrating on the effect of the morphology of particles and root bark. In this study, two types of roots with different morphologies (i.e., ficus microphylla and heptapleurum heptaphyllum) and a local soil (i.e., completely decomposed granite) in Zhuhai City (China) are used as the testing materials to examine the effect of root bark morphology. Two other special granular materials (i.e., PVC and rubber particles) are employed for an examination of the effect of particle morphology. The test results indicate that the root pull-out performance displays three characteristic stages, with the maximum pull-out force increasing with the increase of root diameter and normal pressure. A more rough root bark is responsible for a larger root-soil interface friction force and thus a greater root pull-out force. Also, the rubber particles tend to give rise to a more noteworthy interface friction and a larger pull-out force as compared with PVC particles, given otherwise similar conditions, but the shear strength of root-rubber composite is comparatively low. Furthermore, some conceptual models are proposed to account for the effect of root bark and particle morphologies on the mechanical behavior of the root-soil matrix.

**Title:**

Cyanobacteria as a Novel Nutrient Source for Biomineralization

**Author:**

Thora Arnardottir<sup>1</sup>, Jamie Haystead<sup>2</sup>, Martyn Dade-Robertson<sup>1</sup>, Pierre Reitzer<sup>3</sup>, Robin New<sup>3</sup>, Samantha Bryan<sup>3</sup>, Meng Zhang<sup>2</sup>

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**Abstract:**

Microbially Induced Calcium Precipitation (MICP) is a promising biotechnological process with substantial applications in bioremediation (Wang et al. 2023), soil stabilization (Gowthaman et al. 2023), and bioconstruction (Farajnia et al. 2022). The efficiency of MICP largely depends on the availability of a suitable nutrient source to cultivate the bacteria responsible for the biomineralization process. Traditional nutrient sources often have limitations in cost, sustainability, and environmental impact (Whiffin 2004). In an innovative approach, this study investigates the potential of cyanobacteria as an alternative nutrient source for the MICP bacteria. We evaluated the growth and biomineralization capacity of eleven ureolytic bacteria strains in minimal media supplemented with 1.5% (w/v) cyanobacterial biomass, including species such as *Anabaena*, *Leptolyngbia* sp., and *Synechococcus* sp. PCC 11901. The results showed that in the presence of the cyanobacteria in the minimal media, *Bacillus cereus*, *Bacillus subtilis*, and *Sporosarcina pasteurii* displayed enhanced growth. Remarkably, sand columns treated with the strains maintained their MICP activity and exhibited binding properties, suggesting a strong potential for practical applications. The integration of cyanobacterial biomass as a nutrient source could revolutionise the MICP field by reducing costs, increasing sustainability, and leveraging the synergistic relationship between cyanobacteria and MICP bacteria. The findings open avenues for eco-friendly and efficient biomineralization practices, paving the way for future research into the full-scale application of this technology in the environmental and construction industries.



**Title:**

Study on large-scale preparation of supplementary cementitious materials by steel slag powder carbon fixation of microbial acceleration

**Author:**

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**Abstract:**

Industrial sources such as cement and steel have large CO<sub>2</sub> emissions and low utilization. Steel slag has large annual emissions, high total inventory, and low added value of traditional utilization methods. Microorganism can accelerate the fixation of industrial CO<sub>2</sub> by steel slag powder, and prepare supplementary cementitious materials efficiently, which is helpful to achieve the purpose of carbon neutralization. This report takes the large-scale reaction of steel slag and kiln tail flue gas in a cement plant as an example, introduces the conditions of kiln tail flue gas, and expounds the process of large-scale preparation of carbon-fixing cementitious materials by microbial synergistic waste residue. The stability of carbon fixation was studied. The phase, chemical composition and microstructure of waste residue before and after carbon fixation were analyzed. The soundness and activity of carbon fixation waste residue as cement supplementary cementitious materials were considered. The research results will promote the high value, scale and industrialization of CCUS technology, and have important practical significance and high promotion value for realizing the efficient utilization of solid waste and the green development of carbon reduction.

**Title:**

Mechanical properties and stability of zinc-contaminated red clay cured by MICP synergistically activated MgO

**Author:**

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**Abstract:**

Microbially Induced Carbonate Precipitation (MICP) technology offers an innovative approach for the solidification and stabilization of heavy metal-contaminated soils; However, the mechanical strength and long-term stability of this remediation method have not been thoroughly investigated. This study introduces an innovative curing and stabilization technique using MICP-activated MgO to address the geotechnical challenges posed by zinc ion-contaminated soils. To investigate the effect of zinc ions on soil and the optimal efficacy of MICP-activated MgO in curing zinc contamination, experiments were conducted on zinc-contaminated soils with varying zinc ion concentrations (0.05%, 0.1%, 0.5%, 1.0%), dry densities (1.35, 1.4, 1.45, 1.5 g/cm<sup>3</sup>), and activated MgO admixtures (1%, 2%, 5%, 10%). The effectiveness of MICP-activated MgO was evaluated through macroscopic analysis and stability tests, including unconfined compressive strength tests, direct shear tests, and the Toxicity Characteristic Leaching Procedure (TCLP). The results indicated that zinc ions disrupted soil particle cementation, enlarged inter-particle pores, and significantly reduced both unconfined compressive strength and shear strength. The optimal dosage of MICP-activated MgO for curing zinc-contaminated soil was determined to be 10%, resulting in an unconfined compressive strength of 1.196 MPa and a Zn<sup>2+</sup> leaching concentration of 0.1414 mg/L. The combined actions of MICP-activated MgO facilitated the formation of alkaline magnesium carbonate, calcium carbonate, and magnesium hydroxide. These compounds filled the inter-particle pores of zinc-contaminated soil, encapsulating and co-precipitating zinc ions, thereby enhancing the soil's strength and stability. These findings establish a theoretical foundation for the engineering application of MICP-activated MgO in the remediation of zinc-contaminated soils.

**Title:**

Differences in carbon sequestration rates of granular  $\gamma$ -C2S,  $\beta$ -C2S and C3S minerals and their microscopic mechanisms

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**Abstract:**

Mineral carbon sequestration is a significant area of current research due to its environmental and industrial importance. This study delves into the relationship between the carbon fixation rates of three minerals -  $\gamma$ -C2S,  $\beta$ -C2S, and C3S - and their reaction contact surface and microscopic evolution. The research uncovers the microscopic mechanism behind the varying carbon fixation rates. The findings indicate that: (1) Both  $\gamma$ -C2S and  $\beta$ -C2S exhibit similar carbon fixation rate development patterns following the BoxLucas1 model. The calcium carbonate crystals predominantly consist of calcite on the surfaces of these particles, with a preference for the (1 0 4) thermodynamically stable surface. (2) The calcium carbonate growth on  $\gamma$ -C2S particles progresses from mantle to epitaxial growth, resulting in a larger reaction contact surface and sustained reactivity. Conversely,  $\beta$ -C2S grows epitaxially into the mantle, leading to denser nucleation and more stable grain size development. (3) C3S demonstrates the fastest carbon fixation reaction initially, but stagnates after 5 minutes, aligning with the shrinking core reaction model. Acicular aragonite and cubic calcite grow intertwined on the surface of C3S in the early stages, forming a dense structure that hinders further carbon fixation. Over time, aragonite transforms into calcite as the reaction progresses.

**Title:**

Alternative scaffold materials for photosynthetic and ureolytic MICP

**Author:**

Robin New

**Affiliation:**

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**Abstract:**

Ureolytic MICP has become of great interest in recent years as a promising, low emission alternative to cementitious binders in construction materials. Some studies [C. Heveran et al., 2020] have shown that photosynthetic MICP can be used in combination with other additives to produce aggregate-gel-cell-carbonate hybrids, or living building materials (LBM's), that are stronger than the sum of their parts. These studies used gelatine as a matrix forming agent to provide a scaffold on which the cyanobacteria can grow and precipitate calcite. While gelatine is cheap and readily available, it's derivation from animal bones and cartilage comes with both ethical and scaling concerns. Construction materials are typically produced and sold in tonnes, and sourcing gelatine on that scale would significantly increase global demand. It is therefore necessary to look for other, readily available alternatives. Rheological testing of 8 different matrix forming agents (MFA's), namely gelatine, EPS, agar, pectin, cellulose, lignin, starch and sodium alginate) was performed to determine the optimum concentration for mixing. Compressive testing cubes of aggregate/MFA composites were produced and mechanically tested to compare the impact of MFAs on macro material properties and, finally, the effect of those MFAs on the growth of a range of different photosynthetic and ureolytic organisms was evaluated using optical density and CFU measurements. Testing is currently ongoing, but has already shown that gelatine is not the best matrix option for either supporting growth of microorganisms or conveying mechanical strength to the material. The feasibility of materials other than gelatine to provide a scaffold for cellular growth and mineralisation significantly fortifies the case for MICP as a practical, scalable technology, and their potential to convey additional, novel properties is worthy of further exploration.

## Session 4

**Title:**

Optimization of bio-cementation for soil stabilization and erosion control

**Author:**

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**Abstract:**

Bio-cement is a type of cementitious materials by adopting natural biological processes for geotechnical and construction applications. Bio-cementation is usually achieved through microbially- or enzyme-induced carbonate precipitation (MICP or EICP). The use of soybean urease can be a cost-effective solution for carbonate precipitation and bio-cementation, which are named SICP. The produced calcium carbonate can cement soil particles and bring considerable strength improvement to soils. The optimization of bio-cementation can be made by changing the treatment materials and procedures, such as using lysed cells, low pH, and the salting-out technique. The objectives are to improve treatment uniformity and efficiency. Using cheap and waste materials for bio-cement treatment and bacterial cultivation can also be an alternative way to reduce cost and environmental impacts. Studies on the mechanical behaviour and erosion control performances of bio-cemented soil show that the resistances to wind and hydraulic erosions can be improved significantly through the bio-cement treatment. In addition, the use of optimized method and additives such as xanthan gum and fibers can further enhance the strength, treatment uniformity or ductility of the bio-cemented soils. Attention should be paid that wind forces with saltating particles has much stronger destructive effect than pure wind, which should be considered in laboratory tests. Field studies indicate that bio-cement can improve soil surface strength and erosion resistances effectively. Besides, local plants can germinate and grow on bio-cemented soil ground with low-concentration treatments.

**Title:**

Enhanced Effect of Ice Nucleation Active Bacteria on the Strength of Warm Permafrost

**Author:**

Liyun Tang

**Affiliation:**

School of Architecture and Civil Engineering, Xi'an University of Science and Technology

**Abstract:**

Ice nucleation active (INA) bacteria are capable of triggering ice formation close to 0°C, but their ability of increasing ice content in warm permafrost remain unknown. Ice content is vital because it determines the bearing capacity of warm permafrost. Through nuclear magnet resonance and direct shear device, we found that adding INA bacterium *Pseudomonas syringae* with a concentration of 1 g/L in warm frozen soil can result in 64% increase in the shear strength and 27% increase in ice content. Warm frozen soil with *P. syringae* exhibits brittle failure under normal stresses of 100 kPa to 300 kPa and plastic failure under 400 kPa. The shear strength increment can be regulated by the concentration of *P. syringae* which exponentially relates to ice content and linearly to shear strength. This emerging strategy reveals the importance of INA bacteria in cooling permafrost, and provides a new vision for confronting permafrost degradation and subsequent infrastructure instability.

**Title:**

Improving Erosion Resistance of Mine Tailing Dams with EPS-aided Biocementation

**Author:**

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3. Department of Mineralogy and Petrology, University of Granada, Spain.
4. School of Molecular and Life Sciences, Curtin University.

**Abstract:**

Mining is the backbone of the global economy. However, with the immense contribution to technological advancement and the economy, mining also puts the geoenvironment under great threat with its byproduct, termed Mine Tailings (MT). MT are often dumped in engineered dams for their safe disposal. However, prolonged exposure to the environment induces instability in the MT depositions, which is often initiated by erosion. The failures of MT dams that happened at (1). the Baia Mare Aurul gold mine in Northwestern Romania, resulting in a toxic cyanide spill in river Tisza (2000) and (2). the Brumadinho dam disaster in Brazil (2019), killing 270 people, are a few of the threatening examples. In this paper, we discuss the potential of microbially-aided stabilisation technology for improvement in the mechanical properties of mine tailings. The MT samples were obtained from local mining industries in Western Australia and treated with the extracellular polymeric substance (EPS)-producing ureolytic culture of *Bacillus subtilis* capable of microbially induced calcium carbonate precipitating (MICP) via spraying strategy in multiple stages to develop an erosion-resilient surficial crust after characterising the virgin MT for its physicochemical (pH, electrical conductivity, mineralogy, grain size) characteristics. The surficial strength characteristics of the developed crust were investigated using a needle penetrometer in dry and wet conditions considering field conditions. The hydraulic erosion resistance of the specimens was investigated using a pocket erodometer for the treated samples in wet conditions. The results demonstrate that the erosion resistance of the MT material improved drastically with EPS-aided biocemented MT specimens despite lesser strength and CaCO<sub>3</sub> content than only MICP treatment. The results from the current study can provide alternate solutions to provide an additional layer of safety in the closure of Mine Tailings.



**Title:**

Experimental Study on Microbial-cemented Tailings under Different Calcium Salts

**Author:**

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**Abstract:**

The storage of tailings continues to increase with the development of mining industry, featuring large land occupation, small particle size, and low comprehensive utilization rate. Therefore, the resource utilization of tailings is of great significance and urgently needed. Calcium salt is an important contributing factor for calcium-based biomineralization. To study the effect of calcium salt on microbial-cemented tailings, the calcium salts, including the calcium chloride ( $\text{CaCl}_2$ ), calcium acetate ( $(\text{CH}_3\text{COO})_2\text{Ca}$ ) and calcium nitrate ( $\text{Ca}(\text{NO}_3)_2$ ), were used to prepare the biotreatment solution to carry out the biomineralization tests in this paper. Two series of biomineralization tests in solution and sand column, respectively, were conducted. Scanning electron microscopy (SEM) and X-ray diffraction (XRD) were performed to determine the microscopic characteristics of the precipitated calcium carbonate ( $\text{CaCO}_3$ ) crystals. The experimental results indicate that the biomineralization effect is best for the  $\text{CaCl}_2$  case, followed by  $\text{Ca}(\text{NO}_3)_2$ , and worst for  $(\text{CH}_3\text{COO})_2\text{Ca}$ . The mechanism for the effect of the calcium salt on the microbial-cemented tailings mainly involves: 1) inhibition of urease activity; and 2) influencing on the crystal size and morphology of  $\text{CaCO}_3$ . Calcite is the main calcium carbonate crystal induced by  $\text{CaCl}_2$  and  $\text{Ca}(\text{NO}_3)_2$ , and the calcium carbonate crystals induced by  $(\text{CH}_3\text{COO})_2\text{Ca}$  are mainly vaterite. The difference in biomineralization among the three cases is strongly correlated with the crystal morphology of the precipitated  $\text{CaCO}_3$ .

**Title:**

Mechanical Characterization of Stabilized Dredged Marine Deposits with High Water Content Using Industrial Wastes and Vacuum Preloading

**Author:**

Rundong ZHAO, Wenbo CHEN, Chao ZHOU

**Affiliation:**

Hong Kong polytechnic university

**Abstract:**

Due to the high water content and poor compressive strength, dredged sediment cannot be recycled directly. Alkali-activated industrial wastes can serve as an alternative to cement for stabilizing sediment, offering the benefits of lower costs and reduced carbon emissions. However, pure chemical stabilization often shows limited effectiveness in treating sediments with high water content. This study develops a mixed binder including incinerated sewage sludge ash (ISSA) and ground granulated blast-furnace slag (GGBS) to stabilize Hong Kong marine deposit (HKMD) together with vacuum preloading. The research evaluates the new binder's effectiveness in treating HKMD with water content up to 200% and compares the performance with and without vacuum preloading. The results demonstrate that ISSA and GGBS can be used as cement alternatives to stabilize dredged sediment, and the composite stabilization method (VPCSM) significantly outperforms the pure chemical stabilization method (CSM). The strength and stiffness of VPCSM increase at least five times compared to CSM. Additionally, the treated HKMD exhibits a larger elastic range than pure clay and a smaller stiffness degradation rate than cemented clay. The treated HKMD exhibits crossanisotropy in stiffness, which is caused by the anisotropic micro-structural fabric and is further enhanced by vacuum preloading. The coupling effect of vacuum preloading and chemical solidification includes two main aspects: (1) vacuum preloading reduces the water content of the dredged sediment, which lowers the water-cement ratio and densifies the soil; (2) the solidification reaction increases the permeability coefficient of the mud in the early stage, thereby accelerating the efficiency of vacuum preloading.

**Title:**

Effect of urease activity on the cohesion of deposited calcium carbonate

**Author:**

Rui Zhang

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**Abstract:**

The biogenic  $\text{CaCO}_3$  based on microbial induced calcium carbonate precipitation (MICP) is a promising bio-cement, which has been applied in many fields of concrete self-healing, existing crack repair, modification of recycled aggregates, soil consolidation, etc. Ureolytic bacteria has been most widely used, which have high urease production, resulting in a rapid precipitation rate of  $\text{CaCO}_3$ . However, the excessive rate usually leads to the rapid formation of  $\text{CaCO}_3$  blocking the flow channel of precipitate precursors. In addition, the rapid precipitated  $\text{CaCO}_3$  particles are relatively loose and have low bonding strength with matrix, which greatly limits the utilization efficiency of biogenic  $\text{CaCO}_3$ . The cohesion among  $\text{CaCO}_3$  and its bonding with aggregates are extremely important, which have been found to be closely influenced by the precipitation rate. While in the ureolytic process, urease activity is the key to control the precipitation process. Therefore, taking the modification of recycled aggregates as an example, in this study, the precipitation process was controlled by regulating the release rate of urease activity and its effect on the mechanical properties of the deposited  $\text{CaCO}_3$  was studied. The results showed that controlling the initiation time of induced bacterial urease and the concentration of precipitated precursors (calcium and urea) could effectively slow down the release rate of initial urease activity, thus regulating the precipitation rate. It was found that the bacteria cultivated without substrate urea can slowly release urease activity, and the urea decomposed process can be sustained until two weeks, and the best cohesion of biogenic  $\text{CaCO}_3$  could be obtained at 0.5 M of calcium and urea. It was concluded that slow precipitation process was beneficial to enhance the cohesion of  $\text{CaCO}_3$  and the bonding strength with aggregates, which was of great significance to improve the application efficiency of biogenic  $\text{CaCO}_3$  in civil engineering.

**Title:**

Transcriptome analyses reveal the metabolic mechanisms of *Sporosarcina pasteurii*

**Author:**

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**Abstract:**

Recently, *Sporosarcina pasteurii* (*S. pasteurii*) has become one of the most popular bacteria in MICP. However, the metabolic mechanism related to biomineralization of *S. pasteurii* has not been clearly elucidated. In this paper, the growth and gene expression of *S. pasteurii* under three different culture conditions were compared through transcriptome analyses. It revealed that both ammonium and urea were direct nitrogen sources of *S. pasteurii*, and the presence of ammonium might promote the synthesis of intracellular ATP and enhance the motility of the bacteria.

**Title:**

Study on the properties and mechanism of sand reinforced by ARTP mutagenized calcified microorganisms

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**Abstract:**

Microbial-induced calcium carbonate precipitation (MICP), as a low-carbon and environmentally friendly soil improvement method, has emerged as the most dynamic innovation direction in environmental geotechnical engineering. However, the low mineralization efficiency and the byproduct ammonia limit its wide spread application of urea-hydrolysis based MICP technique. Here, we propose a new technique termed “modified bacteria-MICP” to enhance mineralization efficiency and mitigate ammonia emissions. This technique utilizes atmospheric pressure room temperature plasma (ARTP) mutagenesis to select modified urea-hydrolyzing bacteria with enhanced urease activity and combines with MICP technique. By using modified bacteria to efficiently catalyze urea hydrolysis, the working concentration of urea (a contributor to waste ammonium liquid) is reduced, and simultaneously, the quantity and crystal form of microbially precipitated calcium carbonate (a binder of biocement) are improved. To understand the new technique, we test the stability of urease activity in the modified bacteria after six generations, the effects of urea concentration, calcium acetate concentration, and inoculation ratio on calcium carbonate production and ammonium concentration in effluent are investigated using response surface methodology. Additionally, an efficient and controllable biocementation process is given to mitigate ammonium pollution. On this basis, the modified bacteria-MICP technique is employed to reinforce the sand by two phase injection method to assess its performance in ammonium emission, cementation strength, permeability and erosion control. Furthermore, the microscopic properties of modified bacteria-MICP reinforced sand are characterized, including microstructure, mineral composition and pore structure. The results of the study aim to offer a novel perspective on the sustainability of biogeotechnical engineering.

## Session 5

**Title:**

Application of *Aspergillus Oryzae* Fungi for increasing shear strength of loose sand

**Author:**

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**Abstract:**

The purpose of this research is to examine the possibilities of utilizing fungi, namely *Aspergillus Oryzae* Sp, to increase the shear strength of loose sand. This study is carried out in a laboratory using an elemental scale, such as a cylindrical shape. Some factors that can improve the shear strength of the treated sample are being investigated, including inoculum dose, incubation method, incubation period, and supplementary nutrition (soybean flour and rice flour). The unconfined compression test is used to determine the shear strength of treated materials. Some findings are the outside incubation method yields a better growth of Fungi and produces higher shear strength. Either 10% or 15% of water content is suggested to prepare the sample. At 5% water content, the Fungi could not grow properly, while 10% to 15% water content yields close shear strength of treated soil at the 28-day curing period. Moreover, additional soybean flour has a better effect than additional rice flour in the outside incubation method. The shear strength of the treated samples could increase by double. However, in the inside incubation method, additional soybean flour to the treated sample has a negative effect, and the shear strength drops significantly. Finally, soil treated with *Aspergillus Oryzae* Sp has a lower shear strength than soil treated with *Rhizopus Oligosporus* Sp and *Rhizopus Oryzae*. With the 10% inoculum dosage, 5% soybean flour dosage, and 10% water content, the shear strength of *Aspergillus Oryzae* Sp fungi-treated soil could have about 1.5 times lower shear strength than *Rhizopus Oligosporus* Sp and *Rhizopus Oryzae* treated soil.

**Title:**

Cracks repair by use of microbially induced carbonate precipitation: progress and challenges

**Author:**

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**Abstract:**

In recent years, cracks repairing based on microbial induced carbonate precipitation (MICP), has attracted much attention as an environmentally friendly and compatible repair method for cementitious materials. In-situ precipitation of biogenic  $\text{CaCO}_3$  is especially beneficial for repairing of micro-cracks, since the repairing solution has a low viscosity and therefore easy to penetrate through the whole crack, and hence a homogenous distribution of healing products can be formed inside the crack. So far, most research has been focused on the theory of precipitation in ideal bulk solution. Yet, practical applications still encounter many problems, such as long repair time, low strength recovery and so on. The healing efficiency is much lower than that in ideal experimental conditions. The basic reason is the significant difference between the real crack environment and the ideal bulk solution environment, in the aspects of physical and chemical conditions, resulting in a low volume yield of the bio-precipitates. One most important issue, the way of applying healing agents to the crack, that is, the repair technology which greatly influences the instant concentrations and distribution of bio-reaction agents in the crack, was ignored. Therefore, this study conducted a comprehensive investigation on the influence of repair technology on healing efficiency of micro-cracks, specifically, the injection rate, the pre-sealing length of the crack and the injection position. Healing efficiency was evaluated in terms of crack healing depth, crack volume filling rate and strength regain. Meanwhile, the correlation among the repairing parameters and healing rate was semi-quantified, which could provide critical insights to further optimize the MICP based crack repair technique.



**Title:**

Development of a pH-responsive hydrogel with high moisture absorption for bacteria-based self-healing concrete

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**Abstract:**

Self-healing concrete by bacteria, based on microbial induced carbonate precipitation (MICP), has attracted much attention as an environmentally friendly and compatible repair method with concrete. Carbonate precipitating bacteria are added into mortar specimens to in-situ heal concrete cracks when cracking occurs. Due to the high alkalinity and small pore sizes of concrete, bacteria cannot be added directly. Therefore, an immobilization process is preferable. In our previous study, hydrogel has shown its superiority over other carriers due to its excellent biocompatibility and water absorption and retention property. However, also due to its water absorption or swelling property, hydrogel absorbs water and swells during the mixing process, while release the water and shrinks during the hydration process, resulting in the formation of macropores inside cementitious matrix, and hence a significant decrease of strength. To solve this problem, a novel preparation strategy of chitosan-carrageenan composite hydrogel was proposed for the protection of bacterial spores and applied to the crack self-healing of cement-based materials. The pH sensitivity and hygroscopicity of the composite hydrogel were investigated by swelling and hygroscopicity tests. The bacteria spores entrapping property of chitosan-carrageenan composite hydrogel was investigated by means of a leakage test. The self-healing efficiency of the concrete with/without chitosan-carrageenan composite hydrogel was studied quantitatively by stereo microscope. The results indicated that the swelling of the composite hydrogel has an excellent pH responsive property with low swelling i.e. 17.55-28.67 g/g at pH 12.5 solution as well as cement filtration slurry and high swelling i.e. 43.50-65.38 g/g at pH 10, pH 11 solution as well as deionized water. The escape rate of the spores after solidification by the composite hydrogel ranged from 0.20 % to 0.30 %. In the mortar specimen mixed with composite hydrogel loaded with bacteria spores, the maximum crack width healed was 374  $\mu\text{m}$  after 28 d. The compressive strength reductions were no significant decrease when the hydrogel addition of 0.5~1% by cement mass. It can be concluded that the new chitosan-carrageenan composite hydrogel with bacterial spores can effectively improve the self-healing efficiency of cement-based materials.

**Title:**

Study on the impact of real crack environments on biogenic CaCO<sub>3</sub> precipitation process in microbial self-healing concrete

**Author:**

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**Abstract:**

Microbial-induced carbonate precipitation (MICP) has been proven to be an effective way to seal early cracks of concrete without human intervention. However, it was found in most studies that the distribution of remediation products in the fracture zone following microbial deposition is uneven, which in turn limits the applicability of the technology. In order to figure out the relationship between real crack environments and microbial mineralisation process, in this study, on the basis of the self-healing system by means of urea hydrolysis in combination with hydrogel as carriers, the physicochemical characteristics along the depth of cracks were studied in the aspects of dissolved oxygen, pH and precipitation precursors (Ca<sup>2+</sup> and urea). Results showed that in all groups, dissolved oxygen displayed a decreasing tendency along the depths of cracks after 24h and as time progresses, the oxygen content within the fracture zone declines gradually. The pH values exhibited a range of approximately 11 to 13.2 within a depth interval of 40 mm when specimens were exposed to atmospheric environment for non-calcium series and the values could be lower in the presence of doped calcium source. Moreover, for precipitation precursors, the concentration of Ca<sup>2+</sup> from crack surface to 10mm were decreased gradually under the effect of diffusion and airborne CO<sub>2</sub>, much lower than that at the bottom part regardless of shapes of cracks. A positive linear relationship is observed between urea concentration and dosage, as well as crack zone volume. In combination with the factors affecting the process of microbial mineralisation deposition, this study will illustrate the effect of real crack environments on the precipitation process of biogenic CaCO<sub>3</sub>.

**Title:**

Utilization of pH Responsive Hydrogel as Bacterial Protector in Manufacturing Self- Healing Mortar

**Author:**

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**Abstract:**

A smart material that responsive to environment pH such as hydrogel has found its way in mortar application. The ability of hydrogel to absorb a high amount of water in certain pH made this material become a promising bacteria carrier that can later mitigate micro-cracks in concrete/mortar. However, the high swelling of commercial hydrogel has a negative effect to the mechanical properties of resulting mortar. Thus, in this research, two novel hydrogels generated from carboxymethyl-cellulose (CMC) and xanthan gum that would swell less in high pH were developed. Spores of *B. Sphaericus* were encapsulated into novel hydrogels and acted as healing agent. The swelling capacity of hydrogel, the workability of fresh mortar, the compressive strength of mortar, the capillary water uptake of the mortar and the healing capacity of mortar were observed. The result shows that introducing spores encapsulated into xanthan gum hydrogel proved to increase the mechanical and physical properties of the resulting mortar. The healing and sealing performance of mortar containing xanthan gum-based hydrogel were also higher than mortar containing commercial hydrogel and CMC-based hydrogel. The maximum crack width in mortar that could be entirely closed by introducing spores impregnated into xanthan gum-based hydrogel was 0.53 mm.

**Title:**

A hydrogel-assisted EPS@(Ca-P&C) hybrid coating on biomedical magnesium alloy via microbial-induced mineralization

**Author:**

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**Abstract:**

Magnesium and its alloys have been investigated as biodegradable orthopedic implants owing to their facile corrosion, favorable biocompatibility, near-bone density and elastic modulus. However, uncontrolled degradation limits their clinical applications. Surface coatings have been utilized to mitigate dysfunction. In this work, via microbial-induced mineralization, a hybrid coating consisting of extracellular polymeric substance (EPS) and calcium phosphate / carbonate (EPS@(Ca-P&C)) was in situ constructed on hydrogel-pretreated ZK60 alloy. This approach employed bacteria as builders, making the surface structure of the coating resemble bone tissue. The coating bulk exhibited a “brick-and-mortar” architecture, with hydrogel and EPS as “mortar” and calcium phosphate & carbonate as “brick”, favoring the denseness of the coating and reinforcing its resistance to solution penetration. This type of developed coating with excellent biodegradability and osteoblastic activity helpfully advances the application of Mg-based bone implants.

**Title:**

Experimental study on the effect of microbial consortia-enhanced recycled concrete aggregates on the self-healing performance of concrete cracks

**Author:**

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**Abstract:**

Microbial induced carbonate precipitation can achieve the crack self-healing in concrete. Bacterial carriers can effectively improve the survival rate of bacteria in concrete matrix and thus improve the self-healing performance of concrete cracks. However, potential decrease in mechanical properties, poor compatibility with the cementitious matrix and relatively high cost of the current bacterial carrier cannot be ignored. The self-healing concrete based on enhanced recycled concrete aggregates (RCAs) as bacterial carrier is proposed in this study. The effect of enhancement time of RCAs on the compressive strength and self-healing capacity of concrete was studied to determine the reasonable mineralization enhancement time of RCAs. The enhancement mechanism of RCAs and self-healing mechanism of concrete were subsequently revealed. The test results show that the reasonable enhancement time of recycled concrete aggregates was 7 d. The water absorption and crushing index of enhanced RCAs decreased by 20.5% and 9.5%, respectively. The compressive strength of concrete prepared with the enhanced RCAs increased by 8.6%. After 56d of curing and healing, the average value of healed crack widths and completely healing percentages of the concrete prepared with enhanced recycled concrete aggregates were 0.44 mm and 73%, respectively. Moreover, the surface precipitations on the enhanced RCAs exhibited regular cubic shapes and the crystals of these precipitations were calcite. In addition, the crack-filling precipitation crystals of the self-healing concrete exhibited regular cubic shapes and cluster shape. The crystals of these precipitations were calcite and aragonite.

**Title:**

Investigation into the type of nutrients on the unconfined compressive behaviour of fungal composites

**Author:**

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**Affiliation:**

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**Abstract:**

Recent research has demonstrated that the growth of fungi inside soil can modify hydraulic and mechanical behaviour namely by: a) transformation of the soil towards a more water-repellent state, and b) the formation of a mycelial network that effectively binds soil particles. These modifications to soil behaviour are controlled by the extent of fungal growth achieved within the soils, which is influenced by both the specific fungal species employed and the soil treatment methodology employed. In pursuit of optimizing the methodology for treating soil with Basidiomycota fungal species, we here investigate the influence of various nutrient sources (beechwood chips, wheat bran, oat, barley, rice, corn and coffee) on mycelium growth and the unconfined compressive strength (UCS) of mycelium composites. In addition to UCS testing, Basidiomycota fungal growth was observed in petri dishes and the carbon to nitrogen ratio (C: N ratio) of each nutrient source was measured to understand the growth of Basidiomycota fungi under different nutrient conditions. An unconfined compressive strength of ~21 kPa was achieved when mycelium composites were prepared with beechwood chips only and left to grow for 4 weeks. Replacing 10% of the beechwood chips with oat and wheat bran increased the UCS value by 40% and 47%, respectively. Whereas 10% rice, barley and coffee in the substrate showed only a minor increase (in the range 3% - 6%). Whereas the addition of corn kernels showed a decrease in the UCS value.

Our results demonstrate that the mechanical behaviour of mycelium composites is influenced by the nutrient source provided, due to the fungal hyphal network growth pattern, accessibility of the nutrition and C: N ratio. The findings in this study provide novel insights into the mechanical response of fungal-treated porous media.

**Title:**

Investigation of fungal induced carbonate precipitation (FICP) using basidiomycota fungi

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**Abstract:**

Over the last 15 years microbially induced carbonate precipitation (MICP) has gained increasing attention in the field of geotechnical engineering. Most studies have focused on the use of bacteria and in particular the microorganism *Sporosarcina pasteurii*. Fungi are also known to contribute to biomineralisation processes in the natural environment either by actively contributing via enzyme activity or passively providing a site for surface nucleation of minerals. However, to date very limited research has been conducted on fungal biomineralisation processes for engineering applications. In this study we explore the ability of basidiomycota fungi to induce carbonate precipitation (FICP) via the ureolysis pathway. In a series of batch experiments we investigated the influence of (1) the ratio of urea concentration to calcium chloride concentration, (2) the total concentrations of urea and calcium chloride and (3) the amount of fungal biomass on the rate of ureolysis and the mass of calcium carbonate precipitated. Based on the batch experiment results, the treatment strategy was designed and applied to sand columns. The effect of treatment cycle duration and number of treatment cycles on the mechanical behaviour of the FICP-treated sand columns was also investigated. Scanning electron microscopy was conducted on samples from the FICP treated sand columns.

Our results show that the ratio of urea to calcium chloride has an important influence on solution pH and therefore on the mass of calcium carbonate precipitated. In the sand columns, we demonstrated that increasing the number of treatment cycles, increases the mass of calcium carbonate precipitated. SEM images highlight that calcium carbonate crystals are precipitated on the fungal hyphal network, indicating that it can be an effective scaffold for precipitation

**Title:**

Frozen enzyme EICP method for more effective soil improvement

**Author:**

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**Abstract:**

Enzyme-induced calcite precipitation (EICP) is one of the emerging soil improvement methods. However, when plant based enzyme is used, the urease enzyme harvested from plants cannot be stored long. This affects large-scale applications of this method. This paper presents a new method that not only enables urease enzyme to be stored for a long duration, but also improves significantly the effectiveness and efficiency of EICP for soil improvement. In this method, the storage duration of soybean derived urease enzyme is prolonged by storing it at negative 20 degrees. The experimental results indicated that the frozen-stored urease enzyme had an activity of 326% higher than that of fresh enzyme. The shear strength of a fine sand treated using the frozen-stored enzyme is 238.8% higher than that using a normal EICP method. Thus, the frozen method not only overcomes the enzyme storage problem, but also offers a much-improved EICP method. The reasons for the higher urease activity and improved strength enhancement are also explained.



## Session 6

**Title:**

Research on the Suppression of Surface Powdering and Cracking at Zhouqiao Earthen Site using EICP Technology

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**Affiliation:**

Henan University, China

**Abstract:**

The infiltration of ground water and surface water is an important reason for the drying shrinkage of soil and the cracking of soil surface. The change of soil water environment will pose a threat to the stability of soil. Taking Kaifeng Zhouqiao site in China as the research object, using enzyme induced carbonate precipitation (EICP) technology, we carried out the free shrinkage test of the site soil, and investigated the change rule of the volume of the site soil in the process of drying and wetting under the concentration of different cementing liquids from a macroscopic point of view; and we carried out the mercury compression test to investigate the characteristics of the distribution of the micropores of the site soil from a microscopic point of view. We carried out the free shrinkage test to study the volume change rule of site soil in the process of drying and wetting under different binder concentrations from a macroscopic point of view; conducted the mercury pressure test to study the microporosity distribution characteristics of site soil from a microscopic point of view, and analyzed the mechanism of inhibiting the surface chalking and cracking of the site soil by combining with SEM and XRD tests. The results show that: after EICP curing, the shrinkage rate of the site soil decreases, the pore ratio decreases rapidly with the reduction of water content and then tends to stabilize, and the free shrinkage curve shows multi-stage characteristics; from the micro level, it shows that there is a bimodal pore structure in the site soil after EICP curing, and the change of pore distribution is obvious, with a reduction in the number of medium-sized and large pore spaces, which makes the structure denser compared with that of the plain soil. The research results can reveal the internal pore distribution characteristics of the site soil after EICP curing from a microscopic point of view, and provide an important reference basis for inhibiting the surface layer pulverization and cracking of the soil site.

**Title:**

Microbially influenced concrete corrosion inhibition in marine environments based on the bio-mineralization technique for sustainable coastal cities

**Author:**

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**Abstract:**

Microbially induced corrosion (MIC) on concrete poses a significant challenge, compromising the durability of concrete structures. However, unlike the concrete-based wastewater networks, insufficient attention has been paid to the impact of MIC on marine concrete degradation, and the importance of protection against MIC remains largely unrecognized. The existing strategies for inhibiting concrete corrosion also exhibit certain limitations. The bio-mineralization method was proposed in this study to form a bio-mineralized film on concrete surfaces for marine concrete protection. Laboratory seawater corrosion experiments were conducted under various conditions including chemical corrosion (CC), MIC, and bio-mineralization for corrosion inhibition. During the corrosion, the newly formed biofilms were sampled to conduct a genotypic-based investigation, aimed at screening out the corrosive bacteria from the total bacterial communities and investigating the related functional microorganisms and genes involved in the corrosion process. Moreover, the results of metagenomic data were combined with the concrete property results (e.g., mass loss and strength reduction) to uncover the potential forces influencing the corrosion inhibition effect of bio-mineralization in marine environments. The results show that MIC resulted in much higher proportions of corrosive bacteria than CC. However, the bio-mineralization treatment effectively inhibited corrosion because the formed bio-mineralized film decreased the proportion of corrosive bacteria, as well as the relative abundance of functional genes involved in the sulfate reduction. Our study contributes to the potential application of bio-mineralization for corrosion inhibition to achieve long-term sustainability for major marine concrete structures.

**Title:**

Preparation of high-strength microbial mortar

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**Abstract:**

The sand consolidation using microbial cement has been extensively researched. Nonetheless, the resulting strength of the consolidated sand columns tends to be low, which significantly hinders the broad implementation and utilization of this technology. This study delves into the functions of three different types of sand within microbial mortar and provides a comparative analysis of the sand consolidation effects based on dry density, water absorption, permeability coefficient, compressive strength, and micromorphology. The results indicate that microbial mortar incorporating dolomite sand achieves the highest dry density and the lowest water absorption rate. However, its compressive strength peaks at only 44.56 MPa. Microbial mortar with coral sand demonstrates poor impermeability and unstable compressive strength. In contrast, microbial mortar made with zeolite sand has the lowest dry density and the highest water absorption rate. Despite this, it can achieve a permeability coefficient as low as  $2.41 \times 10^{-7}$  cm/s, and its compressive strength can reach up to 74.32 MPa, with the entire consolidation process completed in just 1.5 days. The sand column attains its strength as the precipitated calcium carbonate adheres to the surface of the sand particles, forming a robust calcium carbonate shell that encapsulates the sand, thus imparting strength. Among the tested groups, the zeolite sand group develops the thickest and most compact calcium carbonate shell. Consequently, zeolite sand proves to be the most effective and durable aggregate for the preparation of microbial mortar.

**Title:**

Experimental study on the reinforcement mechanism and wave thumping resistance of EICP reinforced sand slopes

**Author:**

Shixia Zhang, Chuangzhou Wu\*

**Affiliation:**

Zhejiang University

**Abstract:**

Sand slope is an important part of coastal zone and islands, which is severely affected by wave erosion and causes problems such as degradation of coastal zone and reduction of island area. Enzyme-induced calcium carbonate precipitation (EICP) technology is a new reinforcement technology with environmental friendly and excellent effect, which has been widely studied in the field of geotechnical engineering in recent years. In this research, we focus on the coastal or reef sand slopes in marine environments. The EICP reinforcement of representative sand slope units and large scale flume wave thumping experimental study are conducted indoors. By analyzing the physical and mechanical properties, erosion resistance, and microstructure of EICP-reinforced sand slopes, the mechanism of EICP reinforced sand slopes is revealed, the feasibility of EICP reinforced sand slopes is confirmed, and a feasible solution for EICP reinforced sand slopes is finally obtained. Results show that: (1) EICP reinforcement effectively enhances the surface strength and erosion resistance of sand slopes. Higher calcium carbonate content in the sand slopes corresponds to greater surface strength and improved erosion resistance. When the calcium carbonate content is similar, using low-concentration reinforcement twice is more advantageous than using high-concentration reinforcement once due to its superior uniformity. (2) The intensity of waves, the angle of the sand slope, and the severity of erosion damage are interrelated. Higher wave intensity, steeper sand slope angles, and more serious erosion damage require stronger reinforcement measures. (3) Scanning Electron Microscope (SEM) image analysis reveals that the reinforcing effect of sand slopes primarily depends on the amount of calcium carbonate crystals cemented between sand particles. A higher content of calcium carbonate crystals leads to better erosion resistance in the sand slope.

**Title:**

Investigating the effects of microbial-induced calcite precipitation on clay's hydro-mechanical properties

**Author:**

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**Abstract:**

Microbial-induced calcite precipitation (MICP) has been a recent biogeotechnical approach studied for improving soil properties. Its application in clay remains largely unexplored as MICP is more commonly practiced with sandy soils. Clay soil's smaller void space hinders the efficiency of bacterial spread; further, limited research has been conducted on MICP efficacy on field clay strength improvements. This study aims to investigate the impact of MICP on enhancing the shear strength and hydromechanical properties of field clay.

Currently ongoing, the testing and research phase evaluates the shear strength and hydromechanical behaviours through direct shear and consolidation testing. Bacterial strain, *Bacillus pasteurii*, is selected as the MICP agent due to its catalysed ureolysis process and high calcium carbonate (calcite) yield. With these considerations and rigorous experimentation, results are anticipated to reveal enhancements in the mentioned clay properties of shear strength and permeability.

Overall, this research helps advance understanding regarding MICP as a sustainably natural solution for clay soil stabilisation. By targeting specific sustainable development goals such as environmental and infrastructure development, MICP paves the way for innovative stabilisation techniques; broadening the universal understanding of addressing challenges posed by clay within the geotechnical engineering field.

**Title:**

Exploration of airborne bacteria for high-efficiency microbial induced carbonate precipitation

**Author:**

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**Abstract:**

Microbial induced carbonate precipitation (MICP) is a promising bio-cementation process, in which the formation of calcium carbonate occurs as a consequence of microbiological activity via several pathways. The MICP via urea hydrolysis is a versatile technique that attributes to the most energy efficient solution for many engineering applications including soil improvement, environmental remediation, and construction restoration. Despite its evolvment over the past two decades, the efficient use of soil microorganisms has often been a major challenge, particularly, their survival and absolute performance are unforeseeable when confronted with complex environmental/field conditions. Since the airborne bacteria tend to abide in dynamic environments, they are believed to possess remarkable survivability and adaptability in harsh environments, thus holding more potential for engineering applications compared to soil bacteria. This study focuses on the exploration of airborne bacteria that are capable of precipitating calcium carbonate, aiming to establish a robust approach that can effectively stabilize diverse soils. Due to their wide dispersal, these bacteria traverse various locations, and selected strains from their population can contribute to the broad implementation of microorganisms. In this investigation, airborne bacteria that have been collected from sites with featured climate are subjected to a series of characterization tests under complicated conditions to evaluate their effectiveness for the technique. The results demonstrate that a large number of airborne bacteria can precipitate calcium carbonate, suggesting that the air can serve as an ideal bacterial isolation source for MICP applications.

**Title:**

Stress Sensitivity of Permeability in High-permeability Sandstone Sealed with Microbially-Induced Calcium Carbonate Precipitation

**Author:**

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**Abstract:**

Microbially induced carbonate precipitation (MICP) catalyzed by *S. pasteurii* has attracted considerable attention as a bio-cement that can both strengthen and seal geomaterials. We investigate the stress sensitivity of permeability reduction for initially high-permeability Berea sandstone (initial permeability ~110 mD) under various durations of MICP-grouting treatment. The results indicate that after 2, 4, 6, 8 and 10 cycles of MICP-grouting, the permeabilities incrementally decrease by 87.9%, 60.9%, 38.8%, 17.3% then 5.4% compared with before grouting. With increased duration of MICP-grouting, the sensitivity of permeability to changes in stress gradually decreases and becomes less hysteretic. This stress sensitivity of permeability is well represented by a power-law relation with coefficients representing three contrasting phases: an initial slow reduction, followed by a rapid drop culminating in an asymptotic response. This variation behavior is closely related to the movement and dislocation of the quartz framework controlled by the intergranular bio-cementation strength. Imaging by scanning electron microscopy (SEM) reveals the evolution of the stress sensitivity for permeability associated with evolving microstructures following MICP-grouting. The initial precipitates of CaCO<sub>3</sub> are dispersed on the surfaces of the quartz framework and occupy the pore space, which are initially limited in controlling and reducing the displacement between particles. As precipitates continuously accumulate, the intergranular slot-shaped pore spaces are first bonded by bio-CaCO<sub>3</sub>, with the bonding strength progressively enhanced with the expanding volume of bio-cementation. At this stage, the intergranular movement and dislocation caused by compaction are reduced, and the stress sensitivity of permeability is significantly reduced. As these slot-shaped pore spaces are progressively filled by the bio-cement, the movement and dislocation caused by compaction become negligible and thus the stress sensitivity of permeability is minimized.



**Title:**

Effect of (in)organic additives on microbially induced calcium carbonate precipitation

**Author:**

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**Abstract:**

Calcium carbonate (CaCO<sub>3</sub>) exists in a range of different crystalline morphologies which each have useful applications. Chemical methods of CaCO<sub>3</sub> precipitation have been established to produce specific CaCO<sub>3</sub> morphologies which include the use of different organic and inorganic additives. Microbially induced CaCO<sub>3</sub> precipitation (MICP) is another method of producing CaCO<sub>3</sub> that uses bacterial cells and their associated biological activities to mediate the formation of CaCO<sub>3</sub>. Limited studies have been conducted with MICP alongside additives to understand their impact on CaCO<sub>3</sub> morphology and precipitation rate, therefore this warranted further research. During this study, MICP was monitored in the presence of organic and inorganic compounds using confocal microscopy to observe CaCO<sub>3</sub> formation from the point of nucleation. Additional measurements by scanning electron microscopy, energy-dispersive X-ray spectroscopy and X-ray diffraction were performed to assess CaCO<sub>3</sub> at later stages of crystal development. The results displayed differences in CaCO<sub>3</sub> precipitation in the presence of additives. These differences included quantity of crystals, size of crystals, unique morphologies and delayed precipitation. The work demonstrates the effect of several additives on MICP and sets the stage for further tests to understand additive effects on MICP and achieve controlled CaCO<sub>3</sub> precipitation.

**Title:**

A Study on Sand Behavior of Injection Method on Multiple Cycle MICP Treatment

**Author:**

Amalia Ula Hazhiyah, Yu-Syuan Jhuo, Louis Ge

**Affiliation:**

National Taiwan University

**Abstract:**

The study focuses on the mechanical behavior of the sand specimens that were treated with multiple cycles of MICP (microbial induced carbonate precipitation) treatment. An injection method was proposed single-phase treatment solution into triaxial sand specimens with a falling head injection pressure was applied. The effect of treatment cycle and injection pressure on treatment uniformity were determined by acid digestion. A series of consolidated drained (CD) triaxial tests were performed to investigate the mechanical behaviors of MICP-treated specimens varying with number of treatment cycles. The results showed that injecting multiple cycles of a single-phase treatment solution doesn't affect MICP-treated uniformity, and the calcium carbonate content increases with each treatment cycle. Injection pressure, however, is a critical factor that influences treatment uniformity under the same number of treatment cycles. The increased calcium carbonate content in MICP-treated specimens reflects higher peak strength at the same confining pressure. Multiple cycles of treatment also result in significant increases in shear strength parameters.

**Title:**

Microbial mineralization technology applied in self-healing of marine concrete

**Author:**

Jing Xu

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School of Materials Science and Engineering, Tongji University

**Abstract:**

In marine industry, infrastructures are facing serious issues of deterioration stem largely from concrete cracking due to the hostile seawater environment. Microbial mineralization is an effective and environmentally friendly way to achieve self-healing of concrete cracks. However, the formation and structure of biomineralized products in seawater remain unclear. Besides, there is a lack of assessment on self-healing effectiveness of concrete under multiple damage cycles in marine environment. To close the research gaps, this work first investigated the evolution of aqueous species as well as the phase assemblages and microstructures of bio-minerals in simulated seawater by experimental and thermodynamic approaches. The coupling effects of  $Mg^{2+}$  and bacteria on bio-mineralization kinetics result in significant discrepancy between experimental results and thermodynamic outputs in terms of phase assemblages, yet the morphology depends on  $Mg^{2+}$  rather than bacteria. A comparative analysis on the healing products collected from bio-concrete cracks in seawater revealed a fast abiotic precipitation of brucite followed by the biomineralization process. After multiple cracking cycles, the microbial specimens still exhibit strong crack healing ability.

## Session 7

**Title:**

Micromechanical Properties and Bonding Fracture of EICP-reinforced Sand Analyzed Using Microindentation Test

**Author:**

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**Abstract:**

Enzyme-induced carbonate precipitation (EICP) has emerged as an environment-friendly solution for soil improvement. As a composite material, it is challenging to determine the micromechanical properties of EICP-reinforced sand using common macromechanical tests. In this work, a systematic study was conducted to determine the micromechanical properties of EICP-reinforced sand. The development of the micromechanical properties obtained from indentations along the route of “sand particle-CaCO<sub>3</sub>-sand particle” was examined. The width of interfacial transition zone (ITZ) in EICP-reinforced sand was investigated. The effect of reaction environment on ductility (i.e., ratio of elastic modulus over hardness) of CaCO<sub>3</sub> was investigated. The experimental results have identified that the width of ITZ in EICP-reinforced sand ranges from 0 to 180 μm, which is significantly influenced by the crystallinity or crystal morphology of CaCO<sub>3</sub>. The presence of porous media (i.e., sand particle) leads to the decrease of impurities content in crystal formation environment, resulting in the lower ductility of CaCO<sub>3</sub> accordingly. The mean value of fracture toughness of CaCO<sub>3</sub> precipitation was identified to be the lowest one among sand particles, CaCO<sub>3</sub> precipitation, and sand particles-CaCO<sub>3</sub> interface. The lowest fracture toughness of CaCO<sub>3</sub> indicating the failure of biocementation is derived from the CaCO<sub>3</sub>-CaCO<sub>3</sub> breakage.

**Title:**

An efficient microbial sealing of rock weathering cracks using bio-carbonation of reactive magnesia cement

**Author:**

Zhi-Hao Dong, Xiao-Hua Pan, Chao-Sheng Tang, Dian-Long Wang, Rui Wang, Bin Shi

**Affiliation:**

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**Abstract:**

Bio-carbonation of reactive magnesia cement (RMC) was proposed as a promising strategy to stabilize the weathered stone grains for the efficient sealing of rock weathering cracks near surface. The physical and mechanical properties of the sandstone grains stabilized by bio-carbonation of RMC was first investigated to verify the feasibility of applying the method to the rock weathering cracks which always subjected to compression strength from hundreds of kilopascals to several megapascals. Except initial water content, RMC content and urea concentration, two new effects including bacteria concentration and bacteria solution composition on the complex underlying stabilization mechanism were systematically studied. Four types of bacteria solutions such as bacteria solution with different concentrations, inactivated bacteria solution, liquid supernatant and nutrient solution were considered. The experimental results showed that bio-carbonation of RMC can effectively stabilize the sandstone grains. The highest unconfined compressive strength (UCS) of bio-carbonated sample was up to 5.5 MPa, which was 7.8 times higher than that of the sample stabilized by RMC hydration only and could meet the site requirement of compressive strength resistance. The UCS increases as the RMC content and bacteria concentration increase, while the effects of initial water content and urea concentration is opposite. The dry density, resistance of water absorption, electrical resistivity and P-wave velocity are in proportion to the UCS. The analysis results of the effect of bacteria solution composition indicated that the well stabilization performance on sandstone grains can be attributed to the coupling effect of the bacteria and organic matter such as biomolecules. The bacteria and biomolecules inhibited the air-based carbonation but facilitated the bacteria-based bio-carbonation and made the microstructure more denser, especially subjected to high bacteria concentration. Hydrated magnesium carbonates (HMCs), varying types of products produced during bacteria-based bio-carbonation process not only fill the micro-pore spaces and cement the sandstone grains and amorphous brucites together, but also can form a stable spatial network structure with high strength when the amount of HMCs is large. Finally, 15~20% RMC content, 0~2 M urea concentration and high concentrated bacteria solution with a reasonable initial water content is suggested to be the optimal bio-carbonation strategy for the well-graded coarse sandstone grains.

**Title:**

Soil improvement via polymer-assisted soybean crude urease carbonate precipitation technique

**Author:**

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**Abstract:**

Biocementation using soybean crude urease carbonate precipitation (SCU-CP) technique has become an innovative and economical method for soil improvement. However, it encounters obstacles such as low solution viscosity and inconsistency carbonate precipitation, which result in inadequate calcium carbonate distribution. In order to eliminate this shortcoming, this study presents a series of experiments performed to improve uniform distribution of carbonate precipitation. By incorporating polymers, a biodegradable polymer that enhances solution viscosity, this research optimizes SCU-CP. In this study, the pH and precipitated mass are measured to examine the effectiveness of biocementation. Furthermore, the mechanical properties of the treated soil specimens are investigated through unconfined compressive strength (UCS) tests. Carbonate content measurements on treated samples are also conducted. Then, scanning Electron Microscopy with Energy Dispersive Spectroscopy (SEM-EDS) tests are performed to observe the particle morphology and composition of the precipitated material on treated sample. Finally, this study elucidated that polymer-assisted soybean crude urease carbonate precipitation technique has a potential to be an effective technique for soil improvement.

**Title:**

Evaluating the effect of soil grading on UCS of MICP treated sandy soils

**Author:**

Reena N. Hora<sup>1</sup>, Md Mizanur Rahman<sup>2</sup>, Md Rajibul Karim<sup>2</sup>, and Simon Beecham<sup>2</sup>

**Affiliation:**

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<sup>2</sup> UniSA STEM, University of South Australia, Mawson Lakes, SA 5095

**Abstract:**

Several previous studies have investigated the unconfined compressive strength (UCS) behaviour of Microbial Induced Calcite Precipitation (MICP) treated sandy soils and have developed empirical relationships to predict strength improvement. While UCS of MICP treated soils can be affected by several factors such as chemical concentration, temperature, and biochemistry, it has been found recently that particle size is one of the most important contributing factors, which none of the previously published studies have considered. This study applied MICP on three different variants of AI clean sands with different grain size distributions to evaluate their effect on UCS and Calcium Carbonate (CaCO<sub>3</sub>) precipitation. To better understand the influence of particle size, this study also collected data from the literature on UCS, CaCO<sub>3</sub> content, and selected soil grading properties. A regression analysis was performed on the combined data (literature and experimental) to establish a correlation between UCS, CaCO<sub>3</sub> content and the soil grading properties. Good agreement between the data points and model prediction was observed. An empirical relationship was also established between UCS, CaCO<sub>3</sub> content and d<sub>10</sub>.



**Title:**

Micro-Mechanism of Bio-Cementation Based on Micro-CT Image Analysis

**Author:**

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**Affiliation:**

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**Abstract:**

Under the influence of climate change, extreme weather such as rainstorms may cause geological disasters. Green and low-carbon engineering measures such as bio-cementation could be used to mitigate these hazards. In addition to the concentration of calcium carbonate, the degree of effective cementation between soil particles is a key factor in MICP efficiency. More effective cementation can be produced when MICP is under low saturation with the same amount of calcium carbonate. Therefore, it is of great significance to investigate the micro-mechanisms in bio-cemented sands under different saturations to improve the application of MICP. In this study, the micro-mechanical properties of bio-cemented sand were studied based on CT scanning technology and related image analysis methods. A local CT scanning of the sample was performed to investigate the micromechanical properties of bio-cemented sand first. To get complete information on the microstructure, the global CT scanning tests were also carried out using the miniaturized samples (diameter 10mm, height 20mm). The samples under different saturation conditions were made based on the microfluidic experiment system. The spatial distribution of effective cementation was obtained through image processing on the original image matrix obtained by CT scanning. The main conclusions are as follows: In the unsaturated MICP process, low saturation conditions are more likely to provide effective cementation, but the saturation should not be as low as 20%. After calcification the particle contact coordination number would increase and the contact between soil particles keeps isotropy.

**Title:**

Miniaturized device to measure urease activity in the soil interstitial fluid using Wenner method

**Author:**

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**Abstract:**

The paper presents a microdevice developed to measure electrical conductivity of a liquid or a saturated porous medium using Wenner method. It is basically a squared reservoir (area 5 x 5 cm<sup>2</sup> and 0.5 cm depth) printed in polypropylene using a 3D printing machine, incorporating copper electrodes in its base. This microdevice was used to measure urease activity in the soil interstitial fluid to understand if bacterial activity could be affected by the presence of the particles and tortuosity from pore geometry. This is a novel aspect, as urease activity is not measured in the pore fluid but in solution. Such analysis is important to understand biocementation mechanisms inside the soil and can help to improve the design of such treatment solutions. The electrical resistivity was computed adopting Wenner method, by connecting 4 electrodes to a PCB and using Arduino Nano for measuring the voltage when an AC current of 1 mA was applied. Both square and sinusoidal waves with 5kHz frequency were selected among other frequencies. The measurements were adjusted during the calibration of the microdevice, done using standard salt solutions with known electrical conductivity measured using an electrical conductivity probe. For the bacterial activity measurements, the bacterial and urea solutions were added to a uniform-graded size quartzitic sand (average diameter 0.3 mm) placed inside the microdevice and covering completely the electrodes. Bacterial activity was not much affected by the presence of the sand, which confirms that this treatment is effective for this type of soils.

**Title:**

Evaluating the performance and durability of concrete paving blocks enhanced by bio-cement posttreatment

**Author:**

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**Abstract:**

Concrete pavement often experiences accelerated deterioration due to water and chemical ingress through micro-cracks and surface voids. Particularly, the ingress of aggressive agents into the concrete matrix results in irreversible changes and deterioration on its endurance. Numerous studies unveiled that hydrophobic surface protection could be an inexpensive and effective way of enhancing the durability of concrete. This research work aims to assess the feasibility of bio-cement posttreatment for facilitating hydrophobic surface protection, thus enhancing the performance and durability of concrete blocks. Enzyme induced carbonate precipitation (EICP) is one of the promising bio-cement methods. Concrete blocks casted in four different grades were subjected to EICP treatment with different treatment schemes and recipes of cementation media. The treated blocks were tested for water absorption, ultrasonic pulse velocity (UPV) measurements, unconfined compressive strength (UCS), thermal performance, and scanning electron microscopy (SEM). The results indicated that the concrete blocks subjected to EICP posttreatment showed over a 55% reduction in water absorption, a 15% higher UCS and a 6.7% higher UPV when compared with control blocks. The analysis suggested that the EICP posttreatment could enhance the durability of concrete paving blocks by enabling

a layer of calcite on the surface and by plugging the transport pore channels of the concrete. Although most of the posttreatment strategies investigated herein were found to be operative, a better response was seen in the posttreatment by spraying scheme with 0.5 mol/L cementation media (CM). With the successful demonstration, the EICP treatment prior to the use of concrete blocks can be recommended to the pavement construction industry.

**Title:**

Fast Biomineralization to Inhibit Corrosion on Steel via Urease-Producing Bacteria

**Author:**

Xuanhua Feng

**Affiliation:**

Southeast University, Jiangsu, China

**Abstract:**

Corrosion of steel remains a challenging issue, particularly in marine environments. Traditional anti-corrosion methods such as coatings, inhibitors, cathodic protection, and alloying are often associated with high costs, frequent maintenance requirements, and environmental pollution. Consequently, microbially influenced corrosion inhibition (MICI) technology has emerged as a promising alternative in recent years. This study proposes using a urease-producing bacterium to prevent steel corrosion. By immersing steel in urease-producing bacteria (UPB) solution and its mineralization fluid (MF) to form a protective layer, followed by corrosion testing in simulated seawater (SW), we compared the performance of organic gel layers formed in UPB and organic-inorganic composite layers formed in MF. The results demonstrated that the mineralized layer formed through biomineralization offered rapid, stable, and effective corrosion protection. The organic component was primarily extracellular polymeric substances (EPS) from bacteria, while the inorganic component was mainly calcite. Furthermore, the urease action facilitated the formation of a dense mineralized layer within just one day, providing sustained corrosion resistance. This study introduces a novel rapid mineralization-based anti-corrosion technology, offering new insights for corrosion protection of metallic materials and advancing the field of MICI.

**Title:**

Electrical resistivity method for monitoring the microbially induced calcium carbonate precipitation (MICP) soil stabilization processes

**Author:**

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**Abstract:**

Microbially induced calcium carbonate precipitation (MICP) is a bio-mediated soil stabilization technique with extensive applicability and ecological benefits. However, since the soil is a porous medium with complex properties, the heterogeneity in the distribution of both treatment solution and precipitated calcium carbonate are major factors restricting the effect of MICP treatment. Obtaining the above information in real-time during the MICP treatment process is an important challenge. This study proposed a novel scheme for monitoring the distribution of both treatment solution and precipitated calcium carbonate in MICP treatment using the electrical resistivity method (ERM). Electrical resistance measurements and calcium carbonate assessments were employed to monitor the bio-cementation process of a sand-packed column. The differences in electrical resistivity of sand treated with and without bacteria were compared. The relationship between electrical resistivity and precipitated calcium carbonate content was discussed and established. Results suggest that the resistivity distribution can intuitively characterize the three-dimensional distribution of treatment solution in the sand. The presence of bacteria decreases the sand resistivity slightly. Changes in the resistivity indicate the spatial variability of the urea hydrolysis and  $\text{CaCO}_3$  precipitation during the MICP treatment. The precipitation of  $\text{CaCO}_3$  increases sand resistivity in two ways: one is narrowing the electric channel, and the other is cutting off the electric channel. The final resistivity distribution corresponds to the distribution of precipitated  $\text{CaCO}_3$ , and there is a positive relationship between electrical resistivity and  $\text{CaCO}_3$  content. The study verifies the feasibility of the electrical resistivity method in evaluating the effect of the MICP treatment process. The proposed method is expected to be applied in the in-situ MICP treatment.

**Title:**

Long-term Performance on Drought Mitigation through a Bio-approach: Evidence and Insight from both Field and Laboratory Tests

**Author:**

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**Abstract:**

Soil drought is a serious global environmental issue that causes widespread vegetation mortality, reduced terrestrial carbon uptake, and even land degradation. This study proposed a new bio-approach: microbial induced carbonate precipitation (MICP) to mitigate soil drought. The long-term performance of MICP on drought mitigation was investigated at field and laboratory scales. Seven in-situ slopes treated with different MICP rounds and cementation solution concentrations were subjected to 16-month weathering. The evaporation characteristics, water retention ability and CaCO<sub>3</sub> content of soil were tested. Then a series of laboratory soil samples were further prepared to provide evidence related to underlying weathering mechanisms. The results show that MICP has a time-dependent performance on drought mitigation. After MICP treatment, soil performs a remarkable evaporation suppression ability and the evaporation rate can decrease by 50%. This is attributed to the soluble salts which increase soil water retention capability and dense hard crust which inhibits water vapour migration into the atmosphere. However, the soluble salts and hard crust are sensitive to weathering thus leading to degradation of MICP. Suffering 16-month weathering, the MICP-induced CaCO<sub>3</sub> decreased by more than 60%. The evaporation rate of soil increases with MICP treatment rounds and cementation solution concentration and can reach nearly two times of untreated soil. MICP-treated field soil exhibits weaker water retention capacity than untreated soil because MICP process changes the soil microstructure-expands macropores and decrease the volume of micropores. Macropores connect with each other and then act as favourable evaporation channels and accelerate soil water evaporation. To ensure the MICP long-term effects, periodical treatments and regular maintenance can be employed. Considering MICP treatment rounds and cementation solution concentration, the most effective MICP treatment scheme is four to six treatment rounds and 1.0 M cementation solution.

## Session 8



**Title:**

Exploring Root-Inspired DEM Simulation for Evaluating Root-Soil Complex Shear Strength

**Author:**

Wengang Zhang

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**Abstract:**

The study of root-soil composite faces challenges such as the invisibility of roots and complicated anchorage mechanisms, making it difficult to accurately measure physical quantities and thereafter hinders intricate numerical modeling. Traditional models generally treat the root-soil composite as homogeneous medium, neglecting the random distribution of roots within the soil. To address this issue, this study adopts the characteristics of root growth towards geotropism and low resistance, utilizing the Discrete Element Method (DEM) to establish a root-soil composite model. Through simulation of pullout tests, the study evaluates the pullout strength of different root systems. The model considers the characteristics of root growth and the mechanical interaction between roots and soil mass, offering a novel approach to investigating root-soil complex shear strength. Simulation results reveal that physical properties such as root diameter, density, and strength induce varying degrees of bending during growth, significantly affecting root-soil complex shear strength. This research provides new insights into the study of root-soil interactions and holds significant implications for engineering applications in slope reinforcement.

**Title:**

Seawater-based Soybean Urease Extraction and its Biomineralization of Calcareous Sand

**Author:**

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**Abstract:**

Calcareous sand, a type of common building materials in tropical islands, usually requires reinforcement due to its poor engineering properties. Enzyme induced carbonate precipitation (EICP) process based on crude soybean urease solution is a promising method for this purpose. However, it is a great challenge for the application of the freshwater-based EICP method in the tropical island areas where freshwater is scarce. Therefore, this study proposed to use seawater as a solvent for the extraction of crude soybean urease solution from soybean powder and the preparation of EICP treatment solution to overcome this challenge. The feasibility of seawater-based EICP treatment of calcareous sand was investigated through conducting a series of tests including crude soybean urease extraction test, solution test, and sand column treatment test. Test results indicate that the turbidity of the crude soybean urease solution extracted with seawater is obviously lower than that with deionized water, although its urease activity is slightly lower. Nevertheless, the biomineralization process catalyzed by the seawater-based urease solution, as well as the microscopic characteristics of the precipitated calcium carbonate crystals, is hardly affected compared with that of the deionized water-based urease solution. Moreover, the lower turbidity of seawater-based urease solution is beneficial to improve the uniformity of EICP treated calcareous sand, and thus improve the biomineralization efficiency and strength enhancement. Seawater-based EICP treatment will be a great promising method, and it can be widely applied in the freshwater-scarce tropical island areas.

**Title:**

Effects of combined red mud and phosphogypsum on strength and microscopic characteristics of cement-admixed clay

**Author:**

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**Affiliation:**

Institute of Geotechnical Engineering, School of Transportation, Southeast University, Nanjing, 210096, China.

**Abstract:**

Red mud (RM) and phosphogypsum (PG) are known as two main industrial by-products in China. This study investigates the role of combined RM and PG in improving cement-treated clay. A series of unconfined compressive strength (UCS) tests and isotropically consolidated undrained triaxial (ICUT) tests were conducted to explore the effects of RM/PG ratio on strength behavior of cement-treated clay. Moreover, a series of microscopic tests were conducted to survey the microstructural evolution. The results indicate that the RM and PG contribute to the cement-based hydration reactions collaboratively. The UCS at an early curing stage nearly doubles with an optimal proportion of 7.5%RM+2.5%PG, but the UCS increase is less remarkable after a longer curing time. A high content of RM results in a higher maximum deviator stress and a brittle undrained shearing behavior. As the PG content increases, the peak strength decreases but the ductility of the specimen increases remarkably. The combined RM and PG not only accelerates pozzolanic reactions and produces increasing amounts of cementitious C-S-H, but also brings out a principally denser microstructure due to pore-filling effects of generated ettringite. However, some extruded pores can be caused among large aggregates arising from the expansion potential of ettringite.

**Title:**

Efficient stabilization of dredged sludge through the bio-carbonation of reactive magnesia cement method

**Author:**

Rui Wang<sup>1</sup>, Chao-Sheng Tang<sup>1</sup>, Xiao-Hua Pan<sup>1</sup>, Dian-Long Wang<sup>1</sup>, Zhi-Hao Dong<sup>1</sup>, Xi-Ying Zhang<sup>2</sup>, Xian-Cai Lu<sup>1</sup>

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1 School of Earth Sciences and Engineering, Nanjing University, Nanjing, China

2 Qinghai Institute of Salt Lakes, Chinese Academy of Sciences, Xining, China

**Abstract:**

The poor physical-mechanical properties of dredged sludge, such as high water content, low strength, and low permeability, make it difficult to use in engineering construction for resource utilization. In this paper, based on a novel urea pre-hydrolysis strategy, the improved bio-carbonation of reactive magnesia cement (RMC) method was employed for dredged sludge stabilization. A series of experimental tests on pre-hydrolyzed urea content and unconfined compressive strength were conducted to investigate the effects of pre-hydrolysis duration (T), urease activity (UA), and curing age (CA) on strength enhancement of bio-carbonized samples. The results show that the proposed method could successfully achieve the efficient stabilization of dredged sludge with 80% water content. A stable strength increment of up to about 1063.36 kPa was obtained for the bio-carbonized samples after just 7 days of curing, which was 2.64 times higher than that of the 28-day cured ordinary Portland cement-reinforced samples. Both elevated T and UA significantly affect the stabilization performance of dredged sludge, with too high values impairing the strength enhancement due to the negative effects of high supersaturation. The optimum formula was determined to be the case of T = 24 h and UA = 10 U/ml. Moreover, a 7-day CA was enough for bio-carbonized samples to obtain stable strength, albeit slightly affected by UA. The benefits of high efficiency and water stability presented the potential of this method in achieving dredged sludge stabilization and resource utilization. This study provides some new ideas and insights for the application of advanced biotechnological approaches in the field of dredged sludge stabilization and reuse.

**Title:**

Experimental study on solidification of graphite tailings sand by MICP under the regulation of glutinous rice slurry

**Author:**

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**Affiliation:**

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**Abstract:**

Microbially induced calcium carbonate precipitation (MICP) technology has the potential to enhance the strength of sandy soils, however, there remains significant room for further improvement. This study investigates the influence of glutinous rice slurry (GRS) on the mechanical characteristics of MICP-solidified graphite tailings (GT) sand. In the aqueous solution tests, we examine the impact of varying concentrations of GRS on bacterial proliferation, the formation of  $\text{CaCO}_3$ , and alterations in solution pH. Additionally, we analyze the impact of GRS on the morphology of  $\text{CaCO}_3$  crystals using scanning electron microscopy (SEM) and X-ray diffractometer (XRD). Experiments were carried out to control MICP-solidified GT sand with different concentrations of GRS and then tested the unconfined compressive strength (UCS) and  $\text{CaCO}_3$  content of the bio-cement samples. The research findings demonstrate that, compared to the control group employing traditional methods, the introduction of GRS leads to a notable increase in bacterial count and  $\text{CaCO}_3$  precipitation. Furthermore, it induces an alkaline environment within the solution. While conventional MICP results in irregularly shaped crystals, GRS-controlled MICP yields more stable rhombic hexahedron-shaped  $\text{CaCO}_3$  crystals. This indicates the biological macromolecules present in glutinous rice slurry exert regulatory control over the crystallization and growth of  $\text{CaCO}_3$ . This regulation results in a certain degree of order in the size and morphology of the calcite crystals. GRS can enhance the mechanical properties of GT, and MICP technology can also improve the mechanical performance of GT. However, when both are applied simultaneously, a synergistic effect is observed, resulting in a combined impact greater than the sum of their contributions. These findings hold substantial implications for practical engineering applications.

**Title:**

Engineering carbonic anhydrase as a route to biostability and CO<sub>2</sub> capture

**Author:**

Katie Gilmour, Prakriti Sharma Ghimire, Jennifer Wright, Jamie Haystead, Martyn Dade-Robertson, Meng Zhang, Paul James

**Affiliation:**

Living Construction, HBBE, Applied Sciences, Northumbria University

**Abstract:**

Microbial-induced calcium carbonate precipitation (MICP) has been widely studied for use to produce bioconcrete among other applications within the built environment. A typical pathway by which bacteria can precipitate calcium carbonate is through urease via ureolytic bacteria such as *Sporosarcina pasteurii*, however, high volumes of ammonia are produced as a by-product which is polluting. An alternative pathway is via carbonic anhydrase: an enzyme which catalyses the conversion of CO<sub>2</sub> to bicarbonate ions. Under alkaline conditions, it leads to precipitation of carbonate minerals in the presence of calcium. Here, several novel enzymes from soil bacteria suspected to be alpha- or beta-carbonic anhydrases were ligated into a chemically inducible vector (pHT253) and transformed into *Bacillus subtilis* 168 – a model organism from soil. A phenol red assay was used to show enzyme activity and ability to hydrolyse CO<sub>2</sub>. Expression of the enzyme when *B. subtilis* was grown in a biomineralization media resulted in mineral formation, and similarly when the enzyme was activated in a high CO<sub>2</sub> environment, sequestration of CO<sub>2</sub> coupled with storage as calcium carbonate was observed. *S. pasteurii* was included as a positive control for carbonate precipitation and an empty vector was used as a negative control. A decrease in CO<sub>2</sub> was observed on induction of carbonic anhydrase and minerals recovered from these experiments were identified as calcium carbonate minerals (calcite and vaterite) using X-ray diffraction. Although the mass of carbonate produced by novel carbonic anhydrases on their own was not as great as *S. pasteurii*, experiments which combined both the cell free extract for recombinant carbonic anhydrase and *S. pasteurii* resulted in higher carbonate precipitation and a significant decrease in CO<sub>2</sub>. Through this approach, we are able to produce cementitious materials with significant carbon sequestration ability, potentially this could contribute to the carbon negative/zero construction materials and processes.

**Title:**

Regulating the microbially induced calcium carbonate precipitation (MICP) process through the application of electric fields

**Author:**

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**Abstract:**

Non-uniform cementation challenges can arise when using microbially induced calcium carbonate precipitation (MICP) technology to treat problematic soils. Electrokinetic methods show potential to enhance the MICP process by mobilizing negatively charged urease-producing bacteria and promoting the migration of reactant ions. However, the behavior of bacteria and the characteristics of CaCO<sub>3</sub> precipitates under direct current (DC) electric fields remain poorly understood. This study utilizes microfluidic chips to simulate sandy soil matrices and examines the real-time MICP reaction process under an optical microscope, both with and without a DC electric field. We analyzed the influence of the electric field on bacterial behavior at different locations (attached and suspended bacteria) and the morphology and distribution of CaCO<sub>3</sub> crystals. The results reveal that initially injected bacteria adhered to pore surfaces due to interfacial cohesion, while subsequently injected bacteria remained suspended and migrated towards the anode under the electric field, where they aggregated. Most Ca<sup>2+</sup> ions precipitated near the cathode, forming larger crystals, whereas fewer Ca<sup>2+</sup> ions precipitated at the anode, resulting in smaller crystal particles. This highlights the critical role of Ca<sup>2+</sup> movement in determining the distribution of CaCO<sub>3</sub>. Under a DC electric field, the precipitated CaCO<sub>3</sub> crystals were non-uniform in size, with an overall smaller average size and more complex morphologies. These findings provide valuable insights for regulating the MICP process using electric fields.

**Title:**

Metre-scale sand improvement using microbially induced carbonate precipitation

**Author:**

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**Affiliation:**

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**Abstract:**

Despite the growing interest in microbially induced carbonate precipitation (MICP) for geotechnical applications, reports on meter-scale MICP trials for soil improvement remain limited. This study presented a meter-scale improvement of a poorly-graded sand (initial dry density: 1581 kg/m<sup>3</sup>, porosity: 40%) through MICP in a cylindrical cell (diameter: 1 m; thickness: 15 cm). We adopted a radial flow injection strategy, injecting fluids radially using a single well located at the center while maintaining a constant hydraulic head at the outer boundary. Nine cycles of a two-phase MICP treatment were applied: Phase 1- injection of 0.7 pore volumes (PVs) of bacterial solution and 1-L water pulse; Phase 2- injection of 1.4 PVs of 0.5M cementing solution in two stages (i) 0.7 PV injection two hours after the bacteria were injected, and (ii) a further 0.7 PV injection the following morning after an overnight static reaction period.

The permeability increased slightly from  $1.4 \times 10^{-11}$  m<sup>2</sup> (before treatment) to  $2.0 \times 10^{-11}$  m<sup>2</sup> in the first five treatment cycles, likely due to the hydraulically induced flow channels near the outer boundary. After the final MICP treatment, the permeability dropped to  $6.9-8.9 \times 10^{-12}$  m<sup>2</sup> due to CaCO<sub>3</sub> precipitation. The unconfined compressive strengths (UCSs) of the cores drilled within the 30 - 80 cm diameter range were 1.2-6.8 MPa with CaCO<sub>3</sub> contents of 0.08-0.17. We also observed non-uniform CaCO<sub>3</sub> precipitations along the depth and the distance from the central well, which were induced by the decreasing flux towards the outer boundary under the radial flow pattern, together with the influences by layered packing and hydraulically induced flow channels. Nevertheless, the study demonstrates a good cementation efficacy in the target zone without causing well clogging or a significant permeability drop. The two-phase treatment strategy with radial flow injection can be applied in field implementations of MICP for soil improvement.



**Title:**

The Investigation of Microbial Induced Calcium Carbonate Precipitation for Soil Improvement

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**Abstract:**

Microbially induced calcium carbonate precipitation (MICP) is a process to produce  $\text{CaCO}_3$  through bacterial cells and their biological activities. One application is soil improvement, where precipitated  $\text{CaCO}_3$  can bind adjacent soil particles and fill the pore spaces of soils to increase mechanical properties. Due to the high number of factors involved in MICP, computational models can be utilised to predict the outcome of different treatment strategies, which is important for implementation into the field. There are limitations with these models which include use of assumptions either to reduce computational complexity or from lack of existing data. To improve the accuracy of these models or to verify that the assumptions made are correct, laboratory data needs to be collected to address these data gaps. The objectives of this research were to investigate MICP from microbiology and biochemistry aspects in greater detail, as a means of improving understanding of MICP processes for greater efficient application of this technique and for addressing computational model data gaps. Firstly, several factors were investigated to understand the effects on *Sporosarcina pasteurii* growth and urease activity in both whole cell and cell free extract conditions. Then reaction rates of ureolysis and  $\text{CaCO}_3$  precipitation were measured at a range of reactant concentrations. This was followed by cell tests in sand columns which focused towards understanding the impact of using bacteria cultures of different growth phases as well as understanding in greater detail the distribution profile of both *S. pasteurii* and  $\text{CaCO}_3$  in sand columns. Finally, the impact of charged additives on  $\text{CaCO}_3$  was explored, to determine if morphological changes of  $\text{CaCO}_3$  had an effect on binding properties and unconfined compressive strength of sand columns. Overall, a variety of data was collected and the knowledge from this work is beneficial to the advancement of MICP technology for soil improvement.

**Title:**

Physical Property of MICP-Treated Calcareous Sand under Seawater Conditions by CPTU

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**Abstract:**

MICP (Microbially induced calcite precipitation), an environmentally friendly soil improvement technique, has great potential in ocean engineering due to its ability to promote the precipitation of calcium carbonate through microbial activity to enhance the engineering properties of geomaterials. In this study, piezocone penetration test (CPTU) is used to evaluate the effectiveness of MICP treatment in calcareous sand. The change of physical properties (relative density  $D_r$  and total unit weight  $\gamma_t$ ) of MICP treated calcareous sand is investigated by conducting CPTU on the geomaterials prepared in a series of mini calibration chambers (25cm×50cm). Results indicate that CPTU (tip stress, sleeve friction, and porewater pressure) measurements can be used to interpret the physical characteristics of calcareous sand treated with MICP under seawater conditions. Additionally, a relationship between CPTU measurements, physical parameters (relative density  $D_r$  and total unit weight  $\gamma_t$ ) of MICP treated calcareous sand is proposed and calibrated. The findings of the research extend the implementation of in-situ testing techniques such as CPTU towards physical property evaluation of bio-treated geomaterials in ocean environment and demonstrate the potential of scaling up MICP techniques for broader engineering application.

**Title:**

A novel urease gene structure of *Sporosarcina pasteurii* with double operons

**Author:**

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**Abstract:**

*Sporosarcina pasteurii* (*S. pasteurii*) has been used to induce calcium carbonate production due to their efficient urease activity. However, applications were limited by the lack of basic theoretical studies, especially lacking the research on the structure and expression regulation mechanism of urease genes. Therefore, the growth and urease gene expression of *S. pasteurii* under three different culture conditions were studied for the first time in this research, by RNA-Seq. Afterwards, the urease gene structure of *S. pasteurii* was predicted by bioinformatics approaches, which was further verified by molecular biology method. In summary, it was found that the urease gene of *S. pasteurii* is a double operons structure. The discovery of the double operons structure is also of great significance for genetic engineering studies of urease.

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