

Article

Study on the Combined Behaviour of Montmorillonite and Carbonate Mineralizing Bacteria on Lead Retention and Fixation

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Abstract: Heavy metal pollution management is a global environmental problem that poses a great threat to the ecological environment. Adsorption and biomineralization are considered to be the two most promising heavy metal pollution remediation techniques among the numerous available ones. In this work, a combined heavy metal removal system was constructed employing the adsorption of montmorillonite and the mineralization of carbonate mineralizing bacteria to expand their application potential for controlling heavy metal water pollution. Analysis of changes in pH, CO_3^{2-} concentration, Pb^{2+} concentration in the liquid phase, and changes in microscopic morphology, mineral phase, and functional group on sedimentary minerals was done to study the retention and fixation behaviour of montmorillonite and carbonate mineralizing bacteria on Pb^{2+} . The results show that the liquid phase pH may be slightly altered by the ions dissolved in montmorillonite. Based on the conditions of montmorillonite and carbonate mineralizing bacteria functioning separately, the elimination of Pb^{2+} may reach 35.31% and 45.75%, respectively. However, when montmorillonite is combined with carbonate mineralizing bacteria, which is the heavy metal removal system constructed in this study, montmorillonite can buffer part of the Pb^{2+} rapidly and reduce its biotoxicity. Subsequently, these buffered Pb^{2+} are gradually desorbed by carbonate mineralizing bacteria and removed by the effect of mineralized deposition. Results from SEM, FTIR, and XRD indicate that Pb^{2+} is primarily removed from the liquid phase as rod-shaped PbCO_3 . It is worth noting that this process is able to significantly increase the removal of Pb^{2+} up to 90.06%. In addition, the presence of carbonate mineralizing bacteria can increase montmorillonite's desorption rate to over 81%, greatly enhancing its capacity for reuse. Therefore, our research work contributes to expanding the potential of montmorillonite and carbonate mineralizing bacteria in the treatment of heavy metal-polluted waters.

Keywords: biomineralization; carbonate mineralizing bacteria; montmorillonite; adsorption; heavy metals; retention and fixation behaviour



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1. Introduction

Lead (Pb), a heavy metal with a high ecological threat, is found in the environment largely in the form of divalent compounds [1]. The nuclear industry, the petrochemical industry, mineral mining and smelting, coal combustion, metal plating, etc., are all significant anthropogenic sources supplementary to natural sources such as the weathering and dissolution of rocks and minerals [2,3]. It is worth noting that lead is highly bioavailable, which means that it is easily absorbed and collected by organisms. Nonetheless,

this bioaccumulation effect can exert a certain level of toxicity on biological organs and tissues [4,5]. Lead in the blood, for instance, has a significant detrimental influence on adults' cardiovascular, neurological, and internal organs once a certain concentration level is reached. Similarly, it also has a bad impact on children's cognitive capacities, physical development, and hearing levels [6–11]. Furthermore, lead is an important trigger for cell carcinogenesis; although they do not directly cause cancer, they are a mutagen for cancer development [12–14]. Early studies have shown a strong association between the development of lung cancer and chronic exposure to lead [15,16]. For example, Anttila [17] et al. found that the probability of developing lung cancer was eight times greater in those with blood lead levels at or above 1.0 μM than in the general population. Therefore, the treatment of lead pollution is very critical work.

Adsorption has been identified by a variety of researchers as an effective pollution management technique for the treatment of diverse heavy metal-contaminated wastewater for years of effort [18–20]. In their extensive research endeavours, researchers have discovered an array of natural adsorbent materials with excellent capabilities. One of the minerals of interest is montmorillonite (MMT), which is formed through the weathering of volcanic ash, boasting both a large specific surface area and superior cation exchange capacity while also being widely and affordably available [19,21–23]. In early research work, researchers found that montmorillonite performed well in the removal of toxic metals such as Cu, Fe, As, Cr, Co, Pb, Ni, Zn, Cd, Mn, etc. [21]. For example, Kahkha [24] et al. reported that sodium montmorillonite with appropriate modification treatment could remove almost total Cd, Zn, Pb, and Ni from wastewater; Essebaai [25] et al. found that the adsorption capacity of montmorillonite for Cr(III) could reach 7.5 mg/g in laboratory conditions. Apart from adsorption, biomineralization has also been widely researched for the treatment of heavy metal pollution [26,27]. Some studies have shown that practically all microorganisms have the capacity to biomineralize, and their presence is crucial for the emergence of natural minerals [28,29]. Recently, mineralizing microorganisms have been found to convert various toxic and harmful heavy metal ions (e.g., Cd, Pb, Zn, etc.) in their surroundings from ionic to solid compounds under the appropriate environment, which significantly reduces their transportability and bio-availability in the ecosystem, resulting in a reduction in their ecological threat to the environment and organisms [30–32]. In addition, the operation, cost, ecology, and efficiency of this biomineralization process are also very favourable, allowing for its widespread use in a variety of disciplines, such as the remediation of heavy metal contamination [26,33–35]. Among numerous mineralizing microorganisms, carbonate mineralizing bacteria have demonstrated excellent removal efficacy for various heavy metal ions, making them a promising candidate for the treatment of heavy metal pollution [36]. Some examples include the removal of Zn, Pb, and Cd up to 94.83%, 98.71%, and 97.15%, respectively, by carbonate mineralizing bacteria isolated from calcareous soil environments and the removal of Cd and Ni up to 96% and 89%, respectively, by carbonate mineralizing bacteria screened in farm soil environments [30,37].

The combined remediation of heavy metal pollution by montmorillonite and carbonate mineralizing bacteria has rarely been reported, despite the fact that numerous research works have focused on the modification of montmorillonite to improve the adsorption efficiency of heavy metals and the remediation effect of various carbonate mineralizing bacteria on heavy metal pollution in various environmental factors. Therefore, this study aims to construct a composite system of montmorillonite and carbonate mineralizing bacteria using the rapid adsorption and buffering effect of montmorillonite and the efficient fixation effect of carbonate mineralizing bacteria to explore its retention and fixation behavior and removal effect on Pb^{2+} , as well as to preliminarily evaluate the application potential of the composite system of montmorillonite-carbonate mineralizing bacteria (MMT-CMB) in the treatment of heavy metal pollution.

2. Materials and Methods

2.1. Montmorillonite and Its Purification

The Altai region in Xinjiang provided the montmorillonite utilised in the research. It was purified by continuously stirring and centrifugation in a deionized water solution before the experiments. After being purified, the samples were dried in a drying oven (DHG-9140A Shanghai, China) at 60 °C before being ground to 200 mesh [38,39].

2.2. Experimental Strains and Cultures

The strain used in this experiment is *Klebsiella* sp. CRR1-81_WR13A, which was selected from the purple soil of the Sichuan basin in China. The medium for selecting and cultivating carbonate mineralizing bacteria consists of the following components: urea (20 g/L), protein peptone (10 g/L), and NaCl (5 g/L). All of the aforementioned ingredients, with the exception of urea, were combined with deionized water for the cultivation of microorganisms. The pH was subsequently corrected to 7.0. The urea solution was added to the medium via a water filtration membrane (0.22 µm) after being sterilised and cooled. Finally, the experimental bacterial strain was inoculated into the medium at a volume ratio of 1:100 (v:v) and cultured at 30 °C in a shaking incubator (ZWY-211C, Zhicheng, Shanghai, China) at 120 rpm for subsequent experiments.

2.3. Pb²⁺ Retention and Fixation by Montmorillonite and Carbonate Mineralizing Bacteria

For the experiment, protein peptone (10 g/L), NaCl (5 g/L), and Pb²⁺ 500 mg/L (Pb(NO₃)₂ 0.7993 g) were weighed in deionised water. Urea was then added using the above-mentioned procedure after the mixture had been autoclaved and cooled. At the same time, montmorillonite (20 g/L) was added to the medium, and the experimental group was inoculated with the carbonate mineralizing bacteria reaching the logarithmic growth phase at a volume ratio of 1:100 (v:v), while the control group remained non-inoculated state. Finally, they were placed in a constant temperature shaking incubator (at 30 °C, 150 rpm).

During the experiment, samples were collected from the medium at 0 h, 2 h, 1 d, 2 d, 3 d, 5 d, 7 d, 15 d, and 30 d and were centrifuged at 4000 r/min for 1 min. Firstly, the Multiparameter tester (Seven Excellence, Mettler Toledo, Greifensee, Switzerland) was used to test the pH of the supernatant; secondly, Pb²⁺ concentrations of the supernatant were measured by inductively coupled plasma optical emission spectroscopy (ICP-OES ICAP6500, Thermo Fisher Scientific, Waltham, MA, USA), with a detection limit of 10⁻⁹–10⁻⁶ g/L; finally, the CO₃²⁻ concentration in the liquid phase was determined by titration (DZT 0064.49-1993). Moreover, the precipitate obtained after centrifugation was washed three times with deionised water and dried in a drying oven at 60 °C. Subsequently, Pb²⁺ dissolved by ion exchange was determined via the method described above.

In the end, appropriate samples were collected and processed by drying and grinding (100 mesh). Following this, the samples were examined by X-ray Diffraction (XRD, X'Pert PRO, PANalytical, Almelo, Netherlands) in the range 3–80°, and the data were analysed by Jade 6.0 to determine the change in mineral phases before and after the experiment; Fourier transform infrared spectroscopy (FTIR, Nico-let5700, Thermo Electron Corporation, Waltham, MA, USA) was used to record and analyse the changes in mineral functional groups over the range 4000–400 cm⁻¹ before and after the experiment. In addition, a scanning electron microscope (SEM, Ultra55, Zeiss, Oberkochen, Germany) was used to determine and analyse the microscopic morphology of the sedimentary minerals.

2.4. Analysis of Data

The rate of pH change (n_1), Pb²⁺ removal rate (n_2), Pb²⁺ removal rate (n_3), montmorillonite retention rate (n_4) and carbonate mineralizing bacteria mineralization rate (n_5) were calculated during the experiment as follows:

$$n_1 = \frac{\Delta_{pH}}{\Delta_t} = \frac{pH_{t_2} - pH_{t_1}}{t_2 - t_1} \quad (1)$$

$$n_2 = \frac{\Delta C}{\Delta t} = \frac{C_{t_2} - C_{t_1}}{t_2 - t_1} \quad (2)$$

$$n_3 = \frac{C_0 - C_1}{C_0} \times 100\% \quad (3)$$

$$n_4 = \frac{C_0 - C_2}{C_0} \times 100\% \quad (4)$$

$$n_5 = 1 - n_1 - n_2 \quad (5)$$

where Δt is the unit time of action, Δ_{pH} is the change in pH per unit time, ΔC is the change in Pb^{2+} concentration per unit time, C_0 is the initial Pb^{2+} concentration in the liquid phase, C_1 is the Pb^{2+} concentration in the liquid phase during the experiment, and C_2 is the Pb^{2+} concentration adsorbed by montmorillonite in Section 2.2.

3. Results

3.1. Analysis of pH Changes

The variation of liquid phase pH is shown in Figure 1. It is visually observable that the pH of the liquid phase increases to varying degrees throughout the entire reaction (Figure 1a), whether carbonate mineralizing bacteria is added or not. Meanwhile, all samples show an increase in the pH of the liquid phase to around 7.9 at 1 d. The pH becomes stable when montmorillonite works alone, while it shows an increasing trend from 1–7 d when montmorillonite and carbonate mineralizing bacteria work together and stabilizes at around 8.54. This means that montmorillonite has a moderating effect on pH; it is noteworthy that the rate of pH change (Figure 1b) increases to some extent from 1 d when carbonate mineralizing bacteria is inoculated. Especially from 3 to 7 d, indicating that the effect of montmorillonite on the pH of the liquid phase is primarily reflected in the early stages of the experiment, whereas the effect of carbonate mineralizing bacteria is primarily observed in the middle of the experiment. This is largely caused by the low number of microbial cells in the initial period. However, in earlier research (Figure 1c), it was found that without the addition of Pb^{2+} , the liquid phase pH stabilized at around 9.5 after only 60 h. This stabilization not only occurred more quickly than the combined effect of montmorillonite and carbonate mineralizing bacteria but also resulted in a significantly higher pH level after stabilization. This indicates that the growth of carbonate-mineralizing bacteria may have been affected to a certain extent.

3.2. Analysis of the Variation of CO_3^{2-} Concentration

The concentration of CO_3^{2-} in the liquid phase environment is an effective predictor of the experimental strains' mineralization characteristics. Based on the fact that CO_3^{2-} created by the carbonate mineralizing bacteria is continually mineralized with Pb^{2+} during the mineralization process, the data shown in Figure 2. depict the residual concentration of CO_3^{2-} . Compared to the control group, the concentration of CO_3^{2-} increases rapidly and reaches 105.35 mg/L at 7 d following the inclusion of carbonate mineralizing bacteria, while it increases to 115.03 mg/L slowly later, indicating that the main source of CO_3^{2-} is produced by carbonate mineralizing bacteria. This also means that when microorganisms reach a certain population level, they dramatically enhance their metabolic rate and are able to produce enormous amounts of CO_3^{2-} for mineralization. At the end of the experiment, mineralization deposition is finished, but some CO_3^{2-} is still present in the liquid phase. This may be caused by nutrient restrictions that make it difficult for microbes to continue decomposing urea to produce CO_3^{2-} .

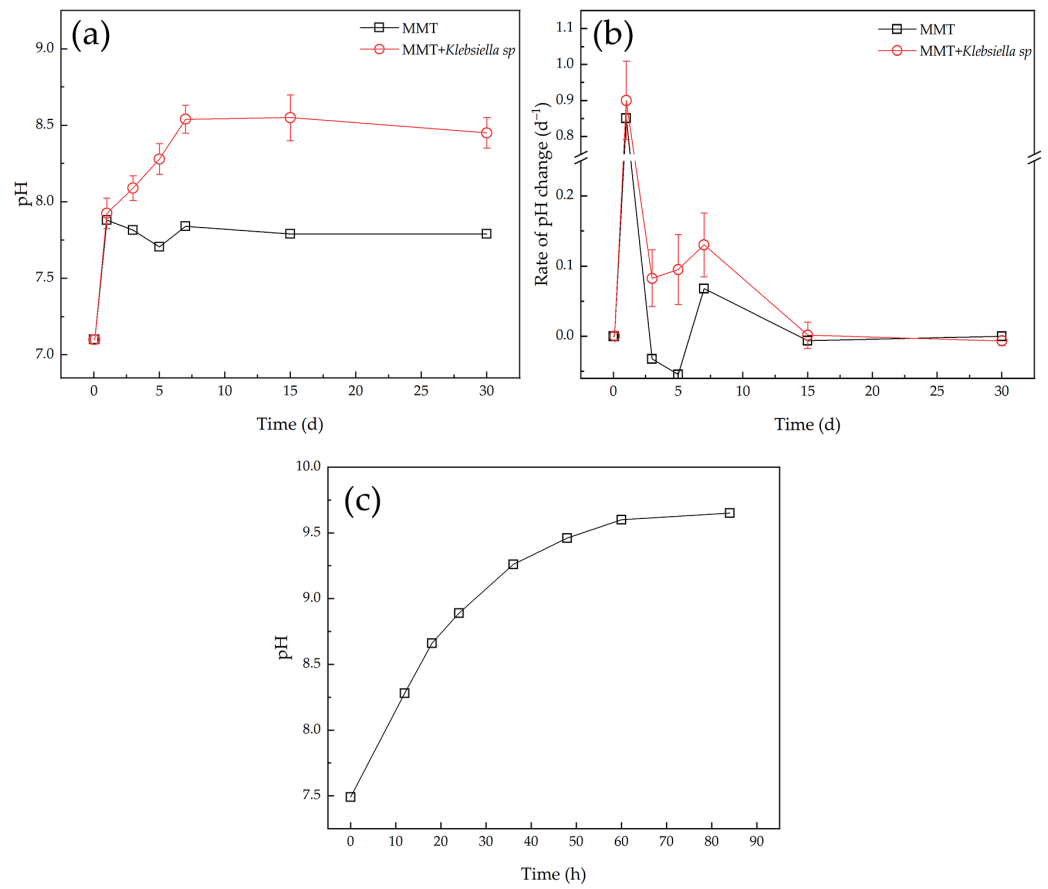


Figure 1. Variation of liquid phase pH (a), rate of pH (b), and variation of liquid phase pH (c) for carbonate mineralizing bacteria without Pb^{2+} in early studies.

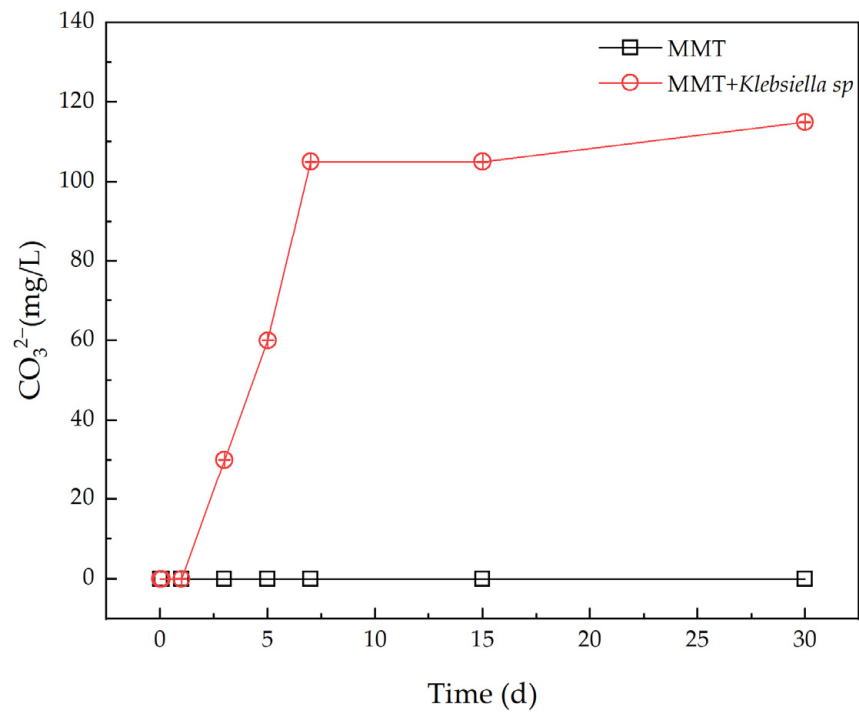


Figure 2. Variation of liquid phase CO_3^{2-} concentration.

3.3. Analysis of the Variation of Pb^{2+} Concentration

The change in Pb^{2+} concentration in the liquid phase environment is the primary factor used to assess the performance of the montmorillonite-carbonate mineralizing bacteria system built for this study. Montmorillonite shows a moderate effect in removing Pb^{2+} , while the addition of carbonate mineralizing bacteria improves its removal efficacy, as seen in Figure 3a. Due to the flocculation of organic material in the medium, the initial concentration of Pb^{2+} determined is 327.4 mg/L, which is lower than the initial concentration of 500 mg/L planned for the experiment. At this initial concentration, when working alone, montmorillonite makes the concentration of Pb^{2+} gradually reduce and stabilise at around 216.40 mg/L after 7 d. However, when both montmorillonite and carbonate mineralizing bacteria are present, the Pb^{2+} concentration can be significantly reduced to 32.52 mg/L. The experimental procedure (Figure 3b) shows that the highest rate of Pb^{2+} adsorption by montmorillonite, which reached 815.43 $mg/L \cdot d^{-1}$, was achieved at 2 h, regardless of whether or not carbonate mineralizing bacteria were included. This is the highest rate of Pb^{2+} adsorption observed for montmorillonite. Furthermore, the addition of carbonate mineralizing bacteria results in a rapid decrease in the concentration of Pb^{2+} within 0.083-7d. Meanwhile, the removal rate of Pb^{2+} is just 35.31% in the control group, and it has been progressively declining since 15 d of the experiment. However, the removal rate of Pb^{2+} in the montmorillonite-carbonate mineralizing bacteria system keeps increasing, and the removal rate of Pb^{2+} is up to 90.06% at 30 d. However, previous research (Figure 3c) had shown that when the initial Pb^{2+} concentration was at 414.2mg/L, the removal rate of Pb^{2+} was less than 50% after 168 h of the only action of carbonate mineralizing bacteria. However, during the same time frame, the addition of montmorillonite to the combination of carbonate mineralizing bacteria resulted in an 80% removal rate of Pb^{2+} , indicating a significant enhancement in Pb^{2+} removal rate with the addition of montmorillonite.

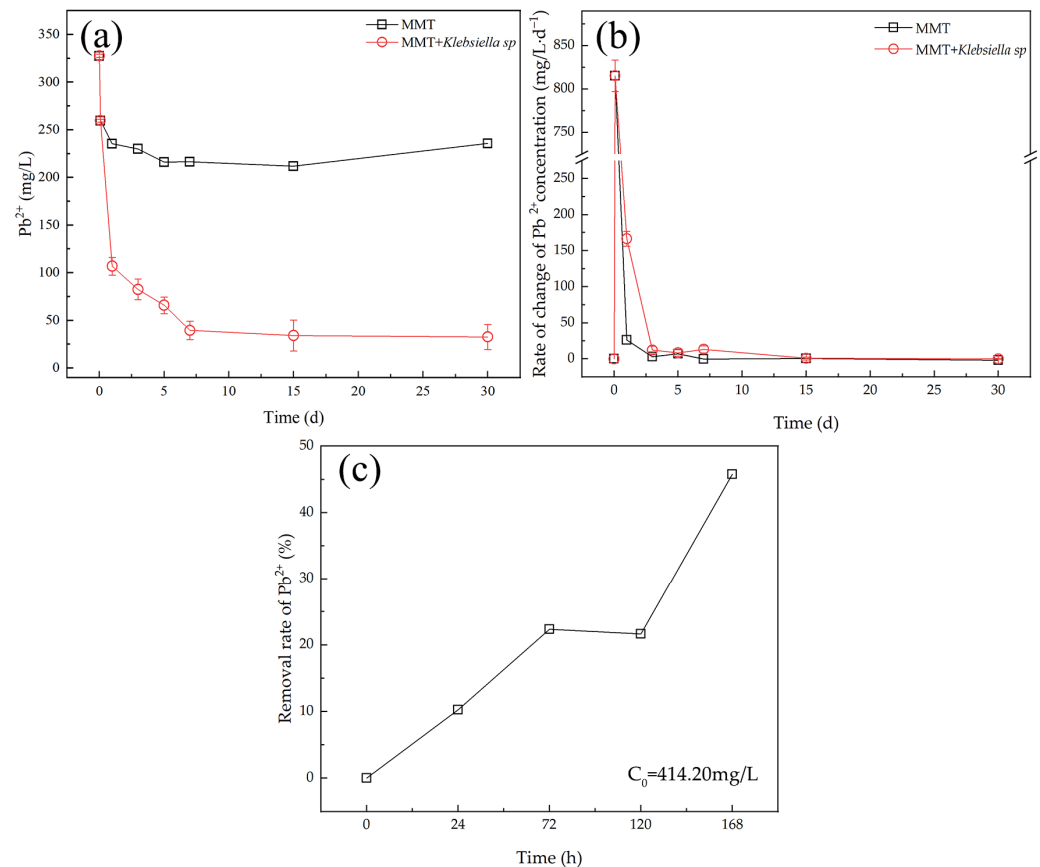


Figure 3. Variation of Pb^{2+} concentration (a), rate of change of Pb^{2+} concentration (b), and the trends in Pb^{2+} removal rate (c) under the action of carbonate mineralizing bacteria in early studies.

3.4. Analysis of the Variation of Pb^{2+} Fugitive Content in MMT-CMB Systems

In the montmorillonite-carbonate mineralizing bacteria system constructed in this study, Pb^{2+} can be divided into three components: the residual component in the liquid phase, the retained component in montmorillonite, and the mineralized component in carbonate mineralizing bacteria. Figure 4 shows the variation of Pb^{2+} amounts in each fraction with time. In terms of the overall effect, the retention effect of montmorillonite on Pb^{2+} is markedly lower than the mineralizing bacteria, which means that carbonate mineralizing bacteria dominate the removal process of Pb^{2+} . Furthermore, the amount of Pb^{2+} remaining in the liquid phase and the retention of montmorillonite gradually decreased as the experiment progressed, while the proportion of Pb^{2+} mineralized by carbonate mineralizing bacteria showed a gradual improvement. The components of the residual, the retained, and the mineralized are 2.69%, 85.29%, and 12.02%, respectively, at 7 d, which means that part of the retained fraction of montmorillonite is transferred to the mineralized fraction by carbonate mineralizing bacteria. This indicates that the carbonate mineralizing bacteria can not only remove Pb^{2+} from the liquid phase but also desorb most of the Pb^{2+} adsorbed by montmorillonite and remove it by mineralization deposition.

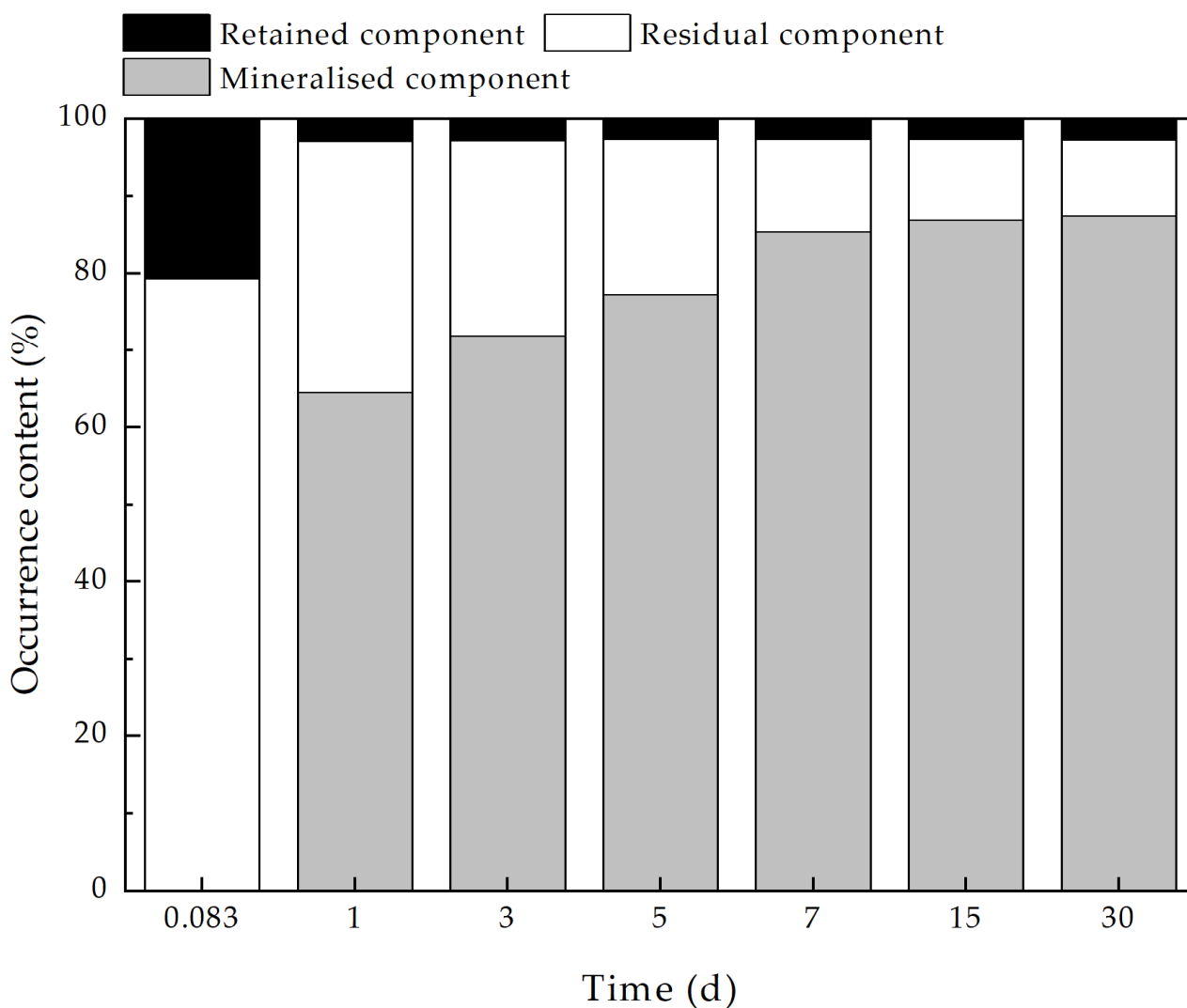


Figure 4. Variation in Pb^{2+} fugitive content in the montmorillonite-carbonate mineralizing bacteria system.

3.5. Analysis of Sediment SEM in the MMT-CMB System

The SEM patterns of the sediments after the work are shown in Figure 5a–c. Compared to the control group, without the action of carbonate mineralization bacteria (Figure 5a), some bars and grains of crystals appear in the sediment (Figure 5b,c), with a size of 1–3 μm , and the surface of these crystals is relatively smooth. This result is relatively consistent with our earlier study on the mineralization products of carbonate mineralizing bacteria (Figure 5d).

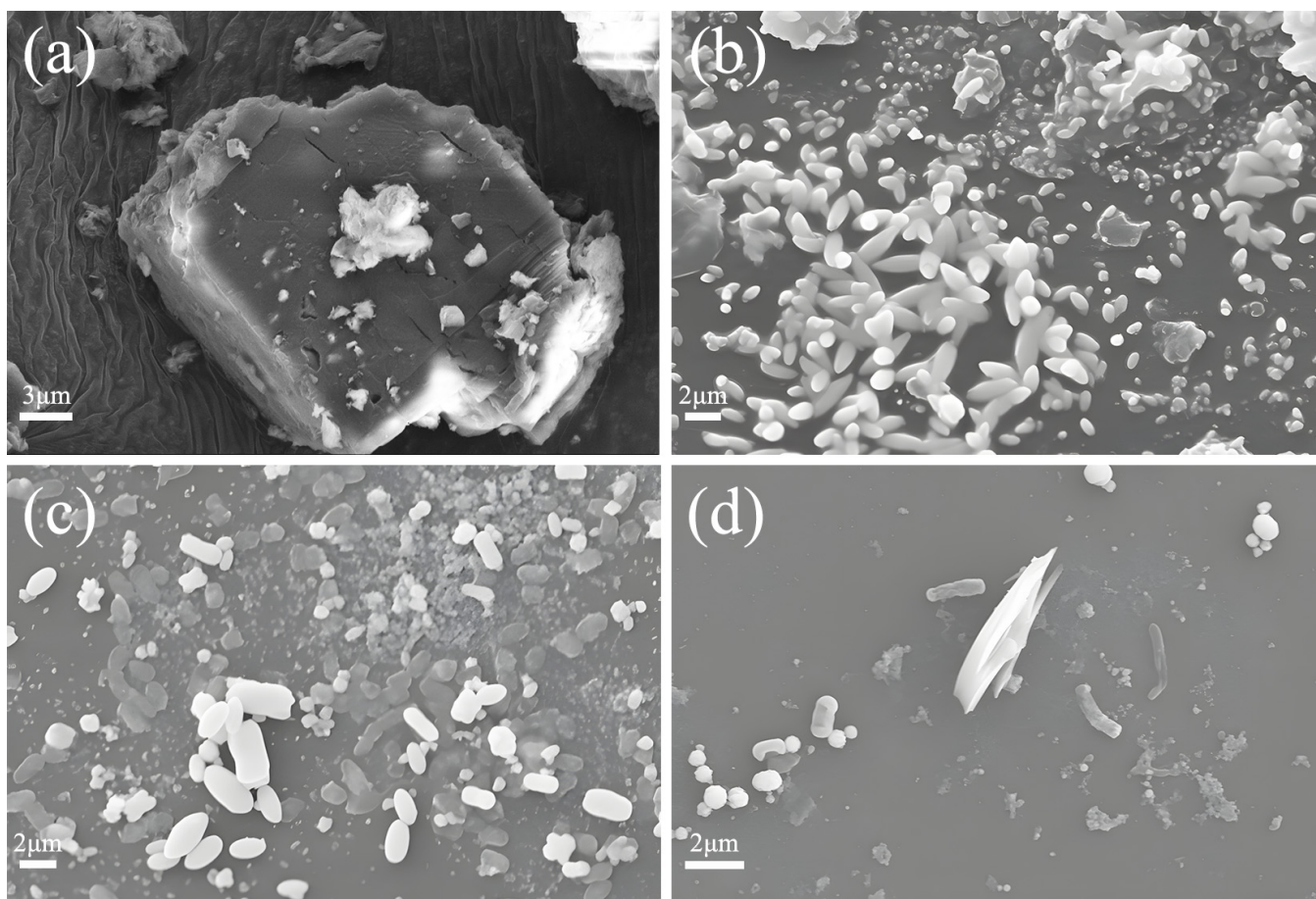


Figure 5. SEM profiles of sedimentary minerals. (a) SEM profile of sedimentary minerals from the control group; (b,c) SEM profiles of sediments from the montmorillonite-carbonate mineralizing bacteria system; (d) SEM profile of products from earlier studies of the interaction of carbonate mineralizing bacteria with Pb^{2+} .

3.6. Analysis of FTIR and XRD Profiles of Sedimentary Minerals

3.6.1. FTIR Analysis

FTIR of the deposited minerals (Figure 6a) shows that, compared to the control group, new absorption peaks appear around 1472 cm^{-1} , 850 cm^{-1} , and 798 cm^{-1} for the experimental montmorillonite minerals, corresponding to the anti-symmetric stretching vibration absorption peak, the out-of-plane bending vibration absorption peak and the in-plane bending vibration absorption peak for CO_3^{2-} , respectively [40,41].

3.6.2. XRD Analysis

Similarly, the XRD patterns of the sedimentary minerals show that the characteristic diffraction peaks of PbCO_3 are present in the sedimentary minerals after Pb^{2+} has been treated with montmorillonite and carbonate mineralizing bacteria. This result further

indicates that the Pb^{2+} in solution has been converted to carbonate by the carbonate mineralizing bacteria and then removed from the liquid phase.

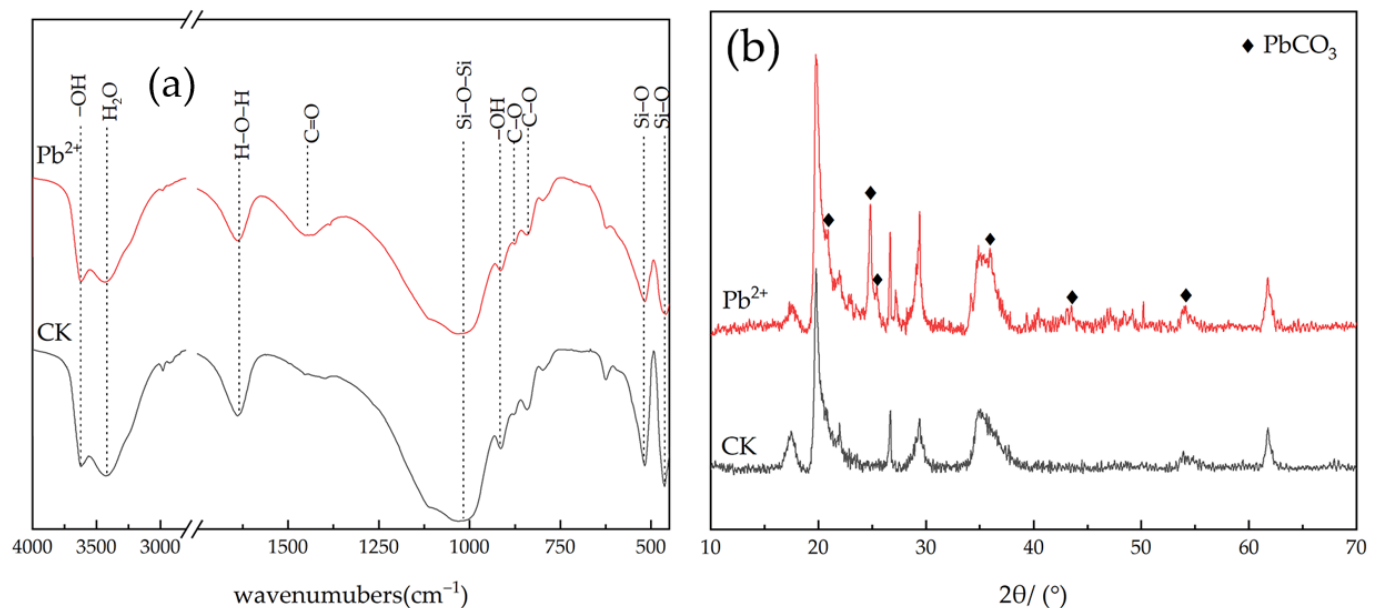


Figure 6. FTIR (a) and XRD (b) profiles of sedimentary minerals.

4. Discussion

4.1. Montmorillonite Structure and Adsorption Properties

Montmorillonite is a typical dioctahedral structure, which contains a quantity of Na^{2+} , Mg^{2+} , Al^{3+} , Fe^{3+} , Ca^{2+} and other cations inside [42–44]. They have a certain hydrophilicity and are capable of hydration, as well as hydrolysis in an aqueous environment. When montmorillonite enters a water environment, the cations contained within it are able to enter the aqueous environment. Due to the hydrolysis of the cations, especially the Al^{3+} , some amount of hydroxide can be released, making the water alkaline. As shown in Figure 1a of this study, the addition of montmorillonite raises the pH of the liquid environment to around 7.79.

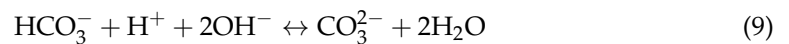
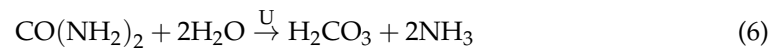
The isomorphous substitution of ions occurs on the tetrahedra and octahedra in montmorillonite; for example, Al^{3+} can replace Si^{4+} and Mg^{2+} in the tetrahedral and octahedral lamellae, respectively, which gives montmorillonite a permanent negative charge or charge layer and gives it a certain adsorption capacity [18,44,45]. However, under certain conditions, the effectiveness of natural montmorillonite in removing heavy metal ions through adsorption is not very high. Moreover, the distinct chemical properties of heavy metal ions, including their charge and ionic radius, can result in variability in the adsorption effectiveness of montmorillonite for different metal ions. For instance, Zhao [46] et al. found that at an initial concentration of 1.6g/L of montmorillonite and 100 ppm Sr^{2+} concentration, the Sr^{2+} removal rate was only 27.15%. Wang [47] et al. investigated the fixation behaviour of natural Ca-montmorillonite and Na-montmorillonite on heavy metals in tailings, and the results showed that the fixation of Pb^{2+} was around 5% and 50%, respectively. Similarly, in this study, as shown in Figure 3a, under conditions where the initial Pb^{2+} concentration was 327.4 mg/L, the maximum removal rate of Pb^{2+} by montmorillonite was only 35.30%. Hu [48] et al. studied the adsorption properties of montmorillonite in the presence of various heavy metal ions and showed that the adsorption capacity of montmorillonite for Pb^{2+} , Cu^{2+} and Cd^{2+} was $\text{Pb}^{2+} > \text{Cu}^{2+} > \text{Cd}^{2+}$ as a result of electronegativity. Moreover, as shown in Figure 3a, it can be deduced that the concentration of Pb^{2+} in the liquid phase experienced a certain degree of reduction under the influence of montmorillonite. However, the concentration of Pb^{2+} exhibited an

increasing trend from 15 d, indicating that the adsorption of Pb^{2+} by montmorillonite lacks long-term stability and is susceptible to desorption.

4.2. Mechanism of Combined Removal by Montmorillonite-Carbonate Mineralizing Bacteria

In the current study, the combined effects of montmorillonite and carbonate mineralizing bacteria are mostly responsible for the removal of Pb^{2+} , and the main mechanism is shown in Figure 7. Montmorillonite has a large amount of negative charge in the layer sheet, which has a strong adsorption effect on Pb^{2+} in the liquid phase. Meanwhile, montmorillonite has strong ion exchange properties. When montmorillonite enters the water column, the Ca^{2+} and Na^+ contained within it will experience ion exchange reactions with Pb^{2+} , and the interlayer ions are transferred to the liquid phase environment while Pb^{2+} enters the montmorillonite interlayer domain.

At the early stage of the experiment, the contribution of carbonate mineralizing bacteria in the reduction of Pb^{2+} is relatively small due to the poor biomass and delayed metabolic activity of the microorganisms. However, once the population of carbonate mineralizing bacteria reached a certain level, they participated in the process of Pb^{2+} removal rapidly. At this moment, montmorillonite has reached adsorption saturation equilibrium, and the carbonate mineralizing bacteria begin to carry out the following reactions:



Firstly, carbonic acid and ammonia are produced by the decomposition of urea during the development of carbonate mineralizing bacteria (Equation (6)); subsequently, ammonia dissolves in water and transforms into NH_4^+ with the release of OH^- , which results in the liquid phase environment to increase in alkalinity (Equation (7)); then, the HCO_3^- ionised from H_2CO_3 is continuously converted to CO_3^{2-} in an alkaline environment (Equations (8) and (9)), which reacts in the reaction of the CO_3^{2-} with the Pb^{2+} in the environment as well as those adsorbed on the surface of the carbonate mineralizing bacteria to form carbonate precipitates (Equation (10)) [49–51]. During this period, the CO_3^{2-} generated by the above reaction is also continuously combined with the Pb^{2+} adsorbed on the surface of the montmorillonite and then deposited, thus allowing the montmorillonite to be desorbed. However, various functional groups are present in the microorganism and their metabolites, including carboxyl, ammonia, hydroxyl, sulfhydryl and phosphate [52–54]. When they come into contact with montmorillonite, they can indirectly modify the treatment of montmorillonite, causing it to have more adsorption sites and stronger adsorption capacity, thus enhancing the adsorption of heavy metal ions by montmorillonite. Moreover, the increase in pH within the environment also enhances the increased adsorption of metal ions by montmorillonite [55,56]. Hence, at the end of the experiment, the montmorillonite still contained some Pb^{2+} .

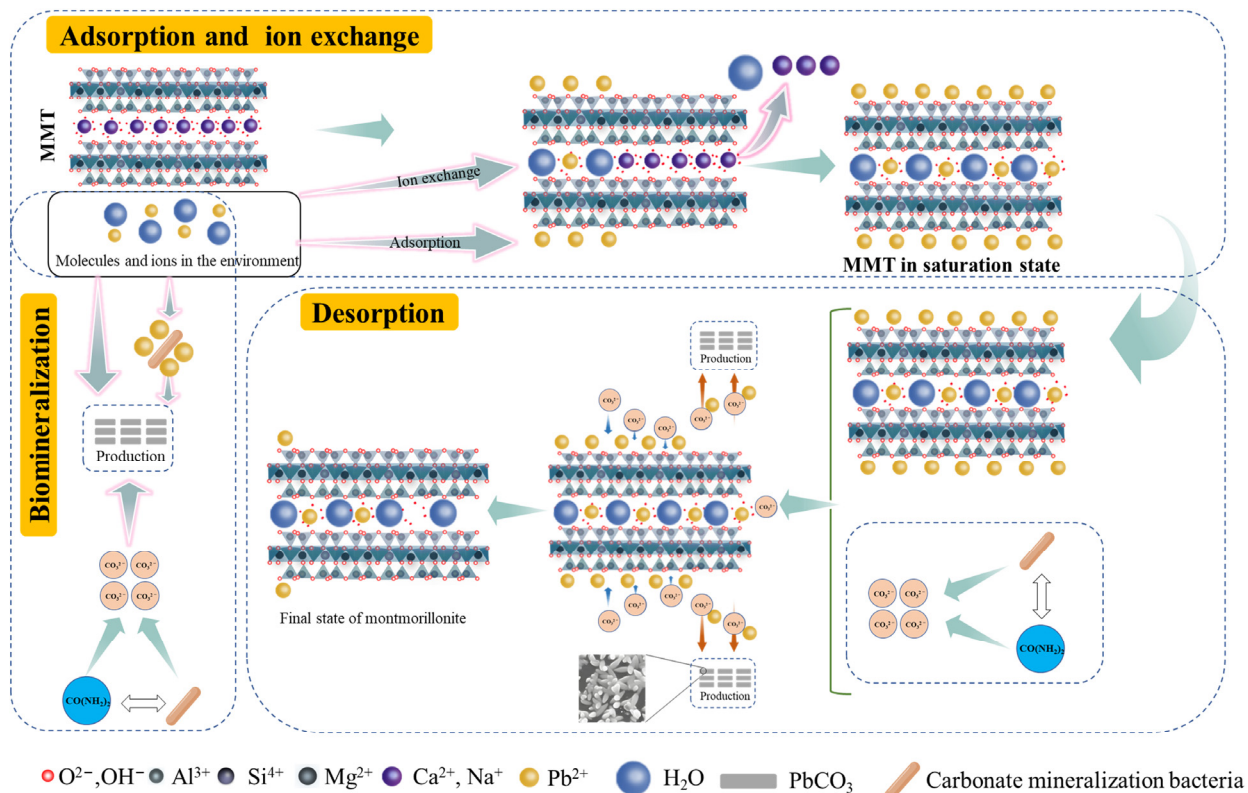


Figure 7. Mechanism of Pb^{2+} removal by montmorillonite-carbonate mineralizing bacteria system.

4.3. The Influence of Montmorillonite on Carbonate-Mineralizing Bacterial Growth

It can be seen from the above results that montmorillonite performs a crucial buffering function in the Pb^{2+} removal experiments. In other words, montmorillonite reduces the concentration of heavy metal ions to a certain extent in a short time through its adsorption and ion exchange. Subsequently, the continuous mineralization and deposition by carbonate mineralizing bacteria reduce the concentration of the Pb^{2+} , while the continuously produced CO_3^{2-} combines with the Pb^{2+} adsorbed by the montmorillonite, converting the Pb^{2+} to PbCO_3 and desorbing the montmorillonite. High concentrations of heavy metal ions (especially Pb^{2+}) have been proven to have certain harmful effects on organisms in a large number of early investigations [50,57]. In previous studies, we found that when exposed to a liquid environment containing Pb^{2+} , the removal rate of Pb^{2+} by carbonate mineralizing bacteria alone was less than 50% (Figure 3c). However, in the montmorillonite-carbonate mineralizing bacteria system, the removal rate of Pb^{2+} reached 90.06%, with a maximum contribution rate of 87.34% by the carbonate mineralizing bacteria (Figure 4). This result confirms that montmorillonite can effectively decrease the biotoxicity associated with Pb^{2+} , highlighting the merits of the montmorillonite-carbonate mineralizing bacteria system in terms of Pb^{2+} removal. Furthermore, compared to the normal growth conditions where carbonate mineralizing bacteria can raise the pH of the culture medium to around 9.5 (Figure 1c), the liquid phase pH in this study only slightly elevates to around 8.5 (Figure 1a). This may be attributed to two factors: the inhibitory effect of Pb^{2+} toxicity on the growth of carbonate mineralization bacteria and the consumption of HCO_3^- due to its transformation to CO_3^{2-} . The SEM profile (Figure 5b,c) illustrates the presence of numerous crystals in the sedimentary minerals, and XRD results (Figure 6b) also indicate the occurrence of PbCO_3 . This further confirms that Pb^{2+} is ultimately transformed from its ionic state to a solid state through the mineralization process of carbonate-mineralizing bacteria and thus removed from the liquid phase environment. This indicates that the montmorillonite-carbonate mineralizing bacteria system constructed in this study allows

for the re-recovery of montmorillonite as well as improving the mineralization performance of the carbonate mineralizing bacteria.

4.4. Performance Evaluation of MMT-CMB System for Heavy Metal Pollution Treatment

Based on the aforementioned research, the system constructed with montmorillonite and carbonate mineralizing bacteria has demonstrated a superior removal effect on Pb^{2+} in comparison to their individual effects. Modification and intercalation of montmorillonite are currently key research areas which have demonstrated a marked enhancement in the adsorption ability of montmorillonite through these processes. For example, Wang [47] et al. found that the removal efficiency of Pb^{2+} and Mn^{2+} increased from about 5% and 8% initially to 80% and 28%, respectively, after montmorillonite was modified by Na_2CO_3 . Ma [55] et al. found that montmorillonite modified with sodium lignosulfonate could increase the adsorption of Pb^{2+} from an initial 40 mg/g to about 52.5 mg/g, while the adsorption of Cu^{2+} increased from an initial 6.5 mg/g to about 7.5 mg/g. It is worth noting that while the montmorillonite modification treatment and intercalation treatment can significantly improve its capacity to adsorb heavy metal ions, both the cost of treatment (as mentioned for sodium lignosulfonate) and the modification treatment process could further increase the cost of heavy metal pollution treatment. Moreover, according to the results of this research, it can be inferred that the long-term stability of Pb^{2+} subsequent to montmorillonite adsorption is relatively inferior. In this study, it was shown that the combined action of montmorillonite and carbonate mineralizing bacteria offers a relatively cost-effective and simpler approach to heavy metal pollution remediation. This approach tackles the risk of environmental re-pollution caused by Pb^{2+} desorption following montmorillonite adsorption by transforming Pb^{2+} into insoluble carbonates. Moreover, the desorption of Pb^{2+} in montmorillonite can enhance its potential for repeated use. Therefore, the utilization of montmorillonite and carbonate mineralizing bacteria for constructing a heavy metal removal system holds promising application prospects. However, considering that the growth of microorganisms and the efficiency of heavy metal ion removal are significantly affected by various environmental factors, such as the co-existence of a large number of other ions in polluted wastewater, which may influence the removal rate of the target ion, this is still an important issue to be addressed in future work. Additionally, the combination of modified montmorillonite or other clay minerals with carbonate mineralizing bacteria to treat heavy metal pollution is another potential direction for further research.

5. Conclusions

In the co-removal of Pb^{2+} by montmorillonite and carbonate mineralizing bacteria, montmorillonite provided an important buffering role, reducing Pb^{2+} concentrations and biotoxicity in a short time. The carbonate mineralizing bacteria can not only contribute to the mineralized deposition of Pb^{2+} in the liquid phase but also release the Pb^{2+} gradually adsorbed by montmorillonite and remove it. In this study, the Pb^{2+} removal rate was only 35.31% and 45.75% for montmorillonite and carbonate mineralizing bacteria, respectively. The composite system using montmorillonite and carbonate mineralizing bacteria could significantly increase the Pb^{2+} removal rate to 90.06% while allowing montmorillonite to be recovered and reused. The results of this study demonstrate significant potential for addressing the problem of heavy metal pollution and thus represent a valuable contribution to the field of environmental research. This study can serve as a useful reference for policy-makers, researchers, and others who are engaged in the search for effective solutions to the problem of heavy metal pollution.

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