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Leaching of metal ions from black shale by organic acids produced by *Aspergillus niger*

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ABSTRACT

The main concern of the present study is to find a more feasible and economical method to extract metal ions from low grade discarded ores like black shale by *Aspergillus niger*. *A. niger* exhibited a good potential in generating varieties of organic acids effective for metal ions solubilization. The effectiveness of organic acids was enhanced when sulphuric acid was added to the medium. Different agricultural wastes as substrates were evaluated. Maximum solubilization of copper (68.5%), zinc (49.0%) and cobalt (60.4%) was achieved in the media containing mango peel, rice bran and glucose as substrates. The extraction of low concentrations of metal ions from this ore indicated that this low grade discarded ore may be a potential source for metal ions in the future.

1. Introduction

Worldwide reserves of high-grade ores are diminishing at an alarming rate due to a rapid increase in the demand for metals. However, there exist large stockpiles of low and lean grade ores yet to be mined. The recovery of metal ions from low and lean grade ores, such as shales and schist, by conventional techniques is very expensive due to high energy and capital inputs. Moreover, the conventional technologies are associated with environmental hazards. As environmental standards regarding toxic wastes continue to establish, the costs for ensuring environmental protection continue to rise (Devasia and Natarajan, 2004; Pradhan et al., 2006).

Due to rapid technological and industrial developments, many industrial sites are contaminated with heavy metals and organic compounds, which are toxic to any kind of living organisms, particularly human beings. Therefore, industries and public offices are obliged to implement the concepts of structured environmental management system more strictly. Moreover, reliable remediation techniques are required to recycle these industrial wastes like electronic scraps and used catalysts and clean up the site (Bayraktar, 2005). As a result metal production has to be met more often from lower grade or complex ores, from mines and industrial wastes (Rawlings, 2004).

The application of biotechnology in mining has been investigated in various research organizations and industries and became a reality in the 1950s with the advent of copper bioleaching (Brierley and Brierley, 2001; Ehrlich, 2001).Today, several technologies have been exploited commercially in well-mechanized and engineered systems, which can be grouped under the term biohydrometallurgy (Brierley and Brierley, 2001; Rawlings, 2004; Tang and Walix, 2006).

Microbially metal-extraction processes are usually more economical and ecofriendly than physicochemical processes (Rawlings, 2004; Akcil, 2004). They do not use large amounts of energy as compared to roasting and smelting and do not produce sulphur dioxide, another harmful gas (Mishra et al., 2004). Microbial technology offers an economic alternative for the mining industry, at a time when high-grade mineral resources are being depleted (Rawlings, 2004). Generally, bioleaching refers to the conversion of metals into their water soluble forms by microorganisms (Rawlings et al., 2003; Olson et al., 2003; Ndlovu, 2008). Black shales undergo chemical leaching and the metal dissolution processes are also mediated by microorganisms. Microorganisms like heterotrophs require carbon as an energy source, and this requirement can be fulfilled by using organic wastes. Acidolysis is the principal mechanism in bioleaching of metals by *Aspergillus niger*. The fungus produces organic acids such as citric, oxalic, malic and gluconic acids during bioleaching (Mulligan et al., 2004; Johnson, 2006). Prediction of metal release from black shale material is an important aspect in pollution abatement. Black shale is a very fine-grained sedimentary rock which has been formed by the consolidation of beds of mud, clay or silt along with residues of algae, bacteria and other life forms that lived in the sea. They are composed chiefly of clay minerals, quartz, and illite (Bohacs et al., 2000). Pakistan has huge reserves of black shale deposits i.e., the Foothills of Himalayas at Lagarbon, in Karakoram valley at Shishi Gol and South of Mirkhani, Kakul in Hazara District, the Chitral areas as well as the Turbela areas (Ahmad, 1969).

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The main concern of the present study is to investigate bioleaching as an economical, environment friendly process and to determine the ability of microbes like *A. niger* to extract copper, zinc and cobalt from black shales using different organic wastes as substrates.

2. Materials and methods

All chemicals and reagents used in the present study were of analytical grade. Pure standards of organic acids (citric, oxalic, malic and tartaric acids) were obtained from Sigma-Aldrich Co. (St Louis, MO) USA.

2.1. Black shale sample

A representative black shale ore sample was obtained from the Pakistan Institute of Engineering and Applied Sciences (PIEAS), Islamabad, Pakistan. It was alkaline (pH 7.3) and insoluble in water. The coloring material in the individual shales might be carbon, hydrocarbon or finely divided iron sulphides. The sample was oven dried at 105 °C and ground to generate particles 74 μm by sieving. The elemental analysis of raw sample by atomic absorption spectrophotometry showed that it is mainly composed of Fe 3761, Cu 39.1, Co 18.3, Zn 31.9 and Ni 8.3 (mg/kg).

2.2. Fungal strain and growth conditions

A. niger was isolated from the black shale ore itself and then cultivated for purification on slants of potato dextrose agar (PDA) medium (3.9% m/v) as described by Bousshard et al. (1996). Briefly, slants were incubated (Incubator, Sanyo, Germany) for 72 h at 25 °C to produce an adequate number of spores. Afterward, the spores were counted using a Petroff-Hausser counting chamber. For growth in liquid medium, the culture medium was composed of (g/L): KH_2PO_4, 5.0; NH_4NO_3, 2.0; $(NH_4)_2SO_4$, 4.0; $MgSO_4.7H_2O$, 0.2; peptone, 2.0; trisodium citrate, 2.5; yeast extract, 1.0 and made volume up to 1000 mL with distilled water (Bhatti et al., 2007). Nine sets of 250-mL flasks containing 100 mL of liquid medium, were prepared each in triplicate samples. Medium in each flask was autoclaved. After sterilization, 5% (m/v) of the given substrate was added in each flask except for the control, the flasks were then inoculated with 1-mL of *A. niger* spore suspension as inoculum (approximately 2.4×10^8 spores mL^{-1}). All the flasks were sealed with removable cotton and incubated in an orbital shaker (Gellen Kamp, England) at 28 °C and 120 rpm for 15 days of growth period.

2.3. Leaching of metal ions

To evaluate the effectiveness of different organic acids in metal dissolution leaching tests were performed with organic acids like citric, malic, oxalic and tartaric acids in different concentrations. Four different concentrations (w/v) 0.05, 0.1, 0.5, and 1% of citric, malic, oxalic and tartaric acids with 2% (pulp density) black shale in triplicate samples were subjected to shaking for the period of 30 days. pH was monitored on every 2nd day of leaching period. Finally, the samples were filtered and analyzed for metal ions dissolved by atomic absorption spectrophotometry (AAS).

2.4. Pretreatments of substrates

Before the addition of substrates in the media, substrates were subjected to pretreatment process. Glucose was the substrate in medium 1. A diluted solution (50% v/v) of molasses was used as substrate in medium 2. Mango peel, seed cake and rice bran were first oven dried at 105 °C, ground and added to media 4, 6 and 8. For media 3, 5 and 7, these agricultural wastes were immersed in sulphuric acid of pH 2 for 24 h. The control medium contained no substrate. The composition of different media and pretreatments is shown in Table 1.

2.5. Leaching experiments

After 15 days of microbial growth, all the culture media in flasks were autoclaved, centrifuged ($8000\times g$ for 10 min at 15 °C) and filtered before HPLC analysis for the determination of organic acid metabolites. Culture supernatants containing organic acid metabolites like gluconic, citric, oxalic, tartaric and malic acids were recovered and then used to leach the metal ions from shale residue. 2% (m/v) of pulp density was added in each medium of flasks containing culture supernatants and incubated on an orbital shaker to keep everything in a homogeneous slurry form at 28 °C and 120 rpm for leaching period of 36 days. Samples were collected after every 3rd day and analyzed for metal ions.

2.6. Analysis of organic acids

Analysis of organic acid metabolites of all media was performed by following the modified HPLC method as described by Escobal et al. (1996). After centrifugation ($8000\times g$ for 10 min at 15 °C) and filtration, samples were vortexed before HPLC analysis. The mobile phase consisting of acetic acid (0.25%) solution was filtered and sonicated to remove the suspended particles. An HPLC (Sykam GmbH, Kleinostheim, Germany) equipped with 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 the filtrate was injected into 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 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 a wavelength of 254 nm. The organic acids were identified by comparing the retention times and quantified on the basis of peak areas.

2.7. AAS analysis

Samples collected every third day of leaching, were subjected to metal ions analysis. At the end of leaching residue samples were washed with water three times then oven dried. Residues (1 g) are dissolved in 40 mL HNO_3 (1:1) and heated at 80–90 °C for 5 h. After cooling, filtrates are evaporated to dryness and the residue was again diluted with 5 mL HNO_3 (0.5 N) by addition of 20 mL distilled water. The volume was made to 200 mL with distilled water. The solution was used for AAS analysis.

2.8. Statistical analysis

All the experiments before and after the leaching process were performed in triplicate (Steel et al., 1997).

Table 1
Substrates and pretreatments.

Medium no.	Substrate	Pretreatment
1	Glucose (5% m/v)	Filtration
2	Molasses (5% of 50% v/v diluted)	Autoclaving (120 °C, 5 min)
3	Mango peel (5% m/v)	Grinding/sulphuric acid (pH 2)
4	Mango peel (5% m/v)	Grinding/autoclaving (120 °C, 5 min)
5	Seed cake (5% m/v)	Grinding/sulphuric acid (pH 2)
6	Seed cake (5% m/v)	Grinding/autoclaving (120 °C, 5 min)
7	Rice bran (5% m/v)	Grinding/ sulphuric acid (pH 2)
8	Rice bran (5% m/v)	Grinding/ autoclaving (120 °C, 5 min)