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RESEARCH ARTICLE

Roles of adhered *Paenibacillus polymyxa* in the dissolution and flotation of bauxite: a dialytic investigation

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Abstract Bio-related techniques have been proved to be efficient and specific in eliminating impure minerals such as goethite, hematite and kaolinite from aluminum hydroxides in bauxite processing. In this study, the bacterium *Paenibacillus polymyxa* (*P. polymyxa*) mediated dissolution and flotation of bauxite were experimentally investigated. To disclose the contribution of adhered bacteria to these two processes, comparative experiments were designed, with one (and the other not) being dialyzed to prevent cells from contacting with bauxite. The results show that all the release rates of Al, Fe and Si are accelerated by the involvement of bacteria during 11 experimental days. More Al, Si and especially Fe are leached out in contact trial than in dialysis trial, and simultaneously, a large amount of Si-enriched flocs are formed. Further analysis indicates that with the adhesion of *P. polymyxa* and high molecular weight metabolites, Fe minerals are much more dissolvable than kaolinite. However, kaolinite can be floated easily with the mediation of adhered bacteria and metabolites. This study suggests that in bauxite biobeneficiation, sufficient contact between microbes and bauxite can facilitate the elimination of impurities such as iron and silicon.

Keywords bauxite, *Paenibacillus polymyxa* (*P. polymyxa*), dialysis, dissolution, biobeneficiation

1 Introduction

Bauxite is the most important raw material in producing aluminum and related derivatives. In its beneficiation, the key step is to eliminate numerous impurities such as iron minerals, silicates and carbonates from aluminum hydroxides. To achieve this goal, several biologic as well as physical and chemical methods have been extensively explored and applied (Groudeva and Groudev, 1983; Anand et al., 1996; Deo and Natarajan, 1999; Groudev, 1999; Vasan et al., 2001). For example, the bacterium *Paenibacillus polymyxa* (*P. polymyxa*) has been reported to be efficient in removing Fe and Ca from bauxite (Anand et al., 1996; Vasan et al., 2001), while "silicate bacteria" such as *Bacillus circulans* and *Bacillus mucilaginosus* are considered to be potent for Si elimination (Groudeva and Groudev, 1983; Groudev, 1999). In these processes, microbes and metabolites are considered to be effective in bauxite purification through changing the behaviors of dissolution, flotation and flocculation of minerals contained in bauxite.

In aqueous environments where microbes and minerals co-exist, microbes and metabolites can either adhere onto the surface of minerals by forces such as van der Waals, hydrophobics, electrostatics and hydrogen bonds (Grantham and Dove, 1996; Lower et al., 2000; Lower, 2005), or just inhabit freely in aqueous solution. These two types of microbes and metabolites can change the dissolution, precipitation, flotation, flocculation and redox reactions of minerals. Taking mineral dissolution as an example, we could find that some minerals can be dissolved mainly by adhered microbes and metabolites (which is called "direct mode), while some others can be dissolved mainly by bulk microbes and metabolites (which is called "indirect mode") (Ehrlich, 1992). For bauxite purification concerned here, Vasan et al. (2001) have shown that the bacterium *P. polymyxa* can dissolve calcium carbonates which are contained in bauxite through both direct and indirect modes. While hitherto the dominant modes for the removal of other impurities, especially Fe and Si, are still in controversy.

In this study, we present a dialysis-based method to investigate the dissolution and flotation of minerals in bauxite with the mediation of *P. polymyxa*. This method

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has recently been proved to be efficient in investigating interfacial interactions between bacteria and minerals (Wightman and Fein, 2004; Buss et al., 2007). In previous dialysis-related studies, usually, either bacteria or minerals are encased into dialysis materials. Such encasement can prevent cells and high-molecular-weight (HMW) metabolites from contacting with minerals. Subsequently, by contrasting the results of non-dialyzed and dialyzed experiments, the contribution of adhered bacteria to the dissolution of impure minerals can be distinguished. Based upon this concept, we deem that the mechanisms of the dissolution and flotation of impure minerals which are mediated by adhered bacteria and HMW metabolites can be revealed through dialysis experiments.

2 Materials and methods

2.1 Bauxite

Gibbsitic bauxite was obtained from the Interfacial Biogeochemistry Laboratory of the University of Wisconsin-Madison, USA. The bauxite is commonly shallow yellow, with some dark flecks and narrow veins being presented on the fresh surfaces. This shows that some impure matters are contained in bauxite. Scanning electronic microscopy (SEM) result shows that gibbsite crystals lead to a primary shape of pseudohexagonal slab, with about 0.1 to 0.5 μm both in length and width (Fig. 1).

Bauxite was ground and sieved to collect the fraction with size between 75 and 150 μm for use. To remove the organic matter, the collected bauxite was peroxided for 24 h and then boiled to remove residual peroxide. After this process, ultrasonic wash was performed for 5 times with each 10 min. Subsequently, it was bathed in HCl solution with pH 4 for 1 h to prevent initial parabolic kinetics dissolution (Welch and Ullman, 1993). The acid-treated bauxite was washed with double deionized water (DDW) for 10 times until the supernatant was clear, and simultaneously, its pH got to 7.0. At last, the treated bauxite was dried at 70°C.

By using X-ray fluorescence spectrometry (XRF) and X-ray diffraction (XRD) techniques, the chemical composition and mineral assemblage of bauxite were measured, respectively. XRF results show that Al_2O_3 dominates the bauxite, with fewer SiO_2, Fe_2O_3 and TiO_2 being presented as the main impurities (Table 1). Through the XRD result, only gibbsite is detected. Furthermore, diffuse reflectance spectroscopy (DRS) was applied to detect iron minerals (Deaton and Balsam, 1991). The result shows that goethite and hematite are the two dominant iron minerals contained in bauxite (Fig. 2). The specific surface area of bauxite is measured to be 11 $m^2 \cdot g^{-1}$ by BET method with N_2 adsorption on a Micromeritics ASAP2010.

Table 1 Chemical composition of bauxite before and after contact trial /(% wt.)

	initial sample	settled solid	floated solid
CaO	0.05	0.05	0.04
MgO	0.37	0.37	0.35
K_2O	0.03	0.03	0.03
Na_2O	0.23	0.20	0.22
MnO	0.17	0.22	0.09
TiO_2	3.22	3.45	2.24
P_2O_5	0.13	0.25	0.98
SO_3	0.25	0.17	0.00
Fe_2O_3	2.58	2.56	1.90
SiO_2	1.94	1.45	2.01
Al_2O_3	58.66	58.21	49.45
LOI*	32.45	32.15	41.55
total	100.20	99.20	99.00
Fe_2O_3/Al_2O_3	0.044	0.044	0.038
SiO_2/Al_2O_3	0.033	0.025	0.041

Note: *, Lost of ignition

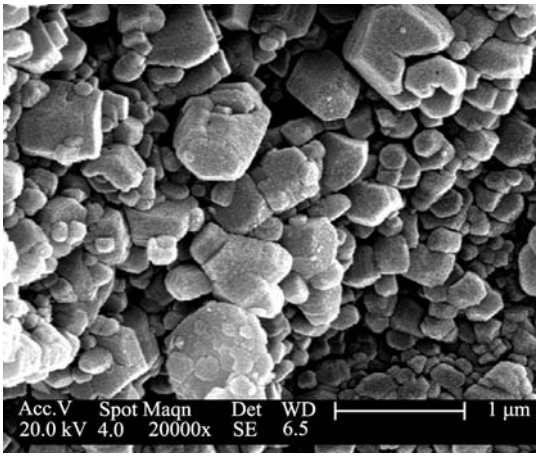

Fig. 1 SEM micrograph of bauxite, showing that gibbsite is the dominant phase

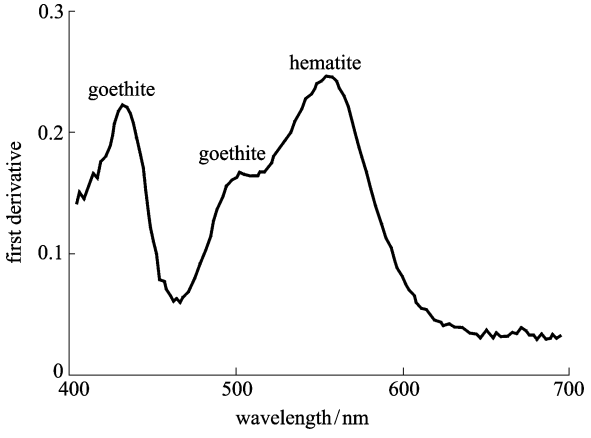

Fig. 2 First derivative of the diffuse reflectance spectrum (DRS) of bauxite

2.2 Bacterium

A strain of *P. polymyxa* was purchased from China General Microbiological Culture Collection Center (CGMCC). The frozen-dried bacteria were revived and incubated at 30°C with modified Bromfield medium (sucrose 5 $g \cdot L^{-1}$, yeast extract 0.15 $g \cdot L^{-1}$, KH_2PO_4 0.5 $g \cdot L^{-1}$, $(NH_4)_2SO_4$ 1 $g \cdot L^{-1}$, $MgSO_4 \cdot 7H_2O$ 0.2 $g \cdot L^{-1}$) (Bromfield, 1954; Vasan et al., 2001). Growth test shows that the static stage is from 18 to 48 h. During this time duration, the amount of *P. polymyxa* keeps steady, with the highest cell concentration of about 1×10^7–2×10^7 cells $\cdot mL^{-1}$.

2.3 Dialysis bags

Dialysis bags (Cellulose Ester Membrane, 14000 Da) were used. According to Santhiya et al. (2002), the molecular weights of extracellular polysaccharides (EPS) produced by *P. polymyxa* are about 17000 Da. It is confirmative that the dialysis bags adopted here can insulate bacteria cells and HMW metabolites such as EPS from contacting with bauxite. Dialysis bags were boiled with 2% (w/v) $NaHCO_3$ and 1 mmol $\cdot L^{-1}$ EDTA at pH 8.0 for 10 min. After cooling and washing, they were boiled again with 1 mmol $\cdot L^{-1}$ EDTA at pH 8.0 for 10 min. At last, the pretreated dialysis bags were washed with DDW.

2.4 Batch experiments

Three experimental sets of bauxite dissolution, including blank control, contact and dialysis trials, were conducted in batch reactors. First, nine portions of bauxite (2 g per portion, with three of which being encased into dialysis bags and then tied with rubber bands), along with nine conical flasks (three of which were 150 mL DDW loaded and the others 150 mL MBM loaded, separately), were prepared and sterilized at 121°C for 20 min. Second, three portions of non-encased bauxite were added to DDW-contained flasks (control) and the other six portions to MBM-contained flasks, respectively (contact and dialysis). Third, MBM cultured bacteria solution of 5 mL was extracted and centrifuged at 5000 rpm for 10 min. After centrifugation, the supernatant was removed, and the cell pellet was resuspended with DDW and centrifuged again. After repeating this process 5 times, the residual cell pellet was inoculated into one of the MBM-contained flasks. The other five MBM-contained flasks were inoculated with *P. polymyxa* by the same procedure. At last, all the flasks were fixed onto a rotary shaker and cultured at 90 rpm and 30°C for 11 d.

2.5 Measurement of element concentrations

10 mL solution was extracted from each flask at 9, 18 h and 2, 4, 7, 11 d. The pH values of solutions were determined immediately after sampling. Each solution sample was divided into two equal parts, with one being acidified to $1 < pH < 2$ by 10% (w/v) HNO_3. All solution samples were centrifuged at 10000 rpm for 10 min. After centrifugation, supernatants were extracted for measuring the concentrations of dissolved Al, Fe and Si. Inductively coupled plasma atomic emission spectrometry (ICP-AES) was employed to fulfill this task.

According to Wightman and Fein (2004), bacteria and metabolites complexed Al, Fe and Si can be released by acidification. Hereby, in this study, it is hypothesized that the quantities of bacteria and metabolites complexed ions can be gained by investigating concentration differences between acidified and non-acidified samples. Two groups of samples, with one from the contact trial and the other from the dialysis trial, were selected for this investigation.

2.6 Residual solid analyses

After being incubated for about one week, some floc-like matters emerged in the contact trial. The flocs subsequently assembled to form floating fluffy spheres (FFSs) with about 1 cm in diameter (Fig. 3). At the end of the experiments, the FFSs were separated carefully from the settled solids. After being dried at 60°C, the two parts of the solid matter were analyzed by ICP-AES and XRD.

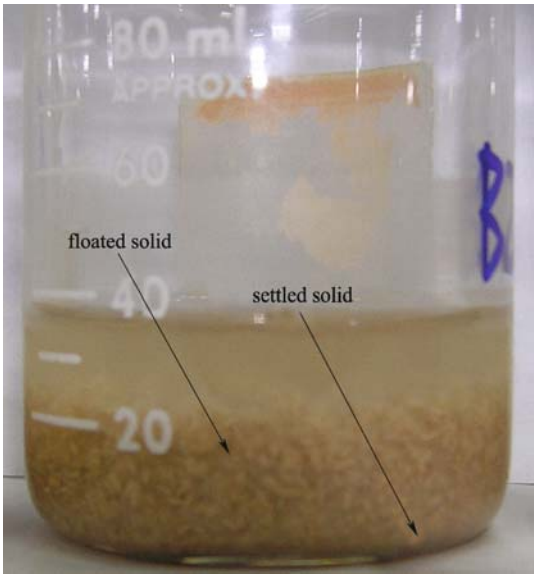

Fig. 3 Floc-like complexes in contact-mode trial

3 Results

3.1 pH values of solutions

Figure 4 shows distinctive pH changes for three trials. For two bacteria-contained trials, pH decreased sharply from 7.0 to about 4.0 in the first 18 h, and the decrease rates slowed down thereafter. For the dialysis trial, pH decreased

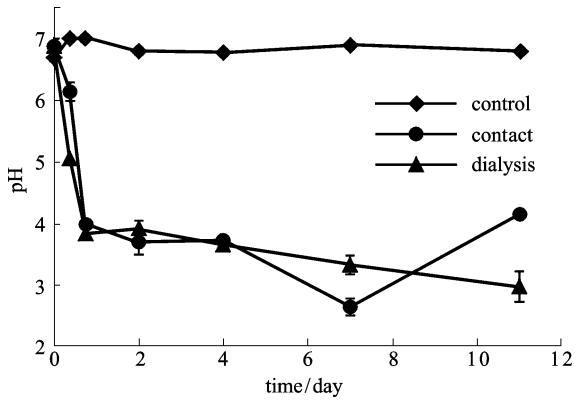

Fig. 4 Variations of the pH values during experimental periods

throughout the whole experiment, while for the contact trial, it began to increase at day 7. At the end of the experiments, the pH value is about 4 for the contact trial and 3 for the dialysis trial. For the control trial, the solution kept neutral all along the 11 d.

3.2 Al, Fe and Si concentrations in acidified solutions

In the control trial, the concentrations of Al, Fe and Si maintained at low levels all along the experimental time (Fig. 5). In bacteria-contained trials, the concentrations of the three elements began to increase after the 4th day. The evident concentration increase for Al was in days 4–7 and days 7–11 for contact and dialysis trials, respectively. While for Fe and Si, no obvious stage characteristic was observed for their concentration increases. At the end of the experiments, all the elements have achieved much higher concentrations in the contact trial than in the dialysis trial. Such difference in concentration is especially great for Fe.

3.3 Al, Fe and Si concentrations in non-acidified solutions

The alteration patterns of Al, Fe and Si concentration in non-acidified solutions are similar to those in acidified solutions (Fig. 6). Moreover, from Fig. 6, it is observed that the concentrations of all three elements are always lower in non-acidified solutions than in acidified solutions. Based on the hypothesis mentioned previously, the concentration differences are due to the complexation of bacteria. In the contact trial, the amounts of bacteria complexed elements have an order of Al > Fe > Si. In the dialysis trial, this order is obscure (Fig. 6). Compared to the dialysis trial, more Al and Fe were complexed with bacteria in the contact trial. However, in two bacteria-contained trials, the amounts of bacteria complexed Al, Fe and Si remained nearly unchanged after 18 h.

3.4 Characteristics of floated and settled solids

Table 1 shows the composition of the newly formed flocs and settled solids. It is observed that in flocs, Si and the lost of ignition (LOI) were enriched, and Ti was depleted. While in settled solids, both Ti and Fe were enriched, and Si was depleted.

The XRD result shows that for the settled solids, gibbsite is still the dominant phase, with only a few other minerals coexisted (Fig. 7). One weak peak which indicates to the kaolinite is identified in the XRD pattern of flocs (but not in settled solids or the initial sample). The identification of kaolinite agrees well with the Si content increase in the flocs.

4 Discussion

4.1 Direct mode in the dissolution of Fe (hydr)oxides

In the experiments, the mechanisms for bacteria-promoted mineral dissolution can be divided into direct and indirect modes. The bacteria-mediated dissolutions of goethite and hematite have been extensively investigated (Hersman et al., 1995; Forsythe et al., 1998; Maurice et al., 2000; Yoshida et al., 2002; Kraemer, 2004). Some studies found that bacteria and metabolites, such as siderophores, can promote the dissolution of Fe-(hydr)oxides mainly through surface attachment (direct mode) (Forsythe et al., 1998;

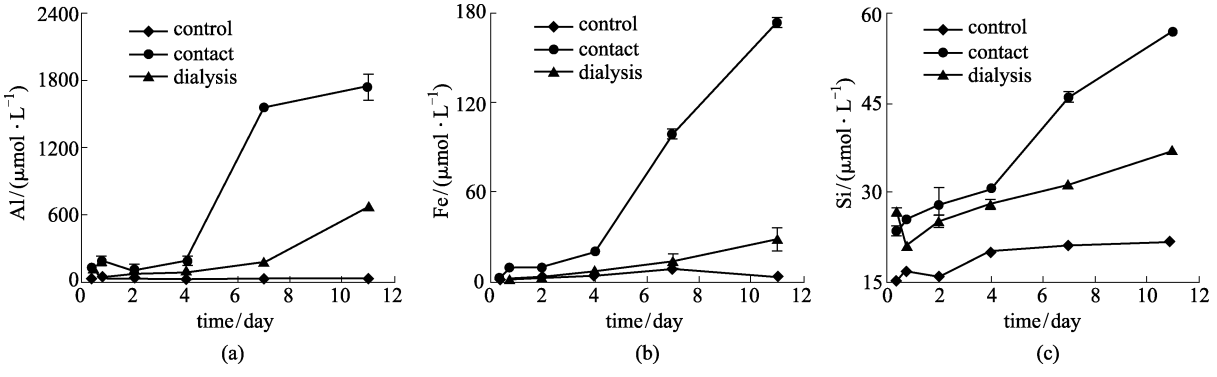

Fig. 5 Concentrations of Al, Fe and Si in acidified solutions

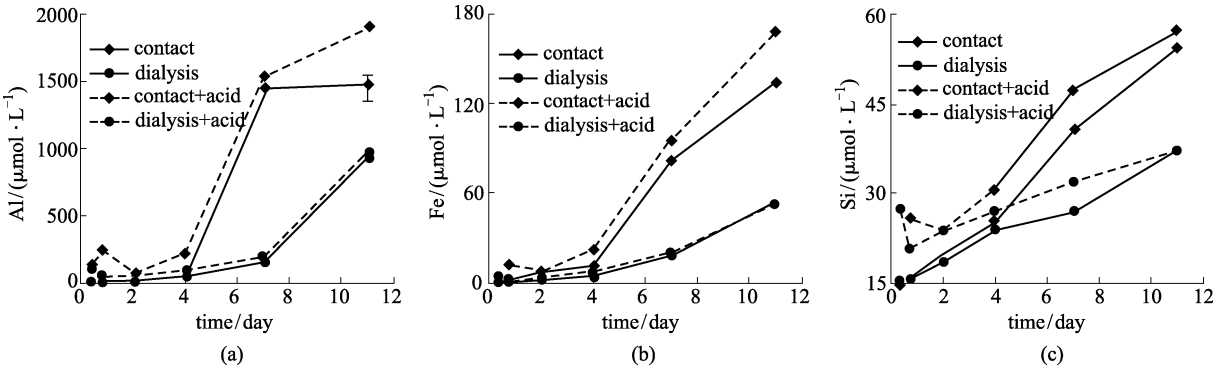

Fig. 6 Comparisons of Al, Fe and Si concentrations in acidified and non-acidified solutions

Yoshida et al., 2002; Wiederhold et al., 2006). Whereas some others supported the indirect mode, through which minerals are dissolved by bacteria and metabolites, such as organic acids, without any contact (Zinder et al., 1986; Kraemer, 2004). In this study, since much more Fe was released in the contact trial than in the dialysis trial (Fig. 5(b)), it is suggested that iron (hydr)oxides were dissolved mainly through the direct mode. The attached *P. polymyxa* cells and HMW metabolites on the mineral surface have played the dominant role in the dissolution of iron (hydr)oxides.

It has long been realized that iron (hydr)oxides can be dissolved both by proton- and ligand-promoted mechanisms (Zinder et al., 1986; Kubicki et al., 1999; Cheah et al., 2003; Kraemer, 2004; Persson and Axe, 2005; Wiederhold et al., 2006). The former mechanism means that mineral is dissolved through the replacement of cations by protons. While the latter one means that mineral is dissolved through the complexation of surfacial elements by ligands (Kraemer, 2004; Persson and Axe, 2005) or through the reaction kinetics alteration which is derived from the complexation of dissolved elements by ligands (Kubicki et al., 1999; Cheah et al., 2003). In this study, it is considered that protons have contributed little to the dissolution of Fe minerals because even when the pH in the dialysis trial has decreased to about 3, the concentration of Fe was still low. In the contact trial, even inadhered bacteria and metabolites have complexed more Fe than that in the dialysis trial (Fig. 6(b)), but the Fe concentration was still high. This implies that such complexation has hardly affected the saturation of Fe, and thus, the kinetics alteration of ligand resulted reaction is negligible. Therefore, it is reasonable that in the contact trial, most of the dissolved Fe was released by adhered bacteria and HMW metabolites.

For bacterial cells and metabolites, no matter what species they are, they often possess a large amount of ligands such as hydroxy, carboxyl and phosphoryl. When bacteria and metabolites are adhered onto the mineral surface, two possibilities will happen. One is that the operating distances between these ligands and mineral will be shortened, and the other is that the ligand concentration near the mineral surface will increase. Due to these two factors, the dissolution of mineral can be promoted by the complexation of ligands.

In this study, it is uncertain whether the proton-promoted mechanism has also been enhanced with the adhesion of bacteria and HMW metabolites. However, since other studies have discovered that the pH near bacteria adhered mineral surface is often lower than that of the bulk solution (Barker et al., 1998; Liermann et al., 2000), it is deduced that this enhancement also exists.

4.2 Direct and indirect modes in the dissolution of gibbsite and kaolinite

From Fig. 5(a), it is observed that at the end of the experiments, both in contact and dialysis trials, considerable gibbsite was dissolved by bacteria and metabolites. This implies that gibbsite can be dissolved by bacteria via both direct and indirect modes. From Fig. 6(a), it is observed that in the dialysis trial, few Al was complexed by bacteria and metabolites. However, in the contact trial, far more Al was complexed by bacteria and metabolites. So it is implied that in the contact trial (but not in the dialysis trial), likely the free bacteria and metabolites have changed the dissolution kinetics of gibbsite through altering the saturation of dissolved Al.

As Fig. 7 has shown, kaolinite is the main silicate mineral in bauxite. So it is considered that the dissolved Si was released mostly from kaolinite. The fact that Si concentrations increased both in dialysis and contact trials indicates that both direct and indirect modes can promote the dissolution of kaolinite. Whereas, compared with those of iron (hydr)oxides and gibbsite, the dissolution of kaolinite is relatively weak both in dialysis and contact trials. This implies that kaolinite is more stable than gibbsite, goethite and hematite in *P. polymyxa* existing environment.

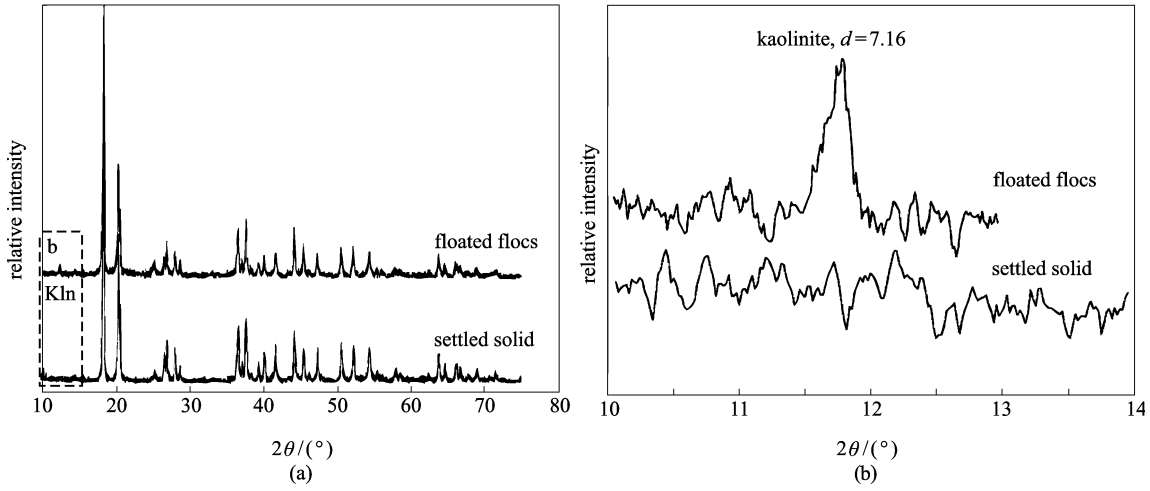

Fig. 7 XRD patterns of residual solids. The enclosed part in (a) is amplified as (b)

4.3 Role of adhered bacteria and HMW metabolites in the removal of kaolinite

Deo and Natarajan (1998) have proposed that some special proteins produced by *P. polymyxa* can adsorb onto kaolinite and make it more hydrophobic and floated. Our study here has validated their viewpoints because we also have obtained kaolinite-enriched flocs. The difference is that, in our study, besides proteins, bacterial cells and other HMW metabolites such as EPS have also participated in the floatation of kaolinite. Combined with Deo and Natarajan's conclusion (Deo and Natarajan, 1998), it is further deduced that the proteins excreted by *P. polymyxa* may be larger than 15000 Da because in our study, no protein has penetrated into dialysis bags and made kaolinite floating.

4.4 Implications for biobeneficiation of bauxite ores

The ratios of Fe and Si to Al for the initial sample, flocs and settled solids are listed in Table 1. Compared to the initial sample, the ratio of Fe to Al kept unchanged in settled solids but decreased slightly in flocs. This indicates that Fe was removed from the total solids due to bacteria-mediated dissolution. The ratio of Si to Al increased in flocs, but decreased in settled solids. This implied that Si mineral (kaolinite) can be floated through the adhesion of bacteria and HMW metabolites. A similar phenomenon also has been reported by Natarajan and Deo (2001).

As a whole, *P. polymyxa* and its metabolites can help in purifying bauxite by promoting the dissolution of Fe (hydr) oxides and floatation of silicates. Since this study has demonstrated that surface adhesion of bacteria and HMW metabolites can highly promote both the dissolution and flotation processes, it is advised that sufficient contact between bauxite and bacteria is crucial in biobeneficiation of bauxite.

5 Conclusions

In aqueous environments, where bauxite and *P. polymyxa* coexist, minerals such as gibbsite, goethite, hematite and kaolinite can be dissolved through both proton- and ligand-promoted mechanisms. The adhesion of bacteria onto mineral surfaces can promote these two processes. The extent of promotion varies with the minerals. Adhered bacteria and HMW metabolites can highly promote the dissolution of Fe (hydr)oxides but are relatively deficient in gibbsite and kaolinite dissolution. Besides dissolution, adhered bacteria and HMW metabolites can also improve the flotation of kaolinite contained in bauxite.

Since adhered *P. polymyxa* and HMW metabolites can promote the dissolution of Fe (hydr)oxides and the floatation of kaolinite, it is deduced that *P. polymyxa* is a strain of useful bacterium in bauxite beneficiation, and furthermore, to increase the beneficiation efficiency of bauxite, sufficient adhesion between bacteria and bauxite must be considered.

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