

# Biosorption Studies using *Pseudomonas Aeruginosa* Bacteria for Depolluting Nickel Contaminated Soil

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## Abstract

Nickel is considered as ubiquitous metal in nature and it is distributed uniformly throughout the soil profile. The principal natural form of nickel oxide occurs in admixture with nickel sulfides in varying proportions in weathered ore. However, it typically accumulates on the surface from deposition caused due to industrial and agricultural activities. Nickel may present a major problem in land near towns, in industrial areas, or even in agricultural land receiving concentrated wastes from solid and liquid waste disposal, water and wastewater treatment sludge and accidental spillage. Decrease in soil pH through various natural and anthropogenic activities can increase the rate of mobility of nickel through the soil and leaching to deeper layers of soil eventually increasing the groundwater nickel content. There are various in-situ and ex-situ remediation methods applied to contaminated site. Among the in-situ methods bio remediation is the most reliable and feasible method for soil depollution. Keeping all the above facts in view, the present study is contemplated on carrying out bio sorption studies using *Pseudomonas Bacteria* for depolluting the nickel contaminated soil. The site soil is a well graded clayey soil with high affinity towards nickel. It is evident from the adsorption study that 85-95% of nickel is adsorbed by the soil easily within 7.5h if the nickel concentration is varies from up to maximum of 750mg/L The optimum dosage of biomass (*Pseudomonas*) is found to be 0.5mL (1.472mg/mL) with minimal or nutrient media of 10mL. The R<sup>2</sup> value of the isotherm model is found to be agreeing with pseudo second order kinetics. Which indicates the rate of adsorption is due to chemo sorption involving valence forces through sharing or exchange of electrons between adsorbent and adsorbate. Bio sorption using *Pseudomonas* with nutrient media is a promising bio remediation method for depolluting nickel contaminated sites.

**Keywords: Biosorption, Environment, Metal, Media, Nickel and Pseudomonas**

## I. INTRODUCTION

Nickel is considered as ubiquitous metal in nature. It is twenty fourth most abundant metal in the earth crust and fifth most abundant element by weight after iron, oxygen, magnesium and silicon, constituting about 2-3% of the earth composition. It is naturally occurring element that exists in various mineral forms. Nickel in its elemental form it is silver white in colour, hard and glossy. While in powder form it is reactive when exposed to atmosphere and ignites spontaneously [1]. It distinguished from other metals by its physicochemical properties: density of 8.9 g/cm<sup>3</sup> @ 25°C, melting point 1455°C and boiling point 2732°C. It merely exists in the 0 and +2 oxidation states and less frequently in the -1, +1, +3 and +4 oxidation states. Nickels property of conducting high electrical and thermal energy makes it resistant to electrical erosion, oxidation and corrosion at temperatures of -20 to +30°C [2].

Naturally, nickel distributed uniformly throughout the soil profile. The principal natural form of nickel oxide occurs in admixture with nickel sulfides in varying proportions in weathered ore. Its content in soil varies in a wide range from 3 to 1000 mg/kg [3, 4]. Nickel can exist in soils in several forms: inorganic crystalline minerals or precipitates, complexed or adsorbed on organic cation surfaces or on inorganic cation exchange surfaces, water soluble, free-ion or chelated metal complexes in soil solution [5, 6]. However, it typically accumulates on the surface from deposition caused due to industrial and agricultural activities. It is used widely in production of coins, catalysts, cement, disinfectants, ink and dye, jewelry, magnets, electroplating and smelting, alloys, stainless steel, spark plug and other igniting materials, electrical resistant heaters, batteries, paints and varnishes, bathroom and kitchen fittings, cables and wires, motor vehicles, food processing, edible oil, desalination plant etc.

Nickel may present a major problem in land near towns, in industrial areas, or even in agricultural land receiving concentrated wastes from solid and liquid waste disposal, water and wastewater treatment sludge, accidental spillage etc. The accumulation of nickel concentration in soil may occur due to other wide range of non-point and point sources. The mobility of nickel in soil can occur when the pH of the soil varies. Decrease in soil pH through reduced use of liming (agriculture), Acid Rain, Acid Mine Drains etc. can increase the rate of mobility of nickel through the soil and leaching to deeper layers of soil eventually increasing the groundwater nickel concentration beyond safe limit (10µg/L). There are various in-situ and ex-situ remediation methods applied to contaminated site. Among the in-situ methods bio remediation is the most reliable and feasible method for soil depollution [7].

Keeping all the above facts in view, the present study is contemplated on carrying out bio sorption of using *Pseudomonas* Bacteria for depolluting the contaminated soil. The study is carried out according to the specific objectives: i) Soil sampling and Characterization, ii) Growth media Synthesis and Bacteria culturing, iii) Synthesis of Synthetic Nickel Sample iv) to carry out batch bio sorption study for removal of nickel and v) to conduct kinetic study for the sorption data.

## II. MATERIALS AND METHODOLOGY

### A. Soil Sampling and Analysis

Soil characterization is carried out to determine the various properties that can affect the growth of bacteria. The soil used for batch bio sorption studies were collected from Sri Jayachamarajendra College of Engineering campus, near dump yard, Mysore. The Top layer of soil was initially scrapped to remove debris after which it was dug up to a depth of 15cms. The stones were parted from soil and a mass of 10kg was collected in a polythene bag. The soil sample was sieved through 2mm IS sieve to remove the coarse debris and dust particles. Then the soil was thermally treated in hot air oven for 24hrs at 180 °C to kill the indigenous microorganisms.

The soil samples were tested for pH, moisture content, porosity, specific gravity, particle size distribution and organic content, according to standard procedure given in IS: 2720.

### B. Bacterial Culture

The *Pseudomonas* Bacteria employed in the presented study is cultured in lab using Sterilized petri plates with Nutrient Agar as the growth supporting media. Streak plate method was adopted for culturing bacteria (Figure 1).

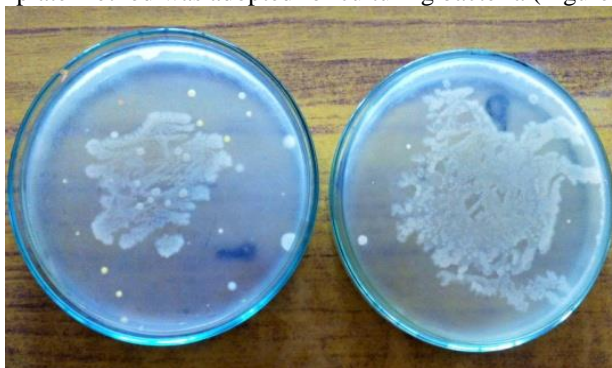


Fig. 1: *Pseudomonas* Bacteria (24hrs incubation period)

In order to increase the bacterial density, the plate culture is inoculated to nutrient broth and incubated for 24hrs at 37°C. After anticipated incubation period the broth is centrifuged at 8000rpm for 15min to collect the bacterial cells. The collected bacterial cells were stored at 4°C and used for batch experiments.

### C. Bacterial Density

Microbial parameters often need to be defined as mass rather than colony forming unit (CFU) or optical density (OD) which is easily measurable using plate counting method or UV-Vis spectrophotometer, especially for any attempt in modeling of contaminant transport and biological growth or degradation within the system. The Optical Density method is used in the present study to determine the biomass of *Pseudomonas* and converted to required unit (mg/mL) using the equation (1). The OD is measured at wave length of 600nm.

$$Y \text{ (mg/mL)} = 2.0087 \times (\text{OD}_{600}) + 0.0764 \quad (1)$$

Where, Y is the Biomass Concentration

### D. Synthesis of Minimal and Nutrient media

In order to support the bacterial growth in the batch studies two media were selected. The effectiveness of these media, in the progress of bacteria was examined by varying the dosage of media. The composition of the media is furnished in Table 1.

Table – 1  
Composition of Minimal and Nutrient Media

Chemicals	Concentration (g/L)
<i>Minimal Media</i>	
<i>Sodium phosphate</i>	64
<i>Potassium phosphate</i>	15
<i>Sodium chloride</i>	2.5
<i>Ammonium chloride</i>	5
<i>Nutrient Media</i>	
<i>Peptone</i>	5
<i>Sodium chloride</i>	5
<i>Beef extract</i>	1.5
<i>Yeast extract</i>	1.5

**E. Preparation of Synthetic Nickel solution**

Analytical grade Nickel Sulphate (NiSO<sub>4</sub>) of 0.479, 0.957, 1.436, 2.393 and 3.588g is dissolved in deionized water to fetch 100, 200, 300, 500 and 750mg/L concentrations nickel solution. After it completely dissolves the solution is acidified with 10 mL HNO<sub>3</sub> and made up to 1L with deionized water.

**F. Batch Study**

The nickel sample of 100mL having concentrations 100, 200, 300, 500 and 750mg/L were taken in conical flask and pretreated soil of 100g only was added to the each flask. The prepared conical flasks were placed in a rotatory shaker at 150rpm. Samples were drawn at every 1.5h up to 7.5h from the start of batch study. The samples drawn were passed through whatman filter paper and analyzed for nickel content remaining in the solution.

The bacterial cultures (0.1ml, 0.3ml and 0.5ml) as well as minimal and nutrient media of different dosages (4, 6, 8 and 10mL each) were added to the conical flasks containing 100mg/L of nickel concentration. The uptake of metal ions from the bacteria *Pseudomonas* was observed for 5th days. The optimal biomass concentration and minimal and nutrient media was determined from this study and was adopted for further studies.

The study was continued by varying the metal ion concentration for the obtained optimal biomass concentration and media dosages along with 100g of pretreated sand. The samples were drawn every 24h till 120h to know nickel concentration remaining in the solution. Mean time 1g of soil was also taken and 0.05M acetic acid (extracting solution) of 10ml in the ratio 1:10 (W/V) was added, stirred well and allowed to rest for about 15min and analyzed for Nickel using ICP - MS (Inductive Coupled Plasma Mass Spectroscopy).

**G. Langmuir and Freundlich Isotherm**

It describes quantitative formation of a monolayer pollutant adsorbate on the outer surface of the biomass or soil adsorbent, and after that no further adsorption takes place. Therefore, the Langmuir represents the equilibrium distribution of metal ions between the solid and liquid phases. Model is assumption is: i) it is valid for monolayer adsorption ii) uniform energies of adsorption exists on the surface and iii) no transmigration of adsorbate occurs on the surface. Whereas the Freundlich isotherm describes the adsorption characteristics for the heterogeneous surface. The governing equation of the isotherm and its terminologies are Furnished in the Table 2 and 3 respectively.

The essential features of the Langmuir isotherm may be expressed in terms of equilibrium parameter R<sub>L</sub>, which is a dimensionless constant referred to as separation factor or equilibrium parameter. The value depicts that the adsorption nature is either unfavorable, linear or favorable if R<sub>L</sub>>1, R<sub>L</sub>=1, or 0<R<sub>L</sub><1 respectively.

$$R_L = 1/(1+(KLC_0)) \quad (2)$$

Table - 2  
Governing equations of Langmuir and Freundlich isotherm models

Isotherm models	General Equation	Linear Equation	Plot	
			x	y
Langmuir isotherm	- $q_e = \frac{Q_0 b C_e}{1 + b C_e}$	$\frac{C_e}{q_e} = \frac{1}{b Q_0} + \frac{C_e}{Q_0}$	$C_e$	$\frac{C_e}{q_e}$
		$\frac{1}{q_e} = \frac{1}{Q_0} + \frac{1}{b Q_0 C_e}$	$\frac{1}{C_e}$	$\frac{1}{q_e}$
		$q_e = Q_0 - \frac{q_e}{b C_e}$	$\frac{q_e}{b C_e}$	$q_e$
		$\frac{q_e}{C_e} = b Q_0 - b q_e$	$q_e$	$\frac{q_e}{C_e}$
Freundlich isotherm	$q_e = K_F C_e^{1/n}$	$\log q_e = \log K_F + \frac{1}{n} \log C_e$	$\log C_e$	$\log q_e$

Table – 3  
Terminologies of Isotherm models

Isotherms	Linear Equation	Terminology
Langmuir isotherm	$\frac{C_e}{q_e} = \frac{1}{bQ_0} + \frac{1}{Q_0}$	$C_e$ = equilibrium concentration of adsorbate (mg/L) $q_e$ = amount of ion adsorbed per gram of adsorbent at equilibrium (mg/g) $b$ = Langmuir isotherm constant (L/mg) $Q_0$ = maximum monolayer coverage capacity (mg/g)
Freundlich isotherm	$\log q_e = \log K_F + \frac{1}{n} \log C_e$	$q_e$ = amount of ion adsorbed per gram of adsorbent at equilibrium (mg/g) $K_F$ = Freundlich isotherm constant (mg/g) $n$ = adsorption intensity $C_e$ = equilibrium concentration of adsorbate (mg/L)

### III. RESULTS

#### A. Soil Characteristics

The effectiveness of the process depends on the physico-chemical characteristics soil. The soil sample obtained from the site is as presented in Table 2. The grain size analysis showed that the soil in the site is a well graded clayey soil with organic content of 4%. And the pH of the soil is found to be above the neutral value (pH 7.56) and which is an unfavorable condition for nickel mobility. The soil temperature was 27.7°C during the time of sampling, and it had void ratio and porosity of 0.358 and 0.264 respectively.

#### B. Batch Study

Firstly, the effect of varying Nickel concentration on the adsorption capacity of the soil is studied (Figure 2). For which Nickel concentration of 100, 200, 300, 500 and 750mg/L is used. The samples drawn at equal intervals and corresponding percentage of nickel removed from the solution is presented in the Figure 3.

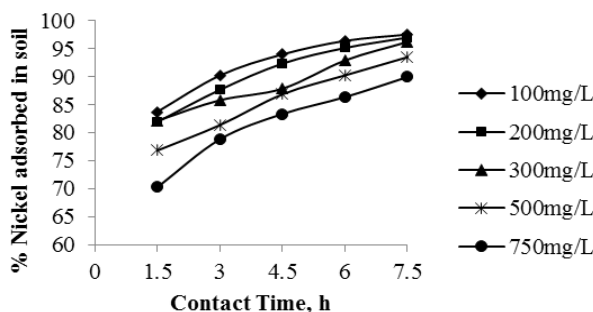


Fig. 2: Effect of different initial Nickel concentrations in soil

From the Figure 3 it is evident that, as the concentration is increased the rate of adsorption of Nickel on to Soil surface reduces. With the initial concentration of 100mg/L, the nickel removal of 83.76% is achieved for contact time of 1.5h. Whereas, for initial concentration of 750mg/L in order to reach 83.37% the contact time dragged up to 4.5h. It is evident enough the well graded clayey soil have great affinity towards the adsorption of nickel ions. Further, it can be said that the soil can adsorb 85-95% of the nickel within 7.5h if the nickel concentration varies up to maximum of 750mg/L

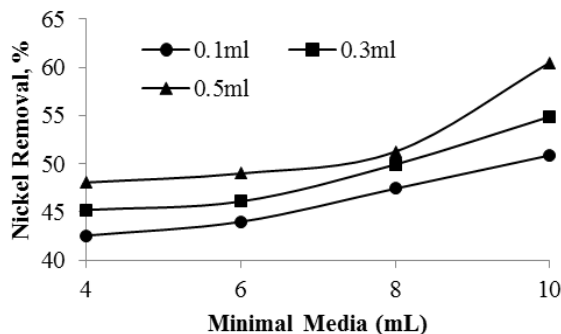


Fig. 3: Effect of biomass and Minimal media dosage in the removal of Nickel

The effect of biomass and minimal media dosage is presented in the Figure 3. It is observed that with increase in dosage of biomass and minimal media there was increase in removal of nickel. Similar trend is observed when the Nutrient media was used (Figure 4). However, the removal efficiency is observed to be more while using nutrient media than minimal media. Maximum

removal was observed to be with the 0.5 and 10mL of biomass and minimal/nutrient media respectively. Hence, it is considered as optimum dosage which is used in further studies.

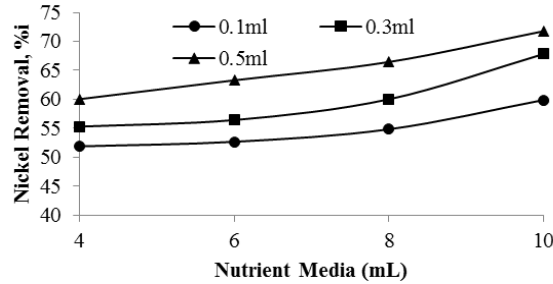


Fig. 4: Effect of different biomass and Nutrient media dosage in the removal of Nickel

After determining the optimum dosage the study is continued to understand the effect of varying initial concentration on bio sorption using bacterial biomass with the media. Figure 5 and 6 show the trend on the effect of varying nickel concentration on the optimum dosage of biomass with minimal and nutrient media respectively.

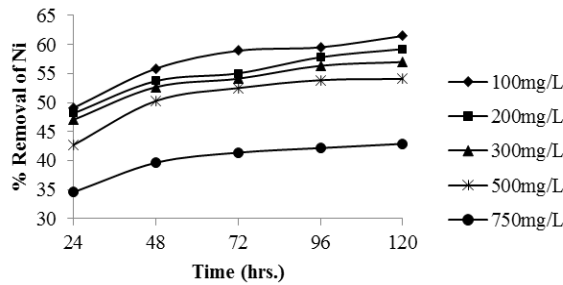


Fig. 5: Effect of Initial concentrations on the biosorption using Biomass with Minimal media

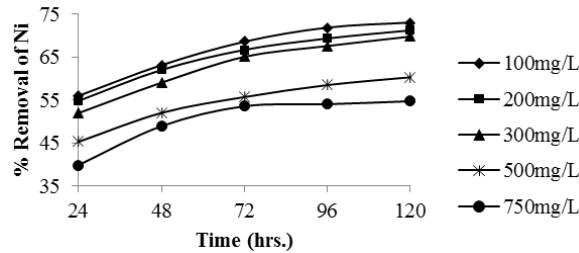


Fig. 6: Effect of Initial concentrations on the biosorption using Biomass with Nutrient media

From figure 5 and 6 it is clear that the removal efficiency increases with increase in contact time. Whereas, when the initial concentration was increased the removal of nickel was found to decrease with respect to time. This phenomenon is may be due to decrease in the rate of uptake by the biomass with reference to the initial concentration.

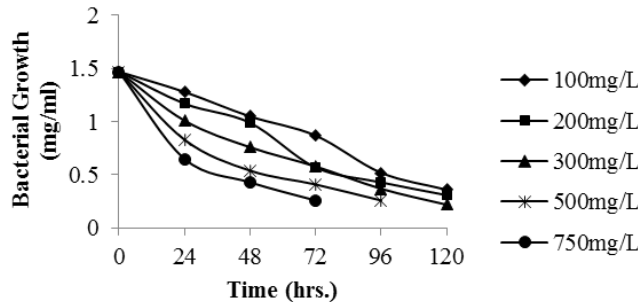


Fig. 7: Effect on the Growth of bacteria with Minimal Media for different initial concentrations of Nickel

The effect of initial nickel concentration on the bacteria is also studied. Figure 7 and 8 is the effect of initial concentrations of nickel on bacterial growth with minimal and nutrient media respectively. The optimum biomass concentration of 0.5mL (1.47mg/mL) is considered to evaluate the effect of initial concentration on the biomass. From the study observation it can be said that as the concentration is increased the bacterial growth was inhibited significantly in both cases (i.e. minimal and nutrient media). There was significant different in the bacterial growth when nutrient media was used. When the nickel concentration was 100 and 200mg/L there was initial rise in the biomass concentration followed by significant deterioration. Whereas in case of

minimal media the bacterial growth is observed to be down trend. Hence, from the study it is evident that the nutrient media is better growth supporting media while removing the nickel from the source.

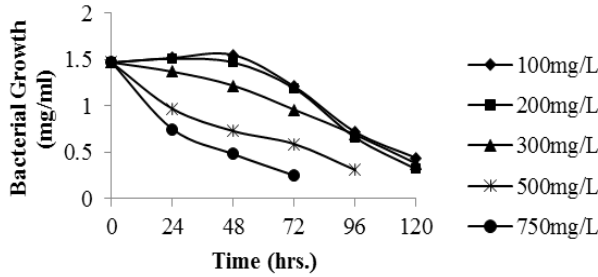


Fig. 8: Effect on Growth of bacteria with Nutrient media for different initial concentrations of Nickel

**C. Isotherms Models**

The computed values of the various model parameters and constants are presented in Table 4. The  $R_L$  value in Langmuir Isotherm is above 0 and less than 1 which indicates that the sorption condition is favorable. And the  $R^2$  value is 0.9948 which means the model is well fitted with the sorption data. From the Freundlich isotherm it can be observed that  $1/n$  and  $n$  value indicates that the adsorption is normal and favorable. And the  $R^2$  is 0.9895 indicates the Freundlich model also is well fitted with the sorption data. The linear plot fitted with both isotherm for sorption data is as given in figure 9 and 10. Isotherm and Kinetics study is done for the only the optimum biomass concentration with nutrient media.

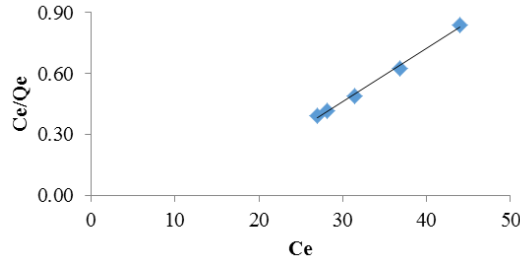


Fig. 9: Langmuir Isotherm Model for Biosorption of Ni

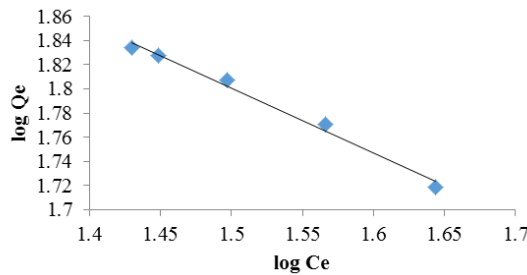


Fig. 10: Freundlich isotherm model for Biosorption of Ni

Table - 4  
Langmuir isotherm constants for Ni Biosorption

Metal Conc. (mg/L)	Langmuir isotherm model					
	$m(\text{slope})$	$b$	$C(\text{intercept})$	$Q_0$	$R^2$	$RL$
100	0.0261	0.082	0.3181	38.314	0.9948	0.109
	Freundlich isotherm model					
	$m(\text{slope})$	$n$	$C(\text{intercept})$	$k_f$	$R^2$	
	0.5377	-1.859	2.607	404.576	0.9895	

**D. Biosorption Kinetic Studies**

Kinetic models were employed to analyze the adsorption rates of nickel metal ions. The experimental batch Biosorption study data was modeled using pseudo first order and pseudo second order kinetics. The plot of first and second order kinetics with different concentrations of metals is shown in the Figure 11 and 12 respectively.

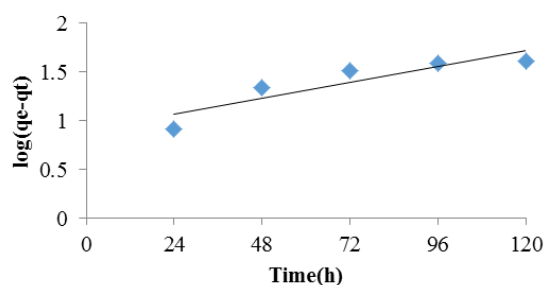


Fig. 11: Pseudo first order plot for Biosorption of Ni

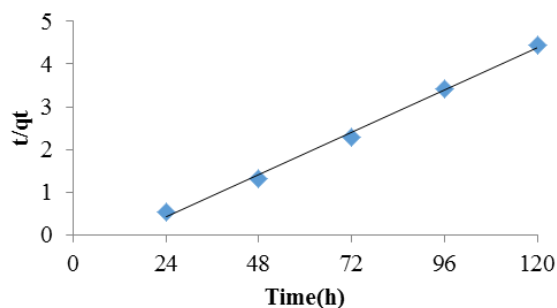


Fig. 12: Pseudo second order plot for Biosorption of Ni

From Table 5 it can be observed that correlation coefficient  $R^2$  for pseudo second order kinetics is much higher than pseudo first order kinetics. These indicate that the adsorption data are better represented by second order kinetic model, which is based on the assumption that rate of adsorption is due to chemo sorption involving valence forces through sharing or exchange of electrons between adsorbent and adsorbate.

Table - 5  
First order and second order sorption rate constants for Ni adsorption

Metal Conc. (mg/L)	Pseudo first order			Pseudo second order		
	k1	qe	R2	k2	qe	R2
100	0.0157	8.0556	0.8134	0.0029	24.155	0.9955

#### IV. CONCLUSIONS

From the present Study following conclusions are drawn: The site soil is a well graded clayey soil with high affinity towards nickel. It is evident from the adsorption study that 85-95% of nickel is adsorbed by the soil easily within 7.5h if the nickel concentration is varies from up to maximum of 750mg/L. The optimum dosage of biomass (*Pseudomonas*) is found to be 0.5mL (1.472mg/mL) with minimal or nutrient media of 10mL. Effects of initial concentration on removal efficiency of nickel using optimum dosage of biomass and media are observed to decrease with increase in nickel concentration. Nutrient media is found to better growth supporting media for *Pseudomonas* when employed of removal of nickel. The bacterial growth observed to follow down trend when minimal media is used. Whereas, the bacterial growth is observed to initially increase in case of nutrient media. The  $R^2$  value indicated that these models were well fitted with sorption data. The  $R^2$  value of the isotherm model is found to be very near to the  $R^2$  value of pseudo second order kinetics. These indicate that the adsorption data are better represented by second order kinetic model, which is based on the assumption that rate of adsorption is due to chemo sorption involving valence forces through sharing or exchange of electrons between adsorbent and adsorbate. Hence, from this study it can be concluded that bio sorption using *Pseudomonas* with nutrient media is a promising bio remediation method for depolluting nickel contaminated sites

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