The effect of interaction between *Bacillus subtilis* DBM and soil minerals on Cu(II) and Pb(II) adsorption

Jun Bai¹,², Yuanqing Chao¹,³,⁴,*, Yanmei Chen¹, Shizhong Wang¹,³,⁴,*, Rongliang Qiu¹,³,⁴

1. School of Environmental Science and Engineering, Sun Yat-sen University, Guangzhou 510275, China
2. School of Public Health, Southwest Medical University, Luzhou 646000, China
3. Guangdong Provincial Key Laboratory of Environmental Pollution Control and Remediation Technology, Sun Yat-sen University, Guangzhou 510275, China
4. Guangdong Provincial Engineering Research Center for Heavy Metal Contaminated Soil Remediation, Sun Yat-sen University, Guangzhou 510275, China

**Abstract**

The effects of interaction between *Bacillus subtilis* DBM and soil minerals on Cu(II) and Pb(II) adsorption were investigated. After combination with DBM, the Cu(II) and Pb(II) adsorption capacities of kaolinite and goethite improved compared with the application of the minerals independently. The modeling results of potentiometric titration data proved that the site concentrations of kaolinite and goethite increased by 80% and 30%, respectively, after combination with DBM. However, the involvement of functional groups in the DBM/ mineral combinations resulted in lower concentrations of observed sites than the theoretical values and led to the enhancement of desorption rates by NH₄NO₃ and EDTA-Na₂. The DBM-mineral complexes might also help to prevent heavy metals from entering DBM cells to improve the survivability of DBM in heavy metal-contaminated environments. During the combination process, the extracellular proteins of DBM provided more binding sites for the minerals to absorb Cu(II) and Pb(II). In particular, an especially stable complexation site was formed between goethite and phosphodiester bonds from EPS to enhance the Pb(II) adsorption capacity. So, we can conclude that the DBM-mineral complexes could improve the Cu(II) and Pb(II) adsorption capacities of minerals and protect DBM in heavy metal-contaminated environments.

© 2018 The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Published by Elsevier B.V.

**Keywords:**
*Bacillus subtilis* DBM, Goethite, Kaolinite, Cu, Pb

**Introduction**

Heavy metals are natural constituents of the earth’s crust. In recent decades, human activities such as mining, industrial processing, and the use of pesticides and chemical fertilizers have caused widespread heavy metal contamination in soil (Liang et al., 2017a; Liu et al., 2013). As potentially harmful and non-biodegradable pollutants which may accumulate through the food chain, heavy metals can cause serious problems to ecosystems and pose a huge threat to human health (Yang et al., 2018). Copper (Cu) is an important trace element for living things, but has negative impacts on the environment and humans at higher concentrations (Yao et al., 2010). Lead (Pb) is a non-bioessential heavy metal pollutant of environmental concern, and is toxic to living things even at low concentrations (Naik and Dubey, 2013).
The bioavailability and mobility of heavy metals in contaminated soil can affect their entry into the food chain (Luo and Christie, 1998; McBride, 1994). Many remediation techniques have been applied to change the mobility or availability of heavy metals in soils or aqueous solutions (Li et al., 2017b; Tang et al., 2018; Yang et al., 2018). Among these techniques, microbes have been effectively used to immobilize heavy metals in soil in many studies (Achal et al., 2011; Fan et al., 2012; Jiang et al., 2009). However, the heavy metals could cause a decline in soil microbial biomass and change the bacterial community structure (Zhang et al., 2016). Still, there are a few natural microbial strains, including bacteria, which can exist in heavy metal-contaminated environments and employ a variety of protective mechanisms to survive in very high levels of heavy metals without any impact on their growth and metabolism (Naik and Dubey, 2013). They can also change heavy-metal speciation, bioavailability, and mobility by biosorption, organic matter production, or oxidation and reduction reactions (Gadd, 2004). These effects directly limit heavy-metal uptake by plants (Geebelen et al., 2003).

We found in our previous studies that a Bacillus subtilis strain designated “DBM” isolated from a multi-metal-contaminated rice paddy soil might significantly decrease the bioavailability of Cu and Pb in soil by biosorption, bioaccumulation, and conversion of Cu and Pb into more stable forms in heavy metal-contaminated soil (Bai et al., 2014, 2017). In fact, the interactions between bacteria and heavy metals in aqueous solution are not sufficient to explain their actual role in soil. Soil is a very complex system, and bacteria do not exist independently. More than 90% of the total mass of soil solid phase consists of soil minerals, which are also the main inorganic components of soil (Huang et al., 2000). It has been pointed out that 80%–90% of the soil microbes attach to the soil minerals or form mineral-organic complexes (Nannipieri et al., 2003). It is important to study the adsorption of heavy metals by microbes and mineral complexes to gain a further understanding of the mechanism of heavy metal immobilization by soil microorganisms.

Microbial cell surfaces contain a rich array of functional groups such as hydroxyl, carboxyl, and phosphate which can bond with hydroxyl groups on mineral surfaces to form complexes (Yee and Fein, 2003). Extracellular polymeric substances (EPS) contain proteins, polysaccharides and nucleic acids that accumulate on the cell wall (Guibaud et al., 2008). EPS play an important role in the attachment of bacteria and minerals by hydrogen bonds and some other chemical bonds (Fang et al., 2012; Tsuneda et al., 2003). There is still controversy regarding the impact on adsorption capacities of the formation of bacteria–mineral complexes. Some researchers suggested that the formation of the complexes could improve the adsorption capacities of the minerals for heavy metals (Chen et al., 2008; Huang et al., 2005), while some others thought that the formation of the complexes could block some of the surface functional groups and result in reduction of the adsorption capacities (Fang et al., 2010).

Kaolinite and goethite, which are abundant in the subtropical paddy soil of China, were purchased and synthesized, respectively and used as the minerals in the present study. Adsorption, desorption, potentiometric titration and infrared spectrum analysis were used to compare the adsorption of Cu(II) and Pb(II) by Bacillus subtilis DBM, minerals and DBM–mineral complexes. The aim was to understand the effect of interaction between Bacillus subtilis DBM and soil minerals on Cu(II) and Pb(II) adsorption.

1. Materials and methods

1.1. Cell cultivation and preparation of suspended bacteria cells

The bacterium Bacillus subtilis strain DBM was isolated from a multi-metal-contaminated rice paddy soil (Bai et al., 2014). It was grown in a beef extract-peptone medium (beef extract 5.0 g, peptone 10.0 g, NaCl 5.0 g, water 1 L, pH 7.2) for 24 hr (early stationary phase of the strain) in a shaking incubator at 30°C at 160 r/min. Bacterial cells were harvested by centrifugation at 4000×g for 10 min at 4°C, the pellets were washed twice with Milli-Q water, and the cells were resuspended in Milli-Q water to OD_{600} = 1.0.

1.2. Preparation of minerals and DBM–mineral complexes

Kaolinite used in this study was purchased from Sigma-Aldrich (St. Louis, MO, USA). Goethite was synthesized by a modified method according to Atkinson et al. (1967). Briefly, 50 g Fe(NO₃)₃·9H₂O was dissolved in 825 mL deionized water and a 200-mL KOH solution of 2.5 mol/L was added with a speed of 5 mL/min and vigorous stirring to form a suspension with approximate pH 12.0. After aging for 48 hr at 60°C, the precipitated fraction was obtained by centrifugation and washed twice with a pH 9.0 KOH solution and three times with deionized water and 95% ethanol. The mineral sample was screened before use through a 100-mesh sieve after being dried for 24 hr at 110°C.

The purchased kaolinite and synthesized goethite were identified by X-ray diffraction (XRD, Empyrean, PANalytical, Holland) analysis. Diffraction patterns were collected in the angular range from 10 to 80° with a step size of 0.02° and analyzed using Jade 5.0 software (Materials Data, Inc., Irvine, CA) and the JCPSD library of compounds.

The best proportions of bacteria to minerals were obtained from a preliminary experiment (data not shown). Kaolinite (15:1, W/W) and goethite (12:1, W/W) were added to the resuspended cell suspension (OD_{600} = 1.0). The pH of the reaction systems was adjusted to 5.0, which was be chosen according to the soil pH and absorption pH determined in a previous study (Bai et al., 2014), and incubated 2 hr in a shaking incubator at 30°C at 200 r/min. The DBM–mineral complexes were harvested by centrifugation at 8000 r/min for 10 min at 4°C.

1.3. Scanning electron microscope observation

Scanning electron microscopy (SEM) was used in the morphological observation of minerals and DBM–mineral complexes. The SEM observation samples of DBM–mineral complexes were prepared according to the method described by Bai et al. (2017). After plating with Au on a membrane under vacuum, the complex samples and mineral samples were observed by a scanning electron microscope (SEM, Model Quanta 400, FEI, Holland).
1.4. Adsorption and desorption

The suspensions containing DBM-mineral complexes were centrifuged at 8000 r/min for 10 min at 4°C. The precipitates were resuspended in 100 mg/L Cu(II) (Cu(NO_3)_2, AR grade, Sigma–Aldrich, USA) at pH 5.0 or 125 mg/L Pb(II) (Pb(NO_3)_2, AR grade, Sigma–Aldrich, USA) at pH 4.0. All Cu(II) and Pb(II) solutions were prepared in 0.01 mol/L KNO_3 to maintain a constant ionic strength. Adsorption conditions including pH, contact time and sorbent dosages were used according to the previous studies (Bai et al., 2014, 2017). The suspension was incubated at 30°C for 2 hr with shaking at 160 r/min and then centrifuged at 8000 r/min for 10 min at 4°C. The Cu(II) and Pb(II) concentrations in the supernatants were determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 5300 DV, PerkinElmer, USA) to calculate the adsorption capacities of the DBM-mineral complexes. The same masses of minerals corresponding to those in the complexes were also used as sorbents to determine the adsorption capacities.

After the adsorption experiments were conducted as described above, the suspensions were centrifuged and the precipitates were resuspended in one of two desorption reagents: 1.0 mol/L NH_4NO_3 (AR grade, Sigma–Aldrich, USA) or 0.1 mol/L EDTA–Na_2 (AR grade, Sigma–Aldrich, USA). The suspensions were incubated at 30°C for 2 hr with shaking at 160 r/min and then centrifuged at 8000 r/min for 10 min. The Cu(II) and Pb(II) concentrations in the supernatants were determined by ICP-OES.

1.5. Potentiometric titration

An acid–base potentiometric titration of surface functional groups on the sorbents was accomplished using an automatic potentiometric titrator (Metrohm 905 Titrando, Switzerland) according to Yee et al. (2004). A 40-mL suspension of minerals or DBM–mineral complexes in a range of concentrations from 10.3 to 14.5 g/L in 0.01 mol/L KNO_3 was added to the titration cup, and high-purity N_2 was bubbled through it for 30 min at a constant temperature of 25°C. The pH of the titration system was first lowered with 0.1 mol/L HCl to approximately pH 3.0, and after stabilization for 30 min, a calibrated NaOH solution (0.194 mol/L) was used to titrate the reaction system to pH 11.0. All of the titration systems were kept under an N_2 atmosphere during the experiment. As a control, a similar volume of 0.01 mol/L KNO_3 was titrated under the same conditions.

1.6. FT-IR analysis

Prior to analysis by Fourier transform infrared (FT-IR) spectroscopy (EQUINOX 55, Bruker, Germany), freeze-dried samples of minerals or DBM–mineral complexes before and after Cu(II) and Pb(II) adsorption were mixed with KBr powder (1:100, W/W), ground in an agate mortar, and compressed in a hydraulic press. Data for all samples were recorded for wavenumbers ranging from 400 to 4000 cm⁻¹, with a resolution of 4 cm⁻¹ and a scan rate of 0.2 cm/sec.

1.7. Statistical analysis

All experiments were carried out in triplicate, and the reported results represent the mean of three values ± standard deviation. Analysis of variance and Duncan’s multiple range test (DMRT) at p < 0.05 were used to compare treatment means. All the statistical analyses were carried out using SPSS 17.0.

2. Results

2.1. The purity of minerals

The purity of minerals was analyzed by XRD. The results in Fig. 1 show that the diffraction peaks of kaolinite and goethite used in this study were a match with the standard diffraction peaks of kaolinite (JCPDS 01-080-0886) and goethite (JCPDS 00-
29-0713) in the JCPDS library. Thus, the kaolinite and goethite had high purity and could be used for the subsequent tests.

2.2. SEM images of minerals and DBM–mineral complexes

Minerals and DBM–mineral complexes were observed by SEM. As shown in Fig. 2, kaolinite appeared as lamellar crystals and goethite as fine acicular crystals. After combination with the DBM strain, the agglomeration degree of both kaolinite and goethite increased. For kaolinite, DBM was weakly bound on the surface of the lamellar structure. For goethite, however, DBM could not only bind to the surface but also into the gaps of the loose aggregates to form tighter complexes.

2.3. Adsorption and desorption of minerals and DBM–mineral complexes

The metal-binding quantity per unit minerals was used as the adsorption capacity to assess the impact of DBM on the metal adsorption capacities of minerals. The metal adsorption capacities and desorption of DBM, minerals and their complexes are shown in Table 1. Compared with the DBM complexes, kaolinite and goethite showed a very low adsorption capacity toward Cu(II) and Pb(II). The Cu(II) and Pb(II) adsorption capacities of kaolinite were 1.94 and 4.73 mg/g, and the adsorption capacities of goethite were 5.44 and 12.05 mg/g. After combination of DBM with kaolinite, the Cu(II) and Pb(II) adsorption capacities were increased to 2.92 and 7.84 mg/g. However, the adsorption capacities were smaller by 32.72% and 22.15%, respectively compared with the theoretical values. The adsorption capacities of the DBM–goethite complex for Cu(II) and Pb(II) were 5.77 and 12.87 mg/g and were decreased by 32.98% and 29.59%, respectively, compared with the theoretical values.

The desorption rates of kaolinite-adsorbed Cu(II) and Pb(II) were 42.77% and 29.72% by NH4NO3, 46.33% and 33.11% by EDTA–Na2, and the desorption rates of goethite-adsorbed Cu(II) and Pb(II) were 8.51% and 11.63% by NH4NO3, 71.87% and 62.93% by EDTA–Na2. After the combination of DBM with kaolinite, the desorption rates were 47.05% and 62.56% by NH4NO3, 64.80% and 75.87% by EDTA–Na2. For the DBM–goethite complex, the desorption rates were 17.25% and

Fig. 2 – Scanning electron microscopy images of minerals and their complexes with DBM. (a) Kaolinite; (b) DBM–kaolinite; (c) goethite; (d) DBM–goethite.
33.32% by NH₄NO₃, 82.19% and 98.74% by EDTA–Na₂. All the observed desorption rates of complexes were higher than the theoretical values, except for the Cu(II) desorption rate by NH₄NO₃.

2.4. Binding site concentrations of minerals and DBM–mineral complexes

Potentiometric titration curves of minerals and DBM–mineral complexes are shown in Fig. 3. Both minerals and DBM–mineral complexes displayed a weak buffering capacity over a wide pH range from 3.0 to 11.0 compared with the DBM cells (Bai et al., 2017). The method used to calculate the binding site concentrations was described in Bai et al. (2017). As the data in Table 2 show, the site concentrations of kaolinite and goethite were 0.05 and 0.10 mmol/g, which were extremely low. After combination with DBM, the site concentrations increased to 0.09 and 0.13 mmol/g, respectively, which were 18.18% and 27.78% lower than the theoretical values.

2.5. Functional groups for Cu(II) and Pb(II) adsorption on minerals and DBM–mineral complexes

FT-IR spectra of kaolinite and goethite were recorded both before and after Cu(II) and Pb(II) adsorption and are shown in Fig. 4. For goethite, the band around 3128 cm⁻¹ corresponds to the presence of dissociative hydroxyl (Russell and Fraser, 1994). The peak around 1651 cm⁻¹ is due to the bending vibration of O–H (Ruan et al., 2001). The peak around 1383 cm⁻¹ was assigned to the bending vibration of adsorbed water and the peaks around 889 and 795 cm⁻¹ were due to the deformation vibration of Fe–OH (Ruan et al., 2002a, 2002b). The peak around 638 cm⁻¹ was indicative of the FeO₆ lattice (Russell and Fraser, 1994). After the Cu(II) and Pb(II) adsorption, some peaks showed subtle changes. The peak shifts from 3128 to 3134 cm⁻¹, 1383 to 1385 cm⁻¹ and 889 to 891 cm⁻¹ indicated the bonding of Pb(II). The single peak shift from 1383 to 1385 cm⁻¹ indicated the bonding of Cu(II). For kaolinite, the peak around 3697 cm⁻¹ was due to the stretching vibration of
inner –OH. The peak around 1630 cm$^{-1}$ was assigned to the bending vibration of adsorbed water. The peaks around 1032 and 912 cm$^{-1}$ represented the bending vibrations of Si–O and Al–OH. The peaks around 795 and 698 cm$^{-1}$ were indicative of Si–O stretching (Adebowale et al., 2006; Yuan et al., 2008). The peaks at 3620, 3450, 1115 and 538 cm$^{-1}$ represented the stretching vibration of structural hydroxyl, adsorbed water, Si–O from quartz and Al–O–Si. The peak at 469 cm$^{-1}$ was due to the deformation vibration of Si–O–Si or the stretching vibration of Fe–O (Eren and Afsin, 2008). The peaks around 1007 and 428 cm$^{-1}$ were assigned to symmetrical stretching of Si–O–Si and stretching of Al–O (Saikia and Parthasarathy, 2010). However, after the adsorption of Cu(II) and Pb(II), only the shift of the peak at 3450 to 3446 cm$^{-1}$ was observed, which indicated the bonding of Pb(II) by adsorbed water.

FT-IR spectra of DBM-kaolinite and DBM-goethite complexes were also recorded both before and after Cu(II) and Pb(II) adsorption and shown in Fig. 5. Comparing the spectra of the minerals (Fig. 4) and DBM (Bai et al., 2014), it can be seen that the functional groups with peaks at 2960, 2929, 1655, 1545, 1454, 1400, 1236, 1159 and 1082 cm$^{-1}$ from DBM bond with the functional groups at 3450, 1630 and 1115 cm$^{-1}$ from kaolinite. The functional groups at 1655, 1545, 1454, 1400, 1236, 1159 and 1082 cm$^{-1}$ from DBM bond with the functional groups at 3128, 1383, 889 and 638 cm$^{-1}$ from goethite. The functional groups at 3421 and 1117 cm$^{-1}$ from kaolinite and 2391, 1653 and 1539 cm$^{-1}$ from DBM in the DBM–kaolinite complex contributed to the bonding of Cu(II) and Pb(II). The functional groups at 1387 and 638 cm$^{-1}$ from goethite and 1539 and 1232 cm$^{-1}$ from DBM in the DBM–goethite complex contributed to the bonding of Cu(II). The functional groups at 1387 cm$^{-1}$ from goethite and 1232 and 1047 cm$^{-1}$ from DBM in the DBM–goethite complex contributed to the bonding of Pb(II).

### 3. Discussion

As mentioned earlier, bacteria are able to bind heavy metals, including Cu and Pb. This ability makes them suitable candidates for use in bio-immobilization of heavy metal-contaminated soil. However, this ability of the bacteria in soil has not been easily explained. Soil is a very complex system and soil minerals are the main soil solid phase, so it is

<table>
<thead>
<tr>
<th>Strain</th>
<th>Total site concentration$^a$</th>
<th>Theoretical concentration$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goethite</td>
<td>0.10</td>
<td>–</td>
</tr>
<tr>
<td>Goethite + DBM</td>
<td>0.13</td>
<td>0.18</td>
</tr>
<tr>
<td>Kaolinite</td>
<td>0.05</td>
<td>–</td>
</tr>
<tr>
<td>Kaolinite + DBM</td>
<td>0.09</td>
<td>0.11</td>
</tr>
</tbody>
</table>

$^a$ The data got from actual experiments (mmol/g).

$^b$ The data calculated from the weighted average of DBM and minerals (mmol/g).

Fig. 4 – Fourier transform infrared spectra of goethite and kaolinite before and after the adsorption of Cu(II) and Pb(II) (CK: Control).
important to study the adsorption of heavy metals on microbes and mineral complexes to gain further understanding of the mechanism of heavy metal immobilization by soil microorganisms. In this study, kaolinite, goethite and their complexes with *Bacillus subtilis* DBM strain isolated from a multi-metal-contaminated rice paddy soil were used to evaluate the effect of the interaction between bacteria and soil minerals on Cu(II) and Pb(II) adsorption.

As shown by the results in Table 1, kaolinite and goethite showed very low adsorption capacities for Cu(II) and Pb(II) compared with the DBM complexes. This is a well-known phenomenon because of the smaller number of functional groups on the soil mineral surfaces (Kurek et al., 1982; Walker et al., 1989). The modeling of potentiometric titration data demonstrated the low site concentrations on the surfaces of kaolinite and goethite (Table 2). The FT-IR analysis proved that only the hydroxyl group was bound with Cu(II) and Pb(II) on the surface of kaolinite and goethite (Fig. 4).

According to Bai et al. (2014), the physically adsorbed and ion-exchanged fraction of Cu(II) and Pb(II) could be desorbed by NH₄NO₃, while the complexed fraction could be desorbed by EDTA–Na₂ but not by NH₄NO₃. The remaining fraction that could not be desorbed by EDTA–Na₂ was considered to be accumulated inside the cells. For minerals, however, those fractions were the physically adsorbed and ion-exchanged fraction, the fraction with complexed hydroxyl groups and the more stably bonded fraction (i.e., metals combined in the lattice of the minerals). For kaolinite, the aluminum hydroxyl, silicon hydroxyl and Lewis acid hydroxyl groups of the lamellar structure edge were strong heavy-metal binding sites; but the low degree of isomorphous substitution could provide fewer binding sites for heavy metals (Davis and Kent, 1990). As shown in Table 1, 42.77% and 29.72% of the Cu(II) and Pb(II) adsorbed by kaolinite were the physically adsorbed and ion-exchanged fraction, and 46.33% and 33.11% of the adsorbed Cu(II) and Pb(II) were bonded with various hydroxyls. The small differences in desorption rate observed between EDTA–Na₂ and NH₄NO₃ might be because of ternary compound formation between kaolinite and EDTA–metals (Güçlü and Apak, 2003; Hizal and Apak, 2006; Liu and Gonzalez, 1999; Nowack and Sigg, 1996). The strongly adsorbed proportion of Cu(II) and Pb(II), which could not be desorbed by EDTA–Na₂, might also be affected by the pH used for adsorption. A study showed that coordination began to dominate only when pH > 6.5 (Wu et al., 2005).

Different kinds of hydroxyls in goethite, which could be formed due to the different numbers of Fe(III) bonded with O, had different capacities in combination with heavy metals (Davis and Kent, 1990; Hiemstra et al., 1989a, 1989b). The fraction of adsorbed Cu(II) and Pb(II) desorbed by NH₄NO₃ was the part physically adsorbed on the surface of goethite. The fraction desorbed by EDTA–Na₂ was bonded with Lewis acid hydroxyl and one Fe(III)-combined hydroxyl group. The fraction that could not be desorbed by EDTA–Na₂ was bound more stably. However, some studies indicated ternary compound formation when EDTA was present (Güçlü and Apak,
and the FeO$_6$ lattice of goethite and hydroxyl and Si-kaolinite and goethite. On the other hand, hydroxyl groups peptidoglycan were involved in the complexes of DBM with carboxyl and P=O from teichoic acids as well as P-amide I and II bonds and C.

are beneficial for the attachment of bacteria on soil minerals and nucleic acids are the three major components of EPS, and soil minerals (Tsuneda et al., 2003). Proteins, polysaccharides, into DBM cells. Similar results were observed for the kaolinite–DBM complex, which might due to the loose complexation of some Cu(II) and Pb(II) in the goethite–DBM complex. The observed rates of Cu(II) and Pb(II) desorption by EDTA–Na$_2$ was lower than the theoretical value. This might lead to a lower desorption rate and could underestimate the fraction of adsorbed Cu(II) and Pb(II) desorbed by EDTA–Na$_2$.

As shown in Table 1, after the minerals, especially kaolinite, combined with the DBM strain, the Cu(II) and Pb(II) adsorption capacities increased. However, the observed values were lower than the theoretical values. The total concentrations of functional groups followed the same patterns (Table 2). During the combining of minerals and DBM, some of the functional groups might be covered. Similar results were observed by Fang et al. (2010), but the study of Chen et al. (2008) obtained the opposite results. The ratios of minerals to DBM used in our study could form the optimal combination, but minerals and bacteria were just simply mixed in the ratio of 1:1 in Chen et al. (2008), and this could have resulted in a less-than-optimal combination.

The observed rate of Pb(II) desorption by NH$_4$NO$_3$ was higher than the theoretical value, which revealed that a larger proportion of functional groups was involved in the goethite–DBM complex. The observed rates of Cu(II) and Pb(II) desorption by EDTA–Na$_2$ were also higher than the theoretical value, especially the Pb(II) desorption rate. The high desorption rates by EDTA–Na$_2$ proved that the goethite–DBM complex could prevent Cu(II) and Pb(II) from entering the DBM cells. Similar results were observed for the kaolinite–DBM complex, but the rates of desorption by EDTA–Na$_2$ in the kaolinite–DBM complex were lower than for the goethite–DBM complex, which might due to the loose complexation of kaolinite with DBM and the entrance of some Cu(II) and Pb(II) into DBM cells.

EPS play an important role in the attachment of bacteria on soil minerals (Tsuneeda et al., 2003). Proteins, polysaccharides, and nucleic acids are the three major components of EPS, and are beneficial for the attachment of bacteria on soil minerals (Ding and Henrichs, 2002; Omoike et al., 2004). From the results of FT-IR analysis shown in Fig. 5, it can be seen that amide I and II bonds and C–H from extracellular protein, carboxyl and P–O from teichoic acids as well as P–O from peptidoglycan were involved in the complexes of DBM with kaolinite and goethite. On the other hand, hydroxyl groups and the FeO$_6$ lattice of goethite and hydroxyl and Si–O of kaolinite were also involved in the complexes of DBM with kaolinite and goethite. Almost all these functional groups could bond with Cu(II) and Pb(II). The most important difference was the change in the Pb(II) bond with phosphodiester in the goethite–DBM complex compared with the bond on the DBM cell surface. The study of Omoike et al. (2004) demonstrated that a stable monodentate complex was formed by the binding of phosphodiester in extracellular nucleic acids with the central iron atoms on the goethite surface. The monodentate complex might have stronger binding capacities toward Pb(II). The bands for amide II at 1545 cm$^{-1}$ in DBM, Fe–OH at 889 cm$^{-1}$ and FeO$_6$ lattice at 638 cm$^{-1}$ and phosphodiester at 1236 cm$^{-1}$ in the goethite–DBM complex. The Fe–OH at 889 cm$^{-1}$ could not bond with Cu(II) anymore in the goethite–DBM complex, and this result explained why the observed rate for Cu(II) desorption by NH$_4$NO$_3$ was lower than the theoretical value. For the kaolinite–DBM complex, the extracellular protein of DBM could provide more binding sites for Cu(II) and Pb(II) adsorption.

The Bacillus subtilisDBM strain was isolated from heavy metal contaminated-soil and could survive in this contaminated environment (Bai et al., 2014). If applied into the contaminated soil, the DBM cells could absorb onto the mineral surfaces to protect themselves and improve the immobilization of heavy metals, according to the findings mentioned above. This process can provide a potential application for the remediation of heavy metal-contaminated soil.

4. Conclusions

Compared with the DBM cells, kaolinite and goethite showed very low adsorption capacities toward Cu(II) and Pb(II). However, after combination with the DBM stain, the binding site concentrations were increased and resulted in the promotion of the adsorption capacities. During the combination process, the extracellular protein of DBM provided more binding sites for the adsorption of Cu(II) and Pb(II) by the minerals. In particular, an especially stable complexation site was formed between goethite and the phosphodiester bond from DBM EPS to enhance the adsorption capacity toward Pb(II). The combination of DBM and minerals might also help to prevent heavy metals from entering DBM cells, which could improve the survivability of DBM in heavy metal-contaminated environments.

Acknowledgments

This work was supported by the National Key R&D Program of China (No. 2018YFD0800700), the Natural Science Foundation of China (No. 41225004 and No. 41671313), the 111 Project (No. B18060), and the Natural Science Foundation of Guangdong Province, China (No. 2015A030313159).

REFERENCES


envelopes and Bacillus subtilis cell walls with two clays and ability of the composite to immobilize heavy metals from solution. Appl. Environ. Microbiol. 55 (11), 2976–2984.


