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Bioremoval of Nickel Using the Bacterial Strain, *Pseudomonas Oleovorans* MTCC617

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Abstract: *The rapid developments of industries areposing a threat to our surroundings resulting in polluted environment. The effluent from refineries, waste treatment plants, chemical industries dischargea variety of heavy metals inlotic water systems causing hazards to human and animals. Nickel is a ubiquitous element and 24th most abundant element in our earth's crust. Microbes play a considerable role in the process of bioremediation of heavy metals like nickel, zinc, cadmium and chromium in contaminated waste water and soil. The passive uptake of metals by biomaterials can be defined as Biosorption. This method is cost effective, eco-friendly and highly efficient in treating the contaminated substances. Hence in the present work an attempt has been made to study the biosorption of nickel using Pseudomonas oleovorans MTCC 617.The bioremoval of nickel ions by P.oleovorans was studied for a period of eight days by exposing to selected concentrations of nickel (250, 500, 750 and 1000ppm). Atomic absorption spectrophotometric (AAS) analysis was carried out for the samples at an interval of two days to find out the amount of nickel removed.Maximum removal of nickel reached after six and eight days of treatment at 1000 ppm concentration. To study the effect of dead cells and sugar supplements on biosorption of nickel, experiments were designed. Among the tested sugars, glucose exhibited the highest biomass.The results of this study indicate the applicability of P.oleovorans in the removal of nickel contaminationin the environment caused by industrial pollutants.*

Keywords: *Bioremediation, Biosorption, Heavy metals, Nickel, Pseudomonas oleovorans.*

INTRODUCTION

Heavy metals are among the environmental pollutants that are not subjected to degradation process by bacterial attack and resulting in additions to our environment. When they accumulate in the food chains in the environment, they can profoundly disrupt biological processes (Ahmad et al., 2006). The toxic metal ions released in the environment often constitute serious health hazards due to their accumulation and non-degradability. Metals are directly or indirectly implicated in all aspects of microbial growth, differentiation and metabolism. Metals such as K, Mg, Fe, Ca, Mn, Cu, Ni, Na, Co, Zn and Mo are essential for biological functions. All these elements have the ability to interact with microbial cells and accumulate as a result of physico-chemical mechanisms and transport systems of varying specificity, directly or indirectly depending on their metabolism (Gadd, 1990). Global nickel consumption is about one million tons. The chief producers of nickel are Russia followed by

Australia, Canada, and Indonesia representing over 65 % of total world production. It finds its usage in various industries such as electrical, engineering, electronics, infrastructure, automobile components and batteries. Nickel toxicity is comparable to cobalt but its toxic effect on humans is better documented and up to 20 % of the populations in industrially developed countries are positive in epidermal testing (Das, 2009).

Biological methods such as Bioaccumulation and Biosorption used in the removal of heavy metal ions are providing alternative to physico-chemical methods (Kapoor et al., 1995). Nickel and chromium removing efficiency of bacteria such as, *Bacillus sp.*, *Pseudomonas fluorescens* and *Azotobacter chroococcum*from the sewage waste water was reported and it was found that percentage of Ni and Cr (VI) removal decreased with increasing metal concentration. Bioaccumulation of heavy metals was observed to be in the order of *Pseudomonas fluorescens>Bacillus sp.>Azotobacter chroococcum* (Parameswari et al., 2009). Nickel is classified as an important inorganic pollutant, with permissible levels under 0.04 mg L^{-1} in human drinking water. Higher concentrations of nickel tend to affect the normal flora present in ecosystems and also human beings (Malkoc et al., 2010). Biosorption is a passive non-metabolically-mediated procedure of metal binding by biosorbent. Algae, yeasts, bacteria and fungi have been extensively used as biosorbents in the removal of heavy metals from contaminated environment (Gupta, 2003).

Organisms such as algae, bacteria, fungi and yeast are coming from the natural habitats and are excellent sources of biomass. Fast growing organisms that are specifically cultivated for biosorption purposes includecrab shells, yeasts, seaweeds, molds and bacteria which have been tested for the ability of metal biosorption with encouraging results. Water bodies are being overwhelmed with bacteria and waste matter. Among toxic substances in such systems heavy metals are reaching hazardous levels (Zouboulis et al., 2004). Gram positive *Micrococcus* sp. and Gram negative bacteria such as *Pseudomonas* sp. were isolated from activated sludge and tested for biosorption ability against nickel, copper, lead, zinc and chromium by immobilisation methods and the results showed more copper uptake by 61 % (Rani et al., 2010).Heavy metal adsorptions of four *Streptomycetes* bacterial strains were compared with each other. Among those tested strains, *Streptomyces viridochromogenes* showed the most efficient metal binding activity (Manohar et al., 2002). *Pseudomonas fluorescens*isolated from the soil samples of the electroplating industry has shown higher percent removalof nickel in various concentrations (Husain et al., 2013).

Physiological and genetic features of *Pseudomonas* bacteria make them as a potential tool for utilization in the fields like agriculture, biotechnology and also in environmental bioremediation processes. Quite a numberof strains of *P. fluorescens* were shown to play a significant role in the bioremediation of pesticides and heavy metals. The ability of *Pseudomonas sp.* isolated from an industrial area to remove hexavalent chromium has been proved efficient (Devi et al., 2012). The indiscriminate release of heavy metals into the water streams and soils is a major global health concern, as they cannot be broken down to non-toxic forms and have long-lasting effects on the ecosystem. Most of them are toxic even at low concentrations; arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, zinc are not only cytotoxic but also mutagenic and carcinogenic in nature (Dixit et al., 2015).

A study involving the nonliving biomass of Pseudomonas oleovorans was used to remove Cu and Ni from their aqueous solution and waste water samples at normal optimum conditions which showed consistence results (Singh, 2012). Biosorption of heavy metals using individual and mixed cultures of bacteria such as *Bacillus subtilis* and *P.aeruginosa* showed 90.4% biosorption of mercury, while 78.5% biosorption was observed for individual cultures and 99.3% for mixed cultures (Tarangini, 2009). Hence in the present work an attempt has beenmade to study the biosorption of nickel by the

bacterium, *Pseudomonas oleovorans* (MTCC 617). Experiments have also been designed to study the impact of dead cells and sugars on the biosorption of nickel ions.

Materials and Methods

Bacterial Strain

The bacterial strain used in the present study, ***Pseudomonas oleovorans*** was procured from Microbial Type Culture Collection [MTCC 617], IMTECH, Chandigarh, India. The obtained culture was maintained onto nutrient agar slants and stored at 4°C.

Estimation of Metal Tolerance

The tolerance of nickel by ***P. Oleovorans*** was determined by inoculation of the selected bacterial strain onto the nutrient agar medium containing wide choice 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 for further experiments.

Bioremoval of Nickel

From the overnight culture maintained in nutrient broth the organism was inoculated (0.1ml) into nutrient broth (100ml) containing the selected concentrations of nickel (250, 500, 750 and 1000ppm) in 250ml Erlenmeyer flasks. The flasks were incubated at 37°C on a shaker for intermittent mixing and the samples were then subjected to the estimation of residual nickel concentration after two, four, six and eight days of treatment period.

Estimation of Optical Density

Two ml of the sample from the culture flask was taken in sterile tubes after centrifugation and with the help of colorimeter optical density values were recorded at 600nm. This procedure was performed after two to eight days of treatment period.

Determination of pH

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

Biomass Estimation

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

Preparation of Dead Cells

For obtaining the dead cells, bacterial culture (24 hours) in nutrient broth was autoclaved at121°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 (10^9) were inoculated separately and samples were taken after five minutes up to eighty minutes for the analysis of nickel concentration.

Nickel Removal with Live Cells

Ten ml of the sample from 250, 500, 750 and 1000ppm concentrations of nickel after five minutes upto eighty minutes were centrifuged at 2500rpm for 15 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.

Supplementation of Sugars

The efficiency of the bacterium for the sorption of nickel was tested by supplementing different carbon sources like fructose, dextrose, lactose, sucrose and glucose 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.

Statistical Analysis

Two way analysis of variance (ANOVA) was performed for the factors, percent removal of nickel and biomass of *P. Oleovorans* 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.

Results

The bacterial strain *Pseudomonas oleovorans* was tested for metal tolerance with wide range of nickel concentrations from 50, 100, 500, 1000, 2000, 3000 and 4000 ppm. The results indicated that after 24 hours incubation, the strain grew well up to 1000ppm nickel concentration. 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.

Figure 1 illustrates the percent removal of nickel after treatment with *Pseudomonas oleovorans*. 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.oleovorans* are shown in Fig. 2. Increase in optical density values during the treatment period was observed which shows the growth of bacterium in the culture medium. Highest optical density valuewas noted after six days at1000ppm nickel concentration.

Figure 3 illustrates the biomass of *P.oleovorans* during nickel treatment. For eight days of treatment highest biomass was noticed in1000ppm nickel. Highest biomass was obtained for all the concentrations after six days with respect to treatment period. Figure 4 illustrates the percent removal of nickel after treatment with dead cells of *P.oleovorans.* Highest percent removal was noticed at1000ppm nickelafter twenty minutes of exposure.

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

Table 1 represents the two way analysis of variance for the factors with the variables, treatment period and concentration of nickel for *P. oleovorans.* Variation in the percent removal of nickel due to treatment period was statistically not significant and for nickel concentration it was statistically significant. Variations in the biomass due to treatment period and nickelconcentration were statistically significant. Variations in the percent removal for dead cells due to treatment period were statistically not significant, while they were statistically significant for nickel concentration.

Discussion

Nickel, chromium and cobalt belong to the group of rare metals and every change of the chemical balance in the natural environment causes not only instability in the growth and development of fauna and flora, but also human health. Heavy metals observed in the environment originate from two anthropogenic sources, one connected with the human activity and the other concerned with the natural cycle of the metals throughout nature. Significant part of nickel finds its way into the

environment as a result of burning of diesel containing nickel. In nature, it usually occurs at an oxidation level of +2, but its valence may change from - 1 to +4. They easily formquite stable chelate compounds as well as complex cations and anions (Barałkiewicz et al., 1999).

The use of adsorbents of biological origin has been emerging in the last decade as one of the most promising alternatives to conventional heavy metal management strategies. Biosorption of heavy metals by microbial cells has been documented as a prospective alternative to existing technologies for recovery of the heavy metals from industrial waste water. Majority of the studies on biosorption for metal removal have involved the use of either laboratory-grown microorganism or biomass generated by food processing industries, pharmaceutical and wastewater treatment plants (Hussein et al., 2004). Bacteria have been proved efficient metal sequesters like *Pseudomonas ambigua*, *Enterobacter cloacae* Ho-1, *Desulfovibrio vulgaris, Dinococcus radiodurans*R1 and *Alcaligenes eutrophus* (Igwe et al., 2006). Different species of *Pseudomonas*, *Sporophyticus, Aspergillus, Bacillus* and *Phanerochaete* have been reported as proficient nickel and chromium reducers (Vijayaraghavan et al., 2007).

Several research works have been reporting the mechanism and the efficiency of bacteria to eliminate different metal ions. Polarisable groups observed on the bacterial surfaces are mainly capable of interacting with and accountable for reversible metal binding capacity. Such groups include carboxyl, hydroxyl, amino and phosphate groups. The bacterial cell wall adsorbs metal cations through a range of mechanisms such as Vanderwaal's forces, electrostatic interaction and covalent bonding (Sankarammal et al., 2014).

Microbial biomass offers an inexpensive choice for removing the heavy metals by biosorption methods. The highest metal tolerance capacity exhibited by Gram negative bacteria is due to the precipitation of metals in their peptidoglycan layers. The nature of lipopolysaccharide in the outer membrane is being accountable for their efficient metal binding capacity (Jadhav et al., 2010). When different concentrations of nickel were used, the biomass of *P. oleovorans* was the highest for 1000ppm**.** The biomass was directly proportional to the concentration of nickel. When dead cells of *P. oleovorans* were exposed to nickel, highest percent removal was observed after twenty minutes at 1000ppm nickel. Marine algae such as *Sargassum* and *Ascophyllum* species which were immobilised exhibited efficient removal of Ni, Zn, Cu, Cd and Pb and the highest percent uptake was shown by both the algal species for nickel and lead (Vieira et al., 2010).

During the addition of different carbon sources like lactose, dextrose, sucrose, fructose and glucose) in 500ppm of nickel, disaccharides enhanced the biomass of *P. oleovorans*. The biomass obtained was more in the case of glucose and less in lactose. When compared with live cells and dead cells of *P. oleovorans*, dead cells were equally efficient in removing nickel.

Conclusion

Bioremediation methods are widely employed to treat the municipal wastes, industrial and mining wastes including the effluents of chemical spills and heavy metal contamination in the environmental systems. Biosorption offers various advantages including high efficiency, regeneration of biosorbent with possibility of maximum metal recovery, cost effectiveness and minimization of biological/chemical sludge. From this study it can be inferred that bacterial biosorbents can be used effectively for heavy metal removal from the contaminated environment. *Pseudomonas oleovorans* can serve as an efficient biosorbent in the removal of nickel from industrial effluents. Bioremediation can be used as an economical and eco-friendly technology to keep our environment safe without toxic metal contamination.

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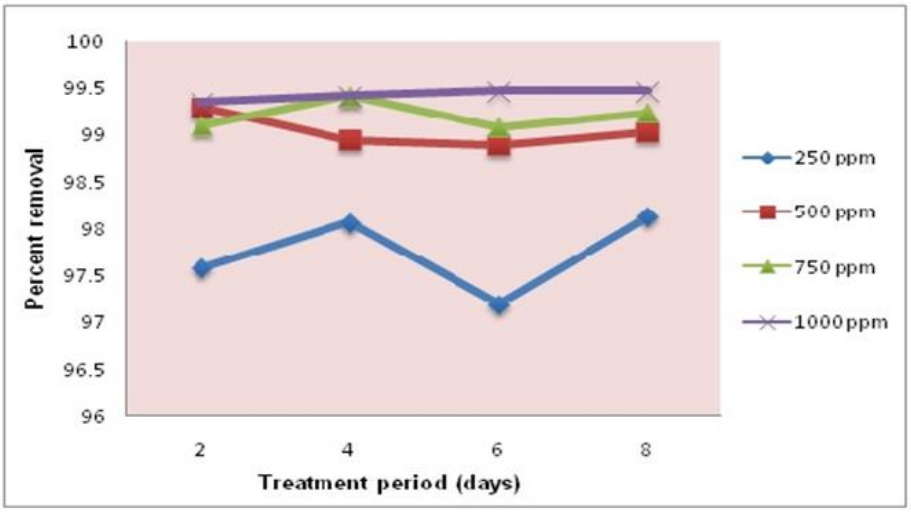

Figure 1. Percent removal of nickel after treatment with ***Pseudomonas oleovorans***

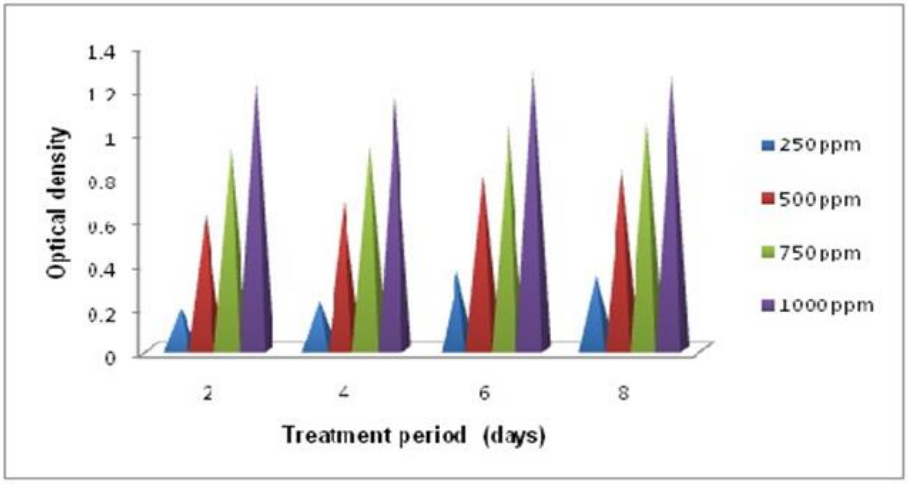

Figure 2. Optical density values obtained during treatment with ***Pseudomonas oleovorans***.

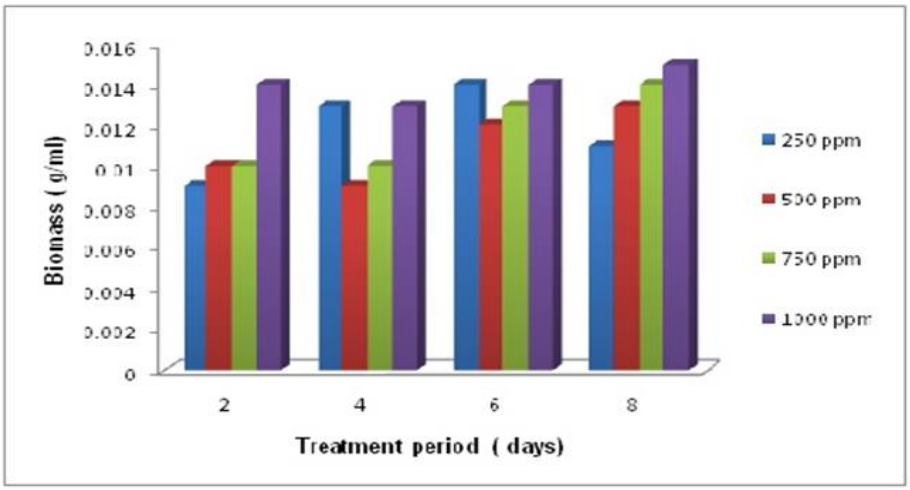

Figure 3. Biomass (g/ml) of ***Pseudomonas oleovorans*** during nickel treatment

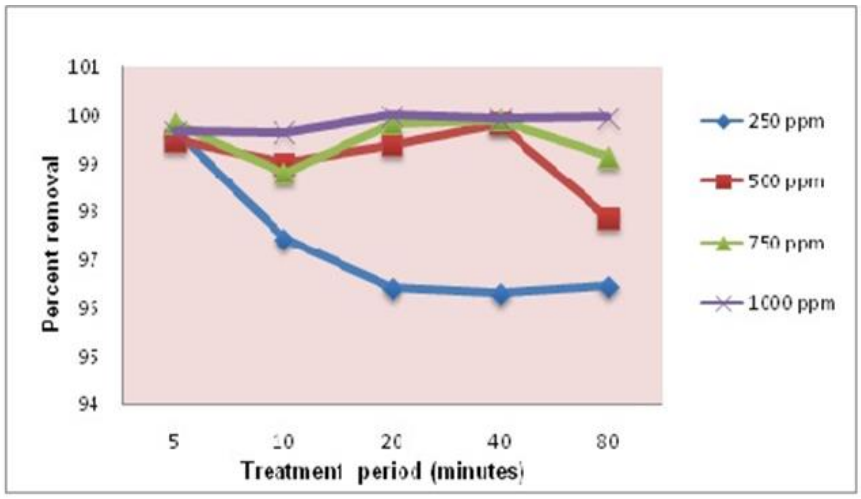

Figure 4. Percent removal of nickel after treatment with dead cells of ***Pseudomonas oleovorans***

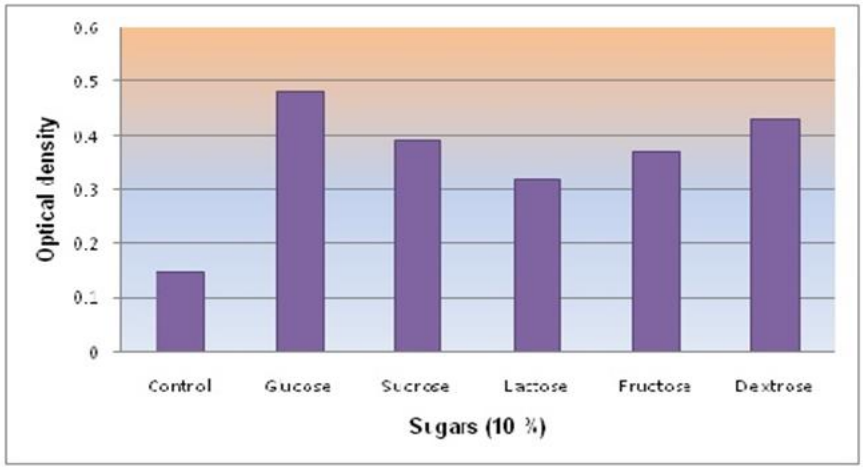

Figure 5. Influence of sugars on the optical density values obtained during nickel treatment (500 ppm) after two days with ***Pseudomonas oleovorans***

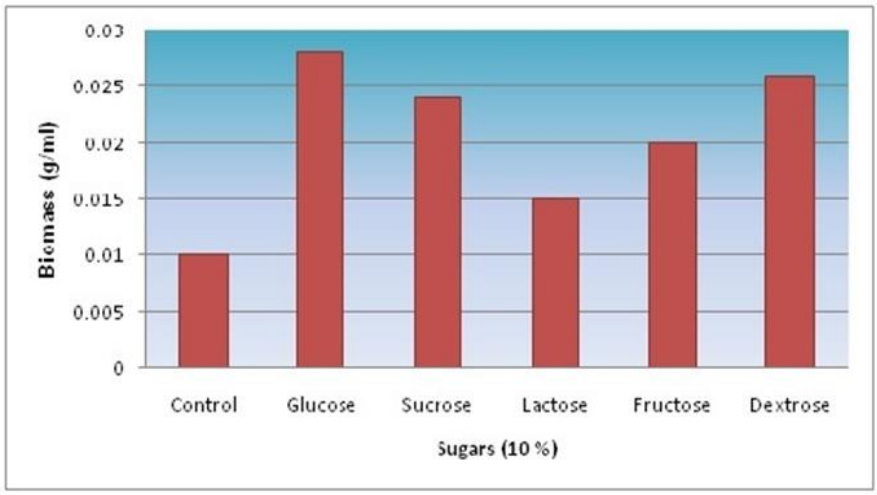

Figure 6. Influence of sugars on the biomass (g/ml) of ***Pseudomonas oleovorans*** during nickel treatment (500 ppm) after two days

Table 1. Two way analysis of variance for the factors with the variables, treatment period and nickel concentration for ***P. oleovorans***

Factor	Source of Variation	*SS*	*df*	*MS*	Calculated F value	F Table value	Level of significance
Percent removal of nickel	Treatment period	0.247569	3	0.082523	1.465608	3.862548	Not Significant
	Nickel concentration	6.898719	3	2.299573	40.84046	3.862548	Significant
Biomass	Treatment period	0.0000281	3	9.39583	4.214953	3.862548	Significant
	Nickel concentration	0.0000231	3	7.729166	3.46729	3.862548	Significant
Dead cells percent removal of nickel	Treatment period	3.64023	3	0.910058	1.395835	3.259167	Not Significant
	Nickel concentration	19.86228	3	6.62076	10.15484	3.490295	Significant