

Bioleaching Studies of Rock Phosphate Using *Aspergillus niger*

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Abstract: The bioleaching behavior of rock phosphate (Jordan imported) was studied using different strains of *Aspergillus niger* (567, 1737 and NRRL 605). X-ray diffraction analysis revealed the presence of fluorapatite [$\text{Ca}_2(\text{PO}_4)_3\text{F}$] as the main source of phosphorus (P). Average content of phosphorus in test ore was 33.6%. Scanning electron microscope showed the presence of significant amount of phosphorus (P). Decrease in pH was observed due to organic acids produced by *Aspergillus niger* strains during growth on liquid media containing glucose. Maximum of 30% P O and 34% U O was solubilized having rock phosphate pulp density 2%.

Key words: Bioleaching, rock phosphate, *Aspergillus niger*, citric acid, oxalic acid, XRD, SEM-EDX, Pakistan

Introduction

Rock phosphate is commonly called "rock" in sedimentary deposits and apatite in igneous deposits. Rock phosphate consists of insoluble calcium phosphate [$\text{Ca}_3(\text{PO}_4)_2 \cdot \text{CaF}_2$] generally known as apatite (Habashi, 1980). Phosphorus is an important element of rock phosphate. It is used in electric furnaces with calcined phosphate rock, silica and coke. Elemental phosphorus (P) is used to produce high purity compounds like sodium, calcium, potassium and ammonium phosphates (Ahmed and Siddiqui, 1992). Phosphorus plays vital role in plant nutrition (Hayman, 1975).

Recently, efforts are being made to utilize acidophilic heterotrophs (bacteria and fungi) for metals extraction from oxides ores for those ores which contain calcite (CaCO_3) and dolomite [$\text{CaMg}(\text{CO}_3)_2$] as gangue minerals. *Aspergillus niger* 3 2 has been found to produce organic acid (e. g. citrate, oxalic acids) that can serve as leaching agents for the solubilization of metals. Bioleaching processes are based on the ability of microorganisms (bacteria and fungi) to transform solid compounds resulting in soluble and extractable elements, which can be recovered (Torma, 1988).

The aim of present studies is to observe the leaching behavior of phosphorus, uranium and some other associated metal ions from rock phosphate with *Aspergillus niger*. The relative objectives are: a) Mineralogical and elemental analysis of rock phosphate b) Identification of organic acids in fermented media c) Shake flask leaching studies of rock phosphate d) Analysis of leach liquor.

Materials and Methods

Rock phosphate: A representative sample of rock phosphate was obtained from Institute of Engineering and Fertilizers Research (IEFR), Jaranwala Road, Faisalabad (2000). The sample was oven dried and was ground to # 300-mesh particle size. The practical work was done in the Department of Chemistry, University of Agriculture, Faisalabad.

Mineralogical and elemental analysis of rock phosphate: X-ray diffraction technique (XRD) was used to determine the mineralogical composition using a Rigaku Rint 300 Series diffractometer and the JCPDS diffraction software. A finely ground rock phosphate was analyzed as top fill mounts using Cu-K α -radiation and a wide range goniometer equipped with a diffracted beam monochromator and a compensating slit. 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 the surface of ore sample.

Fungal strains: Three strains of *Aspergillus niger* (NRRL 567, 605 and 1737) were kindly provided by Dr. S.W. Peterson, Microbiologist, Northern Regional Research Laboratory, Peoria, Illinois 61604, USA.

Microbiological growth media: The media composition for growth kinetic studies of *A. niger* is as follows: (all g/dm³) 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 15 psi. The pH of medium was adjusted to 5.5 with HCl. Inoculation of *A. niger* strains (NRRL 1737 and 605) was made in the shake flasks containing growth media. These flasks were incubated at 30 °C and 150 rpm for 21 days.

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, 0.5 ml/min.

Bioleaching studies of rock phosphate: Bioleaching experiment was carried out in 250 ml Erlenmeyer flasks containing 100 ml of metabolite having rock pulp density 2%. 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 100 rpm for 24 hours. In the time course, sample were removed at intervals and centrifuged to remove solid suspension. Supernatants were analyzed for monitoring pH and dissolved elements.

Elemental analysis of leach liquor: Phosphorus (P) was determined in rock phosphate sample and leach solutions by a standard spectrophotometric method using Barton's reagent (Jackson, 1962). Phosphorus forms a golden yellow complex having a λ_{max} 430 nm. Dissolved uranium contents in leach suspension were determined by spectrophotometric method using Arsenazo-III as chromogenic reagent (Bhatti *et al.*, 1991). An intense pink violet complex of chromogenic reagent with uranyl ion (UO_2^{2+}) can be read at λ_{max} 655nm.

Results and Discussion

Mineralogical and elemental analysis of rock phosphate sample reflected the presence of fluorapatite with other accessory minerals such as illite, gypsum, kaolinite etc. The XRD data for the rock phosphate sample is summarized in Table 1. Bhatti *et al.* (1999) reported that autinite was uranium mineral with mineral formula of [$\text{Ca}(\text{PO}_4)(\text{UO}_2)_2$] present in this rock sample. The sample contained a water insoluble phosphate mineral. The elemental analysis of uranium content in rock phosphate sample was found to contain 0.012%, U_3O_8 and 33.6% P_2O_5 . The elemental analysis of the exposed surface of the rock phosphate (P) under the SEM showed that phosphorus (P) oxygen (O), calcium (Ca) and silicon (Si) are apparently present elements in the test material (Table 2). The spectrum of elemental analysis is shown in Fig. 1.

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

Change in fungal morphology during growth: During the growth of these fungal strains in liquid media, different sizes of beads of white color in large number were formed in the inoculated flasks. In case of *A. niger* NRRL 605 smaller beads were formed than *A. niger* NRRL 1737

under similar experimental conditions. During the growth studies, a slight change in morphology of fungal strains was reflected due to appearance of abnormally short, multiple branched, bulbous hyphae, after 12 day of incubation.

Changes in pH during growth: The accumulation of organic acids by *Aspergilli* is markedly influenced by pH. Periodic decrease in pH was

Table 1: Mineralogical analysis of rock phosphate sample

Mineral identified	Mineral formula
Fluorapatite	$\text{Ca}_5(\text{PO}_4)_3\text{F}$
Albite, orderd	$\text{NaAlSi}_3\text{O}_8$
Illite	$\text{K}(\text{OH})_2(\text{SiAl})_4\text{O}_{10}$
Gypsum	$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$
Kaolinite	$\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$
Talc-2M	$\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2$
Illite-1M (ferroan)	$[(\text{NH}_4)\text{K}]\text{SiAl}$
Dolomite	$\text{CaMg}(\text{CO}_3)_2$
Quartz	SiO_2
Halite, potassium, syn.	$\text{K}_2\text{NaO}_6\text{Cl}_2$
Clinochlor-1M	$\text{Mg}_5\text{Al}(\text{SiAl})_4\text{O}_{10}$
Clinochlore-1 M (ferroan)	$(\text{MgAl})_5(\text{SiAl})_4\text{O}_{10}$
Muscovite	$(\text{K}, \text{Na})\text{Al}_2(\text{Si}, \text{Al})_4\text{O}_{10}(\text{OH})_2$
Biotite	$\text{K}(\text{Mg}, \text{Fe}^{2+})_3(\text{Al}, \text{Fe}^{3+})\text{Si}_3\text{O}_{10}(\text{OH})_2$
Biotite	$\text{KMg}_3(\text{Si}_3\text{Al})\text{O}_{10}(\text{OH})_2$
Halite, syn	NaCl
Aluminium Oxide	Al_2O_3

Table 2: Elements presents in rock phosphate (untreated)

Element identified	Intensity (Counts)	Energy (Kev)
Oxygen (^{16}O)	1278	0.521
Silicon (^{28}Si)	978	1.742
Phosphorus (^{32}P)	5860	2.016
Calcium (^{40}Ca)	23865	3.695

Table 3: Bioleaching studies of rock phosphate conditions: weight of ore sample = 2g (2% pulp density) volume of metabolite 100 ml, temperature 22.25°C. (Leaching time 24-hr)

Strains	Results (mg/L)			
	K_2O	Na_2O	MnO	Fe_2O_3
<i>Aspergillus niger</i> NRRL 587	4.0	29.3	0.52	5.11
<i>Aspergillus niger</i> NRRL 605	3.5	28.1	0.62	5.92
<i>Aspergillus niger</i> NRRL 1737	4.1	28.7	0.52	4.14
Control	0.06	-	N.D.	N.D.

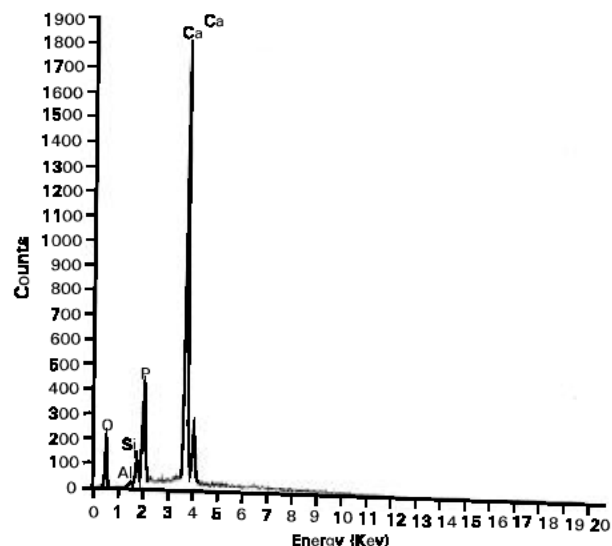
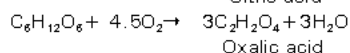
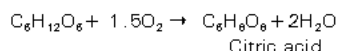


Fig. 1: Elemental composition of rock phosphate. The sample was analyzed by energy-dispersive X-ray (EDX) analysis.

observed during these studies. pH decreased upto 16 day of incubation from 5.5 to 2.2 in case of *A. niger* NRRL 1737 and from 5.5 to 2.4 in case of *A. niger* NRRL 605. According to Bossahard *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 and oxalic acid

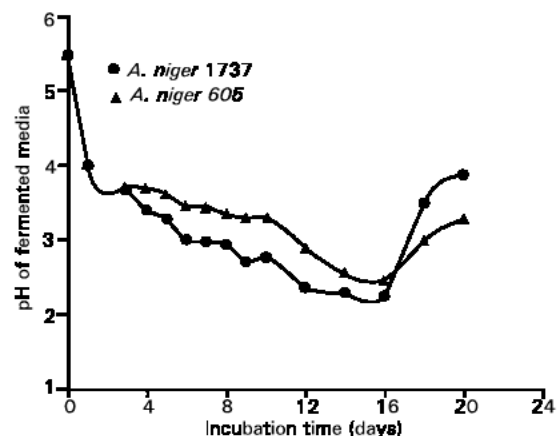


Fig. 2: pH profiles during growth of fungal strains of *Aspergillus niger* for its growth.

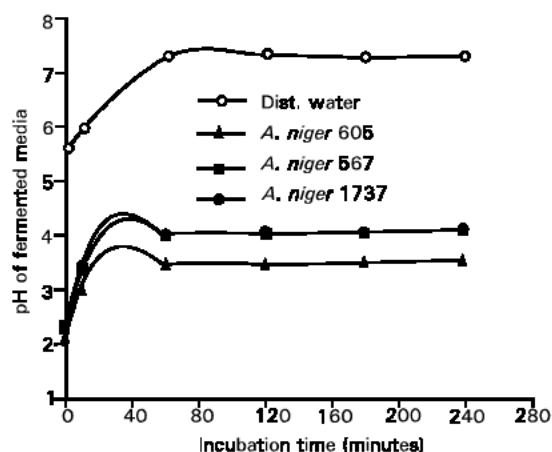


Fig. 3: pH profiles of leach suspension during leaching of rock phosphate with metabolite of *A. niger* strains

was 14.35 and 0.258g/L for *A. niger* NRRL 1737 respectively. As pH of fermented media was in the range of 2-3, so these conditions were more favorable for citric acid production (Fig. 2). pH profile of glucose fermented media in shown in Fig. 2.

After 16th day of incubation, pH of fermented media went on increasing upto 21 days. 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 requirement.

Bioleaching studies of rock phosphate: During leaching studies an acidulation reaction occurred between organic acids and calcite (CaCO_3) and dolomite [$\text{CaMg}(\text{CO}_3)_2$] minerals present in the ore-matrix with

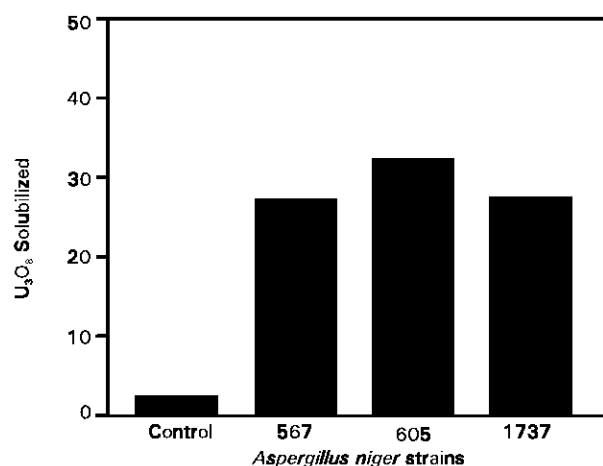
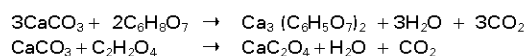


Fig. 4: Uranium solubilization during leaching of rock phosphate with metabolites of *A. niger* strains

effervescence of CO according to the following reactions.



Above chemical reactions were deemed responsible for consumption of H⁺ ions during leaching process. The pH values of the leach suspensions were progressively increased in all treatments (Fig. 3).

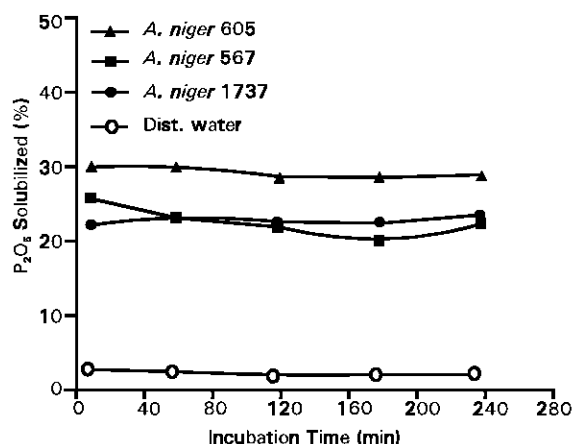
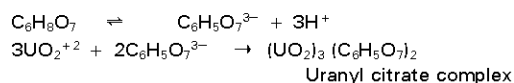


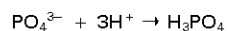
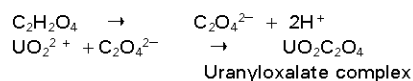
Fig. 5: Phosphorus solubilization during leaching of rock phosphate with microbiologically produced citric and oxalic acids.

Citric and oxalic acids dissociation and solubilization of P and U: Citric acid contains three carboxyl groups (pK_{a1} = 3.13 and pK_{a2} = 4.76 and pK_{a3} = 6.39) and one hydroxyl group (pK_{a2} = 10.82). The possible complex is.



Similarly oxalic acid contains two carboxyl groups (pK_{a1} = 1.20 and

pK_{a2} = 4.20) at 25°C so the possible complex of uranyl ion (UO₂²⁺) with oxalate ion is



In this leaching system, a maximum uranium recovery of 33% U₃O₈ of the total uranium content present in the ore sample was observed during 8hr of leaching (Fig. 4). The dissolution of phosphorus from rock phosphate was as a formation of phosphoric acid (H₃PO₄). The leaching data are shown in (Fig. 5).

The attack of organic acids on the minerals present in the ore-matrix involved both the release of phosphorus (PO₄³⁻) and uranium (UO₂²⁺) and some other associated metal ions (Al³⁺, Ca²⁺, Mg²⁺, Mn²⁺, V⁵⁺, Fe²⁺, Fe³⁺, Na⁺, K⁺ etc.) solution and simultaneously, the complexation/chelation of dissolved metals. The anions and the protons of an organic acid are able to leach metals by acidolysis and complexolysis phenomena. During leaching studies, some other metal ions (Mn, Fe, Na and K) were also dissolved from the rock sample (Table 3).

This is of great advantage that organic acids increase the solubility of metal ions at neutral pH by complexation and thereby, precluding the precipitation of metals as their hydroxides successfully. Among the several organic acids used in the extraction of metals, citric acid is the most preferred organic acid due to its natural multi-dentate complex formation with metal ions and its efficiency in removing metals is relatively consistent (Dodge and Francis, 1997).

This study demonstrates the possibility to explore the application of a bioleaching process mediated with microbiologically produced organic acids for the solubilization of P, U and other associated metal ions from sedimentary-type rock phosphate sample.

References

- AOAC, 1990. Official methods of analysis. The association of official analytical chemists, Arlington, Virginia, USA.
- Ahmed, Z. and R. A. Siddiqui, 1992. Minerals and Rocks for industry. Vol. 3. Geological Survey of Pakistan, 611: 622-623.
- Bhatti, T. M., A. Mateen, M. Amin, K. A. Malik and A. M. Khalid, 1991. Spectrophotometric determination of uranium (U) in bacterial leach liquors using Arsenazo 3. J. Chem. Technol. Biotechnol., 52: 331-341.
- Bhatti, T. M., T. Yasmin, M. Amin and A. Mateen, 1999. Leaching of uranium from rock phosphate by Thiobacillus ferrooxidans and Thiobacillus thiooxidans. In: Proceedings of the International Symposium of Biohydrometallurgy, (IBS'99) June 22-25, Madrid, Spain.
- Bossahard, P. P., R. Bachofen and H. Braudl, 1996. Metal leaching of fly ash from municipal water incineration by *A. niger*. Sci. Technol., 30: 3066-3070.
- Dodge, C. J. and A. I. Francis, 1997. Biotransformation of binary and ternary citric acid complexes of iron and uranium. Environ. Sci. Technol., 31: 3062-3067.
- Habashi, F., 1980. The recovery of uranium from phosphate rock. Progress and problems. In Proceedings of the 2nd International Congress on Phosphorus compounds. Institute Mondial du Phosphate, Paris, pp: 629-660.
- Hayman, D. S., 1975. Phosphorous cycling by soil microorganisms and plant roots. In Soil Microbiology. ed. N. W. Walker, Butterworth, London, pp: 67-91.
- Jackson, M. L., 1962. Soil Analysis. Constable & Co. Ltd. UK.
- Torma, A. E., 1988. Leaching of metals. In: Biotechnology, Vol. 6b (Rehm, H. J. and Reed G. Eds), pp: 367-369. Verlag Chemie, Weinheim.