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ORIGINAL PAPER

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Mechanisms for solubilization of cobalt, copper and nickel from Indian Ocean nodules at near neutral pH by a marine isolate

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Abstract Polymetallic ocean nodules offer an alternative source for extracting valuable strategic metals like Cu, Co and Ni. A novel biodissolution process was carried out, employing the cell-free spent growth medium from a marine organism (*Bacillus* M1) isolated from nodules; and Cu, Co and Ni solubilization from the nodules was observed to be beyond the theoretical solubility limits at near neutral pH. Different characterization techniques revealed the presence of phenolic substances in the spent growth medium, which might have formed soluble complexes with the transition metals. The low prevailing E_h redox value in the medium suggested a strong reducing environment, favoring the reductive dissolution of the oxides. A correlation study of dissolution of Cu, Co and Ni with that of Mn and Fe in the nodules was made to investigate the mechanisms of metal solubilization by the marine isolate. Under the influence of a strong reducing environment coupled with complexation by a phenolic substance present in the spent growth medium, Mn and Fe oxides were solubilized from the nodules, resulting in concomitant dissolution of Cu, Co and Ni associated with them in the nodules.

Introduction

Bioleaching of ocean nodules using a marine isolate, to recover strategic metals like Cu, Ni and Co, is relevant with respect to ongoing research for different environment friendly methods to exploit unconventional mineral resources.

Manganese and ferromanganese deposits are found extensively as nodules, crusts and pavements in present-day oceans. These ocean nodules consist of oxides of manganese and iron, together with various valuable metals such as copper, nickel and cobalt. One of the important prerequisites for extracting metals from these nodules is the solubilization of the desired metals. Since the valuable transition metals are often mineralogically associated with insoluble manganese and/or ferric oxide phases, prior dissolution of oxides is necessary to solubilize them.

The biological reduction of Mn (IV) oxide by marine isolates is a well studied area. All the Mn (IV)-reducing bacteria isolated so far from seawater, marine sediments and ferromanganese concretions have proved to be heterotrophs. Most of the isolates are aerobes which can reduce Mn (IV) aerobically or anaerobically. Ehrlich [4] successfully isolated and characterized a Mn (IV)-reducing organism (*Bacillus* 29) from Atlantic Ocean nodules. Growing cultures of *Bacillus* 29 were able to reduce MnO_2 both aerobically and anaerobically, using glucose as electron donor. However, a component of the electron transport system involved in MnO_2 reduction in this culture was only inducible under aerobic conditions. Both Mn^{2+} and MnO_2 can serve as inducers [5, 16, 17]. MnO_2 reducibility has been found to be inducible in all marine cultures tested so far by Ehrlich [6]. However, the microbial flora associated with Indian Ocean nodules has not been studied extensively, nor has the ability of members of this flora to extract metals from nodules been tested until now.

Recently, Mukherjee et al. [9, 10] studied biologically mediated metal extraction from Indian Ocean nodules

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using a marine isolate, *Bacillus* M1. The spent growth medium from the cultures of the organism solubilized metals from the nodules at near neutral pH and room temperature. Almost 45% Co and 25% Cu and Ni were obtained in about 4 h at an operating pulp density of 10 g/l [9]. The major objective of our present work was to study the mechanism of Cu, Co and Ni solubilization from the nodules by spent growth medium of cultures of the marine isolate.

Materials and methods

Ocean nodule

Ocean nodule samples were collected from the bed of the Indian Ocean by the National Institute of Oceanography, Goa, India. These were ground in a pre-sterilized mortar and pestle and sieved to obtain appropriate size fractions. All the leaching experiments were performed with the 50–75 µm size fraction of nodules, containing around (wt%): 21.83% Mn, 6.4% Fe, 1.14% Cu, 1.15% Ni and 0.17% Co. One gram of nodules per 100 ml of leach pulp (1% pulp density) contained 44.64 mM Mn, 11.42 mM Fe, 1.79 mM Cu, 1.96 mM Ni and 0.3 mM Co.

Microorganism

Bacillus M1, isolated from Indian Ocean nodules [9] was grown in 250-ml baffled Erlenmeyer flasks at 30°C on a rotary shaker (200 rpm), using 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 $MgCl_2{\cdot}7H_2O$, 5 g peptone and 3 g beef extract.

Bioleaching studies

Ten percent of an active inoculum (from the late exponential phase) containing at least 10^9 cells/ml was added to the sterilized ASWNB medium. To obtain the cell-free spent growth medium, a fully grown batch culture (after 10 h of growth) of *Bacillus* M1was centrifuged at 17,700 *g* for 10 min at 5°C followed by pressure filtration through a sterile 0.2-µm Millipore filter. The absence of any cells in the resultant spent growth medium was confirmed by examination under a phase contrast microscope.

After completion of the leaching treatment, the pulp residue was separated from the spent medium by filtration through Whatman 42 paper and digested in 1:1 HCl at 60–70°C. The resultant filtrate was analyzed for Cu, Co, Ni, Mn and Fe by inductively coupled plasma spectrometry.

UV spectroscopic studies

Cell-free spent medium was prepared from growing cultures of the marine isolate. UV difference spectra were recorded for the spent growth medium at pH 10, 11 and 12 (obtained by addition of 0.01 N KOH solution), using a Shimadzu model UV-260 UV-visible spectrophotometer. Each spectrum was run with sterilized ASWNB at the respective pH value as the reference.

A total of 20 ppm of Cu, Ni and Co and 400 ppm of Mn and Fe were added to the spent growth medium using a standard AAS solution of the metal ions (supplied by Thomas Baker, Bombay). After 4 h of incubation at 27°C, spent medium with the added metal ions was centrifuged at 17,700 *g* and UV spectra were run with spent medium at pH 8.2 as a reference.

The leached solutions collected after specified time intervals during a bioleaching experiment with ocean nodules were centrifuged at 17,700 *g*. UV spectra were recorded for the supernatant solutions using spent medium at pH 8.2 as a reference.

Mass spectroscopy

A total of 10 ml of spent medium was evaporated to dryness by heating. A volume of 2–3 ml of chloroform was mixed with the residue. The portion of the residue not dissolved by the chloroform was dried and rinsed several times with 2–3 ml of methanol. It formed a yellow-orange colored solution. This solution was evaporated to dryness and the dry sample was used for mass spectral analysis by matrix-assisted laser desorption/ionization.

Redox potential measurements

Redox potentials of liquid samples were measured using a saturated calomel electrode as reference.

Results

Cu, Co, Ni, Fe and Mn solubilization from the nodules in spent growth medium of *Bacillus* M1

Table 1 shows the extent of dissolution of Cu, Co, Ni, Mn and Fe in the spent growth medium at pH 8.2 for different pulp densities after 4 h. Maximum dissolution for all the metals occurred at 10% pulp density. The extent of dissolved Fe (49.0 mM) was greater than that of Mn (44.6 mM) at 10% pulp density. The extent of Cu, Ni and Co dissolution at 10% pulp density was 3.5, 4.5 and 1.2 mM, respectively. Taking into account the low concentration of Co (3.9 mM) compared with Cu (18.0 mM) and Ni (19.6 mM) in the nodules, the fraction of Co that was dissolved was greater.

Table 1 Metal solubility in the spent growth medium at different pulp densities

Metal	Pulp density (%)	Starting concentration (mM)	Dissolution (%)	Soluble concentration (mM)
Mn	1	44.64	25	11.15
	5	223.3	24	53.60
	10	446.4	10	44.64
Fe	1	11.42	45	5.13
	5	57.14	43	24.60
	10	114.2	43	49.10
Co	1	0.3	45	0.14
	5	1.5	40	0.60
	10	3	40	1.20
Cu	1	1.79	22	0.40
	5	8.97	23	2.06
	10	17.9	20	3.58
Ni	1	1.96	25	0.49
	5	9.81	24	2.35
	10	19.6	23	4.50

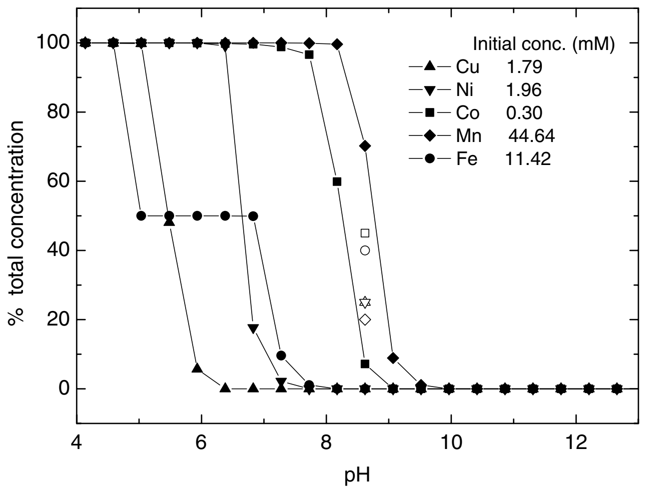

Fig. 1 Solubility of Cu, Co, Ni, Mn and Fe as a function of pH. *Open symbols* Typical metal dissolution in spent growth medium at 1% pulp density.

Following the basic thermodynamic principles of solubility, equilibrium solubility diagrams for Cu, Co, Ni, Fe and Mn were prepared with the help of Environmental Research MINEQL^{+} ver. 2.22 software. The initial concentration of metal ions was chosen to correspond to the typical chemical composition of nodules at 1% pulp density. Figure 1 sums up the theoretical aqueous solubility of the five metals. At the operating pH range of nodule leaching, the theoretical solubility of metal ions tends to follow a sequence: $Mn^{2+} > Co^{2+} > Fe^{2+} > Ni^{2+} > Cu^{2+}$. Next, we superimposed the metal dissolution values obtained in a typical bioleaching experiment of ocean nodules with the spent growth medium of the isolate (1% pulp density, 50–75 μm size fraction) in the same figure. The open symbols signify the dissolved metal concentrations in the spent growth medium. In the case of Cu, Ni, Co and Fe, the dissolved metal concentration was observed to be above the respective metal solubility line. The dissolved Mn concentration was within the thermodynamic solubility limit.

Table 2 Changes in UV spectra of spent growth medium with change in pH. Absorbance values are given in parentheses. *ND* No peaks detected

pH of spent growth medium	Peaks recorded (nm)		
	220–230	250–290	290–300
8.0	233.8	ND	308.2
10.5	ND	ND	298.8 (0.551)
11	ND	ND	297.4 (0.449)
11.5	ND	ND	298.7 (0.642)
12	ND	ND	299.0 (0.849)

Spectral study of the spent growth medium

A kinetic study with the spent liquor to observe changes in the UV spectra during the growth of the marine isolate was reported [9]. The spent growth medium of cultures of the isolate had a p K_a value in the range 11.5–12.5, suggesting a change in the structure of metabolite(s) present therein. To confirm the structural changes, UV-visible spectra of the spent growth medium were recorded at pH values above 10.0. The results are summarized in Table 2, which clearly shows the emergence of a peak near 298 nm. The spent growth medium at pH 8.0 had two peaks, one near 230 nm and another near 310 nm. These spectral results indicate a structural change in the metabolites with increasing pH in the spent growth medium. The UV band near 298 nm in the highly alkaline pH range suggests the presence of phenolic substances having attached carboxylic acid groups, like catechol and di-hydroxybenzoic acid (DHBA). Standard phenolic compounds, catechols and para-hydroxybenzoic acid in aqueous solution and spent growth medium reveal a strong band at 280–300 nm at the same pH.

The mass spectra of partially purified spent growth medium gave a distinct peak at 377.8 nm.

Spectral analysis during leaching

The UV-visible spectra of spent growth medium from cultures of marine isolate collected after 10 h of growth revealed two bands, one near 225 nm and another near 310 nm. To investigate the structural changes taking place during the leaching experiment using crushed nodules in the spent medium, leaching flasks were examined under UV after specified time intervals. The results are summarized in Table 3. It can be seen that the band near 310 nm showed an up-shift in peak position with an increase in absorbance for 3 h, followed by a further slight up-shift in peak position, but a small drop in absorbance over the next hour. A band appeared near

Table 3 Changes in UV peaks of spent growth medium during leaching with nodules

Time (h)	Peaks recorded (nm)			
	220–230	250–275	300–315	> 315
1	ND	263.9 (0.928)	307.2 (0.577)	ND
2	ND	256.0 (0.447)	ND	433.6 (0.432)
3	ND	261.4 (0.861)	309.0 (0.783)	423.0 (0.744)
4	ND	262.6 (0.083)	310.4 (0.614)	ND

Table 4 UV spectra of spent growth medium containing dissolved pure metal ions

Ionic species in spent growth medium	Peaks recorded (nm)		
	250–275	250–300	300–350
Cu (20 ppm)	246.0 (0.175)	294.8 (0.880)	ND
Co (20 ppm)	261.0 (0.195)	295.8 (1.007)	ND
Ni (20 ppm)	255.8 (0.175)	295.2 (0.717)	ND
Mn (400 ppm)	260.0 (0.062)	ND	312.2 (1.299)
Fe (300 ppm)	248.6 (0.207)	ND	342.2 (3.978)
Cu + Co + Mn + Fe + Ni	256.2 (0.560)	ND	302.8 (1.758)

425 nm in the second hour of the experiment, followed by a down-shift in position during the third hour and disappearance in the fourth hour. The absorbance of this band increased from the second to the third hour. The band near 425 nm signifies a Fe (III) siderophore complex [11]. Depending on the ligand–metal ratio, the wavelength value may vary within a range.

Standard solutions of Cu, Co, Ni, Mn and Fe were added individually and together to the spent growth medium, to observe changes in the structure of metabolites therein; and the results are summarized in Table 4. From Table 1, we can observe that the added metal ion concentration in the spent growth medium approximated that of the dissolved metal concentration after 4 h at a 1% pulp density of nodule substance. The added Cu, Ni, and Co each produced two peaks, one near 295 nm with strong absorbance and one in the range 245–260 nm with weak absorbance.

The absorbance increased in the order $\mathrm{Ni_{abs}} < \mathrm{Cu_{abs}} < \mathrm{Co_{abs}}$. Dissolved Mn in spent growth medium gave a very weak band near 260 nm and a stronger peak at 312.2 nm. Two bands, one at 248.6 nm and another at 342.2 nm, were resolved in the case of Fe in the spent growth medium. The absorbance of the Fe peak at 342.0 nm was almost double that of the Mn peak at 312.2 nm. Strong Fe complexation in the spent growth medium may have caused this difference.

Changes in redox value during leaching

The changes in E_h redox values during leaching by spent growth medium are shown in Fig. 2. In the first hour, the E_h of the leach pulp increased from 37 mV to 46 mV. Around 25% of the Mn from nodules was dissolved in that time period. After 1 h, the E_h in the system again decreased, before stabilizing at 32 mV.

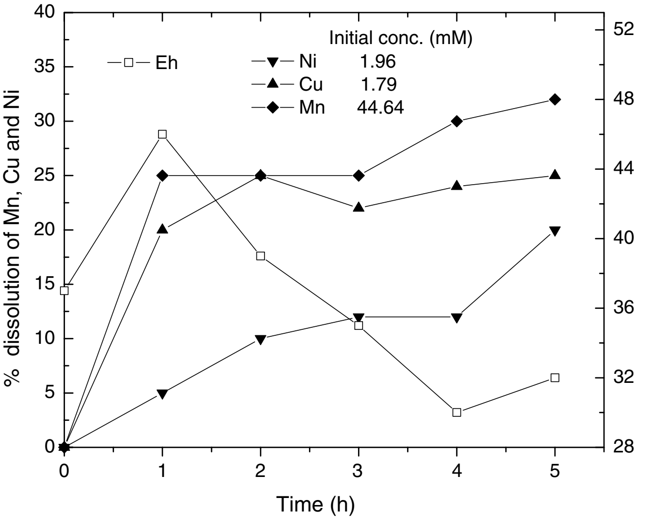

Fig. 2 Change in E_h (*Eh*) during leaching of Cu, Ni and Mn from ocean nodules by the spent growth medium

Discussion

From Table 1 and Fig. 1, it can be concluded that the metal dissolution in spent growth medium at pH values near 8 is enhanced in the presence of certain complexing agents in the spent medium of *Bacillus* M1. To look into this phenomenon, characterization studies were undertaken using the spent growth medium of *Bacillus* M1 and the leached liquor collected after leaching.

Two absorption peaks near 220 nm and 308 nm were noted in the spent growth medium from fully grown cultures [9]. Chakravarty et al. [3] isolated and partially characterized a catechol-type siderophore compound produced by *Pseudomonas stutzeri* grown under iron-depleted conditions. The purified siderophore gave a UV spectrum (bands at 250 nm, 330 nm) which was found to be identical with that of 2,3-DHBA. In another characterization study with siderophores, O'Brien and Gibson [12] isolated five compounds from culture media of *Escherichia coli* and *Aerobacter aerogenes* under conditions of iron deficiency. The compound (2,3-dihydroxy-*N*-benzoylserine) gave two distinct UV peaks in water, one near 246 nm and the other near 308 nm, as shown in Table 5. The absorption peaks in the UV spectra of spent growth medium that were obtained in the present work occurred at similar locations in the above-cited work, suggesting that they were caused by compounds resembling dihydroxybenzoate. However, the spectra in the cited studies were obtained with solutions of purified siderophores. The shift of the 310-nm band in the UV spectrum of spent medium at high alkaline pH (moving to 298 nm) can be explained as an ionization effect.

Table 5 Comparison of the UV bands of the spent growth medium with those of siderophores from previous work. *ND* Not determined

Microbial strain	Type of siderophores	Characterization	UV peaks (nm)	References
Pseudomonas stutzeri	Catecholate	2,3 DHBA and arginine	250 and 330	[3]
Escherichia Coli	Catecholate	2,3 DHBA-*N*-benzoylseine	246 and 308	[12]
Bacillus M1	ND	ND	224.8 and 308.2	Current study

The solution infrared (IR) spectra of the spent growth medium revealed the following bands (in decreasing order): 3,099.0, 2,923.0, 2,853.17, 1,639.2, 1,456.96, 1,376.93, 1,336.43 and 1,108.87 [9]. These bands are suggestive of a phenolic compound with an attached carboxyl group. IR spectra of fresh ASWNB medium (control) did not reveal any of these specific bands.

Table 6 compares the mass spectrum of spent ASWNB medium with the mass spectra of known siderophores cited in the literature. The catecholate-type siderophores have a dihydroxybenzoic acid structure with amino acid fragments attached. Persmark et al. [13] isolated and purified a catechol-type siderophore (chrysobactin) from the phytopathogenic bacterium *Erwinia chrysanthemi*. Mass spectral analysis of the triethylammonium salt of chrysobactin revealed a m/z peak at 370.1614 in the cationic detection mode, which was assigned to [MH+]. In anionic detection mode, a peak was observed at $m/z=368$ and was assigned to $[\mathrm{M}]^{-1}$. Chrysobactin therefore had a M_r of 369. They concluded that chrysobactin consisted of one DHBA, one lysine and one serine molecules. Comparing the mass spectra of the spent growth medium of marine isolate with these results, a difference of 8.8 in m/z values is noted. This difference may arise for two reasons. Either, the exact structure of the siderophore(s) may not be identical in both cases, or the difference in amino acid fragments may give the difference in mass. Persmark et al. [13] isolated chrysobactin and purified the compound before taking mass spectra. In our case, the spent growth medium of the marine isolate may have a number of metabolites. So, the mass spectra of the partially purified spent growth medium of the marine isolate resembled that of standard siderophores from literature, confirming the presence of complex-forming phenolic substances.

The bioleaching system of the nodules was a complex one, where the spent growth medium containing a number of metabolites interacted with a mixture of transition metal oxides.

Table 6 Comparison with mass values of standard compounds

Mass	Suggested structure	Reference
154.0266	DHBA ($C_7H_6O_4$)	[14]
105.0426	Serine ($C_7H_6NO_3$)	[14]
146.1056	Lysine ($C_6H_{14}N_2O_2$)	[14]
369	M-H_2O, Chrysobactin	[13]
377.8	DHBA with amino acid fragments	Current study

Comparing the UV spectra of the leached liquor from Table 3 with that of synthetic solutions of metal ions in the spent growth medium as shown in Table 4, we can infer that the spent growth medium of cultures of marine isolate was able to solubilize Cu, Co, Ni, Mn and Fe ions. The spent growth medium might contain some complexing ligands capable of forming soluble transition metal complexes. These complexes absorbed UV rays and revealed characteristic peaks.

A correlation of Co dissolution with Fe and Mn dissolution by spent culture medium produced by the marine isolate is shown in Fig. 3. The data collected from the bioleaching tests of nodules with the marine isolate at different pulp densities, as shown in the Table 1, are plotted in the diagram. The straight line signifies the linear fit of the corresponding data in both cases. In the figure, the linear regression analysis of Fe and Co dissolution data showed $r=0.97$, while that of Mn and Co dissolution revealed $r=0.89$. From Fig. 3, it is observed that the correlation between Co and Fe dissolution is better than that between Co and Mn dissolution.

The results from a mineralogical study of nodules by Glasby [7] revealed a stable iron phase consisting of limonites (Fe oxyhydroxides), in which the valence state

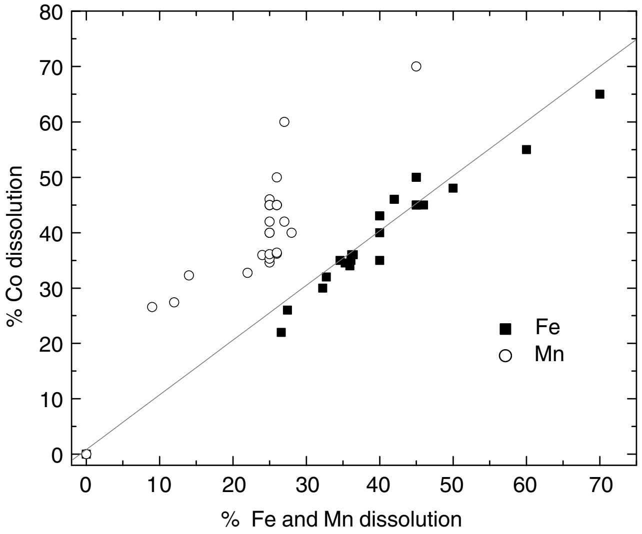

Fig. 3 Correlation of Co dissolution with Fe and Mn dissolution by the spent growth medium

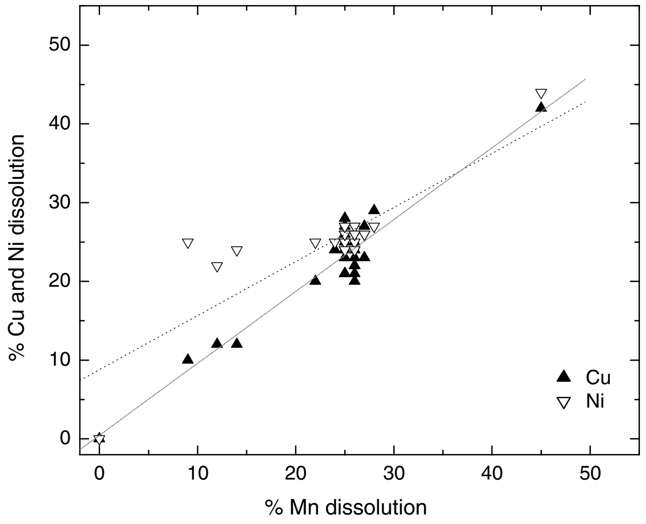

Fig. 4 Correlation between Mn, Cu and Ni dissolution in leaching of ocean nodules by the spent growth medium

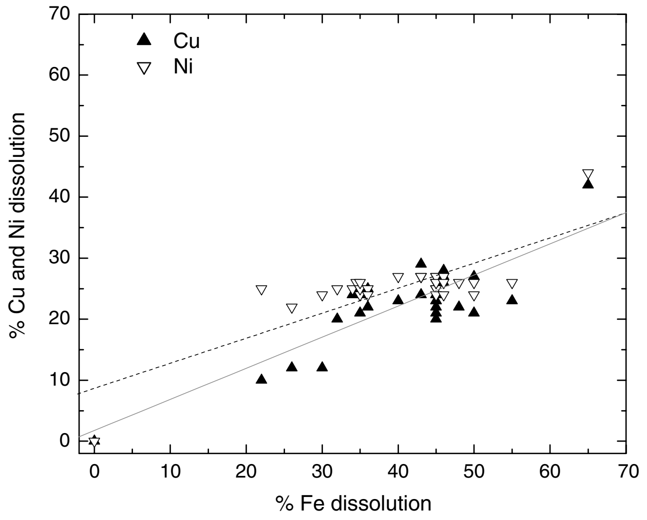

Fig. 5 Correlation between Fe, Cu and Ni dissolution in leaching of ocean nodules by the spent growth medium

of Fe was $+3$. Burns and Brown [1] found that the Fe in nodules had an oxidation state of $+3$, using Mossbaur spectroscopy. At near neutral pH, the thermodynamic solubility of Fe (III) is very low ($-\log K_s = 38$ [15]). In the presence of atmospheric oxygen, Fe (II) is rapidly oxidized to Fe (III) and the latter forms an insoluble ferric hydroxide precipitate.

We inferred the presence of phenolic compounds in spent medium from cultures of *Bacillus* M1 in the present work. These compounds may have complexed the Fe(III) and thereby enhanced its solubility. A comparison of the experimental results shown in Figs. 3, 4 suggests that such a complexing mechanism occurred in this case.

Figures 4, 5 show the correlation of Cu and Ni dissolution with Mn and Fe, respectively, in the leaching experiments with the spent growth medium. The dotted line in both figures represents the line of best fit for Ni, and the solid line in both figures represents Cu. A linear regression analysis shows that the r-value for the Cu-Mn line was 0.96 while that for Ni-Mn was found to be 0.85. So, Cu dissolution was more dependent on Mn solubilization, compared with that of Ni. The linear regression analysis (Fig. 5) shows that the Fe-Cu r-value was 0.84 and Fe-Ni gave a value of 0.79. A comparison between these two figures suggests that both Cu and Ni dissolution had a better correlation with Mn solubilization. This dependence on Mn dissolution can be related to the reducing environment in the spent growth medium.

In a 6-h kinetic study of leaching with the spent growth medium, the pH did not change greatly, confirming that dissolution by acid was not responsible for leaching. The low prevailing E_h during leaching (Fig. 2) signifies a reducing environment in the spent growth medium. In the nodules, we had two major oxide phases: Mn (IV) oxide and Fe (III) oxide. Mn (IV) would be more affected by the reducing environment due to a greater abundance of Mn (IV) in the nodules compared with Fe (III). From our chemical analysis, we observe that the ratio of Mn (IV):Fe (III) was 4:1.

$$\text{a) } \frac{1}{2}\delta\mathrm{MnO_2(s)} + 2\mathrm{H}^+ + 2e^- \rightarrow \frac{1}{2}\mathrm{Mn}^{2+} + 2\mathrm{H_2O} \qquad E^0 = 1.23\,\mathrm{V} \tag{1}$$

$$\text{b) } \frac{1}{2}\mathrm{FeOOH(s)} + 3\mathrm{H}^+ + e^- \rightarrow \mathrm{Fe}^{2+} + 2\mathrm{H_2O} \qquad E^0 = 0.67\,\mathrm{V} \tag{2}$$

From the above two equations, it can be noted that MnO_2 is a more powerful oxidant than Fe (III). Therefore, Mn (IV) oxide would be more reducible by the spent growth medium than the Fe phase.

Godtfredsen and Stone [8] studied the solubilization of Cu bound by Mn dioxide by naturally occurring low-molecular-weight organics, which included hydroxy aromatic moieties. They found that natural organic compounds with only reducing characteristics dissolved Cu by reductive dissolution of Mn, while compounds capable of forming stable Cu complexes in solution caused additional Cu release that could surpass the release by reduction processes alone. One of the formation processes of the ferromanganese nodules is inorganic scavenging of metals from the basinal water on active surfaces through selective chemisorption and autocatalytic oxidation [1]. So, a part of Cu and Ni may be chemisorbed onto the Mn (IV) oxide surface in the nodule.

The spent growth medium of cultures of the isolate was a strong reducing agent; and, in addition, it contained phenolic complexing agents, as we observed from the characterization studies. Therefore, we can extend Godtfredsen and Stone's findings in the case of nodule leaching by spent growth medium.

Reduction of MnO_2 to Mn (II) by metabolites present in the spent growth medium may be written as:

$$MnO_2(s) + PH_2(aq) + 2H^+ \rightarrow Mn^{2+} + P + 2H_2O \quad (3)$$

where PH_2 is the reducing metabolite present in the spent liquor and P is the oxidized form of PH_2

According to this reaction, metabolites may bring about Cu and Ni release indirectly by generating Mn^{II} (which competes with Cu and Ni for metal-sorbing sites on residual MnO_2) and directly by consuming metal-sorbing sites as a result of the reductive dissolution of MnO_2.

An additional mechanism of Cu and Ni dissolution may have been operating in our experiments, namely the formation of Cu, Ni complexes in solution. Butler [2] discussed the solubilization of transition metals other than Fe by microorganisms. In sea water, many of the transition metals are partially or fully complexed by yet-undefined organic ligands. The initial evidence pointed to metal specific organic ligands, distinct from organic ligand or ligand classes complexing Fe (III) in the ocean. So, ligands for Cu and Ni might be different from Fe (III) complexants. In Table 4, showing changes in UV bands during leaching, we observed that the presence of Cu and Ni ions in the spent growth medium gave distinct bands. Complexation of Cu and Ni by the metabolites might be responsible for these specific bands.

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