

## Bioleaching Studies of Bauxite Ore Using *Aspergillus niger*

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**Abstract:** The bioleaching behaviour of low-grade bauxite ore of pail deposits (Khushab), Pakistan using different strains of *Aspergillus niger* (NRRL567, NRRL1737 and NRRL605). X-ray diffraction examination of ore revealed the presence of a number of silicate and aluminosilicate minerals. Average content of alumina in test ore was 40%. Decrease in pH i.e. in the range of 2-2.4 was observed due to organic acids produced by *A. niger* strains during growth on liquid media containing glucose. Fungal nutrient requirement was unsatisfied at final media glucose concentration of 5%. Maximum of ~4g/L  $Al_2O_3$  was solubilized having bauxite pulp density 5%. Scanning electron microscopy along with electron disperse X-ray analysis (SEM-EDX) reflected significant alumina solubilization.

**Key words:** Bauxite, bioleaching, *Aspergillus niger*, citric acid, oxalic acid, XRD, SEM-EDX

### Introduction

The term bauxite is used to designate commonly occurring substances i.e. a mixture of several hydrated aluminum oxides with considerable variations in alumina content. Bauxite is much valuable mineral which is commonly used in the extraction of aluminum, manufacture of refractories, ceramics and chemicals (Bateman, 1956).

Aluminum is the latest metal to find general large scale use in modern industry. The world production of aluminum is based on well known Bayer's Process. However, only high grade ores can be treated by this method (Groudev and Groudeva, 1987). The use of bioleaching processes under these circumstances seems promising.

*Aspergilli* utilize at least two different pathways for glucose catabolism i.e. EMP (Embden Meyerhof Parnas) pathway and pentose-phosphate shunt pathway. The formation of di- and tricarboxylic acids mainly occur via glycolysis. Dissolution of mineral is mainly based upon degradative action of acid protons complemented by the formation of stable complexes of organic acids and hence is a combination of acidolysis and complexation phenomena.

The main objective of this work is to utilize acidophilic heterotrophic fungus, *Aspergillus niger* capable of producing organic citric and oxalic acids from oxidation of carbon source by its metabolic activity for alumina solubilization from low-grade Pail-deposits (Khushab) of bauxite ore. The relevant objectives are:

Chemical and mineralogical studies of bauxite sample.

Identification of organic acids in fermented media by HPLC.

Shake flask leaching studies of bauxite.

Elemental analysis of leach liquor.

Evaluation of technico-economic importance of bioleaching for metal solubilization.

This work will open up a new era to benefit bauxite through biotechnological route in Pakistan.

### Materials and Methods

**Bauxite ore:** The representative bauxite ore sample was of Pail deposits. It was obtained from centre for minerals and metallurgy, PCSIR Laboratories, Lahore. It was ground to ≤ 100 mesh particle size.

**Mineralogical and elemental analysis of bauxite:** X-ray diffraction technique (XRD) was used to determine the mineralogical composition of bauxite ore using Rigaku Rint 300 Series diffractometer. Finely ground bauxite ore sample

was analyzed with a glass slide mount using  $Cu.K\alpha$  radiation. A wide range goniometer was used. The diffractometer was equipped with curved graphite monochromator and  $\theta$  compensating slite.

Elemental analysis of ore was carried out (after acid digestion of ore in nitric acid) by standard method (AOAC, 1990). SEM-EDX analysis was conducted to observe the element present at surface of ore sample.

**Fungal strains:** Three strains of *Aspergillus niger* (NRRL567, NRRL605 and NRRL 1737) were kindly provided by Dr.S.W.Peterson, Microbiologist, Northern Regional Research Laboratory, Peroria, Illinois 61604, USA.

**Microbiological growth media:** The media composition for growth kinetic studies of *A.niger* is as follows (all g/dm<sup>3</sup>) glucose, 50;  $NaNO_3$ , 1.5;  $KH_2PO_4$ , 0.5;  $MgSO_4$ , 0.025; KCl, 0.025; and yeast extract, 0.1. Both glucose and mineral salt solutions were sterilized separately at 121°C and 15psi. The pH of medium was adjusted to 5.5 with HCl.

Inoculation of *A.niger* strains (NRRL1737, NRRL605) was made in the shake flasks containing growth media. These flasks were incubated at 30°C and 150rpm for 21days.

**Characterization of organic acids in fermented media:** The concentration of organic acids produced by *A.niger* strains was determined by High Performance Liquid Chromatography technique. Separation of citric acid and oxalic acids was carried out in an Aminex HPX 87H cation exchange column; mobile phase, 5mM  $H_2SO_4$ ; flow rate, 0.5ml/min<sup>-1</sup>;

Table 1: Mineralogical analysis of Bauxite Ore

Minerals identified	Chemical formula
Albite-disordered	$Na(Si_3Al)O_8$
Biotite-2M#1	$KMg_3(Si_3Al)_{10}(OH)_2$
Enstatite-Syn	$MgSiO_3$
Illite-2M#2	$KA_2(Si_3Al)O_{10}(OH)_2$
Kaolinite-1A	$Al_2Si_2O_5(OH)_4$
Microcline ordered	$KAlSi_3O_8$
Muscovite-2M#2	$(K,Na)Al_2(Si,Al)_4$
Orthoclase	$KAlSi_3O_8$
Tridymite-20H-Syn	$SiO_2$
Trona	$Na_3H(CO_3)_2 \cdot 2H_2O$
Wollastonite-1A	$CaSiO_3$
Wollastonite-1A. ferroan	$(Ca,Fe)SiO_3$
Wollastonite-1A. Syn	$CaSiO_3$
Wollastonite-2M	$CaSiO_3$

Table 2: Bioleaching studies of Bauxite Leaching conditions: Weight of bauxite sample = 5g (5% pulp density), Volume of metabolite = 100ml, Temperature = 22-25°C (room temperature), Leaching time = 24 hours (with shaking)

Strains	Organic acids (g/l)		pH of solution before adding bauxite	pH of solution after 24 hours	Al <sub>2</sub> O <sub>3</sub> (g/l)	Fe <sub>2</sub> O <sub>3</sub> (g/l)	Na <sub>2</sub> O (g/l)
	Citric acid	Oxalic acid					
<i>A. niger</i> NRRL 1737	16.13	28.42	2.33	4.65	1.882	0.221	0.938
<i>A. niger</i> NRRL 605	33.45	45.13	2.10	3.03	1.921	0.243	0.268
<i>A. niger</i> NRRL 567	19.65	39.48	2.25	5.71	3.967	0.214	1.072

Table 3: SEM-EDX analysis of bauxite

Elements present	Intensity (counts)	Energy (Kev)
Bauxite ore		
O	109	0.158
Al	157	1.486
Si	172	1.743
Bauxite leach residue		
Ti	-	0.452
O	5232	0.521
Br	5928	1.488
Si	6747	1.746
Ti	1105	4.608

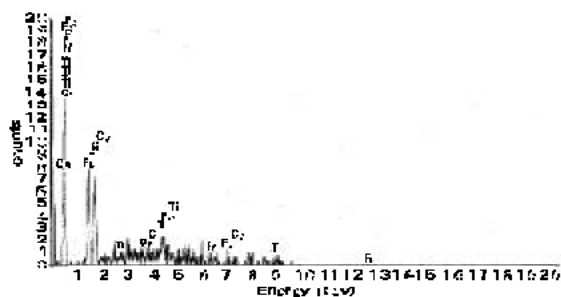


Fig. 1: Spectrum of elemental analysis of bauxite ore

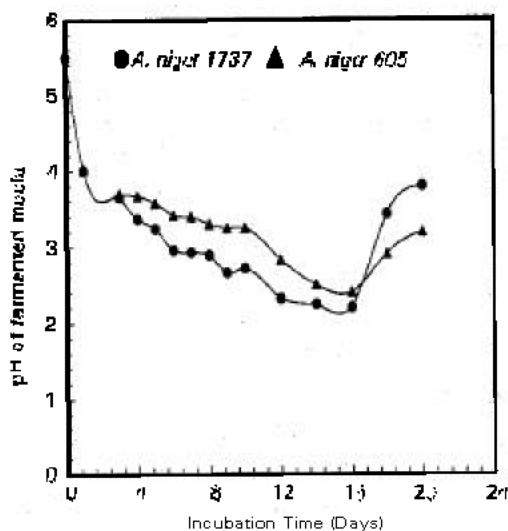
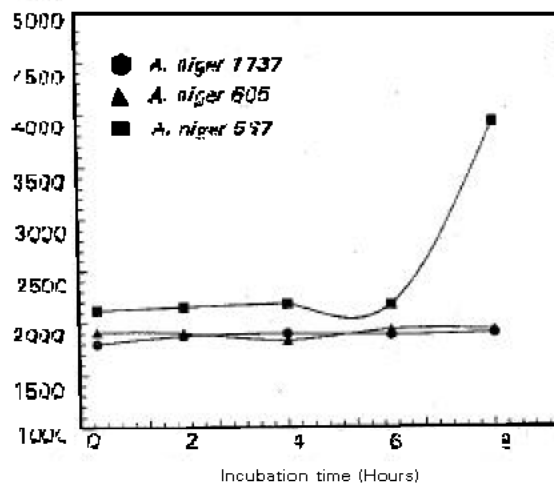
Fig. 2: pH profile glucose fermented media by *A. Niger* temperature, 30°C; detection, UV detector at 213nm

Fig. 3: Al solubilization from bauxite with fermented media in shake flask stud

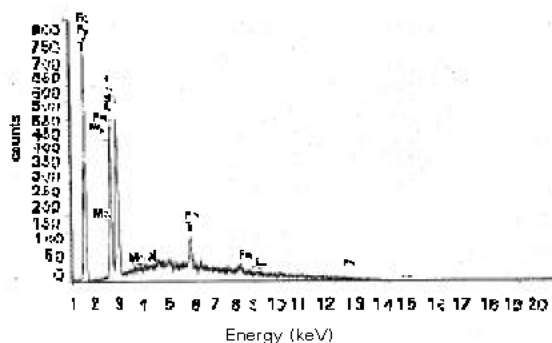


Fig. 4: Spectrum of elemental analysis of leach residue

**Bioleaching studies of bauxite ore:** Bioleaching experiment was carried out in 250ml Erlenmyer flasks containing 100ml of metabolite having bauxite pulp density 5%. The initial pH of metabolite was in the range of 2-2.6. In contrast, chemical sterile control flasks were also included in leaching experiment. All flasks were incubated on shaker at 100rpm for 24 hours. In the time course, samples were removed at intervals and centrifuged to remove solid suspension. Supernatants were analyzed for monitoring pH and dissolved elements.

**Elemental analysis of leach liquor:** Dissolved alumina content in leach solution was determined by spectrophotometric method using eriochrome cyanine R ( $C_{23}H_{16}Na_3O_9S$ ) as chromogenic agent. The 1:1 violet-red complex of aluminum with eriochrome cyanine R can be read at  $\lambda_{max}$ , 540nm (Snell,

1972). Iron (Fe) and sodium (Na) solubilized during bioleaching were analyzed by standard methods (AOAC, 1990).

SEM-EDX analysis of leach residue was conducted to observe the effect of bioleaching on ore concentrate. It was carried out at accelerating voltage of 20keV and beam current of 1 amp.

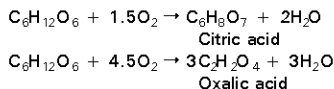
## Results and Discussion

**Mineralogical and elemental analysis of bauxite ore:** The mineralogical analysis of bauxite ore sample reflected the presence of a number of silicate and aluminosilicate minerals. The mineralogical analysis data of ore sample is shown in Table 1. Triplicate samples of bauxite ore were analysed using standard spectrophotometric method. It was found that average content of alumina ( $\text{Al}_2\text{O}_3$ ) in the test ore was 40%. Among other elements present in bauxite ore sample, iron (Fe) and manganese (Mn) were analyzed by atomic absorption spectroscopic method. Their relative abundance in sample was found to be 0.554% ( $\text{Fe}_2\text{O}_3$ ) and  $2.84 \times 10^{-2}\%$  ( $\text{MnO}_2$ ) respectively. Scanning electron microscopy along with electron dispersive X-ray analysis was carried out to observe the elements present at surface of particular area of ore concentrate. The major elements identified were oxygen, aluminum and silicon. Their relative energy and intensity counts have been shown in Table 3. Spectrum of elemental analysis have been shown in Fig. 1.

**Growth kinetic studies of *A. niger*:** Growth kinetic studies of *A. niger* strains was carried out on liquid media containing glucose as carbon and energy source.

**Changes in fungal morphology during growth:** Soon after first day of incubation, yellowish beads in large number appeared in all flasks containing *A. niger* strains, NRRL605 and NRRL1737. Beads in both cases became greater in number and grew larger as time passed upto 7th day. On 12th day, a delicate physiological change of mycelium is reflected in its morphology characterized by appearance of abnormally short, multiply branched, bulbous hyphae in both cases which remained persistent upto 16th day of incubation. Subsequently citric acid formation under these conditions proceeds rapidly (Roehr *et al.*, 1983).

**Changes in pH during growth kinetic studies:** The accumulation of organic acids by *Aspergilli* is markedly influenced by pH. Periodic decrease in pH was observed during these studies. pH decreased upto 16th day of incubation from 5.5 to 2.20 in case of *A. niger* NRRL1737 and from 5.5 to 2.40 in case of *A. niger* NRRL605. According to Bosshard *et al.* (1996), pH was taken an indicator of growth. Decrease in pH was observed due to organic acid production via incomplete oxidation of glucose by *A. niger* strains as:



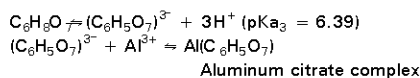
The HPLC results of 6th day (144 hours) of incubation indicated that among organic acids produced, concentration of citric acid and oxalic acid was 14.35g/L and 0.258g/L for *A. niger* NRRL605 and 14.17g/L and 8.43g/L for *A. niger* NRRL1737 respectively. As pH of fermented media was in the range of 2-3, so these conditions were more favourable for citric acid production. pH profile of glucose fermented media is shown in Fig. 2.

After 16th days of incubation, pH of fermented media started increasing and went on increasing upto 21 days, then studies were terminated. The reason for increase in pH was that the fungal strains grew actively and consumed all glucose, then they started utilizing organic acids produced in order to satisfy their nutritional requirements. So an increase in pH was

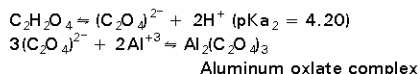
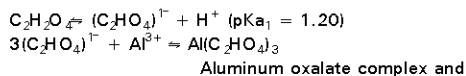
observed.

**Bioleaching studies of bauxite:** pH value has progressively increased during bioleaching due to alumina solubilization and became maximum upto 8 hours. After 8 hours, decrease in pH was observed. Such an effect can be justified that increase in pH the ability of metabolite to dissolve alumina. Thus after 8 hours, 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 hours. The concentration of organic acids in metabolites produced by all the three strains of *A. niger* have been characterized by HPLC. It was observed that concentration of oxalic acid was more than citric acid. It is proposed that reduced glucose flux through glycolysis is cause of shift from citrate to oxalate accumulation. The reason for this shift remains unclear but is currently being addressed (Leangon *et al.*, 1999).

For all three strain employed, the alumina solubilization increased with time and reached at its maximum value after 8 hours of shaking. The result of maximum alumina solubilization is consistent with pH change observed during bioleaching experiment. The fungal micro-organisms 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 ( $\text{H}^+$ ) at 25°C (Sillen and Martell, 1964). When aluminum cations ( $\text{Al}^{3+}$ ) are present in system and citric acid is fully dissociated in aqueous solution, a complexation reaction may take place:



Similarly oxalic acid contains two carboxylic groups ( $\text{pK}_{\text{a}1} = 1.20$  and  $\text{pK}_{\text{a}2} = 4.20$ ) at 25°C. So the possible complexes of aluminum cation with oxalate anion are:



Citric and oxalic acids have proved to be much 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 differences in their optical properties. Among other elements analysed by atomic absorption spectroscopic method are iron (Fe) and sodium (Na). Comparison of different fungal strains after 24 hours of leaching is presented in Table 2. Alumina solubilization for different fungal strains during first 8 hours is presented in Fig. 3.

SEM-EDX analysis of leach residue revealed the presence of significant amounts of titanium (Ti), oxygen (O), bromine (Br) and silicon (Si). The intensity counts for aluminum metal were not detected. EDX can only detect metals within a particular area if their relative abundance is greater than 0.1%. Aluminum was found to be overlapped with significant quantities of silicon (Si) and bromine (Br) within that particular area as indicated by the spectrum (Fig. 4). Intensity counts and

relative energies of elements identified are shown in Table 3

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