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# Bioleaching of heavy metals from red mud using *Aspergillus niger*

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ABSTRACT

Red mud (bauxite residue) is the main waste product of the alkaline extraction of alumina from bauxite with high amounts of metals. In this study, bioleaching of heavy metals from red mud by using the fungus *Aspergillus niger* was investigated. Bioleaching experiments were examined in batch cultures with the red mud at various pulp densities (1–5%, w/v) under various bioleaching conditions (one-step, two-step and spent medium bioleaching). It was shown that the main lixiviant excreted by *A. niger* was citric acid. The highest leaching ratios of most various heavy metals were achieved under spent medium leaching at 1% pulp density. The increase in red mud pulp densities resulted in a general decrease in leaching ratios under all bioleaching conditions. However, in the case of the spent medium leaching the decrease in leaching ratios was lowest. The Toxicity Characteristic Leaching Procedure (TCLP) tests showed that the leaching toxicity of the bioleaching residue was far below the levels of relevant regulations. The micromorphology of the red mud particles were changed by the fungal activity during bioleaching process.



## 1. Introduction

The production of alumina results in the generation of bauxite refinery residue known as red mud, a highly saline and alkaline waste material, which represents the main disposal problem in the alumina industry (Clark et al., 2011). Producing one ton of alumina will consume approximately four metric tons of raw bauxite and simultaneously generate above two metric tons of red mud (Ghosh et al., 2011). The storage volume of red mud in the whole world is estimated to be over 2.7 billion tons (Power et al., 2011). Furthermore, it is increasing with an annual rate of 120 million tons according to the latest reports (Klauber et al., 2011). The management in the future of this kind of waste residue is of increasing environmental concern.

The raw red mud or after processed could potentially be used in various environmental or industrial fields (Klauber et al., 2011). The main attempts for the effective application of red mud are: soil amendment to prevent nutrient loss and reduce heavy metal availability (Alva et al., 2002); adsorbents for removal of heavy metal ions and metalloid ions (Cengeloglu et al., 2006; Zhang et al., 2008); absorbents for hydrogen sulfide and sulfur dioxide in gas purifiers (Fois et al., 2007); building materials as bricks and cements additive (Somlai et al., 2008); pigments and paints (Liu et al., 2009); catalysts (Liu et al., 2009; Wang et al., 2008).

Though red mud could be potentially applied in many fields, the environmental risk of heavy metals leaching from red mud has never been thoroughly evaluated (Ghosh et al., 2011; Milacic et al., 2012). It is believed that the concentration of heavy metals (e.g., V, Cr, Ni, Cu, Zn and As) are elevated in red mud and are approximately 20 folds comparing with the surrounding soil (Kutle et al., 2004). The concentration of various heavy metals generally accounts for 0.01% to 1% respectively of the total weight according to the relevant studies (the concentration of Fe is even over 10%) (Akinci and Artir, 2008; Ghosh et al., 2011; Kutle et al., 2004). It is also reported that the concentration of heavy metals such as Cd, Cu, Ni and Zn in the red mud are 3 orders of magnitude more than the related regulation of Sediment Quality Guidelines Developed for the National Status and Trends Program enacted by National Oceanographic and Atmospheric Administration (NOAA) (Ghosh et al., 2011). Maybe the leaching toxicity of heavy metals is low for the raw red mud due to the high alkaline characteristic itself. However, the leaching toxicity would be likely to increase after the change in surrounding environment or any process for various application purpose. It will have a harmful effect on plants, animals, aquatic life and humans once the heavy metals leach from red mud. Therefore, it is important to decrease the heavy metals contents in red mud before storage or application for the environmental safety.

Biohydrometallurgical approaches (bioleaching) are generally considered as a 'green technology' with low-cost and low-energy requirement (Wu and Ting, 2006). It can complete two aims simultaneously in one process: (1) recover some valuable heavy metals and (2) reduce the leaching toxicity of heavy metals from waste materials (Klauber et al., 2011). Some species of heterotrophic fungus (e.g., *Aspergillus* and *Penicillium*) have shown potential for metal bioleaching of various waste materials, such as fly ash (Bosshard et al., 1996; Wu and Ting, 2006), spent catalysts (Amiri et al., 2011;

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Santhiya and Ting, 2005) and electrical waste (Brandl et al., 2001). The most important mechanism of metal leaching by heterotrophic fungus is an indirect process with microbial production of metabolites, such as amino acids and organic acids (Burgstaller and Schinner, 1993). Due to the strong adaptability, high metabolic activity and high production of organic acids, *Aspergillus niger* becomes the one of the most widely used fungi in bioleaching (Aung and Ting, 2005; Ren et al., 2009).

Although the process of metal bioleaching using *A. niger* seems promising, only few studies have been performed (Ghorbani et al., 2008; Vachon et al., 1994). Therefore, the focal point of this study was chosen to the bioleaching of heavy metals from red mud by using *A. niger*. The chemical characteristic of the red mud and the growth characteristics of the fungus (biomass dry weight, pH and excreted organic acids) in the pure culture were determined before bioleaching. Thereafter, the pH change and heavy metal leaching efficiency under various bioleaching conditions (one-step, two-step and spent medium bioleaching) and pulp densities (1–5%, w/v) were analyzed. Finally the Toxicity Characteristic Leaching Procedure (TCLP) tests, which are designed to determine the leaching toxicity of heavy metals from solid wastes, were conducted and the results were compared with the relevant regulations.

## 2. Materials

### 2.1. Leaching fungal strain

*A. niger* (GenBank accession number is JF909353) was provided by Research Center For Bio-Resource & Technology, Institute of Geochemistry, Chinese Academy of Sciences.

### 2.2. Red mud

The red mud samples were collected from the storage area of bauxite residue (26°41′N, 106°35′E) which belongs to Chinalco in Guiyang. The semi-arid samples were collected by sterile steel containers. When transported to the library, the red mud samples were dried to constant weight in the oven at 80 °C, and ground by using a porcelain pestle and mortar and screened through 74 μm sieves.

### 2.3. Chemical reagents

The chemical reagents used in our experiments were all analytical reagent (AR). All the aqueous solutions were prepared using deionized distilled water.

## 3. Methods

### 3.1. Characterization of red mud

For analyzing the chemical composition of red mud, the process according to US EPA SW 846 Method 3050B was used to totally digest the samples (Wu and Ting, 2006). The metal ions containing in the digestive supernatant were analyzed using a Quadrupole Inductively Coupled Plasma Mass Spectrometry (Q-ICP-MS, PerkinElmer, ELAN DRC-e) and an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, VistaMPX). The pH value of red mud samples were determined by using a digital pH meter (PHS-3C). The electrical conductivity (EC) was determined by EC meter (DDSJ-308A). The acid neutralizing capacity (ANC) was determined by a standard procedure of titration (endpoint of pH was 4.5).

### 3.2. TCLP tests

The TCLP tests were conducted based on U.S. Environmental Protection Agency (EPA) SW 846 method 1311(Aung and Ting, 2005). The extraction supernatant was determined by using Q-ICP-MS and ICP-OES after filtering through a 0.45 μm glass fibre filter.

### 3.3. Bioleaching of red mud by A. niger

*A. niger* was cultivated on potato dextrose agar plates at 30 °C for 7 days in an incubator. The mature spores were harvested with a sterile solution of physiologic saline. The spores suspension was diluted and the number of spores was counted by using a haemocytometer and standardized to approximately $1 \times 10^7$ spores/mL. In order to observe the characteristics of fungal growth in the absence of red mud, two milliliter of spore suspension was inoculated into 100 ml of sucrose medium (autoclaved at 121 °C for 15 min) in 250 ml Erlenmeyer flasks and cultivated in an orbital shaking incubator at 30 °C and 120 rpm. The composition of sucrose medium is shown in Table 1.

Bioleaching studies were carried out using 250 mL Erlenmeyer flasks in 100 mL of sucrose medium (autoclaved at 121 °C for 15 min) with the sterilized red mud at various pulp densities. Three different bioleaching conditions were investigated. In one-step bioleaching, the fungus was incubated together with the sucrose medium and red mud. In two-step bioleaching, the fungus was first incubated in sucrose medium in the absence of red mud for 3 days, after which the sterilized red mud was added. In spent medium bioleaching, the fungus was first incubated in sucrose medium for 10 days. Then the sterilized red mud was added into the cell-free spent medium which was obtained by centrifugation (3000 rpm) and membrane filtration (0.2 μm, Whatman) of the fungal culture. The cultures were incubated in an orbital shaking incubator at 30 °C and 120 rpm. Control experiments were carried out using fresh sucrose medium and deionized distilled water. All the experiments were conducted in triplicate. Two milliliters of samples were withdrawn at regular intervals for analyzing the sugar concentration, organic acids concentration, pH value and heavy metals concentration. The biomass dry weight was also examined.

### 3.4. Analytical methods

The concentration of sugars and organic acids were determined using High Performance Liquid Chromatography (HPLC, Agilent 1200) with a refractive index detector (RID) for analyzing the sugars, and the variable wave-length detector (VWD) for the organic acids. The pH value and heavy metals concentration in the leachate was determined as described in Section 3.1 above. The percentage of metal extraction ratio was calculated through the concentration in the filter liquor divided by the total concentration in red mud. The residue (biomass with bioleached red mud) obtained from the filter paper was dried at 80 °C for 24 h, followed by ashing at 500 °C for 4 h to determine the biomass dry weight (Aung and Ting, 2005). All experiments were performed in triplicate.

The micromorphology of the fungi and red mud was observed using a Scanning Electron Microscopy (SEM, Shimadzu-SS550, 25 kV, 0.25 nA). The samples were prepared by membrane filtration to remove redundant water. Then washed for 1 h with 2% glutaraldehyde solution in order to protect the intact appearance of microbial cell. After that a series of washings with mixtures of water and

**Table 1**
Composition of bioleaching medium.

| Ingredients | Concentration (g/L) |
|---|---|
| Sucrose | 100 |
| $KNO_3$ | 0.5 |
| $KH_2PO_4$ 0.5 | 0.5 |
| Yeast extract | 2.0 |
| Peptone | 2.0 |

ethanol were conducted for the purpose of cell dehydration. The samples were coated with gold and submitted for SEM and EDS analysis.

## 4. Results and discussion

### 4.1. Characteristic of red mud

The pH of the red mud samples was 12.9. Electrical conductivity was 21.8 mS/cm. The ANC of red mud was 3.53 mmol $H^+$/g. The weight percent concentration of major elements containing in the red mud (wt. %): Al (3.27); Ca (11.85); K (0.95); Mg (0.37); Na (5.30); Si (4.53). The weight concentration of heavy metals in red mud (unit: ppm): As (125); Ba (590); Cr (848); Cu (182); Fe (84200); Ni (169); Pb (332); Zn (670); Zr (2070). The heavy metals which weight percent concentrations were below 0.01% were not shown.

The high pH and ANC value is owing to abundant alkaline anions ($OH^-$, $CO_3^{2-}/HCO_3^-$, $Al(OH)_4^-/Al(OH)_3$) dissolved from red mud (Gräfe et al., 2011). The high EC value is due to high ion strength in the red mud leachate (Gräfe et al., 2011). The high pH, ANC and EC value are indicative of the extreme alkalinity and salinity of the red mud. Furthermore, the high concentration of heavy metals makes the characteristic of red mud severely unfavorable for microorganisms to live in (Krishna et al., 2008). The extremely scarce of organic carbon, nitrogen and micronutrients will also limit the microbes to growth (Gräfe and Klauber, 2011; Hamdy and Williams, 2001; Thiyagarajan et al., 2009). Therefore, we chose the heterotrophic fungus *A. niger* which has a prominent tolerance of unfavorable condition (Brandl et al., 2001; Krishna et al., 2005; Ren et al., 2009) and an ability of producing high volume of organic acids as the leaching strain for the further bioleaching research.

### 4.2. Characteristic investigation of A. niger growth in pure culture

Before the bioleaching, the pure culture experiments (in the absence of red mud) of *A. niger* were conducted until it reached the stationary phase in order to determine the optimum time for addition of red mud into fungal culture in two-step bioleaching, as well as to determine the optimum time to obtain the cell-free medium in spent medium bioleaching.

Fig. 1a shows the variation of sugars and biomass concentration during 40 days incubation. The concentration of sucrose drastically decreased to 32.4 mg/L within 3 day, and finally completely hydrolyzed to glucose and fructose through the invertase action after 10 days incubation. With a decrease in sucrose, the concentration of biomass increased to the maximum of 28.6 mg/L along with the glycometabolism at the tenth day, then had a slight decrease during the rest time of incubation, which is probably due to the toxicity of secondary metabolism accumulating in the medium (Amiri et al., 2011).

Fig. 1b shows the change in pH value and organic acids concentration. The concentration of citric acid drastically increased to the maximum of 82.3 mmol/L within 10 days of incubation. The concentration of gluconic acid and oxalic acid also increased to the maximum of 16.9 and 18.3 mmol/L during 20 and 10 days incubation respectively. The increase in organic acids was accompanied by a decrease in pH value. The pH value decreased to the minimum of 1.8 at the tenth day. Thereafter, with the decrease in organic acids, the pH marginally increased after the tenth day. It showed a high correlation ($r = 0.995$, $p < 0.001$) between an increase in biomass dry weight and a decrease in pH value, which indicates that the activity of the *A. niger* is the uppermost factor affecting the pH in the bioleaching culture.

There are several mechanisms involved in the bioleaching of metals when use heterotrophic microbes as the leaching strains. However the most important mechanism is the acidolysis. The surface of metal compound covering by the oxygen atoms are protonated rapidly, thus the metal and water combine with the protons and oxygen is separated from the surface of metal compound (Burgstaller and Schinner, 1993). The most important substances involved in the acidolysis secreted by fungus are the organic acids (Burgstaller and Schinner, 1993). Through Fig. 1b we can clearly see that the citric acid is the main leaching agent (the highest production among all the organic acids) for bioleaching of red mud by the fungi.

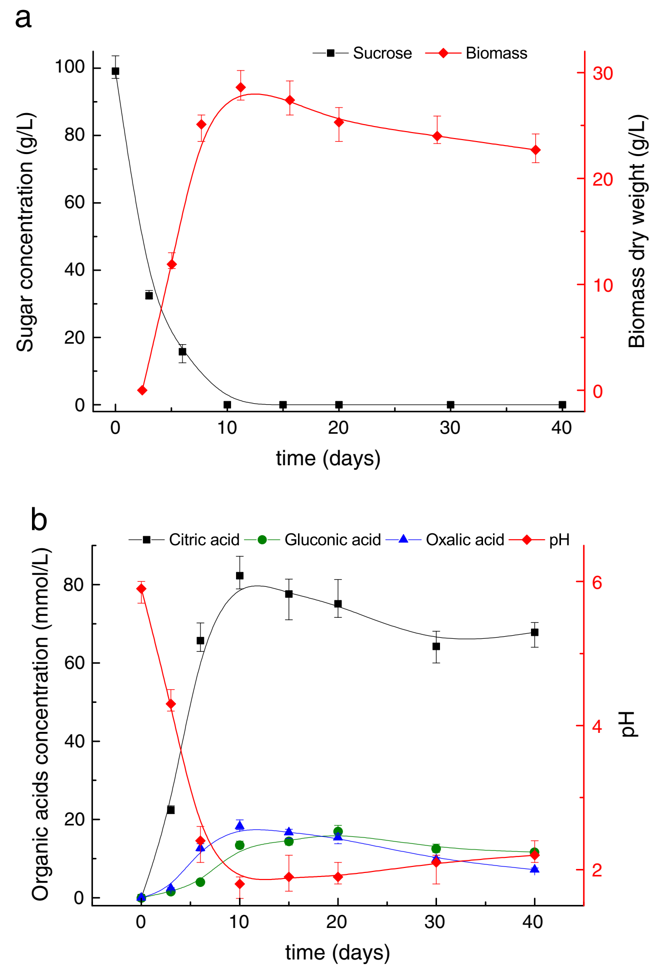


**Fig. 1.** Variation of (a) sugars and biomass concentration and (b) organic acids concentration and pH as a function of time in the pure culture of *A. niger* during 40 days incubation. The experiments were performed in triplicate.

The related reactions between the different organic acids and metal ions are listed as below ($M^{n+}$ represents the metal ions with certain valence):

$$\text{Gluconic acid}: C_6H_{12}O_7 \rightarrow C_6H_{11}O_7^- + H^+ (Pk_a = 3.86) \quad (1)$$

$$n[C_6H_{11}O_7^-] + M^{n+} \rightarrow M[C_6H_{11}O_7]_n (\text{Gluconic metallic complex}) \quad (2)$$

$$\text{Oxalic acid}: C_2H_2O_4 \rightarrow C_2HO_4^- + H^+ (Pk_{a1} = 1.25) \quad (3)$$

$$C_2HO_4^- \rightarrow C_2O_4^{2-} + H^+ (Pk_{a2} = 4.14) \quad (4)$$

$$n[C_2HO_4^-] + M^{n+} \rightarrow M[C_2HO_4]_n (\text{Oxalic metallic complex}) \quad (5)$$

$$n[C_2O_4^{2-}] + 2M^{n+} \rightarrow M_2[C_2O_4]_n(\text{Oxalicmetalliccomplex}) \quad (6)$$

$$\text{Citricacid}: C_6H_8O_7 \rightarrow C_6H_7O_7^- + H^+(Pk_{a1} = 3.09) \quad (7)$$

$$C_6H_7O_7^- \rightarrow C_6H_6O_7^{2-} + H^+(Pk_{a2} = 4.75) \quad (8)$$

$$C_6H_6O_7^{2-} \rightarrow C_6H_5O_7^{3-} + H^+(Pk_{a3} = 6.40) \quad (9)$$

$$n[C_6H_7O_7^-] + M^{n+} \rightarrow M[C_6H_7O_7]_n(\text{Citricmetalliccomplex}) \quad (10)$$

$$n[C_6H_6O_7^{2-}] + 2M^{n+} \rightarrow M_2[C_6H_6O_7]_n(\text{Citricmetalliccomplex}) \quad (11)$$

$$n[C_6H_5O_7^{3-}] + 3M^{n+} \rightarrow M_3[C_6H_5O_7]_n(\text{Citricmetalliccomplex}) \quad (12)$$

The nearly complete hydrolysis of sucrose and the maximum production of glucose and fructose at the third day indicate that the *A. niger* is in the active growth phase. Therefore after 3 days incubation, the red mud added into the fungal culture under two-step bioleaching. The maximum biomass and minimum pH value were reached at the 10th day of incubation. Therefore the cell-free medium was obtained through filtering the culture after 10 days incubation under spent medium bioleaching.

### 4.3. The change in pH value during various bioleaching conditions

The pH value is a very important parameter in determining the bioleaching efficiency. Therefore, the pH change at different pulp densities under one-step, two-step and spent medium bioleaching were examined (Fig. 2).

In the one-step bioleaching with 1% (w/v) red mud pulp density (Fig. 2a), the initial pH (the value was approximately 9.1) of the suspension gradually decreased to the lowest value of approximate 2.0 after 15 days incubation, and then remain constant for up to 40 days. In the two-step bioleaching containing 1% (w/v) red mud, the initial pH was 5.1 when the red mud added into the three days culture. The pH drastically decreased to the lowest value of 1.9 after 6 days incubation, and then remained constant in the rest time. The change in pH value during spent-medium process was much smaller than other bioleaching process, which is because there is no obvious metabolism activity in the leaching medium. The pH gently increased from 2.6 to 3.2 during 40 days incubation after the red mud (1%) added into the cell-free culture. The slowly increase of pH value in spent medium bioleaching is due to the continuous release of alkaline anions from red mud (Khaitan et al., 2009).

With an increase in red mud pulp densities from 1% to 5% (w/v), the pH value during all the three bioleaching conditions increased. However, the increase extent in each bioleaching process was different. The minimum pH value in one-step bioleaching increased from 2.0 at 1% pulp density to 5.0 at 2% pulp density. The increase extent of pH in two-step bioleaching was lower than that in one-step bioleaching. The minimum pH value increased from 1.9 at 1% pulp density to 3.9 at 5% pulp density. Furthermore, the time to reach the lowest value of pH in two-step bioleaching was also shorter than that in one-step bioleaching at all red mud pulp densities. These phenomena indicate that the production activity of organic acids by leaching fungus was obviously inhibited in one-step bioleaching, especially at high red mud concentration. The higher organic acids production in two-step bioleaching is probably because that the pre-culture of fungus in the absence of red mud is conducive for the production of organic acids and fungal growth (Amiri et al., 2011; Bosshard et al., 1996; Yang et al., 2008). The increase extent of pH value in spent medium bioleaching was the lowest among all the three bioleaching process. The minimum pH value increased from 2.6 at 1% pulp density to only 3.5 at 5% pulp density (though the pH value increased in some degree with the leaching time prolonging). And in the presence of 5% (w/v) red mud, the final pH value was lower than that in one-step bioleaching.

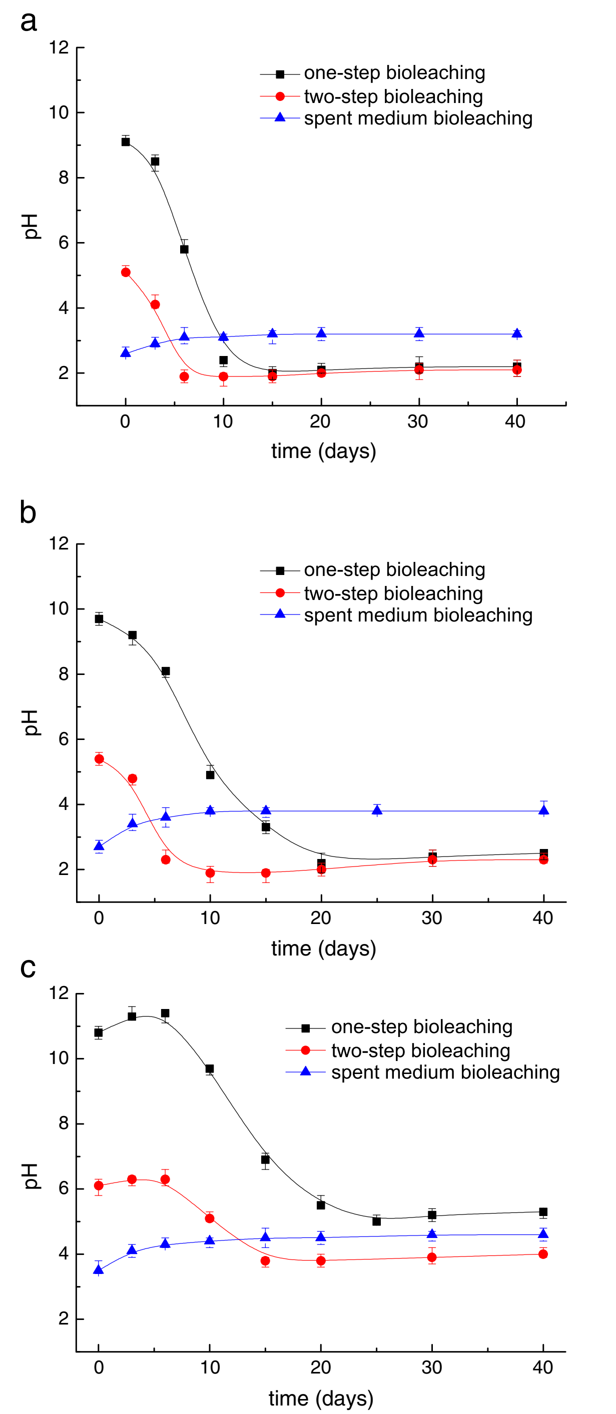


**Fig. 2.** Variation of pH as a function of time during various bioleaching conditionsat different pulp densities of red mud: (a) 1% (w/v), (b) 2% (w/v) and (c) 5% (w/v).The experiments were performed in triplicate.

The previous studies considered that the red mud of 2% concentration will exert severe toxicity to organisms (Pagano et al., 2002). Our results showed that the fungus had a favorable growth condition and organic acids production in the presence of 5% (w/v) pulp density. Therefore, *A. niger* has a potential application for bioleaching of red mud.

### 4.4. Leaching efficiency of heavy metals from red mud under different bioleaching conditions

The leaching ratios of heavy metals under different bioleaching conditions and different red mud pulp densities are shown in Fig. 3. The optimum pulp density in one-step, two-step and spent medium bioleaching were all 1%. The highest leaching ratios of most heavy metals were achieved under spent medium bioleaching at 1% pulp density, with leaching ratios at over 80% of Pb and Zn, 67% of Cu, 50% of Ni, 44% of As, 31% of Ba, 26% of Cr and about 11% of Fe and Zr. The leaching ratios data of fresh sucrose medium and deionized distilled water were not shown due to the negligible extraction.

Different bioleaching conditions have different leaching efficiency orders of heavy metals. The leaching efficiency in descending order for the one-step, two-step and spent medium bioleaching can be roughly arranged as below, respectively, with slightly different.

$$\mathrm{Zn} \approx \mathrm{Ni} \approx \mathrm{Pb} > \mathrm{Cu} \approx \mathrm{As} > \mathrm{Ba} > \mathrm{Fe} > \mathrm{Zr} \approx \mathrm{Cr} \tag{13}$$

$$\mathrm{Zn} > \mathrm{Pb} \approx \mathrm{Ni} > \mathrm{Cu} \approx \mathrm{As} > \mathrm{Fe} \approx \mathrm{Ba} > \mathrm{Zr} \approx \mathrm{Cr} \tag{14}$$

$$\mathrm{Pb} > \mathrm{Zn} \approx \mathrm{Cu} > \mathrm{Ni} > \mathrm{As} > \mathrm{Ba} > \mathrm{Cr} > \mathrm{Fe} \approx \mathrm{Zr} \tag{15}$$

The different leaching ratios between different heavy metals under various leaching conditions are due to: (1) the physical and chemical properties of heavy metals themselves; (2) the solubility of the complexes formed by organic acids and metal ions (Burgstaller and Schinner, 1993); (3) the biosorption and bioaccumulation of heavy metals by the leaching fungus during bioleaching process (Yang et al., 2009); (4) the precipitation of heavy metals to the surface of leaching materials (Wu and Ting, 2006).

From Fig. 3 it can be found that the leaching efficiencies of Pb, Cu and Cr in the spent medium bioleaching were obviously higher than that in one- and two-step bioleaching. This is possibly because the adsorption capacities for the fungus of these heavy metals are comparatively higher than other heavy metals (Volesky and Holan, 1995). They will be highly adsorbed by the fungus after leaching from the red mud. However, the biosorption will not occur frequently in spent medium bioleaching due to the scarce biomass in the leaching medium. Maybe this is an important mechanism that the spent medium bioleaching had better leaching efficiency of heavy metals than other bioleaching conditions. This is inconsistent with Wu's study (Santhiya and Ting, 2005) that the leaching efficiency of spent medium bioleaching is lower when bioleaching of fly ash, but is consistent with Amiri's study (Amiri et al., 2011) when using spent hydrocracking catalyst as the leaching materials.

The increase in red mud pulp densities result in a general decrease in leaching efficiency under each bioleaching process. This is probably due to two reasons: (i) the high concentration of red mud results in high pH value in leaching solution; (ii) the fungal growth is inhibited by the toxicity from red mud under one- and two-step bioleaching, which results in a decrease of metabolites (e.g., organic acids) produced from leaching strains (Aung and Ting, 2005). Precisely because the second reason, with an increase in pulp densities, the decrease extents of leaching efficiency between the three bioleaching conditions were different. The highest decrease extent was occurred in one-step bioleaching, next was the two-step bioleaching. The decrease extent of spent medium bioleaching was the lowest.

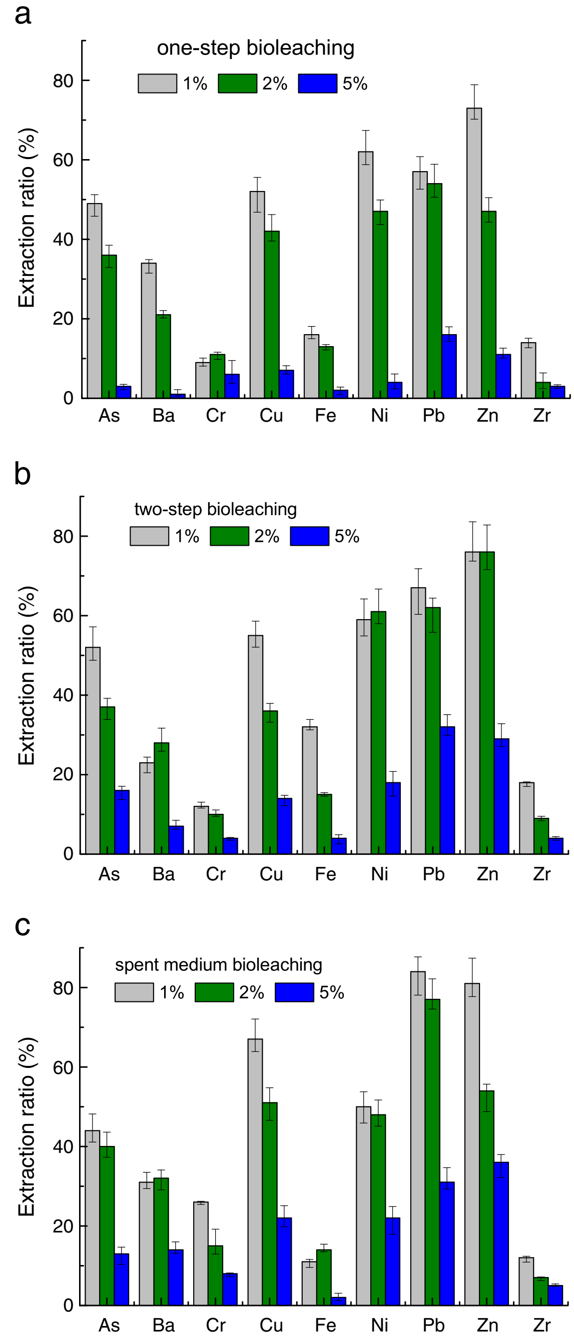


**Fig. 3.** Extraction efficiencies of heavy metals at various pulp densities of red mud under (a) one-step bioleaching, (b) two-step bioleaching and (c) spent medium bioleaching. The experiments were performed in triplicate.

According to our results, the leaching efficiency of spent medium bioleaching was the highest among all the bioleaching conditions, regardless of low or high pulp densities of red mud. Furthermore, the spent medium bioleaching also has other advantages, such as short processing time, easy handling and convenient optimization (Aung and Ting, 2005). Therefore, it can be concluded that the spent medium bioleaching probably is the best choice for leaching heavy metals from red mud when using *A. niger* as the leaching fungus.

### 4.5. TCLP tests of the bioleaching residue

Table 2 shows a comparison of the TCLP test results of the red mud before and after bioleaching process against the identification standard for hazardous wastes enacted by National Environmental Agency of China, recommended acceptance criteria for suitability of industrial wastes for landfill disposal enacted by National Environment Agency of Singapore, and TCLP regulatory level enacted by U.S. EPA, respectively.

The concentration of As in the extract of raw red mud by TCLP tests was found to exceed the regulatory level. In contrast, the concentration of heavy metals in the extract of the bioleaching residue by TCLP tests was reduced to well below the regulatory level. This indicates that the bioleaching process has a prominent effect on reducing the leaching toxicity of heavy metals from red mud. Therefore, the red mud after bioleaching process can probably be disposed of safety or reused in other application fields (e.g., construction materials). However, considering the certain amount of heavy metals leached from raw red mud, it is urgency to develop relevant regulations on the storage and disposal of red mud in order to assess the environment risk as well as to guarantee human health.

### 4.6. Micromorphology analysis of red mud particles and *A. niger*

The micromorphology of red mud particles and mycelium is shown in Fig. 4. The size of individual raw red mud particles is largely different, which is ranging from nano-scale to micron-scale (Fig. 4a). The amorphous and poorly crystalline structure is predominant in raw red mud particle which appear as fluffy aggregates.

However, the morphology of the bioleaching residue particles is much different from the raw particles. More fine grained particles appear, and crystalline structures also occur (Fig. 4b). This is due to a comprehensive effect induced by fungal activity (Lian et al., 2008; Xiao et al., 2012). First, the hyphae will penetrate red mud particles through physical destruction force after the spores germinate and the hyphae elongate. Secondly, the organic acids and amino acids secreted by the hyphae will slowly erode red mud particles through chemical corrosive action. Thirdly, the $CO_2$ produced by respiration during metabolism activity will form carbonic acid when it reacts with water molecule. The carbonic acid can also have corrosive effect on red mud particles like other mineral weathering (Xiao et al., 2012). The final result is the large particles will be split and destroyed to more fine-grained particles through these comprehensive effects of physical destruction and chemical erosion. After bioleaching, the crystalline structures occur, while the non-crystalline structures disappear to some extent in red mud particles. Therefore it is speculated that the non-crystalline structures of the red mud tend to be eroded or damaged easily by the fungal activity, but the crystalline structures are difficult to erode.

When bioleaching was carried out at 1% pulp density (Fig. 4c), most fine red mud particles adhere to the surface zone of mycelium. But if the red mud particle is large, the mycelium can penetrate through the whole particle (marked by the white arrow). This is beneficial for the leaching of metals since it can expand the contacting area between fungus and red mud particles. Furthermore, the large particles have a greater tendency to be broken and turn into small ones by the physical destruction of mycelium, which is also conducive for the bioleaching.

When bioleaching was carried out at 5% pulp density (Fig. 4d), the red mud particles cover almost the whole mycelial surface, which possibly form a thick barrier to impede the fungal metabolic product (e.g., organic acids) escaping to the external space of the fungus, as well as reduce the speed rate to reach the chemical equilibrium and homogeneous state in solution. Therefore, the pH value inside this barrier will possibly decrease faster comparing to the outside when the fungal begins to secrete organic acids. Finally, due to the lower pH in the microenvironment, the actual concentration of heavy metal ions around the spores or the mycelium is probably higher than the measured value of that in solution. The fungal metabolic activities will be fiercely restrained by high concentration of heavy metals inside this red mud barrier during bioleaching process. That is possibly one of the important reason that the fungi can't grow well at high concentration of red mud. However, enhancing the mass transfer rate is possibly a good way to alleviate this negative effect.

## 5. Conclusion

This work has shown that heavy metals from red mud can be mobilized by leaching with *A. niger*. The main lixiviant excreted by the fungi was the citric acid. The highest leaching ratios were achieved under spent medium bioleaching at 1% pulp density. According to our results, the spent medium bioleaching probably was the best choice for leaching heavy metals from red mud when used *A. niger* as the leaching fungus. The TCLP tests showed that through the bioleaching process, the leaching toxicity of red mud decreased obviously. The micromorphology analysis indicates that the appearance of red mud particles is changed by the fungus activity during bioleaching process.

## Acknowledgment

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**Table 2**
TCLP test results of red mud (before and after bioleaching) in comparison with the various regulatory levels.

| Heavy metals | Metal concentration in extraction fluid (mg/L) | | | | |
|---|---|---|---|---|---|
| | Raw red mud | Bioleaching residue[a] | Regulatory levels (China)[b] | Regulatory levels (Singapore)[c] | Regulatory levels (U.S.A.)[d] |
| As | 8.60 ± 0.22 | 0.84 ± 0.03 | 5 | 5 | 5 |
| Ba | 4.83 ± 0.17 | 0.38 ± 0.04 | 100 | 100 | 100 |
| Cr | 5.19 ± 0.09 | 0.10 ± 0.01 | 15 | 5 | ns |
| Cu | nd | nd | 100 | 100 | ns |
| Fe | 2.42 ± 0.21 | 0.31 ± 0.02 | ns | 100 | ns |
| Ni | 3.43 ± 0.10 | 0.09 ± 0.01 | 5 | 5 | ns |
| Pb | 0.61 ± 0.02 | 0.16 ± 0.01 | 5 | 5 | 5 |
| Zn | 15.00 ± 0.85 | 1.30 ± 0.11 | 100 | 100 | ns |
| Zr | 0.96 ± 0.07 | nd | ns | ns | ns |

nd: not detected; ns: not stated in regulation.
[a] Bioleached residue under one-step bioleaching at 1% pulp densities after 40 days.
[b] Identification standard for hazardous wastes—identification for extraction procedure toxicity, National Environmental Agency, China (GB5085.3-2007).
[c] Recommended acceptance criteria for suitability of industrial wastes for landfill disposal, National Environment Agency, Singapore.
[d] "Identification and listing of hazardous waste" U.S. Code of Federal Regulations (CFR), title 40, Chapter 1, Part 261, U.S. Environmental Protection Agency.

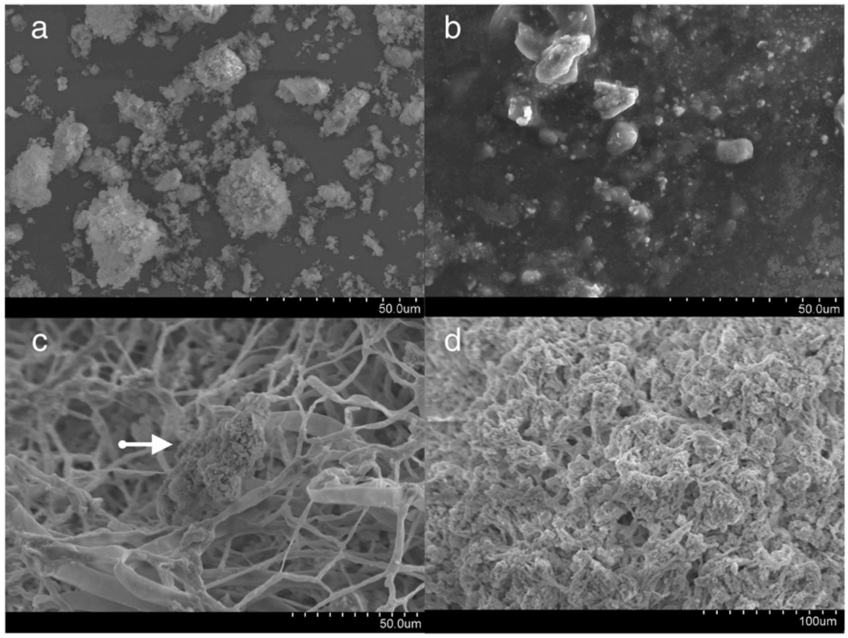


**Fig. 4.** SEM images of (a) raw red mud particles, (b) red mud particles processed after 40 days under one-step bioleaching at 1% pulp density, (c) red mud particles adhering to mycelium at 1% pulp density under one-step bioleaching, and (d) mycelium covering by red mud particles at 5% pulp density under one-step bioleaching. The experiments were performed in triplicate.

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