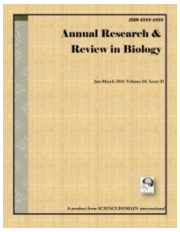

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Bioremoval of Nickel Using *Pseudomonas aeruginosa*

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Authors' contributions

This work was carried out in collaboration between all authors. Author AJT designed the study and wrote the protocol. Author MVD managed the analyses and performed statistical analyses. Author DR made literature survey and prepared the first draft. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Soil samples were collected from an electroplating industry located at Tirunelveli in Tamil Nadu and a nickel resistant bacterial strain was isolated and identified as *Pseudomonas aeruginosa* which can remove nickel effectively from aqueous solutions. When 200, 400, 600 and 800 ppm of nickel concentrations were tested for ten days, more than 90% removal was observed in 1000 ppm nickel concentration. Among the different cell preparations tested, immobilized cells exhibited highest removal followed by free cells and autoclaved cells after fifty minutes.

Keywords: Nickel; Pseudomonas aeruginosa; biosorption; free cells; immobilized cells and autoclaved cells.

1. INTRODUCTION

Heavy metals are discharged into environment from various industries, such as textile, pigments, plastics, mining, electroplating and metallurgical processes [1,2]. They are considered as persistent environmental contaminants [3]. Due to their toxic effects and accumulation tendency throughout the food chain, they represent an important problem with

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serious ecological and human health consequences. Hence, it is desirable to remove the heavy metals from industrial wastewaters. Nickel is an important environmental inorganic pollutant, with allowed levels under 0.04 mg/L in drinking water. Higher concentrations affect normal flora in ecosystems and even human beings [4].

One of the ideal solutions for pollution abatement is bioremediation and it is the most effective innovative technology which uses biological systems in a cost effective manner for the treatment of contaminants [5]. Microorganisms and biological materials have the ability to remove heavy metals from the polluted sites. Microorganisms remove metals through biosorption and bioaccumulation [6]. Toxic metals are classified as environmental pollutants but their oxidation state can be changed to another less toxic state by microorganisms. Thus, bioremediation of heavy metals aims at sequestering the metals to make them unavailable in the ecosystem or mobilizing them for reuse or safe disposal [7]. Bioremediation using natural biomaterials is a promising alternative to conventional methods [8]. Hamza [9] reported natural process employing microorganisms as an effective and eco-friendly method of decontamination. Heavy metals in effluents must be reduced to acceptable limits before discharging into environment to avoid threats to living organisms [10,11]. Use of microbial resources is one of the most promising and economical strategies for removing environmental pollutants [12,13]. Heavy industrialization and disposal of toxic substances have made the natural attenuation process inadequate to reduce the quantity of toxic substances released into the environment. The problem has to be solved by the application of alternate bioremediation methods [14]. Hence in the present work an attempt has been made to study the removal of nickel ions by *Pseudomonas aeruginosa*. Experiments also have been designed to study the efficiency of immobilized, live and dead cells on the biosorption of nickel ions.

2. METHODOLOGY

2.1 Collection of Sample

Soil samples were collected from a site where wastes were disposed from a nickel plating company at Tirunelveli, in sterile containers and immediately brought to the laboratory for analysis.

2.2 Isolation of Bacteria

The collected soil samples were serially diluted up to 10^{-6} dilution and 0.1 ml was taken from 10^{-6} dilution and plated onto nutrient agar plates using spread plate technique. The plates were then incubated at 37ºC for 24 hours.

2.3 Isolation of Nickel Resistant Bacteria

The grown bacterial colonies were selected and tested with different concentrations (100, 200, 400, 600, 800, 1000 and 2000 ppm) of nickel prepared from nickel sulphate for their resistance. The plates were then incubated at 37ºC for 24hours and from the nickel resistant colonies, one was chosen for further experiments.

2.4 Identification of Bacteria

The resistant bacterial strain selected was identified as *Pseudomonas aeruginosa* by adopting Bergey's Manual [15].

2.5 Estimation of Metal Tolerance

Based on the growth of *P. aeruginosa,* concentrations like 200, 400, 600, 800 and 1000 ppm of nickel were selected for further experiments.

2.6 Bioremoval of Heavy Metals

From the overnight culture maintained in nutrient broth, the organism was inoculated (0.1 ml) into 100ml minimal broth in 250 ml Erlenmeyer flasks containing the selected concentrations, 200, 400, 600, 800 and 1000 ppm of nickel. The flasks were incubated at room temperature on a shaker for intermittent mixing and five ml samples were centrifuged and the supernatants were subjected to the estimation of residual nickel concentration after every two days up to ten days.

2.7 Biomass Estimation

The pellet from the above step was collected from each concentration and suspended into a Petri dish. The Petri dish containing pellet was dried in a hot air oven at 80ºC for three hours and the final dried biomass was weighed, to get the biomass in g/l.

2.8 Preparation of different Cell Types

In order to obtain immobilized cells, the seed culture of the bacterium was grown in nutrient broth and the cells were prepared in beads [16]. Such beads were washed with sterile distilled water and used for biosorption study. In order to obtain dead cells, the bacterial culture in nutrient broth was autoclaved at 121ºC for 30 minutes. For the preparation of live cells, overnight culture of *Pseudomonas aeruginosa* in nutrient broth was taken and subjected to biosorption, experiments.

2.9 Atomic Absorption Spectrophotometer Analysis

The samples from the culture flasks were centrifuged at 2500g for fifteen minutes. Samples were taken after every thirty minutes up to 150 minutes. The clean supernatant was used for Atomic Absorption Spectrophotometeric (AAS) analysis using Varian Spectra AA220 (lamp current 12 mA; wavelength 232 nm and slit width 0.2 nm).

2.10 Statistical Analysis

Two way analysis of variance (ANOVA) was performed with reference to live cells for the factors percent removal of nickel and biomass of *P. aeruginosa* during nickel treatment with the two variables, nickel concentration and treatment period. It was also performed on the percent removal of nickel by different cell preparations with the two variables, treatment period and cell types using Microsoft MS-Excel package.

3. RESULTS

The nickel resistant bacterium isolated in the present study was identified as *P. aeruginosa* based on its characteristic appearance on nutrient agar medium and the biochemical tests (Table 1). The organism was positive to Citrate, Catalase and Gelatin liquefaction and negative for Indole, Methyl red, Voges Proskauer, and Lactose tests.

Fig.1 illustrates the percentage removal of nickel after treatment with *P. aeruginosa.* It indicated a gradual increase in the metal uptake from the second day till tenth day in all the concentrations. Highest removal was obtained for all the concentrations on sixth day with respect to treatment period. Fig. 2 indicates the biomass of *P. aeruginosa* during nickel treatment. Nickel concentration and biomass were directly proportional to each other. Fig. 3 divulges the percentage removal of nickel after treatment with *P. aeruginosa* of different cell preparations. Live cells exhibited efficiency in removing nickel ions while the dead cells showed the least removal. But immobilized cells showed a gradual increase in the sorption of nickel and it was maximum after forty and fifty minutes.

The variations due to treatment period and nickel concentration were statistically significant at 5% level for the percent removal of nickel with live cells. For the biomass of live cells variations due to nickel were statistically significant but they were not significant due to treatment period. Variations due to cell types were statistically significant while they were not significant due to nickel concentration for the percent removal of nickel with different cell types of *P. aeruginosa* (Table 2).

Table 1. Biochemical tests used for the identification of the isolated organism

S.No	Biochemical Tests	*Pseudomonas aeruginosa*
1.	Colony character	Smooth Wrinkled
2.	Colony size	Medium
3.	Cell type	Rod
4.	Gram reaction	-
5.	MR test	-
6.	VP test	-
7.	Indole test	-
8.	Catalase test	+
9.	Citrate test	+
10.	Gelatin Liquefaction	+
11.	Cellobiose	-
12.	Lactose	-
13.	Maltose	-
14.	Sucrose	-
15.	D-xylose	-
16.	Trehalose	-
17.	Sorbitol	-
18.	Malonate	+
19.	D-Arabinose	-
20.	Glycerol	+

Note: + Positive,
- Negative

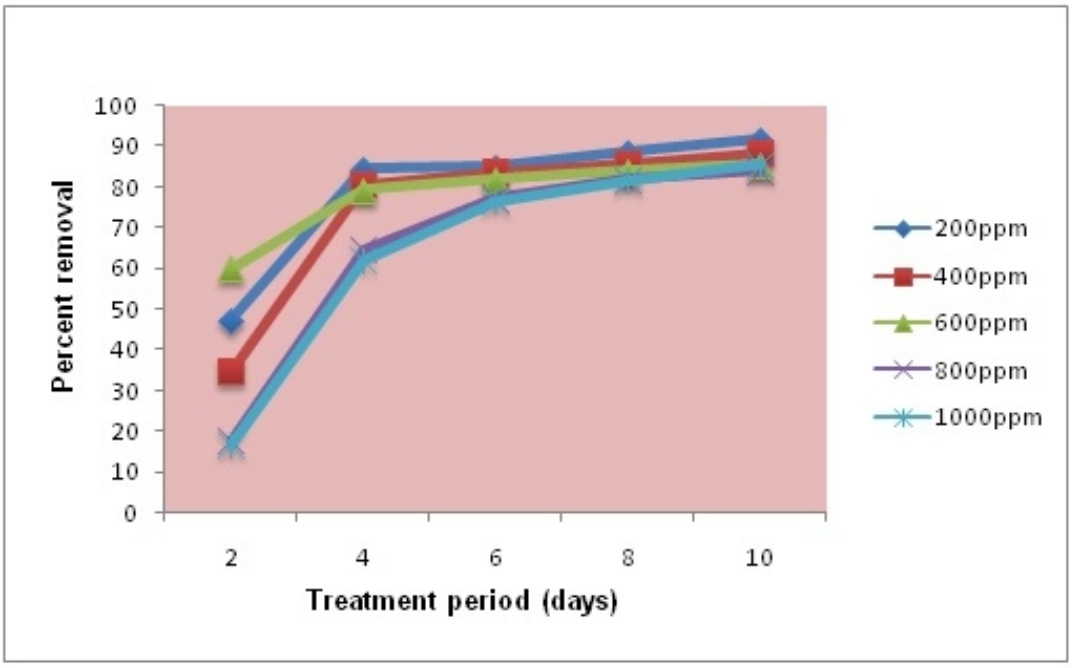

Fig. 1. Percent removal of nickel after treatment with live cells of *Pseudomonas aeruginosa*

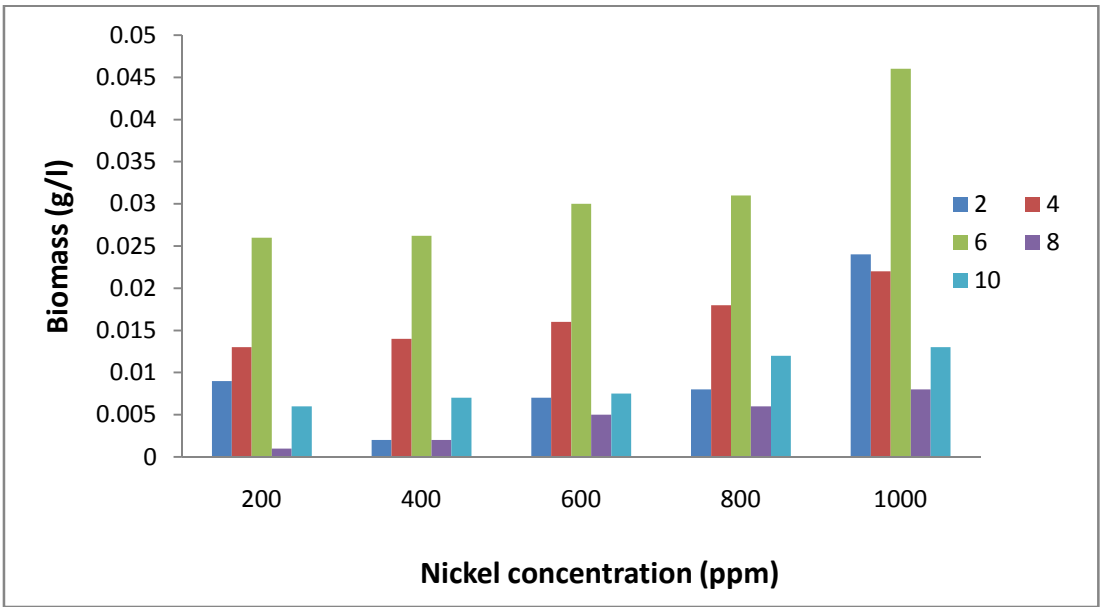

Fig . 2. Biomass of live cells of *Pseudomonas aeruginosa* during nickel treatment

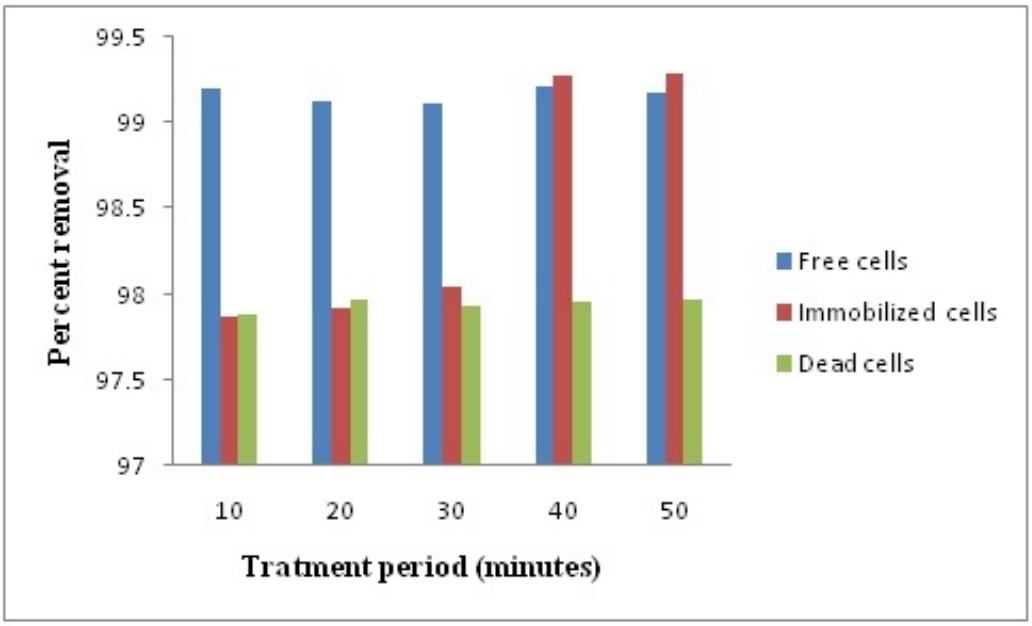

Fig. 3. Percent removal of nickel from 1000 ppm of nickel concentration after treatment with *Pseudomonas aeruginosa* of different cell preparations

Table 2. Two way analysis of variance for the factors with the variables, treatment period and nickel concentration and cell types

Factor	Source of variation	df	MS	Calculated F value	Table F value	Level of Significance at 5% level
Percent removal of nickel with live cells	Treatment period	4	2256.249	38.504	3.0069	Significant
	Nickel concentration	4	250.451	4.27	3.0069	Significant
Biomass of live cells	Treatment period	4	0.0001	2.834	3.0069	Not Significant
	Nickel concentration	4	0.0004	7.811	3.0069	Significant
Percent removal of nickel with different cell types	Treatment period	2	1.857	10.907	4.4589	Significant
	Nickel concentration	4	0.203	1.192	3.8378	Not Significant

4. DISCUSSION

Industries using metals such as metal plating and tanneries have generated large amount of aqueous effluents that contain high levels of heavy metals. Nickel is frequently encountered in raw waste water streams from industries such as non-ferrous metal, mineral processing, paint formulation, electroplating and steam electric power plants.

The metal removing ability of microorganisms has been studied extensively [16]. Biosorption of heavy metals by microbial cells has been recognized as a potential alternative to existing technologies for recovery of heavy metals from industrial waste streams. Biosorption of metals has involved the use of either laboratory grown microorganisms or biomass generated by pharmaceutical and food processing industries [17].

Biosorption of nickel by *Pseudomonas* sp is effective as most of the metal ions are sequestered very fast from solution within first ten minutes and almost no increase in the level of bound metals have occurred after this time interval [18].

In the present study, the ability of the isolated bacterium to accumulate nickel was tested by allowing the bacterium to grow under different concentrations of nickel and the organism was able to resist even 1000ppm concentration of nickel. 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. When the cell wall is in direct contact with environment, negatively charged groups are able to attract and bind to metallic cations based on electrostatic forces, without cellular energy consumption which is favored by high surface volume ratio in bacteria [4].

It has been stated that nickel can bind to *Pseudomonas* sp as much as 556mg/g [18]. Nickel adsorption by dried cells of *Enterobacter agglomerans* SM38 was found at optimum pH and the removal reached 25.2% while for *Bacillus subtilis* WD90, nickel removal was 27% [19]. The complex structure of microorganisms implies that there are many ways for metals to be taken up by microbial cell. The biosorption mechanisms are various and are not fully understood [17].

The biomass of *P. aeruginosa* observed was more in 1000ppm nickel concentration. Optimum pH values for bacterial biosorption are acidic since cell wall keeps negatively charged. The pH values above five are known to result in nickel precipitation. At low pH values functional anionic groups could be bound to hydronium ions($H_3O^{+)}$ leading to restriction of cation uptake as a result of charge –repulsion forces, which become strong as pH decreases [20,21].

Different cell preparations of *P. aeruginosa* namely live cells, dead cells, and immobilized cells have effectively removed nickel within the first ten minutes. When the live cells were exposed to 1000ppm concentration of nickel, they showed the highest percent removal followed by immobilized cells. Dead cells exhibited the least among the different cell preparations.

The bacterium isolated from the contaminated soil was confirmed as *P. aeruginosa*. Pyocyanin is a bluish green phenazine pigment soluble in water and chloroform and it is only produced by *P. aeruginosa.* From the results it has been inferred that nickel ions can be effectively taken up by *P aeruginosa.* Atomic absorption spectrophotometeric analysis of nickel indicated that the absorption of nickel by *P. aeruginosa* was high during initial treatment thereby indicating the effective uptake of nickel by the species.

Previous studies reveal that an increase in the biomass resulted in an increase in biosorption due to the increase in the surface area of the biosorbent, which in turn increases the number of binding sites [22].The results of the present study show the highest removal obtained for all the concentrations after six days of treatment in which biomass was maximum.

The biosorption is basically at laboratory scale inspite of its development for decades [23]. In the present study, the dead cells of *P aeruginosa* were used as the biosorbent for the adsorption of nickel. *Pseudomonas* aeruginosa can be considered to be the most effective biosorbent because of its high adsorption capacity. Previous studies reported that the maximum adsorption of heavy metals reached up to 88% with *Pseudomonas* sp. [24].

In this study immobilized cells were highly efficient in removing nickel, while the activity of dead cells were found to be lesser. It was due to the dead bacterial cells having small particles with low density, poor mechanical strength and little rigidity [25]. The immobilized bacterial cells showed greater biosorption than the dead bacterial cells. Hence the biomass can be immobilized before being subjected to biosorption. The immobilized biomass offers many advantages including better reusability, high biomass loading and minimal clogging in continuous flow systems [26].

5. CONCLUSION

Pseudomonas aeruginosa can be used for nickel removal effectively. Live cells of *P. aeruginosa* can take up nickel effectively above 80% within four days. Generally increase in biomass was noticed upto six days of treatment. In bioremediation programmes, immobilized cells of *P. aeruginosa* can be used more than that of dead cells.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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