Available online at www.sciencedirect.com
SCIENCE DIRECT®
Hydrometallurgy 73 (2004) 205–213
hydrometallurgy
www.elsevier.com/locate/hydromet

# Bioprocessing of polymetallic Indian Ocean nodules using a marine isolate

Amitava Mukherjee[a], Ashok M. Raichur[a,*], Jayant M. Modak[b], K. A. Natarajan[a]

[a] *Department of Metallurgy, Indian Institute of Science, Bangalore 560012, India*
[b] *Department of Chemical Engineering, Indian Institute of Science, Bangalore 560012, India*

Received 7 November 2002; received in revised form 25 August 2003; accepted 25 October 2003

**Abstract**

Polymetallic Indian Ocean nodules offer a lucrative resource for valuable strategic metals like Cu, Co and Ni. A novel bioleaching process to recover those metals from the nodules at near-neutral pH and ambient temperature is presented in this paper. The significant role of in situ microorganisms and their by-products in solubilization of valuable trace metals from the nodules was studied and is discussed. A marine organism was isolated from the nodules and characterized, both physiologically and morphologically. The isolate was observed to grow well in artificial seawater medium, at neutral pH, 30 °C and 0.25 M NaCl concentrations and showed $MnO_2$ reducing activity. The growing culture as well as the cell-free growth supernatant of the isolate was employed in bioleaching studies of the nodules, and leaching efficiency was observed to follow the same trend in both the cases. It can be inferred that the metabolites produced during growth of the microorganism played a primary role in the bioleaching process. The initial concentrations of Co, Cu and Ni in the polymetallic ocean nodules were 17, 114 and 115 ppm, respectively, for 1% pulp density. Around 45% Co and 30% of Cu and Ni were dissolved at pH of 8.2 in 10 h. Co recovery by bioleaching was comparable to that obtained in concentrated acidic solutions. It was also found that increasing the pH of the growth supernatant to about 13 markedly improved the leaching efficiency.


*Keywords:* Bioprocessing; Polymetallic Indian Ocean nodule; Marine isolate

## 1. Introduction

In view of continuous depletion of the land-based resources along with increasing consumption of valuable metals in India, development of environmentally friendly technologies for tapping alternative sources of metals has gained importance lately. One of them is recovery of the strategic metals Cu, Ni and Co from polymetallic Indian Ocean nodules by biological processing. Fuerstenau and Han (1983) extensively reviewed processing and extraction of valuable metals from manganese nodules touching upon both hydro- and pyrometallurgical routes. The high porosity of the nodules resulting in quite high moisture content, coupled with polluting effluent gases pose major difficulties in pyrometallurgical treatment of the nodules. Thus, in the last two decades, hydrometallurgy has emerged as a potentially viable route for extraction of metals from nodules. However, often, slow

* Corresponding author. Tel.: +91-80-3608025; fax: +91-80-3600472.
*E-mail address:* amr@met.iisc.ernet.in (A.M. Raichur).

0304-386X/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.hydromet.2003.10.007

kinetics and poor recovery with dilute acids and corrosiveness of the concentrated ores restrict this route of extraction. Researchers have studied the addition of reducing agents, with mineral acids and ammonia media. The reducing environment enhances leaching by breaking up the matrix of the nodules into which the valuable metals are supposed to be occluded (Niinae et al., 1996; Das et al., 1986; Kanungo and Jena, 1988a,b; Jana et al., 1999; Trifoni et al., 2001; Zhang et al., 2001). These processes ended up with varying degrees of success. However, most of them often require high-temperature pretreatment and/or costly, corrosive reagents to obtain a sizeable amount of metal recovery with a favorable kinetics. As the nodules are low-grade ores of Cu, Co and Ni, use of costly chemical reagents as reducing agents may not be economically viable for large-scale operations.

Ehrlich (1963) isolated and characterized Mn-reducing organism *Bacillus* 29 from the Atlantic Ocean nodules, and the organism was able to reduce $MnO_2$ aerobically and anaerobically using glucose as electron donor. However, a component of electron transport system involved in $MnO_2$ reduction in the culture is only aerobically inducible. Both $Mn^{2+}$ and $MnO_2$ can serve as inducers (Ehrlich, 1966; Trimble and Ehrlich 1968, 1970). $MnO_2$ reducing ability has been found inducible in all marine cultures tested so far by Ehrlich (1973). Despite differences in the electron pathway from the donor to the acceptor, the overall reaction involving $MnO_2$ reduction appears to be the same in all the marine organisms tested so far by Ehrlich et al. (Ehrlich et al., 1972; Ehrlich 1973). However, microbial ecology of the Indian Ocean nodules has not been studied in detail to date. Utilizing microorganisms isolated from the nodules themselves to leach out valuable metals still remains an unexplored route.

In the recent past, researchers have started looking into bioprocessing as an alternative route of metal recovery from the nodules. Konishi et al., (1997) showed leaching by acidiphilic sulphur oxidizing bacteria and thermophilic *Acidianus brierleyi*. Kumari and Natarajan (2001) have been able to extract valuable metals by electro-bioleaching using acid producing chemolithotrophs like *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*, but these processes often employed highly acidic environment supplemented with thermal energy or electrical energy for recovering a sizeable amount of metals.

The major objectives of the present work were as follows:

1. Isolation and characterization of marine organism(s) from polymetallic Indian Ocean nodules.
2. Dissolution of Co, Cu and Ni from the ocean nodules using the growing cells and metabolic by-products of the marine organism(s) and comparison with that in acidic leaching.
3. Effect of pH and reducing agents on the biodissolution of Co, Cu and Ni from the nodules.

## 2. Experimental

### 2.1. Materials

The Indian Ocean nodule sample was collected from the beds of Indian Ocean by the National Institute of Oceanography, Goa, India. The as-received sample was ground in a mortar and pestle and sieved to obtain appropriate size fractions. All the leaching experiments were done with $-50$, $+75$-μm size fraction of nodules consisting of around 21.8% Mn, 6.4% Fe, 1.14% Cu, 1.15% Ni and 0.17% Co (all values in wt.%). The various phases revealed in the X-ray diffraction pattern of nodules included Asbolan ($NiMn_2O_3(OH)_4$), cobalt manganese oxyhydroxide ((Co,Mn)OOH) and quartz ($SiO_2$).

### 2.2. Microorganisms

#### 2.2.1. Isolation of the unidentified marine species

The marine bacteria occurred inherently on the nodules as a spore former. To get rid of surface contaminants and germinate the spores, the as-received nodules were boiled in water for 30 min (Ehrlich, 1963). After boiling, water was removed by decantation. The nodules were transferred to an autoclaved porcelain mortar (outer diameter, 150 mm) in an inoculating hood and pulverized with a sterilized pestle. About 1 g of this nodule powder was added aseptically to 20 ml of sterilized artificial seawater nutrient broth (ASWNB) which had the following components per liter of distilled water: 28.13 g NaCl;

0.77 g KCl; 1.60 g $CaCl_2 \cdot 6H_2O$; 4.8 g $MgCl_2 \cdot 6H_2O$; 0.11 g $NaHCO_3$; 3.5 g $MgCl_2$, 7 $H_2O$; 5 g peptone and 3 g beef extract.

ASWNB with the nodule powder was incubated at 30 °C for 24 h. From this enrichment media, a loopful of the inoculum was streaked onto the artificial seawater nutrient agar plates and incubated at 37 °C for 24 h. The next day, round and smooth surface colonies were found growing on the medium. A single colony was picked from the plate and subcultured several times on the same medium to finally obtain the pure strain of the marine isolate. Periodic streaking was done to check for the purity of the isolated strain.

*2.2.2. Growth of the marine isolate*

The marine isolate was grown in ASWNB in 250-ml baffled Erlenmeyer flasks at 30 °C on a rotary shaker (200 rpm). A 10% (v/v) sample of an active inoculum (from the late exponential phase) containing at least $10^9$ cells/ml was added to the sterilized ASWNB medium. The growth of the microorganism was monitored by measuring the cell count using a Petroff–Hauser counter employing phase-contrast microscopy. The sodium chloride concentration was kept at 0.25 M, which was arrived at by tests at different salt concentrations. Growing cells as well as the cell-free growth supernatant containing metabolites produced during growth were used as bioleaching agents.

*2.2.3. Manganese reducing activity of the marine isolate*

For this study, reagent-grade $MnO_2$ (obtained from Loba Chemie, Mumbai, India) was washed 10 times in 200-ml volumes of distilled water to remove fine, colloidal particles of oxide from the larger ones. It was then dried in an oven and used for experiments thereafter.

A 1-g sample of $MnO_2$ was steam sterilized separately and added to 90 ml of sterilized ASWNB. Inoculum of 10% v/v of marine isolate containing $10^9$ cells/ml was added therein to start the growing cell experiment. A control experiment was run without inoculation for comparison. Cell count and Mn analysis were performed after appropriate time intervals. Soluble Mn was determined by means of inductively coupled plasma (ICP) spectrometer.

*2.3. Methods*

*2.3.1. Chemical leaching experiments*

In order to generate baseline data to compare with the bioleaching results, chemical leaching experiments were carried out in 250-ml Erlenmeyer flasks on an incubated rotary shaker (200 rpm) at 30 °C. HCl, $HNO_3$ and $H_2SO_4$ solutions of 2.5 M concentrations were employed as leaching agents. Glucose (20%) was added to 2.5 M HCl, while sodium thiosulphate in 1:4 molar ratio with Mn(IV) content in nodules was added to pH 2 $H_2SO_4$ solutions to introduce reducing atmosphere in the leach liquor. In all the cases, the nodule/liquid ratio was kept at 1:100 (w/v), and to optimize different parameters, the duration of leaching was fixed at 6 h.

The leach liquor was filtered using Whatman 42 filter paper, and the residue was digested in 1:1 HCl at 60–70 °C. The resultant solution was analyzed for Cu, Co, Ni, Mn and Fe with the help of AN ICP spectrometer after proper dilutions were made. All the chemicals used were of reagent grade.

*2.3.2. Bioleaching experiments*

*2.3.2.1. Leaching with the uninoculated media.* To verify the effect of media constituents on bioleaching of nodules, control tests were carried out with ASWNB and different constituents of ASWNB separately. A 1-g sample of pulverized nodules was added to 100 ml of media, and initial pH was measured to be 7.6–7.8.

*2.3.2.2. Leaching with growing culture.* A 1-g sample of pre-sterilized pulverized ocean nodule was taken in 90 ml of sterilized ASWNB media in 250-ml conical flasks. A 10% v/v actively growing culture (having a cell count of $10^9$ cells/ml) of the marine isolate was added as the inoculum to this suspension. Leaching was carried out in an incubated shaker at 220 rpm at room temperature for 10 h. The flasks were taken out after appropriate time intervals for analyzing leached metal content in the solution.

*2.3.2.3. Leaching with cell-free growth supernatant.* To obtain cell free growth supernatant, fully grown culture (after 10 h of growth) was centrifuged at 10,000 rpm for 15 min followed by pressure

filtration using a Millipore ultra-filtration unit. The absence of any cells in the resultant supernatant was assured by observing the same under a phase-contrast microscope.

A 1-g sample of pulverized ocean nodules was added to 100 ml of the growth supernatant. The effect of time and pH on leaching of all the five metals was studied. pH adjustments were done using 0.01N $H_2SO_4$ and 0.01N NaOH solutions.

In all the above cases, leach liquor was collected after appropriate time intervals by filtration using a Whatman 42 filter paper. The residue was washed several times in water followed by mild acidic solutions (10% $HNO_3$) and then digested in 1:1 HCl at 60–70 °C. The resultant solution was analyzed by ICP. Reproducibility of all the analysis results was found to be within an error limit of 3–5%.

## 3. Results and discussion

### 3.1. Characterization of the marine isolate

A marine bacterium was isolated from the nodules following the procedure discussed in Section 2.2. The isolated strain when observed under a scanning electron microscope was seen to be rod shaped and was 3–4 μm in length and 0.5–0.75 μm in diameter. It was found to be Gram-positive and tested positive for the following characteristics: catalase activity, oxidase activity and aerobic metabolism. More importantly, it displayed the ability to reduce Mn(IV) to Mn(II).

### 3.2. Growth characteristics of the marine isolate

The typical growth curve of the marine isolate is illustrated in Fig. 1. The figure also shows the variation of pH and redox potential (Eh) measured against a saturated calomel electrode (SCE) during growth. After a negligible lag phase, cells started growing rapidly in the logarithmic phase. This phase lasted for about 7 h beyond which growth was very slow. The typical plateau observed after 6–7 h was due to the stationary phase of the bacterium, a characteristic of microbial growth in batch cultures.

The drop in redox potential from 120 to −10 mV during growth might be due to the production of reducing substance(s) by the marine bacteria. The metabolism of the organism caused the drop in Eh (since no such drop in redox potential was observed in an uninoculated control). The identity of these reducing metabolic species depends on the culture and the growth conditions (medium ingredients, etc.)

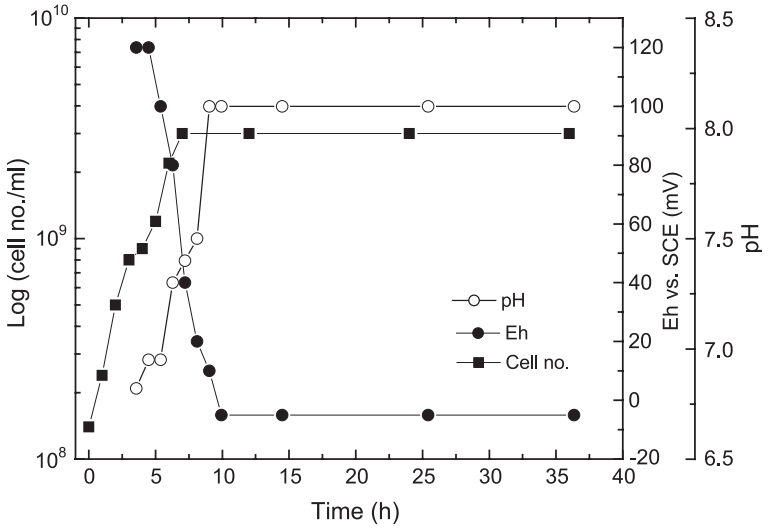


Fig. 1. Growth characteristics of the marine isolate in ASWNB medium, and changes in Eh and pH during growth of the marine isolate.

employed. The growth was also accompanied by increase in pH of the medium from 6.8 to 8.1.

The growth rate of the cells was calculated to be 5.4 $h^{-1}$ and mean doubling time 0.13 h. This was a relatively high growth rate for a bacterium.

### 3.3. Mn(IV) reduction by the marine isolate

Mn was present in the complex oxy-hydroxide matrix of the nodules mostly in the oxidation states of +3 and +4. Since higher oxidation states were quite improbable for solubilization, Mn must be reduced to the +2 state to obtain an easily soluble form. Mn solubilization by the growing culture of the marine isolate was studied using pure $MnO_2$, and the results are shown in Fig. 2. A constant rise in concentration of soluble Mn was noted in the growth flask after the first day of incubation, while no soluble Mn was detected in the supernatant of the uninoculated control flask. After 72 h, both cell count and soluble Mn in the growth flask decreased.

The inoculum used for $MnO_2$ reduction tests consisted of wild cells of the marine isolate. A 12% Mn dissolution signifies around 300 ppm of Mn(II), which might be toxic enough to decelerate the bacterial metabolism and growth process. This might have caused the decline in cell count. If the isolates were further subcultured in fresh media with solid $MnO_2$, more Mn dissolution could have been obtained.

The decrease in concentration of dissolved Mn after 72 h can be due to one or a combination of both of the following reasons:

(a) Decrease in cell number caused a significant slowdown in the bacterial oxygen consumption and pH increased to 8.0 (Fig. 2). Thus, the conditions in the medium became more oxidizing due to a rise in oxygen concentration. This might have caused autooxidation of Mn(II) to Mn(IV) resulting in precipitation of Mn(IV) oxide.
(b) The Mn solubilization by the growing cells of the isolate may be due to metabolic production of reducing substances. The metabolite production might have ceased after 80 h since the cell count had decreased significantly. This decrease in concentration of reducing substances rendered the medium more oxidizing leading to reoxidation of Mn(II).

The initial Eh was noted to be 120 mV, which decreased to around 40 mV in the first 80 h, and at the end of 7 days, the Eh again rose to 140 mV. The changes in Eh prove that reducing conditions pre-

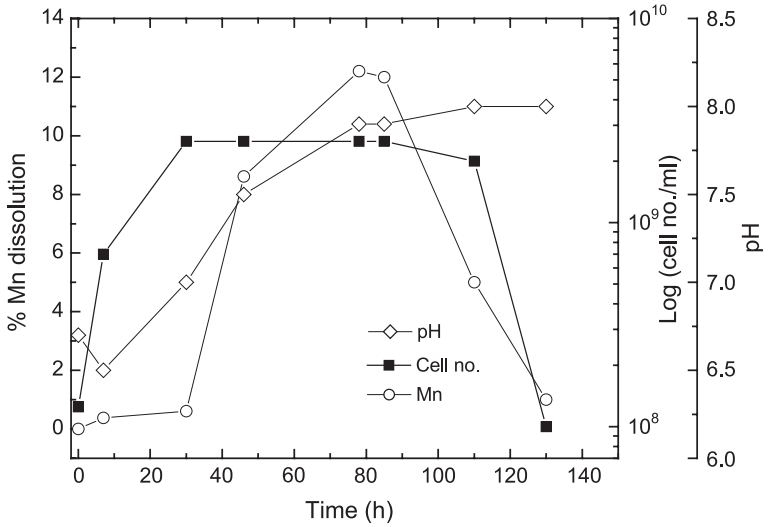


Fig. 2. Manganese reduction tests with the growing cells of the marine isolate.

vailed in the medium in the first 80 h, and then the solution became more oxidizing.

These results proved that the growing culture of the marine isolate was able to solubilize Mn from $MnO_2$. The isolate had the ability to reduce Mn(IV) to Mn(II). Control tests with uninoculated medium were not able to dissolve any Mn during the same time period.

### 3.4. Chemical leaching experiments

Baseline leaching tests of the nodules were carried out using HCl, $HNO_3$ and $H_2SO_4$ of 2.5 M concentrations, and the results are summarized in Table 1. Around 80% Cu and 55% Ni were dissolved by these acids. While HCl solubilized 30% of Co and Mn, no significant dissolution could be achieved in $HNO_3$ and $H_2SO_4$. Low Co recovery in acid leaching studies may be related to low Mn dissolution. A part of Co in nodules is supposed to be occluded in the $MnO_2$ matrix, so disintegration of the matrix becomes an essential prerequisite for Co solubilization. Therefore, adding a reducing agent to HCl solutions remarkably enhanced the recovery of both Mn and Co. With the addition of 20% glucose in 2.5 M HCl solution, 50% Co was dissolved, while Mn extraction increased to 80%. This disparity in Co and Mn dissolution suggests that all of the cobalt present in the nodules might not be directly associated with Mn.

Since 2.5 M acid solution is not well suited for industrial applications due to its corrosiveness and handling hazards, dilute acidic solutions having pH of 2.0 were used for leaching the nodules. We can observe from Table 1 that 25% Co, 25% Ni and 40% Cu could be leached along with 10% Mn and 40% Fe with pH 2 $H_2SO_4$ solution. With sodium thiosulfate added to the pH 2 $H_2SO_4$ solutions, metal dissolution went up by 5–10%. In this case also, adding a reducing agent in the leaching medium enhanced the recovery of metals.

Table 1
Leaching of ocean nodule using chemical reagents

| Conditions of leaching | % Co | % Cu | % Ni | % Mn | % Fe |
|---|---|---|---|---|---|
| 2.5 M HCl | 30 | 80 | 55 | 30 | 60 |
| 2.5 M HCl +20% glucose | 50 | 85 | 85 | 80 | 65 |
| 2.5 M $H_2SO_4$ | <5 | 85 | 55 | <5 | 60 |
| 2.5 M $HNO_3$ | <5 | 80 | 50 | <5 | <5 |
| pH 2 $H_2SO_4$ | 25 | 40 | 26 | 10 | 40 |
| pH 2 $H_2SO_4$ + thiosulphate | 35 | 45 | 40 | 20 | 50 |

Table 2
Effect of constituents of growth media on leaching of ocean nodules

| Leaching agents | % Cu | % Ni | % Co | % Mn | % Fe |
|---|---|---|---|---|---|
| ASWNB | 12.54 | 2.36 | – | 1.46 | – |
| ASWNB-Peptone | 11.99 | 1.99 | – | 2.23 | – |
| ASWNB-Beef extract | 10.90 | 1.00 | – | 1.16 | – |
| ASW | 5.22 | – | – | 0.84 | – |

### 3.5. Biological leaching experiments

The results of a few control-leaching experiments carried out to verify the effect of the constituents of uninoculated media, i.e., ASWNB are shown in Table 2. Though around 10–12% Cu was leached, dissolution of other metal ions was negligible. The noticeable dissolution of Cu by the uninoculated media may have occurred due to complexation by peptone and beef extract present therein (Ehrlich et al., 1973).

A 180-h bioleaching study with nodules was carried out using growing cells of the isolate. Most of the metal recoveries were stabilized within first 10 h. Thus, leaching studies were performed using the growing culture as well as the cell-free growth supernatant of the marine isolate for 10 h, and the results are shown in Figs. 3 and 4 respectively. Almost 35% Co and Fe dissolution were achieved in growing culture, while Cu, Ni and Mn solubilization were 16%, 12% and 25%, respectively.

In Mn(IV) reduction experiments with the growing cells of the marine isolate, we observed a decline in soluble Mn concentration after 80 h due to reoxidation and subsequent precipitation of $MnO_2$. However, 180-h bioleaching experiment with the growing culture did not reveal any such decrease. The probable causes behind this different behavior can be as follows:

- Since the marine organism was isolated from nodules, its growth and metabolism pattern in the presence of nodules would be different from that in $MnO_2$ suspension. Therefore, cell growth was not decelerated after 80 h in the presence of nodules, and production of metabolites was also not hindered. The metabolites are supposed to play an

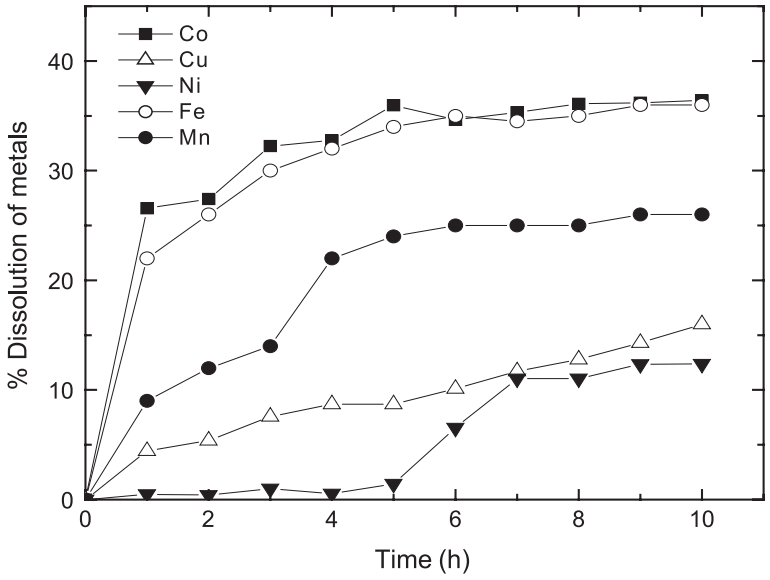


Fig. 3. Dissolution of metals during leaching by the growing cells of the marine isolate.

active role in keeping the reducing atmosphere in the medium.

- No increase in Eh was noted in the growing culture in the presence of nodules after 80 h as in case of $MnO_2$. Thus, the reducing atmosphere prevailed throughout, preventing any reoxidation and subsequent precipitation of Mn.

Almost 10% increase in dissolution of all the five metals was noted in the growth supernatant compared

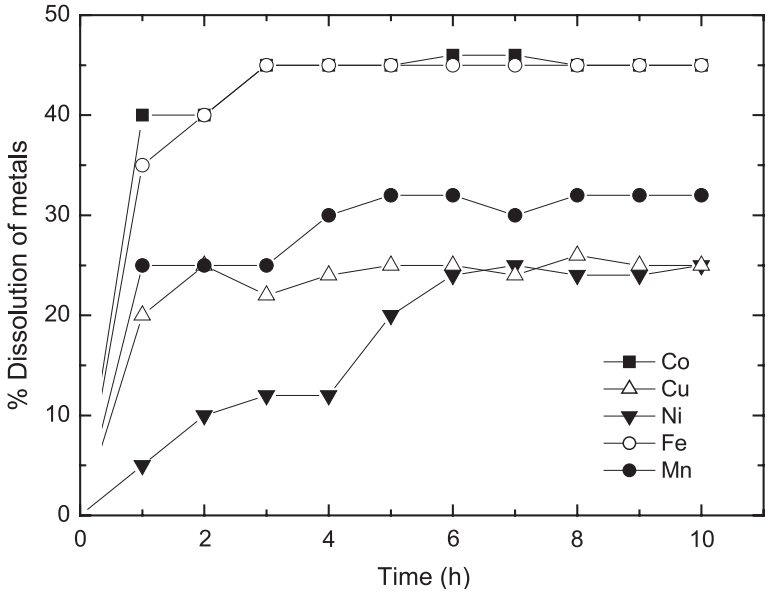


Fig. 4. Dissolution of metals during leaching by the cell-free growth supernatant of the marine isolate.

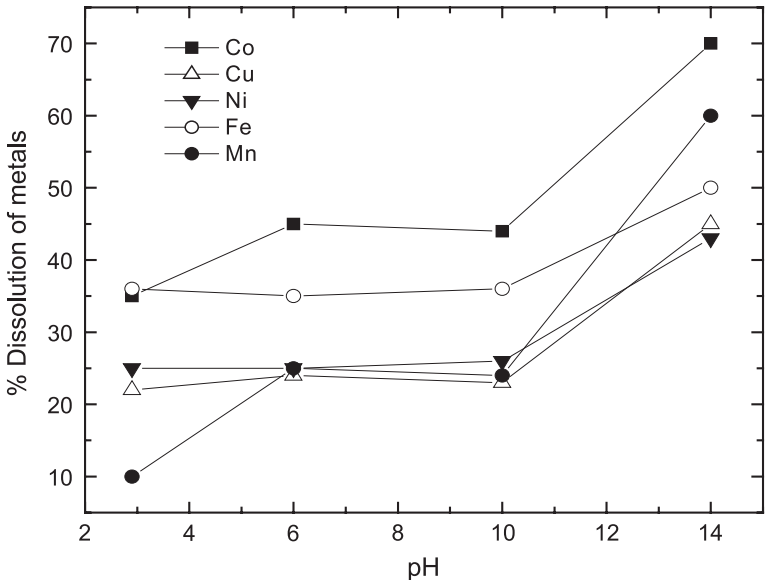


Fig. 5. Leaching by the cell-free growth supernatant of the marine isolate at different pH.

to that in growing culture of the isolate (Fig. 4). Assuming metabolites to be primarily responsible for leaching, this enhancement can be related to greater concentration of metabolites in the growth supernatant collected from a fully grown culture than that in a growing culture.

It is important to mention that in both the cases, the solubilization of both Co and Fe is greater than that of Cu, Ni and Mn. This can be attributed to the positive correlation of Co with Fe in the nodules (Burns and Fuerstenau, 1966; Cronan and Tooms, 1967). It should be emphasized that the pH of the medium was 8.1–8.5. The theoretical solubility of all the metals concerned would be negligible in that pH range. However, the solubility of the transition metals in the complexed state could be markedly different from that in uncoordinated state. Thus, the marine isolate might be producing certain organic substances during growth and metabolism which complexed the ions, thereby increasing their solubility near neutral pH and produced a reducing condition in the media. The exact compounds responsible for this phenomenon are being identified.

Comparing the results of chemical leaching by 2.5 M HCl (Table 1) and bioleaching by the growth supernatant of the marine isolate (Fig. 4), some interesting conclusions may be drawn. Although in the case of bioleaching Cu and Ni dissolution were only 25%, the amounts of Co, Mn and Fe solubilized were quite comparable. Thus, in spite of operating at near-neutral pH, metal dissolution similar to that of highly acidic conditions could be achieved. Presently, acidic dissolution under highly corrosive conditions offers one of the extraction routes for nodules used in large-scale operations, so the bioprocess developed here holds enormous potential as an environmentally safe alternative route.

Leaching experiments were carried out for 10 h using the growth supernatant in the pH range of 3–14, and the results are shown in Fig. 5. When the pH of the medium was increased from 3 to 6, Mn and Co solubilization increased by 10–15%. In the pH range of 6–10, no change in metal dissolution was noted. The dissolution of all the five metals was enhanced by almost 20–25% when the pH of the growth supernatant was increased to about 13.0 from 8.0. This may be attributed to a change of the structure of the metabolites present in the growth supernatant of the marine isolate, which intensifies the complexation effect in the leach solution. A titration of the growth supernatant against 0.1 M NaOH showed that the $pK_a$ value for the growth supernatant lies between 11.5 and 12.5, confirming the deprotonation of the metabolites above that pH.

## 4. Conclusions

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

1. A marine organism was isolated from the polymetallic Indian Ocean nodules was found to grow well in artificial seawater nutrient broth at near-neutral pH and had the ability to reduce Mn(IV) to Mn(II).
2. The growing cells as well as the cell-free growth supernatant were able to leach out Co, Cu and Ni from the nodules.
3. Significant dissolution of Co and Fe (about 45%), comparable to chemical leaching by 2.5 M HCl, could be achieved by leaching with the growth supernatant of the marine isolate at near-neutral pH.
4. Metabolites produced during the growth of the isolate probably solubilized transition metals at neutral pH by complexation.
5. Leaching was enhanced in the highly alkaline growth supernatant having a pH over 12.0, which could be attributed to a probable change in structure of the metabolites present therein.

## Acknowledgements

The authors are grateful to the Department of Science and Technology, Government of India for providing necessary financial assistance for carrying out the above work.

## References

Burns, R.G., Fuerstenau, D.W., 1966. Am. Mineral. 51, 891–902.
Cronan, D.S., Tooms, J.S., 1967. Deep-Sea Res. 15, 215–223.
Das, R.P., Anand, S., Das, S.C., Jana, P.K., 1986. Hydrometallurgy 16, 335–344.
Ehrlich, H.L., 1963. Appl. Microbiol. 16, 197–202.
Ehrlich, H.L., 1966. Dev. Ind. Microbiol. 7, 43–60.
Ehrlich, H.L., 1973. Interuniversity Program of Research of Ferromanganese Deposits on the Ocean Floor. Phase I Report, Seabed Assessment Program, International Decade of Ocean Exploration. National Science Foundation, Washington, pp. 217–219.
Ehrlich, H.L., Ghirose, W.C., Johnson II, G.L., 1972. Dev. Ind. Microbiol. 13, 57–65.
Ehrlich, H.L., Yang, S.H., Mainwaring Jr., J.D., 1973. Z. Allg. Mikrobiol. 13, 39–48.
Fuerstenau, D.W., Han, K.N., 1983. Miner. Process. Technol. Rev. 1, 1–83.
Jana, R.K., Pandey, B.D., Premchand, 1999. Hydrometallurgy 53, 45–56.
Kanungo, S.B., Jena, P.K., 1988a. Hydrometallurgy 21, 23–39.
Kanungo, S.B., Jena, P.K., 1988b. Hydrometallurgy 21, 41–58.
Konishi, Y., Asai, S., Sawada, Y., 1997. Metall. Mater. Trans. 28B, 25–32.
Kumari, A., Natarajan, K.A., 2001. Hydrometallurgy 62, 125–134.
Niinae, M., Komatsu, N., Nakahiro, Y., Wakamatsu, T., Shibata, J., 1996. Hydrometallurgy 40, 111–121.
Trifoni, M., Toro, L., Veglio, F., 2001. Hydrometallurgy 59, 1–14.
Trimble, R.B., Ehrlich, H.L., 1968. Appl. Microbiol. 16, 675–702.
Trimble, R.B., Ehrlich, H.L., 1970. Appl. Microbiol. 19, 966–972.
Zhang, Y., Liu, Q., Sun, C., 2001. Miner. Eng. 14, 525–537.