

# Bioremoval of Nickel Using *Pseudomonas fluorescens*

R. S. Akram Husain<sup>1,\*</sup>, A. J. Thatheyus<sup>2</sup>, D. Ramya<sup>2</sup>

<sup>1</sup>PG Department of Immunology and Microbiology, The American College, Madurai, India

<sup>2</sup>PG and Research Department of Zoology, The American College, Madurai, India

\*Corresponding author: [jthatheyus@yahoo.co.in](mailto:jthatheyus@yahoo.co.in)

Received December 29, 2012; Revised May 08, 2013; Accepted May 10, 2013

**Abstract** The bioremoval of nickel ions by *Pseudomonas fluorescens* was studied for the period of eight days by exposing to different concentrations of nickel (250, 500, 750 and 1000ppm). Experiments were designed to study the effect of dead cells and sugars on the biosorption of nickel ions. Atomic absorption spectrophotometric (AAS) analysis was carried out for the samples at an interval of two days. The highest nickel uptake was at 1000ppm concentration. Metal binding by bacteria has been shown to be profoundly influenced by the concentration of the metal.

**Keywords:** nickel, *Pseudomonas fluorescens*, bioremediation, atomic absorption spectrophotometry

## 1. Introduction

The contamination of heavy metals in the environment is a major global problem due to their toxicity and the industrial activities have contributed to the accumulation of metal pollutants in the environment. The dispersion of the metal ions in the water bodies leads to their biomagnifications through the food chain and results in toxicity [1]. Many industries such as electroplating and metal finishing discharge heavy metal-laden effluents into the environment, being one of the major contributors to heavy metal pollution in surface waters [2]. In this type of effluents, there is usually a high concentration of nickel ions ( $\text{Ni}^{2+}$ ) [3]. In large doses like 0.5g, some forms of nickel may be acutely toxic to humans when taken orally. Toxic effects of oral exposure to nickel usually involve damage to kidneys and lungs [4].

There are several methods employed for the removal of heavy metals from waste waters and effluents. They are Reverse osmosis, Electrodialysis, Ultrafiltration, Ion exchange, Chemical precipitation and Phytoremediation. Microbial biomass can bind heavy metals either actively or passively or by a combination of both processes. The passive phenomenon of 'biosorption' has several advantages over the active phenomenon of 'bioaccumulation'. It is a passive non-metabolically-mediated process of metal binding by biosorbent. Bacteria, yeasts, fungi and algae have been used as biosorbents of heavy metals [1].

Bacteria make excellent biosorbents because of their high surface to volume ratios and a high content of potentially active chemo sorption sites such as teichoic acid in their cell walls. Bacterial cell walls are negatively charged under acidic conditions and the cell wall functional groups display a high affinity for metal ions in the solution. Hence in the present study an attempt has been made to study the biosorption of nickel ions by *Pseudomonas fluorescens* (natural isolate). Experiments were also designed to study the effect of dead cells and sugars on the biosorption of nickel ions.

## 2. Materials and Methods

### 2.1. Sample Collection

The soil samples were collected from an electroplating industry, near Jaihindpuram, Madurai. Samples were collected in pre - sterilized glass bottles covered with aluminum foil to avoid contamination.

### 2.2. Bacterial Strains

The bacterial strains isolated from the soil by serial dilution method were maintained in agar slants. Among them one strain was selected and tentatively identified according to morphological and biochemical criteria such as the gram reaction, Indole, Methyl Red, Voges Proskauer, Citrate utilization and Catalase tests as *Pseudomonas fluorescens*.

### 2.3. Estimation of Metal Tolerance

The tolerance of nickel by *P. fluorescens* was determined by inoculation of the selected bacterial strain onto the nutrient agar medium containing wide range of nickel concentrations (50, 100, 500, 1000, 2000, 3000 and 4000ppm). The plates were incubated at 37 °C and observed for growth after 24 hours. Based on the growth, 250, 500, 750 and 1000ppm of nickel concentrations were selected.

### 2.4. Bioremoval of Nickel

From the overnight culture maintained in nutrient broth the organism was inoculated (0.1ml) into minimal broth containing the selected concentrations of nickel (250, 500, 750 and 1000ppm). The flasks were incubated at room temperature on a shaker for intermittent mixing and the samples were then subjected to the estimation of residual metal concentration after every two days up to eight days.

### 2.5. Estimation of Optical Density

Two ml of the sample from the culture flask was taken and with the help of colorimeter optical density values were taken at 450nm. It was performed from two to eight days of treatment.

## 2.6. Determination of pH

The pH of the medium after treatment was determined using pH meter and pH 7 was observed throughout the treatment period.

## 2.7. Biomass Estimation

Pellet from the above step was collected and poured in a Petri dish. Then the Petri dish containing pellet was dried in a hot air oven at 80 °C for three hours. The final dried biomass was weighed and the dry biomass was determined.

## 2.8. Preparation of Dead Cells

For obtaining dead cells, the bacterial culture (24 hours) in nutrient broth was autoclaved at 121 °C for thirty minutes and used for the study. For testing the biosorption of dead cells, 100ml of minimal broth containing 250, 500, 750 and 1000ppm of nickel in 250ml Erlenmeyer flasks was prepared. To such flasks dead cells were inoculated individually and samples were taken after five minutes up to eighty minutes.

## 2.9. Atomic Absorption Spectrophotometry

Ten ml of the sample from the 250, 500, 750 and 1000ppm concentration of nickel were centrifuged at 2500rpm for fifteen minutes, after five minutes up to eighty minutes. The clear supernatant was used for AAS analysis. The values so obtained by AAS analysis represent the residual concentration of nickel in the solutions.

## 2.10. Supplementation of Sugars

The efficiency of the bacterium for the sorption of nickel was tested by supplementing different carbon sources like dextrose, fructose, glucose, lactose and sucrose at 10% concentration in minimal broth containing 500ppm concentration of nickel and the inoculum ( $10^9$  cells). The flasks were incubated at 37 °C on a shaker and the optical density and biomass were estimated after two days by performing centrifugation at 2500rpm for fifteen minutes, followed by drying in a hot air oven at 80 °C for three hours.

## 2.11. Statistical Analysis

Two way analysis of variance (ANOVA) was performed for the factors, percent removal of nickel and biomass of *P. fluorescens* during nickel treatment for the two variables namely nickel concentration and treatment period. It was also performed for the factor, percent removal of nickel for dead cell preparations with two variables namely treatment period and nickel concentration, using Microsoft MS- Excel Package.

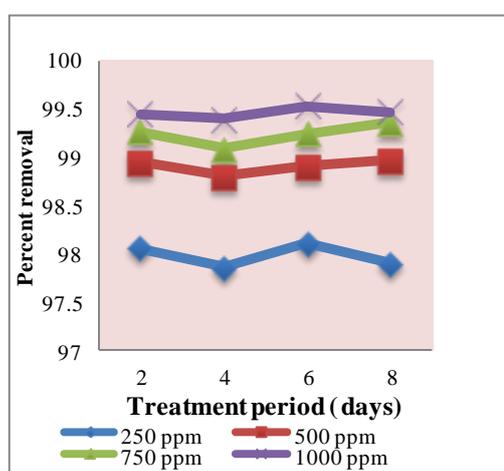
## 3. Results

The bacterial strain *Pseudomonas fluorescens* was tested for metal tolerance with wide range of nickel concentrations (50, 100, 500, 1000, 2000, 3000 and 4000 ppm). The results indicated that after 24 hours incubation, the strain grew well up to 1000ppm concentration of nickel. Based on the metal tolerance level the strain was subjected to different concentrations of nickel (250, 500, 750, 1000ppm) for sorption up to eight days.

When different concentrations of nickel were plated with nutrient agar, *P. fluorescens* were able to resist up to 1000ppm of nickel. The biochemical tests are shown in Table 1. Figure 1 illustrates the percent removal of nickel after treatment with *Pseudomonas fluorescens*. It indicates that among all treatments, highest percent removal was for 1000ppm concentration of nickel after six days of treatment. The optical density values obtained during the treatment of *P. fluorescens* are shown in Figure 2. Increase in the optical density values during the treatment period was observed. This shows the growth of bacterium in the minimal broth. Highest optical density was observed after six days for 1000ppm of nickel concentration.

**Table 1. Morphological, cultural and biochemical characteristics of *Pseudomonas fluorescens***

| Characteristics             | Tests             | Results       |
|-----------------------------|-------------------|---------------|
| Colony and Cell Morphology  | Colony size       | large         |
|                             | Surface           | Irregular     |
|                             | Opacity           | Opaque        |
|                             | Color             | Yellow green  |
|                             | Motility          | Motile        |
|                             | Cell shape        | Rod           |
|                             | Cell size         | Small         |
| Biochemical characteristics | Gram's staining   | Gram negative |
|                             | Citrate           | Positive      |
|                             | Indole            | Negative      |
|                             | MR                | Negative      |
|                             | VP                | Negative      |
|                             | Oxidase           | Positive      |
|                             | Catalase          | Positive      |
|                             | TSI               | Positive      |
|                             | Nitrate Reduction | Positive      |
| Gelatin liquefaction        | Positive          |               |



**Figure 1.** Percent removal of nickel after treatment with *Pseudomonas fluorescens*

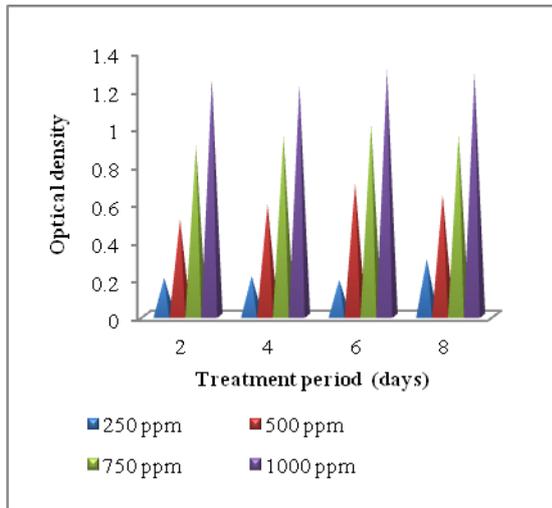


Figure 2. Optical density values obtained during treatment with *Pseudomonas fluorescens*

Figure 3 illustrates the biomass of *P. fluorescens* during nickel treatment. For eight days of treatment highest biomass was observed with 1000ppm concentration of nickel. It indicates that nickel concentration and biomass are directly proportional to each other. Highest biomass was obtained for all the concentrations at the sixth day with respect to treatment period. Figure 4 illustrates the percent removal of nickel after treatment with dead cells of *P. fluorescens*. It indicates highest percent removal for 1000ppm concentration of nickel for twenty minutes.

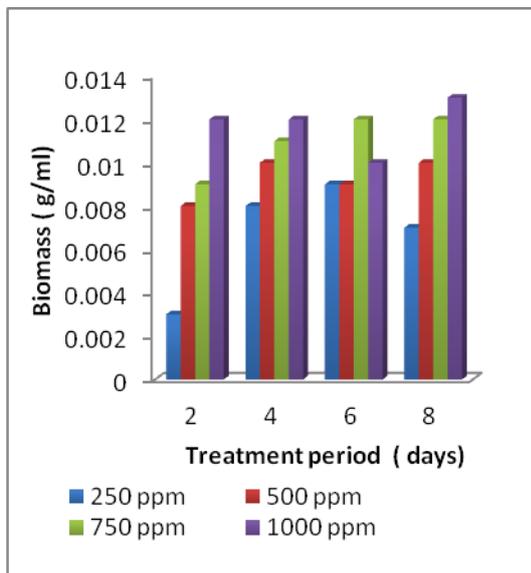


Figure 3. Biomass (g/ml) of *Pseudomonas fluorescens* during nickel treatment

Influence of sugars at 10% concentration on the biomass of *P. fluorescens* during (500ppm) nickel treatment is exhibited in Figure 5. It indicates that the biomass being highest in the case of lactose followed by sucrose, dextrose, glucose and fructose respectively. The trend indicates that the increase in the biomass was due to the influence of disaccharides (lactose and sucrose) while with monosaccharide (dextrose, glucose and fructose) the biomass decreased. Figure 6 shows the optical density values obtained during treatment with *P. fluorescens* after two days of nickel treatment. Highest value was obtained

for lactose followed by sucrose, dextrose, fructose and glucose respectively.

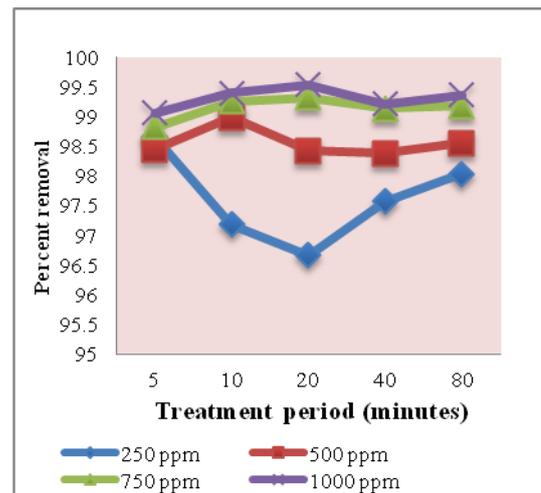


Figure 4. Percent removal of nickel after treatment with dead cells of *Pseudomonas fluorescens*

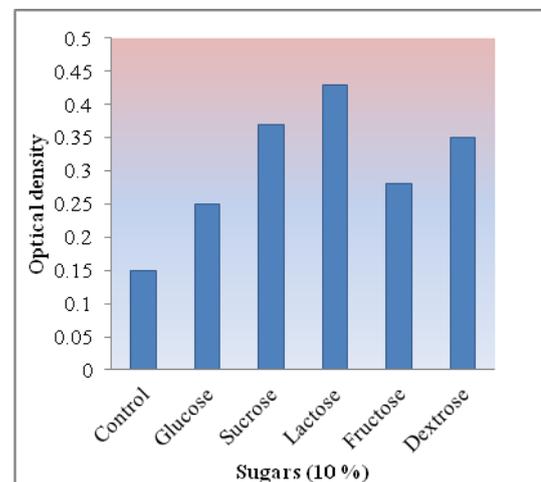


Figure 5. Influence of sugars on the optical density values obtained during nickel treatment (500ppm) after two days with *Pseudomonas fluorescens*

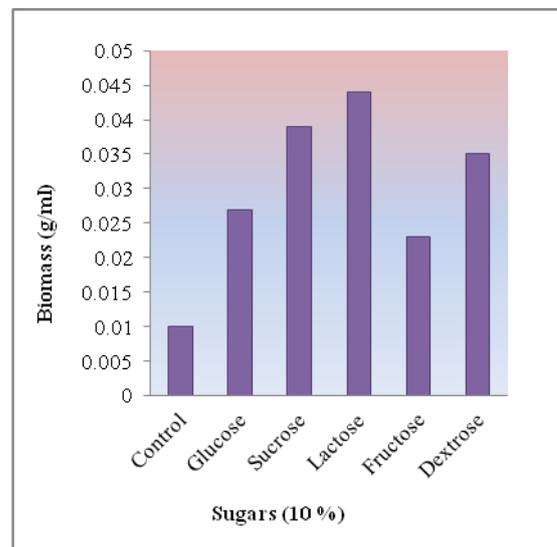


Figure 6. Influence of sugars on the biomass (g/ml) of *Pseudomonas fluorescens* during nickel treatment (500ppm) after two days

Table 2 represents the two way analysis of variance for the factors with the variables, treatment period and nickel concentration for *P. fluorescens*. The variations in the percent removal of nickel due to treatment period and nickel concentration were statistically significant. The variations in the biomass due to treatment period were

statistically not significant but statistically significant for nickel concentration. The variations in the percent removal for dead cells due to treatment period were statistically not significant, while they were statistically significant for nickel concentration.

**Table 2. Two way analysis of variance for the factors with the variables, treatment period and nickel concentration for *P. fluorescens***

| Factor                               | Source of variation  | df | MS         | Calculated F value | Table F value | Level of Significance |
|--------------------------------------|----------------------|----|------------|--------------------|---------------|-----------------------|
| Percent removal of nickel            | Treatment period     | 3  | 0.021156   | 5.187298           | 3.862548      | Significant           |
|                                      | Nickel concentration | 3  | 1.70219    | 417.3596           | 3.862548      | Significant           |
| Biomass                              | Treatment period     | 3  | 5.23       | 2.535354           | 3.862548      | Not significant       |
|                                      | Nickel concentration | 3  | 0.00001972 | 9.565657           | 3.862548      | Significant           |
| Dead cells Percent removal of nickel | Treatment period     | 3  | 0.06125    | 0.279094           | 3.259167      | Not significant       |
|                                      | Nickel concentration | 3  | 2.922618   | 13.31732           | 3.49025       | Significant           |

## 4. Discussion

The high incidence of heavy metal contamination in aquatic environments is known to cause severe damage to aquatic life. In addition, these metals kill microorganisms during biological treatment of wastewater with a consequent delay of the process of water purification [5]. Most of the heavy metal salts are soluble in water and form aqueous solutions and consequently cannot be separated by ordinary physico-chemical means of separation. Biological methods such as biosorption/bioaccumulation for the removal of heavy metal ions may provide an attractive alternative to physico-chemical methods [6].

Bacteria express a wide range of complex molecules on their cell wall, which confer anionic net charge to the cell surface at acidic pH values [7]. In Gram negative bacteria, the lipopolysaccharide, a highly anionic structure, has been identified as the main binding site for metals. When the cell wall is in direct contact with the environment, negatively charged groups are able to attract and bind metallic cations based on electrostatic forces, without cellular energy consumption, an effect that is favored by the high surface-volume ratio in bacteria [8]. Bacteria have been proved to act as a very good metal sequester like the bacterial strains; *Pseudomonas ambigua*, *Desulfovibrio vulgaris*, *Enterobacter cloacae* Ho-1 *Alcaligenes eutrophus*, and *Dinococcus radiodurans* R1 [9].

Nickel is an important environmental inorganic pollutant, with allowed levels under  $0.04\text{mg L}^{-1}$  in drinking water. Higher concentrations affect normal flora in ecosystems and are toxic to human beings. When different concentrations of nickel were plated with nutrient agar, *Pseudomonas fluorescens* was able to resist up to 1000ppm of nickel.

On exposure of *P. fluorescens* to nickel (250, 500, 750 and 1000ppm) with a treatment period of two to eight days, the maximum percent removal of nickel was observed at 1000ppm and minimum at 250ppm. It can be indicated that at higher concentration of nickel, the uptake by *P. fluorescens* was efficient. The important role of

negatively charged carboxyl groups in heavy metal biosorption by acetone washed biomass of *Saccharomyces uvarum* was demonstrated [10].

The optical density value seems to be increasing during the days of treatment for the strain in the minimal broth. This indicates the growth of bacteria in the medium which can be compared to biomass during treatment.

When different concentrations of nickel were used, the biomass of *P. fluorescens* was highest for 1000ppm. The biomass is directly proportional to increase in nickel concentration. When dead cells of *P. fluorescens* were exposed to 250, 500, 750 and 1000ppm of nickel, highest percent removal was at twenty minutes for 1000ppm concentration. Two immobilised marine algae *Ascophyllum nodosum* and *Sargassum fluitans* were used for the removal of Ni, Pb, Cu, Cd and Zn and highest percent uptake was shown by both algae for lead and nickel [11].

In the addition of 10% concentration of different carbon sources (Lactose, Sucrose, Dextrose, Fructose and Glucose) for 500ppm of nickel, disaccharides enhanced the biomass of *P. fluorescens*. The biomass obtained was more in the case of lactose and less than that of fructose. The optical density values increased with the same type of sugar supplements for *P. fluorescens*. When compared with live cells and dead cells of *P. fluorescens*, the dead cells showed higher percent removal. Biomass is also observed to be influenced by increasing nickel concentration.

## 5. Conclusion

Bioremediation is one of the most promising new technologies for treating industrial wastes, municipal/urban wastes, and mining wastes which include effluents containing heavy metals, chemical spills and hazardous wastes. However the process of biosorption is enhanced by various other factors. By this study it can be inferred that bacterial biosorbents can be used for heavy metal removal effectively. One can protect and maintain the environment, free of pollution and contamination by which future generations can be free from toxicity of heavy metals.

## Acknowledgments

The authors thank the University Grants Commission (UGC Major Research Project F. No.40-368/2011(SR), New Delhi for financial assistance and the authorities of the American College for facilities and encouragement.

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