

Bioleaching of Low Grade Manganese Ore with *Penicillium citrinum*

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ABSTRACT

A native microorganism, *Penicillium citrinum* was isolated from the top soil of a manganese mine. Based on its efficiency for manganese solubilisation, it was utilized for the leaching of a low-grade manganese ore. The effects of various parameters such as pulp density, particle size, sucrose concentration, inoculum size and bioleaching duration on manganese ore were studied. The optimised conditions for maximum solubilization of the manganese ore (64.58% Mn) were : a particle size of 45 μ m; a pulp density of 2% (w/v); a sucrose concentration of 10% (w/v); an inoculum dosage of 10% (v/v); and a 30 day duration. © 2002 SDU. All rights reserved.

Keywords: Manganese ore; *Penicillium citrinum*; Leaching

1. INTRODUCTION

India has been recognized as one of the leading manganese producing countries in the world. Considering the environmental problems related to conventional processes, both pyrometallurgical and hydrometallurgical, its recovery through bioleaching utilizing different kinds of microorganisms has been studied extensively by several workers (Baglin *et al.*, 1992; Gupta and Ehrlich, 1989; Srimekanond *et al.*, 1992). Leaching of manganese by heterotrophic microorganisms is caused by metabolites such as organic acids (Baglin *et al.*, 1992). The proposed mechanisms for the bioleaching of manganese dioxide include both direct or indirect (Ehrlich, 1976; Toro *et al.*, 1988). In the former case, the microbes are said to be capable of utilising the MnO₂ as a final acceptor of electrons in the respiratory chain of their metabolism instead of oxygen (Ehrlich, 1987). In the latter case, the reductive process is associated with the formation of reductive compounds (citric acid) resulting from their metabolism (Toro *et al.*, 1988).

The following communication provides the preliminary evidence of the mechanism for the dissolution of manganese from a manganese ore using a native strain of *Penicillium citrinum*. Studies on isolation, identification, and leaching characteristics of this fungus are described in this paper.

2. MATERIALS AND METHODS

2.1. Substrate

Manganese ore was obtained from Joda East manganese mines of Tata Iron & Steel Company Ltd. (TISCO) in Keonjhar District in Orissa. The ore used in this study was of low grade, the manganese content ranging from 22-29% (Table 1). The XRD analysis of the ore indicated the presence of pyrolusite (MnO₂), chalcophanite (ZnMn₃O₇.3H₂O), hematite (Fe₂O₃) and goethite (FeOOH). For leaching experiments, the bulk ore was ground and sieved to get

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different size fractions. After acid digestion, the elemental analysis of ore was carried out using a Perkin Elmer-3100 Atomic Absorption Spectrophotometer.

Table 1
Chemical analysis of manganese ore

| Average Particle size (microns) | Element (%) | | | | | | | |
|---------------------------------|-------------|-------|------|------|------|------|------|------|
| | Mn | Fe | Zn | Cd | Pb | Cu | Ca | Mg |
| 2812 | 29.08 | 34.92 | 0.01 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 |
| 1831 | 24.54 | 26.24 | 0.00 | 0.00 | 0.00 | 0.01 | 0.01 | 0.00 |
| 500 | 22.96 | 23.18 | 0.02 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 |
| 112.5 | 25.72 | 24.94 | 0.02 | 0.00 | 0.00 | 0.01 | 0.02 | 0.01 |
| 60 | 23.99 | 22.55 | 0.00 | 0.00 | 0.03 | 0.01 | 0.01 | 0.01 |
| 45 | 23.04 | 22.06 | 0.48 | 0.05 | 1.07 | 0.02 | 0.02 | 0.01 |

2.2. Microorganism

2.2.1. Isolation

The microorganisms were isolated from the top soil of a manganese mine. A total of eight isolates was obtained and those isolates were used for selection of the best species for manganese solubilisation. Colony growth of fungal and bacterial species were observed using agar plates. Individual colonies, which grew well on solid media were selected on the basis of colour, size, shape and dominance.

2.2.2. Screening

The colonies were purified by single colony streaking and then each organism was restreaked on solid media impregnated with sterile, sub-micron, ball-milled manganese ore. The species which removed suspended ore particles and produced halos around colonies growing on agar plates were sub-cultured for taxonomic identification (Burgstaller and Schinner, 1993).

Submerged leaching was also carried out using all of the eight isolates at 2% (w/v) pulp density at an initial pH of 6.5 with a 500 microns particle size and 10% (v/v) inoculum dosage for a period of 10 days. The mineral salt media used for leaching contained the following constituents in 1 liter of distilled water, 3g NH_4NO_3 , 1g KH_2PO_4 , 0.5g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 100g Sucrose. After the preliminary screening, a fungal culture was selected on the basis of maximum manganese solubilisation from the ore.

2.2.3. Identification

The fungal culture was identified by the Institute of Microbial Technology, Chandigarh, India. This fungus was further used for bioleaching of manganese ore.

2.3. Bioleaching studies

Leaching experiments were carried out in 250ml Erlenmeyer flasks containing 90ml salt medium and 2-10% (v/v) (10^6 spores/ml) inoculum, 2-10% (w/v) sucrose at 2-10% (w/v) pulp density of ore and particle sizes of manganese ore ranging from 2812 microns to 45 microns for 90 days. The flasks were incubated at room temperature (32°C) while shaking at 140rpm. Experiments were performed in duplicate. At the termination of each set of experiment, the flasks were sterilised. The contents of the flasks were filtered. The final pH was measured. The biomass and the ore which remained on the filter paper were thoroughly washed with distilled water and then with dilute sulphuric acid. The washings were added to the filtrate. The overall manganese extractions thus stated here is the sum of acid desorbed and soluble

manganese at the termination of each set of experiment. The soluble/acid desorbed concentrations of manganese/iron were calculated as (Vegliò *et al.*, 1997):

$$\text{Manganese (or iron) extraction yield (\%)} = \frac{C - C_i}{C_o} \times 100 \quad (1)$$

or recovery

where, C = total manganese/iron recovered after 30 days treatment (%),
 Co = initial manganese / iron content in the leaching system (%),
 Ci = Mn²⁺ due to inoculum (%), (Here, Ci was taken as zero)

3.RESULTS AND DISCUSSION

3.1. Influence of particle size

Various size fractions ranging from 2812 microns to 45 microns were used for the study. The microbial leaching of manganese ore with heterotrophic microorganisms like fungi generally follows an indirect mechanism (Abbruzzese *et al.*, 1990; Toro *et al.*, 1993) where manganese solubilization is due to reduction.

Table 2 shows that the total manganese extraction yield is highest (64.58%) for the finest particle size of 45 microns due to the accessibility of the ore particles to the fungus. Soluble manganese in the culture fluids ranged from 4.13% to 19.88% for the different granulometric size ranges.

Table 2
 Influence of particle size on bioleaching of manganese ore

| Conditions: Pulp density: 2% (w/v) Duration: 30 days | | Inoculum: 10% (v/v) pH initial: 6.5 | | Temperature: 32°C Sucrose: 10% (w/v) | | Shaking: 140rpm | |
|---|----------|---|------|---|-------|----------------------|-------|
| Average Particle size (microns) | Final pH | Soluble manganese and iron extraction yield (%) | | Acid desorbed manganese and iron extraction yield (%) | | Total extraction (%) | |
| | | Mn | Fe | Mn | Fe | Mn | Fe |
| 2812 | 5.95 | 4.13 | 0.08 | 8.08 | 2.90 | 12.21 | 2.98 |
| 1831 | 5.85 | 4.81 | 0.08 | 9.78 | 3.88 | 14.59 | 3.96 |
| 500 | 5.76 | 13.72 | 0.09 | 19.25 | 6.69 | 32.97 | 6.78 |
| 112.5 | 5.70 | 15.01 | 1.28 | 41.99 | 9.02 | 57.0 | 10.3 |
| 60 | 5.62 | 16.80 | 0.26 | 42.93 | 10.02 | 59.73 | 10.28 |
| 45 | 5.60 | 19.88 | 0.14 | 44.70 | 12.24 | 64.58 | 12.38 |

There is not a strong pH decrease as can be seen from Table 2. Similar observations were made by Paponetti *et al.* (1989) while leaching MnO₂ using *Aspergillus niger*. They proposed the indirect mechanism of dissolution where organic acids produced during the process (oxalic and citric acids) were responsible for reduction and solubilization of the manganese ore. *Penicillium sp.* is also said to produce citric and oxalic acids (Silverman and Munoz, 1970; Tzeferis, 1994). There seems to be greater evidence for the indirect mechanism and hence, it was assumed that dissolution of manganese ore in our case with *P. citrinum* also followed indirect reaction mechanism. During the bioleaching process, manganese may precipitate. Also, manganese ions adsorb on the surface of the MnO₂ ore particles at pH>5 (Marshall, 1979) which may limit further leaching (Buys *et al.*, 1986). As manganese oxide has negligible solubility in dilute sulphuric acid at ambient temperature (Imai, 1978), the adsorbed manganous ions were re-dissolved in the dilute sulphuric acid wash. Therefore the total Mn²⁺ extracted is the sum of acid desorbed manganese extraction yield and soluble manganese extraction yield at the end of each batch of experiment.

The iron dissolution was also highest for the finest particle size of 45 microns corresponding to the peak in manganese extraction. The maximum concentration of soluble manganese in the culture fluids (19.88%) at the 45 microns size was not as high as expected. It may be due to the influence of the mineralogy of the manganese ore. There is a trend towards slower microbial leach rates on oxides with more ordered or crystalline structures like pyrolusite. XRD data of the manganese ore in our investigation indicated the presence of more pyrolusite than other minerals like cryptomelane, rhodochrosite, calcite, etc. Manganese oxides containing relatively more pyrolusite have been shown to be less susceptible to microbial attack than those with less (Srimakanond *et al.*, 1992).

3.2. Influence of pulp density

A series of experiments were carried out with different pulp density suspensions ranging from 2 to 10% (w/v) manganese ore and the data is summarized in Table 3. A constant particle size of 500 microns was used in the experiments. The highest dissolved or solubilized manganese concentration (13.8%) was achieved with a 2% (w/v) pulp density at 32°C (Table 3). There was no appreciable change in pH which again indicates a non-enzymatic reduction of manganese by *Penicillium citrinum*. Higher Mn²⁺ extraction yields are achieved with lower pulp densities. Similar observations were made by Vegliò *et al.*, 1997. The total manganese recovery or manganese extraction yield after 30 days treatment was evaluated in a similar fashion to previous tests, adding the soluble (Mn²⁺) and the precipitated manganese after acid treatment of the solid leached residue.

The maximum total recovery of 33% was achieved for the 2% (w/v) pulp density. Iron extraction was also maximized at this pulp density, 6.78%. As the pulp density increased, the manganese extraction yields decreased. This may be due to a decrease in the availability of oxygen or nutrients for the fungus. MnO₂- reduction in an acid solution under anoxic conditions represented by the following reaction:



is not very likely due to the high energy of activation (Ehrlich, 1996).

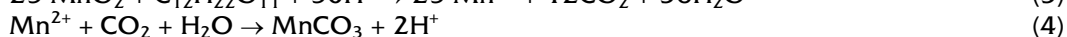
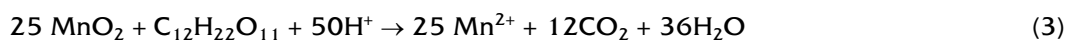
Table 3
 Influence of pulp density on bioleaching of manganese ore using *P. citrinum*

| Conditions: Sucrose: 10% (w/v) Shaking: 140rpm Particle size: 500 microns | | Inoculum: 10% (v/v) Duration: 30 days | | Temperature: 32°C pH initial: 6.5 | | | |
|---|----------|---|------|---|------|----------------------|------|
| Pulp density (% w/v) | Final pH | Soluble manganese and iron extraction yield (%) | | Acid desorbed manganese and iron extraction yield (%) | | Total extraction (%) | |
| | | Mn | Fe | Mn | Fe | Mn | Fe |
| 2 | 5.76 | 13.72 | 0.09 | 19.25 | 6.69 | 32.97 | 6.78 |
| 4 | 5.80 | 12.54 | 0.08 | 17.51 | 4.66 | 30.05 | 4.74 |
| 6 | 5.82 | 7.40 | 0.04 | 16.77 | 4.40 | 24.17 | 4.44 |
| 8 | 5.85 | 7.06 | 0.02 | 15.81 | 4.28 | 22.87 | 4.30 |
| 10 | 5.95 | 4.75 | 0.35 | 12.63 | 3.54 | 17.38 | 3.89 |

3.3. Influence of sucrose concentration

The experiments conducted to observe the influence of sucrose concentration on the bioleaching of manganese ore indicated that there is a direct relationship between manganese extraction and the total amount of sucrose present in the media. Heterotrophs like *Penicillium sp.* were found to solubilize MnO₂ by producing citric and oxalic acids in a culture medium containing sucrose (Silverman and Munoz, 1970). This type of media is commonly utilized in microaerobial monitored conditions. It was previously mentioned that strong evidence exists for

the reduction of MnO_2 by fungi through the indirect mechanism or non-enzymatically (Paponetti *et al.*, 1989). Fungi produce metabolic products like oxalic or citric acids by utilising sucrose. These acids are strong enough reductants for MnO_2 . Since the electron transport mechanism in fungi, which are eukaryotic organisms is located in mitochondrial membranes and not in plasma membrane, as in procaryotic cells, fungi cannot be expected to reduce MnO_2 enzymatically (Ehrlich, 1978), except possibly by extracellular enzymes. The overall indirect reaction utilizing sucrose can be expressed as follows :



Sucrose consumption was greater in the presence rather than in absence of manganese (Toro *et al.*, 1991). The growth of the microorganism began with a short lag phase which was followed by an exponential growth phase when manganese was extracted from ore, hence utilising the sucrose.

In these investigations, it was observed that a maximum extraction of manganese of 32.97% was obtained with 10% sucrose with a particle size of 500 microns (Table 4). The manganese extraction yields were increased as the sucrose concentration in the culture media increased. Similar observations were made by (Vegliò *et al.*, 1995). Correspondingly, iron extraction was also peak (6.99%) with a 10% sucrose concentration. In the control (media without sucrose) there was a low recovery of Mn^{2+} (6.98%) and iron (0.9%). In the absence of energy substrate like sucrose *Penicillium citrinum* could not produce organic acids to reduce the MnO_2 and hence, a low extraction was observed.

Table 4
 Influence of sucrose concentration on the bioleaching of manganese ore using *P. Citrinum*

| Conditions: Pulp density: 2% (w/v) Inoculum: 10% (v/v) Temperature : 32°C Particle size: 500 microns Shaking: 140rpm Incubation: 30 days pH initial: 6.5 | | | | | | | | |
|--|----------|---|------|---|------|----------------------|------|--|
| Sucrose concentration (% w/v) | Final pH | Soluble manganese and iron extraction yield (%) | | Acid desorbed manganese and iron extraction yield (%) | | Total extraction (%) | | |
| | | Mn | Fe | Mn | Fe | Mn | Fe | |
| 2 | 5.90 | 10.83 | 0.09 | 12.63 | 5.82 | 23.46 | 5.91 | |
| 4 | 5.85 | 11.59 | 0.09 | 12.84 | 6.04 | 24.43 | 6.13 | |
| 6 | 5.83 | 12.68 | 0.17 | 15.03 | 6.13 | 27.71 | 6.30 | |
| 8 | 5.80 | 13.66 | 0.13 | 16.6 | 6.47 | 30.26 | 6.60 | |
| 10 | 5.76 | 13.72 | 0.30 | 19.25 | 6.69 | 32.97 | 6.99 | |
| Control | 6.35 | 6.42 | 0.04 | 0.56 | 0.86 | 6.98 | 0.90 | |

3.4. Influence of inoculum size

It was observed from these studies that the product formation (manganese reduction) was related closely to the microbial inoculum or growth (product associated to the growth). The greater the inoculum concentration, the greater the production of organic acids which will eventually lead to manganese reduction. A dosage of 10% (v/v) inoculum gave the maximum total manganese extraction of 32.97% as shown in Figure 1. One of the major drawbacks with submerged cultivation technique employing fungus is that the leaching material is adsorbed to fungal mycelium (Burgstaller and Schinner, 1993). As stated above, the growth of the fungus was enhanced in presence of the manganese ore as manganese is an essential element for growth and differentiation of the fungus (Auling, 1989). Greater than 10% (v/v) inoculum was not employed for this investigation as it would have caused clumping of the mycelia and the adsorption of the leached manganese to the mycelia. The control with no fungal spore inoculum under the similar experimental conditions produced a manganese extraction of only 1.18% and only after acid washing. The soluble manganese extracted into the culture media was only 0.003%, the final pH (6.45) of the culture media also reflects the lack of extraction. In general,

the manganese reduction is enhanced as the inoculum concentration increases to the point where mycelia clumping and adsorption becomes problematic.

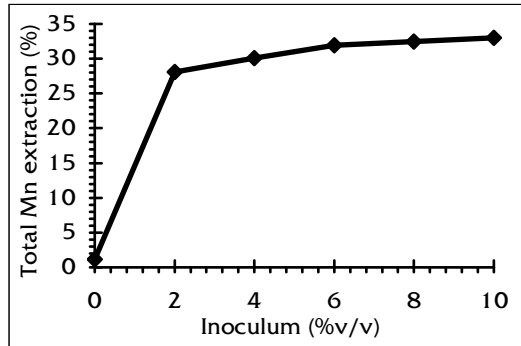


Figure 1. Influence of inoculum size on Manganese extraction

3.5. Influence of time period

The effect of leach duration on the extraction of manganese with *P. citrinum* was studied. A maximum period of 90 days was employed using a finer particle size of 112.5 microns. The studies were conducted with an initial pH of 6.5 at 32°C. It appears that the manganese was extracted from the ore by indirect mechanism or non-enzymatically by the fungus *P. citrinum*. This is supported by a decrease in the pH of the leach liquor. The pH decreased to 5.25 in 30 days giving a total extraction of 58% [16.1% of soluble manganese and 41.9% of acid washed manganese in the leach system (Figure 2)]. There was a net acid consumption at the time of maximum Mn^{2+} reduction. In the acidic medium (organic acids released by the fungus), the iron was also reduced. A total recovery of 10.30% iron was achieved within a period of 30 days. There was an increasing trend of total manganese extraction up to 45 days (68.3%). After 45 days, there was a decrease in the total manganese extraction (59.48%) as well as iron (6.01%). It has been suggested that the manganese which is leached out gets accumulated on the surface of some structure of the fungal hyphae (Ghiorse and Ehrlich, 1992). It was observed that the leaching was growth independent and most likely the result of microaerobic fermentation where the reductive process was associated with the production of reductive compounds (Madgwick, 1993). The reduction in the recovery of manganese after 45 days may be due to :

1. the accumulation of leached material (manganese) on the surface of fungal hyphae (Ghiorse and Ehrlich, 1992).
2. the production of volatile metabolites instead of organic acids and decay of the enzymes of the fungus following its death phase (Harrison and Pirt, 1967) as a result of which there is a slight rise in pH of the media.

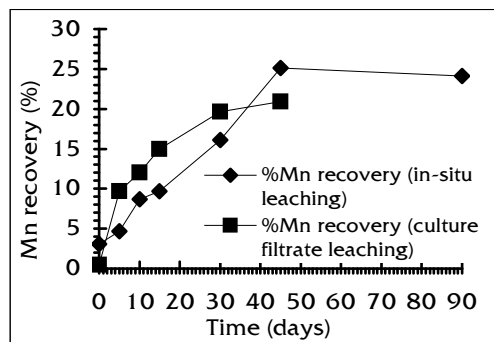


Figure 2. %Mn recovery in 1) in-situ leaching (soluble manganese) 2) culture filtrate leaching

The experiments were terminated at the end of 90 days following a reduction in the manganese extraction yield.

4. CONCLUSIONS

The fungus *Penicillium citrinum*, a native organism from the top soil of a manganese ore mine, was found suitable for the leaching of a low grade manganese ore. A maximum manganese extraction of 64.58% was achieved with a particle size of 45 microns, a pulp density of 2% (w/v), a sucrose concentration of 10%(w/v), an inoculum dosage of 10% (v/v) and a duration of 30 days.

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