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Aluminum solubilization from red mud by some indigenous fungi in Iran

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ABSTRACT

Objective: The biological leaching of aluminum (Al) by fungi isolated from red mud, the main waste product of the alkaline extraction of Al from bauxite was studied.
Methodology and results: Biological leaching experiments were carried out using indigenous fungal isolates, *Aspergillus niger* and *Penicillium notatum*. Sabouroud Dextrose Chloramphenicol Agar (SDA) was used as medium for culturing the selected fungi. All microorganisms were tested for acid-production and leaching capabilities of aluminum from red mud. Leaching tests were performed in 250 ml Erlenmeyer flasks at 28°C and 150 rpm under aseptic conditions. Heating of red mud and its impact on the leaching process was also investigated. Indigenous specimen fungi were the most efficient with 2082 mg of Al_2O_3/l solubilized at 15% pulp density of red mud. The metal content of leaching solution was determined using wet chemical and atomic absorption spectrophotometer.
Conclusion and application of findings: Significant amounts of Aluminum and Titanium were obtained through bioleaching processes thus demonstrating that some Iranian fungal isolates have potential application in extraction of metals. Further research could lead to discovery of more efficient isolates and improvement in efficiency of the bioleaching technology.

Key words: Biological leaching, red mud, waste, Bayer process, *Aspergillus niger*, *Penicillium*, fungi.

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INTRODUCTION

Red mud is a chemical waste produced during the alkaline extraction of alumina from bauxite, an ore with a high concentration of aluminium compounds, in a procedure referred to as Bayer process (Dias *et al*., 2004). To produce primary aluminium, the compounds in the bauxite are first dissolved chemically, using caustic soda, in an alumina refinery to produce aluminium oxide. Red mud is a slurry containing natural substances that are originally present in the bauxite. The ore residues, with a residual amount of alkali, are left over after the process. The high concentration of iron compounds gives the waste product its characteristic red colour, and the amount of red mud produced depends on the aluminium content of the bauxite. Bauxite ores with a high aluminium content result in lower ore residues (Yunus *et al*., 2001).

Typical compositions of industrially used bauxite are Al_2O_3 (40- 60%), combined H_2O (12-30%), Fe_2O_3 (7- 30%), SiO_2 free and combined (1-15%), TiO_2 (3-4%), F, P_2O_5, V_2O_5 and others (0.0-5-0.2%) (Josnamayee *et al*., 1998). The caustic insoluble bauxite minerals found in the red muds are typically hematite (Fe_2O_3), which gives the characteristic red colour to mud, and aluminium goethite ((Fe, Al) OOH), along with titanium dioxides (anatase and rutile) and occasionally some boehmite (AlOOH). The presence of silica in red mud ore reduces the extractable amount of aluminium due to the formation of a highly insoluble reaction product, the sodium aluminium silicate, called Bayer-sodalite: $(3(Na_2O.Al_2O_3.2SiO_2.nH_2O).Na_2X)$ where X represents CO_3^{2-}, SO_4^{2-}, $2OH^-$, $2Cl^-$, or a mixture of all, depending on the types of impurities in the digesting ligour (which is sodium hydroxide of industrial grade). Sodalites are zeolite-type compounds with an extremely high ion exchange capacity, which makes red mud a good adsorbent for heavy metals (as oxyanions) and influences the surface properties of red mud slurries (Maria *et al*., 2002).

Different methods of disposing red mud are practiced throughout the world but none of them are known to be environmentally innocuous. For example, in Germany and France, many aluminum plants pump red mud directly into the sea. Studies have demonstrated that a "dead zone" is established in the center of the red mud deposit at the sea bottom. Consequently, only organisms that are resistant to red mud survive near this zone (Maria *et al*., 2002). Another disposal method is to dump red mud into large settling ponds near the alumina plant to form a "red lake", which is commonly practiced in Canada and India. Little information is available about the environmental impact of this disposal method, except that the large content of free alkali in red mud can adversely affect the fertility of adjacent soil (Hulya & Jens, 2002).

Many attempts have been made to find environmentally safe methods of disposing or using red mud. Thakur and Sant, as cited by Hulya and Jens (2002) and Tsakiridis *et al*. (2002) have listed a number of uses for this waste, such as absorbents to remove H_2S from industrial emissions; constituents in building materials such as bricks, ceramics, cement, concrete, and road materials' coagulants to remove phosphate in wastewater treatment; catalysts in coal hydrogenation or in the preparation of anticorrosive materials and pigments particularly for use in marine environments.

More recently, red mud has been used in columns to remove bacteria and viruses from secondary effluents (Tsakiridis *et al*., 2002); as a pH modifier in heap leaching of gold bearing ores and as a neutralizing agent for acid wastes such as those obtained from the production of gypsum or titanium dioxide. All these processes can utilize only a small fraction of the total amount of red mud produced (Hulya & Jens, 2002). Some workers have also tried to recover re-usable substance and/or valuable metals from red mud by using diffrent chemical extraction processes. However, due to their high complexity and /or their elevated cost, none have been feasible on an industrial scale (Nalini & Sharama, 2002). Under these conditions, the use of bioleaching process seems promising.

Many metabolic processes of fungi are similar to a great extent to those of higher plants, with the exception of carbohydrate synthesis. The glycolytic pathway converts glucose into a variety of products including organic acids (Vasan *et al*., 2002). Bioleaching processes are mediated by chemical attack of the ores by the extracted organic acids. 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 organo-metallic complexes (Kawatra & Natarajan, 2002).

The main objective of this study was to utilize indigenous fungal isolates, *Aspergillus niger* and *Penicillium notatum* for alumina solubilization from red mud at the Jajarm Alumina Plant (Iranian Alumina Co.) main waste site. The main objectives were to carry out mineralogical and elemental analysis of red mud samples, characterization of organic acids in fermented media by HPLC, shake flask leaching studies of red mud and chemical analysis of leach liquor. This work would open up new ways of managing red mud wastes in Iran through application of bioleaching technology .

MATERIALS AND METHODS

Mud: Red mud samples were obtained from the Jjajarm Alumina Plant (Iranian Alumina Co.). The mud was dried to constant weight, and after blending, divided for chemical and mineralogical analysis.

Fungi: Strains of indigenous fungi (*Aspergillus niger* and *Penicillium* sp.) were isolated from soil and water samples (Fig. 1, 2, 3) using sabouroud dextrose choramphenicol agar (SDA) as solid nutrient media. The media composition is (g/L): peptone, 10; D(+) Glucose, 45; chloramphenicol, 0.5; and agar, 15. The strains were characterized based on color using methylene blue ($C_{16}H_{18}ClN_3SXH_2O$) and further identification done at the Institut Pasteur d' Iran. *A. niger* isolated from soil was named PTCC 1001 and the *Penicillium* strain isolated from water PTCC 1002.

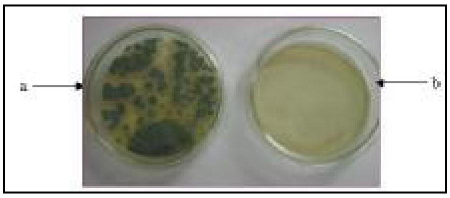

Figure 1: Isolation from water sample on SDA medium. Dilutions are a = 10^{-1}; b= control sample.

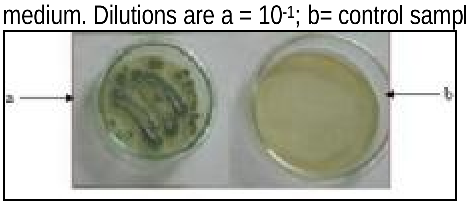

Figure 2: Isolation from soil sample on SDA medium. Dilutions are a = 10^{-1}; b= control sample.


Figure 3: Slant culture of water sample (left) and soil sample (right) on SDA medium.

Acid production process: The ability of fungi to produce acid was tested. The medium composition for kinetic studies of *Aspergillus niger* is (%) glucose, 50; sodium nitrate, 1.5; potassium dihydrate phosphate, 0.5; potassium chlorate, 0.025; magnesium sulfate.7H_2O, 0.025; and yeast extract, 1.6. For *Penicillium* medium composition is (%) glucose, 50; sodium nitrate, 1.5; potassium dihydrate phosphate, 0.5; potassium chlorate, 0.025; magnesium sulfate, 0.025; and yeast extract, 0.1. All the salts were of analytical grade and the mineral salt solution was sterilized by autoclaving at 121°C for 15 min. Glucose solution was sterilized at the same temperature for 5 min. The pH of mineral medium was adjusted to 5.4 with sodium hydroxide (NaOH) using digital pH meter. Inoculum of *Aspergillus niger* and *Pencillium* were made in shake flasks containing growth media, 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.

Heating of red mud: Previous research has shown that solubilization of Aluminum is increased considerably by heating the clays at 600 - 650°C for 1 - 2 h. The treatment causes changes of the raw material due to separation of water from the hydroxylic groups in the crystalline structures of the clay minerals. Heat treatment not only enhances aluminum leaching but also it inhibits iron leaching. The latter effect is considered very important, as iron impedes the subsequent extraction of aluminum from the solution. In this investigation heating of red mud and its impact on the bioleaching process was studied.

Bioleaching studies: Bioleaching experiment was carried out using 250 ml Erlenmeyer flasks containing 100 ml of metabolite having red mud pulp density of 5%. The initial pH of metabolite was in the range of 2 - 2.5. For comparison, chemical sterile control flasks were also included in the leaching experiment. In the sterile control flasks, 5ml of a methanol solution containing 2% thymol were added instead of the inoculum. All flasks were incubated on a 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 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

The chemical and mineralogical composition of red mud was determined (Table 1). Regarding fungal growth characteristics, light yellow colored, tiny beads appeared after about 20h of incubation in shaking flasks. The size and number of beads increased in both *Pencillum* and *Aspergillus niger* cultures by the 7th day of incubation. In the flasks containing *Penicillum* the hyphae were branched, scale-like with knobby ends. On the condensed mass, small hyphae appeared and the color of hyphae changed from white to bluish green after three days. The bluish green color was of conidia because the hyphae of penicillium are colorless (Murad *et al*., 2001).

Changes in fungal morphology during growth of *Aspergillus niger* were also observed. On the 12th day, a change of mycelium occurred as reflected in its morphology, characterized by appearance of abnormally short, multiple-branched, bulbuls hyphae that remained persistent up to 16th day of incubation. Citric acid formation under these conditions has been reported to proceed rapidly (Murad *et al*., 2001). In the *Penicillium* culture a decrease in pH from day 12-18 was observed in the range of 3.6-3.2 (figure 4). pH decreased up to the 16th day of incubation from 5.5 to 2.2 in the case of *Aspergillus niger* (figure 4).

Table 1: Chemical and mineralogical composition of red mud.

Chemical composition	Weight (%)	Mineralogical composition	Weight (%)
Al_2O_3	17	Katoite	38
Fe_2O_3	29.19	Hematite	22
CaO	15.14	Sodium Aluminum Silicate	30
SiO_2	14.52	Anatase	8
TiO_2	9.24		

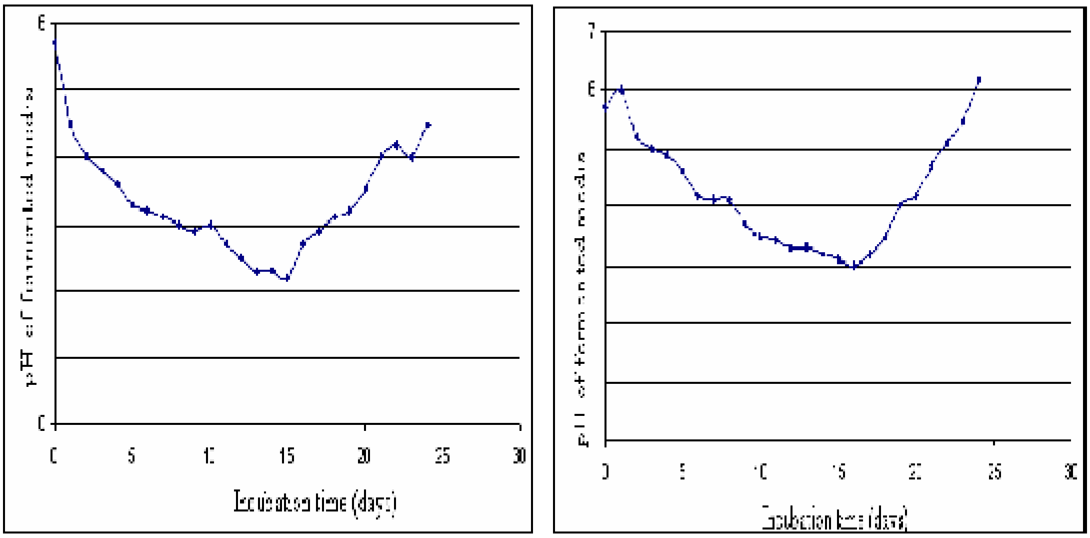

Figure 4: pH profile in glucose media fermented by *A. Niger* (left) and Penicillium (right).

Citric and oxalic acids were mainly produced by the fungi using glucose as energy source (Murad et al., 2001). The decrease in pH was observed due to the organic acid production via incomplete oxidation of glucose by the fungi species as Eq. 1 and 2:

(1) $C_6H_{12}O_6 + 4.5\ O_2 \rightarrow 3C_2H_2O_4$ (Oxalic acid) $+ 3\ H_2O$

(2) $C_6H_{12}O_6 + 1.5\ O_2 \rightarrow 3C_6H_8O_7$ (citric acid) $+ 2\ H_2O$

High performance liquid chromatography (HPLC) was used for the determination of organic acid concentration in fungal metabolite (Figures 5 & 6). After maximum decrease of pH over about 15-16 days, increase in pH was observed (Figure 4). The increase in pH after 15 – 16 days growth could be explained by the fact that after complete utilization of glucose, the fungi started using their own metabolites.

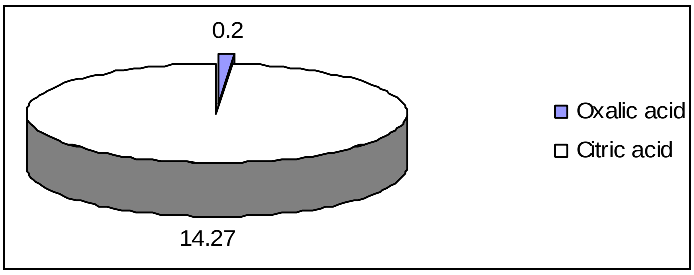

Figure 5: Organic acid concentration(g/l) metabolites of *Aspergillus niger.*

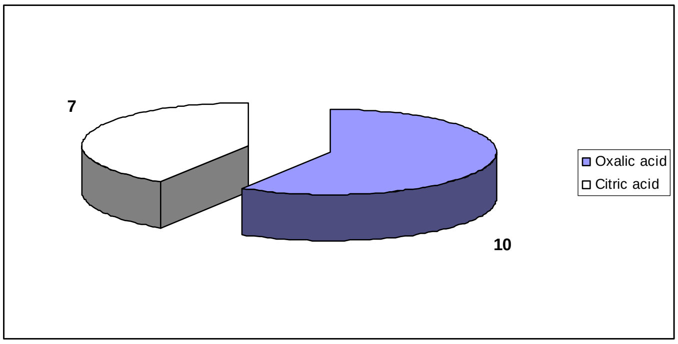

Figure 6: Organic acid concentration(g/l) in metabolites of *Pencillium sp.*

The pH progressively increased during bioleaching due to alumina solubilization, reaching a maximum after 8h, and then it decreased. Increase in pH results in the ability of metabolites to dissolve alumina. Thus after 8h no complexing reaction occurred between aluminum and organic acids, and therefore protons of organic acid produced as a result of acidolysis, which were free, caused a decrease in pH after 8 h. *Penicillium* produced more oxalic acid than citric acid. It is proposed that reduced glucose flux through glycolysis causes a shift from citrate to oxalate accumulation. The reason for this shift remains unclear but it is currently being studied. For the two strains that were studied, the alumina solubilization increased with time and reached its maximum after 8h of shaking.

The result of maximum alumina solubilization is consistent with pH changes observed during the bioleaching expriment. The fungi are able to leach metals by acidolysis and other chemical processes. Citric acid is a tricarboxylic acid that contains three carboxylic groups and one hydroxyl group as possible donors of protons (H^+) at 25°C. When aluminium cations (Al^{+3}) are present in the system and citric acid is fully dissociated in aqueous solution, a complexing reaction may take place:

$C_6H_8O_7$ à $(C_6H_5O_7)^{3-}$ $+3H^+$ (pKa_3=6.39)
$(C_6H_5O_7)^{-3}$ $+Al^{3+}$ à 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:

$C_2H_2O_4$ à $C_2HO_4)^{1-}$ $+H^+$ (pKa_1=1.20)
$3(C_2HO_4)^{-1}$ $+Al^{3+}$ à Al $(C_2HO_4)_3$ [Aluminum oxalate complex] and

$C_2H_2O_4$ à $(C_2O_4)^{2-}2H^+$ (pKa_2=4.20)
$3(C_2O_4)^2$$+2Al^{+3}$ à Al_2 $(C_2O_4)_3$ [Aluminum Citrate complex]

Citric and oxalic acids have proved to be efficient leaching agents for alumina solubilization when conditions are optimum. The differences between strains in alumina solubilization efficiency were more likely due to variability in environmental adaptations or difference in their optical properties. Other elements analyzed by atomic absorption spectroscopic method were Iron (Fe), titanium (Ti) and silicon (Si). A high concentration of aluminum (20.82 g of Al_2O_3 /L) was obtained after bioleaching with a mixture of choleric acid and fungal metabolites (Table 2 – 5). Dissolution of titanium in bioleaching of red mud was about 4 g/L. The latter effect of heating is considered tobe very important in concentration and purification section since iron impedes the subsequent extraction of aluminum from the pregnant solution. Mixing of choleric acid with fungial metabolites increases Aluminum solubilization while mixing nitrate acid with fungal metabolites decreases aluminum solubilization.

Table 2: Elements solubilized from red mud in bioleaching studies using indegenous fungal isolates in Iran.

	Aspergillus niger				*Penicillium*			
	Al_2O_3	TiO_2	SiO_2	$Fe_2O_2$3	Al_2O_3	TiO_2	SiO_2	$Fe_2O_2$3
Common	13.1	0.43	0.14	1.25	18.33	6.8	0.35	5.93
heating	13.44	0.27	0.29	0.38	13.95	0.67	0.18	0.99
Mixture with nitric acid	2.15	0.13	0.1	0.09	7.21	0.38	0.3	0.4
Mixture with choleric acid	19.17	3.9	0.22	12.69	20.82	4.44	0.14	19.74

REFERENCES

Joiret B, Keddam S, Nóvoa M, Pérez XR, Takenouti H, 2004. Passivity of iron in red mud water solutions. Electrochemical Acta 49 (17-18): 3039-3048.

Yunus Ç, Esengul K, Mustafa E, 2001. Recovery and Concentration of Al (III), Fe (III), Ti IV), and Na (I) from Red Mud. Journal of Colloid and Interface Science 244: 342-346.

Josnamayee P, Yasobanta D, Suren D, Rarindra ST, 1998. Adsorption of phosphate from aqueous solution using activated red mud. Journal of Colloid Interface Science 204: 169-172.

Maria TOP, Konstantions SHB, Leonidas NM, Constantion ES, 2002. Pilot- Plant Investigation of the Leaching Process from the Recovery of Scandium from Red Mud. Indy, Egg, Chew 41: 5794-5801.

Hulya G. and Jens C, 2003. Absorption of Arsenate from Water using Neutralizid Red Mud. Journal of Colloid and Interface Scince 264: 327- 334.

Tsakiridis PE, Agatzini-Leonardouu S, Oustadakis P, 2004. Red Mud addition in the Raw Material Production of Portland Cement Clinker. Journal of Hazardous Materials 116 (1-2): 103-110.

Nalini J. and Sharama DK, 2004. Biohydrometallurgy for nansulfidic minerals. Geomicrobiology Journal 21: 135-144.

Vasan SS, Jayant M, Natarajan KA, 2001. Some recent advances in the bioprocess of Bauxite. Int. J. Miner process 11: : 173-186.

Kawatra SK. and Natarajan KK, 2001. Microbial Aspect of Mineral Beneficiation, Metal Extraction and Environmental Control. Mineral Biotechnology 37-54.