African Journal of Biotechnology Vol. 6 (11), pp. 1284-1288, 4 June 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Use of some isolated fungi in biological Leaching of Aluminum from low grade bauxite

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Accepted 17 January, 2006

In this investigation the biological leaching of aluminum by isolated fungi from low grade bauxite (<50% Al_2O_3.) was studied. X-ray diffraction investigation indicated that silicate and aluminosillicate minerals were major mineral composition. Wet chemical and electron disperse X-ray analysis showed the presence of aluminum, iron, silica and titanium. Biological leaching tests were carried out using indigenous specimen fungi, *Aspergillus niger* and *Penicillium notatum*. Savored dextrose chloramphenicol agar (SDA) was used as medium for the selected fungi. All microorganisms were tested for acid-production and leaching capabilities of aluminum from low grad bauxite. Leaching experiments were performed in 250-ml Erlenmeyer flasks at 28°C and at 150 rpm agitation under aseptic conditions. Pry heating of bauxite and its effect on leaching process was studied. Indigenous specimen fungi were the most efficient of the fungal cultures; 7080 mg of Al_2O_3/l was solubilized at 15% pulp density of bauxite. The metal content of leaching solution was determined by using wet chemical and atomic absorption spectrophotometer.

Key words: Bioleaching, low grade bauxite, organic acids, *Aspergillus niger*, Penicillium.

INTRODUCTION

The use microorganisms in leaching and beneficiation of lead grade ores and minerals are widely considered an efficient, economical and environmentally benign alternative to conventional hydrometallurgical operations. Biohydrometallurgy is commercially exploited in the recovery of copper and uranium, and is also used for increasing the recovery of finely disseminated gold from refractory ores like pyrite and arseopyrite (Brierly, 2000). Bacterial oxidation of sulphide ores using chemoauto-trophic bacteria such as *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* is a well-known process in biohydrometallurgy, and thought to occur by a combination of direct and indirect mechanisms (kawatra and Natarajan, 2001). These bacteria attach themselves to the sulphide ore particles and from etch patterns on the mineral surface. This is known as direct attack mechanism. *Thiobacillus* also oxidizes ferrous ions in the solution to produce ferric ions, which indirectly oxidizes the sulphides. This is called indirect mechanism because the bacterium is not directly involved in the leaching process. Biohydrometallurgy of non-sulphidic minerals has received little attention in the past compared to bioleaching of sulphidic minerals by *Thiobacilli* that dominates the the literature. There are several examples of such microbe-mineral systems including bioleaching of aluminum from aluminosilicates, biological removal of iron from sands, kaolins (Groudev et al., 1985), bioleaching of nickel from laterites (Kar et al., 1995), and biobeneficiation of bauxite to remove impurities like silica, calcium and iron (Vasan, 2001). Biobeneficiation differs from bioleaching in that it refers to the selective dissolution of undesirable mineral component(s) from the ore by direct or indirect action of the microbes, thereby enriching the desirable mineral content in the ore.

Bauxite is a heterogeneous material having hydrated aluminum oxide especially gibbsite ($Al_2O_3.3H_2O$) and diaspora (Al_2O_3-H_2O). Bauxite is commonly used in the commercial production of aluminum in the Bayer's process (Henry, 1995). But only high grade bauxite ore can be used in this method (Groudev et al., 1985). Aluminum is virtually in all economic segments but its principal uses have been developed in five major industries

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Table 1. Chemical and mineralogical composition of –200+300 mesh bauxite ore.

Chemical composition	Weight (%)	Mineralogical composition	Weight (%)
Al_2O_3	39.18	Diaspora	30-36
Fe_2O_3	24.91	Goethite	4-6
CaO	0.33	Hematite	7-10
SiO_2	18.56	Calcite	6-9
TiO_2	3.9	Quartz	2-3
		Anatase	3-5
		Rutile	1-2
		Illite	2-4
		Albite	4-6
		Kaolinite	5-10

namely transportation, construction, electrical, containers packing and mechanical equipments (Karavaiko et al., 1989). The use of bioleaching process under these conditions seems promising.

The metabolic process of fungi is similar to a great extent to those of higher plants with the exception of carbohydrate synthesis. The glycol tic pathway converts the glucose into variety of products including organic acids (Nalini and Sharma, 2004). Bioleaching processes are mediated due to the chemical attack by the extracted organic acids on the ores. The acids usually have dual extracted organic acids on the ores. The acids usually have dual effect of increasing metal dissolution by lowering the pH and increasing the load of soluble metals by complexion/chelating into soluble organic-metallic complexes (Vasan et al., 2001). The main objective of this study was to utilize indigenous specimen fungi, *Aspergillus Niger* and *Penicillium notatum* for alumina solubilization from low grade Jajarm Alumina Plant (Iranian Alumina Co.) of bauxite ore. The relevant objectives are mineralogical and elemental analysis of bauxite sample, characterization of organic acids in fermented media by HPLC, shake flask leaching studies of bauxite ore and chemical analysis of leach liquor. This work will open up a new era to beneficiate bauxite through biotechnological route in Iran.

MATERIALS AND METHODS

Ore

Bauxite ore samples were obtained from Jajarm Alumina Plant (Iranian Alumina Co.). The raw bauxite was crushed, ground and screened to select the –200+300 mesh (53-74 m) size fraction, with the chemical and mineralogical composition shown in Table 1.

Fungi

Strains of indigenous fungi (*Aspergillus niger* and *Penicillium*) were isolated from soil and water samples by using sabouroud dextrose choramphenicol agar (SDA) as a solid nutrient media. The media composition is as follows (g/l): peptone, 10; D(+)Glucose, 40; chloramphenicol, 0.5; and agar, 15. The strains were characterized by the colored methods using methylene blue. The *A. niger* isolated from soil sempsum was named PTCC 1001 and the penicillium fungi isolated from water sempsum PTCC 1002.

Acid production process

The ability of fungi in production of acid was tested. The medium for kinetic studies of *Aspergillus niger* is (%) glucose, 50; sodiums nitrate, 1.5; potassium de hydrate phosphate, 0.5; potassium chlorate, 0.025; magnesium sulfate.7H2O, 0.025; and yeast extraction, 1.6. For *Penicillium* it is (%) glucose, 50; sodiums nitrate, 1.5; potassium dihydrate phosphate, 0.5; potassium chlorate, 0.025; magnesium sulfate, 0.025; and yeast extraction, 0.1. All the salts were of analytical grade mineral salt solution was sterilized by autoclaving at 121°C for 15 min and glucose solution was sterilized at same temperature for 5 min. The pH of mineral medium was adjusted to 5.4 with sodium hydroxide (NaOH) using digital pH meter.

Inoculums of *Aspergillus niger* and *Pencillium* were made in the shake flasks containing growth media. These flasks were incubated at 30°C and 150 rpm for 15 days.

Characterization of organic acids in fermented media

The concentration of organic acids produced by *Aspergillus niger* and *Pencillium* strains was determined by high performance Liquid chromatography (HPLC). Separation of citric and oxalic acids was carried out in an CLC-C825 CM caption exchange column; mobile phase, 90% H_2O and 10% CH_3OH; flow rate, 1 ml/min and temperature 35°C.

Pry heating of bauxite

Researches have showed the susceptibility is increased considerably by heating the clays at 600 to 650°C for 1 to 2 h. The treatment caused amotphication of the raw material due to the separation of water form the hydroxylic groups in the crystalline structures of the clay minerals. The heat treatment not only enhances the aluminum leaching also inhibits the iron leaching. The latter effect is considered very important, as iron impedes the subsequent extraction of aluminum from the pregnant solution. In this investigation pry heating of bauxite and its effect on leaching process was studied.

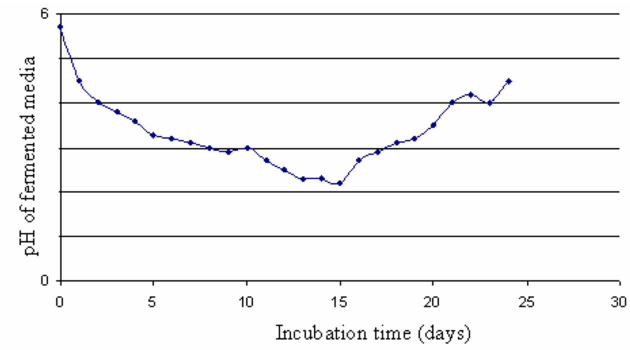

Figure 1. pH profile glucose fermented media by *A. Niger*

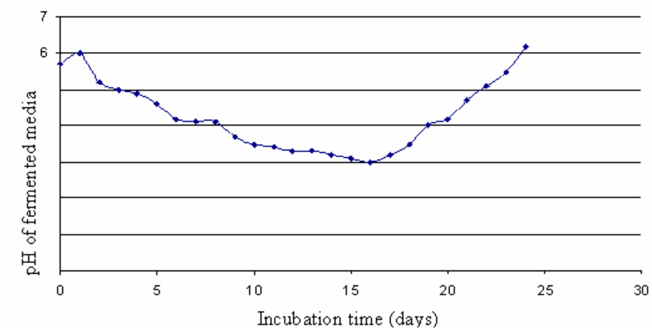

Figure 2. pH profile glucose fermented media by *pencillium.*

Bioleaching studies of bauxite ore

Bioleaching experiment was carried out 250 ml Erlenmeyer flasks cantoning 100 ml of metabolite having bauxite pulp density of 5%. The initial pH of metabolite was in range of 2-2.5. In contrast, chemical sterile control flasks were also included in leaching experiment. All flasks were incubated on shaker at 150 rpm for 24 h. In the time course, samples were removed at intervals and centrifuged to remove solid suspension elements. Soluble content of metal were determined by using atomic absorption spectrophotometer and wet chemical methods. In the bioleaching experiment, the mixture of chlorate and nitrate acids with metabolite was tested.

RESULTS AND DISCUSSION

Fungal growth characteristics

Light yellow colored and tiny beads were appeared after about 20 h of incubation in shaking flasks. Size and number of beads increased in both pencillum and *Aspergillus niger* on 7th day of incubation, In the flasks contain *Penicillum* the hyphae were branched, scale-like with knobby ends. On the condensed mass, small hyphae appeared and the color of hyphae was changed from white to bluish green after three days. This bluish green color was of conidia because the hyphae of penicillium are colorless (Murad et al., 2003)

Changes in fungal morphology during growth of *Aspergillus niger* was also observed. On 12th day, a delicate physiological change of mycelium is reflected in its morphology characterized by appearance of abnormally short, multiply branched, bulbuls hyphae in both cases which remained persistent up to 16th day of incubation. Subsequently citric acid formation under these conditions proceeds rapidly (Murad et al., 2003).

In *Penicilium* decrease in pH from day 12-18 was observed in the range of 3.6-3.2 (Figure 1). pH decreased up to 16th day of incubation from 5.5 to 2.2 case of *Aspergillus niger* (Figure 2). Citric and oxalic acids were mainly produced by the fungi using glucose as energy source (Murad et al., 2003). Decrease in pH was observed due to the organic production via incomplete oxidation of glucose by *Penicillium* species in HPLC was

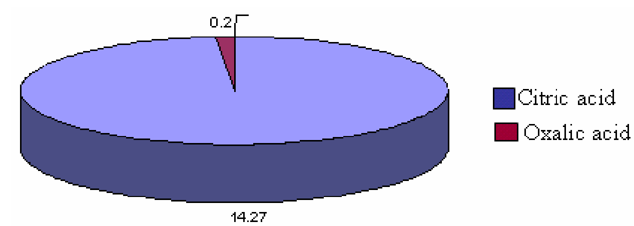

Figure 3. Organic acid concentration in fungal metabolite (*Aspergillus niger*).

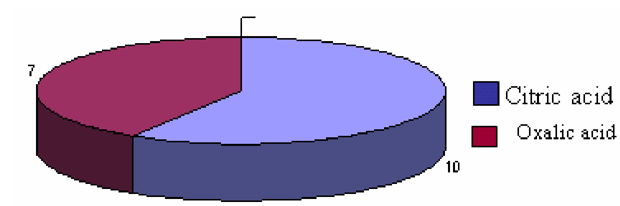

Figure 4. Organic acid concentration in fungal metabolite (*pencillium*)

as:

$$C_6H_{12}o_6 + 4.50_2 \rightarrow 3C_2H_2O_4 + 3H_2O$$
$$\text{oxalic acid}$$

$$C_6H_{12}O_6 + 1.5O_2 \rightarrow C_6H_8O_7 + 2H_2O$$
$$\text{Citric acid}$$

And by *Aspergillus niger* strain as

$$C_6H_{12}O_6 + 1.5O_2 \rightarrow C_6H_8O_7 + 2H_2O$$

$$\text{Citric acid}$$

$$C_6H_{12}O_6 + 4.5O_2 \rightarrow 3C_2H_2O_4 + 3H_2O$$
$$\text{oxalic acid}$$

used for the determination of organic acid concentration in fungal metabolite (Figures 3 and 4). After maximum decrease of pH for about 15-16 days, increase in pH was observed (Figure 1 and 2). This was due to the reason

Table 2. Result of aluminum dissolved in bioleaching by indigenous fungi.

Strain	Organic acids (g/l)		Al_2O_3 (g/l)			
	Citric acid	Oxalic acid	Common	Pry heating	Mixture with nitric acid	Mixture with choleric acid
Aspergillus niger	14.27	0.2	1	2.4	3	7.08
penicillium	10	7	2	6.2	3.	5.01

Table 3. Result of Titanium dissolved in bioleaching by indigenous fungi.

Strain	Organic acids (g/l)		TiO_2 (g/l)			
	citric acid	Oxalic acid	Common	Pry heating	Mixture with nitric acid	Mixture with choleric acid
Aspergillus niger	14.27	0.2	0.13	0.19	0.23	1.9
Penicillium	10	7	0.1	0.25	0.18	0.19

Table 4. Result of Silicon dissolved in bioleaching by indigenous fungi

Strain	Organic acids (g/l)		SiO_2 (g/l)			
	Citric acid	Oxalic acid	Common	Pry heating	Mixture with nitric acid	Mixture with choleric acid
Aspergillus niger	14.27	0.2	6.1	7.9	2.7	2.8
penicillium	10	7	4.1	4	4.8	3

that after complete utilization of glucose, the fungi started to use its own metabolite.

Bioleaching studies of bauxite

pH progressively increased during bioleaching due to alumina soulbilization and became maximum at 8 h. After 8 h, decrease in pH was observed. Such an effect can be justified that increase in pH results in the ability of metabolite to dissolve alumina. Thus after 8 h, no complexation reaction occurred between aluminum and organic acids, and protons of organic acid produced as result of acidolysis were free to cause decrease in pH after 8 h. Penicillium produced oxalic acid than citric acid. It is proposed that reduced glucose flux through glycolysis cause of shift from citrate to oxalate accumulation. The reason for this shift remains unclear but is currently being studied.

For the two strains employed, the alumina solubilization increased with time and reached at its maximum values after 8 h of shaking. The result of maximum alumina solubilization is consistent. The fungi are able to leach metals by acidolysis and complication phenomena. Citric acid is a tricarboxylic acid and contains three carboxylic groups and one hydroxyl group as possible donor of protons (H^+) at 25°C. When aluminum cations (Al^{+3}) are present in system and citric acid is fully dissociated in aqueous solution, a complexation reaction may take place:

$$C_6H_8O_7 \rightarrow (C_6H_5O_7)^{3-} + 3H^+ (pKa_3 = 6.39)$$

$$(C_6H_5O_7)^{3-} + Al^{3+} \rightarrow Al(C_6H_5O_7)$$

Aluminum citrate complex

Similarly oxalic acid contains two carboxylic groups (pKa_1=1.20 and pKa_2=4.20) at 25°C. So the possible complexes of aluminum action with oxalate anion ore:

$$C_2H_2O_4 \longrightarrow (C_2HO_4)^{1-} + H^+ \ (pKa_1=1.20)$$

$$3(C_2HO_4)^{-1} + Al^{3+} \longrightarrow Al\ (C_2HO_4)_3$$

Aluminum oxalate complexes and

$$C_2H_2O_4 \longrightarrow (C_2O_4)^{2\cdot}2H^+ \ (pKa_2=4.20)$$

$$3(C_2O_4)^2 + 2Al^{+3} \longrightarrow Al_2\ (C_2O_4)_3$$

Aluminum citrate complex

Citric and oxalic acids have proved to be efficient leaching agents for alumina solubilization within the optimum conditions. The individual strain differences in the amount of alumina solubilization (g/L) were due to their variability caused by different environmental adaptations or difference in their optical properties. Among other elements analyzed by atomic absorption spectroscopic method are iron (Fe), titanium (Ti) and silicon (Si). Comparison of different fungal strains after 24 h of leaching and aluminum (Al) solubilization is presented in Tables 2 to 5.

The following major Conclusions could be made based on this study. The high concentration of aluminum (7.08 g of Al2O3 /l) was obtained in bioleaching with mixture choleric acid and fungi metabolites. Dissolution of titanium in bioleaching of low grad bauxite was very low (less than 0.5 g/l). The latter effect of pry heating is considered very

Table 5. Result of iron dissolved in bioleaching by indigenous fungi.

Strain	Organic acids (g/l)		Fe_2O_3 (g/l)			
	Citric acid	Oxalic acid	Common	Pry heating	Mixture with nitric acid	Mixture with choleric acid
Aspergillus niger	14.27	0.2	2	1.2	1.2	5.2
penicillium	10	7	4.2	4.2	1.8	3.8

important, as iron impedes the subsequent extraction of aluminum from the pregnant solution. Mixture choleric and nitrate acids with fungi metabolites increases the aluminum solubilization.

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