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Bioleaching of Bauxite by *Penicillium simplicissimum*

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Abstract: The research work deals with the examination of the dissolution of aluminum from low grade bauxite of pail deposits (Khushab) with different penicillium strains. X-ray diffraction data indicated the presence of silicate and aluminosilicate minerals. Scanning electron microscopy and electron disperse X-ray analysis showed the presence of aluminum iron silica and titanium. Decrease in pH in the range of 3.40 was observed in liquid growth media inoculated with *Penicillium* strains (*Penicillium simplicissimum* and *Pinicillium* sp. UAF1) due to the production of organic acids. About 4.6 g l^{-1} Al_2O_3 was solubilized having bauxite pulp density 5%.

Key wards: Bauxite, *Penicillium*, bioleaching, XRD, SEM-EDX

Introduction

Bauxite is a heterogeneous material having hydrated aluminum oxide especially gibbsite ($Al_2O_3.3H_2O$) and diaspora ($Al_2O_3.H_2O$) (Kurtz, 1980). Bauxite is commonly used in the extraction of aluminum and most popular method for the commercial production of aluminum is Bayer's process (Pearson, 1955). But only high-grade bauxite ore can be used in this method (Groudev and Groudeva, 1987). Aluminum is virtually in all economic segments but its principal uses have been developed in five major industries namely transportation, construction, electrical, on containers packing and mechanical equipments (Khan and Hussain, 1970). 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 exception being the process of carbohydrate synthesis. The glycolytic pathway converts the glucose into variety of products including organic acids (Nawaz, 1992). Bioleaching processes are mediated due to the chemical attack by the 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 complexing/chelating into soluble organo-metallic complexes (Burgstaller and Schinner, 1993).

The main objective of this study was to utilize *P. simplicissimum* for alumina solubilization from low grade pail deposits (Khushab) 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 Pakistan.

Materials and Methods

Bauxite ore: The research work was conducted from 15th June to 30th October 2000 in Physical Chemistry and Hi-tech Laboratory of University of Agriculture Faisalabad (UAF). The representative bauxite ore sample was of Katha Pail deposits. It was obtained from the institute of Geology, University of Punjab, Lahore. The sample was ground to ≤100 mesh particle size.

Chemical and mineralogical analysis of bauxite: Chemical analysis of bauxite ore sample was carried out (after acid digestion of ore in nitric acid) by standard method (Anonymous, 1990). SEM-EDX analysis was conducted to observe the elements present at the surface of ore sample. X-ray diffraction technique (XRD) was used to determine the mineralogical composition of bauxite ore by using Rigaku Rint 300 series diffractometer and JCPDS diffraction software. A finally ground dried sample was analyzed as glass slide mounts using the Cu-Kα-radiation 40 Kv and 20 mA and a wide range goniometer equipped with a diffracted beam curved graphite monochromator and 20 compensating slit.

Fungal strains: A strain of *P. simplicissimum* was obtained from National Institute of Biotechnology and Genetic Engineering, culture collection and P*enicillium* sp. UAF1 was locally isolated.

Growth media: The medium for the growth kinetic studies of *Penicillium* strains were based on the composition which is as follow: (all g dm^{-3}) glucose, 50; $NaNO_3$, 1.5; KH_2PO_4, 0.5; $MgSO_4.7H_2O$, 0.025; KCl, 0.025 and yeast extract, 1.6. 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 about 5 min. The pH of mineral medium was adjusted to 5.4 with sodium hydroxide (NaOH) using digital pH meter.

Inoculum of *Penicillium* strains (*P. simplicissimum* and *Penicillium* sp., UAF1) was made in the static and shake flasks containing growth media. Both these flasks were placed at 30°C and shaking flasks were placed at the 150 rpm at room temperature.

Growth kinetic studies of *Penicillium* sp.: Growth of fungal species of *Penicillium* in liquid media was studied in shaking and static conditions using glucose as energy source.

Characterization of organic acids: The concentration of organic acids produced by *Penicillium* sp. was determined by high performance liquid chromatography (HPLC). Separation of citric acid ($C_6H_8O_7$) and oxalic acid ($C_2H_2O_4$) was carried out in an Aminex HPX 87H cation exchange column, mobile phase 5 mM H_2SO_4, flow 0.5 ml min^{-1}.

Shake flask bioleaching studies: Metabolite of fungal growth medium (*P. simplicissimum*) was used for shake flask bioleaching studies of bauxite. The pH of metabolite was 2.69 and Erlenmeyer flask containing 100 ml metabolite, having bauxite ore pulp density 5% was used.

Chemically, sterile control flask was also included in leaching experiment. Both the flasks were incubated on shaker at 100 rpm for 24 h. In the time course, samples were removed at regular intervals and centrifuged to remove solid suspension. Supernatants were analyzed for monitoring pH and dissolved elements.

Chemical analysis of leach liquor: Dissolved contents of different metals in leach solution were determined by using spectrophotometer. Alumina contents were determined by using UV visible spectrophotometer (HITACHI, UV-280, Japan). Eriochrome cyanine R ($C_{23}H_{15}Na_3O_9S$) was used as chromogenic agent which form stable water soluble complex with aluminum.

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The 1:1 violet red complex of aluminum with eriochrome cyanine R can be read at λ_{max} 540 nm (Snell, 1972). Soluble iron (Fe), sodium (Na) and potassium (K) content were determined by standard method (Anonymous, 1990) using atomic absorption spectrophotometer (HITACHI, 2-8200, Japan).

Results and Discussion

Chemical and mineralogical analysis of bauxite ore: The major elements identified at the surface of bauxite ore were oxygen (O), aluminum (Al) and silicon (Si) (Table 1). Spectrum of EDX analysis is shown in Fig. 1. Elemental analysis of ore by atomic absorption spectrophotometer showed the presence of ore by atomic absorption spectrophotometer showed the presence of different metal elements like Fe, Na and K. The concentrations of these metal elements in sample were 2771.42, 634.76 and 56.9 ppm, respectively.

X-Ray diffraction data indicated that bauxite ore contained oxide minerals, silicate minerals like enstatite syn ($MgSiO_3$) and alumino-silicate minerals like orthoclase ($KalSi_3O_8$) (Table 2).

Morphological changes: Light yellow coloured and tiny beads were appeared after about 24 h of incubation in shaking flasks. Size and number of beads increased in both *P. simplicissimum* and *Penicillium* sp. UAF1 on 7th day of incubation, small hyphae appeared on the surface of beads. The hyphae were branched, scale like with knobby ends, growth of mycelia was more prominent in *Penicillium* sp. UAF1. In static conditions filamentous mass was formed in both species after 24 h, which became condensed later on subsequently citric acid formation under these conditions proceeds rapidly (Roehr *et al*., 1983). On the condensed mass small hyphae appeared and the colour of hyphae was changed from white to bluish green, after 72 h. This bluish green colour was of conidia because the hyphae of *Penicillium* are colourless (Nawaz, 1992).

pH changes during fungal growth: Noting the pH of media from time to time followed the growth of fungus. In *P. simplicissimum* (static and shaking) decrease in pH from 12-18 days was observed in the range of 3.6–3.2 (Fig. 2). During the growth of *Penicillium* sp. UAF1, pH decrease was from 5.4 to 3.4 in about 15 days (Fig. 3). Citric and oxalic acids were mainly produced by the species of *Penicillium* by using glucose as energy source (Groudev and Groudeva, 1983). Decrease in pH was observed due to the organic acid production via incomplete oxidation of glucose by *Penicillium* species as.

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

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

HPLC was used for the determination of organic acid concentration in fungal metabolite (Fig. 4,5). After maximum decrease of pH for about 15 days, increase in pH was observed (Fig. 2,3). This was due to the reason that after complete utilization of glucose, fungus started to use its own metabolite.

Bioleaching studies of bauxite ore: The twenty day metabolite had pH, 3.4 (Fig. 2). The concentration of $C_6H_8O_7$ and $C_2H_2O_4$ was 14.14 and 7.67 g l^{-1}, respectively (Fig. 5). The pH value has progressively increased during bioleaching due to the consumption of organic acids for alumina (Al_2O_3) solubilization. Decay *et al.* (1981) reported that wide range of microbes are capable of degrading rock and non-sulphide minerals generally due to the production of organic acids. The pH decreased for about 8 h, after 24 h increase in pH was observed, this was due to mineralogical alterations.

Table 1: EDX analysis of bauxite ore sample

Elements identified	Intensity (count)	Energy (Kev)
Aluminum (Al)	100	1.426
Oxygen (O)	84	0.527
Silicon (Si)	90	1.749

Table 2: Mineralogical analysis of bauxite sample Silicate

Mineral identified	Mineral formula
Albite disordered	$Na(Si_3Al)O_8$
Biotite-2M#1	$KMg_3(Si_3Al)O_{10}(OH)_2$
Diaspora	$Al_2O_3.H_2O$
Enstatite Syn	$MgSiO_3$
Gibbsite	$Al_2O_3.3H_2O$
Illite 2M#2	$KAl_2(Si_3Al)O_{10}(OH)_2$
Kaolinite-1A	$Al_2Si_2O_5(OH)_4$
Microcline ordered	$KAlSi_3O_8$
Muscovile-2M#2	$(K,Na)Al_2(Si,Al)_4O_{10}(OH)_2$
Orthoclase	$KAlSi_3O_8$
Tridymite-20H Syn	SiO_2
Wallastonite	$CaSiO_3$
Wallastonite-1A	$CaSiO_3$
Wallastonite-1A ferroan	$(Ca,Fe)SiO_3$
Wallastonite-1A Syn	$CaSiO_3$
Wallastonite-2M	$CaSiO_3$

Table 3: Solubilization of different element oxides during bioleaching with time

Time (h)	pH	Fe_2O_3 (mg L^{-1})	Na_2O (mg L^{-1})
0	6.76	54.34	607.50
2	7.02	47.19	580.50
4	7.12	48.62	621.00
6	7.14	58.63	634.50
8	7.20	51.48	607.50
24	5.88	58.63	594.00

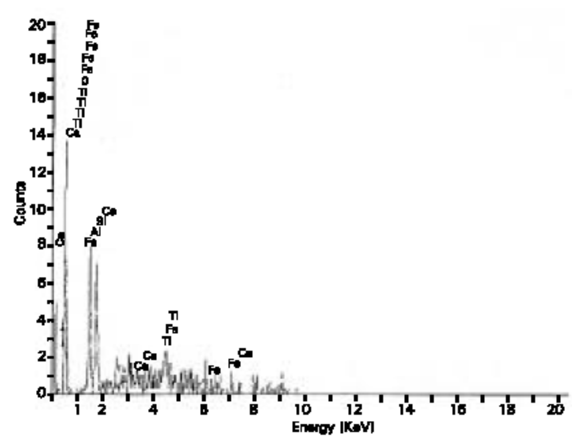

Fig.1: EDX spectra of bauxite ore sample

Fungus is able to leach metals by acetolyses and complexation reactions. Citric acid contains three carboxylic groups (Pka_1= 3.13, Pka_2= 4.76, Pka_3= 6.39) and one hydroxyl group (Pka_4= 13.82) at 25°C as possible donor of proton [H^+]. When $C_6H_8O_7$ was dissociated in aqueous solution and aluminum ions (Al^{+3}) present in the system, a complexation reaction may take place. The overall chemical reaction is as follow:

$$C_6H_8O_7 \rightarrow (C_6H_7O_7)^{-1} + H^+ \quad (Pka_1 = 3.13)$$
$$(C_6H_7O_7)^{-1} \rightarrow (C_6H_6O_7)^{-2} + H^+ \quad (Pka_2 = 4.76)$$
$$(C_6H_6O_7)^{-2} \rightarrow (C_6H_5O_7)^{-3} + H^+ \quad (Pka_3 = 6.39)$$
$$(C_6H_5O_7)^{-3} + 3Al^{+3} \rightarrow (C_6H_5O_7)_3Al$$
Aluminum citrate

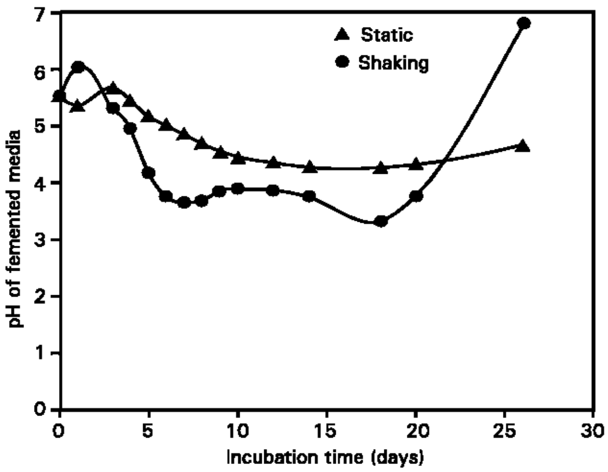

Fig. 2: pH profile during the oxidation of glucose by *P. simplicissimum*

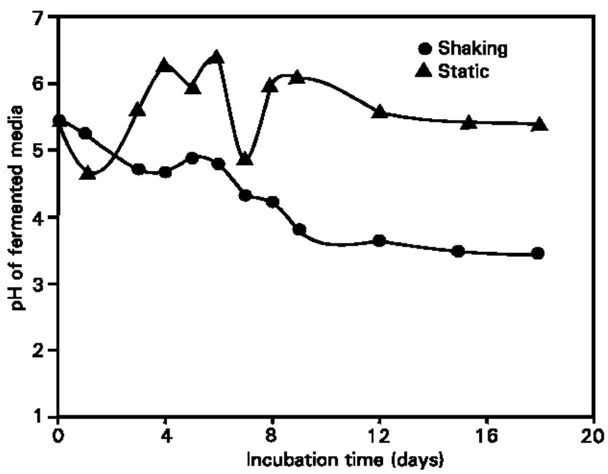

Fig. 3: pH profile during the oxidation of glucose by *Penicillium* sp. UAF1

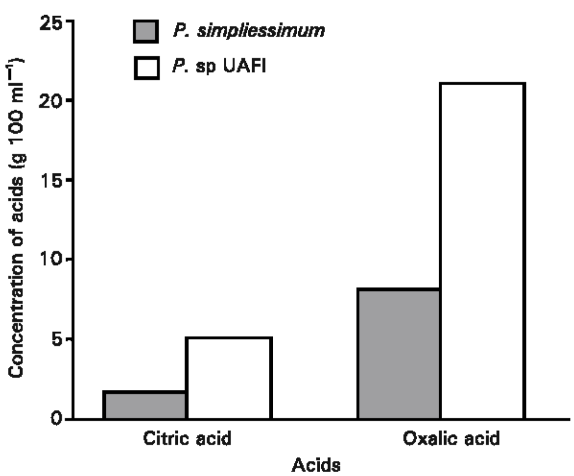

Fig 4: HPLC analysis data for organic acid characterization in *Penicillium* strains (6 day metabolite)

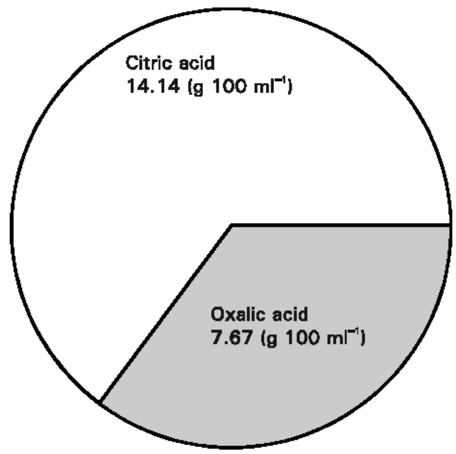

Fig. 5: HPLC analysis data for organic acid characterization in metabolite of *P. simplicissimum* (20 day metabolite)

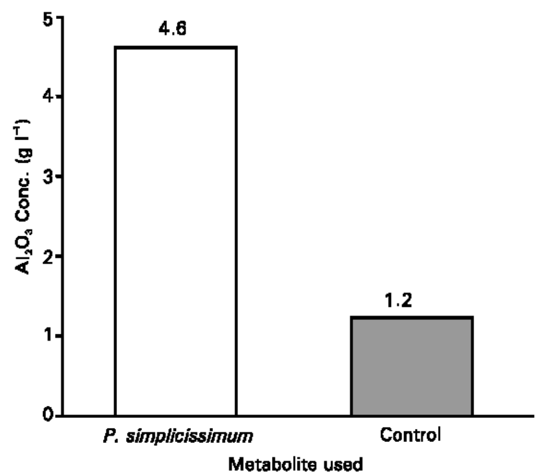

Fig.6: Concentration of Al_2O_3 during bioleaching of bauxite by *P. simplicissimum*

Oxalic acid contains two carboxylic groups ($Pka_1 = 1.2$ and $Pka_2 = 4.2$) at 25°C as possible donor of proton (H^+). The chemical reaction which cover the dissolution of aluminum from ore in leaching system is

$$C_2H_2O_4 \rightarrow (C_2HO_4)^- + H^+ \ (Pka_1 = 1.2)$$
$$(C_2HO_4)^- \rightarrow (C_2O_4)^{-2} + H^+ \ (Pka_2 = 4.2)$$
$$3(C_2O_4)^{-2} + Al^{-3} \rightarrow Al_2(C_2O_4)_3$$

Aluminum oxalate

These acids have proved to be good leaching agents for the alumina solubilization. Concentration of solubilized alumina is shown in Fig. 6.

Atomic absorption spectrophotometric analysis: Concentrations of different metal elements (mg l^{-1}) like Fe, Na and K were determined by atomic absorption spectrophotometric analysis (Table 3).

Reasonable amount of alumina and other elements were leached out from low grade bauxite ore. This is easy and cheap method for metal solubilization. So this will open up a new era to beneficiate bauxite through biotechnological route in Pakistan.

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