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# Bioremediation of Ammonia and Nickel from Solutions by Viable, Killed and Immobilized Non-pathogenic Microorganisms

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Bioremediation of ammonia and nickel from artificially introduced solutions was determined by viable, killed and immobilized non-pathogenic microorganisms. The concentration of the metals used was 100 mg/mL. The concentration of sorbent used in biosorption technique was 5.0 mg/mL. The live *Staphylococcus* species could remove 90.7% of ammonia and 56.5% nickel at pH 7. It could remove 91.5% ammonia and 36% of Ni by biosorption and 95% ammonia and 74.3% Ni by immobilization technique indicating that it was a very potential microorganism that can remove ammonia very efficiently. There was highest percentage removal of ammonia (93.3%) by *E. coli* through immobilization. Live *Bacillus* species (BS1) could remove 78.3% ammonia and 61.4% Ni, 94.36% ammonia and 79.8% Ni by immobilization technique. Live *Bacillus megaterium* could remove 84.3% ammonia and 24.4% Ni, 100% ammonia and 79% Ni through biosorption technique at pH 7. The effect of pH on bioremediation was studied. The results were subjected to chi-square test to determine whether the percentage removal of these pollutants was significant or not. These results clearly indicate that bioremediation of ammonia and nickel was very efficient by all microorganisms used and they can be utilized in the treatment of industrial, agricultural and domestic wastes, where there was plenty of ammonia and nickel.

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

## INTRODUCTION

Indiscriminate disposal of industrial, municipal and agricultural wastes is the major source of environmental pollution[1-3]. Water pollution due to various hazardous pollutants has become a major global concern. Refineries, steel plants, coke manufacturing units, pharmaceuticals and various other phenol processing industries discharge effluents which contain phenols associated with various levels of nitrogen. Nitrogen is available in the form of ammonium ion in these effluents. Ammonia is harmful beyond the permissible limit of 5 mg/L to the terrestrial and 1.2–3 mg/L to aquatic life. The desired limit of ammoniacal nitrogen for fish culture is 1.2 mg/L. The toxic effects of ammonia and ammonium salts can be very high. Even at pH values below 9 ammonia can be corrosive to certain metals and materials of construction and cause trouble in chlorination of water. Nickel is an essential micronutrient but when present beyond the environmental permissible limit, creates problems by entering into the food chain and causes bio-magnification. Electroplating industrial effluents are a rich source of Ni pollution. Ni toxicity on land, in water, on microorganisms, eukaryotic plants, animals and humans has

been reported to be carcinogenic, mutagenic, teratogenic, genotoxic, allergenic and immunomodulatory[4–6]. Therefore, it is of prime importance to protect our environment by employing various technologies where bioremediation is one such eco-friendly technique which uses biological organisms. So far several algae, fungi, plant materials were used for this purpose[7–10] but reports on the use of bacteria were scanty[11, 12].

Recently, it has been reported that the bacterium which has been isolated by us (*Bacillus* species, BS1) in its viable form was able to remove 100% ammonia, 92.5% nickel and 88.5% chromium from untreated sterile effluent of Visakhapatnam steel plant[13]. There was 76% of nickel removal by *Bacillus megaterium* by biosorption technique[14]. Therefore, an attempt was made to study the potency of non-pathogenic microorganisms such as *Staphylococcus aureus*, *E. coli*, *Bacillus megaterium* and *Bacillus* species to remove ammonia and nickel from artificially introduced solutions under laboratory conditions.

## EXPERIMENTAL

**Microbial culture conditions:** One microorganism was isolated from the industrial effluent of Hindustan Zinc Industry, Visakhapatnam, India and the other bacteria were isolated earlier[13]. Later, the microorganisms were identified up to genus level. The microorganisms used in the present study were *Staphylococcus* species, *Bacillus* species (BS1), *Bacillus megaterium* and *E. coli*. The slants of these bacterial cultures were maintained in a refrigerator at 4°C and fresh subcultures were prepared from them for all experiments.

**Bioremediation of ammonia by viable bacteria**: Fresh bacterial cultures inoculated separately in basal salt medium ($10^6$ cells/mL), with different pH such as 2, 4, 6, 7, 8 and 10, containing ammonium chloride stock (100 mg/L) were incubated at 37°C for 24 h. Later the samples were spun at 5000 rpm and the supernatant was collected to assess the ammonia content. Ammonia standard curve was plotted by taking various concentrations of ammonium chloride. A control without bacterial inoculum but with ammonia stock was simultaneously incubated. Ammonia content in the control and samples was assessed by Nessler's method of Ramteke and Moghe[15].

**Bioremediation of nickel by viable microorganisms**: Each fresh, microbial culture was inoculated in basal salt medium ($10^6$ cells/mL of medium), with different pH separately, into which 100 mg/L concentration of Ni stock solution (nickel sulphate) 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 cultured supernatant was collected to estimate nickel content. Nickel was estimated[15] using dimethyl glyoxime. Ni content was obtained from the standard curve. 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 ammonia and nickel 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 ammonia or Ni 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 percentage removal of ammonia and Ni in comparison with controls which possessed only stock solutions of either ammonia or Ni at similar concentrations. The dosage of sorbent was determined as 5 mg/mL. So the distilled water samples with different pH such as 2, 4, 6, 7, 8 and 10 were separately inoculated with 5 mg/mL sorbent of a single microorganism and incubated at 37°C for 2 h in orbital shaker (at 100 rpm shaking for all experiments). Later the solution was spun and the supernatant was collected to estimate the toxicants. The controls were simultaneously incubated with respective stock solutions but without sorbent whose pollutant concentration was estimated. The percentage removal of toxicants was calculated in relation to control. Similarly, all microorganisms were tested for metal removal at different pH.

**Bioremediation of ammonia and nickel by immobilization technique[13]:** 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 the beads in sterile calcium chloride solution. One gram of beads was incubated/2 mL of basal salt medium (pH 7) which contained 100 mg/L concentration of each metal, at 37°C for 24 h in orbital shaker. Later the solution was used to estimate pollutant concentration. Control beads were prepared without the microorganisms. Then the percentage removal of metal ion was calculated. Each set of 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.

Live *S. aureus* could remove 67.3–92% of ammonia at various pH. *Bacillus* species (BS1) could remove 78.3–85.9% of ammonia. Viable *B. megaterium* and *E. coli* showed 68.8–94.6% and 66.6–93% of ammonia respectively. These results indicate that all organisms used were potential in removing ammonia efficiently. The viable *Staphylococcus* species (Table-2) showed 44.8–73.5% of nickel removal, highest removal at pH 2 and least at pH 6.

TABLE-1
PERCENTAGE REMOVAL OF AMMONIA BY VIABLE MICROORGANISMS

| pH | Organisms | | | |
|---|---|---|---|---|
| | *Staphylococcus* spp. | *Bacillus* (BSI) spp. | *B. megaterium* | *E. coli* |
| 2 | 78.5 | 81.2 | 90.4 | 93.0 |
| 4 | 91.5 | 79.1 | 82.6 | 92.0 |
| 6 | 67.3 | 80.0 | 68.8 | 71.6 |
| 7 | 90.7 | 78.3 | 84.3 | 68.3 |
| 8 | 92.0 | 83.1 | 75.3 | 69.0 |
| 10 | 91.1 | 85.9 | 94.6 | 66.6 |

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

TABLE-2
PERCENTAGE REMOVAL OF NICKEL BY VIABLE MICROORGANISMS

| pH | Organisms | | | |
|---|---|---|---|---|
| | *Staphylococcus* spp. | *Bacillus* (BSI) spp. | *B. megaterium* | *E. coli* |
| 2 | 73.5 | 53.0 | 24.4 | 56.7 |
| 4 | 52.8 | 55.5 | 59.2 | 59.0 |
| 6 | 44.8 | 47.6 | 52.8 | 66.9 |
| 7 | 56.5 | 61.4 | 46.5 | 65.5 |
| 8 | 60.5 | 81.6 | 24.0 | .63.0 |
| 10 | 51.0 | 77.5 | 37.0 | 82.0 |

Viable *Bacillus* species could remove 47.6–81.6% of nickel, highest removal at pH 8 and lowest removal at pH 6. *B. megaterium* could remove a maximum of 59.2% of Ni at pH 4 and *E. coli* could remove 82% of nickel at pH 10. These results indicate that live bacteria could remove higher percentage of Ni neal neutral or alkaline pH except *Staphylococcus* species. The biosorption technique (Table-3) showed that *Staphylococcus* species could remove 90.4–95% of ammonia, *Bacillus* species could remove 81.7–85.6%, *B. megaterium* from 94–100% and 92.3–94.6% of ammonia.

TABLE-3
PERCENTAGE REMOVAL OF AMMONIA BY BIOSORPTION TECHNIQUE

| pH | Organisms | | | |
|---|---|---|---|---|
| | *Staphylococcus* spp. | *Bacillus* (BSI) spp. | *B. megaterium* | *E. coli* |
| 2 | 95.0 | 83.8 | 94.0 | 94.6 |
| 4 | 94.6 | 85.6 | 98.9 | 94.2 |
| 6 | 92.8 | 81.7 | 100.0 | 93.2 |
| 7 | 91.5 | 83.3 | 100.0 | 92.8 |
| 8 | 90.8 | 82.4 | 100.0 | 92.3 |
| 10 | 90.4 | 82.6 | 100.0 | 91.3 |

TABLE-4
PERCENTAGE REMOVAL OF NICKEL BY BIOSORPTION TECHNIQUE

| pH | Organisms | | | |
|---|---|---|---|---|
| | *Staphylococcus* spp. | *Bacillus* (BSI) spp. | *B. megaterium* | *E. coli* |
| 2 | 34.4 | 36.5 | 44.4 | 56.5 |
| 4 | 34.6 | 34.4 | 46.3 | 58.5 |
| 6 | 35.6 | 36.9 | 46.7 | 64.5 |
| 7 | 36.0 | 43.6 | 47.7 | 50.4 |
| 8 | 34.8 | 40.3 | 46.5 | 49.1 |
| 10 | 38.5 | 42.5 | 50.8 | 47.9 |

The biosorption technique was more efficient in removing ammonia than other techniques. The percentage removal was highest at all pH and was more at acidic pH. The percentage removal of Ni by *Staphylococcus* species through biosoption technique (Table-4) was found to be less at all pH ranging from 34.4–36%. *Bacillus* species could remove 34.4–43.6%, with highest removal at pH 7 and lowest removal at pH 4. *B. megaterium* could remove 44.4–50.8% of Ni. *E. coli* could remove 64.5% of Ni at pH 6.

The immobilization technique (Table-5) showed that *Staphylococcus* species could remove 90–96.9% of ammonia, highest percentage removal at pH 4 and lowest at pH 10. *Bacillus* species could remove 91.3–97.1% of ammonia. *B. megaterium* could remove 88.5–95.1% of ammonia by immobilization technique. *E. coli* could remove 91.3–96.9% of ammonia, highest percentage removal at pH 2 and lowest at pH 10. These results indicate that all bacteria used could efficiently remove ammonia at all pH through immobilization technique. The percentage removal of nickel by immobilization technique (Table-6) showed that *Staphylococcus* species could remove 69–76.3%. *Bacillus* species 71.2 –3.2%, *B. megaterium* 74.3–96.6%, *E. coli* 0.0–79.4%. These results indicate that the organisms used were very efficient in the bioremediation of ammonia and nickel which can be used in the treatment of industrial, agricultural and domestic waste waters.

TABLE-5
PERCENTAGE REMOVAL OF AMMONIA BY IMMOBILIZATION TECHNIQUE

| pH | Organisms | | | |
|---|---|---|---|---|
| | *Staphylococcus* spp. | *Bacillus* (BSI) spp. | *B. megaterium* | *E. coli* |
| 2 | 93.3 | 97.1 | 95.1 | 96.9 |
| 4 | 96.9 | 95.1 | 94.3 | 96.0 |
| 6 | 95.7 | 94.3 | 93.0 | 93.6 |
| 7 | 95.0 | 93.2 | 90.2 | 93.2 |
| 8 | 99.3 | 92.5 | 89.2 | 92.8 |
| 10 | 90.0 | 91.3 | 88.5 | 91.3 |

Control beads: 10% removal not deducted from the values.

TABLE-6
PERCENTAGE REMOVAL OF NICKEL BY IMMOBILIZATION TECHNIQUE

| pH | Organisms | | | |
|---|---|---|---|---|
| | *Staphylococcus* spp. | *Bacillus* (BSI) spp. | *B. megaterium* | *E. coli* |
| 2 | 69.0 | 71.2 | 74.5 | 64.9 |
| 4 | 73.1 | 75.3 | 74.3 | 77.6 |
| 6 | 74.1 | 74.9 | 96.6 | 0.0* |
| 7 | 74.3 | 79.8 | 79.0 | 0.0* |
| 8 | 76.3 | 83.1 | 78.6 | 79.4 |
| 10 | 72.2 | 74.7 | 76.9 | 75.3 |

*Insignificant; rest of the chi-square values are the tabulated values, *i.e.*, 3.8 and $p < 0.05$. Control bead for Ni = 10%.

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