Biochemical Leaching of Metals from Indian Ocean Nodules by *Aspergillus Niger*

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Abstract

Bioleaching of valuable metals from Indian Ocean polymetallic nodules was carried out using a pure culture of *Aspergillus niger*. Parameters such as pH, pulp density (PD), particle size, time were optimised for the biorecovery of metals. At pH 4.5, 35 °C temperature and 5% PD and in 30 days the biorecovery of the metals was found to be 97% Cu, 98% Ni, 86% Co, 91% Mn and 32% Fe whereas in control experiment 4.9% Cu, 8.2% Ni, 27% Co, 6.3% Mn and 7.1% Fe were dissolved. The interesting aspect of this investigation is that a good amount of leached metals are adsorbed on fungal mycelia which can be recovered by acid wash. Fall in pH rom 7 to 4.6 and rise in Eh from 140 mV to 380 mV indicated that *A. niger* followed indirect leaching mechanism by releasing some organic acids which in turn converted higher oxides to lower oxide phases as identified by XRD phase analyses.

1. Introduction

With the gradual loss of land based mineral resources and increasing consumption of valuable metals across the globe, efforts are being made to explore alternate metal resources. The polymetallic ferro- manganese deposits are generated continuously on sea bed in the form of nodules. The nodules comprise mainly of manganese and iron oxides together with valuable metals such as copper, nickel, cobalt and zinc [1]. As these transition metals are often locked up inside the manganese and iron phases rather than in separate identifiable mineral phases within the nodules, reduction of the host oxides is thus a prerequisite to release and dissolve the valuable metals [2]. Many researchers have studied the chemical dissolution of metals in mineral acids/ ammonia with/ without pretreatment of ocean nodules. Mention may be made of a few of them viz., glucose - ammonia leaching [3], reduction – roast- ammonia leaching [4, 5], ammonia sulphur dioxide- pressure leaching [6], and sulphuric acid leaching at elevated temperature [7] for the recovery of valuable metals. These processes consume high energy and are often associated with excessive corrosion and impart negative impact on environment.

As regards microbial extraction of metals from low grade oxide ores, leaching of valuable metals from ocean nodules by a marine isolate [8], leaching of manganese from manganese ore by *Penicillium citrinum* [9] and nickel form lateritic nickel ore by heterotrophic microorganism are reported [10]. Metal dissolution characteristic on fungal activity using *A. niger* to leach copper and zinc from an oxidised ore [11] and treatment of domestic low grade lateritic ore of limonitic and haematitic type by *Aspergillus* sp. and *Penicillium* were studied where low metal recoveries were obtained (70% - 50%) even after acid wash of fungal biomass [12]. *A. niger* and *Penicillium* were used for bioleaching of zinc and nickel from silicates viz., calamine and garnierite [13]. Mulligan et al [14] studied biodissolution of copper and other metals embodied in low grade mining residues using *A. niger*. Bioleaching is an ecofriendly process and is very much useful specially to leach out metals from low grade ores. In present investigation the feasibility of using a fungus viz., *Aspergillus niger* for bioleaching of metals from the Indian ferromanganese ocean nodules has been explored.

2. Materials and Methods

Aspergillus niger was obtained from Microbial Culture Collection Centre, IMTECH Chandigarh, India and revived at 37°C using Czapek Dox medium (Sucrose 30g, sodium nitrate 3g, Dipotassium phosphate 1g, Magnesium sulfate 0.5g, KCl .5g, ferrous sulfate .01g). Sea nodules in the air dried form were provided by NIO, Goa. All the chemicals used were of Anala R grade. The sea nodule sample was crushed, ground and passed through a sieve of 300μm. A representative sample was prepared by coning and quartering method and was analysed by atomic absorption spectrometer (AAS). The nodules contained 0.89% Cu, 0.996% Ni, 0.12% Co, 6.44% Fe, 18.31% Mn, 0.09% Zn, 0.04% Mo, 0.72% C, 16% SiO_2 and 8% Al_2O_3. A known amount of the nodule powder was also passed through different sieves to get the sieve fraction analyses for bioleaching experiments. Chemical analyses of the sieve fractions are given in Table 1.

Table 1. Composition of different size fractions of sea nodules

Particle size (μm)	Fraction retained, %	Cumulative fraction retained, %	Composition (%)				
			Cu	Ni	Co	Mn	Fe
300 – 150	27.33	27.33	0.884	0.989	0.1035	18.30	7.2
150 – 100	36.12	63.45	0.856	0.99	0.105	19.20	7.7
100 – 75	14.97	78.42	0.847	0.850	0.105	19.73	7.98
< 75	21.58	100	0.849	0.981	0.107	18.81	8.13

2.1 Growth and adaptation of microorganism: Lyophilised A. niger culture was revived in C'dox medium and streaked on sterile agar plates to check the purity. The plates containing pure culture were saved in a refrigerator at 4°C and liquid culture was used for adaptation on the substrate. The adaptation was performed in media containing sea nodule powder (5% w/v PD) at pH 4 and 35 °C temperature in incubator shaker. After considerable growth the organism in the supernatant was shifted to the fresh medium containing nutrient and sea nodules for the second time adaptation. In this way adaptation on sea nodule was carried on for four times and adapted culture was stored in refrigerator at 4°C for use in bioleaching.

2.2 Bioleaching experiments: Bioleaching experiments were carried out in conical flasks taking 25g sea nodules of known size at the desired temperature in an orbital motion incubator under shaking condition (100 rpm). Adapted fungal spores were inoculated in nutrient broth in presence of the nodules. Spore inoculum was prepared by washing spores from mature plates of A. niger. Several sets of experiments were run to determine the influence of variables such as temperature, pulp density, pH and particle size of the nodules. Progress of bioleaching of metals was studied by collecting the samples at specific intervals and analysing the metal content by AAS. Redox potential (Eh) against saturated calomel electrode (SCE) was measured and reported at 5 days interval and pH of the supernatant was measured and maintained on alternate days with 10 (N) H_2SO_4.

In order to determine the amount of metals adsorbed in the fungal biomass viz., *A. niger*, the leach residue consisting of the biomass was thoroughly washed with dilute sulphuric acid (pH: 1) up to 2 h and then centrifuged at 10, 000 rpm. The supernatant was collected for metal analysis. A known amount of the leach residue after washing was taken for acid digestion and metal analyses.

3. Results and Discussion

Parameters that were studied for the optimisation of metal biorecovery from the sea nodules using *A. niger* are presented. Experiments were performed in triplicate. Data with less than 5% error were recorded.

3.1 Influence of pulp density: Fig 1 depicts bio-leaching of metals obtained using *A. niger* at various pulp densities (PD). An increase in pulp density resulted in decrease in metal dissolution. At a pulp density of 5% (w/v) about 79% Mn was recovered in 21 days at 4 pH and 35 °C temperature after acid wash along with 89.2% Cu, 79.5 % Ni, 93% Co and 66% Fe. These values subsequently came down to 46.6 % Mn, 37.8% Cu, 39.8 % Ni, 23.7% Co and 4% Fe with the change in pulp density to 20% under the same experimental condition. In the control experiment recovery was, however, very less at all the pulp densities studied. For an instance, the chemical dissolution in sterile experiment at 5% (w/v) PD in 21 days was found to be 10% Cu, 7.8% Ni, 32% Co and 6.3% Mn. So 5% (w/v) PD was considered optimum for the recovery of all the metals of importance. Higher pulp density might cause toxicity to the organism, besides limitation of the microbial growth thereby yielding lower metal recovery.

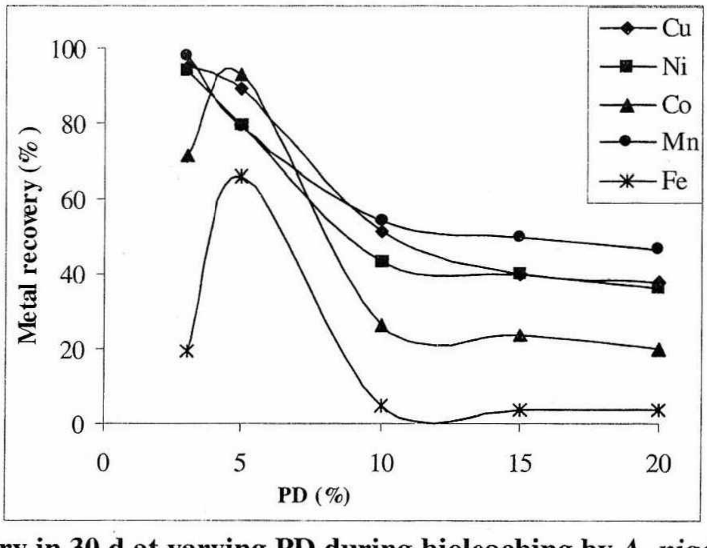

Fig 1. Metal recovery in 30 d at varying PD during bioleaching by *A. niger* at 4 pH , 35 °C

3.2 Influence of particle size: To find out the influence of different particle size of the sea nodules, bioleaching experiments were set at 4 pH, 35 °C temperature and 5% (w/v) PD using *A. niger* along with a set of control experiments. Data on metal recovery vs fraction size of the sea nodules are shown in Table 2. The composition of different size fractions given in Table 1 is not very different. So total recoveries as seen in Table 2 are very similar, excepting that of cobalt, which shows increasing trend with the fineness of the particles. The total biorecovery was found to be 97% Cu, 98% Ni, 38% Co, 93% Mn and 15% Fe for the coarser size of the nodules (300- 150 μm) as against recovery of 15% Cu, 32% Ni, 9% Co, 0.7% Mn and 0.9% Fe under the sterile conditions in 30 days. However, the biorecovery of cobalt from the sea nodules of <75μm size was found as to be 97% along with the dissolution of 96% Cu, 99% Ni 98% Mn and 18% Fe in 30 days.

Table 2. Metal recovery by *A. niger* at varying particle size at 4 pH, 5% PD and 35 °C

Fraction size (μm)/ condition	Days	Biorecovery (%)				
		Cu	Ni	Co	Mn	Fe
	7	26	24	24	28	3
	14	45	52	27	66	3
(300- 150)*	21	58	67	29	67	-
	30	57	78	27	82	3
Acid wash	---	41	20	11	11	13
Total	---	97	98	38	93	15
	7	29	26	20	27	2.3
	14	53	57	35	68	3
(150- 100)	21	48	65	38	62	2
	30	61	74	41	71	---
Acid wash	---	36	19	13	10	16
Total	---	97	93	55	81	16
	7	29	37	34	37	3
	14	73	32	45	58	5
(100- 75)	21	70	82	26	65	2
	30	64	74	38	69	---
Acid wash	---	33	23	14	12	15
Total	---	97	97	52	81	15
	7	29	43	62	48	8
	14	67	89	87	96	3
<75	21	66	73	59	77	4
	30	71	79	79	86	2
Acid wash	---	25	20	18	12	16
Total	---	96	99	97	98	18
	Control leaching					
(300- 150)*	30	15	32	9	0.7	0.9

3.3 Influence of pH: Effect of pH on biodissolution of metals was investigated at 35 °C, 5% (w/v) PD using 4% (v/v) suspension of adapted A. niger spores in a 250 ml leaching medium. Recoveries of metals at different pH in 30 days without acid wash are shown in Fig. 2 (a – d). High metal recovery of 97% Cu, 98% Ni, 86% Co, 91% Mn and 32% Fe was obtained at 4.5 pH whereas Co and Fe recovery was higher at pH 4 (96 and 66% respectively) after acid wash. Decreased recovery at pH 5 in all the cases is attributed to lower solubility and higher precipitation of metal ions. It has been demonstrated that manganese ions are absorbed on fungi and also precipitates at pH >5 [15] and limits further leaching [16].

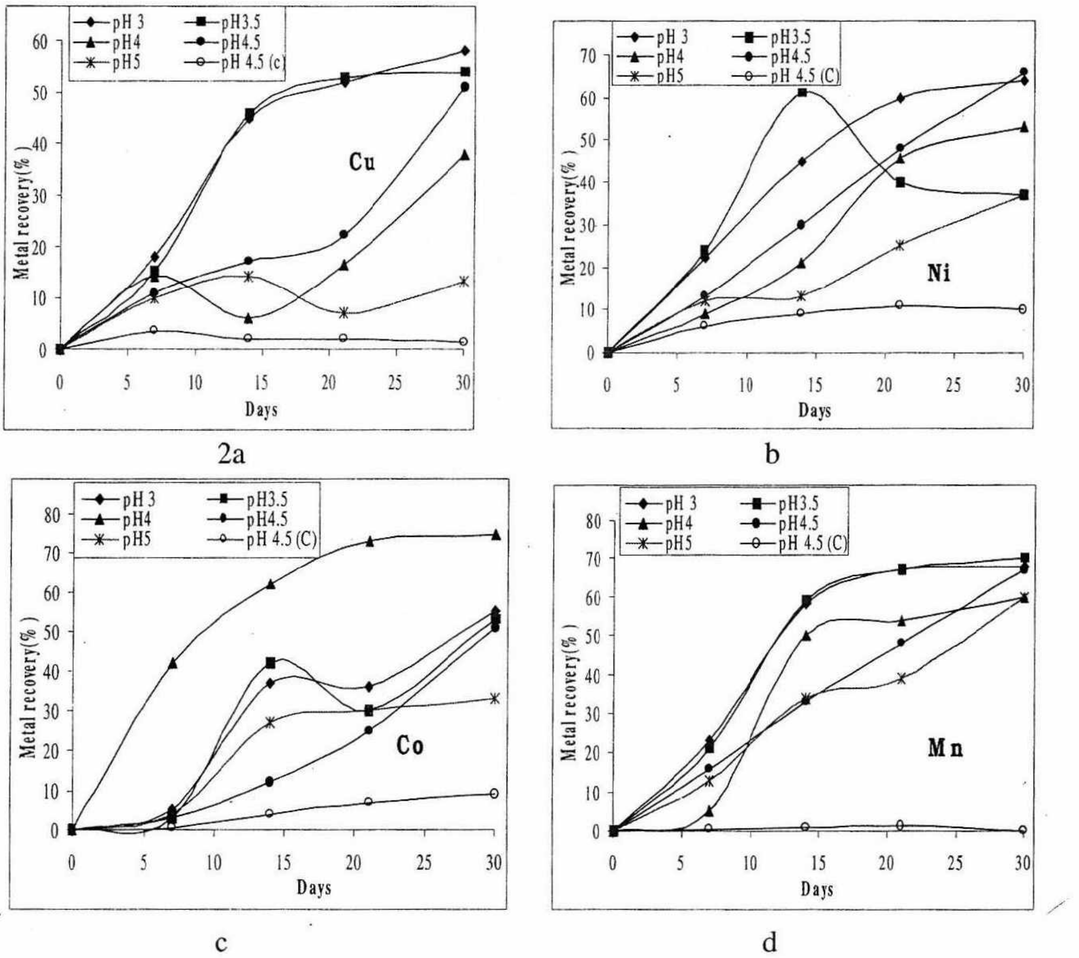

Fig. 2 (a –d). Metal recovery (data without acid wash) at varying pH in 30 days during bioleaching by at 35 °C and 5% PD.

Therefore, after each bioleaching experiment the fungal biomass along with the residue was washed with dilute sulphuric acid to evaluate the acid desorbed manganese and other metals. The total recovery of metals rose by 21- 60%. It may be assumed here that the metal ions were leached out and got accumulated mainly on the surface of the fungal hyphae [17]. This was further substantiated by insignificant recovery of metals during acid wash of leach residue from control experiments at pH 4/ 4.5. For the leaching of metals at 4 pH the variation of pH prior to adjustment and corresponding redox potential monitored for entire period of 30 days at 35 °C are shown in Fig 3. Initially the pH of the leaching medium was high and corresponding redox potential was low. But with time, pH decreased and Eh gradually increased and after 25 days of experiment Eh became stable at 380 mV indicating that the biological activity reached to a saturation level and so was the bioleaching of metals. The fall in pH as the leaching proceeded may be attributed to the secretion of organic acids such as oxalic, citric and gluconic by the fungus.

The microbial leaching of manganese with fungi usually occurs indirectly by non-enzymatic reduction process [18]. *A. niger* releases reductive compounds viz., acids (oxalic/ citric/ gluconic) which in turn converts Fe (III) to Fe (II) and Mn (IV) to Mn (II) [19]. The mechanism of the dissolution process can be represented by reactions 1 and 2. Oxalic acid for example released by *A. niger* first reacts with manganese dioxide and goethite. The metals associated with these two are thus released and get dissolved at lower pH, besides even forming soluble metal complexes. Some of the metals are in turn adsorbed on fungal cell wall and easily desorbed again by acid wash of the biomass.

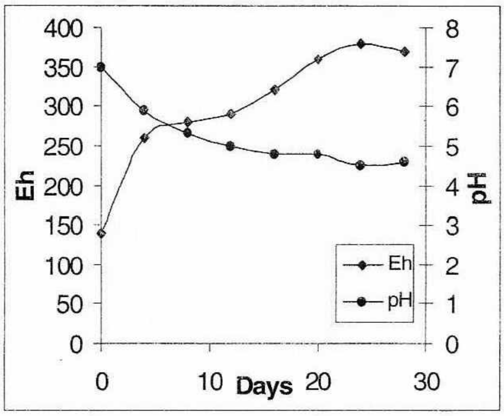

Fig 3. pH & redox potential profile during bioleaching by *Aspergillus niger* in 30 d, 35 °C.

$MnO_2 + HOOC—COOH + 2H^+ \rightarrow Mn^{2+} + 2CO_2 + 2\,H_2O$ ....(1)

$2\,Fe\text{-}O\text{-}OH + (COOH)_2 + 4\,H^+ \rightarrow 2\,Fe^{2+} + 2\,CO_2 + 4\,H_2O$ ....(2)

The phase identification by XRD analyses of leach residue by *A. niger* at pH 3.5 and pH 4.5 and also the nodules is presented in Table 3. Apart from the manganese phases such as birnesite, lithiphorite and silica, formation of hydronium jarosite at pH 3.5 and iron- hydroxide at pH 4.5 as major phases was an interesting feature. Besides, some amount of MnO, γ Mn_2O_3, braunite and maghemite were also formed as minor phases in leach residue at pH 3.5 and goethite was found in that of 4.5 pH. Formation of phases comprising of mixed and lower oxidation states of manganese and iron clearly demonstrates microbial attack of host lattice and release of metals entrapped in them for subsequent dissolution.

Table 3. XRD phase identification of sea nodules before & after bioleaching at 35°C, 30 d

Sample		Phases
Sea nodules		Major: Todorokite, birnesite, lithiophorite, silica
		Minor: Maghemite, goethite, alumina
Bioleach residue	pH 3.5	Major: Birnesite, lithiophorite, silica, hydronium jarosite,
		Minor: MnO, γ Mn_2O_3, braunite, maghemite, alumina
	pH 4.5	Major: Silica, birnesite, lithiophorite and $Fe(OH)_3$
		Minor: goethite, alumina

4. Conclusions

Bioleaching by *A. niger* is an indirect and nonenzymatic process which has easy desorption of adsorbed metals by acid wash of fungal biomass. Approximately 97% Cu, 98% Ni, 86% Co, 91% Mn and 32% Fe can be recovered by *A. niger* by bioleaching of sea nodules at pH 4.5 in 30 days at 35°C temperature, 5% pulp density using the sea nodules of < 300μm size.

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