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BACTERIAL SYNERGY FOR ENHANCED TAILING SAND SOLIDIFICATION VIA MICP

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ABSTRACT: This study proposes a synergistic microbially induced carbonate precipitation (MICP) approach combining *Sporosarcina pasteurii* (*SP*) with indigenous urea-decomposing bacteria to solidify tailing sand and immobilize heavy metals. First, the bio-stimulation solution for indigenous bacteria was optimized using Plackett-Burman design and Central Composite Design. Subsequently, a comparative analysis was conducted among three treatment methods (bio-stimulation alone, *SP* alone, and the combined approach) over 4, 7, and 10 treatment cycles. Evaluation metrics included surface strength, toxicity characteristic leaching procedure (TCLP) tests, and scanning electron microscopy (SEM) analysis. Experimental results indicated that the optimized indigenous culture achieved a urease activity of 6.6 mmol/L urea/min. After 7 treatment cycles, the combined solidification method yielded a surface strength of 1188 kPa, which was 5.3 times that of bio-stimulation and 1.5 times that of the *SP* method. Regarding environmental safety, the combined method significantly reduced the leaching concentrations of Fe, Zn, and Cd by 89.2%, 72.5%, and 87.6%, respectively, compared to untreated samples. SEM analysis revealed that the combined approach generated larger, more numerous calcite crystals with a flaky arrangement that tightly filled inter-particle pores. Furthermore, the economic analysis demonstrated that the combined method reduced material costs to \$23.79 per cycle/m², representing only 55.6% of the cost of the traditional *SP* method. This integrated strategy offers a mechanically robust, cost-effective, and environmentally sustainable solution for tailing sand remediation.

Keywords: Microbially induced carbonate precipitation; Sporosarcina pasteurii; Indigenous urea-decomposing bacteria; Tailing sand

1. INTRODUCTION

Microbially induced carbonate precipitation (MICP) represents a new technology currently utilizing microbes to ameliorate soil conditions and immobilize heavy metals. Its core mechanism is` based on employing urease produced through microbial activity to transform Ca^{2+} and metal ions within the soil into carbonate precipitates, while simultaneously improving the physical attributes related to the soil and immobilizing heavy metal elements [1]. For tailing sand specifically, the target engineering performance criteria generally include achieving a minimum surface strength sufficient to resist wind erosion and support the trafficability of light maintenance vehicles. Regarding the treatment goals, the immobilization of heavy metals is intended primarily for environmental protection to prevent the leaching of toxins into groundwater, whereas the concurrent mechanical strengthening ensures structural safety and surficial stability. How the reaction proceeds can be seen in Eq. (1) and (2), with Me^{2+} representing heavy metal ions [2].

$$\mathrm{CO(NH_2)_2} + \mathrm{Ca^{2+}} + 2\mathrm{H_2O} \rightarrow \mathrm{CaCO_3} \downarrow + 2\mathrm{NH_4^+} \quad (1)$$

$$\mathrm{CO(NH_2)_2} + \mathrm{M}e^{2+} + 2\mathrm{H_2O} \rightarrow \mathrm{M}e\mathrm{CO_3} \downarrow + 2\mathrm{NH_4^+} (2)$$

Sporosarcina pasteurii (*SP*) is the most effective microbes for MICP process through urea hydrolysis [3]. However, tailing usually contains heavy metals [4]. The growth of *SP* is inhibited, and its activity decreases [5] in environments with high heavy metal concentrations, consequently affecting the progress and efficiency of MICP. Moreover, the high cost of cultivating and applying exogenous bacteria, coupled with potential bio-safety and ecological adaptation concerns, limits the field-scale applicability of traditional bioaugmentation strategies. Various methodologies have been documented in academic literature for enhancing soil properties via MICP. The two-step protocol, introduced by Whiffin et al. [6], has become the standard approach in most subsequent bio-cementation research. Building upon the two-phase technique, a variation known as the staged injection method was established [7]. This protocol incorporates a distinct incubation interval following bacterial delivery to facilitate superior microbial attachment. Furthermore, Harkes et al. [8] utilized a three-step process, comprising bacteria, a fixation fluid, and finally the cementation media, to prove that a more uniform distribution of both biomass and calcite could be achieved.

Exploratory research on biostimulation has been conducted in the field of ground improvement [9-11]. Liu et al. [9] successfully reinforced calcareous sand by stimulating the growth of indigenous urea-decomposing microorganisms using YE (yeast extract), ammonium chloride, sodium acetate, urea, calcium chloride, and nickel chloride under various pH conditions. Gomez et al. [10] investigated the effects of stimulating microorganisms for soil reinforcement at different depths; they found that although the stimulation response of urea-decomposing bacteria varied significantly with depth, a certain degree of reinforcement was achieved in all cases. Gat et al. [11] studied the population dynamics of bacteria in soil following biostimulation, and the results indicated that simple nutrients could be used to biostimulate urea-decomposing bacteria even in arid and nutrient-poor environments. Although biostimulation avoids the risk of exogenous bacterial invasion, the activity of the activated urea-decomposing bacteria is relatively low, resulting in consolidation strengths that are generally lower than that achieved by MICP method.

Consequently, a synergistic approach that combines the high urease activity of *SP* with the environmental resilience of indigenous bacteria presents a compelling yet underexplored strategy. Several scholars have proposed combined consolidation methods. For example, Cao et al. [12] combined indigenous urea-decomposing bacteria with enzyme induced carbonate precipitation (EICP) to reinforce land-based hydraulic marine sand fill; Li et al. [13] utilized MICP technology combined with porous silica adsorbent materials for the solidification/stabilization remediation of zinc-lead co-contaminated soil; and Yuan et al. [14] incorporated organic materials, specifically skimmed milk powder, glutinous rice flour, and brown sugar, into the EICP process to reinforce silty soil. But most existing studies focus primarily on the final solidification effect without systematically optimizing the bio-stimulation phase for complex tailing environments. Specifically, standard bio-stimulation approaches often rely on generic nutrient solutions rather than formulations tailored to the specific heavy metal profile of the site. Furthermore, while the technical feasibility of hybrid MICP has been explored, comprehensive quantitative analyses comparing the economic benefits of hybrid methods against traditional bio-augmentation or bio-stimulation alone remain scarce in the literature.

This study posits the hypothesis that a synergistic approach, combining the broad environmental adaptation of indigenous bacteria with the high urease activity of exogenous *Sporosarcina pasteur*i, can overcome the limitations of single-method treatments. Consequently, the primary objective of this work is to develop and validate this hybrid strategy to enhance mechanical strength and heavy metal immobilization efficiency while reducing costs. Specifically, this study first utilizes Plackett-Burman design and central composite design (CCD) to rigorously optimize a bio-stimulation formulation tailored for tailing sand to maximize indigenous bacterial activity. Subsequently, a comprehensive experimental campaign is conducted to compare the solidification efficacy of the combined method against bio-stimulation or *SP* alone across different treatment cycles, focusing on surface strength and the leaching toxicity of Fe, Zn, and Cd. Finally, the study aims to elucidate the synergistic mechanism through scanning electron mcroscopy (SEM) analysis of crystal morphology and to perform a detailed economic analysis to verify the cost-effectiveness of the proposed hybrid technique relative to traditional methods.

2. RESEARCH SIGNIFICANCE

This study presents a novel and synergistic strategy for tailing sand remediation by integrating bio-stimulated indigenous urea-decomposing bacteria with the exogenous bacterium *Sporosarcina pasteurii* (*SP*) within the MICP process. This combined approach uniquely addresses the key limitations of using either method alone: it harnesses the superior environmental adaptation and distribution of native microbes to provide widespread nucleation sites, while leveraging the high urease activity of *SP* to drive efficient carbonate precipitation. The resulting synergy significantly enhances mechanical strength and heavy metal immobilization beyond what either method achieves independently, concurrently reducing treatment costs and ecological risks. The work establishes an innovative, sustainable, and economically viable framework for managing complex mine tailing.

3. MATERIALS AND METHODS

3.1 Tailing Sand

The tailing sand was taken from the tailing reservoir of Fujian Makeng Mining Co., Ltd. located in China. The particle size distribution of the tailing sand was shown in Figure 1. Based on the distribution curve, the characteristic particle sizes were determined as D_{10} = 0.06 mm, D_{50} = 0.17 mm, and D_{90} = 0.50 mm, indicating a poorly graded soil structure. The basic characteristics was shown in Table 1. Referring to "Soil Environmental Quality - Risk Control Standard for Soil Contamination of Agricultural Land (Trial) [15]," and "Technical Specification for Soil Environmental Monitoring [16]," both Cd and Zn in the tailing sand exceed the specified standard limits. Specifically, Cd exhibits moderate pollution, while Zn shows slight pollution.

To ensure the uniformity of the experimental materials and minimize spatial variability, the collected tailing sand was thoroughly air-dried and mechanically homogenized prior to use. Preliminary tests on random sub-samples from different locations within the batch confirmed that Fe, Zn, and Cd were uniformly distributed.

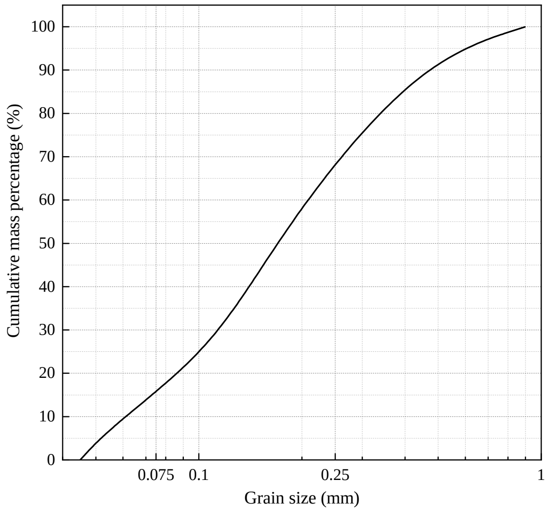

Fig.1 Particle size distribution of tailing sand

Table 1 Basic characteristics of tailing sand

Parameters	Values
Density	2 g/cm^3
moisture content	2.1%
specific gravity	3.5
organic content	5.1%
initial porosity	0.8
pH	9.5
Fe	127.1 g/kg
Zn	0.376 g/kg
Cd	0.0013 g/kg

Note: Determination methods for Fe, Zn and Cd: Samples are digested using a four-acid mixture, and the concentrations are determined by ICP-MS.

3.2 Formulating and Improving a Bio-stimulation Technique Applicable to Tailing Sand

Based on existing research findings [17], the starting formulation of the bio-stimulation solution is created in 1 L of distilled water: 3.5 g of sodium acetate, 5.35 g of ammonium chloride, 20 g of urea, 0.2 g of yeast extract, 0.01 mmol of nickel chloride.

The urease activity exhibited by the urea-decomposing bacteria stands as the key parameter influencing the success of MICP solidification. Consequently, the urease activity of the supernatant following bio-stimulation of tailing sand serves as both the assessment criterion and the outcome variable. The precise steps for improvement are as follows:

3.2.1 Screening of Optimal Carbon and Nitrogen Sources

Commencing with the initial bio-stimulation solution, one-variable experiments are to be performed by altering the carbon or the nitrogen source. A selection of equivalent mass quantities of glucose, fructose, maltose, starch, and molasses are to be used as the carbon sources. peptone, corn steep liquor, soybean meal, ammonium chloride, ammonium sulfate, and sodium nitrate are to be chosen as the nitrogen sources. Ten grams of air-dried tailing sand are to be introduced into a flask holding ninety milliliters of the bio-stimulation solution. The mixture is to be cultivated within an incubator. The urease activity within the conical flask is to be measured at consistent time intervals using the conductivity method, and this measurement is to serve as the evaluation criterion for identifying the most favorable carbon and nitrogen sources.

3.2.2 Identifying Crucial Factors Through PB Design

A Plackett-Burman (PB) design was utilized to examine the optimal carbon and nitrogen sources identified previously, along with other constituents in the bio-stimulation solution and the pH level of the solution. The objective of this design was to pinpoint the components exerting the greatest influence on urease activity, designating these as crucial factors [18]. Following this, single-factor experiments were carried out by altering the concentration of each crucial variable in order to ascertain the most favorable concentration span for every one.

3.2.3 Determining the Optimal Bio-stimulation Solution Formulation

Urease activity was set as the outcome variable, with central composite design (CCD) being conducted employing Minitab16 program [19]. The best concentrations for every crucial variable were determined, thereby yielding the optimal formulation for the bio-stimulation solution intended for tailing sand.

3.3 Bacterial Suspension and Cementation Solution

The bacterium is *Sporosarcina pasteurii* (strain DSM33). The culture medium composition is as follows: 20 g/L yeast extract, 10 g/L ammonium sulfate, 15.8 g/L trisaminomethane. The experiments utilized the bacterial culture after 48 hours of cultivation.

The cementation solution concentration was 1 mol/L, prepared by dissolving 111 g of calcium chloride and 60.06 g of urea in 1 L of deionized water.

3.4 Molds and Methods

The experiments utilized aluminum boxes with a diameter of 10 cm and a height of 5 cm. The tailing sand with an initial water content of 2.1% was placed into the mold in three layers and compacted by vibration layer by layer, resulting in a final density of 2.4 g/cm³. there were some holes in the base of the box to allow waste liquid to drain and a filter cloth was placed over the holes. treatments were applied from the top only. After each treatment cycle was completed, the aluminum box was placed into a slightly larger sealed cylindrical container to ensure full contact between the solution and the tailing sand. Before the next treatment, the waste liquid in the slightly larger sealed cylindrical container surrounding the aluminum box was discarded.

The study was split into three sets: the *SP*, the bio-stimulation, and the combined solidification.

Concerning the *SP* set, the tailing sand underwent initial sterilization at 120 °C inside an oven over a 12-hour period. Based on our previous research findings [20], the one-phase low-pH MICP method was employed. Daily, 100 milliliters of the low-pH bacterial suspension (pH = 3.5) was mixed with 100 milliliters of the cementation solution and then introduced to the sand samples. This constituted one treatment cycle, with a 24-hour interval between each cycle.

Regarding the bio-stimulation set, the tailing sand remained unsterilized. Daily, 100 milliliters of the bio-stimulation solution was introduced as the initial step. Twelve hours later, 100 milliliters of the cementation solution was additionally applied. This constituted one treatment cycle, with a 24-hour interval between each cycle.

For the combined solidification group, the tailing sand was also not sterilized. Daily, 100 milliliters of bio-stimulation solution was first added to the sand specimens, followed after 12 hours by the addition of a mixed solution of 50 milliliters low-pH bacterial suspension and 50 milliliters of the cementation solution. This constituted one treatment round, with a 24-hour interval between each cycle.

The initial pH of the tailing sand was 9.5. Direct addition of calcium sources into such a highly alkaline environment typically leads to rapid, uncontrolled precipitation of calcium and metal hydroxides, causing surface clogging and poor depth uniformity. To mitigate this, a one-phase low-pH MICP method was adopted. For the SP and combined treatments, the bacterial suspension was adjusted to pH 3.5 using dilute hydrochloric acid. This acidic treatment temporarily neutralizes the pore fluid alkalinity, maintaining the solubility of calcium and heavy metal ions during infiltration. Subsequent urea hydrolysis by the bacteria then gradually raises the pH in situ, triggering controlled calcite co-precipitation and heavy metal immobilization.

Three different numbers of treatment cycles (4, 7, and 10) were investigated to study the effect of the number of treatment cycles on the solidification effect. Each treatment cycle was performed at 24-hour intervals. The control group sand samples were treated with 200 milliliters of deionized water.

After multiple treatment cycles, the following assessments were carried out: The penetration resistance of the solidified sand samples was tested using a mini penetrometer. The mini penetrometer used was model WXGR-4.0, equipped with a cylindrical probe with a cross-sectional area of 30 mm². The penetration rate was 1 mm/s, and the penetration depth was 6 mm. Five points were uniformly tested on the top face of every sand specimen, then their average value was taken as the surface strength of the sand specimen. The leaching concentrations of Fe, Zn, and Cd within the sand specimens prior to and following solidification were determined using the Toxicity Characteristic Leaching Procedure (TCLP) method for hazardous waste [21].

4. RESULTS AND DISCUSSION

4.1 Optimal Bio-stimulation Solution Formulation

4.1.1 Best Carbon and Nitrogen Sources

The change in urease activity of the solution within the flasks over time using distinct carbon and nitrogen nutrient sources is illustrated in Figure 2 and Figure 3. Upon employing starch and sodium acetate to provide carbon, the urease activity was relatively high, reaching the peak within a 48-hour period. Considering the range of sources of nitrogen, both inorganic and organic, ammonium sulfate, ammonium chloride, peptone, and soybean meal showed relatively high urease activity. Consequently, these six carbon and nitrogen sources, which showed relatively high urease activity and involved reduced bio-stimulation duration, were designated for the next stage of the PB experiment.

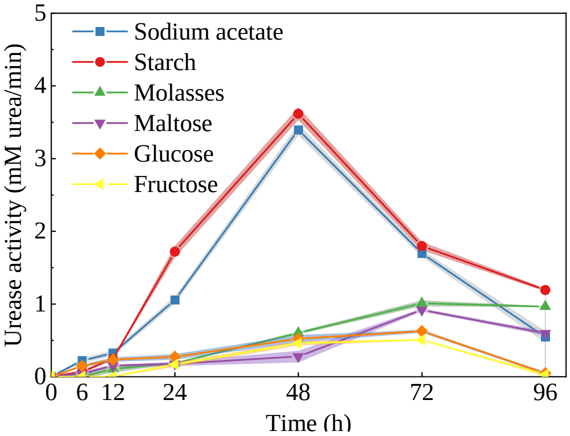

Fig.2 Change in urease activity over time under different carbon sources

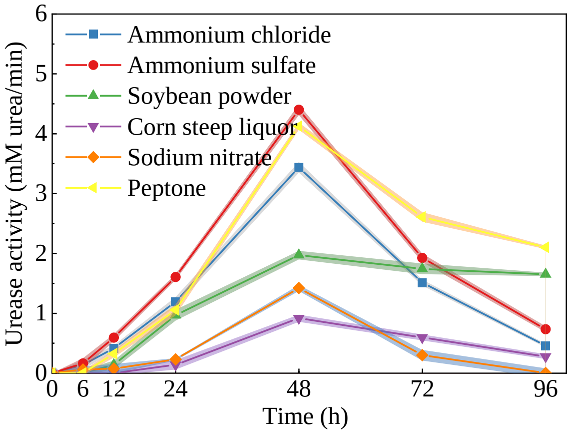

Fig.3 Change in urease activity over time under different nitrogen sources

4.1.2 Crucial Factors

Six carbon and nitrogen sources chosen based on the one-variable testing, accompanying the three additional ingredients present in the bio-stimulation preparation and the solution's pH, served as the 10 variables to be screened. With the urease activity in the suspension following 48 hours of bio-stimulation as the outcome measure, a PB experiment with N=12 trials was executed. The analytical outcomes can be viewed in Table 2.

Table 2 Analysis of PB design

Factor	Level (g/L)		Effect	P-value
	-1	1		
starch	3.5	5.25	0.01	0.90
sodium acetate	3.5	5.25	-0.74	0.04
peptone	5.35	8	0.04	0.53
soybean meal	5.35	8	-0.37	0.07
ammonium chloride	5.35	8	0.10	0.26
ammonium sulfate	5.35	8	1.14	0.02
urea	20	30	0.71	0.04
yeast extract	0.2	0.3	-0.48	0.06
nickel chloride	0.01	0.015	-0.23	0.12
pH	7	9	2.33	0.01

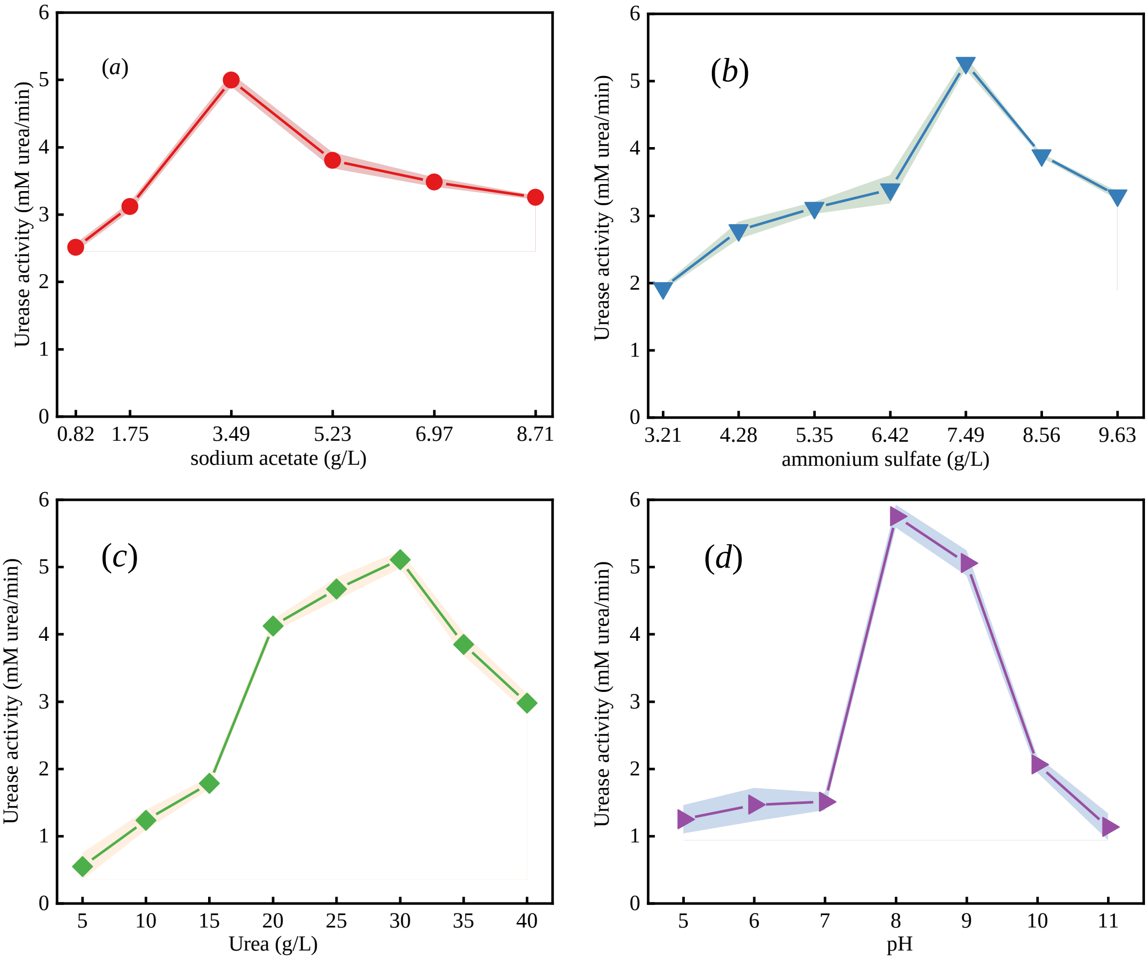

Fig.4 Effect of crucial factor concentration on urease activity

In this analysis, the P-value serves as a metric measuring the difference in the outcome value across varying factor levels. The P-values for sodium acetate, ammonium sulfate, urea, and pH value were all below 0.05, demonstrating that these items represent important influences on urease activity. The P-value for pH value was the smallest, indicating that pH value is the key determinant with the greatest impact on urease activity. These four variables were designated to be the crucial variables for the follow-up central composite design.

4.1.3 Optimal Formulation

The initial bio-stimulation solution composition was adjusted according to the beneficial and adverse influences of every factor shown in Table 2. Factors with negative effects were set at the –1 level, and factors with positive effects were set at the +1 level, resulting in the following composition: sodium acetate 3.5 g/L, $(NH_4)_2SO_4$ 8 g/L, urea 30 g/L, yeast extract 0.2 g/L, nickel chloride 0.01 mmol/L, and pH 9. Based on the formulation, one-variable tests were conducted by sequentially varying the level of each crucial factor while keeping other components constant. The urease activity at 48 hours at different levels of each crucial factor is shown in Figure 4. All four crucial factors possess optimal concentration ranges within which urease activity initially increases and subsequently decreases.

These four crucial factors served as independent variables, with the 48-hour urease activity as the response parameter. A CCD design was conducted. The model predicted a maximum urease activity of 6.54 mmol/L urea/min, and the corresponding optimal bio-stimulation solution composition was: sodium acetate 2.94 g/L, $(NH_4)_2SO_4$ 7.07 g/L, urea 28.86 g/L, yeast extract 0.2 g/L, nickel chloride 0.01 mmol/L, and pH 8.7. A bio-stimulation solution was prepared according to this formulation and used for cultivation with the tailing sand. Three replicates were prepared. The measured 48-hour urease activity was 6.6 mmol/L urea/min, which was close to the predicted value, verifying the accuracy of the model.

4.2 Surface Strength

The unconfined compressive strength（UCS） is commonly used to evaluate the strength of soil after MICP solidification [22]. And the mini penetrometer with a cylindrical probe is routinely used to make quick estimates of the UCS of soils [23]. Figure 5 shows the surface strength of sand specimens after undergoing treatment for 4, 7, and 10 cycles, respectively, using the three methods: *SP*, bio-stimulation, and the combined method. The surface strength of all after treatment was significantly improved in different amounts. When the number of treatment rounds was identical, the surface strength in sand specimens subjected to bio-stimulation was the lowest, while that subjected to combined solidification was the highest

The surface strength of the tailing sand after seven rounds of solidification using indigenous urea-decomposing bacteria was 225 kPa. Field studies on MICP-treated soils have demonstrated that calcium carbonate crusts effectively resist wind erosion at high wind speeds (e.g., 16 m/s) once a coherent surface layer is formed [24]. The bio-stimulation group achieved 225 kPa, which corresponds to a "very stiff" consistency according to geotechnical design standards [25]. This strength is sufficient to prevent dust emission and support pedestrian traffic (human foot contact pressure is typically <100 kPa) without rupture, making it suitable for ecological coverage in non-active zones.

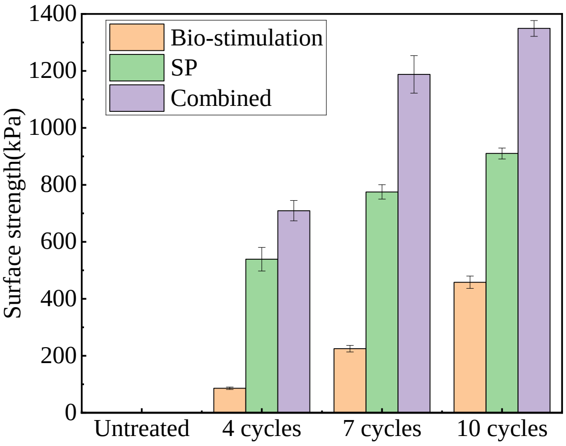

Fig.5 Surface strength of tailing sand

The surface strength of the tailing sand after seven rounds of combined solidification was 1188 kPa, classified as "hard" soil consistency [25]. This strength level significantly exceeds the requirements for mechanical operations. According to the theory of ground vehicles, the ground contact pressure of pneumatic tires is approximately equal to their inflation pressure [26]. Since standard light utility vehicles and pickup trucks typically operate at tire pressures of 200–350 kPa, the combined crust offers a safety factor of over 3.0. This indicates that the combined method enables the tailing surface to serve as a temporary pavement for inspection vehicles and light maintenance machinery, a capability that bio-stimulation alone does not securely provide.

However, the rate of increase in surface strength after 7 cycles of combined solidification decreased rapidly. This suggests that a 7-cycle treatment regimen represents the optimal balance between engineering performance and efficiency for field applications.The solidification process fundamentally alters the mechanical behavior of the tailing sand. Untreated tailing sand typically exhibits ductile behavior with low stiffness, undergoing large deformations under load. In contrast, the MICP treatment transforms the material into a rigid matrix characterized by high stiffness and brittle behavior. The hydrolysis of urea and the subsequent reaction with chloride salts (calcium chloride) precipitate calcite crystals that bridge sand particles. While this cementation mechanism is responsible for the substantial increase in bearing capacity, it also induces a transition from ductile to brittle failure modes, as observed in similar bio-cementation studies [27]. Consequently, while the stiffness of the treated crust is significantly enhanced, the material may exhibit sudden failure at peak loads compared to the gradual yielding of untreated sand.

4.3 Heavy Metal Leaching Toxicity

Figure 6 presents the leaching concentrations of Fe, Zn, and Cd within untreated tailing sand and in samples that underwent 10 cycles using the three methods. After solidification, the leaching concentrations of the three heavy metals were all notably lowered compared to the untreated sand samples. The order of heavy metal leaching concentration was: combined solidification < bio-stimulation < *SP*. The existing urea-decomposing bacteria in the tailing sand have high heavy metal tolerance and are uniformly distributed, providing more nucleation sites for carbonate precipitation. The carbonate ions generated during the MICP process can more easily combine with free heavy metal ions in the tailing sand. While during combined solidification, *SP*, which has higher urease activity, decomposed urea and produced more carbonate ions, further increasing the heavy metal immobilization rate. For the sand samples after combined solidification, the leaching concentrations of Fe, Zn, and Cd showed reductions of 89.2%, 72.5%, and 87.6%, respectively.

The high initial pH (9.5) of the tailing sand naturally favors the formation of metal hydroxides. However, the significant leaching toxicity observed in untreated samples indicates that these forms are not stable or sufficiently immobilized under leaching conditions. The low-pH treatment strategy employed in the *SP* and combined methods played a crucial role in altering metal speciation. By temporarily lowering the pH during treatment, the process prevents the immediate formation of unstable hydroxides on the surface. As the bacterial urea hydrolysis proceeds, the system pH rises, shifting the equilibrium toward the formation of stable metal-carbonate co-precipitates rather than hydroxides. Furthermore, the bio-stimulation solution was optimized to pH 8.7. Although slightly lower than the native tailing pH of 9.5, this value represents the physiological optimum for urease activity as determined by the CCD analysis. The native pH of 9.5 was found to be inhibitory to maximal enzymatic turnover. Thus, buffering the solution to pH 8.7 provided a balance that relieved this alkaline inhibition while remaining close enough to native conditions to prevent pH shock.

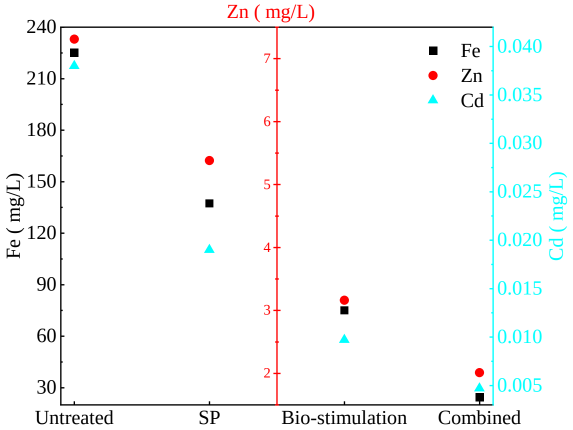

Fig.6 Heavy metal leaching toxicity of tailing sand

4.4 SEM Analysis

In the course of the MICP process, a competitive interaction exists between the nucleation and the growth of calcium carbonate crystals. [28]. Figure 7 shows SEM images of tailing sand before and after 10 cycles of solidification using the three methods. The indigenous urea-decomposing bacteria activated by bio-stimulation are spread out broadly and evenly throughout the tailing sand, which furnish additional nucleation sites. Furthermore, indigenous urea-decomposing bacteria have lower urease activity, inducing slower calcium carbonate formation, thereby facilitating the formation of new calcium carbonate crystals by nucleation. *SP* is an introduced exogenous bacterium with a limited migration and diffusion range. Moreover, *SP* has high urease activity, which can induce the formation of a large amount of calcium carbonate precipitate over a brief duration. It leads to calcium carbonate more readily precipitating on pre-existing crystals. As a result, the calcium carbonate crystal size in Figure 7(b) is smaller than in Figure 7(c). In addition, due to the lower urease activity of indigenous urea-decomposing bacteria compared to *SP*, less calcium carbonate is induced to form. Therefore, the number of calcium carbonate crystals in Figure 7(b) is also less than in Figure 7(c). During combined solidification, the indigenous urea-decomposing bacteria provided more nucleation sites, while the high urease activity *SP* induced the formation of more calcium carbonate. Therefore, both the number and size of calcite crystals in Figure 7(d) are greater than in Figure 7(b) and Figure 7(c). These crystals showed tight flaky arrangements, tightly wrapping around the sand particles, thereby achieving higher solidification strength.

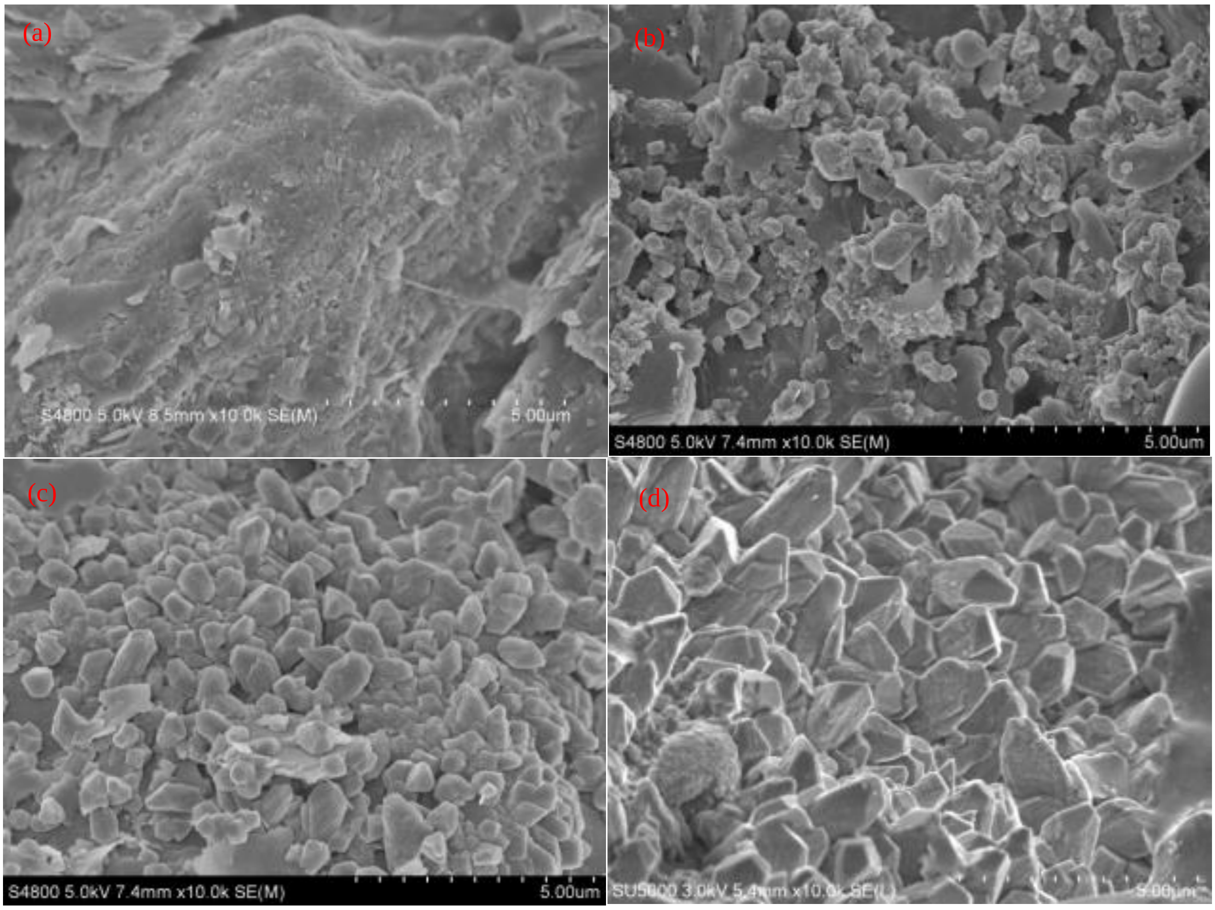

Fig.7 SEM images of tailing sand: (a) Untreated; (b) Bio-stimulation; (c) *SP*; (d) Combined solidification

4.5 Cost Calculation and Sensitivity Analysis

4.5.1 Cost Calculation

The surface area of the tailing sand sample placed in the aluminum box mold was 78.5 cm². For the SP treatment, 100 mL each of the low-pH bacterial solution and the cementation solution were added. For the bio-stimulation treatment, 100 mL each of the stimulation solution and the cementation solution were added. During combined solidification, 100 mL of stimulation solution, 50 mL of low-pH bacterial solution, and 50 mL of cementation solution were added, respectively. And the low-pH bacterial solution was prepared by mixing the cultured original bacterial solution and dilute hydrochloric acid at a ratio of 2:1.

Based on this, the costs of the three MICP methods were calculated as shown in Table 3, with the unit prices of the materials provided by Fujian Chenhong Technology Co., Ltd.

The cost per cycle for *SP* treatment is $42.77, while the cost per cycle for bio-stimulation is only $13.60, which is merely 31.8% of the cost of the *SP* method. The cost per cycle for the combined solidification is $23.79, which is only 55.6% of the cost of the *SP* method. The components of the *SP* culture medium, such as trisaminomethane and yeast extract, are relatively expensive. Bio-stimulation does not require the expensive trisaminomethane, reduces the amount of yeast extract used. Furthermore, indigenous urea-decomposing bacteria exhibit higher heavy metal tolerance, survive longer in the tailing sand, and the urea decomposition process in MICP lasts longer, resulting in a significantly higher heavy metal immobilization rate compared to *SP*. Additionally, by eliminating the need for bacterial cultivation and transportation, bio-stimulation significantly reduces solidification costs, thereby offering better economic and environmental benefits. However, the lower urease activity means that the strength achieved by bio-stimulation remains relatively low even after multiple treatment cycles. After seven cycles of combined solidification, the strength of the tailing sand is 5.3 times that achieved with the same number of cycles of bio-stimulation and 1.5 times that achieved with *SP*. The heavy metal immobilization rate is also the highest among the three methods. Although the combined method still uses *SP*, the halved dosage of bacterial suspension significantly reduces the solidification cost.

Table 3 Cost calculation of three MICP method

Treatement		Component	Concentration (kg/L)	Unit cost ($/kg)	Quantity (L/m^2/ cycle)	Cost calculation ($/m^2)
SP	Bacterial suspension	Yeast extract	0.020	45.03	8.67 (13/3×2)	42.77/cycle
		$(NH_4)_2SO_4$	0.010	4.22		299.39/7 cycles
		Triaminomethane	0.0158	174.47		
	Cementation solution	$CaCl_2$	0.111	5.63	13	427.7/10 cycles
		urea	0.060	4.22		
Bio-stimulation	Bio-stimulation solution	Sodium acetate	0.0029	5.63	13	13.60/cycle
		$(NH_4)_2SO_4$	0.0071	4.22		
		urea	0.0289	4.22		
		Yeast extract	0.0002	45.03		
		$NiCl_2$	1×10^{-6}	33.77		
		NaOH	0.0014	8.44		
	Cementation solution	$CaCl_2$	0.111	5.63	13	
		urea	0.060	4.22		
Combined	Bio-stimulation solution	Sodium acetate	0.0029	5.63	13	23.79/cycle
		$(NH_4)_2SO_4$	0.0071	4.22		
		urea	0.0289	4.22		
		Yeast extract	0.0002	45.03		
		$NiCl_2$	1×10^{-6}	33.77		
		NaOH	0.0014	8.44		
	Bacterial suspension	Yeast extract	0.020	45.03	4.33 (6.5/3×2)	166.53/7 cycles
		$(NH_4)_2SO_4$	0.010	4.22		
		Triaminomethane	0.0158	174.47		
	Cementation solution	$CaCl_2$	0.111	5.63	6.5	237.9/10 cycles
		urea	0.060	4.22		

4.5.2 Sensitivity Analysis

To further evaluate the economic robustness of the combined method, a sensitivity analysis was conducted focusing on three critical variables: the number of treatment cycles, variations in reagent prices, and the dosage of the exogenous *SP* bacteria.

(1) The total cost is directly proportional to the number of treatment cycles. As established in the strength tests, the combined method achieves a surface strength of 1188 kPa after 7 cycles. The cost for 4 cycles of the combined method is $95.16. However, strength gain may be insufficient for heavy loads. The cost of 7 cycles is $166.53. This represents the optimal balance, achieving 5.3 times the strength of bio-stimulation and 1.5 times that of the *SP* method while maintaining a cost 44.4% lower than the *SP* method for the same duration. Increasing to 10 cycles raises the cost to $237.90. Given that the rate of strength increase slows after 7 cycles, the marginal strength gain does not justify the 43% increase in cost from cycle 7 to 10.

(2) The most expensive components in the MICP process are trisaminomethane ($174.47/kg) and yeast extract ($45.03/kg). The *SP* method is highly sensitive to these price changes because it relies entirely on the expensive culture medium containing trisaminomethane. In contrast, the Bio-stimulation method is the most economically stable as it does not require trisaminomethane and uses minimal yeast extract. The combined method demonstrates moderate sensitivity. However, because it halves the dosage of the *SP* suspension, it buffers the impact of raw material price spikes better than the traditional *SP* method.

(3) In the combined solidification protocol, the ratio of *SP* bacterial suspension was set to 50% of the injection volume. Reducing the *SP* dosage further lowers costs significantly. If the *SP* dosage in the combined mix is reduced from 50 mL to 25 mL, the estimated cost per cycle decreases by approximately $8.01. However, this cost-saving must be weighed against potential reductions in urease activity and calcium carbonate precipitation rates. The current 50/50 ratio provides a high safety factor for heavy metal immobilization, but for projects with lower strength requirements, reducing *SP* dosage offers a flexible cost-saving mechanism.

4.6 Scale-up Challenges and Field Implementation

While the combined solidification method demonstrates significant laboratory success, implementing this technology on a field scale presents specific geotechnical and environmental challenges that must be addressed.

In heterogeneous tailing deposits, fluids tend to follow preferential flow paths, potentially leaving low-permeability zones untreated. For the surface crust formation targeted in this study, surface spraying or percolation methods are generally effective. However, to prevent shallow surface clogging that blocks deeper penetration, a staged injection method is recommended. The combined method offers an inherent advantage here: indigenous bacteria are already uniformly distributed within the matrix, reducing the reliance on transport mechanisms compared to the exogenous introduction of *SP* alone.

The combined method utilizes indigenous bacteria with varying urease activities, which promotes a more diffuse precipitation profile compared to the rapid, localized clogging often observed with high-activity *SP* injection. Nevertheless, field implementation should monitor infiltration rates. If clogging occurs, flushing with water or temporarily reducing reactant concentrations may be required to reopen flow paths.

While calcite is relatively stable, it is susceptible to degradation under specific environmental stressors. Repeated freeze-thaw cycles can induce micro-cracks in the cemented matrix, potentially reducing strength over time. The "flaky arrangement" of crystals observed in the combined method (Fig. 7d) 7 may offer better interlocking and resistance to these physical stresses than the smaller, discrete crystals formed by bio-stimulation alone.

The hydrolysis of urea releases ammonium ions, which can be toxic to aquatic life if leached into groundwater. Field applications may require a system to recover effluent or the establishment of vegetation to uptake the generated nitrogen. Nickel is a necessary co-factor for urease but is itself a heavy metal. This study’s optimized formulation strictly limits nickel chloride concentration to 0.01 mmol/L. This trace amount is negligible compared to the massive reduction in the leaching of existing toxic metals achieved by the treatment, resulting in a net positive environmental benefit.

To illustrate the economic feasibility, we estimate the material costs for treating a hypothetical 1,000 m^2 tailing surface area using the optimized 7-cycle combined solidification method. The calculated cost for the combined method is $23.79 per cycle/$m^2$. Total material cost: $23.79 × 7 cycles × 1,000 m^2 = $166,530. To avoid interference from impurities and to facilitate comparisons among groups, analytical-grade reagents were used throughout this study, and the costs were calculated accordingly. In field applications, industrial-grade reagents could be considered to further reduce costs.

5. CONCLUSIONS

This study proposed a method for solidifying tailing sand using indigenous urea-decomposing bacteria combined with *SP*, which improves the physical attributes related to the tailing sand while simultaneously immobilizing the heavy metals within it. The detailed findings are listed hereafter:

(1) The optiml bio-stimulation solution formulation for tailing sand is: sodium acetate 2.94 g/L, $(NH_4)_2SO_4$ 7.07 g/L, urea 28.86 g/L, yeast extract 0.2 g/L, nickel chloride 0.01 mmol/L, and pH 8.7.

(2) After seven cycles of combined solidification, the strength of the tailing sand is 5.3 times that achieved by bio-stimulation in the same cycle and 1.5 times that of the *SP* method. It also shows the highest heavy metal immobilization rate, while the cost is only 55.6% of that of the *SP* method.

(3) The mechanism by which MICP improves mechanical properties is the ultimate filling of pores between tailing sand particles by calcium carbonate in the form of calcite crystals. Both the number and size of calcite crystals generated by combined solidification were greater than for *SP* and bio-stimulation.

The combined MICP approach demonstrated in this study offers significant practical advantages for sustainable mine tailing management, addressing critical environmental risks: (1) Mitigating structural failures of tailing dams through rapid strength gain; and (2) Preventing heavy metal migration into adjacent ecosystems. Moreover, utilizing bio-stimulation reduces reliance on exogenous microbial cultivation and lowers operational costs, while the optimized bio-stimulation solution ensures scalability for field applications. However, long-term stability under environmental stressors (e.g., acid rain, freeze-thaw cycles) warrants further investigation before large-scale implementation.

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