

Effect of mechano-chemical activation on bioleaching of Indian Ocean nodules by a fungus

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ABSTRACT

The effect of mechano-chemical activation of Indian sea nodules, while recording the zeta potential, particle size distribution and surface area, on the bio-dissolution of metals by *Aspergillus niger* has been investigated. Activation is a term used to indicate what takes place when increasing grinding time does not result in significant change in particle size but rather results in the accumulation of energy that may lead to the development of lattice defects within the particles that can aid biological attack. It was observed that the mechano-chemical activation improved the bio-dissolution of metals such as copper, nickel and cobalt from the sea nodules at initial pH in the range 4.0–5.0. With 10 min milling of particles of $\leq 75 \mu\text{m}$ size, 86% material was reduced to $\leq 10 \mu\text{m}$ size with a change in zeta potential from -18 to -34 mV. Above 95% copper, nickel and cobalt each was leached out in 15 days time from the nodules activated for 10 min at 5% (w/v) PD and 35°C temperature with initial pH of 4.5; the biorecovery being almost similar when the material was activated for 30 min. In the case of nodules without activation, $\geq 89\%$ metal bioleaching was achieved in 25 days time at an initial pH of 4.5 under this condition. The mechano-chemical activation of sea nodules has thus influenced the bio-dissolution process, while providing a wider pH range available for processing of nodules with the involvement of organic acids such as oxalic and citric generated from the fungus.

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1. Introduction

Bioleaching of valuable metals from the deep sea nodules (Ehrlich, 1968, 1996, 2001, 2002; Rossi and Ehrlich, 1990; Bosecker, 1997; Li et al., 2000; Li and Feng, 2000; Zhang et al., 2001) has been investigated by several researchers. Some attempts were also made for biorecovery of metals from Indian Ocean nodules (Sukla et al., 1995; Kumari and Natarajan, 2001, 2002; Mehta et al., 2002, 2003; Mukherjee et al., 2003, 2004a,b). In the bioleaching studies, *Acidithiobacillus ferrooxidans* (Mehta et al., 2002, 2003) and microbial isolates from the nodules (Mukherjee et al., 2003, 2004a,b) were used. In general, bioleaching of ocean nodules with microbes is a slow process with metal recovery taking days to months. This problem may be addressed by involving an input energy in the form of ultrasonics or mechano-chemical activation (MA). The application of ultrasonics (Sukla et al., 1995) as a pre-treatment of *Aspergillus niger* for bioleaching of lateritic nickel has resulted in improved nickel extraction. Similarly, the use of ultrasonic waves has improved bio-dissolution of metals such as copper, zinc, cobalt, nickel, iron and aluminium from black shale (Anjum et al., 2010) with *A. niger* because of improved organic acid secretion from the fungus under controlled conditions.

The role of activation of minerals for extraction of metals is well documented in literature (Thacova, 1989; Amer, 1995; Balaz, 2000, 2008; Zhao et al., 2009). MA comprises of those structural and physicochemical changes which are induced in solids by deformation, disintegration and/dispersion due to mechanical energy or which are consequences of such action (Thacova, 1989; Bernhardt, 2000; Butyagin, 2000; Steinik and Thacova, 2000; Boldyrev and Thacova, 2000). This may cause change in surface charge characteristics and introduce defects in the lattice – meaning deviation from the ideal structure (Steinik and Thacova, 2000). The creation of defects often leads to storage of energy in the solids and changing the structural as well as thermodynamic state (Bernhardt, 2000). The change may be in terms of atomic shift from lattice structure, bond length and angle and in some cases the excitation of electron subsystems (Boldyrev and Thacova, 2000). It was thus considered worthwhile to investigate the role of mechano-chemical activation as a pre-treatment method to improve the process of metal biorecovery from Indian sea nodules by *A. niger* and results are presented in this study.

2. Materials and methods

Sea nodules were obtained from NIO, Goa. Phases identified by XRD in the nodules were todorokite, birnesite, and lithiophorite as major Mn(IV) constituents while iron phases being goethite,

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maghemite and ferrihydrite. Copper, nickel and cobalt were a part of manganese oxide and iron phases. The sea nodule sample was crushed and ground to less than 300 μm size. A representative sample obtained by coning and quartering was analysed by AAS and the composition was found as 0.89% Cu, 0.96% Ni, 0.12% Co, 18.31% Mn and 6.4% Fe.

The activation of the sample was accomplished in a high energy attrition mill (PE 075, Netzsch, Germany) under the following conditions: 500 mL volume, 2 kg SS balls of 2 mm dia (86% of volume filled up), 150 g nodules sample ($\leq 75 \mu\text{m}$ size), 250 mL water, 25 °C temperature and 5–30 min of activation at 1000 rpm. To compare the results on bioleaching of activated and non-activated (milled in other unit) material, the sea nodule of $<50 \mu\text{m}$ size particles (25 g) was ground in a planetary mill (Model PM 480 Ritzsch, Germany) with vessel of 125 mL capacity using three balls of tungsten carbide of 20 mm dia at 200 rpm for 45 min in dry condition.

Particle size distribution and specific surface area were determined by laser particle size analyser (Mastersizer, Malven, UK). The zeta potential of the particles after milling was measured by zeta meter (model 501 PENKEM Inc., USA).

A. niger (MTCC 281), collected from IMTECH, Chandigarh was revived at 35 °C using Czapek Dox medium. The 1 mL of the sub-cultured *A. niger* in 100 mL liquid Czapek Dox medium was subjected to grow on 2% (w/v) sea nodules in a flask under incubator at 35 °C and 6.8 pH. In this process – the adaptation, fungi became tolerant to the substrate. The growth was observed to be slow till 24 h in the first adaptation and then increased to a high level with 2×10^8 spores/mL in 36 h. In the second adaptation stage, the full growth of *A. niger* was realised in 18 h and finally within 12 h in the last stage. The four times adapted culture over nodules was used for the bioleaching experiments.

Bioleaching experiments were carried out in conical flasks of 250 mL in an orbital motion incubator shaker at the desired temperature. Adapted fungal culture suspension (5% v/v) was inoculated in the pre-sterilized (at 120 °C for 15 min) Czapek Dox medium (sucrose – 30 g/L, NaNO_3 – 3 g/L, K_2HPO_4 – 1 g/L, MgSO_4 – 0.50 g/L, KCl – 0.5 g/L and FeSO_4 – 0.01 g/L) in the presence of desired amount of sea nodules. Experiments were run to determine the influence of variables such as initial pH and particle size of nodules. The sterile sets were also run under the similar conditions but without fungus. The liquid samples as supernatants were collected at specific intervals and analysed by AAS for metal recovery. The organic acids such as oxalic and citric secreted from the fungus during the experiment was determined by the method described elsewhere (Castro et al., 2000). For this, a known volume of the supernatant sample was drawn from the inoculated set and subjected to centrifugation at 8000g for 10 min followed by filtration, before analysis.

Redox potential was measured against saturated calomel electrode (SCE). All the experiments were carried out in duplicate to check the consistency and variation in metal recovery was found to be within $\pm 2\%$.

To determine the amount of metals adsorbed in the fungal biomass, the leach residue consisting of exhausted ore and the biomass was thoroughly washed with dilute sulphuric acid (pH: 1) for 10 min and then centrifuged (10,000 rpm). The leach residue after washing was taken for metal analysis to check the material balance. Unless specified otherwise, total leach recovery presented in the text was computed for the metal by adding the amount estimated in the leach liquor and that obtained from washing the residue and the biomass.

3. Results and discussion

The mechano-chemically activated nodules were characterized for particle size distribution (Rossi and Ehrlich, 1990), zeta poten-

tial, surface area, etc. Trend in particle size reduction (in terms of d_{50} = median diameter) and increase in specific surface area (SSA) with time of milling are plotted in Fig. 1. It clearly shows typical stages achieved during mechano-chemical activation in relation to milling. In 5 min of milling, the particles pass through Rittinger stage where excessive breakage of particles occurs and SSA increases proportionately to the energy input. In the next stage, between 5 and 20 min, limited breakage continues showing only a minor decrease in d_{50} values (from 6.2 to 3.4 μm) but the proportional increase in SSA is lost due to weak interactions between the particles. Here the input energy is used to activate the material by shear stress with structural disintegration/plastic deformation, defects, transformation etc. in the particles. Beyond 20 min some breakage occurs with the increase in SSA. The SEM photographs of the activated materials during milling with time are shown in Fig. 2 which indicates the pattern of breakage of the particles. Bioleaching was performed with the nodules activated for 5 min, 10 min and 30 min.

In Figs. 3a and 3b, particle size distribution due to milling/activation is shown. Fig. 3a reflects that size of the particles is below 38.5 μm in 10 min of activation, whereas in 20 min, the size is $<28.7 \mu\text{m}$ which is observed to be of $<11.9 \mu\text{m}$ size in 30 min of activation. The sea nodules ground for 45 min at a low speed (200 rpm) in a planetary mill, termed as the non-activated material, exhibit about 95% of the particles below 42.45 μm size. Fig. 3b shows that most of the activated particles lie in the range 0.5–10 μm with hardly any change in chemical composition. Very few of them are of 20–30 μm size and only 0–4% of them are $\geq 30 \mu\text{m}$. Lower particle size and higher SSA offer more reactive area for microbial and chemical dissolution.

Surface charge characteristics of particles in terms of zeta potential (ZP) are expressed in Fig. 4. ZP dropped gradually from –18 mV in case of non-activated sea nodules to –34 mV in 10 min and –47 mV in 15 min of activation. However, ZP did not change much in 5 min of activation. Increase in ZP (–mV) with time of activation indicates the accumulation of the similar charge on the surface and higher electrophoretic mobility of the activated particles in the leaching medium. The activated particles thus gain so less weight that gravitational force becomes low and particles remain dispersed in bulk medium offering more contact for the chemical leaching/microorganism. A plot of zeta potential against specific surface area given in Fig. 5 shows only limited change in surface area with increase in surface charge (ZP) beyond –34 mV. This clearly shows that the milling of material causes activation which is accompanied by accumulation of input energy and is reflected by increase in surface charge characteristics.

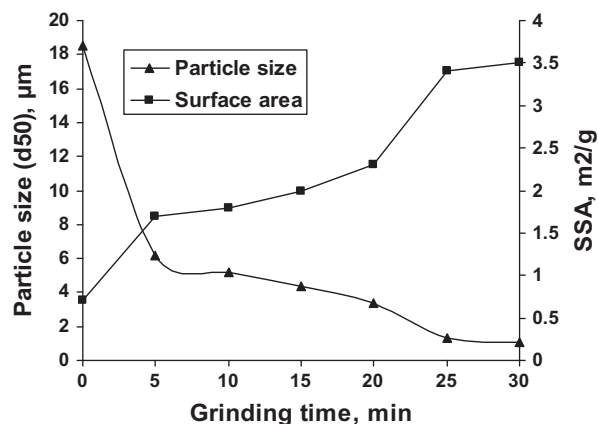


Fig. 1. Size reduction and surface area during grinding of sea nodules.

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