Potential Application of a Heterotrophic Microorganism for Bioleaching of Metals from Indian Ocean Nodules

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Abstract

Bacillus circulans, a heterotrophic microorgansim has been used for the recovery of copper, nickel and cobalt from sea nodules. The organism was revived in nutrient medium and adapted gradually on sea nodules prior to leaching. Bioleaching studies were carried out in shake flasks containing sea nodule powder with growth medium and adapted microbial culture under a range of conditions such as pH, pulp density, particle size of nodules and temperature. Metal recovery was highest in 30 days but the rate of recovery was slow after 25 days of bioleaching. A 92% Cu, 73% Ni, 72% Co, 38% Mn and 17% Fe was recovered at pH 2 in 25 days at 35°C temperature. XRD identification of residue showed the presence of some lower and mixed oxidation phases of manganese and some unaltered iron phases. The biodissolution of metals was direct enzyme leading to reduction of Mn (IV) to Mn (II) and Fe (III) to Fe (II).

Key words: Bioleaching, sea nodules, oxide ore, *Bacillus circulans*

Introduction

The deep sea ferro- manganese nodules are rich and renewable source of non- ferrous metals such as copper, nickel, cobalt and zinc apart from the major components as manganese and iron. The metals are not present in identifiable separate mineral phases within nodules but incorporated in crystal lattices of major oxide/ hydroxide component (Gupta and Mukherjee 1990). The manganese phases often reported are todorokite, birnesite and lithiophorite and iron phases are goethite, maghemite and ferrihydrite in amorphous form (Mehta, Pandey and Mankhand 2002). The valuable metals thus can not be easily recovered unless the crystal lattice is broken by chemical or biological treatments. Although, recent efforts on large scale (500 kg/ d) (Mittal and Sen 2003) hydrometallurgical processing is underway in our country, recovery of metals by bioleaching with microorganisms is an alternative with certain features such as low energy consumption and environmentally cleaner option.

Ehrlich reported the biogenesis of marine ferro- manganese nodule and its reduction by bacteria (Ehrlich 1968). While reviewing the mechanism of bioleaching of metals from oxide ores (Ehrlich 1997) the potential use of bacteria to recover valuable metals (Ehrlich 2001) from ocean nodules was highlighted. Vrind et al used a marine Bacillus sp. to reduce manganese phase of nodules (Vrind, Boogerd and Elisabeth 1986). Recently biodissolution of copper, nickel and cobalt from Indian ocean nodules in presence of pyrite and sulphur using *Thiobacillus* sp. was investigated (Mehta, Pandey and Mankhand 2002). The corresponding reaction kinetics and mechanism were established (Mehta, Pandey and Mankhand 2003) which indicated that manganese and iron oxide/hydroxide lattice were broken by biochemical action leading to biodissolution of valuable metals. Mention may be made of the galvanic interaction (Kumari and Natarajan 2002) of ocean nodules in presence of pyrite and pyrolusite for complete recovery of copper, nickel and cobalt using *Thiobacillus* strains. As regards the use of heterotrophic microorganisms, the leaching of manganese from lowgrade ore and corresponding mechanism were studied by Acharya et al Acharya, Kar and Shukla 2001). Bacillus strains are known to reduce Mn (IV) to Mn (II) by direct involvement of type b and c cytochromes (Vrind, Boogerd and Elisabeth 1986). In this investigation, potential application of a heterotrophic microorganism, viz., a pure culture of *Bacillus circulans* has been attempted for the biodissolution of metals from Indian Ocean nodules, a ferromanganese oxide ore.

Materials and Methods

Bacillus circulans was collected from Microbial Culture Collection Centre, IMTECH Chandigarh and revived at 37°C using nutrient broth medium. Sea nodules in the air dried form were provided by National Institute of Oceanography, 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 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 particles was also passed through different sieves to get the sieve fraction analyses for bioleaching experiments. Chemical analyses of sieve fractions are given in Table 1.

Table 1. Composition of different size fractions of sea nodules

Size (µm)	Metal retained (%)	Cumulative metal 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
Mixed	----	-----	0.89	0.996	0.12	18.31	6.44

Growth and adaptation of microorganisms

Lyophilised microorganisms were revived in appropriate growth 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 at pH 2 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 saved in refrigerator at 4°C for use in bioleaching.

Bioleaching experiments

Bioleaching experiments were set up in conical flasks taking 25g sea nodules of known size at the desired temperature in an incubator under shaking condition. Adapted bacteria were inoculated in nutrient broth in presence of the nodules. Several sets of experiments were run to determine the influence of variables such as temperature, pulp density, pH and particle size of nodules. Samples were collected at the specific intervals and analysed by AAS for metal recovery. Redox potential against saturated calomel electrode (SCE) was measured at 5 days interval and pH of the supernatant was measured and maintained on alternate day with 10 N H_2SO_4.

Results and Discussion

In the bioleaching of metals the pH of the leaching medium plays an important role. The effect of pH on the recovery of metals by *Bacillus circulans* is shown in Fig 1. The experiments were also run under sterile conditions. The recovery of copper, nickel and cobalt was 92%, 73%, 72% respectively at pH 2. The metal recovery decreased to 58% Cu, 59% Ni and 19% Co at pH 1.5 while it was 82% Cu, 73% Ni, 58% Co at pH 2.5. The lower recovery of metals may be related to the lower activity of bacteria at pH 1.5. On the other hand hydronium jarosite formation on the surface of nodule particles may be the reason for somewhat lower recovery of a few metals at pH 2.5, which restricted the diffusion of the leachant with the nodule particles.

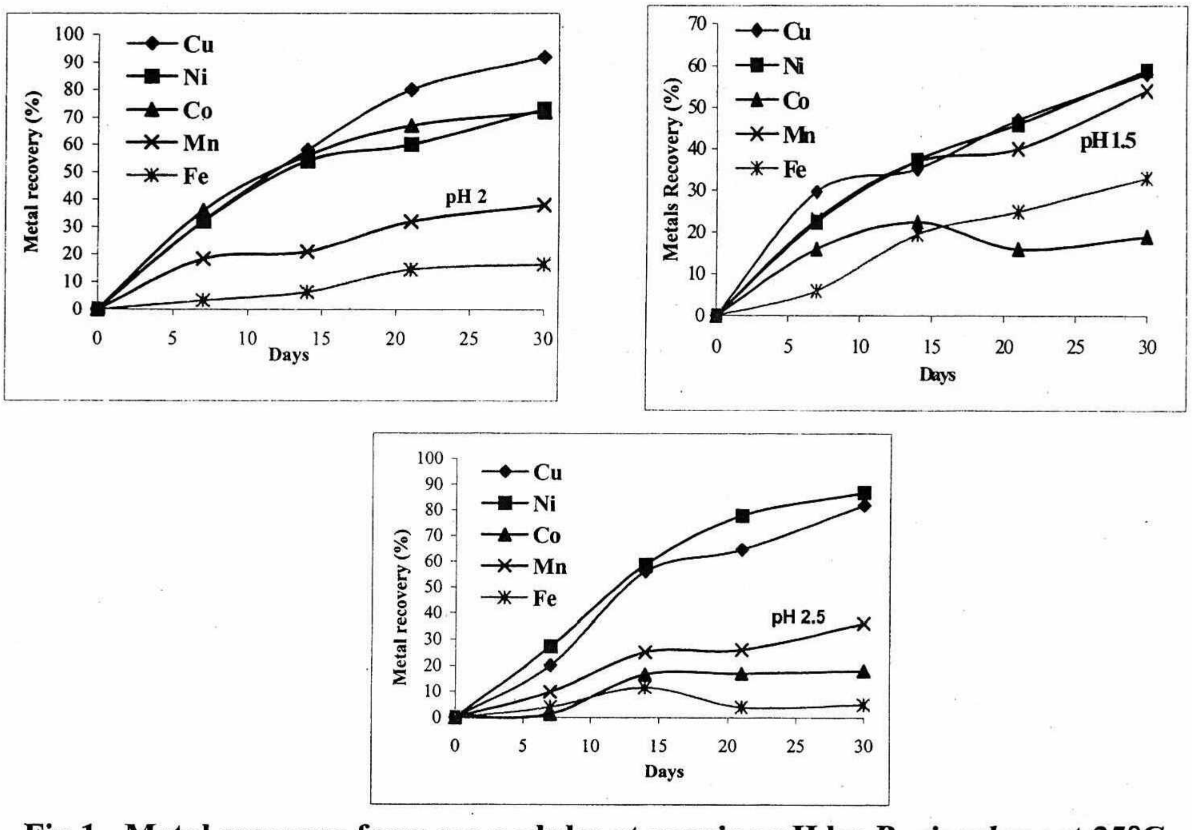

Fig.1. Metal recovery from sea nodules at varying pH by *B. circulans* at 35°C

In case of *B. circulans* highest metal recovery was obtained at pH 2 and 35°C temperature in 25 days with 5% pulp density (w/v) and mixed particles of the nodules. Repeated wash at (pH 2) of bioleached residue did not yield any more metal in solution. Table 2 represents biorecovery of different metals against control experiments. In bioleaching it may be seen that the improved recovery of copper by almost 20% and cobalt by 16% as compared to control leaching, whereas nickel recovery improved only by 6%. High recovery of copper indicated the occurrence of this metal on the manganese oxide in adsorbed mode mostly. The low nickel biorecovery (73%) showed its occurrence in the host lattice, which may be correlated with low manganese leaching (38%).

Table 2. Biorecovery of metals from sea nodules at pH 2, 35°C temperature and 5% pulp density

Days	Cu		Ni		Co		Mn		Fe	
	Bio	Cont	*Bio*	Cont	Bio	Cont	Bio	Cont	Bio	Cont
0	0	0	0	0	0	0	0	0	0	0
05	33	23	42	41	23	18	19	1	3	3
10	56	51	53	45	33	29	26	3	4	4
15	80	63	60	57	48	38	32	3	15	6
20	84	67	67	62	62	46	36	3	16	8
25	92	73	73	67	72	56	38	3	17	10

The influence of particle size of the nodules on bioleaching of metals was examined at 2 pH, 35 °C temperature and 5% PD along with control experiments (without microbes). Data on metal recovery vs fraction size of nodules are shown in Table 3 and metal recovery profile in 25 days using 100 – 75 µm particles are shown in Fig. 2. Copper and manganese recovery was almost consistent in all the cases but nickel recovery was quite higher (82%) for 100 – 75 µm particles. However, experiments on mixed particle size gave best results on overall metal recovery. In this case 92% Cu, 73% Ni, 72% Co, 38% Mn and 17% Fe were recovered. The higher recovery may be due to better permeation of the organisms in the mixed particles in the leaching medium. Fe with the change in pulp density to 20%. In case of 3% pulp density nickel and manganese recovery were more than that of 5% PD. 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% pulp density was 44% Cu, 60% Ni and 1.4% Mn. For the large scale application 5% pulp density may be considered optimum. Higher pulp density might cause toxicity to the organism, besides limitation of the microbial growth thereby yielding lower metal recovery.

Table 3. Biorecovery of metals from varying particle size in 25 days at pH 2, 30°C

Particle size (μm)	Max. recovery (%)			
	Cu	Ni	Mn	Fe
300 – 150	73	73	26	3
150 – 100	78	66	25	3
100 – 75	73	82	25	2.5
< 75	74	71	24	3

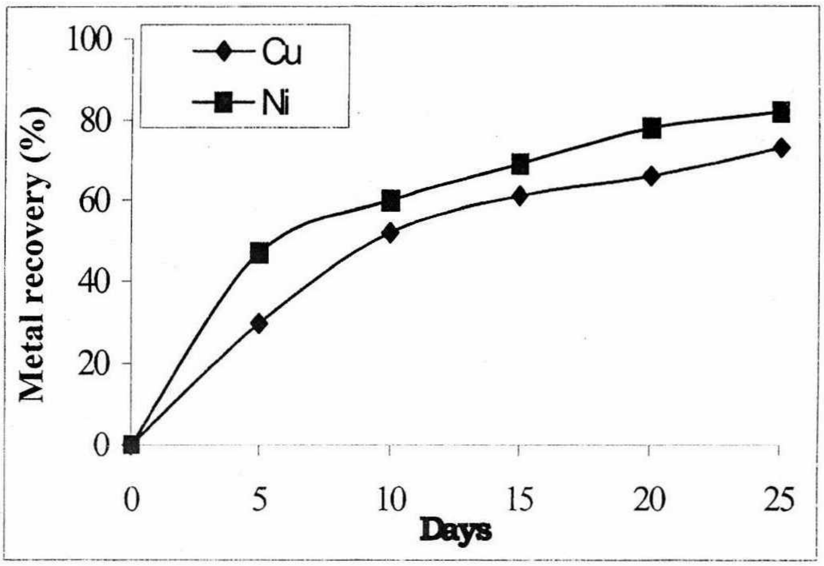

Fig. 2. Metal recovery by *B. circulans* in 25 days from 100- 75 μm particles at 35°C. pH: 2, PD 5%

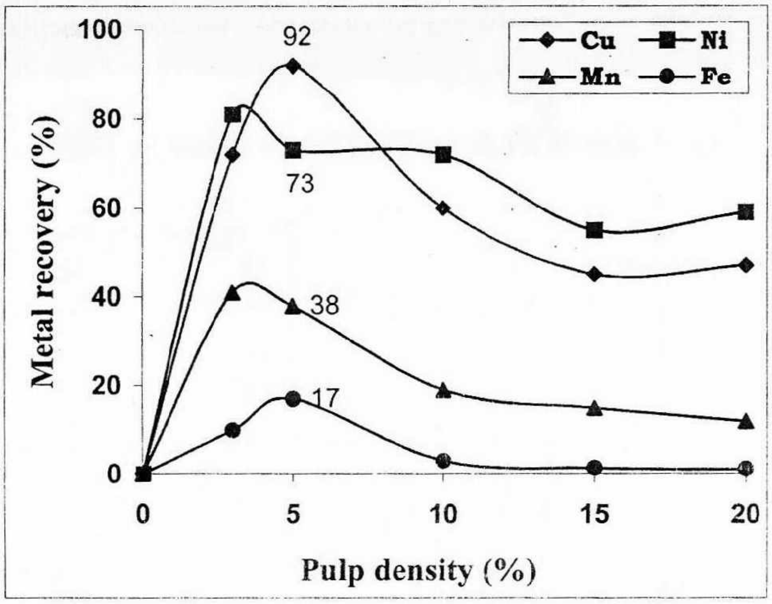

Fig. 3. Recovery of metals at varying pulp densities by *B. circulans* in 25 days at 35°C and pH 2

Under the optimum condition, the pH and redox potential in 21 days at 35°C temperature are shown in Fig 4. Initially the pH of the leaching medium was high and corresponding redox potential was low. But with time pH decreased and Eh gradually increased to 560 mV from 310 mV and after 21 days Eh became stable indicating that the biological activity reached to a saturation level and so was the bioleaching of metals. The data shown in Fig.4 for pH and Eh are the values determined at 5 days interval with the pH adjustment on alternate day.

XRD phase identification of the leach residue obtained in leaching the nodules at 35°C is shown in Table 5. It reveals the presence of γ Mn_2O_3, braunite and some minor amount of todorokite, birnesite and goethite. Formation of the lower/ mixed oxidation phases of manganese led to its lower recovery. Since the host lattice was broken by the microbial action the recovery of copper and nickel was high. However, low dissolution of Fe phases resulted in low cobalt recovery.

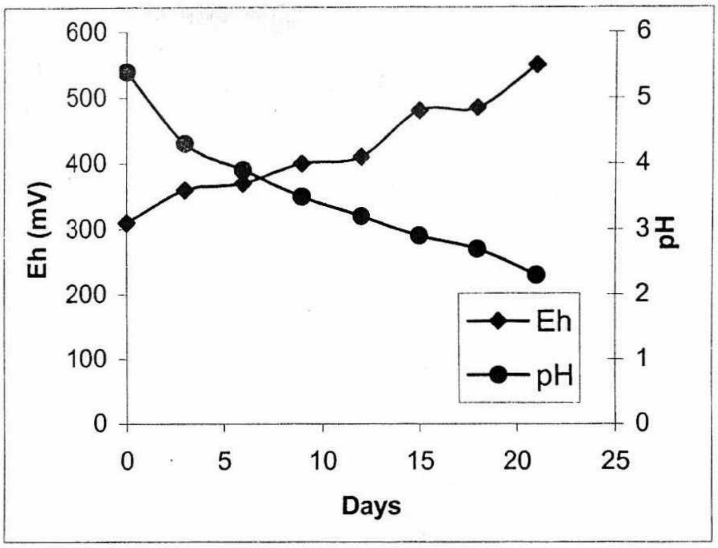

Fig. 4. The pH profile and redox potential of leaching medium during bioleaching by *B. circulans* in 21 days at 30 °C temperature.

Table 5. XRD phase identification of sea nodules before and after bioleaching at 35°C in 25 days

Sample	Mineral phase
Sea nodules	Major: Todorokite, birnesite, lithiophorite, silica
	Minor: Maghemite, goethite, alumina
Residue after bioleaching	Major: γ Mn_2O_3, braunite, goethite
	Minor: Todorokite, birnesite, hydronium jarosite

Bacterial dissolution of metal values from the sea nodules is accompanied by direct action involving enzyme catalysed redox reactions with *B. circulans*. In the process of reduction the metal containing minerals, viz., MnO_2 and goethite present in the nodules act as an electron acceptor, the bacteria conveying electron to it from a soluble electron donor, which may be inorganic or organic substances such as glucose or acetate. The reduction of Mn (IV) to Mn (II) and Fe (III) to Fe (II) resulted in their solubilization. Eqn (1) – (4) showed some possible reactions that led to biodissolution of metals from the nodules by *B. circulans*. The aerobic activity of bacteria is generally associated with a soluble protein fraction called a membrane bound reductase, which utilises NADH/ NADPH (Nicotinamide- adenosine -di -phosphate) to act as electron donor. Reducing power generated within the cell of bacteria upon glucose metabolism is used as below;

$MnO_2 + 2e^- + 4H^+ \rightarrow Mn^{2+} + 2H_2O$ .... (1)

$2 FeOOH + H_2 + 4H^+ \rightarrow 2 Fe^{2+} + 4 H_2O$ .... (2)

Fe^{2+} may be used for the reduction Mn (IV) of the sea nodules.

$MnO_2 + 2 Fe^{2+} + 4H^+ \rightarrow Mn^{2+} + 2 Fe^{3+} + 2 H_2O$ .... (3)

Fe^{3+} thus generated is involved in chemical leaching of valuable metals from their respective oxides.

$MO + Fe^{3+} + 2H^+ + e^- \rightarrow M^{2+} + Fe^{2+} + H_2O$ .... (4)

Where M stands for Cu, Ni and Co.

Metals thus released from the host lattice of Mn (IV) and Fe (III) get dissolved in the leach liquor which may be separated by solvent extraction. So the whole process is biochemical, initially bacterial action through direct mechanism followed by dissolution of valuable metals by the chemical process.

Conclusions

1. Bioleaching by *Bacillus circulans* is a direct process mediated by membrane bound reductase kind of enzyme and utilizes reducing power generated by glucose uptake. Reduction of Mn(IV) to Mn(II) and Fe(III) to Fe (II) by bacterial action leads to release of valuable metals from the host lattice thereby dissolving them chemically.
2. Biorecovery of the valuable metals is maximum at pH 2 because of intense bacterial action which appears to diminish at lower pH of 1.5. At 2.5 pH decrease in biodissolution of a few metals may be attributed to the formation of hydronium jarosite on the surface of nodules, besides lower chemical action.
3. Up to 92% Cu, 73% Ni, 72% Co, 38% Mn and 17% Fe are recovered by *B. circulans* at pH 2 in 25 days at 35°C temperature, 5% pulp density using the sea nodules of $< 300\mu m$ size.

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