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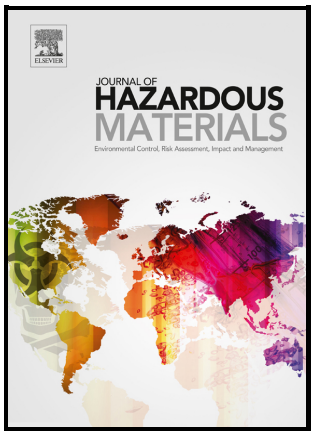

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Inhibition of cadmium releasing from sulfide tailings into the environment by carbonate-mineralized bacteria

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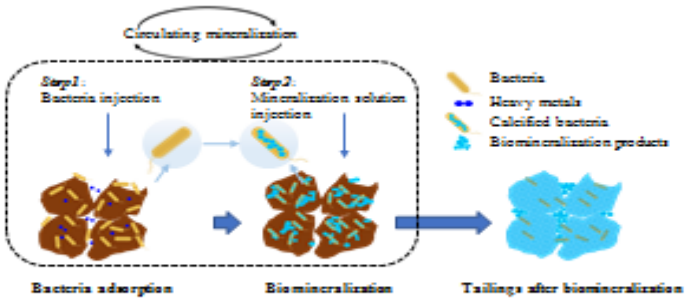
Abstract

Microbially induced carbonate precipitation (MICP) could be a potential green solution to resolve the issue of heavy metal releasing from the sulfide tailings. However, detailed mechanism of heavy metal-biomineralization in sulfide tailings and impact of procedure parameters on in-situ applications remain unexplored. We systematically investigated the biomineralization process in the column tests for a better understanding of the mechanism and effects on the inhibition of cadmium (Cd) releasing from sulfide tailings. Results revealed that uniform and efficient mineralization in the tailings column occurred under bacterial concentration of 1×10^8 cfu mL^{-1}, bacterial retention time of 3 hours, concentration of mineralization solution of 0.25 mol L^{-1}, and flow rate of 1.5 mL min^{-1}. The leachable Cd concentration decreased 80.7% after 7 mineralization cycles. From a suit of characterizations, bacteria can adhere on the tailings and acted as the nucleation sites to induce the mineralization of Ca and Cd (to $(Ca0.67, Cd0.33)CO_3$ and calcite phase); eventually, tailings particles were coated with the growth of mineralized carbonates, resulting in a reduction of exposure for tailings (especially sulfur). And thus, Cd release was inhibited. Results from this study will provide a fundamental basis for future in-situ applications of MICP to mitigate heavy metal pollutions.

Graphical abstract



Keywords: Microbially induced carbonate precipitation (MICP); cadmium (Cd); biomineralization; precipitation; mine tailings

1. Introduction

Mine tailings produced from mine operation, 10 billion tonnes per year worldwide [1], heavily pollute the environment, due to the heavy metals leached such as cadmium (Cd), copper (Cu), lead (Pb), and arsenic (As) [2,3]. In sulfide tailings, sulfide minerals such as pyrite and chalcopyrite can be easily oxidized when exposed to air, water and microorganisms (e.g., *Thiobacillus thiooxidans*, *Thiobacillus ferrooxidans*), in formation of acid containing SO_4^{2-}, Fe^{3+}. This results in increasing of dissolution of metal ions that can migrate into the surrounding environment spreading the heavy metal contamination area [4,5]. Thus, to mitigate their migration, toxicity, and accumulation, heavy metals in this sulfide tailings must be dealt with to protect the health of ecosystem and humans.

Past efforts to treat heavy metals in sulfide tailings include soil capping, chemical precipitation, leaching which may be high cost or environmentally destructive[6]. Biological method such as phytoremediation or microorganism-mediated remediation is a promising technology due to its environmental friendliness[2,7]. However, current microorganism-mediated remediation based on sulfate reducing bacteria (SRB) are limited to anaerobic environments and phytoremediation are limited to long

remediation cycles[8].

Microbially induced carbonate precipitation (MICP), one of the unique processes for heavy metals bioremediation, can mineralize heavy metals such as Cu[9], Pb[10], Cd [11,12], and As[13] by immobilizing heavy metals from the ionic state into a stable carbonate or heavy metals-calcium carbonate co-precipitation in the presence of calcium ions via the inducing of urease-positive microorganisms. As such, the mobility and toxicity of heavy metals are greatly reduced [13,14]. In addition, MICP has the ability to reduce pore size and permeability by forming cementitious calcium carbonate in between the surfaces of loose sand and granules [15,16], which has been used successfully for consolidation of sand columns or repair of cracks in concrete [17,18]. Therefore, this outside layer will inhibit the releasing of toxic heavy metals from sulfide tailings into the environment.

Previous studies on practical application of MICP, including soil improvement[17,18], strengthening of sandy soils[20], and desert aeolian sand solidifying[16], are mainly focused on parametric investigations of soil type, retention times, biomass, effective flow rates and other parameters. Thus far, in-situ MICP process is, however, not well understood for the heavy metals-mineralization in sulfide tailings. Information is lacking on the detailed characterization of the mineral products and the mineralization mechanism.

Therefore, in this study, the release of toxic metals (i.e., cadmium (Cd)) from the sulfide tailings is studied in MICP by urease positive carbonate-mineralized bacteria inoculated in column tests programmed with 2-phase injection biomineralization program. Specifically, we aim to: (1) assess the effects of concentration and retention time of the bacterial suspensions, concentration and injection rate of mineralization solution, and mineralization cycles on the mineralization efficiency and uniformity of the heavy metals in tailings; (2) identify the interaction between bacteria and the tailings and characterize the bacterial behavior and the product formation during the biomineralization process from a suit of characterizations by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction analysis (XRD), and Scanning

Electron Microscope-Energy Dispersive Spectrometer (SEM-EDS); and (3) propose the possible biomineralization mechanism of tailings. This research would present an efficient strategy for the inhibition of the release of toxic metals from the sulfide tailings, and provides the foundation for future large-scale in-situ application.

2. Materials and methods

2.1 Samples, microorganism and mineralization solutions

Samples used for the biomineralization in this study were collected from the Dexing copper mine, located in the Dexing city, Jiangxi province, China. It is the largest typical open pit operation in Asia (with the predominant ore mineral sulfide ores, e.g. $CuFeS_2$)[21], and deposited approximately 1105 t of flotation tailings per day [22]. The screening of heavy metal pollution in tailing pond and surrounding soil of Dexing copper mine revealed that cadmium (Cd) was the main contributor to the potential ecological risk[23,24]. Therefore, Cd was selected in this study to evaluate the biomineralization in tailings.

Tailings were sampled at a depth of 20 cm from the tailings dam of the Dexing Mine. The tailings were dried and powdered at ambient temperature for the biomineralization and characterization. To characterize and classify the soils, modified sieve and hydrometer analyses were performed according to Oualha et al. (2020)[25]. Sieving was performed using sieve openings of 425 μm, 380 μm, 250 μm, 150 μm, 120 μm, 106 μm, 75 μm, 45 μm, 38 μm, and 25 μm to determine the grain size distribution (Fig. S1). The Toxicity Characteristic Leaching Procedure (TCLP) leachable Cd concentration of the tailings was 3.11±0.20 μg L^{-1}.

The urease producing and carbonate-mineralized functional bacterial consortium, UPC, was previously isolated from the soil-tailings mixture of Dexing Copper Mine [12]. The UPC was inoculated in sterilized NBU (nutrient broth with urea: peptone, 10 g L^{-1}; NaCl, 5 g L^{-1}; beef extract, 3 g L^{-1}; urea, 20 g L^{-1}) media at natural pH and cultured in a thermostat shaker for 24 h at 30℃ and 150 rpm. Before use, the cultured bacterial suspensions were diluted by normal saline to predetermined optical densities of 1×10^6, 1×10^7, 1×10^8, 5×10^8, 7.5×10^8, and 1×10^9 cfu mL^{-1}. The mineralization

solution used in the biomineralization tests provided the basic chemicals (as urea and Ca source) for MICP process where molar concentrations of urea and $CaCl_2$ were equal.

2.2 Biomineralization tests

For preparing the specimens, 400 g tailings samples were compacted in three layers in every PVC column (D= 5 cm, H= 15 cm) to a dry density of 1.36 g/cm^3 and porosity of 0.407. Two layers of gauze were placed at each end of the column. And the biomineralization program for the tailings was performed as follows (Fig. 1) [18,26]:

Step1: 120 mL (equal to the pore volume) of bacterial suspensions with different concentrations (*Parameter 1*) were injected into the tailings columns at a flow rate of 1.5 mL min^{-1}, until uniform distribution of the bacterial suspension was achieved in the tailings columns (the time required is *Parameter 2*).

Step2: 120 mL (equal to the pore volume) of *mineralization solution* with different concentrations (*Parameter 3*) were injected into the tailings column at different flow rate (*Parameter 4*), allowing for 7 days (excessive time) of biomineralization.

The 2-phase injection is called a cycle of biomineralization, followed by repeated injection for the circularly mineralization (*Parameter 5*).

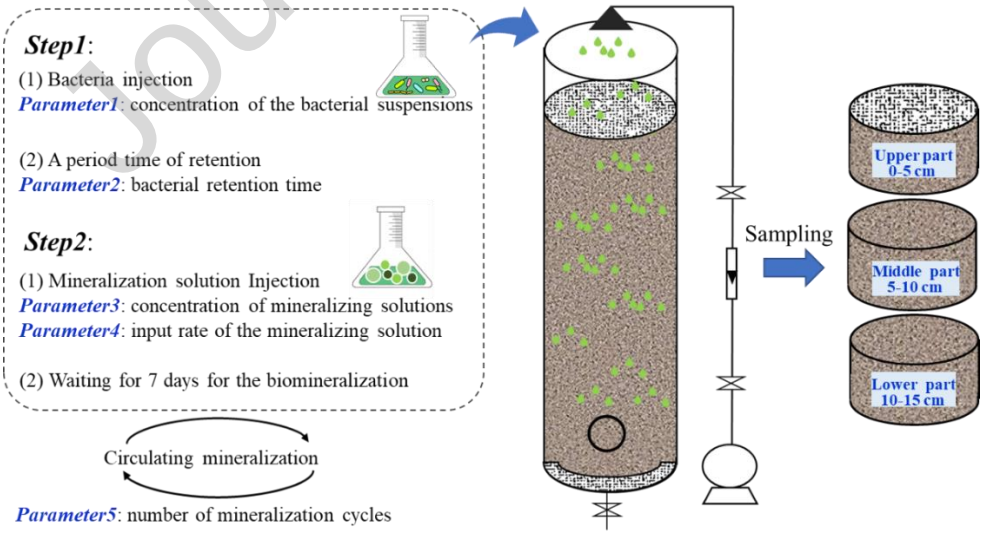


Fig. 1. Schematic diagram of mineralization program and parameters design.

The five selected parameters were screened to determine the optimal conditions

on the biomineralization, as shown in Table 1. All tests were done in triplicate and placed at room temperature (25 ± 3℃). Effect of *Parameter1-Parameter4* were studied under 1 cycle of mineralization. After *Step 1* of the tests for *Parameter 1 and Parameter 2*, samples were collected for the urease activity detection by observing the bacterial suspension's distribution in the tailings columns. After *Step 2* of the tests for all the 5 parameters, all biomineralized tailings columns were divided into 3 parts (upper, middle and lower) (Fig. 1) and sampled for the evaluation of immobilization efficiency and uniformity of Cd in the tailings by the biomineralization.

Table 1. The investigated parameters and the values of their levels.

parameters		levels
Parameter 1	concentration of the bacterial suspensions	1×10^6, 1×10^7, 1×10^8, 5×10^8, 7.5×10^8, and 1×10^9 cfu mL^{-1}
Parameter 2	bacterial retention time	1, 3, 8, and 24 h
Parameter 3	concentration of *mineralization solution*	0.10, 0.25, 0.50, and 1.00 mol L^{-1}
Parameter 4	flow rate of the mineralization solution	0.5, 1.0, 1.5, 2.0, and 2.5 mL min^{-1}
Parameter 5	number of mineralization cycles	1, 2, 3, …, 8, and 9

2.3 Microcosm experiments

Microcosm experiments were designed to characterize the detailed biomineralization process. First, adsorption experiments were carried out to verify the adhering ability of bacteria to tailings by mixing 2 g tailings with the 100 mL of bacteria suspensions of 1×10^8 cfu mL^{-1} at 25±3℃ and 150 rpm for 3 hours. The precipitates of tailings, pure cell of bacteria, and the mixture of bacteria adhered on tailings were collected by centrifugation at 1000 rpm for 5 min and freeze dried for FTIR analysis. And the mixture of bacteria adhered on tailings was also pretreated and characterized by SEM analysis. To avoid the influence of the culture medium on the FTIR, bacteria washed with normal saline for three times were used in the adsorption experiments. And then, to characterize the bacterial behavior and the formation of

products during the biomineralization process, samples of mixture of bacteria-tailings-products in the biomineralization process were pretreated and characterized by SEM-EDS analysis. To characterize the crystal structures of the tailings and mineralization products, samples of tailings before and after mineralization were analyzed by XRD.

2.4 Characterizations and analyses

The bacterial concentration was determined by blood cell counting plate. Electrical conductivity method was adopted to measure the urease activities in tailings [26]. Toxicity Characteristic Leaching Procedure (TCLP) was applied to evaluate the immobilization efficiency and uniformity of Cd in the tailings by the biomineralization [27]. TCLP leachable Cd concentrations were measured by Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS, Perkin Elmer, USA). The $CaCO_3$ in the column after biomineralization was then determined by the titration method using phenolphthalein as an indicator (Eq. 1) [26].

$$\mathrm{CaCO_3\ increment\ (g\ kg^{-1})} = \frac{m_i - m_0}{0.4} \qquad (1)$$

where m_i is the calcium carbonate content of a mineralized tailings column (g); m_0 is the average calcium carbonate content of the control groups; 0.4 is the mass of tailings in a column in this study.

The chemical functional groups of the pure bacteria cells and tailing samples were determined by a Fourier transform infrared spectrometer (FTIR, Nicolet iS50, Thermo Fisher, USA) at wavenumbers from 400-4000 cm^{-1}. Besides, the crystal structures of the tailings and mineralized products were detected using X-ray diffraction analysis (XRD; Bruker, D8 Advance X). The scanning electron microscope with energy dispersive X-ray spectroscopy (SEM-EDS, ZEISS-EVO18, Germany) was used to characterize the surface morphology and elemental distribution of the tailings, mixture of bacteria adhering on tailings, and mixture of bacteria-tailings-products. A modified method according to Warren, et al. (2010)[28] and Qian, et al. (2017)[29] was used to pretreat the mixture of bacteria adhered on

tailings and mixture of bacteria-tailings-products for SEM analysis. Mixtures were washed three times with phosphate buffer and mixed with 2.5% sterile glutaraldehyde in phosphate buffer for 12 h at 4℃ for fixing. Then, mixtures were gradient dehydrated with ethanol (30%, 50%, 70%, 85%, 90% alcohol once, 100% dehydrated twice, standing for 10-15 min in each gradient) and freeze dried for SEM analysis. Phosphate buffer was adjusted to pH 7.2 with 1 M NaOH and sterilized before use. 2.5% sterile glutaraldehyde was prepared with phosphate buffer.

2.5 Statistical analyses

The standard deviation (SD) of the leachable Cd concentration was used to evaluate the mineralization uniformity of the upper, middle and lower parts of the biomineralized-tailings in the column [30]. All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 19.0 software. Statistical analysis was performed using SPSS 25.0 software.

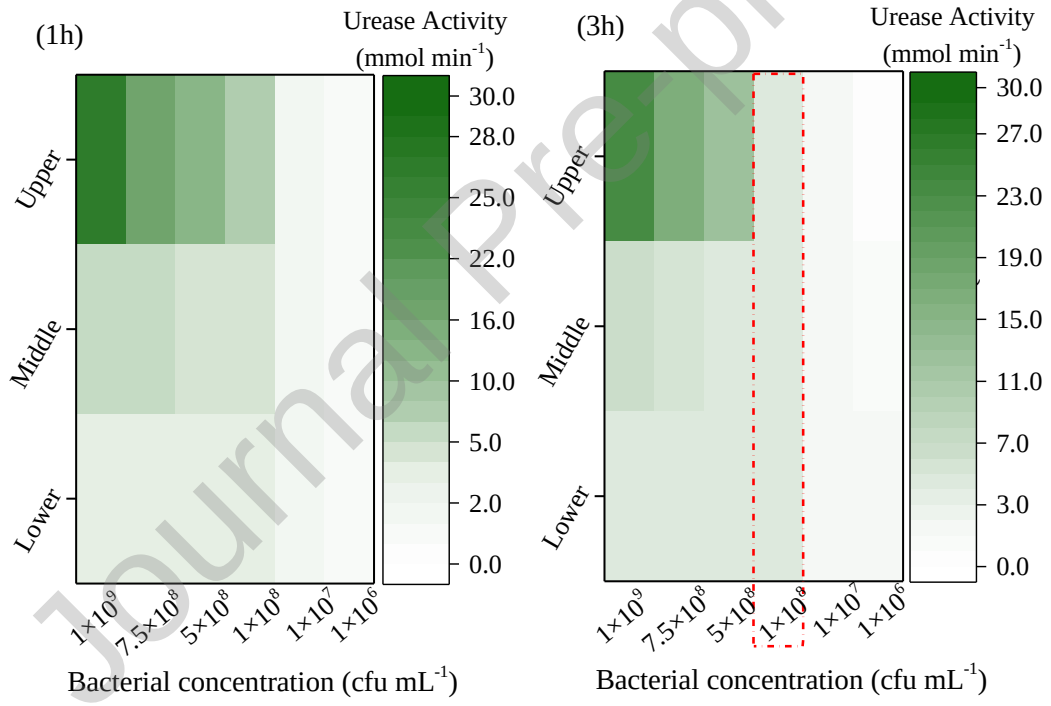
3. Results and discussion

3.1 Effect of concentration and retention time of the bacterial suspensions

Urease activity, an important restrictive factor of the efficiency in MICP [31,32], was first tested because the urease activity of bacterial suspensions is usually positively related to mineralized products. While concentration and residence time of bacterial suspensions could demonstrate a significant influence over the spatial distribution in the column, an excess of bacterial suspensions may yield high calcium carbonate thereby plugging the columns near the injection source [33]. Therefore, it is essential to obtain the maximum urease activity while uniformly distributing the bacteria in the tailing column. To this end, the distribution of the bacteria after *Step 1* and the biomineralization effect after *Step 2* were assessed, respectively, under different concentrations and retention time of bacterial suspensions.

Fig. 2 demonstrates the urease activity that characterizes the bacterial distribution in the column. With increasing retention time (within 3 hours), the bacteria moved downward slightly. The bacterial suspension of 1×10^8 cfu mL^{-1} after retention time of 3 hour (urease activity of 4.51-4.95 mmol min^{-1}), along with lower concentrations

(1×10^7 and 1×10^6 cfu mL^{-1}) after retention time of 1 hours (urease activity of 1.54-1.98, 0.99-0.55 mmol min^{-1}, respectively), was uniformly distributed in the tailings column. When the retention time was increased to 8 hours or 24 hours, the urease activity decreased, which may be attributed to the decay of cells [34]. However, even at 24 hours, most of the bacteria with high-concentration (1×10^9, 7.5×10^8, 5×10^8 cfu mL^{-1}) remained at the upper part of the tailing column and could not achieve uniform distribution. This demonstrated that a high bacterial concentration is not favorable for distribution in the column [26]. Therefore, the concentration of 1×10^8 cfu mL^{-1} and retention time of 3 hours were selected to obtain the higher activity and uniform distribution of urease.



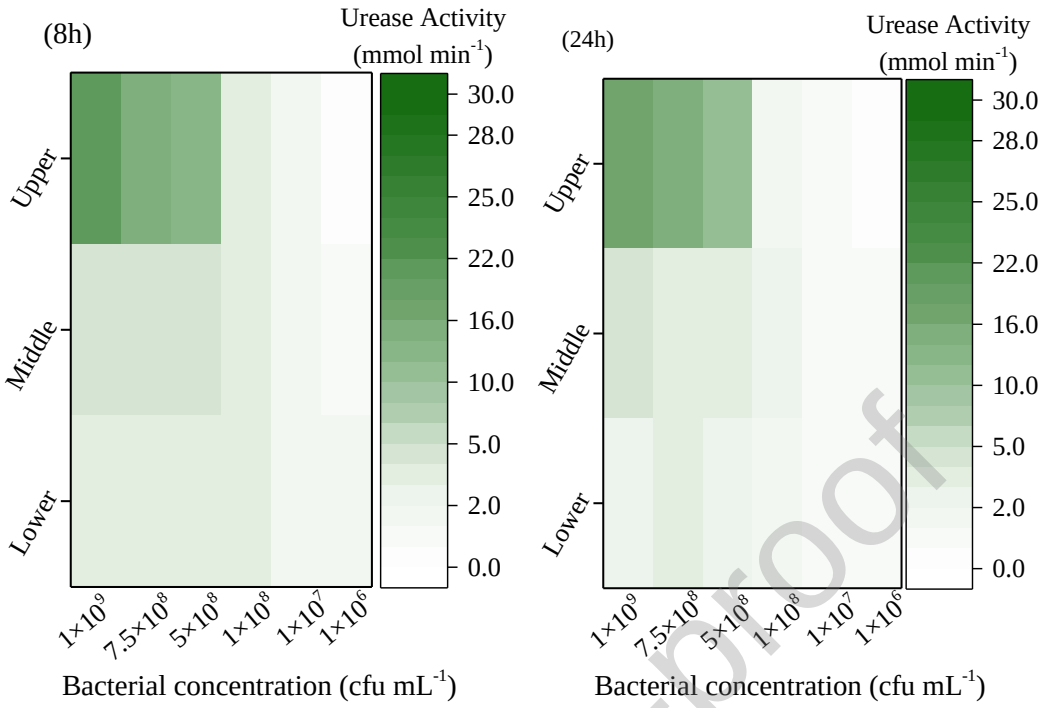


Fig. 2. Distribution of the bacterial suspensions with different concentrations in the tailings columns under different retention times.

To investigate the effect of bacterial concentration on mineralization, the TCLP leaching method was applied to measure the leachable Cd concentration from the biomineralized-tailings under the bacterial retention time of 3 hours (Fig. 3). The standard deviation (SD) of the leachable Cd concentration was used to evaluate the mineralization uniformity of the upper, middle and lower parts of the biomineralized-tailings. The results showed that with the bacterial suspensions of 1×10^8, 1×10^7 and 1×10^6 cfu mL^{-1}, the leaching Cd concentration of the tailings after mineralization were 2.53-2.61, 2.86-2.89, and 2.94-3.03 μg L^{-1} with small SD values (0.03, 0.01, 0.04 μg L^{-1} respectively, < 0.05 μg L^{-1}), indicating that a relatively uniform mineralization in the tailing column. However, when the bacterial suspensions were of high concentration (1×10^9, 7.5×10^8, 5×10^8 cfu mL^{-1}), the leaching Cd concentration of the upper parts after mineralization was significantly less than that of the lower parts, indicating the nonuniformity of the mineralization as indicated by the larger SD value (0.33, 0.25, 0.20 μg L^{-1}, respectively, > 0.05 μg L^{-1}). The length of the boxes indicated the same results. It may be because a large number

of Ca and urea in the mineralization solution could adsorbed by the high-concentration bacteria staying on the upper part [35,36], where mineralization occurs favorably. Consistent with observations of urease activity, bacterial concentration of$\times 10^8$ cfu mL^{-1} and retention time of 3 hours were selected for the Cd leaching tests in this study.

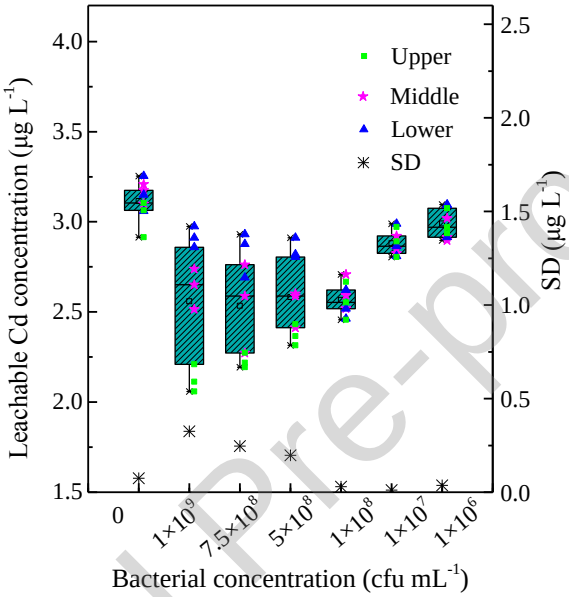


Fig. 3. TCLP leachable Cd concentrations at the upper, middle, and lower parts of the biomineralized-tailings and its standard deviation (SD) at differently bacterial concentrations. The bacterial retention time was 3 hours in this experiment. The horizontal bars within boxes represent median and the point represent leachable Cd concentrations at different parts. The tops and bottoms of boxes represent 75th and 25th quartiles, respectively. The upper and lower whiskers represent the maximum and minimum, respectively. The longer boxes represent a relative lower uniformity of the mineralization, otherwise indicate uniform mineralization.

3.2 Effect of concentration and flow rate of mineralization solution

3.2.1 Concentration of mineralization solution

In Fig. 4a, the uniform mineralization in the tailing column was showed at different concentrations of mineralization solutions. The leachable Cd concentration decreased with the increasing concentration of mineralization solution. When the

concentrations of mineralization solution were 0.1, 0.25, and 0.5 mol L^{-1}, the leachable Cd concentrations after mineralization decreased by 6.93%, 17.59%, and 20.49%, respectively, compared with that before mineralization. Notably, the leachable Cd concentration of the mineralized tailings was only 2.90% lower at 0.5 mol L^{-1} than that of 0.25 mol L^{-1}, indicating that the mineralization efficiency was, however, lower although sufficient mineralized solution was supplied. Mineralization solution of 1.0 mol L^{-1} showed the same trend. We speculate that, when the bacteria cells are exposed to a high dose of salt, adverse impact on enzyme production of the bacteria could be caused resulting in retarded growth [37,38]. Overall, these findings are in accordance with findings reported by Nemati et al. (2005) [39] and Okwadha et al. (2010) [40], that high concentrations (0.5-1.0 M) of urea and calcium chloride generated a significant amount of calcite but with a low efficiency; on the contrary, the formation of calcite at lower concentrations (0.05-0.25 mol L^{-1}) can be more efficient. In the current study, the highest mineralization efficiency of Cd was measured at the mineralization solutions of 0.25 mol L^{-1}.

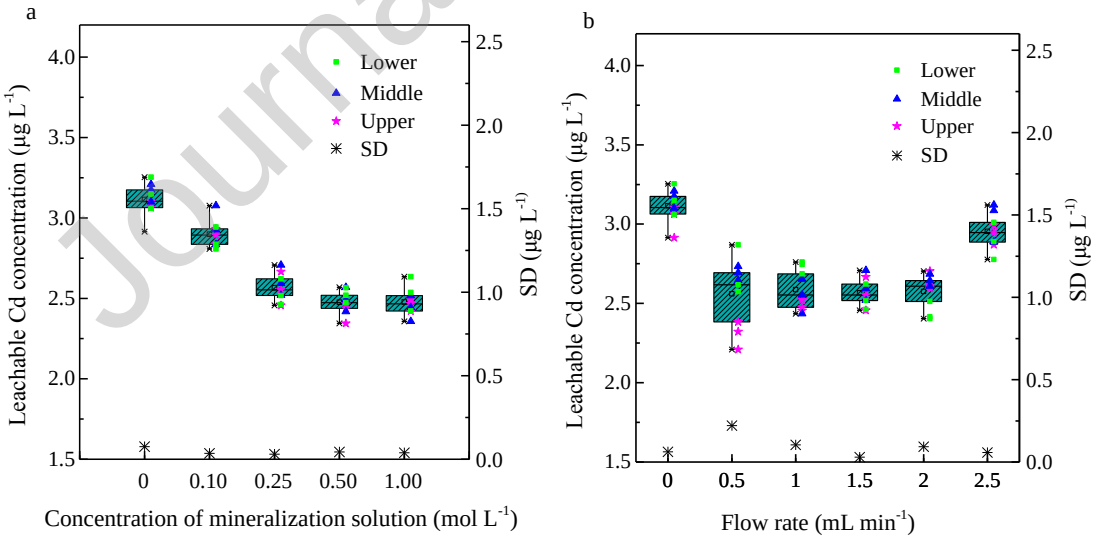


Fig. 4. TCLP leachable Cd concentrations at the upper, middle, and lower parts of the biomineralized-tailings and its standard deviation (SD) under (a) different concentrations and (b) different flow rates of mineralization solution. The horizontal bars within boxes represent median and the point represent leachable Cd

concentrations at different parts. The tops and bottoms of boxes represent 75th and 25th quartiles, respectively. The upper and lower whiskers represent the maximum and minimum, respectively. The longer boxes represent a relative lower uniformity of the mineralization, otherwise indicate uniform mineralization.

3.2.2 Flow rate of mineralization solution

Fig. 4b compared the leachable Cd concentrations at the upper, middle, and lower parts of the biomineralized-tailings with different flow rates of mineralization solution. The results displayed that the 1.5 mL min^{-1} treatment led to relatively uniform mineralization (SD value of 0.03 μg L^{-1}). However, treatment with the lower (0.5, 1.0 mL min^{-1}) or higher (2.0 mL min^{-1}) flow rates showed a low uniformity of the mineralization which was indicated by their larger SD values (0.22, 0.10, 0.09 μg L^{-1}, respectively, > 0.05 μg L^{-1}). When the flow rate was increased to 2.5 mL min^{-1}, more leachable Cd concentration was detected. A similar pattern of results was obtained by Qabany et al. (2012)[41] which showed that the efficiency of MICP decreased at the higher flow rate compared with that of lower. This may be due to the fact that flow rate of the mineralization solution is associated with the transfer of the mineralization solution and mineralized precipitates: a high flow rate can deliver the effective constituent and precipitates to further locations (to the lower parts or out of the column); whereas, low flow rate leads to a contrary result [18,42].

3.3 Cd leaching tests under different mineralization cycles

Nine cycles of mineralization were carried out with the optimal parameters and the variations in TCLP leachable Cd concentration and $CaCO_3$ increment with the number of mineralization cycles were demonstrated in Fig.5a and b. In addition, previous studies have confirmed that Cd can co-precipitate with Ca in MICP [12,43], so a relationship between TCLP leachable Cd concentration and $CaCO_3$ increment was established (Fig. 5c).

While the leachable Cd concentration at different number of mineralization cycles (Fig. 5a) was fitted using the logistic function, a linear straight line was used to fit the $CaCO_3$ increment at different number of mineralization cycles (Fig. 5b). The results

showed that, the leachable Cd concentration decreased significantly as the increasing of the cycles of mineralization, while $CaCO_3$ increment increased. When the number of mineralization cycles ranged from 0 to 7, the TCLP leachable Cd concentration decreased 80.7% (from 3.11 to 0.60 μg L^{-1}), resulting an inverse sigmoidal shape curve. The increment of $CaCO_3$ content increased by 6.19863 g kg^{-1} for each cycle of mineralization, resulting in an approximately linear correlation. The final pH of tailings before and after biomineralization was alkalescent (Table S1). In addition, as the $CaCO_3$ increment increased, the leachable Cd concentration of the tailings decreased, resulting an inverse sigmoidal shape curve. This is expected and consistent with previous studies, because soluble Cd precipitated along with soluble calcium and showed a positive correlation which may due to the co-precipitations [11].

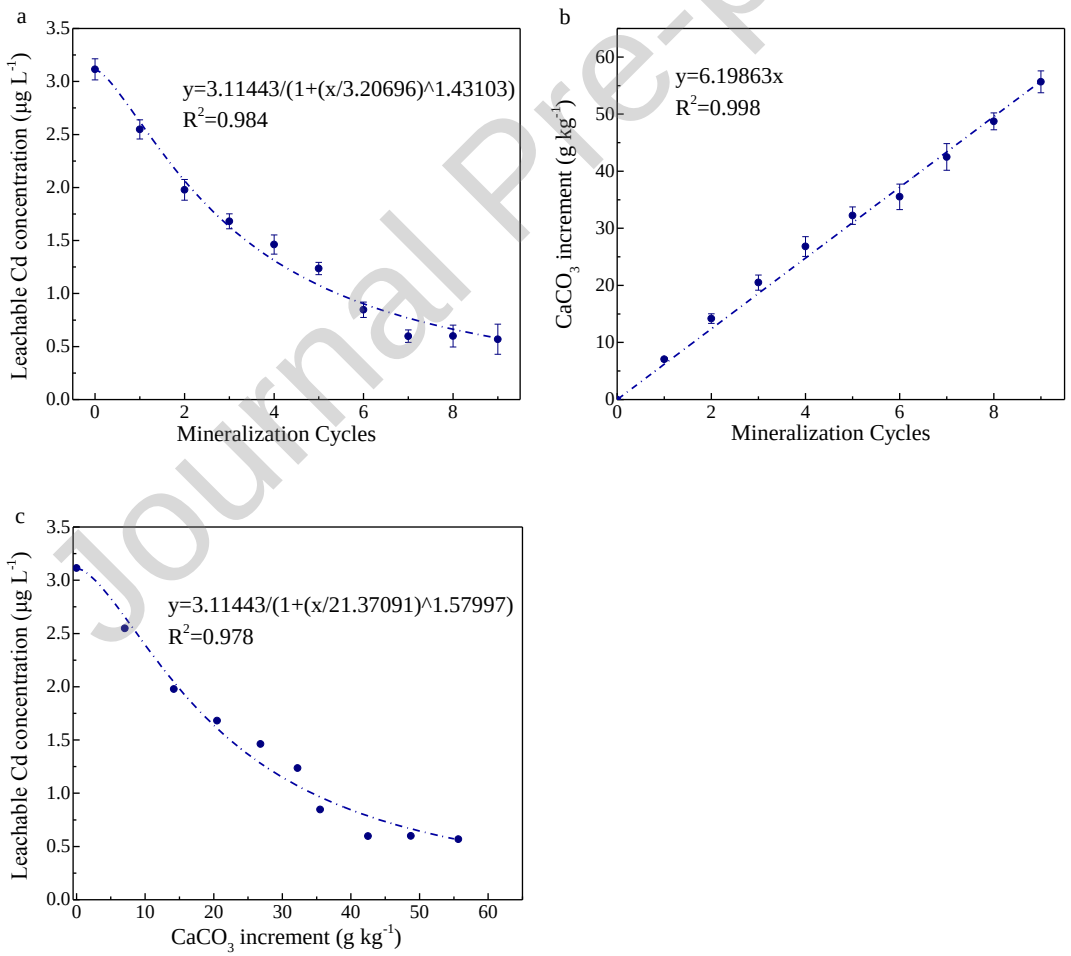


Fig. 5. (a) Variations in TCLP leachable Cd concentration with the number of mineralization cycles. (b) Variations in $CaCO_3$ increment with the number of

mineralization cycles. (c) Relationship between TCLP leachable Cd concentration and $CaCO_3$ increment. Error bars indicate the standard deviation of triplicate samples.

3.4 Biomineralization mechanisms

3.4.1 Bacteria adhered to tailings

Under the bacterial suspension concentration of 1×10^8 cfu mL^{-1} and retention time of 3 hours, the mineralization after the injection of mineralization solution was uniform, which was consistent with the uniform distribution of the bacterial suspensions. In *step 2*, the injection of mineralization solution did not affect the distribution of bacteria in tailings. It is thus rational to hypothesize that bacteria can adhere to the tailings for mineral production, which was verified by the FTIR and SEM analysis.

Fig. 6a-b shows the FTIR spectra of tailings, pure cell of bacteria, and a mixture of bacteria adhered on tailings. The functionalities of each sample revealed by FTIR enable to evaluate the adsorption of bacteria on the mineral. Compared to pure tailings, it can be seen that the characteristic peaks at 2961 cm^{-1} and 2874 cm^{-1} of the pure cell of bacteria which were associated to the asymmetric -CH_3 stretching [44] appeared on the mixture of bacteria adhered on mineral at 2965 cm^{-1} and 2876 cm^{-1}.In addition, peaks at 2921 cm^{-1} and 1619 cm^{-1} of original tailings existed blue-shifts of 10 and 18 cm^{-1} in the asymmetric -CH_2 stretching and C-N/N-H component of amide II bonds, respectively[45] after bacteria adhered. The symmetric vibrations of COO^- from carboxylate at 1423 cm^{-1} of the original tailings existed red-shift of 5 cm^{-1}[29] after bacteria adhered. These results indicate that -CH_3, -CH_2, C-N/N-H and COO^- that from the bacteria mainly played important roles in the adsorption, especially the -CH_3. In addition, it is also obvious that bacteria were adsorbed on the mineral surface of the tailings through the SEM analysis (Fig. 6c).

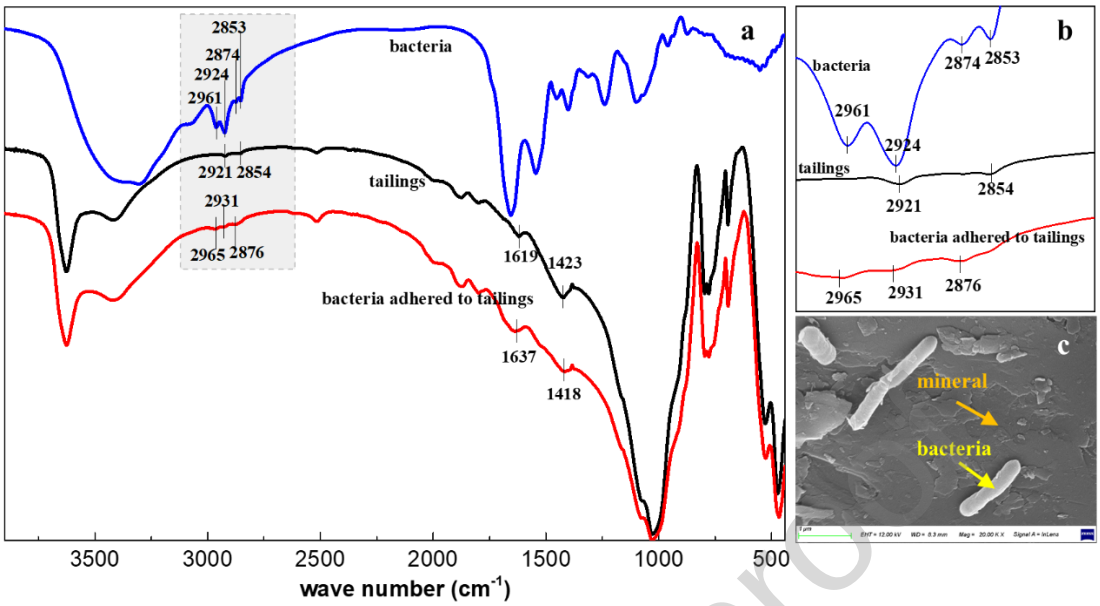


Fig. 6. (a and b) FTIR spectra of bacteria, tailings before and after adsorption; (c) SEM image of bacteria adhered to mineral.

3.4.2 Mineralization of the tailings induced by bacteria

The XRD indicates that major mineralogical phases in the original tailings include calcite, quartz, illite, dolomite, and kaolinite (Fig. 7), and similar mineral compositions of tailings were found in previous study [46]. In addition, SEM-EDX revealed the presence of elements including cadmium (Cd), copper (Cu), iron (Fe), and sulfur (S), which may be greenockite or otavite, chalcopyrite, pyrite and other mineral phases [24]. After mineralization treatment, $(Ca_{0.67}, Cd_{0.33})CO_3$ which is the solid solution of calcite via the replacing of Ca by Cd were detected in the tailings. Additionally, strong peaks of calcite indicate the formation of large amounts of calcite-$CaCO_3$ after mineralization. This result agrees well with previous studies in aqueous solutions [12,43]. It is worth noting that the $CaCO_3$ precipitated by MICP could be stable even under simulated acid rain of pH 3.5 [47].

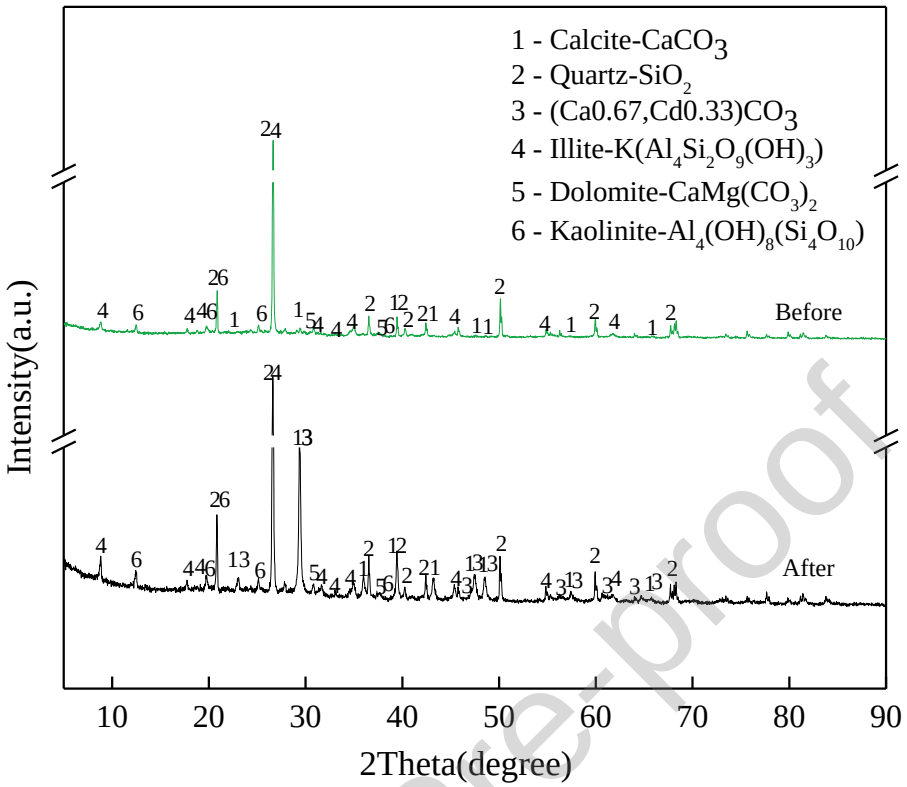


Fig. 7. XRD analysis of tailings before and after biomineralization. 10.0 mg/g Cd ($CdCl_2$) was added herein to obtain the obvious XRD results.

Mixtures of bacteria-tailings-products were examined by SEM to further characterize the bacterial behavior and the products formation during the biomineralization process (Fig. 8). It can be seen clearly that, compared with the smooth bacteria (Fig. 6c) before mineralization (without mineralization solution), there are dense crystals formed on the surface of the bacteria due to the mineralization process (with mineralization solution) (Fig. 8a). EDS analysis (Fig. 8g) represented that Ca, C, O are the main element associated with the bacterial surface (point A), indicating that calcareous shell is formed on bacteria. This result implied that bacteria, as heterogeneous nucleation sites which can therefore reduce the activation energy for nucleation [48], induced the formation of precipitates on its surface. This result has also been confirmed by previous studies, which showed that bacteria can be used as nucleation sites in the process of mineralization[46].

The EDX analysis of the unwrapped tailings (Fig. 8h; point B in Fig. 8d)

confirmed the presence of alumino-silicates and sulfide minerals and other mineralogical phases. With the progress of mineralization, the crystals continued to grow on the calcified bacteria, forming even larger calcareous crystals (Fig. 8b-c and 8i). Subsequently, the mineral particles of tailings were gradually wrapped up in the product formation process (Fig. 8d, 8e). In the parts wrapped by mineralized products, only Ca, C, O could be detected by EDS, indicating that the elements of the tailings were covered. In addition, the mineralization products cemented the mineral particles in the tailings (Fig. 8f), resulting in porosity reduction (from 0.407 to 0.298 after 7 cycles of mineralization) (Table S2). These findings are consistent with previous studies on sand soil consolidation which have shown that the cementation of loose sand grain can reduce porosity and permeability [16,33]. This may lead to the reduction of the access of oxygen and water which needed for sulfide oxidation.

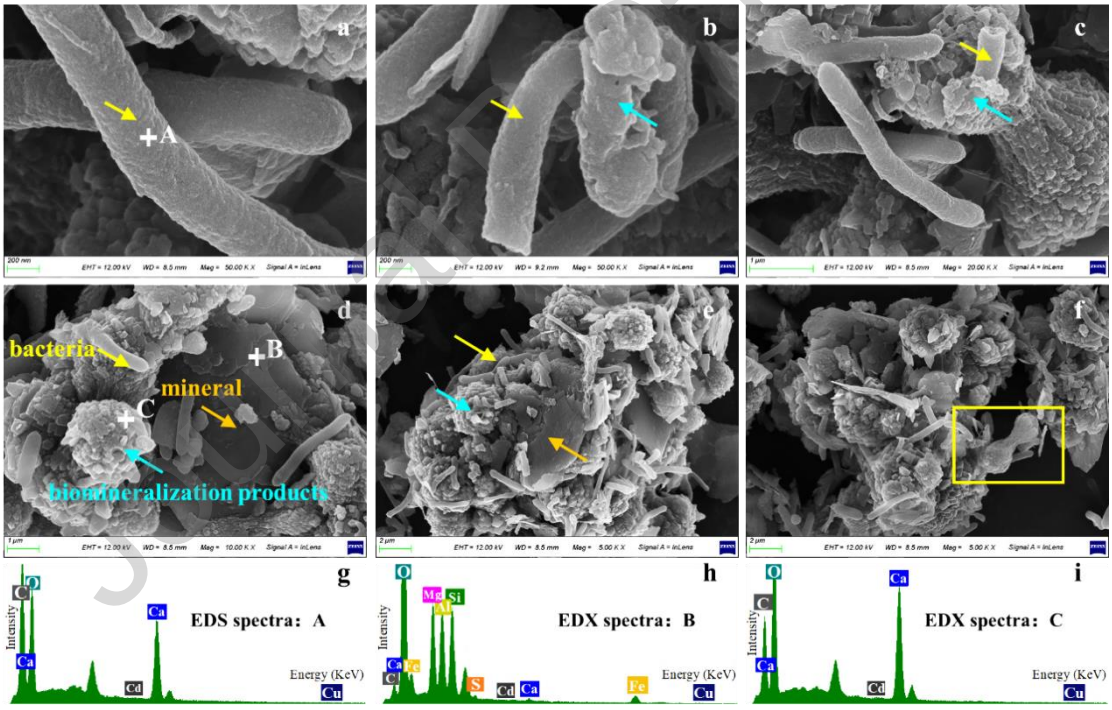


Fig. 8. SEM images (a-f) and DES spectra (g-i) of the biomineralization process induced by bacteria. (a) Biomineralization precipitates form on the surface of bacteria. (b, c) The precipitate on the bacteria grows into larger crystals. (d, e) The mineralized products gradually wrapped the mineral particles in tailings through the induction by the bacteria. (f) The mineralization products cemented the mineral particles in the tailings. (g-i) EDS spectra at the positions of plus sign (A, B, C) respectively.

Fig. 9 presents the SEM images and element mapping analysis of the particles in the tailings before and after biomineralization. It shows that particles in tailings have a smooth surface and some edges and corners before biomineralization (Fig. 9a and 9b). After biomineralization, the surface of particle tailings became rough (Fig. 9f and 9g) due to the coverage of the mineralized products mainly composed of calcite. The phenomenon can be explained by the element mapping images of the Ca which was brighter after biomineralization (Fig. 9j) than before (Fig. 9e). This again confirmed that the formation of calcite roughens the tailings surface. Moreover, the element S and Si of the particles in tailings before biomineralization (Fig. 9c, 9d) were brighter than after (Fig. 9h, 9i), indicating that the calcite densely wrapped the tailing particles and reduced the S and Si exposure.

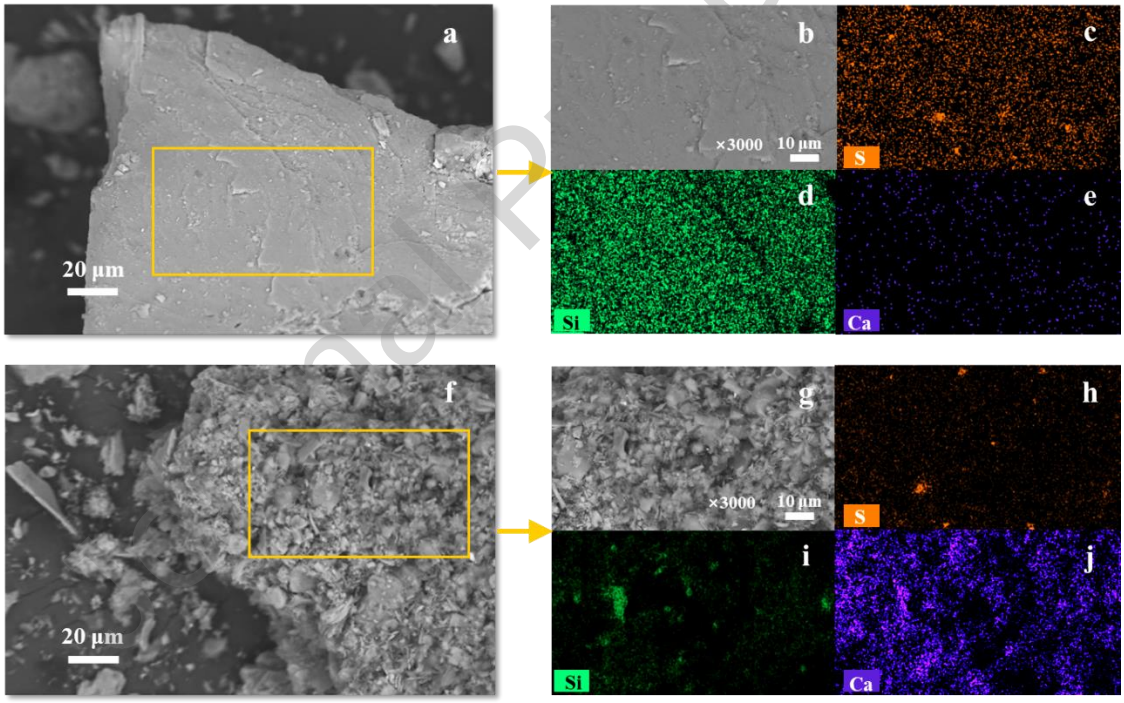


Fig. 9. The SEM images and element mapping analysis of the particles in the tailings before and after biomineralization. (a, b) SEM images before biomineralization. (c, d, e) Element mapping of S, Si, and Ca of the particles in the tailings before biomineralization. (f, g) SEM images after biomineralization. (h, i, j) Element mapping of S, Si, and Ca of the particles in the tailings after biomineralization. The concentration of special element is positive correlation with the brightness. The

sample of tailings after 7 cycles of mineralization was used in this experiment.

To sum up, we believe the overall biomineralization processes of the sulfide tailings based on MICP mainly include: (1) the adsorption of the bacteria by tailings after the bacteria injection; (2) the formation of biomineralization products (to the $(Ca_{0.67}, Cd_{0.33})CO_3$ and calcite phase) on heterogeneous nucleation sites after the injection of mineralization solution and the formation of calcareous shell on bacteria; and (3) the wrapping of the tailings that reduces the sulfur (S) exposure and the cementation of the mineral particles in the tailings along with the growth of larger calcareous crystals on the calcified bacteria. Due to the calcite scale, the access of oxygen and water required for the oxidation of sulfides can be restricted and the acid production of sulfides can be reduced [46]. On the other hand, carbonate precipitation induced by microorganism can resist [47] or neutralize [4] the acid in rainwater or acid from sulfurized tailings. As a result, the release of Cd to the environment will be significantly prevented or delayed.

4. Conclusion

In this study, biomineralization by carbonate-mineralized bacteria was successfully demonstrated for reducing the leaching of Cd from the sulfide tailings into the environment. Our results indicated that the biomineralization relied on concentration and retention time of the bacterial suspensions and concentration and flow rate of mineralization solutions. These critical parameters had significant effects on the mineralization efficiency and uniformity of the heavy metals in sulfide tailings in the column. The TCLP leachable Cd concentration decreased 80.7% after 7 cycles of mineralization, because Cd and Ca were induced to the form of calcite. Tailings particles were eventually wrapped by the mineralization products, resulting in the reduction of tailings exposure (especially sulfur). As such, the release of Cd to the environment will be prevented or delayed. This research presents an efficient strategy for the inhibition of the release of toxic metals from the sulfide tailings, and provides the foundation for future large-scale in situ application.

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Appendix A. Supplementary information

Supplementary material to this article can be found in the file of the Supplementary Material.

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Highlights

- Bacteria induced carbonate mineralization can reduce Cd leaching from tailings.
- Critical parameters affected the mineralization efficiency and uniformity in column.
- The leachable Cd concentration decreased 80.7% after 7 mineralization cycles.
- Cd and Ca were mineralized to the $(Ca_{0.67}, Cd_{0.33})CO_3$ and calcite phase.
- Sulfide tailings were wrapped by products and thus the exposure was reduced.

CRediT Author Statement

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Xi Chen： Formal analysis.

Declaration of interests

☑The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.