Asian Journal of Chemistry Vol. 21, No. 8 (2009), 6029-6040

Bioleaching of Rock Phosphate by *Aspergillus niger*

HAQ NAWAZ BHATTI*, FOZIA ANJUM, SUMERA SAEED and KALIL-UR-REHMAN
Industrial Biotechnology Laboratory, Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad-380400, Pakistan
Fax: (92)(41)9200764; Tel: (92)(41)9200161/3309
E-mail: hnbhatti2005@yahoo.com

Bioleaching process is based on the ability of microorganisms (bacteria and fungi), to transform solid compounds resulting in soluble and extractable elements, which can be recovered. The main objective of the present study is to characterize the solubilization of phosphorus and uranium from rock phosphate with *Aspergillus niger*. An appreciable amount of uranium was also determined in the rock sample (0.012 % U_3O_8). XRD data indicated the presence of flourapatite [$Ca_2(PO_4)_3F$] as the main source of phosphorus. Other accessory minerals like biotite, muscovite, gypsum, aluminium oxide, trona, orthoclase, diopside and albite, *etc.* were estimated in the sample. The fungal strains of *Aspergillus niger* produced citric acid and oxalic acid during fermentation of glucose which resulted in a drastic drop in initial pH of the growth medium. During bioleaching studies of rock phosphate, the dissolution of phosphorus, uranium and other metal ions was observed in shake flasks leaching studies. Solubilization of these metal ions was apparently attributed to pH dependant. The SEM-EDX analysis of rock phosphate revealed that a significant amount of phosphorus, calcium, silicon and oxygen was present in the rock-matrix.

Key Words: Bioleaching, Biooxidation, Biotechnology, pH Control.

INTRODUCTION

The optimal development of crops demands a high, often costly, input of mineral fertilizers. Particularly in case of P-fertilization, natural phosphate-bearing materials represent a less costly alternative. The beneficial effect of rock phosphate (RP) on plant growth and the increased concern for environmental quality has made this material an attractive component for management in agriculture, especially when the availability of soluble phosphate fertilizers is limited[1].

The usage of non-conventional methods in rock phosphate processing has been studied widely. A very attractive approach for rock phosphate solubilization is the application of microorganisms, which are able to produce organic acids as metabolites. *Aspergillus niger* has been utilized for the solubilization of rock phosphates due to production of low-molecular-weight organic acids[2-5]. Rock phosphate is commonly called 'rock' in sedimentary deposits and apatite in igneous deposits. Rock phosphate consists of insoluble calcium phosphate [$Ca_3(PO_4)_2.CaF_2$] generally known as apatite.

Phosphorus is one of the major essential macronutrients for biological growth and development[6]. Phosphorus rocks and other deposits have been recognized as a valuable alternative source for phosphatic fertilizer. In recent years the possibility of practical use of rock phosphates as fertilizers has received significant interest. Phosphate solubilizing microorganisms convert these insoluble phosphates into soluble forms. A series of organic acids are formed by fungal metabolism resulting in organic acidolysis, complex and chelate formation. Proton induced and ligand-induced mineral solubilization occurs simultaneously in the presence of ligands under acidic conditions[7-10]. Filamentous fungi are widely used as producers of organic acids[7] and in particular, *Aspergillus niger* and some *Penicillium* species have been tested in fermentation systems or inoculated directly into soil, in order to solubilize rock phosphate[11-14].

Keeping in view the significance of rock phosphate as source of phosphorus-fertilization, the present study is designed to examine the rock phosphate solubilization by isolates of *Aspergillus niger* and to verify the potential for further applications in solubilization of rock phosphates.

EXPERIMENTAL

Rock phosphate sample: A representative rock phosphate sample (imported from Jordan) was obtained from the Institute of Engineering and Fertilizers Research (IEFR), Jaranwala road, Faisalabad. The sample was oven dried and ground to < 300-particle mesh size and was used for its mineralogical and chemical analysis and for shake leaching experiments.

Mineralogical analysis: X-Ray diffraction studies of rock phosphate sample was carried out to determine the mineralogical composition using a Rigaku Rint 300 Series diffractometer and the JCPDS diffraction software. A finely ground rock phosphate sample was analyzed to top fill mounts using CuK_{α}-radiation and wide range goniometer equipped with a diffracted beam monochromator and a θ compensating slit. Step scans were conducted from 3 to 70º 2θ increments using a 4 s step time[15].

Scanning electron microscopic (SEM) studies: For scanning electron microscopy (SEM), an air-dried rock phosphate sample was mounted on to the aluminium stubs by the standard method to observe the crystal morphology and elemental analysis of the rock sample. The sample was examined under a scanning electron microscope (Hitachi S-2380N) at 20Kv.

Determination of phosphorus contents: Phosphorus was determined in rock phosphate sample and leach solutions by a standard spectrophotometric method using Barton's reagent[16].

In this method, phosphorus forms a golden yellow colour complex with a λ_{max} at 430 nm. The optical density of the complex was measured against a corresponding reagent blank using a 10 mm path cell in a spectrophotometer (Hitachi UV-2001). Finely ground rock sample (1 g) was taken in in beakers and was digested with 50 mL

of HNO_3 (1:1) for *ca.* 4 h. The leached solution was filtered and filter paper was washed thoroughly with dilute HNO_3 solution (1:200). The filtrate was evaporated to moist dryness and the residue was redissolved in HNO_3 to make the volume 100 mL and the normality of this final solution should be 0.5-0.7 N HNO_3.

An aliquote (2 mL) of the above solution was taken in 50 mL of volumetric flask and 5 mL Barton, s reagent was added. The contents of the flasks were shaken thoroughly and then volume was made 50 mL with deionized water. The optical density of the complex was measured (after 10 min) at 430 nm against the corresponding reagent blank using a 10 mm path cell in a spectrophotometer.

P_2O_5 recovery (%) = P_2O_5 leached out × 100/P_2O_5 present in leaching system

Determination of uranium: The contents of the uranium in the leach liquor were determined by reacting the uranium with arsenazi-III, which forms an intense pink-voilet complex with uranyl ion (UO_2^{2+}) having a λ_{max} at 655 nm[17,18].

U_3O_8 recovery (ppm) = U_3O_8 leached out × 50/U_3O_8 present in leaching system

The concentration of iron and manganese in rock phosphate sample and leach solution was carried out by standard atomic absorption spectroscopy (Hitachi Z-8200). For soluble sodium and potassium in leach solution, standard flame photometric technique was used.

Growth studies of *Aspergillus niger*: Fungal strains of *Aspergillus niger* NRRL 567, *Aspergillus niger* NRRL 605 and *Aspergillus niger* NRRL 1737 were obtained from Agriculture Research Service Culture Collection. Northern Regional Research Laboratory, U.S. Department of Agriculture, Illinois, USA. The fungal strains were cultivated on slants containing 3.9 % (w/v) potato dextrose agar (Difco Lab., USA). Incubation time was one week at 30 ºC to produce an adequate number of spores (conidia). The spores were washed off the slants with a sterile solution of 0.2 % (w/v) sodium dodecyl sulfate (SDS). The spores were counted using a Petrof-Hausser counting chamber. The composition of the culture medium was (g/L): Glucose, 50; $NaNO_3$, 1.5; KH_2PO_4, 0.5; $MgSO_4.7H_2O$, 0.025.0; KCl, 0.025 and yeast extract, 1.6. Media was autoclaved at 121 ºC for 15 min. The culture media were inoculated with 1 mL of *Aspergillus niger* spore suspension as inoculum (2.5×10^7 spores/mL) and incubated at 28 ºC and 100 rpm.

Shake flask leaching studies: After 15 d, when substrate was almost consumed in culture media, the suspension was filtered. The filtrate (metabolite) then was used as leaching solution (lixiviant) for metal solubilization from rock phosphate. The leaching studies were performed in triplicate in the 500 mL Erlenmeyer flasks containing 100 mL metabolite supplemented with 2 g rock phosphate of < 200 particle mesh size (2 % wt/v ore pulp density). These flasks were incubated on a rotary shaker at 28 ºC and 100 rpm. At regular intervals, samples of leach suspension were taken for monitoring pH and filtered through 0.45 µm membrane filter for phosphorus, uranium and other metal ions analysis. Sterile growth medium of pH 5.5 was used as control for leaching studies.

High-pressure liquid chromatography: After 15 d of incubation, media was sterilized and centrifuged (12000 rpm for 15 min), then filtered to remove the solids before analysis of metabolites (*e.g.*, citric acid and oxalic acid) by modified HPLC method as described by Kordis-Krapez *et al.*[19]. After centrifugation at 8000 rpm for 10 min at 15 ºC, samples were prepared by dissolving in a 96 % ethanol and double distilled water (volume ratio 10\90) which was referred to as solvent, prior to use, the solvent was sonicated for 5 min in an ultrasonic bath to remove air bubbles. An HPLC (Sykam GmbH, Kleinostheim, Germany) equipped with a S-1121 dual piston solvent delivery system and S-3210 UV/Vis diode array detector and software package for data acquisition was used. A 20 µL of filtered sample was injected in to an analytical Hypersil (Thermo Hypersil, GmbH, Germany) ODS reverse phase (C_{18}) column (250 × 4.6 mm; 5 µm particle size) fitted with a C_{18} guard column. The mobile phase consisted of $H_3PO_4 = 6 \times 10^{-3}$ mol/L (pH = 2.1). The chromatographic separation was performed by isocratic elution of the mobile phase at a flow rate of 1.0 mL min^{-1} at 30 °C. Detection was performed at wavelength of 210 nm. Organic acids were identified by comparing the retention times and quantified on the basis of peak area per cent of the unknowns with those of pure standards of oxalic acid, citric acid, tartaric acid and malic acid (Sigma Chemical Co. (St. Louis, MO). The peak areas were recorded and calculated by a computer with chromatography data acquisition and integration software (SRI Instrument, Torrance, California, USA).

RESULTS AND DISCUSSION

Rock phosphate sample (imported from Jordan) used in these studies was light brown in colour and was of sedimentary type of rock deposits. Moreover, it was alkaline in nature, insoluble in water but soluble to some extent in solutions of acids and carbonates. The rock sample is being used as a feed stock for the manufacturing of various types of commercial phosphate fertilizers.

Mineralogy of rock phosphate (XRD analysis): The XRD data for the finely ground rock phosphates sample are summarized in Table-1. The rock sample was found to contain flourapatite with a general mineral formula of $[Ca_3(PO_4)_2F]$ with the other accessory minerals such as illite, gypsum, kaolinite, talc, clinochlore, muscovite, biotite, dolomite, halite and albite. The diffraction lines for a uranium minerals were below the level of detection defined as < 2 % relative intensity. The chemical analysis of uranium content in rock phosphate sample was found to contain 0.012 % U_3O_8 (120 ppm U_3O_8). A complete elemental analysis of rock phosphate sample is reported in Table-2. These data indicated that the rock phosphate sample was of high grade phosphate containing 33.6 % P_2O_5.

Scanning electron microscopy-electron dispersive X-ray spectroscopy (SEM-EDX): The SEM micrograph showed the crystal morphology of the rock sample (Fig. 1). The mica and silicate minerals are apparent on the crystal surface of the sample. The elemental analysis of the exposed surface of the phosphate under the SEM showed that phosphorus, oxygen, calcium and silicon are apparently present elements in the test material. These results are shown in Table-3.

TABLE-1
MINERALOGICAL ANALYSIS OF ROCK PHOSPHATE SAMPLE

Mineral identified	Mineral formula
Albite	$Ca_3(PO_4)_3F$
Illite	$NaAlSiO_8$
Gypsum	$KOAl_2(SiAl)_4O_{10}$
Kaolinite	$CaSO_4.2H_2O$
Talc-2M	$Al_2SiO_2O_5(OH)_4$
Illite-1M (ammonium)	$Mg_3Si_4O_{10}(OH)_2$
Dolomite	$[(NH_4)K]SiAl$
Quartz	$CaMg(CO_3)_2$
Halite, Potassium, syn	SiO_2
Clinochlore-1M	KO_4NaO_6Cl
Clinochlore-1M (FERROAN)	$Mg_5Al(SiAl)_4O_{10}$
Muscovite	$(K,Na)Al_2(Si, Al)_4O_{10}(OH)_2$
Biotite	$K[Mg,Fe^{2+}]_3(Al, Fe^{3+})Si_3O_{10}(OH)_2$
Biotite	$KMg_3(Si_3Al)O_{10}[OH]_2$
Halite, syn	NaCl
Aluminium Oxide	Al_2O_3

TABLE-2
CHEMICAL ANALYSIS OF ROCK PHOSPHATE SAMPLE

Constituents	Typical analysis (%)	Constituents	Typical analysis (%)
P_2O_5	33.60	CO_2	4.700
CaO	51.75	Fe_2O_3	0.160
K_2O	0.12	Na_2O	1.120
MgO	0.47	F	3.920
SiO_2	1.60	Organic Matter	0.210
MnO	0.02	U_3O_8	0.012
Al_2O_3	0.70		

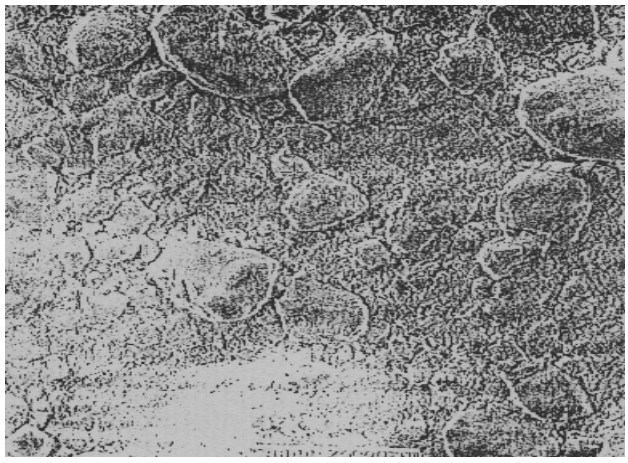

Fig. 1. SEM micrograph of rock phosphate sample

TABLE-3
ELEMENTS PRESENT IN ROCK PHOSPHATE (UNTREATED)

Element identified	Intensity (Counts)	Energy (Kev)
Oxygen ($_8O^{16}$)	1278	0.521
Silicon ($_{14}S^{28}$)	978	1.742
Phosphorus ($_{15}P^{32}$)	5860	2.016
Calcium ($_{20}Ca^{40}$)	23865	3.695

Growth studies of *Aspergillus niger*: Growth studies of fungal strains of *Aspergillus niger* NRRL 567, *Aspergillus niger* NRRL 605, *Aspergillus niger* NRRL 1737 were carried out in batch cultures using glucose and cane-molasses as carbon energy source for these fungi. These studies were performed in shaking and static conditions. During the growth of these fungal strains in liquid media, different sizes of beads of various colours in large number were formed in the inoculated flasks. In case of *Aspergillus niger* NRRL 605 smaller beads were formed than the fungal strains of *Aspergillus niger* NRRL 1737 under similar experimental conditions. It was obvious from these studies that the size of beads of fungal strain of *Aspergillus niger* NRRL 1737 was reduced than the previous days and a thick blackish mat of fungal biomass was observed in the growth medium on the upper surface. This fungal mat was composed of mycelia. During the growth studies, a slight change in morphology of fungal strains was reflected due to appearance of abnormally short, multiple branched, bulbus hyphae, after 12th day of incubation.

Change in pH of growth medium: *Aspergillus niger* is able to produce organic acids *via* its metabolic activity utilizing organic such as carbon and energy source[7,20]. The fungus *Aspergillus niger* is capable of producing di-and tri-carboxylic acids mainly *via* glycolysis. A characteristic feature of *Aspergillus niger* is the presence of pyruvate carboxylase that forms oxaloacetate from pyruvate and CO_2[21]. The oxaloacetic acid enters tricarboxylic acid cycle (TCA. Cycle) and the accumulation of organic acids by *Aspergillus niger* are markedly influenced by the pH.

During the growth of these fungal strains, it was observed that the initial pH of growth medium inoculated with fungal strains of *Aspergillus niger* NRRL 605 and *Aspergillus niger* NRRL 1737 was decreased due to the formation of organic acids like citric acid, oxalic acid, *etc.* by the microbial oxidation of glucose by the metabolic activity[7].

$$C_6H_{12}O_6 + 1.5O_2 \longrightarrow C_6H_8O_7 + 2H_2O \quad (1)$$

The pH of the inoculated media was decreased progressively up to 14th day of incubation (Fig. 2). The decrease in pH was due to the formation of organic acids *via* incomplete oxidation of glucose by the fungal strain of *Aspergillus niger*. So, the initial pH 5.5 of the media was decreased to 2.50 during the 14th day of incubation. According to Matty[7], *Aspergillus niger* was characterized by its intensive production of organic acids mainly citric acid and oxalic acid from sugar containing substrates[2]. It is reported in literature that the formation of organic acids during microbial oxidation

is oxygen limited phenomenon. But, after 16th days of incubation, there was an increase in the pH of the inoculated media which indicated the utilization of organic acids in phosphate solubilization by the *Aspergillus niger*[2,3,22] reported the correlation between the phosphate solubility and the citric acid production during the bioconversion. However, increase in solubility is not related to the increasing of the concentration of the available citric acid in the liquid culture. The presence of maximum citric acid can be explained due to partial neutralization of the citric acid with Ca^{2+} ions, which are liberated due to the decomposition of the phosphate structure as Ca-citrate.

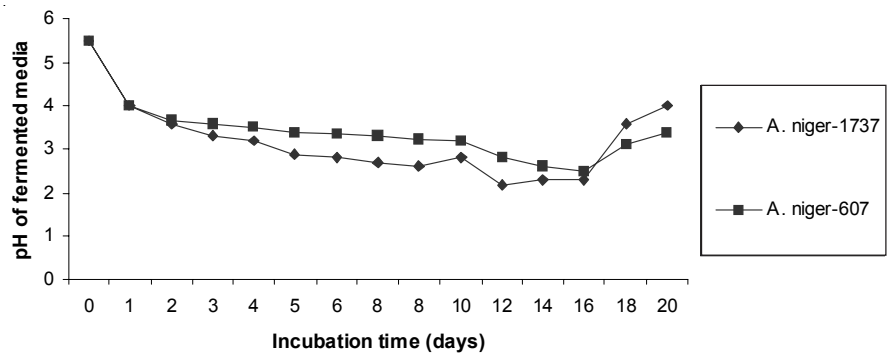

Fig. 2. pH profile during growth of fungal strains of *Aspergillus niger* for its growth

Characterization of organic acids in growth medium: The microbial oxidation of glucose was an acid producing system. During the growth studies of fungal strains of *Aspergillus niger*, the formation of citric acid and oxalic acid were observed during the microbial oxidation of glucose. The HPLC data are shown in Table-4. It was obvious from the HPLC data that the fungal strains of *Aspergillus niger* NRRL 605 produced higher yield of citric acid (45.13 g/L citric acid) as compared with oxalic acid (33.45 g/L oxalic acid) during 25th day of incubation. A similar data of organic acids formation was also observed in the growth medium inoculated with *Aspergillus niger* NRRL 1737 (Table-4). It was also observed that the strains of *Aspergillus niger* NRRL 605 produced higher amount of citric acid and oxalic acid as compared with *Aspergillus niger* NRRL 1737 during the microbial oxidation of

TABLE-4
HPLC ANALYSIS OF FERMENTED MEDIA

Fungal strain	pH of the fermented media	Organic acids (g/L)	
		Citric acid	Oxalic acid
On 6th day of incubation:			
Aspergillus niger NRRL 605	3.42	14.35	0.26
Aspergillus niger NRRL 1737	2.96	14.37	8.43
On 25th day of incubation:			
Aspergillus niger NRRL 605	2.11	45.13	33.45
Aspergillus niger NRRL 1737	2.30	28.42	16.13

glucose under similar experimental conditions. Burgstaller and Schenver[8] reported that *Aspergillus niger* produced citric acid at pH around 2.5, so the present conditions are more favourable for citric acid production.

The growth of fungal strains of *Aspergillus niger* NRRL 567, *Aspergillus niger* NRRL 605 and *Aspergillus niger* NRRL 1737 was also observed in growth medium supplemented with cane-sugar molasses. It was obvious that the initial pH of the inoculated flasks was also decreased during 20th days of incubation under static condition at room temperature. The change in pH profiles of these experiments are shown in Fig. 3. A thick mat was formed on the surface of the inoculated media present in flasks. The pH decrease was due to production of organic acids.

Growth of fungal strain on solid media: During the growth studies of fungal strains of *Aspergillus niger* on solid medium supplemented with glucose as carbon substrate and methyl orange as indicator, the colour of the indicator was changed from orange to reddish after 2 d of incubation. The change in colour was due to change in pH of the medium, which was accompanied due to the formation of organic acids[2]. Reddish colour was found in all inoculated Petri-dishes which were due to the production of citric acid by *Aspergillus niger* which changed the colour of methyl orange indicator. Black colour spores were found during its growth.

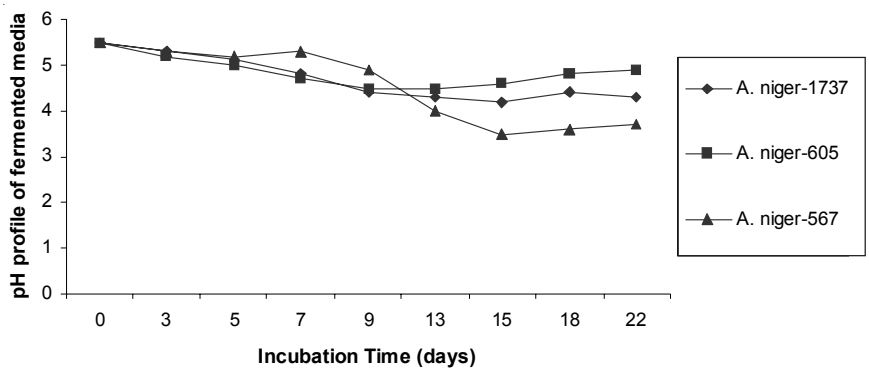

Fig. 3. pH profile of growth medium supplemented with cane sugar molasses inoculated with fungal strains of *Aspergillus niger*

Bioleaching studies of rock phosphate: During the leaching studies, when the rock sample was added into the flasks containing metabolites as leaching solution, an acidulation reaction occurred between organic acids and calcite ($CaCO_3$) and dolomite [$(CaMg)CO_3$] minerals present in the ore matrix with the effervescence of CO_2 according to the following reactions:

$$3CaCO_3 + 2C_6H_8O_7 \longrightarrow Ca_3(C_6H_5O_7)_2 + 3H_2O + 3CO_2 \quad (2)$$

$$CaCO_3 + C_2H_2O_4 \longrightarrow CaC_2O_4 + H_2O + CO_2 \quad (3)$$

The chemical reactions (2-3) were deemed responsible for consumption of H^+ ions during leaching process. Consequently, the pH-values of the leach suspension

were progressively increased in the all treatments (Fig. 4). It was observed that the pH-values of the leach suspension increased from 2.1 to 4.0 during leaching. When the rock phosphate sample was treated with leachate containing organic acids, a chemical reaction takes place in the leaching system. The pH values of all the treatments were progressively increased due to the chemical reaction that occurred in between citric acid and oxalic acid and various metal ions present in the rock matrix. This consumption of acids causes the slightly increase in pH-values of leaching solutions. Same trend has been reported[3-5]. The attack of organic acids on the minerals present in the ore matrix involved both the release of phosphorus (PO_4^{2-}) and uranium (UO_2^{2+}) and some other associated metal ions (Al^{3+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, V^{5+}, Fe^{2+}, Fe^{3+}, Na^+, K^+, *etc.*) in the leach solution and simultaneously, the complexation/chelation of dissolved metals. The anions and protons of an organic acid are able to leach metals by acidolysis and complexolysis phenomena. When citric acid and oxalic acid are fully dissociated in aqueous solution and the uranyl ions (UO_2^{2+}) present in the leaching solution. The following reactions take place accordingly:

Citric acid and oxalic acid dissociation and solubilization of P and U:

$$C_6H_8O_7 \rightleftharpoons C_6H_5O_7^{3-} + 3H^+$$

$$3UO_{26} + 2C_6H_5O_7^{3-} \rightleftharpoons (UO_2)_3(C_6H_5O_7)_2$$

(Uranyl citrate)

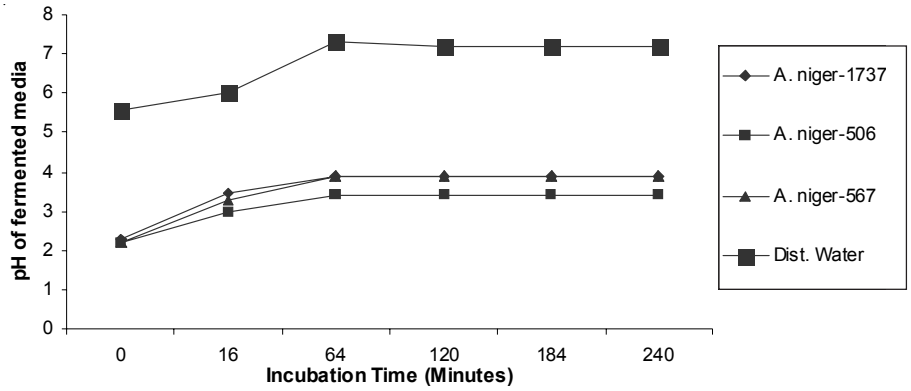

Fig. 4. pH profile of leach suspension during leaching of rock phosphate with metabolite of *Aspergillus niger* strains

$$C_2H_2O_4 \rightleftharpoons C_2O_4^{2-} + 2H^+$$

$$UO_2^{2+} + C_2O_4^{2-} \longrightarrow UO_2C_2O_4$$

(Uranyl oxalate)

$$PO_4^{3-} + 3H^+ \longrightarrow H_3PO_4$$

Citric acid contain three carboxyl groups ($pKa_1 = 3.13$; $pKa_2 = 4.76$ and $pKa_3 = 6.39$) and one hydroxyl group ($pKa_4 = 10.82$) and oxalic acid ($C_2H_2O_4$) contains two carboxyl group ($pKa_1 = 1.20$ and $pKa_2 = 4.20$) at 25 ºC as possible donators of protons (H^+). Under oxidizing conditions, citrate ions $(C_6H_5O_7)^{3-}$ forms a bidentate

complex $(UO_2\text{-citrate})_2^{2-}$ with uranyl ion $(UO_2)^{2+}$ and to a lesser extent a tridentate complex $(UO_2\text{-citrate})^-$. Uranium dissolution from the ore was mainly attributed to formation of soluble uranyl citrate $[(UO_2)_3(C_6H_5O_7)_2]$ and uranyl oxalate $[(UO_2C_2O_4)]$ complexes during the leaching process. About 6 % U_3O_8 of the total uranium contents present in the ore sample was dissolved within the first 10 min of the leaching experiments mediated with the metabolite containing micrbiologically produced citric acid (26 mM) and oxalic acid (55 mM). In this leaching system, a maximum uranium recovery of 33 % U_3O_8 of the total uranium content present in the ore sample was observed during leaching (Fig. 5).

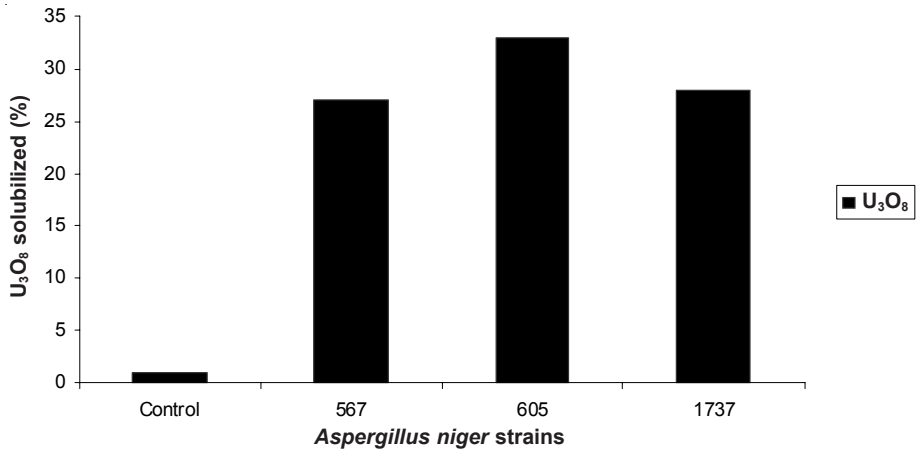

Fig. 5. Uranium solubilization during leaching of rock phosphate with metabolites of *Aspergillus niger* strains

The dissolution of phosphorus from rock phosphate was as a formation of phosphoric acid. Phosphorus was present as flourapatite $[Ca_3(PO_4)_2F]$ mineral in the matrix of rock phosphate. Phosphorus solubilization was observed in the leach solutions as the rock phosphate was treated with microbiologically produced citric acid and oxalic acid. Earlier reports[3-5] show a relationship between the utilization of organic acids and increase in the soluble phosphate concentration, which is in accordance with present results. The leaching data are shown in Fig. 6. It was observed from these results that an appreciable amount of phosphorus was solubilized from rock phosphate within 5-10 min of leaching time at room temperature (22-25 ºC) without shaking the flasks. It was observed that higher amount of phosphorus (29.5 % P_2O_5) was solubilized during 8 h of leaching time. In chemical control (leaching with distilled water), a negligible amount of phosphorus was solubilized under similar experimental conditions. During leaching studies, some other metal ion (Mn, Fe, Na and K) was also dissolved from the rock sample. These results are reported in Table-5.

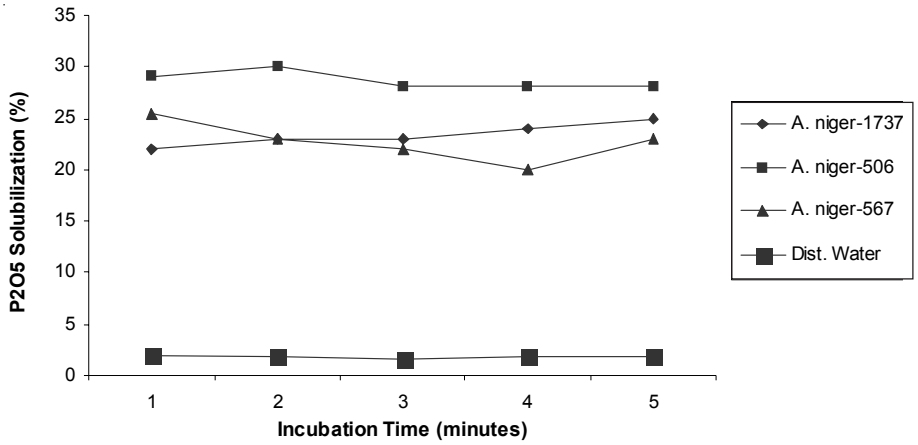

Fig. 6. Phosphorus solubilization during leaching of rock phosphate with microbiologically produced citric and oxalic acids

TABLE-5
SOLUBILIZATION OF METAL IONS DURING LEACHING OF ROCK PHOSPHATE WITH MICROBIOLOGICALLY PRODUCED CITRIC AND OXALIC ACIDS (LEACHING TIME 24 h)

Strains	Results (mg/L)			
	K_2O	Na_2O	MnO	Fe_2O_3
Aspergillus niger NRRL 567	4.00	29.3	0.52	5.11
Aspergillus niger NRRL 605	3.50	28.1	0.62	5.92
Aspergillus niger NRRL 1737	4.10	28.7	0.52	4.14
Control	0.06	-	N.D	N.D

This is of great advantageous that organic acids (citric acid, acetic acid, gluconic acid, oxalic acid, *etc*.) increase the solubility of metal ions at neutral pH by chelation/ complexation phenomena and, thereby, the precipitation of metals as their hydroxides is prevented by this way successfully. This phenomenon is very important in hydrometallurgy. When a high concentration of metal is achieved at neutral or alkaline pH-values. The leaching of metals from raw materials by acidophilic heterotrophs could be economically acceptable only if a cheap and suitable organic source is available as a substrate. Among the several organic acids used in the extraction of metals, citric acid is the most preferred organic acid due to its natural multidentate complex formation with metal ions and its efficiency in removing metals is relatively consistent. This study demonstrates the possibility to explore the application of a bioleaching process mediated with microbiologically produced organic acids for the solubilization of phosphorus, uranium and associated metal ions from sedimentary type of rock phosphate sample.

Conclusion

It has been analyzed that *Aspergillus niger* can be successfully used for the solubilization of phosphorus and uranium from rock phosphate. It might be due to the formation of different organic acids and other factors may also be involved. The results showed that maximum extraction of phosphorus and uranium in soluble form can be achieved by bioleaching, after studying the economic feasibility of competing the traditional rock phosphate processing.

ACKNOWLEDGEMENT

The authors are thankful to Dr. Tariq Mahmood Bhatti, Principal Scientist, Pakistan Institute of Nuclear Science and Technology (PINSTECH), Islamabad for his technical help.

REFERENCES

1. S.S.S. Rajan, W.H. Watkinson and A.G. Sinclair, *Adv. Agron.*, **57**, 77 (1996).
2. D. Bojinova, R. Velkova and R. Ivanova, *Bioresour. Technol.*, **99**, 7348 (2008).
3. D.H. Goenadi and S.Y. Siswanto, *Soil Sci. Soc. Am. J.*, **64**, 927 (2000).
4. M. Reddy, S. Kumar, K. Babita and M.S. Reddy, *Bioresour. Technol.*, **84**, 187 (2002).
5. S. Seshadri, S. Ignacimuthu and C. Lakshminarasimhan, *Chem. Eng. Commun.*, **191**, 1 (2004).
6. H.L. Ehrlich, in eds.: A. Einsele, R.K. Finn and W. Samhaber, Geomicrobiology, VCH Verlagsgesellschaft, Weinheim, edn. 2 (1990).
7. M. Mattey, *Rev. Biotechnol.*, **12**, 87 (1992).
8. W. Burgstaller and F. Schinner, in eds.: A.E. Wey and J.E. Lackshman, Biohydrometallurgical Techniques Torma, The Minerals, Metals and Materials Society, Warrendale, PA, pp. 325-328 (1993).
9. J. Berthelin and W.E. Krumbein, Macmbial Geochemistq. Blackwell, Oxford, pp. 223-262 (1993).
10. D. Mishra, D.J. Kim, J.G. Ahn and J.C. Lee, *KIGAM Bull.*, 48 (2004).
11. R.M.N. Kucey, *Appl. Environ. Microbiol.*, **55**, 2699 (1987).
12. P.E.A. Asea, R.M.N. Kucey and J.W.B. Stewart, *Soil Biol. Biochem.*, **20**, 459 (1988).
13. P.C. Cerezine, E. Nahas and D.A. Banzatto, *Appl. Microbiol. Biotechnol.*, **29**, 501 (1988).
14. J.E. Cunningham and C. Kuiack, *Environ. Microbiol.*, **52**, 1451 (1992).
15. W. Barzik, A. Kowal, A. Pomianowski and A. Rakowska, *Physicochem. Prob. Min. Process.*, **36**, 9 (2002).
16. M.L. Jackson, Soil Chemical Analysis, Constable & Co. Ltd., UK (1962).
17. T.M. Bhatti, A. Mateen, M. Amin, K.A. Malik and A.M. Khalid, *J. Chem. Technol. Biotechnol.*, **52**, 331 (1991).
18. T.M. Bhatti, T. Yasmin, M. Amin and A. Matten, In: Processings of the International Symposium of Biohydrometallurgy, (IBS, 99) June 22-25, Madrid, Spain (1999).
19. M. Kordis-Krapez, V. Abram, M. Kac and S. Ferjancic, *Food Technol. Biotechnol.*, **39**, 93 (2001).
20. D.Y. Bojinova, R.G. Velkova, in eds.: P. Zhang, H. El-Shall, P. Somasundaran and R. Stana, Beneficiation of Phosphates: Fundamentals and Technology, Society for Mining, Metallurgy and Exploration, Inc., Littleton, Colorado, USA, pp. 69-78 (2002).
21. I.M. Casselton, J.L.R. Fietto, R.X. Vieira, M.J.M. Tropia, L.M.M. Campos, E.B. Paniago and R.L. Brandao, *Hydrometallurgy*, **57**, 39 (2000).
22. V. Narsian and H.H. Patel, *Soil Biol. Biochem.*, **32**, 559 (2000).

(*Received*: 11 September 2008; *Accepted*: 22 May 2009) AJC-7576