

Exploring process options to enhance metal dissolution in bioleaching of Indian Ocean nodules

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Abstract: Polymetallic Indian Ocean nodules offer a lucrative resource for valuable strategic metals such as Cu, Co and Ni. A novel bioleaching process using cell-free spent growth medium from a fully-grown culture of a marine organism isolated from the nodules (*Bacillus M1*) dissolved about 45% Co, and 25% Cu and Ni at a the pH of 8.2 in 4 h. To enhance metal dissolution, different modifications in the bioleaching process, such as increasing the pH of the spent growth medium, carrying out leaching in multiple steps, and introducing organic reductant in the leach pulp, were investigated in this study. Increasing the initial pH of the spent growth medium to above 12 resulted in a 25–30% increase in dissolution of Cu, Co and Ni. The pK_a value for the spent growth medium was observed to be in the range of 11.5–12.5. UV-visible spectroscopy of the growth medium at pH values above 10.0 suggested a change in the structure of complexing phenolic substances present therein. A four-step leaching process using the spent growth medium, each step lasting for about 4 h, was able to bring around 60% Cu and Ni and 85% Co in solution. About 85% Co, 90% Cu and 60% Ni were dissolved in two-stage leaching, in which the bioleached residue was treated with the spent growth medium from *Acidithiobacillus thiooxidans* in the second cycle. The effects of concentration of starch (0.1–10%) as an organic reductant to the spent growth medium were also studied. The dissolution of Cu, Co and Ni stabilized at about 80–85% at a starch concentration of 3% and did not increase much thereafter.

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1 INTRODUCTION

In view of the continuing scarcity of land-based mineral resources along with increasing domestic consumption of valuable metals across the globe, development of environment-friendly technologies for exploring some of the alternate resources of metals has become the need of the day. One of these technologies is the use of biotechnology to recover strategic metals such as Cu, Ni and Co from ocean nodules. Manganese and ferromanganese deposits occur extensively as nodules, crusts and pavements in present-day oceans. The nodules are comprised of oxides of manganese and iron together with various valuable metals such as Cu, Ni and Co.¹ Though the economic potential of recovering valuable metals from nodules has long been recognized, still the search is on for an economically favorable as well as environmentally-benign extraction process.

In the recent past, researchers have started looking into bioprocessing as an alternative route of metal recovery from the nodules. A few acidophilic sulfur-oxidizing bacteria and the thermophilic *Acidianus*

brierleyi have been employed for leaching valuable metals from the nodules.² Extracting valuable metals by an electrobioleaching process using chemolithotrophs such as *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* has also been attempted.³ But all these processes often suffer from the limitations of an acidic environment and the application of high thermal energy or electrical energy for recovering a sizeable amount of metals, which makes them economically unattractive.

Since the Mn(IV) oxide phase in the nodules harbors Cu, Co and Ni, bacterial reduction of Mn(IV) oxide would result in solubilization of these metals. All the Mn(IV)-reducing bacteria isolated so far from seawater, marine sediments and ferromanganese concretions have been found to be heterotrophs. Most of the isolates from Atlantic and Pacific Ocean nodules were found to be aerobes, which could reduce Mn(IV) aerobically or anaerobically.^{4–6} The microbial ecology of the Indian Ocean nodules with special reference to metal dissolution has not, up to now, been studied in detail.

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Recently, a marine species, *Bacillus* M1, was isolated from Indian Ocean nodules and found to be able to leach nodules at near neutral pH and room temperature using the spent growth medium of the isolate; 45% Co and 25% Cu and Ni was extracted in about 4 h at an operating pulp density of 10%.⁷ Mild operating conditions coupled with faster kinetics and significant metal recoveries are some of the advantages of this bioprocess. However, considering the very low concentration of Cu, Co and Ni in the ocean nodules and high mining costs involved, enhancing the metal dissolution to the maximum becomes essential.

The main objective of the present work was to investigate different process options for augmenting metal dissolution by bioleaching of Indian Ocean nodules using spent growth medium of the marine isolate. One option studied was the effect of modifying the pH of the spent growth medium on metal dissolution. Another option was the series leaching of nodules in the spent medium in four consecutive steps. In addition, two-step leaching experiments were carried out at pH 2 using H₂SO₄ solutions and spent growth medium from acidophiles such as *A. thiooxidans*. A final option was to test the effect of adding organic agents such as starch to the spent growth medium from the cultures of the isolate.

2 MATERIALS AND METHODS

2.1 Ocean nodules

Ocean nodules, collected from the bed of the Indian Ocean, were supplied by the National Institute of Oceanography, Goa, India. These were ground in a mortar and pestle and sieved to obtain all appropriate size fractions for the leaching experiments. All the leaching experiments were done with a -50, +75 μ m size fraction of nodules containing around 21.83% Mn, 6.4% Fe, 1.14% Cu, 1.15% Ni and 0.17% Co (all values in wt%). One g of nodules per 100 cm³ of leach pulp corresponding to a 1% pulp density contained 44.64 mmol dm⁻³ Mn, 11.42 mmol dm⁻³ Fe, 1.79 mmol dm⁻³ Cu, 1.96 mmol dm⁻³ Ni and 0.3 mmol dm⁻³ Co.

2.2 Production of cell-free spent growth medium

A bacterial strain, labeled *Bacillus* M1, was isolated from the ocean nodules. The details of the isolation and characterization of the bacterium have been reported elsewhere.⁸ An active culture of the organism is being maintained in the laboratory. The culture is streaked periodically to check for contamination. *Bacillus* M1 was grown in artificial seawater nutrient broth (ASWNB) in 250 cm³ Erlenmeyer flasks at 30 °C in an incubated rotary shaker at 200 rpm. An active culture (from the late exponential phase) containing at least 10⁹ cells cm⁻³ was added to the sterilized ASWNB medium amounting to 10% inoculum and was used for subculturing. To obtain cell-free spent growth medium a fully-grown batch culture was

centrifuged at 17 700 g at 10 °C followed by pressure filtration through a sterile 0.2 μ m Millipore filter. The visual absence of any cells in the resultant spent growth medium was confirmed by examination under a phase contrast microscope.

2.3 UV-visible spectroscopic studies with the spent growth medium from *Bacillus* M1

To determine UV spectra of the spent medium at a pH of 8, an uninoculated medium at the same pH value was taken as reference. For studies with the spent growth medium having a pH value of 10, 11 or 12, spent growth medium at a pH of 8.0 was used as the reference to obtain the difference spectra. A Simadzu model UV-260, UV-visible spectrophotometer was employed for all these studies.

2.4 Leaching experiments

For leaching studies, 1 g of pulverized ocean nodules was added to 100 cm³ of the spent growth medium to keep the solid:liquid ratio at 1:100 (w/v), ie 1% pulp density, and leaching was continued for 10 h. The recovery of different metals in the leach liquor was monitored as a function of time.

2.4.1 Effect of pH

The initial pH of the spent growth medium was in the range of 8–8.5. The effect of initial pH on leaching efficiency was investigated by varying the pH using 0.01 mol dm⁻³ H₂SO₄ or 0.01 mol dm⁻³ NaOH solutions.

2.4.2 Series leaching experiments

Multistep leaching experiments with the spent growth medium from the marine isolate culture consisted of four cycles in succession, as shown in Fig 1.

In two-step leaching experiments, the first cycle consisted of leaching with the spent growth medium from the marine isolate culture. After 4 h of the leaching cycle, the residue was filtered, dried and was used as feed for the second stage. The second cycle consisted of leaching in (a) spent growth medium from an *A. thiooxidans* culture and (b) sulfuric acid solution having a pH of 2.0, to get benefit from the low pH value. A strain of *A. thiooxidans* (MCM B41) was used in this study. It was obtained from the Maharashtra Association for Cultivation of Science (MACS), Pune, India. The organism was subcultured in the laboratory using modified 9K basal medium, 1 dm³ of which contained 0.25 g dipotassium dihydrogen phosphate, 0.5 g magnesium sulfate, 2 g ammonium sulfate and 10 g sulfur powder. After 72 h, the culture was centrifuged in a Sorvall RC-5B refrigerated high-speed centrifuge at a force of 17 700g for 10 min at 5 °C to obtain cell-free spent growth medium.

2.4.3 Leaching with spent growth medium to which starch was added

Analytical grade soluble starch (potato) was added directly to the spent growth medium cultures from

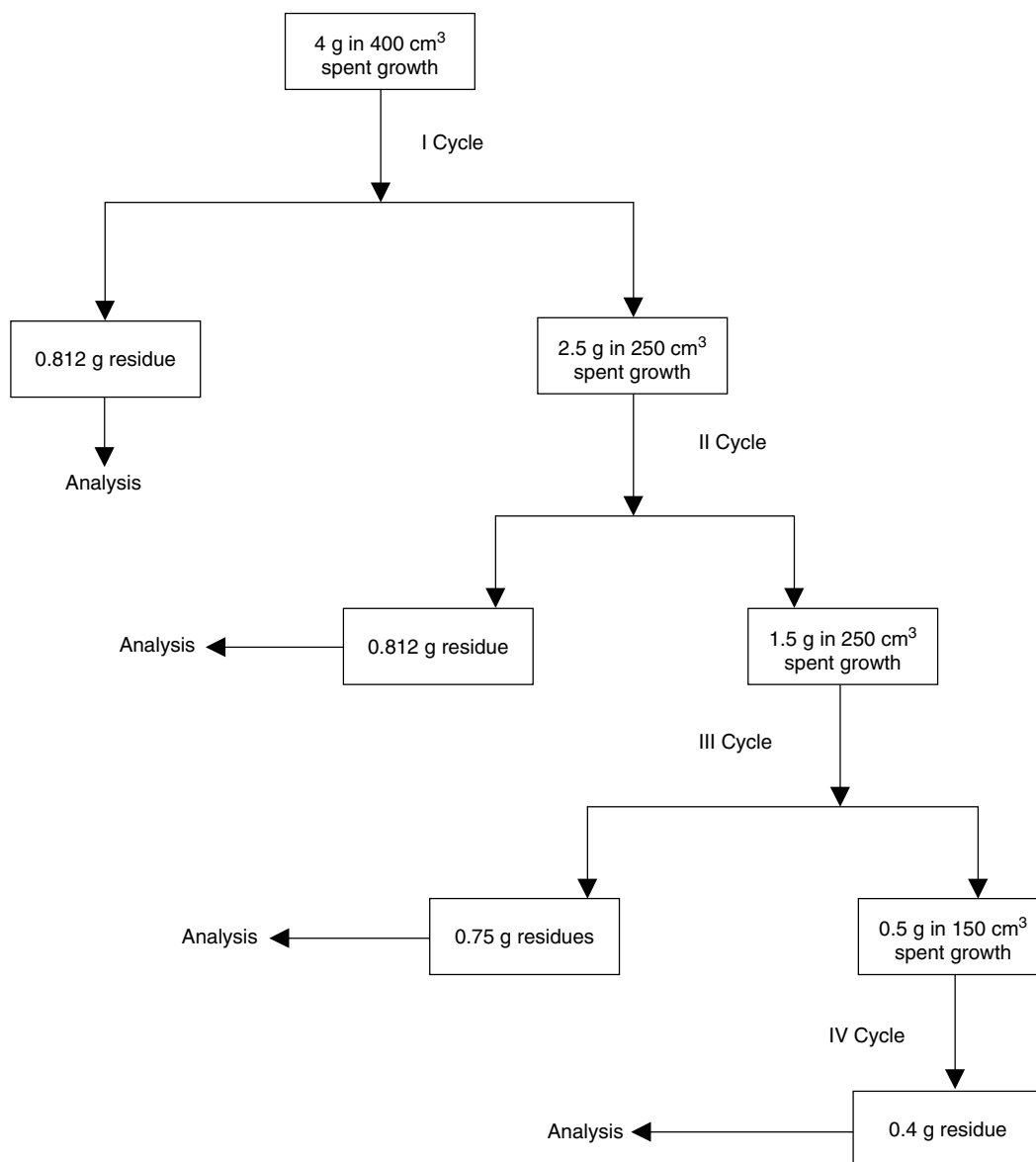


Figure 1. Flow scheme of the four-cycle leaching process of ocean nodules using spent growth medium of the marine isolate.

the *Bacillus* M1 culture in varying amounts and the nodules at 1% pulp density were leached for 4 h.

In all the above cases, leach liquor was collected after appropriate lengths of contact with crushed nodules used for leaching and filtered using Whatman No 42 filter papers. The residue was then digested in 1:1 HCl at 60–70 °C. The resultant solution was analyzed after appropriate dilutions for Cu, Co, Ni, Mn and Fe by using a Jobin Yvon JY 24, inductively coupled plasma spectrometer.

3 RESULTS AND DISCUSSION

3.1 Control leaching experiments with spent growth medium

The result of a control leaching experiment using spent growth medium from the marine isolate having a pH of 8.2 is illustrated in Fig 2. Within 4 h, almost 45% Co and Fe, and 25% Mn had been leached whereas 20–25% Cu and Ni were solubilized in 5–6 h.

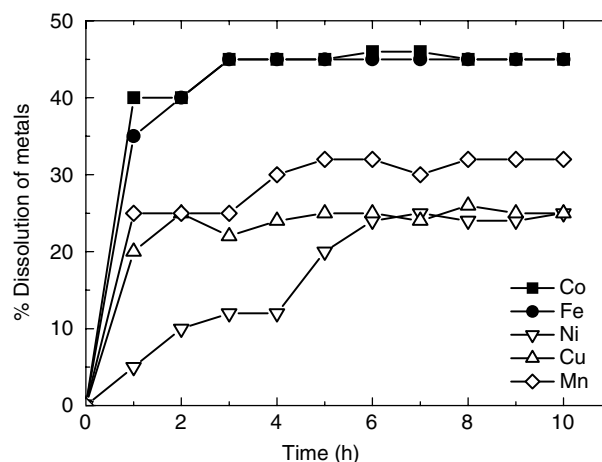


Figure 2. Kinetics of metal dissolution in bioleaching with the spent growth medium from the marine isolate.

Detailed characterization studies on the spent growth medium revealed the presence of phenolic substances

having attached carboxylic acid groups, which are known complexation agents for transition metals.⁸ The probable mechanism of metal dissolution by the spent growth medium at near neutral pH has been discussed elsewhere (Mukherjee A, unpublished).

3.2 Effect of modifications in pH of the spent growth medium from *Bacillus M1* culture

The effects on bioleaching, of gradually increasing the pH of the spent growth medium from 8 to 12 were investigated. No significant changes were observed in metal dissolution in a pH range from 8 to 10, whereas on increasing the pH to 12, a 20–25% increase in the dissolution of Cu, Co and Ni was noted.

UV-visible spectroscopy of the spent growth medium at pH 8 showed two peaks, one near 230 nm and another at 310 nm. Chakravarty *et al*⁹ isolated and partially characterized a catechol-type siderophore compound produced by *Pseudomonas stutzeri* grown under iron-depleted conditions. The purified siderophore revealed UV spectra (230 nm and 330 nm bands) which were found to be identical with that of 2,3-DHBA (dihydroxy benzoic acid). In another characterization study with siderophores O'Brien and Gibson¹⁰ isolated five compounds from culture media of *Escherichia coli* and *Aerobacter aerogenes* under conditions of iron deficiency. The compound named 2,3-dihydroxy-*N*-benzoylserine gave two distinct UV peaks in water, one near 315 nm and the other near 310 nm. Therefore the presence of absorption peaks near 230 and 308 nm strongly suggests a DHB (dihydroxy benzoate) structure.

The enhancement in metal dissolution above pH 12 might be due to a change in structure of the metabolites, which may intensify the complexation effect. To confirm structural changes, UV-visible difference spectra of the spent growth medium from the isolate were recorded at pH values above 10, with respect to spent growth medium having a pH of 8, and the results are summarized in Table 1. Increasing the pH of the spent growth medium clearly caused a change in the UV spectra, suggesting a structural change in the metabolites in the medium. Displacement of a benzene band can be caused either by electron-contributing or by electron-attracting influences in the constituent groups. A shift of the 310 nm band to a lower wavelength of 298 nm in a very high alkaline pH range may be attributed to the ionization of a group(s) attached to the benzene ring, probably diminishing a tendency for electron transfer to or from the ring.

A titration curve of the spent growth medium with 0.01 mol dm⁻³ NaOH is shown in Fig 3. The pK_a value for the spent growth medium was observed to lie between 10.5 and 11.0, confirming a state of deprotonation of the metabolites above that pH range. So the deprotonated structure of the metabolite(s) in the spent growth medium could have enhanced their complexation efficiency.

Table 1. Change in UV spectra of the spent growth medium with increase in pH from 8 to 12

pH of spent growth medium	Recorded peak positions (nm) (absorbance given in parentheses)		
	220–230 range	250–290 range	290–300 range
8	233.8		308.2
10.5	—	—	298.8 (0.551)
11	—		297.4 (0.449)
11.5			298.7 (0.642)
12	—	—	299.0 (0.849)

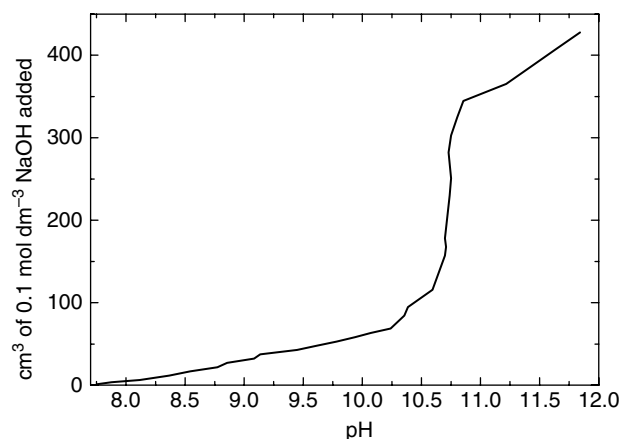


Figure 3. Titration curve of the spent growth medium in the pH range of 8 to 12 using 0.01 mol dm⁻³ NaOH.

3.3 Changes in the process design – series leaching

In order to improve metal dissolution, multistep leaching experiments were conducted using spent growth medium of the marine isolate. The results are shown in Table 2. In these experiments the leached residue from the first cycle of leaching was put through subsequent leaching cycles to determine if fresh spent growth medium could help in recovering the metal values remaining in the residue. After four cycles of leaching, each of a 4 h duration, around 87% Co, 90% Fe, 60% Cu and Ni, and 70% Mn were dissolved.

In multistep leaching experiments with the spent growth medium, recovery values in each cycle signified the fraction of total metal values that was added before the first leaching cycle. If the starting concentration

Table 2. Metal dissolution from ocean nodules using spent growth medium in four-stage leaching process

Conditions of leaching	% Dissolution of metals				
	Co	Cu	Ni	Mn	Fe
Cycle I	45	25	23	27	50
Cycle II	23	17	16	18	24
Cycle III	12	12	12	13	11
Cycle IV	5	9	8	12	5
Cycle I + Cycle II + Cycle III + Cycle IV	85	63	59	70	90

of Co was 100 ppm, the recovery in the first cycle of leaching was 45 ppm, in the second one 23 ppm, the third cycle recovered 12 ppm and in the last cycle, recovery was only 5 ppm, total recovery was 85 ppm at the end of four cycles, signifying 85% recovery.

Although multistep leaching in spent growth medium from the marine isolate dissolved considerable amounts of metal at 1% pulp density, the process has certain limitations, such as:

- Leaching in four steps would take nearly 20 h taking into consideration the time usually spent between two successive leaching cycles.
- Cu and Ni recovery values were below 65%.

In view of the above limitations, studies were performed with fewer re-leaching steps. Some leaching experiments were performed in two steps in which the first cycle in spent growth medium from the marine isolate was followed by re-leaching the residue at a low pH. The low-pH environment was induced by both biological and non-biological means. Low-pH leaching agents such as pH 2 sulfuric acid solutions and spent growth medium from *A thiooxidans* were employed for re-leaching.

The basic idea behind using the second cycle of leaching in acidic media was to:

- Enhance solubility of metal ions in a low-pH environment.
- Use the sulfate ions present in spent growth medium from *A thiooxidans* culture to solubilize metals in nodule material.

The results from such two-step leaching experiments are shown in Table 3. The dissolution of metals was taken as a fraction of total metal content in the nodules (as obtained from the initial chemical composition) at each individual step. The sum of the metal dissolution at stages 1 and 2A, respectively, gives total recovery by a two-step leaching process, when residue from the leaching in spent growth medium of *Bacillus* M1 is re-leached in pH 2 solutions. Similarly, adding up the metal dissolution values in stages 1 and 2B respectively, we obtain the total solubilization, when leaching in spent growth medium of *Bacillus* M1 is followed by that in the spent growth medium of *A thiooxidans*.

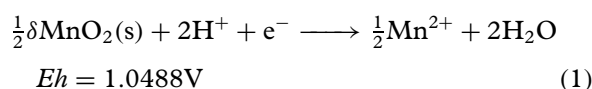
In the case of 1% pulp density, total dissolution after two-step leaching in *A thiooxidans* spent growth

medium were 85% Co, 90% Cu and 60% Ni. Re-leaching in pH 2 solutions yielded less metal dissolutions in two steps compared with that in the spent growth medium from *A thiooxidans*. Notably, both pH 2 solutions and the spent growth medium from *A thiooxidans* dissolved more Fe in comparison to Mn in the re-leaching step. When the pulp density was increased to 5% in the case of two-step leaching, the metal dissolution increased correspondingly.

3.4 Effect of adding starch to the spent growth medium from *Bacillus* M1

In the extensively-discussed hypochlorite oxidation of starch, researchers¹¹ observed that the reaction rate was markedly influenced by pH. The reaction rate was found to be most rapid in slightly acidic and basic solutions while it slowed down in a highly alkaline or acidic pH range. Since the typical pH values of leaching with the spent growth medium fell within the neutral range, starch was chosen as a reductant for enhancing metal dissolution.

Oxidants such as potassium permanganate, persulfate, chlorite and hydrogen peroxide have been used to bleach starch. Mn(IV) oxide is also a powerful oxidant.



The nodules consist of Mn(IV) oxide as a major phase; therefore starch can be applied as a possible agent for reductive dissolution.

The reduction potential of 1% starch solution in the spent growth medium was observed to be -0.045V (wrt Saturated Calomel Electrode). From eqn (1) the Eh value corresponding to Mn(IV) reduction is 1.0488V . This large difference in Eh values ensured the reducibility of MnO_2 by starch in spent growth medium.

Table 4 illustrates the effect of increasing the concentration of starch from 0.1% to 10% in the spent growth medium of *Bacillus* M1 as described below:

- Adding starch to spent growth medium from the marine isolate enhanced dissolution of metals from the nodules. Leaching in unamended spent growth medium alone at 1% pulp density brought about 45% Co and around 25% Cu and Ni into solution. Adding 3% starch to the spent growth

Table 3. Metal dissolution in each step of different two-stage leaching processes

Pulp density	Leaching stage	Leaching medium	% Dissolution of metals				
			Co	Cu	Ni	Mn	Fe
1%	1	<i>Bacillus</i> M1 spent growth medium	45	20	29	25	45
	2A	pH 2 solution	30	30	39	5	40
	2B	<i>A thiooxidans</i> spent growth medium	40	70	38	2	45
5%	1	<i>Bacillus</i> M1 spent growth medium	50	20	25	25	50
	2A	pH 2 solution	30	27	30	8	39
	2B	<i>A thiooxidans</i> spent growth medium	40	70	39	8	45

Table 4. The effect of adding starch to the spent growth medium on leaching of ocean nodules

Leaching reagent	% Dissolution of metals				
	Co	Cu	Ni	Mn	Fe
Spent growth medium only (marine isolate)	45	20	29	25	45
Spent growth medium + 0.1% starch	65	35	35	30	50
Spent growth medium + 0.5% starch	85	70	60	83	80
Spent growth medium + 1% starch	80	80	65	85	86
Spent growth medium + 3% starch	80	83	82	83	85
Spent growth medium + 5% starch	83	85	86	87	84
Spent growth medium + 10% starch	84	88	87	85	87

medium enhanced the dissolution values up to 80–85%.

- (ii) No further increases in metal solubilization were noted above 3% starch in the spent growth medium.

Fe dissolution by starch was almost double the Mn dissolution in all the cases. The reductant to metal oxide molar ratio was higher in the case of Fe since Mn concentration in the nodules was three to four times greater than Fe concentration. According to some schools of thought, Co may be associated with an Fe(III) oxide phase;¹ therefore higher Co dissolution may be a direct consequence of higher Fe dissolution in solution. In the case of leaching with 0.1% starch in growth medium lower recovery of Cu and Ni is corroborated with low Mn recovery. At higher concentrations of starch in the spent growth medium Mn solubilization was enhanced, resulting in a concurrent rise in Cu and Ni dissolution. A direct correlation of Cu and Ni with Mn in the ocean nodules is well supported in the literature.^{1,12,13}

4 CONCLUSIONS

The following major conclusions can be drawn based on the above study:

1. Changing the pH of the spent growth medium from the initial pH of 8 up to pH 10 did not affect metal dissolution. However, increasing the pH of the spent growth medium to above 11 led to a 20–25% increment in dissolution of Co, Cu and Ni. This could be related to a concomitant change in structure of the metabolite products present therein. The pK_a value for the spent growth

medium was found to lie within the range of pH 10.5–11.0.

2. A four-cycle step leaching in spent growth medium from *Bacillus* M1 dissolved about 85–90% Co and Fe, 70% Mn, and 60% Cu and Ni in solution.
3. In a two-step bioleaching process about 80–85% dissolution of Cu, Co and Ni was obtained by re-leaching the leached residue in spent growth medium from *A. thiooxidans*. When pH 2 H_2SO_4 solution was used as leaching agent in the second step, nearly 50%, 75%, 68% dissolution of Cu, Co and Ni respectively was obtained at 1% pulp density.
4. Addition of starch to the spent growth medium enhanced metal dissolution by reductive dissolution of major oxide phases. With 3% starch in the spent growth medium around 80% of Cu, Ni, Co and Mn could be dissolved.

REFERENCES

- 1 Burns RG and Fuerstenau DW, Electron probe determinations of interelement relationships in manganese nodules. *Amer Mineral* **51**:891–902 (1966).
- 2 Konishi Y, Asai S and Sawada Y, Leaching of marine manganese nodules by acidophilic bacteria growing on elemental sulfur. *Metall Mater Trans* **28B**:25–32 (1987).
- 3 Kumari A and Natarajan KA, Electrobioleaching of polymetallic ocean nodules. *Hydrometallurgy* **62**:125–134 (2001).
- 4 Ehrlich HL, Bacteriology of manganese nodules I. Bacterial action on manganese in nodule enrichments. *Applied Microbiol* **16**:197–202 (1963).
- 5 Ehrlich HL, Reactions with manganese by bacteria from marine ferromanganese nodules. *Developments in Industrial Microbiology* **7**:43–60 (1966).
- 6 Ehrlich HL, Ghirese WC and Johnson II GL, Distribution of microbes in manganese nodules from the Atlantic and Pacific Oceans. *Developments in Industrial Microbiology* **13**:57–65 (1972).
- 7 Mukherjee A, Raichur AM, Modak JM and Natarajan KA, Bio-processing of Indian Ocean nodules using a marine isolate—effect of organics. *Minerals Engg* **16**(7):651–657 (2003).
- 8 Mukherjee A, Raichur AM, Modak JM and Natarajan KA, Preferential solubilization of cobalt from Ocean nodules at neutral pH- A novel bioprocess. *J Ind Microbiol Biotechnol* **30**:606–612 (2003).
- 9 Chakravarty RN, Patel HN and Desai SB, Isolation and partial characterization of catechol-type siderophore from *Pseudomonas stutzeri* RC 7. *Curr Microbiol* **20**:283–286 (1990).
- 10 O'Brien IG and Gibson F, The structure of enterochelin and related 2,3 dihydroxy-N-benzoylserine conjugates from *Escherichia coli*. *Biochim Biophys Acta* **213**:393–402 (1970).
- 11 Whistler RL and Schweiger R, Oxidation of amylopectin with hypochlorite at different hydrogen ion concentration. *J Amer Chem Soc* **79**:6460–6464 (1957).
- 12 Cronan DS and Tooms JS, Subsurface concretions of manganese nodules in Pacific sediments. *Deep-sea Res* **14**:355–359 (1967).
- 13 Glasby GP, Mechanism of incorporation of manganese and associated trace elements in marine manganese nodules. *Oceanogr Mar Biol Ann Rev* **12**:11–40 (1974).