Microbiological Leaching of Al from the Waste of Bayer Process by Some Selective Fungi

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ABSTRACT: *In present investigation the biological leaching of aluminum by isolated fungi from red mud, the main waste product of the alkaline extraction of Al from bauxite was studied. Biological leaching experiments were carried out using indigenous, date and orange specimen fungi, Aspergillus Niger, Penicillium simplicissimum 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 red mud. Leaching tests were performed in 250 mL Erlenmeyer flasks at 28 °C and at 150 rpm under aseptic conditions. Preheating of red mud and its impact on leaching process was investigated. Indigenous specimen fungi were the most efficient of the fungal cultures; 2082 mg of Al_2O_3/l was solubilized at 15 % pulp density of red mud. The metal content of leeching solution was determined by using wet chemical and atomic absorption spectrophotometer.*

KEY WORDS: *Biological leaching, Red mud, Waste, Bayer process, Aspergillus niger, Penicillium simplicissimum, Fungi.*

INTRODUCTION

Red Mud is the main waste waste produced by the alkaline extraction of Al from bauxite (Bayer process). Bauxite is an ore with a high concentration of aluminium compounds. This high aluminium content makes bauxite a useful raw material for the extraction of aluminium [1]. To produce primary aluminium, the aluminium compounds in the bauxite is first dissolved chemically, using caustic soda, in an alumina refinery. This produces aluminium oxide. Red mud, which is a slurry containing natural substances originally present in the bauxite. The ore residues with a residual amount of alkali - is left over from the process. The high concentration of iron compounds

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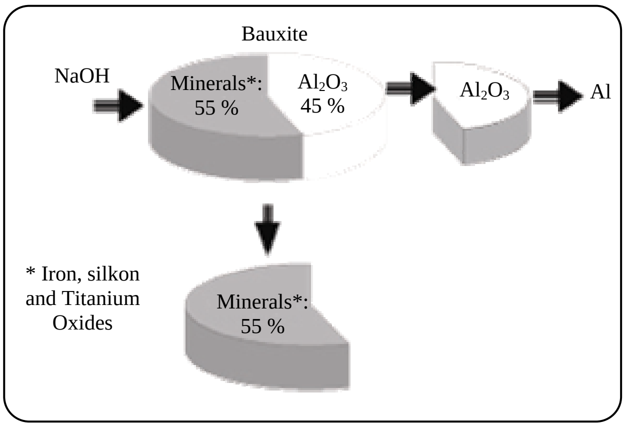

Fig. 1: Aluminium oxide is dissolved out of the bauxite and processed further to aluminium. The residue containing a mixture of naturally occurring minerals is then transferred to a disposal site.

gives the waste product its characteristic red colour. 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 than bauxite ores with a lower aluminium content, see Fig. 1 [2].

Typical compositions for 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, etc(0.0-5-0.2) % [3], the caustic insoluble bauxite minerals composing the red muds are typically hematite , that gives the characteristic colour to red mud, and aluminium goethite, along with titanium dioxides and occasionally some boehmite [4].

Many attempts have been made to find environmentally safe methods to dispose of or use red mud. *Thakur* and *Sant* [5,6], 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 ppreparation of anticorrosive materials and pigments particularly useful in marine environments [6]; 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. Finally, some workers have also tried to recover reausable substance and/or valuable metals from red mud by using diffrent chemical extraction processes but, due to their high complexity and /or their elevated cost, none have been feasible on an industrial scale [7]. 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 [8]. Bioleaching processes are mediated due to the chemical attack by the extracted organic acids on the ores. The acids usually increas metal dissolution by lowering the pH and increasing the load of soluble metals by complexion/chelating into soluble organic-metallic complexes [9]. The main objective of this study was to utilize indigenous specimen fungi, *A. Niger* and *P. Simplicissimum* notatum for alumina solubilization from red mud, Jajarm Alumina Plant (Iranian Alumina Co.) main waste.

The relevant objectives are mineralogical and elemental analysis of red mud sample, characterization of organic acids in fermented media by HPLC, shake flask leaching studies of red mud and chemical analysis of leach liquor. This work will open up a new era to beneficiate red mud through biotechnological route in Iran.

MATERIALS AND METHODS

Red mud

Red mud samples were was obtained from Jajarm Alumina Plant (Iranian Alumina Co.). The red mud was dried to constant weitht, and after blending, divided for chemical and mineralogical analysis. The chemical analysis of red mud sample was carried out by X-ray fluorescence (XEF) and X-ray diffraction technigue (XRD)was used to determin the mineralogical composition of red mud, see table 1.

Fungi

Strains of indigenous fungi (*A. Niger* and *P. Simplicissimum Simplicissimum*) were isolated from soil and water samples of Jajarm Alumina Plant region by using sabouroud dextrose choramphenicol agar (SDA) as a solid nutrient media. The media composition was as follows (g/L): peptone, 10; D (+) Glucose, 45; chloramphenicol, 0.5; and agar, 15. Growth of Strains was studied in static condition as Pry, second and slant strain culture. The strains were characterized by the colored

Table 1: Chemical and mineralogical composition of red mud.

Chemical composition	Wtight (%)	Mineralogical composition	Wtight (%)
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		

methods using methylene blue ($C_{16}H_{18}ClN_3SXH_2O$) and isolated cultures were fungi, was confirmed by Institute Pasteurd' Iran. *A. Niger* isolated from soil sempsum (Dilutions:1/100) was named PTCC 1001 and the *P. Simplicissimum Simplicissimum* fungi isolated from water sempsum PTCC 1002.

Acid production process

The ability of fungi to produce acid was tested. The medium for kinetic studies of *A.Niger* is (%) glucose, 50; sodiums nitrate, 1.5; potassium de hydrate phosphate, 0.5; potassium chlorate, 0.025; magnesium sulfate.$7H_2O$, 0.025; and yeast extraction, 1.6. For *P. Simplicissimum* it is (%) glucose, 50; sodiums nitrate, 1.5; potassium dihydrate phosphate, 0.5; potassium chlorate, 0.025; magnesium sulfate, 0.025; and yeast extrac, 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 *A. Niger* and *P.Simplicissimum* 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 *A. Niger* and *P. Simplicissimum* 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 red mud

Researches have showed Aluminum solubilization 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 [10], in this investigation pry heating of red mud and its impact on bioleaching process was studied and red mud was heated at 650 °C for 1.5 h.

Bioleaching studies of red mud

Metabolite of fungal growth medium was used for shake flaske bioleaching studies of red mud. Bioleaching experiment was carried out in 250 mL Erlenmeyer flasks containing 100 mL of metabolite having red mud 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, in the strile control flasks, 5 mL of a methanol solution containing 2 % thymol were added instead of th inoculum. 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 appeared after about 20 h of incubation in shaking flasks. Size and number of beads increased in both pencillum and *A. Niger* on 7^{th} day of incubation, In the flasks containing Penicillum the hyphae were branched, scale-like with knobby ends. On the condensed mass, small hyphae

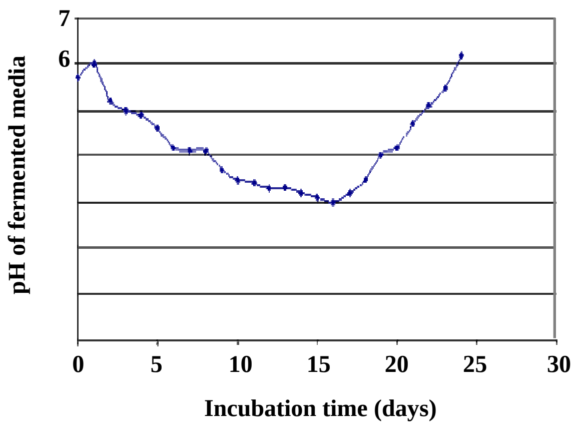

Fig. 2: pH profile glucose fermented media by P. Simplicissimum.

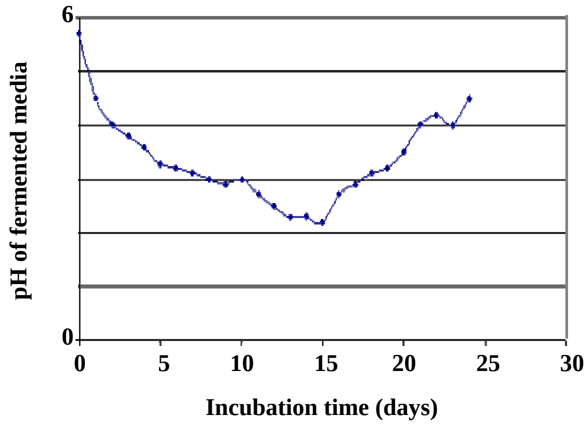

Fig. 3: pH profile glucose fermented media by A. Niger.

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 *P. Simplicissimum* are colorless [4-11] Changes in fungal morphology during growth of *A. 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 [4].

pH Changes during fungal growth

The growth of fungus was followed by noting the pH of media from time to time. In *P. Simplicissimum* decrease in pH from day 12-18 was observed in the range of 3.6-3.2, see Fig. 2. Citric and oxalic acids were mainly produced by the fungi using glucose as energy source [4]. Decrease in pH was observed due to the organic acid production via incomplete oxidation of glucose by *P. Simplicissimum* species which can be formulated as Eqs. (1) and (2):

$$C_6H_{12}O_6 + 4.5\ O_2 \longrightarrow 3C_2H_2O_4 + 3\ H_2O \qquad (1)$$

$$C_6H_{12}O_6 + 1.5\ O_2 \longrightarrow 3C_6H_8O_7 + 2\ H_2O \qquad (2)$$

The accumulation of organic acids by *A. Niger* is markedly influenced by pH. Priodic decrease in pH was observed during these studies. pH decreased up to 16th day of incubation from 5.5 to 2.2 in case of *A. Niger*, see Fig. 3. The equation for production of organic acids by *A. Niger* proceeds through the same mechanism of *P. Simplicissimum*. After maximum decrease of pH for about 15-16 days, increase in pH was observed (Figs. 2 and 3). This was due to the reason that after complete utilization of glucose, the fungi started to use its own metabolite.

The High results for the determination of organic acid concentration in fungal metabolite is shown in Figs. 4 and 5.

Bioleaching studies of red mud

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. *P. Simplicissimum* 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 with pH change observed during bioleacing expriment.

The fungi are able to leach metals by acidolysis and complexation 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.

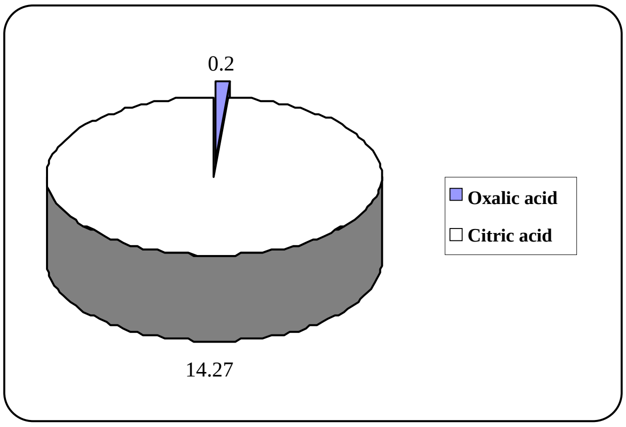

Fig. 4: HPLC analysis data for Organic acidcharactrezation in metabolite of A. Niger (g/L).

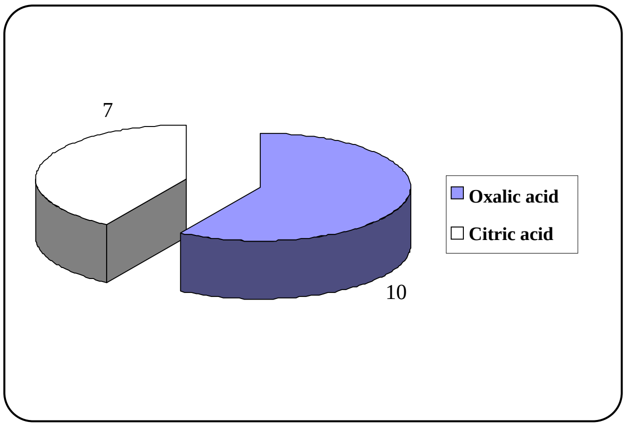

Fig. 5: HPLC analysis data for Organic acidcharactrezation in metabolite of P. Simplicissimum (g/L).

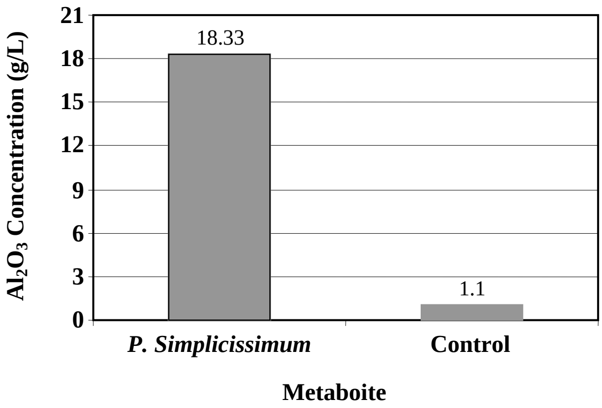

Fig. 6: Concentration of Al_2O_3 during bioleaching of red mudby P. Simplicissimum (g/L).

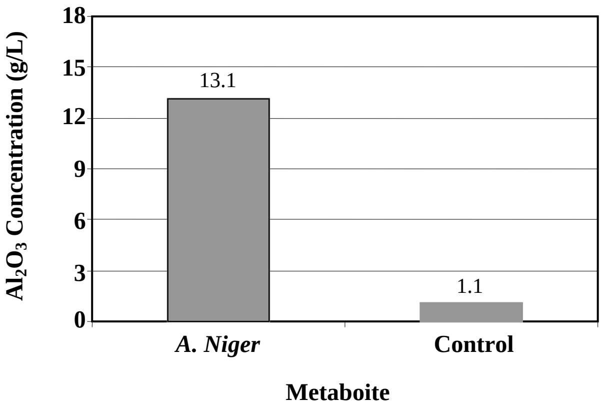

Fig. 7: Concentration of Al_2O_3 during bioleaching of red mud by A. Niger (g/L).

When aluminum cations (Al^{+3}) are present in system and citric acid is fully dissociated in aqueous solution, a complexation reaction may take place can be fprmulated as Eqs. (3) and (4):

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

$$(C_6H_5O_7)^{-3} + Al^{3+} \longrightarrow Al\ (C_6H_5O_7) \quad (4)$$

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 can be fprmulated as Eqs. (5) and (8):

$$C_2H_2O_4 \longrightarrow C_2HO_4)^{1-} + H^+ \ (pKa_1=1.20) \quad (5)$$

$$3(C_2HO_4)^{-1} + Al^{3+} \longrightarrow Al\ (C_2HO_4)_3 \quad (6)$$

Aluminum oxalate complex and

$$C_2H_2O_4 \longrightarrow (C_2O_4)^{2\cdot}2H^+ \ (pKa_2=4.20) \quad (7)$$

$$3(C_2O_4)^2 + 2Al^{+3} \longrightarrow Al_2\ (C_2O_4)_3 \quad (8)$$

Citric and oxalic acids have proved to be good leaching agents for the alumina solubilization (Figs. 6 and 7).

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 typtical 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.

Table 2: Result of Aluminum dissolved in bioleaching of Red mud. Leaching Conditions: Weight of Red mud sample=5 g (5 % pulp density), Volume of metabolite=100 mL, Temprature=22-25 (room temprature), Leaching time= 24 hours (with shaking).

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
A. Niger	14.27	0.2	13.1	13.44	2.15	19.17
P. Simplicissimum	10	7	18.33	13.95	7.21	20.82

Table 3: Result of Titanium dissolved in bioleaching of Red mud. Leaching Conditions: Weight of Red mud sample=5 g (5 % pulp density), Volume of metabolite=100 mL, Temprature=22-25 (room temprature), Leaching time= 24 hours (with shaking).

Strain	Organic acids (g/L)		TiO_2 (g/L)			
	citric acid	Oxalic acid	Common	Pry heating	Mixture with nitric acid	Mixture with choleric acid
A. Niger	14.27	0.2	0.43	0.27	0.13	3.9
P. Simplicissimum	10	7	6.8	0.67	0.38	4.44

Table 4: Result of Silicon dissolved in bioleaching of Red mud. Leaching Conditions: Weight of Red mud sample=5 g (5 % pulp density), Volume of metabolite=100 mL, Temprature=22-25 (room temprature), Leaching time= 24 hours (with shaking).

Strain	Organic acids(g/L)		SiO_2 (g/L)			
	Citric acid	Oxalic acid	Common	Pry heating	Mixture with nitric acid	Mixture with choleric acid
A. Niger	14.27	0.2	0.14	0.29	0.1	0.22
P. Simplicissimum	10	7	0.35	0.18	0.3	0.14

Table 5: Result of iron dissolved in bioleaching of Red mud. Leaching Conditions: Weight of Red mud sample=5 g (5 % pulp density), Volume of metabolite=100 mL, Temprature=22-25 (room temprature), Leaching time= 24 hours (with shaking).

Strain	Organic acids (g/L)		F_2O_3 (g/L)			
	Citric acid	Oxalic acid	Common	Pry heating	Mixture with nitric acid	Mixture with choleric acid
A. Niger	14.27	0.2	1.25	0.38	0.09	12.69
P. Simplicissimum	10	7	5.93	0.99	0.4	19.74

CONCLUSIONS

The following major Conclusions could be made based on this study. The high concentration of aluminum (20.82 g of Al_2O_3 /l) was obtained in bioleaching with mixture choleric acid and fungi metabolites. Dissolution of titanium in bioleaching of red mud was about (4 g/L). The latter effect of pry heating is considered very important in conceitration and purfication section, as iron impedes the subsequent extraction of aluminum from the pregnant solution. Mixture choleric acid with fungi metabolites increases the aluminum solubilizationbut mixture nitrate acid with fungi metabolites decreases the aluminum solubilization.

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