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Bioremediation of Nickel and Iron from Solutions by Non-pathogenic Microorganisms

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Bioremediation of nickel and iron from artificially introduced solutions by various non-pathogenic microorganisms was tested. The concentration of metal used was 100 mg/L. Viable *Pseudomonas* species and *Bacillus* species could remove 56.24 and 56.8% of nickel, respectively. There was 11.1–96.86% removal of iron by viable microorganisms, *Aspergillus* species with highest and *Saccharomyces* species with lowest percentage removal. The biosorption technique showed a range of 54.32–87.2% removal of nickel, with highest removal by *Bacillus* species. The biosorption of iron ranged from 60–100% with highest percentage removal by *Aspergillus* species. The percentage removal of nickel ranged from 54.8–92.4 and iron removal ranged from 73–95.68% by immobilization technique. The results of the present study indicate that these microorganisms are potential to remove nickel and iron from polluted waters, soils, industrial effluents, agricultural and municipal wastes.

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

INTRODUCTION

Rapid industrialization and urbanization have resulted in elevated emission of toxic heavy metals and radionuclides entering into the biosphere, ultimately affecting the health of humans and animals. An inorganic toxicant may be cationic such as metallic ions of mercury, cadmium, chromium, lead, nickel, uranium, etc. Toxic inorganic metal may also be alkylated or aromatized forms of metal ions such as methyl-mercury and phenyl-mercury. Several conventional chemical methods are available for removal of these toxicants which are expensive and inefficient[1–4]. Phyto-remediation of toxic heavy metals was well documented[5–9]. Microorganisms can physically remove heavy metals and radionuclides from polluted waters either by bioaccumulation or by biosorption. Bioaccumulation is the retention and concentration of the metals by an organism. During the phenomenon of bioaccumulation the heavy metals are transported through the membranes of microorganisms from the external environment of the microbial cell into the cytoplasm where the metals are sequestered and immobilized. Biosorption does not require cellular energy as the positively charged metal ions are sequestered by adsorption to the negatively charged ionic groups on microbial cell surfaces. These surfaces may be polysaccharide capsules, slime layers or other binding sites such as carboxyl, phosphate residues, S-H groups and hydroxyl groups[10–12]. There were several reports on bioremediation of heavy metals by microorganisms. But bioremediation of nickel and iron by these particular

microorganisms through viable cells, biosorption and immobilization techniques was not reported to date. Therefore we are interested in this study.

EXPERIMENTAL

Microbial culture conditions: Microorganisms were isolated from industrial effluent of metal plating industry, at Duvvada, Visakhapatnam District (A.P), India, which was expected to contain nickel. Later the microorganisms were identified until genus level according to the method of Cappuccino and Sherman[13]. These microorganisms were *Aspergillus* species, *Saccharomyces* species, *Bacillus* species and *Pseudomonas* species (normal soil isolate). The bacteria and fungi were isolated by plating different aliquots of serially diluted industrial effluent on nutrient agar and sabourauds agar plates and incubating them at 37°C and at RT (29°C), for 18–24 and 24–48 h, respectively. Later they were identified and slants were maintained in refrigerator at 4°C and fresh subcultures were prepared from them for all experiments.

Bioremediation of nickel by viable microorganisms: Each fresh microbial culture was inoculated in basal salt medium (one loopful/mL of medium) as described earlier[11], with different pH separately into which 100 mg/L concentration of Ni stock solution (nickel sulfate) was added and incubated in orbital shaker at 37°C for 24 h. Later the cultures were spun at 5000 rpm for 15 min and the culture supernatant was collected to estimate nickel content. Nickel was estimated according to the method of Ramtek and Moghe[14] using dimethyl glyoxime. Ni content was obtained from the standard curve as described earlier[11]. Simultaneously controls without inoculating with microbes but with same Ni content were also incubated and the percentage removal of Ni was calculated by comparing with that of control. Different pH were set to basal salt medium in order to see the effect of pH on bioremediation. The results obtained were subjected to Chi-square test to show whether the results were significant or not.

Bioremediation of iron by viable microorganisms: Similarly, the percentage removal of iron was calculated by live microorganisms where 100 mg/L concentration of iron was used. The iron stock was prepared by ferrous ammonium sulfate. Iron was estimated by 1 : 10 phenanthroline method[15] as described earlier[11].

Bioremediation of nickel and iron by biosorption technique: Sorbent was prepared by harvesting large amounts of microbial cultures. The cells were dried at 100°C for 1 h and made into fine powder (sorbent). The dosage of sorbent was determined for each culture by incubating sterile distilled water (pH : 7) containing 100 mg/L concentration of either Ni or Fe with various doses of sorbent of each culture such as 2.5, 5, 10 and 20 mg/mL separately for 30 min, 1 h and 1.5 h at 37°C in orbital shaker. Later the samples were centrifuged and supernatant was used to estimate the metals. Then the percentage removals of Ni and Fe were calculated in comparison with controls which possessed only stock solutions of either Ni or Fe at the above concentrations. The dosage of sorbent was determined as 5 mg/mL. So the distilled water with different set pH such as 2, 4, 6, 7, 8 and 10 was separately inoculated with 5 mg/mL sorbent of a single microorganism and incubated at 37°C for 2 h in orbital shaker (100 rpm shaking for all

experiments). Later the solution was spun and the supernatant was collected to estimate the metals. The controls were simultaneously incubated with metals but without sorbent whose metal concentration was estimated. The percentage removal of metal ions was calculated in relation to control. Similarly all microorganisms were tested for metal removal at different pH.

Bioremediation of nickel and iron by immobilization technique: This was performed as described earlier[11]. Sodium alginate beads (3%) were prepared with each microbial culture where the microbes were adsorbed to the beads. Briefly, 5 mL of 3% sterile sodium alginate was mixed with one plateful of each fresh culture, mixed well and dropped into beads in sterile calcium chloride solution. One gram of beads were incubated in 2 mL of basal salt medium (pH : 7) which contained 100 mg/L concentration of each metal separately, at 37°C for 24 h in orbital shaker. Later the solution was used to estimate metal concentration. Control beads were prepared without the microorganisms. Then percentage removal of metal ion was calculated. Each microbial culture beads were incubated in basal salt medium with different pH as mentioned above.

RESULTS AND DISCUSSION

The results are summarized in Tables 1–6. The percentage removal of Ni by viable *Aspergillus* (Table-1) ranged from 7.7–32.5 indicating the lowest % removal at acidic pH, *i.e.*, at 2 and highest at pH 8. Similarly the percentage removal of Ni by *Saccharomyces* spp. showed a range of 4.3–25.0%, with lowest percent removal at pH 6 and highest at 7. The percentage removal of Ni by live *Bacillus* ranged from 47.5–56.8 at pH 7–8, respectively. Live *Pseudomonas* showed a range of 4.7–56.2 indicating lowest percentage removal at pH 2 and highest at pH 6. These results indicate that viable microorganisms were able to remove higher percentage of Ni at higher pH than acidic pH. There was highest percentage removal of nickel by live *Bacillus* species, *i.e.*, 56.8%. Live *Pseudomonas* could remove 56.2% of Ni and 74.1% of iron. However, the percentage removal of Ni by viable cells was less when compared with those of biosorption and immobilization techniques.

TABLE-1
PERCENTAGE REMOVAL OF NICKEL BY VIABLE MICROORGANISMS

pH	Organisms			
	Aspergillus spp.	*Saccharomyces* spp.	*Bacillus* spp.	*Pseudomonas* spp.
2	7.7*	8.5*	51.9	4.7*
4	30.3	17.1	52.3	47.5
6	20.0	4.3*	49.6	56.2
7	19.4	25.0	47.5	48.3
8	32.5	14.8*	56.8	53.6
10	24.0	19.4	53.8	50.1

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

TABLE-2
PERCENTAGE REMOVAL OF IRON BY VIABLE MICROORGANISMS

pH	Organisms			
	Aspergillus spp.	*Saccharomyces* spp.	*Bacillus* spp.	*Pseudomonas* spp.
2	67.8	11.1	76.6	65.9
4	77.3	45.5	82.6	69.5
6	74.8	66.5	71.0	74.1
7	96.8	57.8	61.0	65.0
8	87.1	44.9	82.1	69.0
10	65.3	45.1	72.5	72.0

TABLE-3
PERCENTAGE REMOVAL OF NICKEL BY BIOSORPTION

pH	Organisms			
	Aspergillus spp.	*Saccharomyces* spp.	*Bacillus* spp.	*Pseudomonas* spp.
2	70.9	54.3	75.7	50.0
4	74.4	82.4	83.4	80.5
6	74.5	84.7	84 .7	80.7
7	79.9	82.4	85.5	79.7
8	75.1	77.0	87.7	83.2
10	66.0	81.2	86.5	81.2

TABLE-4
PERCENTAGE REMOVAL OF IRON BY BIOSORPTION

pH	Organisms			
	Aspergillus spp.	*Saccharomyces* spp.	*Bacillus* spp.	*Pseudomonas* spp.
2	89.9	65.4	60.0	63.7
4	90.5	69.0	60.8	64.5
6	92.0	69.5	60.3	66.7
7	94.7	70.4	62.5	68.4
8	99.4	73.5	63.5	71.3
10	100.0	78.2	63.7	70.7

TABLE-5
PERCENTAGE REMOVAL OF NICKEL BY IMMOBILIZATION

pH	Organisms			
	Aspergillus spp.	*Saccharomyces* spp.	*Bacillus* spp.	*Pseudomonas* spp.
2	86.2	67.4	89.5	83.3
4	80.2	88.5	82.5	85.3
6	82.4	79.9	81.5	92.4
7	54.7	71.5	86.4	77.5
8	85.3	84.7	90.2	85.5
10	81.2	85.1	87.8	88.5

Bead control for nickel = 15%

TABLE-6
PERCENTAGE REMOVAL OF IRON BY IMMOBILIZATION

pH	Organisms			
	Aspergillus spp.	*Saccharomyces* spp.	*Bacillus* spp.	*Pseudomonas* spp.
2	88.8	95.7	86.0	82.7
4	87.6	83.8	87.3	83.8
6	84.4	76.1	82.1	86.2
7	93.4	82.0	81.9	92.3
8	90.6	73.7	84.1	94.1
10	88.7	84.0	82.1	94.2

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

There was 96.8% removal of iron by viable *Aspergillus* spp. (Table-2) at pH 7. It showed a range of 65.3–96.8 at pH 10 and 7. *Saccharomyces* spp. could remove a maximum of 66.5% of iron at pH 6. Viable *Bacillus* species could remove 82.6% of Fe at pH 4 and live *Pseudomonas* could remove 74.1% of iron at pH 6. These results indicate that Ni and Fe removal was higher at neutral and alkaline pH but less at acidic pH by viable cells. Howewver, the percentage removal of iron was more by viable microorganisms when compared withi Ni. The removal of iron may be facilitated by siderophores, the iron chelators developed by microorganisms.

The highest percentage removal of Ni and Fe (Tables 3 and 4) by *Aspergillus* spp. through biosorptiuon technique was 79.9 and 100, respectively suggesting the adsorption of metal ions to the negatively charged cellular components. There was 84.7 and 78.2% removal of Ni and Fe by *Saccharomyces* sp through biosorption at pH 6 and 10 respectively. The highest percentage removal of Ni (87.7) and Fe (63.7) by *Bacillus* spp. occurred at pH 6 and 10 pH respectively indicating that this organism was more potential in the removal of Ni than Fe. *Pseudomonas* could bioremediate 83.2 and 71.3% of Ni and Fe respectively at pH 8. Immobilized beads showed (Tables 5 and 6) highest percentage removal of Ni (86.2) and Fe (93.4) by *Aspergillus* species. *Saccharomyces* was able to remove Fe ranging from 73–95.7 and Ni (67.4–88.5). There was 90.2% removal of Ni

and 87.3% removal of Fe by *Bacillus* spp. through immobilization technique. *Pseudomonas* showed 92.4% removal of Ni and 94.2% removal of Fe at pH 6 and 10 respectively. It was apparent from the results that bioremediation through viable cells was less efficient than that of biosorption and immobilization techniques. Of all the microorganisms used viable *Bacillus* spp. was efficient in Ni removal (56.6%) and *Aspergillus* in Fe removal (96.8%). The % removal of metals were less at acidic pH by live cells. Biosorption showed highest % removal of Ni by *Bacillus* spp. (87.6) and Fe by *Aspergillus* spp. (100%). Highest % removal of Ni by immobilization was obtained by *Pseudomonas* (92.4) and Fe by *Saccharomyces* spp. (95.7), indicating that these were the most potential microorganisms which can be utilized in the metal plating or any other industrial treatment plants for treating the effluents.

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