Asian Journal of Chemistry Vol. 18, No. 1 (2006), 186–190

Bioremediation of Nickel and Iron from Solutions by Viable, Killed and Immobilized Microorganisms

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Bioremediation of heavy metals such as nickel and iron from artificially introduced solutions was tested. The microorganisms used in this study were *Bacillus* (BS5), *Nitrosomonas*, *Bacillus* (BS6) and *Pencillium* species. The metal ion concentration used was 100 mg/L. The bioremediation of these metals by live, killed and immobilized microorganisms was assessed. The sorbent concentration used was 5 mg/mL. There was highest percentage removal of Ni (92.5%) and Fe (92.2%) by viable *Nitrosomonas* species, indicating that it was a very potential microbe. Biosorption technique showed 20.8–84% removal of Ni and 65.7–100% removal of Fe by microorganisms used. There was 92.6% removal of Ni by *Bacillus* species and 97.8% by *Penicillium* species through immobilization technique. The effect of pH on bioremediation of these metals was also assessed. The results obtained were subjected to chi-square test and indicate that all the microorganisms and techniques used were efficient in the removal of Ni and Fe and can be used to remediate these metals from industrial, agricultural and domestic wastewaters.

Key Words: Bioremediation, Biosorption, Immobilization, Non-pathogens, Microorganisms, Nickel, Iron.

INTRODUCTION

Industrial, agricultural and municipal wastes are the major sources of environmental pollution[1–4]. The hazardous chemicals introduced into the environment beyond permissible limits will enter into the food chain causing deleterious health hazards in humans and animals. The permissible limit for Ni is 5 mg/L[5] and if it exceeds its level in humans it acts as a carcinogen, teratogen, mutagen and immunomodulator[6, 7]. The permissible limit for Fe is 0.2 mg/L and beyond this level it makes the water non-palatable, causes stains on clothes, more importantly it alters the normal physiology of the entire life on this earth. So the top priority should be given to the environmental cleaning programmes in order to protect it. Several remediation processes are in race for the last one and a half decades, where old conventional chemical methods[8, 9] and recent eco-friendly techniques of bioremediation, employing several plants and a few microbes[10–12] are being

used. But reports on bioremediation of Ni and Fe, particularly by these microbes through biosorption and immobilization, were scanty. Therefore, an attempt was made to study the removal of Ni and Fe from artificially introduced solutions using these microorganisms.

EXPERIMENTAL

Penicillium species and *Bacillus* species (BS6) were isolated from industrial effluent of metal plating industry, Duvvada, Visakhapatnam. Other two bacteria such as *Bacillus* species (BS5) and *Nitrosomonas* were isolated from soil. They were identified as such by performing several biochemical tests[13]. Fungi were cultivated on Saborauds agar and bacteria on nutrient agar. They were preserved in the refrigerator and fresh cultures are prepared from them for all experiments.

Bioremediation of Ni and Fe by viable microorganisms: Basal salt medium containing one of the metals (100 mg/L) was inoculated with one loopful of each culture/mL and incubated at 37°C in the orbital shaker (100 rpm) for 24 h. Later the culture was spun at 5000 rpm and the supernatant was collected in which the metal concentration was estimated. Ni estimation was done by Ramtek and Moghe[14] and Fe by Vogel's method[15]. Standard curves were plotted from which the values were drawn. Simultaneously control experiment was performed without microorganisms. The percentage removal of metal concentrations was determined in comparison with control. Each organism was inoculated separately for each metal and each pH. To assess the effect of pH on biosorption, different pH such as 2, 4, 6, 7, 8 and 10 were set to the media used.

Bioremediation of Ni and Fe by biosorption technique: Sterilized distilled water with different set pH of 2, 4, 6, 7, 8 and 10 containing 100 mg/mL of either Ni or Fe were inoculated with 5 mg/mL of sorbent of each microbial culture separately. This was incubated in orbital shaker at 37°C for 2 h. Later the solution was centrifuged at 5000 rpm for 15 min and the culture supernatant was collected to estimate the metal content. Controls were simultaneously kept but without the sorbent.

Bioremediation of Ni and Fe by immobilization technique[4]: Sodium alginate beads were made with these microorganisms separately. One gram of beads were inoculated/mL of basal salt medium containing either Ni or Fe and incubated at 37°C for 24 h. Later the solution was collected to estimate the amount of metal. Control beads were made without microorganisms and incubated simultaneously with experimental. The percentage removal of metal was calculated in comparison with the control. Metal concentration used was 100 mg/L.

RESULTS AND DISCUSSION

The results are summarized in Tables 1–6. Bioremediation of Ni by viable microorganisms (Table-1) showed a range of 0–92.5% removal with highest removal by *Nitrosomonas* species at a pH of 7, indicating that this bacterium is a potential one in Ni removal. Similarly, Fe (Table-2) removal was highest by

Nitrosomonas, i.e., 92.2%, at pH 7 and lowest removal was by *Bacillus* species at pH 2. These results indicate that the percentage removal of Ni and Fe was less at acidic pH and higher at pH 7 by viable cells. The biosorption assay (Tables 3 and 4) showed a range of 20.8–80.1% of Ni removal and highest percentage removal was by *Bacillus* species (BS6). Cent per cent removal of Fe by biosorption technique was observed by *Bacillus* species (BS6) and *Nitrosomonas* species. The immobilization technique (Tables 5 and 6) showed highest percentage removal of Ni (94.2%) by *Nitrosomonas* species and Fe (97.5%) by *Bacillus* species (BS6). The effect of pH on immobilization was that there was less percentage removal at acidic pH by most of the microbes, which was increased at higher pH.

TABLE 1
PERCENTAGE REMOVAL OF NICKEL BY VIABLE MICROORGANISMS

pH	Organisms			
	Bacillus spp. (BS5)	*Bacillus* spp. (BS6)	*Penicillium* spp.	*Nitrosomonas* spp.
2	38.8	0*	33.3	48.4
4	89.1	14.6*	38.2	37.1
6	71.3	27.9	35.2	0.0*
7	75.5	18.5	29.8	92.5
8	72.6	36.3	1.8*	42.7
10	38.5	18.4	0.0*	0.0*

Control showed 0.0% removal of metals used at different pH for all experiments.
*Insignificant; rest of the Chi-square values are > the tabulated value, *i.e.*, 3.8 and so $p < 0.05$.

TABLE-2
PERCENTAGE REMOVAL OF IRON BY VIABLE MICROORGANISMS

pH	Organisms			
	Bacillus spp. (BS5)	*Bacillus* spp. (BS6)	*Penicillium* spp.	*Nitrosomonas* spp.
2	11.1*	78.8	69.4	70.8
4	95.5	80.3	69.1	84.0
6	66.5	83.0	75.6	91.2
7	57.8	88.6	71.1	92.2
8	44.9	79.8	79.1	88.4
10	45.1	73.9	73.5	87.0

*Statistically insignificant.

TABLE-3
PERCENTAGE REMOVAL OF NICKEL BY BIOSORPTION

pH	Organisms			
	Bacillus spp. (BS5)	*Bacillus* spp. (BS6)	*Penicillium* spp.	*Nitrosomonas* spp.
2	54.2	65.3	54.9	59.7
4	63.6	65.7	30.6	60.0
6	68.1	60.2	27.1	64.7
7	84 .0	73.2	25.0	48.2
8	72.2	71.3	20.8	73.9
10	68.4	80.1	22.5	73.4

TABLE-4
PERCENTAGE REMOVAL OF IRON BY BIOSORPTION

pH	Organisms			
	Bacillus spp. (BS5)	*Bacillus* spp. (BS6)	*Penicillium* spp.	*Nitrosomonas* spp.
2	95.8	98.1	68.7	94.2
4	95.0	99.5	70.6	100
6	95.5	100.0	72.9	100
7	97.0	100	72.7	100
8	96.4	100	65.7	98.9
10	92.9	100	73.5	98.9

TABLE-5
PERCENTAGE REMOVAL OF NICKEL BY IMMOBILIZATION

pH	Organisms			
	Bacillus spp. (BS5)	*Bacillus* spp. (BS6)	*Penicillium* spp.	*Nitrosomonas* spp.
2	85.1	85.3	70.5	86.8
4	84.4	90.8	87.2	89.5
6	89.1	87.2	87.0	91.8
7	86.7	89.0	80.5	94.2
8	91.4	89.5	86.2	88.1
10	92.6	90.8	86.8	87.8

Control bead for nickel = 15%.

TABLE-6
PERCENTAGE REMOVAL OF IRON BY IMMOBILIZATION

pH	Organisms			
	Bacillus spp. (BS5)	*Bacillus* spp. (BS6)	*Penicillium* spp.	*Nitrosomonas* spp.
2	89.3	97.18	86.5	92.1
4	85.6	94.2	90.3	95.9
6	86.3	97.5	80.1	84.3
7	84.4	89.0	83.4	82.7
8	82.9	89.2	83.6	82.6
10	83.1	89.1	90.5	82.1

Control bead for Fe = 10%; not deducted from experimental results.

These results clearly indicate that viable microorganisms were less efficient in the removal of Ni and Fe when compared to those of other techniques. Of all the three techniques used biosorption technique proved to be very efficient in the removal of Ni and Fe.

ACKNOWLEDGEMENTS

One of the authors Dr. K.M. Elizabeth is grateful to UGC, Hyderabad for financial support. Thanks are also due to the management of GITAM for providing laboratory facilities.

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(*Reccived*: 14 December 2004; *Accpeted*: 17 September 2005) AJC-4382