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Bioleaching of rare earth and radioactive elements from red mud using *Penicillium tricolor* RM-10

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HIGHLIGHTS

- Leaching efficiencies of rare earth and radioactive elements from red mud were test.
- There is a decrease in the leaching ratios with an increase in pulp density.
- The bioleached red mud can meet the European and Chinese radioactivity standards.
- Citric acid and oxalic acid play major roles in the bioleaching of red mud.
- A smooth and layered structure appears in the bioleached red mud particles.

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ABSTRACT

The aim of this work is to investigate biological leaching of rare earth elements (REEs) and radioactive elements from red mud, and to evaluate the radioactivity of the bioleached red mud used for construction materials. A filamentous, acid-producing fungi named RM-10, identified as *Penicillium tricolor*, is isolated from red mud. In our bioleaching experiments by using RM-10, a total concentration of 2% (w/v) red mud under one-step bioleaching process was generally found to give the maximum leaching ratios of the REEs and radioactive elements. However, the highest extraction yields are achieved under two-step bioleaching process at 10% (w/v) pulp density. At pulp densities of 2% and 5% (w/v), red mud processed under both one- and two-step bioleaching can meet the radioactivity regulations in China.

1. Introduction

Red mud (bauxite residue) is the main by-product generated by the caustic digestion of bauxite ores during the production of alumina. The global storage of red mud is currently estimated to be over 2.7 billion tons, with an annual growth rate of approximately 120 million tons (Klauber et al., 2011). The major methods of disposal of such huge amounts of red mud include marine disposal, lagooning, dry stacking and dry cake disposal (Power et al., 2011). However, due to highly caustic, alkaline and radioactive nature of red mud, there are potential problems associated with these disposal methods. These include leaching of alkaline solution from the containing barriers and leaking of red mud slurry due to damage of retaining dams. This can lead to serious damage to the environment. For example, pollution to water, soil and air may result in damage of the ecological structure of the surrounding area. The most notorious tragedy of this kind was the collapse of the red mud reservoir in Ajka (Hungary) in 2010, when $\sim 7 \times 10^5$ m^3 of highly caustic red mud slurry flooded into the surrounding agricultural area, Torna stream and the Marcal river. As a result, ten people were killed and hundreds injured (Gelencser et al., 2011).

Clearly, there is an urgent need to develop safe and effective methods of disposal and utilization of the stored and fresh red mud. Red mud has been reported to have several possible applications (Gräfe et al., 2011; Wang et al., 2008): in the field of pollution control (wastewater treatment, absorption and purification of acid waste gases); as a coagulant, adsorbent, and catalyst; in pigments and paints; in ceramic production; for soil amendment and metal recovery; and for construction materials. However, none of these applications has been commercially applied on an industrial scale. In view of the large volume of red mud stored and produced in the world, utilizing red mud in the field of construction materials is probably the best economic and convenient choice. However, the radioactive nature of red mud has restricted its direct application as a construction material since the radioactivity in the product

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may exceed the relevant radioactivity standards for building materials (Akinci and Artir, 2008; Somlai et al., 2008). Subsequently, red mud can only be mixed with concrete or cement in very low ratios unless the radionuclides can be removed or leached out from the mud.

Red mud is believed by metallurgists to be “a polymetallic raw material with a complex content of oxides of aluminum, iron, titanium, silicon and other valuable components, such as scandium, uranium and thorium” (Klauber et al., 2011). Thus, from this point of view, red mud can be regarded as an “artificial ore”. Due to this special coexistence of aluminum with other precious elements in red mud, it is highly valuable to develop the relevant technology to extract and recover such precious elements from red mud. In particular, rare earth elements (REEs), which trade at very high prices on the international markets and are always treated as strategic resources by many countries, are well worth recovering.

The REEs comprise of the 15 lanthanides (with atomic numbers 57–71), plus scandium and yttrium. They are usually classified into two subgroups: light REEs (atomic numbers of 57–63) and heavy REEs (atomic numbers of 64–71 + 39) (d’Aquino et al., 2009). REEs have applications in various fields such as high temperature superconductivity, safe storage, lasers, magnets, nuclear batteries, X-ray tubes, computer memories, neutron capture and magnetic refrigeration (Jorjani et al., 2008).

The extraction of REEs from red mud has already attracted some attention (Klauber et al., 2011; Petropulu et al., 1996). However, all of these studies have used inorganic acids as the leaching agent to extract REEs. Chemical leaching techniques suffer from the main disadvantages of high energy requirements, high costs, and the potential for environmental pollution, as well as the liability associated with hazardous chemical usage during the treatment (Wu and Ting, 2006).

Compared to conventional chemical leaching methods, a biohydrometallurgical approach is generally considered to be a “green technology” for the extraction of metals from solid materials. In this respect, it is an approach that offers many attractive features such as environmental benignity, operational flexibility and low energy requirements and cost (Burgstaller and Schinner, 1993; Krebs et al., 1997). Furthermore, radioactive elements and other hazardous components can also be removed from the leaching materials during the bioleaching process, which leads to a harmless treatment of the waste materials.

There are two different kinds of microbes used in bioleaching: autotrophic and heterotrophic. Autotrophic microbes are not suitable for bioleaching red mud as the red mud greatly increases the pH of the medium and contains no energy sources (sulfur or reduced iron) for the growth of chemolithoautotrophic bacteria (*e.g.,* acidophilic *Thiobacillus* sp.) (Burgstaller and Schinner, 1993). In contrast, heterotrophic microbes have several advantages as far as leaching metals from red mud. First, they can survive in highly alkaline conditions (Wu and Ting, 2006). Secondly, they can excrete metabolites such as organic acids, amino acids and proteins to form complexes with the toxic metal ions contained in red mud, thus reducing the damage to the metabolic activity of the microbes (Burgstaller and Schinner, 1993; Valix and Loon, 2003). Bioleaching of industrial waste such as fly ash (Bosshard et al., 1996; Wu and Ting, 2006), spent catalyst (Amiri et al., 2011; Santhiya and Ting, 2005), and electronic computer scrap (Brandl et al., 2001) using heterotrophic microbes has been well documented. However, similar studies involving red mud are rare and only relate to bioleaching of Al (Ghorbani et al., 2008; Vachon et al., 1994).

There is currently no data available pertaining to fungal bioleaching of REEs and radioactive elements from red mud. The aim of this study is to focus on, (i) the extraction of REEs and radioactive elements from red mud and, (ii) the reduction of the concentrations of radioactive elements in the red mud to acceptable levels to facilitate residue use in construction applications. In this investigation, in order to increase microbial adaptation and bioleaching efficiency of the red mud, the strains which can excrete the maximum volume of organic acids were isolated directly from the mud. In the next stage, the bioleaching efficiency of REEs and radioactive elements from red mud were investigated under various bioleaching processes and pulp densities. The residual activity of the radionuclides in the bioleached red mud using different bioleaching methods was measured in order to evaluate its potential use in construction materials. Finally, the organic acids produced by the strains were test, and the micromorphology and components of the red mud before and after bioleaching were analyzed.

2. Methods

2.1. Red mud collection

Red mud samples were obtained from the bauxite residue storage area (26°41′N, 106°35′E) of Chinalco in Guizhou province. All the samples were collected using sterile equipment and stored in sterile laminated stainless steel containers. At each sampling location, six sub-samples were collected one by one from points within a horizontal distance of 10 m and used to form a composite sample. The first sample was taken 10–30 cm below the residue surface 30 m away from the dike. It was transported to the lab, dried to constant weight in an oven at 80 °C, crushed and powdered to 200 mesh. This sample was subsequently used for component analysis and bioleaching experiments. A second sample was also taken and used only for isolating microorganisms. It was collected 1 m away from the dike and on the residue surface. It was kept at 4 °C until the separate experiments on microorganisms was carried out.

2.2. Microorganism isolation and screening

The second sample was directly plated on Horikoshi media with 2% (w/v) red mud using the method of serial dilution. The Horikoshi medium comprised of 10 g/L glucose, 5 g/L yeast extract, 5 g/L polypeptone, 1 g/L K_2HPO_4, 0.2 g/L $Mg_2SO_4{\cdot}7H_2O$, 10 g/L Na_2CO_3, and 20 g/L agar and was autoclave sterilized at 121 °C for 15 min before use. Sixteen pure strains were isolated from the red mud and then inoculated into sterilized liquid Horikoshi media with 2% (w/v) red mud. By monitoring the changes in the pH values of the media, a filamentous fungi named RM-10 was found to drastically reduce the pH value of the medium from over 10.0 to 3.0 in 200 h. Therefore, it was chosen from the 16 to participate in the follow-up experiments.

2.3. Bioleaching

The strain RM-10 was cultured on a 3.9% (w/v) potato dextrose agar (PDA) slant. Spores were washed from 7 day old cultures using a sterile solution of physiological saline (9 g/L NaCl). The number of spores was counted using a haemocytometer and standardized to approximately 10^7 spores/mL with sterilized physiological saline solution. A 2 mL portion of the spore suspension was added to 100 mL of sterilized sucrose medium in a 250 mL Erlenmeyer flask. The sucrose medium composition was: 100 g/L sucrose, 0.5 g/L KNO_3, 0.5 g/L KH_2PO_4, 2.0 g/L yeast extract, 2.0 g/L peptone, and 20 g/L agar. All the cultures were incubated at 30 °C and 120 rpm.

Bioleaching was carried out at different pulp densities of red mud. Three series of bioleaching processing were performed: (i) incubating the fungus together with the red mud and medium ('one-step bioleaching'); (ii) pre-culturing the fungus in sucrose medium without red mud for 72 h, and after an obvious increase in biomass, the sterilized red mud was added ('two-step bioleaching'); and (iii) the fungus was first cultured in sucrose medium for 10 days, then the suspension was filtered through a filter membrane (0.2 μm, Whatman) to obtain a cell-free spent medium. Sterilized red mud was then added to the filtrate. The control experiments were conducted using fresh sucrose medium. All experiments were performed in triplicate.

2.4. Analytical methods

The dry red mud (100 g) was mixed with 500 mL of deionized water in a 2 L polyethylene bottle with airtight cover for 16 h. The extracts were collected and used to determine the pH and electrical conductivity (EC) by using a digital pH meter (model PHS-3C) and EC meter (model DDSJ-308A), respectively.

The acid neutralizing capacity (ANC) was determined using a standard procedure for soil titration. A long-term titration lasted 60 days was conducted, and the pH value of titration endpoint was 4.5 (Khaitan et al., 2009). The elemental composition of red mud was determined through total digestion according to US EPA SW 846 Method 3050B method (US EPA, 1996). A quadrupole inductively coupled plasma mass spectrometer (Q-ICP-MS, Perkin-Elmer, ELAN DRC-e) was employed and further analysis was also performed using an X-ray fluorescence (XRF) spectrometer (Unique UX-230).

After the required intervals, samples were taken from each flask, centrifuged at 5000 rps for 10 min and filtered through a filter membrane. The filtered liquor was analyzed for pH value, metal ion concentration and organic acids. The concentration of REEs and radioactive elements in the leachate was analyzed using Q-ICP-MS. The percentage of metal extraction was calculated based on the metal concentration obtained from total digestion.

The organic acid components were investigated using high performance liquid chromatography (HPLC, Agilent 1200) using a variable wavelength detector (210 nm) and Dikma-C18 column, mobile phase of 5 mM H_2SO_4, and flow rate of 0.5 ml/min at ambient temperature.

The biomass was determined using the same method as Aung described (Aung and Ting, 2005). The residue (biomass with bioleached red mud) obtained from the filter paper was transferred to a pre-weighed evaporating dish and dried at 80 °C for 24 h, followed by ashing at 500 °C for 4 h. The dry weight of the biomass was calculated from the difference of the weight under the two temperatures respectively.

The micromorphology of the samples were observed using 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. After that a series of washings with mixtures of water and ethanol were conducted for cell dehydration. Finally the samples were coated with gold and submitted for SEM analysis.

To determine the activity concentration of the residual radionuclides in the bioleached red mud, the residue in the bottom of the flask was collected after the bioleaching process was finished. The residue were centrifuged at 5000 rps for 10 min and washed with distilled water to remove the residual mycelium on the top of the centrifuge tube. Then dried to constant weight at 80 °C, and crushed to a powder. After various counting procedures (Somlai et al., 2008), the concentration of the radionuclides (^{40}K, ^{226}Ra, and ^{232}Th) was determined using a high-resolution gamma-ray spectrometer (KANQ Digital PGS-6000G). The coaxial HPGe (Canberra 7229P-7915–30S) detectors had a relative efficiency of 27% and an energy resolution of 1.9 keV at 1333 keV of ^{60}Co. The detectors were shielded to reduce gamma-ray background. The gamma spectra were recorded by a channel analyser (MAC, SEIKO EG&G 7700). Samples were measured for 60,000–80,000 s. Background measurements were taken under the same conditions of sample measurements and subtracted in order to get net counts.

3. Results and discussion

3.1. Basic chemical characteristics and elemental composition of red mud

The pH, EC and ANC value of the first sample were 12.9, 21.8 mS/cm, and 3.53 mmol H^+/g, respectively. The corresponding values for the second sample were 10.1, 9.3 mS/cm and 3.17 mmol H^+/g, respectively. The high pH and ANC of red mud are due to the numerous types of alkaline minerals (*e.g.*, tri-calcium aluminate, sodium–aluminum–silicate and calcite, *etc.*) containing in red mud. Considering that the volume of external red mud in the residue impounded is tiny and that its chemical character is already influenced to some extent by the ambient environment (the pH, EC and ANC of the second sample were all lower than the first sample), we choose the first sample to use as the material for the subsequent bioleaching research.

Table 1 shows the rare earth, radioactive, major and other minor elements of the red mud samples. The red mud is enriched in scandium, yttrium, lanthanides and cerium, with a uniform total concentration of REEs over 0.26%. The individual concentrations of Sc, Y, La, Ce, Nd, Eu and Tb were all in excess of 0.01%. In view of the fact that some uranium ores containing 0.3% REEs are mined and processed economically in the USA, red mud could be treated as a future resource for sourcing REEs (Smirnov and Molchanova, 1997).

The concentrations of the radioactive elements Th and U in the red mud were 201 and 59 mg/kg, respectively. The radioactive concentration of ^{226}Ra, ^{232}Th and ^{40}K were 310.3 ± 21.7, 219.7 ± 12.0 and 423.9 ± 38.2 Bq/kg, respectively. The total activity concentration of ^{226}Ra and ^{232}Th in the red mud samples is 4–6 times larger than the permissible world average value of 50 Bq/kg specified for building materials (Akinci and Artir, 2008; Somlai et al., 2008).

As several radionuclides contribute to the overall dose, the activity concentration index (I) should be taken into account when deciding whether the red mud can be properly used in construction materials. It can be calculated following the radiation protection code formulated by the European Commission (European Commission, 1999):

$$I = C_{Ra}/300 + C_{Th}/200 + C_K/3000, \tag{1}$$

where C_{Ra} is the activity concentration of radium, *etc.* in Bq/kg. Equivalently, for use in China, using the limits for radionuclides in building materials code GB 6566-2010, (AQSIQ, 2001):

$$I = C_{Ra}/370 + C_{Th}/260 + C_K/4200. \tag{2}$$

The activity concentration indices of raw red mud calculated using Eqs. (1) and (2) were 2.27 and 1.78, respectively, both of which exceeded the safety limit value of 1.0. This means that the red mud can not be directly used in construction materials in both Europe and China unless steps are taken to reduce the concentration of the radioactive elements to a suitable level (*i.e.* less than 1.0).

3.2. Screening and identification of strain RM-10

No strains were isolated from the first sample using the same method used to isolate strains from the second sample. It was also found previously that in fresh red mud from the discharge pipe the microbial biomass is close to zero (Banning et al., 2011). This is due

Table 1
Elemental composition of red mud.

	Digestion	XRF		Degestion	XRF
Rare earth elements (mg/kg)			Major elements (%)		
Sc	158 ± 13	139 ± 7	Al	3.27 ± 0.13	4.48 ± 0.09
Y	266 ± 24	113 ± 19	Ca	11.85 ± 0.92	23.85 ± 0.66
La	416 ± 56	454 ± 22	Fe	8.42 ± 0.40	8.01 ± 0.51
Ce	842 ± 16	477 ± 15	K	0.95 ± 0.08	1.40 ± 0.04
Pr	95 ± 11	nt±	Mg	0.37 ± 0.01	0.82 ± 0.03
Nd	341 ± 9	92 ± 5	Na	5.30 ± 0.22	4.63 ± 0.04
Sm	64 ± 4	nt	Si	4.53 ± 0.09	8.48 ± 0.27
Eu	110 ± 10	nt	Minor elements (mg/kg)		
Gd	56 ± 5	nt			
Tb	184 ± 14	nt	As	125 ± 20	119 ± 9
Dy	48 ± 4	nt	Ba	590 ± 11	690 ± 3
Ho	25 ± 3	nt	Cr	848 ± 4	518 ± 9
Er	28 ± 3	nt	Cu	182 ± 12	76 ± 5
Tm	14 ± 1	nt	Ga	570 ± 28	183 ± 4
Yb	28 ± 2	nt	Ni	169 ± 3	59 ± 1
Lu	14 ± 2	nt	Pb	332 ± 30	61 ± 4
Radioactive elements (mg/kg)			Sr	4230 ± 19	1692 ± 78
			V	4220 ± 66	3185 ± 150
Th	201 ± 17	70 ± 8	Zn	670 ± 189	283 ± 97
U	59 ± 5	13 ± 1	Zr	2070 ± 72	1066 ± 44

nt: not tested, element not quantifiable by the instrument.

to the inherently hostile nature of the red mud towards microorganisms, namely, very low nutrient conditions and fierce toxicity (Gräfe et al., 2011). However, 16 different heterotrophic strains were isolated from the second sample indicating that microorganisms can live on the surface of the red mud or near the dike. This is because in these places the substance exchange between the ambient environment and the red mud are frequent and hence the hostile characteristics of the red mud are alleviated.

It is believed that the main mechanism for bioleaching by heterotrophic microorganisms is acidolysis of organic acid (Burgstaller and Schinner, 1993; Krebs et al., 1997). In order to improve bioleaching efficiency, the strain named RM-10 was chosen from the 16 strains for subsequent studies. This is because it can produce large volumes of organic acids (determined by monitoring the minimum pH value in the culture medium). Strain RM-10 is a filamentous fungi that generates blue spores. The RM-10 nucleotide sequencing data were submitted to the GenBank nucleotide sequence database (accession number JF909351). Comparing the 18S rDNA from RM-10 with previously registered sequences using the Basic Local Alignment Search Tool (BLAST), showed it was 99% similar to *Penicillium tricolor*. Furthermore, taking into account the morphological, physiological and biochemical characteristics, we were able to confirm that strain RM-10 was belong to *P. tricolor* (Frisvad et al., 1994).

3.3. Effect of red mud pulp density on the growth of P. tricolor RM-10 and pH value during different bioleaching methods

3.3.1. Biomass and pH change under one-step bioleaching process

Fig. 1(a and b) shows the biomass and pH changes under one-step bioleaching process. It showed that at 2% pulp density the biomass of RM-10 was the largest and the lag phase duration the shortest. The greater the concentration of red mud, the lower the biomass yielded and the longer the lag phase lasted. However, in contrast to other leaching materials (fly ash, spent catalyst, electronic scrap, *etc.*) where the maximum biomass is present at 0% pulp density (Amiri et al., 2011; Brandl et al., 2001; Wu and Ting, 2006), the biomass of RM-10 at 0% pulp density of red mud was *lower* than that at 2% and the lag phase was also marginally longer. RM-10 did not experience a fiercely toxic effect at 2% red mud pulp density but rather a stimulating effect in comparison to Pagano's results (Pagano et al., 2002). This is probably due to the presence of essential metal ions required for microbial metabolic activity in red mud, and also long-term adaptation of strain RM-10 to the hostile environment.

With increasing pulp density, both the starting and end point pH was elevated. This is due to two reasons: (i) the toxicity restraining microbial metabolic activities is more severe at higher pulp densities; and (ii) the ANC of red mud also increases with increasing pulp density. At 5% or higher pulp density, the pH value increased at the beginning of bioleaching before the strain grew abundantly. This same phenomenon (elevated pH) was also observed at the end of the bioleaching process when the strain is going into its death phase. This is possibly due to the continual dissolution of substantial alkaline minerals in red mud, which can take more than 50 days to reach chemical equilibrium under laboratory conditions (Khaitan et al., 2009).

Compared to previous research which clearly indicates that growth inhibition is experienced in the bacilli isolated from red lake when 4% red mud is present (Vachon et al., 1994), the strain RM-10 can grow well at even higher pulp densities (~10%) and thus it has the potential to be used in the bioleaching of red mud.

Taking into consideration the pH and biomass change, after 3 days of incubation (in the exponential phase of strain RM-10) red mud was added to the culture medium under the two-step bioleaching process. Further, after 10 days of incubation, the cell-free spent medium was obtained to deploy the spent medium bioleaching process (since the acid production had reached its maximum value).

3.3.2. Biomass and pH changes under two-step bioleaching process

Fig. 1(c and d) shows the biomass and pH changes under two-step process. As with the one-step process, as the pulp density increased, the maximum biomass decreased and the pH during the whole leaching process was increased in the two-step process. However, the culture during two-step process reaches the stationary phase earlier compared to the one-step process. Although at 2% pulp density the maximum biomass and acid production was lower compared to the one-step process, at higher pulp densities (10%) the maximum biomass and acid production was significantly greater than in the one-step process. A previous study has postulated that the good growth of strains in the two-step bioleaching

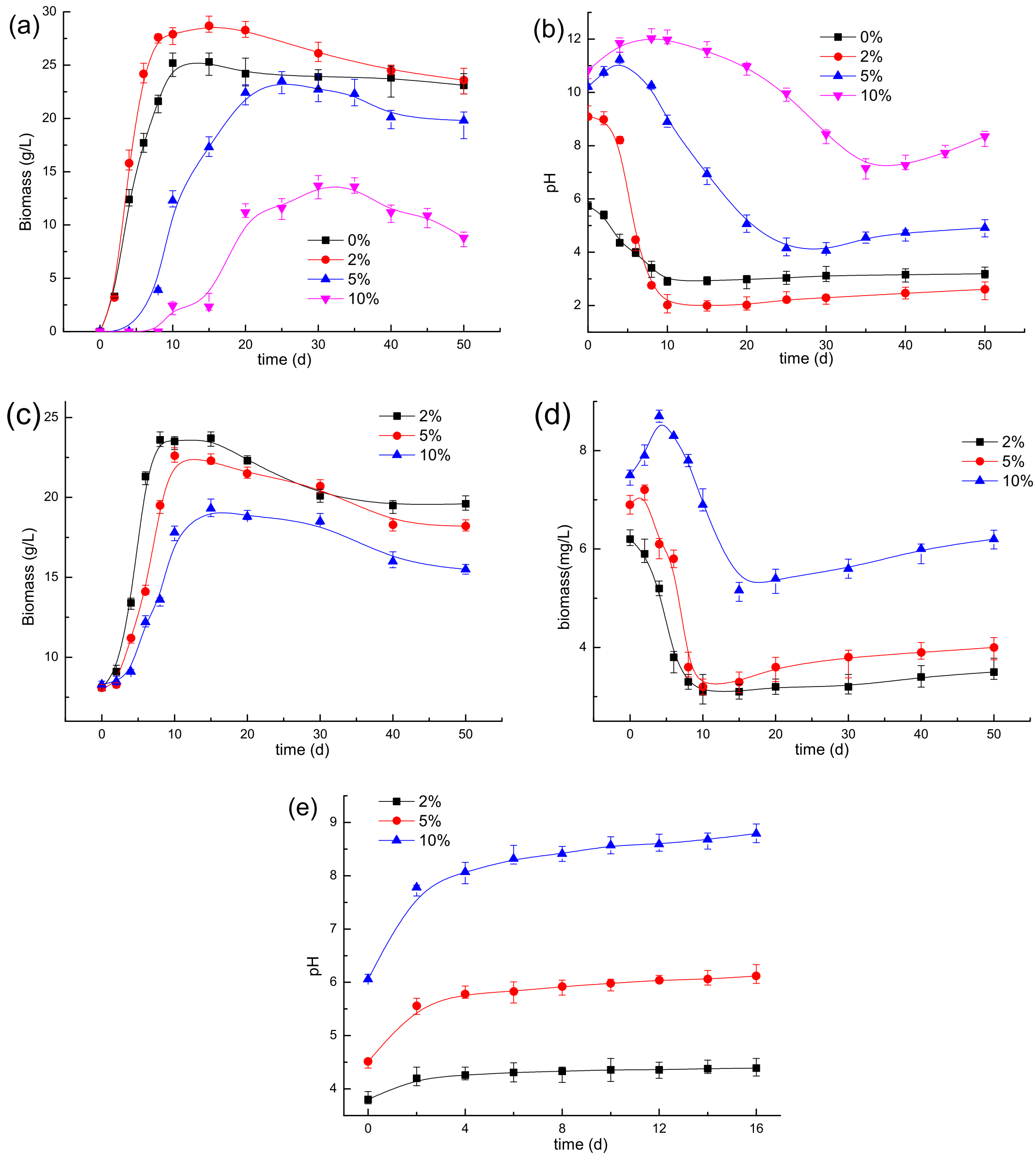

Fig. 1. Change of biomass and pH value at different pulp densities under various bioleaching processes: (a) biomass and (b) pH change in one-step bioleaching; (c) biomass and (d) pH change in two-step bioleaching; and (e) pH change in spent medium bioleaching.

process is due to the higher tolerance of the mycelium to the leaching materials compared to the spores (Yang et al., 2008). This has ignored the fact that the toxicity of the leaching materials can be reduced by metabolic products (citric, oxalic, and amino acids and polypeptides, *etc.*) that have already been secreted by the strains before leaching materials are added to the culture and contact with the strains (Burgstaller and Schinner, 1993). It seems from our results that the two-step bioleaching process is more appropriate for leaching red mud at high pulp densities.

3.3.3. The change of pH value under spent medium bioleaching process

The pH of the spent medium increased throughout the leaching process because of the slow dissolution of the alkaline minerals in the red mud (Fig. 1e). With increasing pulp density, the degree of pH elevation increased. The change in pH of the spent medium was not severe compared to that in the one- and two-step processes. This is due to an absence of strong biotic activity of microbes in the spent medium and thus the amount of metabolic products is tiny.

3.4. Extraction of rare earth and radioactive elements using different bioleaching methods

The leaching ratios of REEs and U and Th under different pulp densities and leaching conditions are shown in Fig. 3. Regardless of the leaching conditions and pulp densities, the leaching ratios generally increased with the atomic number of the REEs except for yttrium and scandium. Also, the ratios for the heavy REEs were clearly higher than those for the light REEs. The reason yttrium has a very high leaching efficiency is that the chemical properties and ionic radius of yttrium are more similar to heavy REEs than to light REEs. The leaching ratios for Sc were also higher than light REEs but lower than Y. The observed leaching pattern is similar to that obtained previously using inorganic acids as the leaching agents (Petropulu et al., 1996). The ascending order of the leaching ratios may result from the gradual decrease in ionic radii of the REEs. The leaching ratios of uranium were higher than thorium's, which is also possibly due to the smaller ionic radius of uranium that makes it more active than thorium.

Table 2
Activity concentrations of residual radionuclides in the red mud after different bioleaching methods at different pulp densities.

Radionuclide activity concentration[a] (Bq/kg)	After one-step bioleaching pulp densities (w/v)			After two-step bioleaching pulp densities (w/v)			After spent medium bioleaching pulp densities (w/v)			Untreated red mud
	2%	5%	10%	2%	5%	10%	2%	5%	10%	
^{226}Ra	102.4	124.1	220.3	108.6	121.0	164.5	142.7	183.1	245.1	310.3
^{232}Th	120.8	136.2	169.2	127.4	132.8	151.6	156.0	171.4	188.9	219.7
^{40}K	114.5	140.0	250.1	122.9	156.8	207.8	190.8	216.2	288.3	423.9
Activity concentration index										
I (Europe)[b]	0.98	1.14	1.67	1.04	1.12	1.38	1.32	1.54	1.86	2.27
I (China)[c]	0.77	0.89	1.31	0.81	0.88	1.08	1.03	1.21	1.46	1.78

[a] Values are the means of three experiments: S.D.s < 10%.
[b] *I* (European): The activity concentration index calculated using Eq. (1), *i.e.* protection 112 code formulated by the European Commission.
[c] *I* (China): The activity concentration index calculated using Eq. (2), *i.e.* following the GB 6566–2010 code formulated by the Chinese Government.

With increasing pulp density, there is a decrease in the leaching ratios of the REEs and radioactive elements in all the leaching methods. The decrease in leaching ratios was highest under the spent medium process and lowest under the two-step process. When 2% and 5% pulp densities were present, the one-step process showed the highest leaching ratios for the REEs. This is probably because as soon as spore germination starts in the one-step process, the strains can start to have an effect on the red mud by secreting metabolic products and causing physical destruction through hyphae growth. Furthermore, previous studies have shown that REEs have a stimulating effect on microbial growth and enzyme activity (d'Aquino et al., 2009). These interactive effects between strains and red mud are more obvious and longer lasting in one-step bioleaching compared to other leaching methods. When 10% pulp density is present, the two-step bioleaching method exhibits the maximum leaching ratios for the REEs. The leaching ratios of the REEs in spent medium bioleaching were almost the same as for one-step bioleaching at 2% pulp density. This is because bio-accumulation and bio-sorption of REEs in microbial cells will not occur obviously in spent medium bioleaching. However, the leaching ratios of the REEs were the lowest compared to one- and two-step bioleaching at 5% and 10% pulp densities, which is in good agreement with a previous study which has postulated that the fungal cells plays an important role in effecting metal extraction (Santhiya and Ting, 2005).

The leaching characteristics of the radioactive elements in the one-step, two-step and spent medium bioleaching were different to those of the REEs. The one-step bioleaching process showed maximum leaching ratios of Th and U at 2% pulp density among all the leaching methods. On the other hand, two-step bioleaching showed the maximum leaching rations at 5% and 10% pulp densities. The spent medium bioleaching process exhibited the lowest leaching ratios. The mechanism for uranium leaching has been attributed to the formation of stable organo-uranyl complexes with citric acid (Nies, 1999).

In general, in one-step process at 2% pulp density, the highest leaching ratios of both REEs and radioactive elements were achieved. It may be that this is conducive to the harmless treatment of red mud to tackle its radioactivity and ensure environmental protection. However, the highest extraction yields of REEs and radioactive elements were achieved under two-step bioleaching at 10% pulp density. This is beneficial for recovering valuable metals for use in metallurgy. The spent medium bioleaching process also showed good leaching efficiency at low pulp densities. It has its own advantages. The red mud will not be polluted by microbial cells and the cultures can be recycled to use in the next leaching process. Also, it can be used at very high red mud pulp densities regardless of the microbial growth. The fresh medium bioleaching process showed the lowest leaching efficiency since there were no functioning strains and thus it is worthless as far as leaching metals from red mud is concerned.

3.5. Radionuclide removal efficiencies using different bioleaching methods

In order to evaluate the removal efficiency of radioactivity by strain RM-10, the radionuclides ^{226}Ra, ^{232}Th, and ^{40}K in the bioleached red mud were determined (Table 2). The residual ratios of the radionuclides' activity concentrations in the bioleached red mud is ^{40}K < ^{226}Ra < ^{232}Th, in ascending order. It means that the radionuclide ^{232}Th is the most difficult to extract and remove from the red mud, and ^{40}K is the easiest. This is probably due to the different groups of the periodic table to which the radionuclides belong (^{226}Ra, ^{232}Th, and ^{40}K belong to the alkaline earth metals, actinides, and alkali metal groups, respectively) and hence different chemical activity.

An increase in pulp density leads to an increase in residual concentration of radionuclide activity for each bioleaching method. At 2% pulp density the best reduction in radionuclide activity was shown by the one-step process, and at 5% and 10% pulp densities the two-step bioleaching was superior. The removal effect under the spent medium bioleaching process was unsatisfactory at all pulp densities.

The residual ratio of ^{232}Th in the bioleached red mud after one-step process at 2% pulp density was approximately 55.0%, which means that the leaching ratio in the filtrate should be 45.0%. However, the actual leaching ratio of Th in the filtrate was only 35.1% (Fig. 2). This implies that approximately 9.9% of the Th was lost (*i.e.* neither in the red mud nor the leaching filtrate). This loss of Th also occurred in the two-step process. However, this phenomenon did not occurred in the spent medium process. Therefore, it can be concluded that the bioaccumulation or biosorption of Th by strain RM-10 is responsible for the Th lost in the bioleaching process. Therefore, bioaccumulation and biosorption probably plays an important role in removing the radioactivity in the red mud during the bioleaching process. There have been several reports relating to bioaccumulation and biosorption of radionuclides (Ra, Th and U) by fungal mycelium and bacterial cells (Volesky and Holan, 1995). However, reports about uptake of radionuclides by microorganisms during bioleaching process are rare and thus the relevant mechanism is not very clear.

If the activity concentration index of a material exceeds the safety limit value of 1.0, it is forbidden to be used directly as a building material. Therefore, only red mud processed after one-step process at 2% pulp density can meet the European criterion. Red mud processed after both one-step and two-step process at 2% and 5% pulp densities can meet the standards required in China.

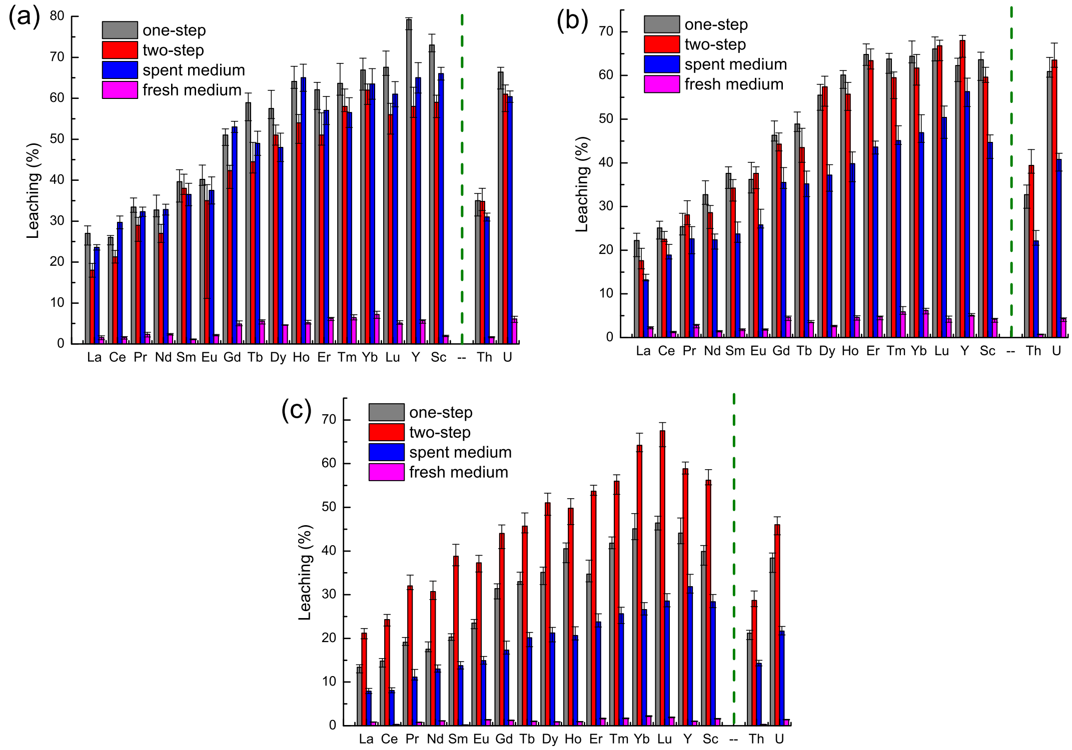

Fig. 2. Leaching ratios of REEs and radioactive elements from red mud under different bioleaching processes and pulp densities (w/v): (a) 2%, (b) 5%, and (c) 10%.

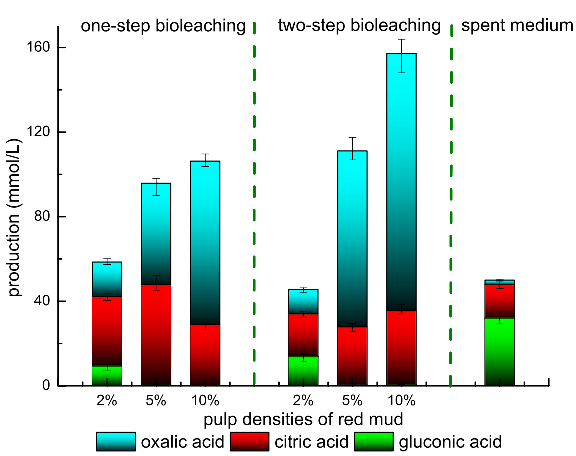

Fig. 3. Organic acids produced by RM-10 under different bioleaching methods at different pulp densities.

3.6. Effect of red mud on the organic acids produced by strain RM-10

Organic acids have two main functions that are very important in bioleaching. First, they can facilitate the dissolution of metals ions from leaching materials through chelation of the metals released in solution and also destabilize the bonds between the surface metal and bulk leaching materials (Gräfe et al., 2011). Secondly, they can reduce the adverse effects metal ions impose on microorganisms through chelation or complexation (Burgstaller and Schinner, 1993). The organic acids produced by RM-10 under different bioleaching processes are shown in Fig. 3.

In spent medium without red mud, the gluconic and citric acid concentrations were 32.0 and 16.7 mmol/L, respectively, but oxalic acid was not found. With increasing pulp density, the citric and oxalic acid concentrations increased but the gluconic acid concentration decreased. This does not accordance with previous studies which have indicated that the citric acid concentration decreases with increasing pulp density if the bioleaching material contains Fe and Mn (Amiri et al., 2011; Burgstaller and Schinner, 1993). The oxalic acid concentration increased to its maximum value of 121.6 mmol/L at 10% pulp density under two-step bioleaching process. This is the highest production amount compared to other bioleaching studies (Amiri et al., 2011; Brandl et al., 2001; Wu and Ting, 2006). It has been reported that the high pH values stimulate oxalic acid production (Bosshard et al., 1996). Oxalic acid can combine with the REEs to form oxalates, resulting in an important detoxification mechanism which promotes further microorganism growth (Burgstaller and Schinner, 1993). The gluconic acid can hardly be detected if pulp density exceeds 5%. This indicates that the glucose oxidase that hydrolyzes glucose to gluconic acid is strongly inhibited by red mud.

The significant increase in the volumes of citric and oxalic acids occurring as the red mud pulp density increases can well explain the phenomenon that even at high red mud pulp densities (5% and 10%) the bioleaching efficiencies of the one-step and two-step processes are prominent. It can be concluded that both citric acid and oxalic acid play major roles in the bioleaching at high pulp densities by using strain RM-10.

3.7. Micromorphology of strain RM-10 and red mud particles

In order to observe the micromorphology of strain RM-10 and red mud, the SEM experiments were conducted. The mycelium pellets of strain RM-10 are homogeneously oval or round with diameters of approximately 500–700 μm The raw red mud are comprised of a multitude of various particles and aggregates in poorly crystalline and amorphous forms, with greatly differing sizes ranging from 0.05 μm to over 50 μm. The smaller particles bond closely to each other and form bigger 'fluffy' aggregates, as described by Gelencser (Gelencser et al., 2011). During the bioleaching process, the red mud particles adhere closely to the mycelium of strain RM-10. However, this phenomenon only occurs on the surface of the mycelium pellet while the internal parts of the mycelium are hardly touched by the red mud particles. After bioleaching, the small and amorphous particles on the surface of the bioleached red mud are severely eroded, leaving a relatively smooth and layered surface with euhedral habits.

4. Conclusions

P. tricolor RM-10 had a favorable survival capability to red mud. The maximum leaching ratios of the REEs and radioactive elements were achieved under one-step bioleaching process at 2% pulp density. However, the highest extraction yields were achieved under two-step process at 10% (w/v) pulp density. Red mud processed under both one-step and two-step bioleaching at 2% and 5% pulp densities met the radioactivity regulations in China. The main lixiviants in the bioleaching process were oxalic and citric acids. After bioleaching, a smooth and layered structure appears in the bioleached red mud particles.

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