Biorecovery of Manganese from Pyrolusite ore using *Aspergillus niger* PCSIR-06

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Summary: Investigations were carried out for biotechnological leaching of manganese from pyrolusite ore using *Aspergillus niger.* Strain of *Aspergillus niger* PCSIR-06 was isolated from pyrolusite ore collected from Wadh area of Khuzdar District, Balochistan, Pakistan. The growth characteristics were investigated in terms of final pH, biomass and total acid produced by the fungus in both shaking and surface culturing conditions. Bioleaching of manganese from low grade pyrolusite ore was carried out in shake cultures by applying *Aspergillus niger* PCSIR-06. Effects of various parameters in situ leaching of pyrolusite ore such as sucrose concentration, pulp density and inoculum size were investigated. Maximum leaching of manganese (85.39 %) was achieved at 10 % sucrose, 1 % pulp density and 10 % inoculum in a period of 12 days.

Introduction

Microorganisms have been in use for recovering metals from low grade ores, mining wastes as well as from metals containing secondary raw materials by promoting biochemical biotransformation of metallic compounds. These prevalently oxido-reductive transformations modify the metal solubility and its distribution in the environment. There are three groups of microorganisms which are used for recovery process: autotrophic bacteria [1-3], heterotrophic bacteria [4] and fungi [5]. The microbial leaching of manganese is generally carried out by using heterotrophic micro organisms which live in micro aerobic conditions and require organic nutrient to serve as energy and carbon source for their growth. The kinetic model for manganese leaching in presence of iron oxidizing bacteria has been reported by Takami *et al.,* [6]. Fungi from genus *Aspergillus, Botrytis, Mucor, Penicillium and Trichoderma* are mostly used for leaching of manganese. Heterotrophic bacteria as well as fungi have the potential for producing acid metabolites that are able to solubilize oxides, silicate, carbonate and hydroxide minerals by reduction, acidolysis and complexation mechanisms [7].

The major accumulating minerals of manganese are oxides, silicates and carbonates. The most important of these are psilomelane, pyrolusite, manganite, rhodochrosite, chondrite, etc. Usually, manganese ores are classified into three grades depending upon the presence of manganese contents, high grade 44-48 % Mn, medium grade 35-44 % Mn and low grade 24 to 35 % Mn contents [8]. Recovery of metals from low grade ores using pyrometallurgical and hydrometallurgical techniques are expensive due to high energy requirements, costs and producing environment polluting compounds. The use of microorganisms for recovery of metals from low grade ores is comparatively better alternative as it holds promise of lowering the fixed capital and reducing environmental pollution. In present studies, strains of heterotrophic micro organisms were isolated and screened for manganese leaching efficiency. Growth and bioleaching behaviour of selected strain were also investigated.

Results and Discussion

Growth Studies

In surface culture conditions, it was observed that the pH of the medium lowered from 6.5 to 2.4 at 30 °C in 12 days. Most of the metabolic products of *Aspergillus niger* PCSIR-06 were found to be organic acids which resulted in lowering pH of the culture broth (Fig. 1). Burgastaller and Schinner [9] have also reported that the end metabolic products of

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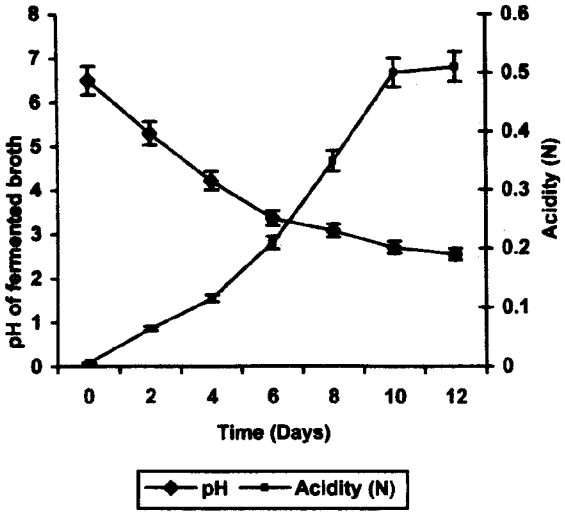

Fig. 1 pH and acidity variations in culture medium during the growth of *Aspergillus niger* PCSIR-06 in surface culture conditions.

fungus are organic acid which reduces pH of the medium. pH of the medium was inversely related to the strength of the acid produced during the growth of fungus. The pH of the medium was decreased during the log phase of growth from 2nd to 10th day where the strength of acids increased from 0.065 to 0.5 N (Fig. 1). Further increase in strength of acid after 10th day was also observed which was not significant. It can be assumed that the acid produced during the specified period is proportional to the increase in the biomass. During the log phase of growth (2nd to 10th day) the biomass increased from 10.32 to 62.08 g/l (Fig. 2). When the cells grow in stationary liquid medium, the nutrients around them are rapidly depleted producing large amount of acids. The aging of such cultures is also rapid [10].

In shaking conditions the growth of *Aspergillus niger* PCSIR-06 was less as compared to surface culture in terms of produced biomass, organic acids and lowering of pH. The maximum biomass and strength of acid produced were 67.4 g/ l (Fig. 3) and 0.5 N respectively (Fig. 1).

The production of acid ceased after the production of sizeable amount of biomass in both the culturing conditions. This observation support the assumption made by Pelczar *et al.*, [11] that amount of acid produced by any fungus under specified conditions was directly proportional to the magnitude of the fungal biomass. In stationary phase the culture metabolize sugars or fats vigorously. Due to formation of abundant mycelium and depletion in oxygen concentration of the environment occurred, which affect the metabolism of cells. The production of biomass and carbon dioxide is replaced by the production / formation of alcohol, butanediol and other volatile metabolites [12]. As a result, there was

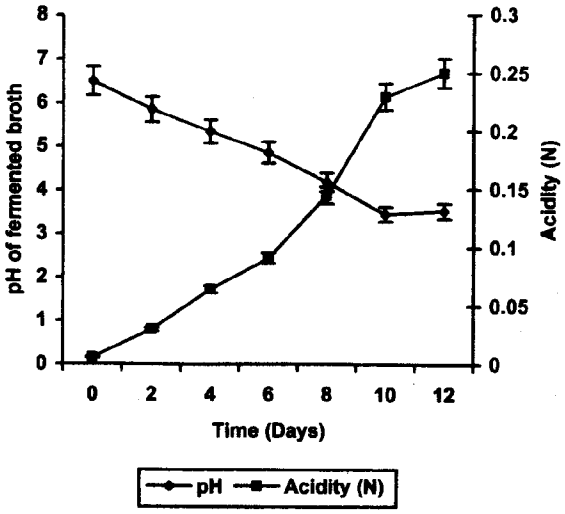

Fig. 2 pH and acidity variations in culture medium during the growth of *Aspergillus niger* PCSIR-06 in shake culture conditions.

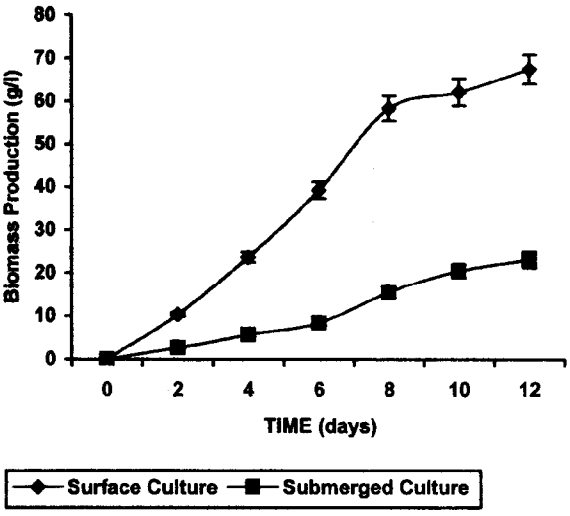

Fig. 3 Biomass production of *Aspergillus niger* PCSIR-06 in surface and shaking culture conditions.

an increase in pH of the growth medium was observed (Fig. 1 and 3). The stationary phase followed by death phase which was observed in our experiments as foaming due to decay of enzymes and proteins.

Bioleaching studies

Bioleaching of oxide ores with heterotrophic micro organism generally follows an indirect mechanism where the reduction mechanism involved for manganese solubilization [13]. In all cases, the biological processes utilize sacchariferous substances. The overall reactions involve in the process are:

$$24\ MnO_2 + C_{12}H_{22}O_{11} + 48H^+ = 24\ Mn^{2+} + 12\ CO_2 + 35H_2O \quad \text{---------- (1)}$$

$$Mn^{2+} + CO_2 + H_2O = MnCO_3 + 2H^+ \quad \text{----------(2)}$$

The studies on the effect of time for the bioleaching of manganese were carried for a period of 25 days. The studies were conducted with an initial pH of 6.5 at 30 °C. The leaching of manganese started at lower pace in start of the experiment which later on boost up after 6th day and reached to its maximum at 12th day. The pH of the leach liquor fell to 4.4 in 12 days giving a total extraction of 62.25 %. There was an increase in manganese contents of the medium beyond 12th day till 20th day but was non significant. After 20th day, a decrease in the manganese contents of leach liquor with an increase in pH was also observed (Fig. 4). This decrease in manganese contents may be due to the accumulation / adsorption on the surface of some structures of fungal hyphae [14] or due to the production of volatile metabolites instead of reductive compounds and decay of enzyme system following death of mycelium [12]. The association of manganese leaching with the production of reductive compounds by the fungus has been reported by Madgwick [15].

Experiments were carried out by varying the pulp densities of manganese ore from 2 to 12 % w/ v in the growth medium. Higher extraction of manganese was observed with lower pulp densities. At 1 % pulp density 85.39 % manganese leached in the medium with fall in pH to 4.12 (Fig. 5). Similar observations i.e., higher extraction with decrease in pH have also been made by Veglio *et al.* [16] and Acharya *et al.*, [17]. The increase in pulp density decreases the extraction of manganese. It may be due

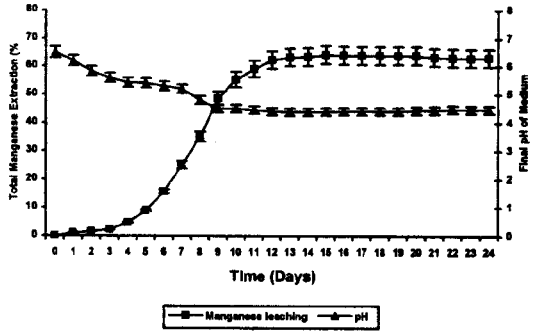

Fig. 4 In situ Bioleaching leaching of manganese from pyrolusite ore by *Aspergillus niger* PCSIR-06.

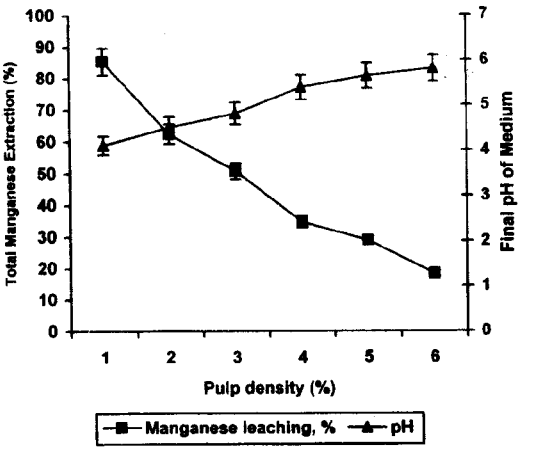

Fig. 5 Effect of pulp density on bioleaching leaching of manganese from pyrolusite ore by *Aspergillus niger* PCSIR-06.

to the unavailability of oxygen and nutrition to the fungus for growth due to the adsorption of ore on the surface of mycelium.

The increase in sucrose concentration of the medium influences the extraction of manganese from pyrolusite ore. The data (Fig. 6) revealed that sucrose had direct relation with the extraction of manganese. The manganese extraction yields were increased as the sucrose concentration in the culture media increased. Heterotrophs were found to solubilize MnO_2 by producing citric and oxalic acids in the culture medium [13]. These heterotrophic micro organisms utilise sugars for the production of organic acids [18]. Castro *et al.*, [19] had also used sucrose

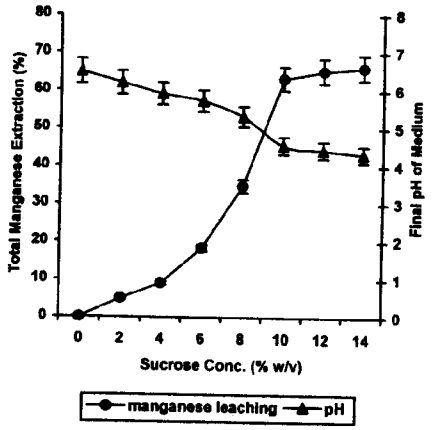

Fig. 6 Effect of sucrose concentration of medium on bioleaching leaching of manganese from pyrolusite ore by *Aspergillus niger* PCSIR-06.

as energy source in culture medium for bioleaching of minerals using heterotrophic micro organisms. A maximum of 66 % recovery of manganese was obtained with 14 % sucrose. It had also been observed that the recovery of manganese was non significant beyond 10 % increase of sucrose in the culture medium. In the absence of energy source (Sucrose) there was a very low recovery of manganese (0.19 %) which showed that *Aspergillus niger* PCSIR-06 did not produce organic acids which could reduce MnO_2 and hence a low recovery was observed.

Fig. 7 show that the reduction of manganese is related to the growth of *Aspergillus niger* PCSIR-06. The more the inoculum, more will be the growth; more will be production of organic acids which lead to more reduction of manganese. An optimum of 10 % (v/ v) inoculum of fungal spores gave maximum yield extraction of manganese (65.54 %). The adsorption of leaching material to the fungal mycelium is one of the major draw back of submerged cultivation of fungus [9]. More than 12 % inoculum was not taken for the investigation because it would lead to improper results due to excessive clumping of mycelium and adsorption of manganese to mycelium.

Experimental

Ore

Manganese ore was collected from Wadh area of Khuzdar District, Balochistan, Pakistan. The ore

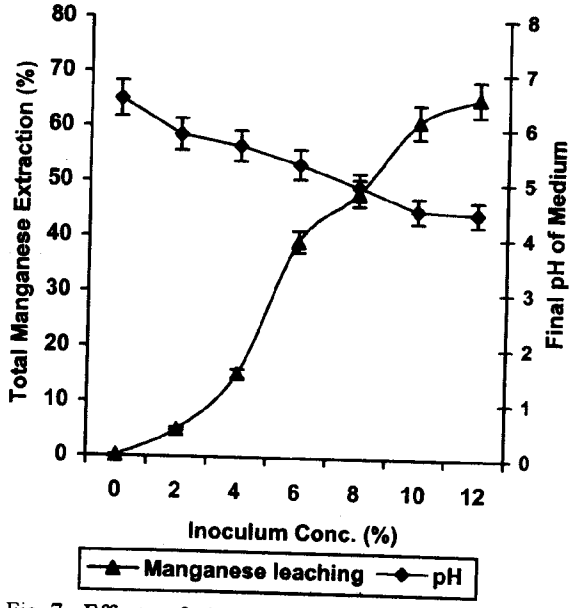

Fig. 7 Effect of inoculum size on bioleaching leaching of manganese from pyrolusite ore by *Aspergillus niger* PCSIR-06.

assayed manganese content 24.57 % (38.87 % as MnO_2). The other contaminants (%) were Fe 3.49, Ca 0.75, Mg 0.03, Zn 0.01, Cu 0.027, Ni 0.03, S 0.16, P 0.23, Si 11.37 and LOI 10.16. The ore was ground to mesh size -150 and used throughout the studies.

Microorganism

Ten *Aspergillus niger* strains were isolated from the cleaved surfaces of ore *on Czapek-Dox medium* and purified by repeated sub culturing and single colony isolation on Czapek-Dox medium.

Screening of isolated strains for leaching efficiency

The isolated *Aspergillus niger* strains were screened for manganese leaching ability by the application of submerged cultivation technique. Mineral Salt medium used for screeing contained (% w/ v) NH_4NO_3 0.3; KH_2PO_4 0.1; $MgSO_4.7H_2O$ 0.05 and sucrose 10, manganese ore 2.0 pH was adjusted to 6.5 with dilute NaOH. 10 ml of inoculum of the isolated strains was added separately to sterilized medium. The leaching studies were carried out in TUNAIR™ cell growth shake flask system of Shelton Scientific-IBI, working volume 100 ml, shaker speed 150 rpm, filter size 0.22 micron nitrocellulose. After 10 days of incubation at 30 °C, the flasks were sterilized. The contents were filtered out using Whattman filter paper and quantified for

manganese. The strain of *Aspergillus niger* which showed maximum solubilization of manganese was chosen for further studies. Further selection of strain was also observed by using direct screening method [9]. The most efficient *Aspergillus niger* strain for manganese solubilization was maintained on Czapek-Dox medium and named as *Aspergillus niger* PCSIR-06. Further, growth and bioleaching studies were performed using *Aspergillus niger* PCSIR-06.

Growth studies

The growth studies of *Aspergillus niger PCSIR 2004* were carried out both in shaking (150 rpm) and surface culturing conditions. The growth of the strain was measured in terms of final pH, total acidity and dried biomass weight. The growth in both cases was measured for 12 days with an interval of 2 days. After 2 days intervals the flasks were removed, sterilized and pH was noted. The contents were filtered through pre weighed filter paper. The biomass remained on filter paper was dried at 100 °C for 1 hour and weighed while the filtrate obtained was used for the determination of total acid strength.

Bioleaching studies

Manganese bio-leaching experiments were carried out using sterilized mineral salt medium containing known quantities of ore in TUNAIR™ Cell growth Shake Flask System of Shelton Scientific-IBI, working volume 1.0 liter shaking speed 200 rpm and filter size 0.22 micron nitrocellulose.

Effects of several parameters i.e., time period for leaching, effects of inoculum size 2 to 12 %, sucrose concentration 2 to 14 % and pulp density 1 to 6 %, were studied.

The leaching vessel was sterilized and final pH was measured at the end of each experiment. Leached solution was analysed after filtration through Whatman No. 1 filter paper. The contents were filtered and biomass and the ore remained on filter paper were washed with de-ionized water and then with dilute sulphuric acid. The washings were added to the filtrate. The overall manganese Leaching was determined at the end of each experiment.

Manganese contents in the leached solution were determined by Atomic Absorption Spectrophotometer M series and using SOLAAR Software manufactured by Thermo Electron corporation UK using standard conditions as mentioned in Table-1.

Table-1: Standard conditions of atomic absorption spectrophotometer for analysis of manganese.

Weavelength	279.5 nm
Band pass	0.3 nm
Lamp current	75 %
Flame	Air/acetylene
Fuel flow	1.0 L/min
Burner type	50 mm
Burner height	7.0 mm

Conclusions

It can be safely concluded from the aforesaid studies, that:

1. The production of organic acids by *Aspergillus niger* PCSIR-06 is proportional to the growth of mycelium.
2. The growth of *Aspergillus niger* PCSIR-06 was better in static conditions than in shaking conditions.
3. Inoculum concentration, sucrose concentration and pulp density play important role in leaching of manganese from pyrolusite ore.
4. Manganese leaching performance of *Aspergillus niger* PCSIR-06 was maximum in a medium containing 10 % sucrose (w/v) with pulp density 1 % (w/v) inoculated by 10% inoculum (v/v).

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