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Improving the Strength and Leaching Characteristics of Pb-Contaminated Silt through MICP

Fusheng Zha, Hao Wang, Bo Kang *, Congmin Liu, Long Xu and Xiaohui Tan

School of Resource and Environmental Engineering, Hefei University of Technology, Hefei 230009, China; geozha@hfut.edu.cn (F.Z.); wanghao2016@mail.hfut.edu.cn (H.W.); liucongmin@mail.hfut.edu.cn (C.L.); xulong_2005@hfut.edu.cn (L.X.); tanxh@hfut.edu.cn (X.T.)

* Correspondence: kangbo@hfut.edu.cn; Tel.: +86-551-6290-1523

Abstract: Microbial-induced carbonate precipitation (MICP) is an effective technology for repairing sites contaminated by heavy metals. In this work, *Sporosarcina pasteurii* was cultured and mixed with a cementing fluid as a binder to remediate Pb-contaminated silt. The effects of varying experimental parameters, including Pb concentration and dry density, were also tested and analyzed. The leaching and strength characteristics and the MICP improvement mechanism of the Pb-contaminated silt were studied. Samples with dry densities of 1.50 g/cm³ and 1.55 g/cm³ exhibited the highest unconfined compression strengths (UCS). Scanning electron microscopy showed that not all CaCO₃ crystals produced a cementation effect, with some filling pores in an invalid cementation form. The results showed that MICP remediation of low Pb²⁺ concentration-contaminated silt could meet the relevant Chinese environmental safety standards. Low Pb concentrations helped improve MICP-treated, Pb-contaminated silt strength, whereas high Pb concentrations significantly reduced this strength. Testing to determine the tolerance of an active microbe, *Sporosarcina pasteurii*, showed that trace amounts of Pb promoted its growth, thus improving the MICP effect, whereas excessive Pb had a toxic effect, which reduced MICP effectiveness. Mercury injection experiments showed that MICP produced CaCO₃; this mainly filled soil mesopores and macropores and, thus, improved the soil UCS. Scanning electron microscopy showed that not all CaCO₃ crystals produced a cementation effect, with some filling pores in an invalid cementation form. MICP was innovatively applied to silt sites with heavy metal pollutants while considering the soil compaction in actual construction, thus broadening the application scope of MICP, optimizing the construction process, and reducing the construction cost.

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1. Introduction

In recent years, with the continuous development and integration of microbiology, environmental engineering, engineering geology, and geotechnical engineering, a microbial-induced calcium carbonate precipitation (MICP) technique has been widely used in enhancing the properties of soil. Compared with traditional technologies, it economizes substantial amounts of energy in producing materials and on-site operation, which can remarkably reduce carbon footprint, resulting in an elimination of potential danger (toxic chemicals, massive carbon dioxide emissions) to the environment [1–3] (Dejong et al. 2013; Kim and Lee, 2019; Peng et al., 2020). The MICP process can be divided into three stages. The first is urea hydrolysis, in which urea is rapidly hydrolyzed to ammonium and carbonate, under catalysis by urease, which is produced through microorganism metabolic activity (Equation (1)) [4]. The second process involves acid–base equilibrium in an aqueous solution, in which CO₃^{2−} is converted to HCO₃[−], which is the more dominant carbonate species, simultaneously resulting in an increase in the pH of the aqueous solution; this leads to the dissociation of NH₄⁺ to NH₃, which continues until equilibrium is reached between NH₄⁺/NH₃ and HCO₃[−]/CO₃^{2−} (Equations (2) and (3)) [5] (pp. 14–26). The third

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process involves formation of CaCO_3 , which takes places as the produced CO_3^{2-} ions precipitate in the presence of Ca ions as calcite crystals, which form cementing bridges between soil grains (Equation (4)) [6,7].

Sporosarcina pasteurii, which is often used as a urease-producing bacterium, can maintain high levels of biological activity under various environmental conditions. During the MICP processes, these bacteria play two roles: providing urease for the hydrolysis of urea and providing nucleation sites for CaCO_3 crystal formation [8,9].

MICP has shown a huge potential in environmental geotechnical applications. In previous studies, a removal rate over 90% was confirmed in MICP remediation of soil with contaminant of Zn(II) (94.83%), Cu(II) (95%), Mo(II) (98%), Se(III) (96.6%), Ni(II) (90.5%), and Cd(II) (99.5%), respectively [10–15]. Moreover, MICP can also effectively improve the strength of contaminated soil and provide strength support for the further development and utilization of heavy metal-contaminated sites [16–20].

In consideration of the high feasibility and the great advantages of the MICP method in the field of contaminated soil improvement, it is a hot topic for geotechnicians to give a clear understanding of the whole cementation process and devote to establish a mature construction practice that can be scaled up to real-site applications. However, its remediation effect on heavy metal-contaminated sites is influenced by many factors. These factors can be generalized in four aspects:

- (1) factors related to bacteria and cementation solution such as cell type and concentration, nutrient, and calcium sources [21,22];
- (2) environmental factors such as oxygen availability, aqueous environment, pH, and temperature [23–25];
- (3) properties of treated soil such as size distribution, density, and saturation degree [17,26–28];
- (4) construction technique such as injection rate and mode, retention time, and number of cycles [18,29].

In the existing four common construction processes of grouting, spraying, slurry mixing, and soaking, slurry mixing can obtain the best effect under the same conditions. The degree of compaction in the process of slurry mixing is very important to the curing effect, but there is no relevant research on the optimal degree of compaction.

Therefore, this test takes the artificially prepared, lead-contaminated soil as the research object and studies the optimal compaction under the existing optimal construction conditions. Under the condition of optimal compaction, the solidification effect of lead-contaminated soil was studied through a series of unconfined compressive strength and leaching characteristics, and its mechanism was revealed as combined by using scanning electron microscopy (SEM) and mercury intrusion porosimetry (MIP) testing, and tests were conducted to determine *S. pasteurii* tolerance to Pb. The experimental results can provide a reliable basis for the selection of construction conditions and process control of micp in contaminated soil remediation.

2. Materials and Testing Methods

2.1. Materials

2.1.1. Microorganisms and Their Growth Medium

The urease-producing bacterium used in this study was *S. pasteurii* (identification number: ATCC 11859), purchased in the form of a freeze-dried powder from the American Standard Bacteria Bank. The ATCC 1376 $\text{NH}_4\text{-YE}$ growth medium, consisting of 1 L deionized (DI) water, 20 g yeast extract, 10 g $(\text{NH}_4)_2\text{SO}_4$, and 0.13 M Tris buffer, was

employed, as recommended by the supplier. The freeze-dried *S. pasteurii* powder was activated and inoculated into a culture solution, at a volume ratio of 1:100, and sterilized using a high-pressure, steam sterilization pot (Shanghai Shen'an DSX-280KB30, Shanghai, China), at 121 °C. It was then incubated continuously, on a constant-temperature, oscillating incubator (Shanghai Yiheng THZ-98A, Shanghai, China). The cell concentration was proportional to the turbidity of the bacterial solution and, therefore, to the optical density. The OD₆₀₀ value of the bacterial solution (the optical density of a sample, measured at 600 nm) was used to indicate the concentration of bacteria and measured every 2 h during the culturing process, using an ultraviolet spectrophotometer (Shanghai Jingke Shangfen 721G, Shanghai, China) to obtain the bacterial growth curve. After incubation for 48 h, the bacterial solution OD₆₀₀ reached 2.0, which was the value required for soil sample preparation.

A cementing solution was used to provide the Ca and C sources necessary for the MICP process. In this experiment, a solution mixed with CaCl₂ and CH₄N₂O was used as the cementing solution, with each liter of this solution containing 1 L DI water, 1 mol CaCl₂, and 1 mol CH₄N₂O.

2.1.2. Soil and Pollutants

Soil samples were collected from a construction site foundation pit in Bozhou, Anhui province, China. The natural soil was dug from depths of 1.5–2.0 m, with a color of yellow-brown and a state of hard and plastic. The basic properties of the soil samples, determined in accordance with the relevant American Society for Testing and Materials standards (ASTM D7263-09; D2216-10; and D4318-17e1) [30–32], are listed in Table 1, and their chemical components, as determined using X-ray fluorescence spectrometry (XRF-1800, Shimadzu, Shanghai, China), are listed in Table 2. Based on these listed results, the soil was classified as a type of silt, with its main components, constituting > 68% of the total, being SiO₂ and CaO. No heavy metals were detected in the soil samples, indicating that there would be no interference with the test results from pre-existing heavy metal content.

Table 1. Basic properties of tested soil.

Water Content (%)	Specific Gravity	Natural Density (g/cm ³)	Liquid Limit (%)	Plastic Limit (%)
25.34	2.69	1.85	17.6	32.9
Mean Particle Size (μm)	Uniformity Coefficient C _u	Coefficient of Curvature C _c	Void Ratio	
13.48	7.06	1.82	0.82	

Table 2. Tested soil chemical components.

Component	CaO	MgO	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃
Content (%)	24.58	1.87	43.52	9.29	13.14
Component	SO ₃	TiO ₂	Na ₂ O	K ₂ O	Others
Content (%)	4.16	0.43	0.30	1.73	0.98

As a typical heavy metal pollutant, Pb (as Pb(II)) was selected as the representative contaminant for this study due to its strong toxicity to the human body and widespread distribution. Pb(II) was mixed in the soil in the form of analytical grade Pb(NO₃)₂·6H₂O.

2.2. Testing Methods

2.2.1. Specimen Preparation

Contaminated soil samples needed to be pre-prepared manually for testing, and so the soil was dried in its natural state, before being crushed through a 0.5-mm sieve. Based on

the pre-determined Pb concentration to be tested (the mass ratio of Pb to dry soil), the silt powder and appropriate amount of $\text{Pb}(\text{NO}_3)_2$ solid were carefully weighed. The $\text{Pb}(\text{NO}_3)_2$ solid was then dissolved in excess distilled water to prepare a $\text{Pb}(\text{NO}_3)_2$ solution, which was mixed into the silt powder until uniform, and then sealed in a constant temperature and humidity incubator, where it was maintained at 97% humidity and 20 °C, for 2 weeks.

The prepared contaminated soil was then dried, crushed, and filtered through a 0.5-mm sieve, before being weighed, based on the designed dry density. *S. pasteurii* (cultured for 48 h) was diluted to a 2.0 OD_{600} value in the culture medium. The same volumes of bacterial liquid and cementing solution were measured, so that the sum of the volumes was equal to the soil liquid volume calculated using the natural moisture content of the sample. The bacterial solution was then added to the contaminated silt, mixed until homogenous, and then sealed for curing.

After 3 h, the same volume of cementing liquid was added, and the mixture was again mixed until homogenous. The sample was then divided into four equal portions and placed in a 39.1 × 80 mm stainless-steel mold. Each mixture was shaved with a fine wire after compacting, to prevent obvious layering, and the samples were then demolded and sealed, before being left for 2 weeks to cure.

The control experiments were set up. In the control experiments, the blank culture medium was substituted for the *S. pasteurii* solution and was mixed into the contaminated silt with the cementing solution in turn. The mixture was eventually made into columnar samples.

2.2.2. Unconfined Compressive Strength Testing

Sample unconfined compressive strengths (UCS) were determined in accordance with ASTM 2000 [33]. The cylindrical specimens to be tested were placed on the base plate, before the load frame was fixed, without applying any stress to the sample. Then, the dial gauge and proving readings were adjusted to zero once the sample was in contact with the top plate. Subsequently, the specimen was pressurized and the strain rate was controlled at 1.5 mm/min. Simultaneously, the axial stress and axial deformation data were recorded by reading the dial gauge.

2.2.3. The Toxicity Characteristic Leaching Procedure (TCLP)

The leachability of Pb^{2+} from the prepared silts was detected using the toxicity characteristic leaching procedure (TCLP), in accordance with US Environmental Protection Agency Method 1311 [34]. The prepared specimen was crushed into pieces < 9.5 mm, and a leachant with $\text{pH } 2.88 \pm 0.05$ was prepared, by diluting 5.7 mL acetic acid (HAC) into 1000 mL DI water. Then, 250 mL of leachant was placed in a polythene bottle, 12.5 g of crushed specimen was added (solid-to-liquid ratio of 1:20), and the mixture was vibrated at 180 rpm for 18 h. After standing and layering, the supernatant was filtered for the measurement of the pH and Pb^{2+} concentration, to reveal the leaching characteristics of the treated specimen.

2.2.4. Testing *S. Pasteurii* Resistance to Pb(II)

A batch of Pb(II) concentration gradients of 0, 50, 100, 200, 400, 600, 800, 1000, and 1200 mg/L of culture medium, containing Pb(II) contaminated up to 200 mL, was prepared, using the continuous dilution method. Activated *S. pasteurii* was inoculated into the contaminated culture medium, at a 1:100 volume/volume ratio [35] (pp. 17–18). The cells were continuously cultured for 24 h in a constant-temperature oscillating incubator, at 30 °C and 200 rpm, with the final culture OD_{600} value measured to record bacterial concentrations, using a spectrophotometer.

2.2.5. CaCO_3 Content Determination

The CaCO_3 content was determined in this study using the acid leaching process [36–38]. The 20-g soil sample to be measured was weighed and placed in a beaker, and HCl was

added gradually until no further bubbles formed in the liquid. The liquid was then filtered, and the remaining soil was dried and weighed. The formula applied to calculating CaCO_3 content can be seen in Equation (5):

$$\omega = \frac{m_1 - m_2}{m_1} \times 100\%, \quad (5)$$

where m_1 indicates the mass of the soil before acid leaching (in our case, 20 g), while m_2 represents its mass after acid leaching. The CaCO_3 production amount is the difference between the CaCO_3 content of the tested sample and that of the original soil.

3. Results and Discussion

3.1. Effect of Compactness on MICP-Solidified, Pb-Contaminated Soil

The UCS results for MICP-treated, Pb-contaminated soil samples of different densities can be seen in Figure 1, which shows that, after MICP curing, all sample UCS characteristics increased significantly. Samples with 1.50 and 1.55 g/cm^3 dry densities exhibited the highest UCS values, indicating that these samples had the best cementation characteristics. The UCS was the lowest for samples with 1.45 g/cm^3 dry density.

Figure 1. Unconfined compression strengths of silt samples with different dry densities.

Silt dry density is determined by the compacting degree at the time of sample preparation and reflects pore volume and distribution within the silt. Pore ratios before and after sample MICP curing, obtained through dry density calculation and MIP testing, respectively, can be seen in Figure 2. Evidently, with increased dry density, the amount of soil particles in the samples increased, the pore volumes decreased, and the initial pore sample ratios decreased. After MICP curing, the pore ratios for samples with a 1.50 g/cm^3 dry density (0.0857) were significantly lower than those of the other three groups. In contrast, the pore ratios of the samples with a 1.45 g/cm^3 dry density were the highest, reaching 0.152, while those of samples with 1.55 and 1.60 g/cm^3 dry densities were 0.125 and 0.139, respectively.

Figure 2. Cumulative pore volumes and diameters for samples with different dry densities (with logarithmic x-axis).

Soil pores are sites for MICP reaction and provide space and adhesion surfaces for CaCO_3 crystallization. In turn, CaCO_3 formation fills soil pores, changing pore volume and distribution, thus affecting soil strength, which made it imperative to study the pore volume and distribution of the solidified soil.

The MIP procedure was used in this study to analyze soil pore distributions. Clays are known to have intra-aggregate, inter-aggregate, and large enclosed pores [39]. A Gaussian function was used to fit and calculate the pore distribution curve, which allowed the pore data from our samples to be quantified, in terms of its intra-aggregate and inter-aggregate pores, its microcracks, pore volumes, and average pore sizes [40]. Gaussian fitting can be expressed as shown in Equation (6):

$$f(D) = \sum_{i=1}^n f_i(D) = \sum_{i=1}^n \frac{a_i}{\sqrt{2\pi}\sigma_i} e^{-\frac{(\log D - \mu_i)^2}{2\sigma_i^2}}, \quad (6)$$

where $f(D) = \frac{dV}{d \log D}$ indicates the volume of mercury intruded into a pore of diameter D at a given pressure increment, in 1 g of the dry soil [41]. Symbol n represents the number of peaks in the PSD curves on a logarithmic scale (1 and 2, for unimodal and bimodal types, respectively), a_i denotes the pore volume in 1 g of dry soil covered by the fitted curve for $f_i(D)$ (mL/g), and σ_i stands for the standard deviation on a logarithmic scale. Symbol μ_i indicates the mean pore diameter in the fitted curve for $f_i(D)$, on a logarithmic scale (μm), which reflects the effective average pore sizes of the intra-aggregate and inter-aggregate pores and the microcracks [40].

3.1.1. Analysis of Gaussian Fitting Results

The pore size distribution curves and Gaussian fitting results for Pb-polluted soils with different dry densities after MICP treatment can be seen in Figure 3. The curves are all bimodal, except when the dry density was 1.45 g/cm^3 , in which case the distribution was unimodal. It was reported that unimodal-type distribution curves are mostly found in coarse, granular soils, in which the pores are mostly a single type [42], while bimodal types are characteristic of some structural soils, such as compacted silts [43], and clays with flocculated structures [44], which represent intra-aggregate and inter-aggregate pores, respectively [45,46]. The Gaussian fitting parameters for our samples are listed in Table 3, where μ_1 and μ_2 reflect the effective pore diameters of intra-aggregate and inter-aggregate pores, respectively, and a_1 and a_2 reflect their respective pore volumes. Inspection of the data listed in this table showed that the sample with a dry density of 1.45 g/cm^3 produced a unimodal curve, indicating that the pores in the soil body were mainly inter-aggregates.

Figure 3. Gaussian function fitting results for samples with different dry densities.

Table 3. Soil sample Gaussian fitting parameters.

Dry Density (g/cm ³)	Fitting Parameters						
	a_1 (mL/g)	μ_1 (nm)	σ_1 (μm)	a_2 (mL/g)	μ_2 (nm)	σ_2 (μm)	R ²
1.45				0.51720	153.978	1.51967	0.93623
1.50	0.04519	30.245	1.99212	0.00854	676.285	0.73795	0.96478
1.55	0.00315	6.780	0.36675	0.04549	193.370	1.72220	0.91746
1.60	0.00444	10.208	0.28925	0.04312	170.809	1.56577	0.92212

Pore distribution areas with a significant influence on the strength of the solidified contaminated soil were concentrated at pore sizes $> 0.05 \mu\text{m}$ [40]. Therefore, pore volume (a_2) and effective pore diameter (μ_2) results were our focus here. The a_2 value of the 1.45 g/cm^3 sample was found to be 10–60 times that of the other three dry density samples, indicating that the pore volume between aggregates in this soil, after MICP solidification, was much higher than it was in the other samples, which also explained its low UCS. The a_2 value of the 1.50 g/cm^3 sample was the lowest; therefore, its strength was the highest. However, the a_2 and μ_2 values of the samples with 1.55 and 1.60 g/cm^3 dry densities were similar, ensuring that their UCS values were also similar.

3.1.2. Pore Volume Distributions in Specimens

The International Union of Pure and Applied Chemistry (IUPAC) advises that there are five soil pore categories: micropores ($< 0.002 \mu\text{m}$), mesopores ($0.002\text{--}0.05 \mu\text{m}$), macropores ($0.05\text{--}5 \mu\text{m}$), air voids ($5\text{--}50 \mu\text{m}$), and pre-existing microcracks ($> 50 \mu\text{m}$) [39,47,48]. The pore types in the four dry-density samples in our study were categorized using these definitions, with the results shown in Figure 4, where it can be seen that only a small number of micropores existed in the soil samples with high dry densities (1.55 and 1.60 g/cm^3), with mesopores and macropores accounting for 76–91% of the totals in these samples. At increased dry densities, the total mesopore and macropore volumes gradually decreased. Macropores represent the bonding strength between the aggregates of solidified, contaminated soil, and can reflect the cementation and filling characteristics of the microorganism-produced CaCO_3 , to the extent that it can be said that the larger the pore size (generally), the lower the bonding strength between the aggregates. In our

work, sample macropores occupied greater volumes than the mesopores, except in the case of the 1.60-g/cm³ sample. The macropore volume in the 1.45-g/cm³ sample reached 0.49 mL/g, accounting for 69.42% of the total pore volume, so that, as expected, its UCS was significantly lower than that of the other three, dry-density samples.

Figure 4. Pore volume distributions for samples with different dry densities.

3.1.3. Discussion: Cementing Models of Soil Particles by MICP

In an unexpected result, it was found that, although the 1.60-g/cm³ dry density sample exhibited the lowest macropore volume, its UCS was not the highest, which may have been due to the CaCO₃ distribution in the pores and the resulting cementation effect. The size of urease-producing bacterial is generally between 0.5–3 µm [49]. In the samples with high dry density, the soil pores are mainly micropores, which resulted in the blocking of migration of bacteria fluid and heterogeneity of the CaCO₃ distribution. Two distinct CaCO₃ distribution modes were observed in soil pores (Figure 5) [49]. One has been referred to as “union” distribution, which indicates that calcite has been precipitated equally thickly all around the soil particles. Consequently, the bonding by calcite between any two particles is relatively weak, and, as a result, insignificant changes in the soil properties can be expected. The other mode has been called “preferential” distribution, indicating that the calcite is precipitated only at particle–particle contact points. Preferential distribution is the desirable engineered spatial distribution, as calcite directly contributes to improving soil properties. However, in practical engineering, CaCO₃ precipitation distribution is the result of a balance between these two extremes. Different distribution models produce different cementing effects, which can be classified into valid and invalid cementation (as shown in Figure 6a), based on the degree of soil particle cementation. Valid cementation occurs when CaCO₃ attaches to soil particle surfaces, contacts are made, and cementation occurs, which can effectively improve soil strength. On the contrary, in invalid cementation, CaCO₃ only attaches to the surface of the soil particles without cementing occurring between them, and so, this cementation type cannot completely fill the original pores, and has no effect on soil strength. SEM imagery illustrating these cementation types can be seen in Figure 6b, which shows that part of the aragonite precipitation produced in the sphere has filled in the pores, in the form of valid cementation, and part of it has filled the pores with invalid cementation. Particularly, when MICP technology is applied to tunnels, retaining walls, and highway embankments, the CaCO₃ crystals produced by MICP could bond the soil–structure interface. However, the CaCO₃ on the structural interface exists with both forms of invalid cementation and valid cementation, resulting in

the limited increase in the strength of a single round of MICP. Therefore, multiple rounds of MICP are required to obtain a better effect [50].

Figure 5. Illustration of calcite distribution alternatives within pore spaces [49] (Dejong et al., 2010).

Figure 6. (a) Schematic and (b) SEM images showing valid and invalid cementation.

Samples with 1.50- and 1.55-g/cm³ dry densities exhibited the highest UCS values, while MIP testing showed that MICP produced CaCO₃, which mainly filled the soil mesopores and macropores. SEM results showed that not all CaCO₃ crystals produced a binding cementation effect, with some filling the pores with invalid cementation. Therefore, when using the slurry method to repair contaminated soil, the compaction degree of 84% (the dry density is 1.55g/cm³) is the most appropriate, which can provide enough space for calcium carbonate crystallization and obtain the best curing effect.

3.2. Evaluation of Repair Effectiveness Based on TCLP Testing

The environmental safety risk inherent in conducting MICP remediation of Pb-contaminated silt was evaluated using TCLP testing, with the results then compared to the leached Pb²⁺ concentration limits specified in Chinese standard GB5085.3-2007 *Identification standards for hazardous wastes- Identification for extraction toxicity* [51]. The leached Pb²⁺ concentrations revealed by the TCLP testing conducted on without-MICP and MICP-repaired soil with different initial Pb²⁺ concentrations can be seen in Figure 7. As can be seen from the figure, leached Pb²⁺ concentration of the contaminated soil without MICP was much higher than that after MICP curing. And the Pb²⁺ leaching concentration increased in line with increasing the initial silt Pb²⁺ concentrations. When the initial Pb²⁺ concentration in the silt was <0.1%, the leached Pb²⁺ concentration was up to 5 mg/L, which was below the limit specified in the standard for hazardous waste disposal in China. For initial silt Pb²⁺ concentrations > 0.1%, however, the leached Pb²⁺ concentrations failed to comply with the standard. This meant that the MICP process had to be improved, in order to ensure that it could be used to render silt contaminated with higher Pb concentrations environmentally safe.

Figure 7. Leachate Pb²⁺ concentrations from silty soil samples with different initial Pb content cured by the microbial-induced carbonate precipitation (MICP) process.

3.3. Effect of Pb Concentration on the Strength of MICP-Cured, Pb-Contaminated Soil

UCS testing was performed on specimens with different initial Pb concentrations before and after MICP treatment. The results are illustrated in Figure 8, which shows that, compared with the control experiment without MICP treatment, the strength of the soil was greatly improved after curing with MICP technology. As the Pb concentration increased, the MICP-cured sample UCS values initially increased and then decreased, with the peak value achieved for the 0.1% Pb specimen, suggesting that the MICP curing effect was stronger in low-concentration Pb and weaker in high-concentration Pb. CaCO₃ contents in the sample with various Pb concentrations were measured after they were broken in the UCS testing. The results, as illustrated in Figure 9, revealed that sample CaCO₃ contents also increased first, with increasing Pb concentration, before decreasing, in a trend similar to that seen for the UCS testing, indicating that CaCO₃ content was the main factor determining sample strength.

3.4. Influence Mechanism of Lead Concentration of MICP-Cured, Pb-Contaminated Soil

Pb influences the effect of MICP by affecting microorganism growth [52–54]. When the Pb concentration was <0.1%, *S. pasteurii* growth was promoted, leading to increased urease production. The MICP process, therefore, produces more CaCO₃, thus increasing the cementation effect and strengthening the soil. When the Pb concentration was >0.1%, *S. pasteurii* growth stalled, the population stagnated, and even died off, thus decreasing its urease production capacity, which led, in turn, to less CaCO₃, resulting in reduced soil strength. This phenomenon motivated our study of *S. pasteurii* tolerance to Pb(II) pollution. *S. pasteurii* concentrations after 24 h in culture solutions with different Pb concentrations can be seen in Figure 10.

Figure 8. Unconfined compression strengths (UCS) of silty soil samples with different initial Pb concentrations, cured using the MICP process.

Figure 9. CaCO₃ content of silty soil samples with different Pb concentrations cured by MICP.

Figure 10. OD₆₀₀ values for bacteria in media having different Pb concentrations (24 h).

3.4.1. Resistance Mechanism and Stimulating Effect of *S. pasteurii* in Low Pb Concentration

The results showed that when the culture medium Pb concentration was <600 mg/L, the bacterial liquid concentration increased with the increasing Pb concentration, indicating that Pb promoted microorganism growth at this concentration. This outcome was probably revealing the mechanism bacteria use to resist heavy metals, and the stimulating effect heavy metals can have on microorganisms.

Heavy metals have strong surface binding affinities with metal-sensitive functional groups, such as amides, mercaptans, or histidines, at trace concentrations [55]. It was reported that the Pb-resistance mechanisms employed by microorganisms include exclusion by means of a permeability barrier, intra- and extra-cellular sequestration, active transport efflux pumps, enzymatic detoxification, and reduction in the sensitivity of cellular targets to metal ions [56–59]. This resistance has been demonstrated many times, including, for example, another study that found that increased soil Pb/Zn/Cd concentrations did not have a negative effect on microorganism numbers in the soil [60]. It was also shown that low levels of heavy metals do not inhibit microorganism growth, due to these resistance mechanisms; rather, the ability to synthesize exopolysaccharide (EPS), in response to the stimulation provided by the heavy metal toxicity, becomes enhanced [61]. This meant that, in such situations, *S. pasteurii* activity and use of energy-providing materials of *Sporosarcina pasteurii* were stimulated [62] (pp. 32–36). Fliebbach (1994) [63] reported that, by adding a sludge containing a low concentration of heavy metal to soil, the microbial biomass of the soil could be improved, and that its microbial activity could be enhanced. Zaborowska et al. (2006) [60] reported that as soil Pb concentration increased from 0 to 1000 mg/kg, the number of microorganisms increased 2.5 times and urease activity increased from 11.88 to 15.87 mg/h (as N-NH₄). In conclusion, low concentration of Pb can promote the growth and reproduction of *S. pasteurii* and make it secrete more urease, thus promoting the effect of MICP.

3.4.2. Toxic Effect to *S. pasteurii* in High Pb Concentration

The results also showed that, once Pb concentrations increased beyond 600 mg/L, the bacterial solution concentration decreased, eventually becoming lower than that of a pollutant-free culture solution. This showed that Pb was toxic to *S. pasteurii*, at this concentration, inhibiting its growth and metabolism, and even causing its death [64]. Analogously, studies have shown that MICP is also inhibited in free Cu culture solution when the Cu

concentrations ranged from 0–1000 mg/kg [65]. It has also been reported that exceeding the level of heavy metals beyond the limit of microbial resistance results in toxicity [66], via a mechanism that presents with two aspects. Firstly, protein (enzyme) functioning is affected, destroying the DNA double-stranded structure, and then normal microorganism metabolic processes are affected [67]. In one such mechanism, toxic metal cations can injure or destroy biological functions by substituting for physiologically essential cations at the binding site of a specific protein, such as an enzyme [68]. Meanwhile, toxic metal cations can also bind to sensitive thiol groups on nascent proteins, which impairs protein folding, or the binding of enzymes by cofactors, thereby deactivating the normal biological activity of these proteins [69]. These reactions frequently require and produce reactive oxygen species, which are transient and highly reactive compounds that can damage biological macromolecules [70]. MICP is initiated through the activity of enzymes, such as urease, carbonic anhydrase, and asparaginase [53], which are easily inactivated by high heavy metal concentrations.

4. Conclusions

Noting the renowned remediation efficiency of the MICP process when applied to heavy metal-contaminated sites, and being mindful of its environmentally friendly reputation, its strengthening attributes and soil improvement mechanism were reviewed in this study, using Pb(II)-contaminated silt as the test material. The main conclusions, which could both reduce MICP costs and optimize the process, are as follows:

- (1) TCLP test results showed that when the initial soil Pb concentration was <0.1%, the leached Pb^{2+} concentration was <5 mg/L, which met Chinese waste disposal standard environmental requirements. When the initial concentration was >0.1%, further MICP process optimization was needed, in order to comply with these requirements;
- (2) As the Pb concentration increased, the UCS of the MICP-cured samples initially increased, and then decreased. The soil $CaCO_3$ content showed the same trend as the Pb concentration, with this found to be because, while low Pb concentrations promoted *S. pasteurii* metabolism, due to a combination of resistance mechanism and stimulus action, higher concentrations were toxic. Testing on the bacteria's Pb tolerance showed that trace Pb amounts promoted *S. pasteurii* growth, thus improving MICP process effectiveness, while excess Pb had an evident toxic effect, thus inhibiting MICP effectiveness;
- (3) Samples with 1.50 and 1.55 g/cm³ dry densities exhibited the highest UCS values, while MIP testing showed that MICP produced $CaCO_3$, which mainly filled the soil mesopores and macropores. SEM results showed that not all $CaCO_3$ crystals produced a binding cementation effect, with some filling the pores with invalid cementation.

An innovative idea was presented in this study: application of the MICP technology to the remediation of silt sites with heavy metal pollutants. The construction design could be optimized according to the concentration of heavy metals in the site, and construction costs could be reduced by controlling the compaction degree of the site. However, the findings of this study are to be seen in light of some limitations. The environment of the field construction site is much more complex than that of the laboratory environment, especially regarding the existence of miscellaneous bacteria in the soil, which are bound to compete with *S. pasteurii* suitable for MICP in terms of the nutrients needed for growth and reproduction, causing uncertainty in the effect of MICP. Therefore, further studies should consider screening urease-producing bacteria existing in the soil to replace *S. pasteurii*, so as to reduce the influence of miscellaneous bacteria on the MICP technology. In addition, the raw materials used in MICP are likely to be replaced by industrial and agricultural wastes, such as soybean urease instead of *S. pasteurii* [71], pig urine instead of industrial urea [72] and oyster shells, scallop shells, and eggshells instead of calcium sources [73]. It was proven to significantly reduce the cost of MICP without weakening the curing effect of MICP for those methods, which also is one of the research focus points in the next stage.

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