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Biosorption of Nickel Using Mixed Cultures of *Pseudomonas aeruginosa* and *Bacillus subtilis*

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ABSTRACT

Biosorption of Ni(II) was investigated in this study using dead biomass of gram positive (*Bacillus subtilis*) and gram negative (Pseudomonas aeruginosa).The effects of pH, initial adsorbent dosage, initial metal ion concentration, contact time and temperature were studied in batch experiment. A contact time of 40 min, pH 5.0 and temperature 30 °C were found to be optimum. Nickel removal decreased from 77 to 45 per cent as the concentration increased from 50 mg/L to 250 mg/L. The Ni(II) removal increased from 45 to 75 per cent as adsorbent dose increased from 0.25 to 1.5 g/L. The Langmuir and freundlich models for dynamics of metal of metal ion uptake proposed in this work fit the experimental data reasonably well. The adsorption capacity (Q_o) calculated from Langmuir isotherm was 89.08 mg for Ni (II).

Keywords: Bacteria; Nickel removal; Biosorption; Kinetics; Isotherms.

1. INTRODUCTION

Pollution of water resources is a common problem being faced today. Heavy metal pollution occurs directly by effluent from industries. Metals are extensively used in several industries, including mining, metallurgical, electronic, electroplating and metal finishing (Ercole et al., 1994) The presence of metal ions in final industrial effluents is extremely undesirable, as they are toxic to both lower and higher organisms. Under certain environmental conditions, metals may accumulate to toxic levels and cause ecological damage. Mainly mercury, lead, cadmium, arsenic, chromium(VI) are regarded as toxic; whereas, others such as copper, nickel, cobalt, zinc are not toxic, but their extensive usage and increasing levels in the environment are of serious concerns (Prasad and Freitas, 2003).

Inorganic micro-pollutants are of considerable concern because they are non-biodegradable, highly toxic and have probable carcinogenic effect (Ahalya et al., 2003). Ni(II) is well known toxic heavy metal, which pose serious threat to the fauna and flora of receiving water bodies when discharged into natural water. Nickel (II) present in the effluents of silver refineries, electroplating, zinc base casting and storage battery industries (Kadirvelu, et al., 2000a). Because of nickel's slow rate of oxidation at room temperature, it is considered corrosion-resistant (Golab and Breitenbach, 1995; Iqbal and Saeed, 2007). In India acceptable limit of nickel in drinking water is 0.01 mg/L and for discharge of industrial wastewater is 2.0 mg/L (Kadirvelu, 1998). At higher concentration, Ni (II) causes cancer of lungs, nose and bone. Dermatitis (Nickel itch) is the most frequent effect of exposure to nickel, such as coins and costume jewelry. Nickel carbonyl [$Ni(CO_4)$] has been estimated as lethal in humans at atmospheric exposures of 30 ppm for 30 min (Namasivayam and Ranganathan, 1994).

At present, various physico-chemical methods, such as chemical precipitation, chemical oxidation or reduction, electrochemical treatment, evaporative recovery, filtration, ion exchange, and membrane technologies have been widely used to remove heavy metal ions from industrial wastewater. These processes may be ineffective or expensive (Volesky and Holan, 1995). Therefore, there is a need to develop new and cost effective methods which are more environmental friendly. Biological methods such as biosorption/ bioaccumulation for the removal of heavy metal ions may provide an attractive alternative to physico-chemical methods (Vijayaraghavan and Yun, 2008). Microorganisms such as bacteria, yeast, fungi and algae uptake metal either actively (bioaccumulation) and/or passively (biosorption) (Kuyucak and Volesky, 1988). 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 (Xie et al., 1996). Hence in the present study an attempt has been made to study the biosorption of nickel ions by mixed biomass cultures of gram positive (*Bacillus subtilis*) and gram negative (*Pseudomonas aeruginosa*).

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Experiments were also designed to study the factors that affect the biosorption process.

2. MATERIALS AND METHODS

2.1 Adsorbent

Bacterial plate culture was obtained from Department of Microbiology, PSG College of Arts and Science, Coimbatore. This was used to prepare broth culture. From this 100 µl of each (*Pseudomonas aeruginosa* and *Bacillus subtilis*) microbial culture was inoculated into the medium for getting mixed culture in laminar air flow chamber. The inoculated flasks were incubated in an orbital shaker (Scingenesis Biotech/ ORBITEK) at 250 rpm at 32 °C for two days to obtain the biomass. Biomass was harvested from the medium by centrifugation (HITACHI-High Speed Refrigerated Centrifuge) at 9000 rpm for 10 min. The supernatant was discarded and the cells were re-suspended in double distilled water for washing and again centrifuged as above to make sure that no media remain on the cell surface. The biomass was heat killed in a conventional hot air oven at 60 °C for 8 h. This biomass was used for the sorption experiments.

2.2 Adsorbate

Stock solution of Ni(II) (1000 mg/L) was prepared by dissolving nickel sulphate in double distilled water. The stock solution was diluted with distilled water to obtain the working solution of different metal concentration from 50 mg/L to 250 mg/L. All experiments were conducted in triplicate. The mean values of the results are given in this paper.

2.3 Batch mode adsorption studies

The studies were carried out at 30±1°C using 100 ml of metal ion solution containing the desired concentration and 150 mg of adsorbent in 250 ml conical flasks and stirring speed of 180 rpm in thermostatic orbital shaker. Samples were withdrawn after predetermined time intervals, and were separated by centrifugation at 4000 rpm for 15 min. Ni(II) was determined by atomic adsorption spectrophotometer (Perkin Elmer-2280).

The metal concentration retained in the adsorbent phase (q_e, mg/g) was calculated by using the following equation.

$$Q_e=(C_o-C_e)\ v/m \qquad (1)$$

Where $\mathbf{C}_o$ and $\mathbf{C}_e$ are the initial and final concentrations of metal ion in solution (mg/L), ***v*** is the volume of solution (ml) and ***m*** is the mass of adsorbent (mg). Adsorption isotherms were performed at 30±1°C, with initial concentrations of Ni(II) from 50 mg/L to 250 mg/L, a solution volume of 100 ml an adsorbent weight of 150 mg.

3. RESULTS AND DISCUSSION

The suspension of dead biomass of *Pseudomonas aeruginosa* and *Bacillus subtilis* was mixed with metal solution, the biosorption of nickel ions occurred due to negatively charged sites on bacterial cell wall. Biosorption capacity of bacterial biomass was studied in batch mode experiments. In our study the optimum conditions of biosorption were a contact time of 2 hrs, biomass concentration of 1.00 g and pH 5.00. Experimental showed the influence of initial metal concentration on the metal uptake for dried biomass. Both the Langmuir and Freunlich adsorption were suitable for biosorption of Ni(II) by *Pseudomonas aeruginosa* and *Bacillus subtilis.*

3.1 Effect of pH

The effect of pH on the adsorption of Ni(II) on bacterial biomass was studied at room temperature by varying the pH from 1.0-7.0. The pH of the aqueous solution is an important controlling parameter in the adsorption process and thus the role of hydrogen ion concentration was examined in the solution at different pH. The plot of metal adsorption percentage versus pH is shown in Fig.1. From the figure, it is observed that adsorption of Ni(II) varies with pH and hence Ni(II) adsorption on biomass is highly pH dependent. Adsorption is high at pH 5.0 and decrease as the pH increases or decreases. At low pH value, the H^+ ions compete with metal cation for the exchange sites in the system there by partially releasing the metal cation (Nies, 1999). pH affects both cell surface metal binding sites and metal chemistry in water. At low pH values, cell wall ligands are closely associated with the hydronium ions and repulsive forces limit the approach of the metal ions with increasing pH, more ligands, such as amino and carbonyl groups, would be exposed leading to attraction between these negative charges and the metals hence increases in biosorption on to the cell surface (Aksu.Z et aI., 1992). The lower uptake at higher pH value is probably due to the formation of anionic hydroxide complexes (Chang ey al., 2003). Because of this effect at higher pH values the ligands such as carboxylate and sulfonate groups could uptake metal ions (Veglio and Beolchini, 1997).

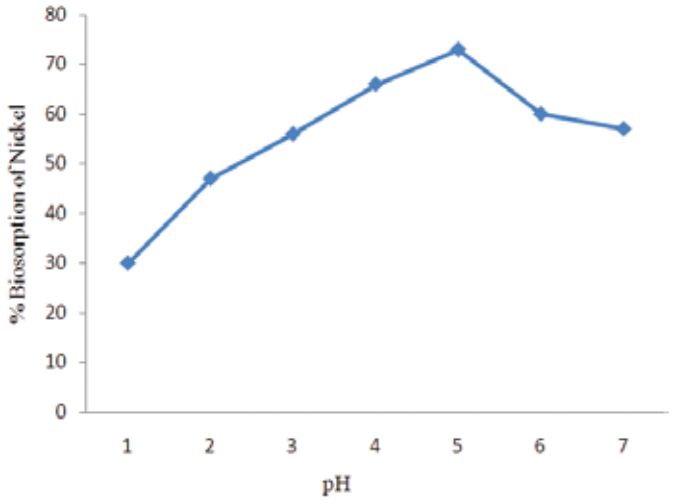

Fig 1: Effect of pH on the adsorption of Ni on bacterial biomass.

3.2 Effect of Adsorbent Dose

The dependence of nickel adsorption on the amount of biomass is studied at room temperature and at pH - 5.0 by varying the adsorbent amount from 0.25 g to 1.50 g while keeping the volume and concentration of the metal solution constant. The result is graphically shown in Fig.2. It is apparent that the percent removal of nickel increase rapidly with increase in the dose of biomass due to the greater availability of the biosorbent. Adsorptions is maximum with 1 g of biomass and

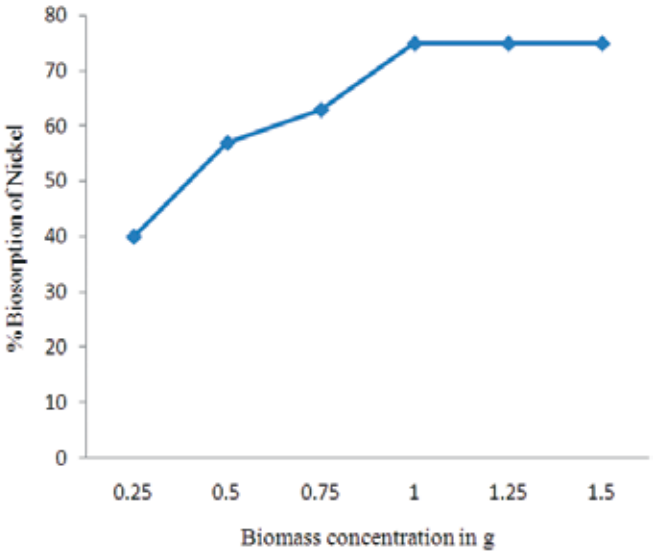

Figure 2. Effect of adsorbent dose on the percent removal of Nickel.

the maximum percentage removal is about 75 per cent. It was observed that there is no further increase in the percentage sorption of nickel with increase of biomass beyond 1g. From that it can be conclude that the 1g of biomass dose was set to be optimum biomass dose (Das, 2009).

3.3 Adsorption Isotherms

Adsorption isotherms of Ni(II) onto bacterial biomass of mixed culture are presented in Fig.1. Two models were used to analyse the adsorption isotherm. Langmuir isotherm was applied for adsorption equilibrium.

$$C_e/q_e = 1/(Q_o b) + C_e/Q_o \quad (2)$$

Where $\mathbf{C}_e$ is the equilibrium concentration, $\mathbf{q}_e$ is the amount adsorbed at equilibrium, $\mathbf{Q}_o$ and $\boldsymbol{b}$ is the Langmuir constants related to adsorption capacity and energy of adsorption. The linear plot of C_e/Q_e versus C_e showed in Fig.3. This shows that

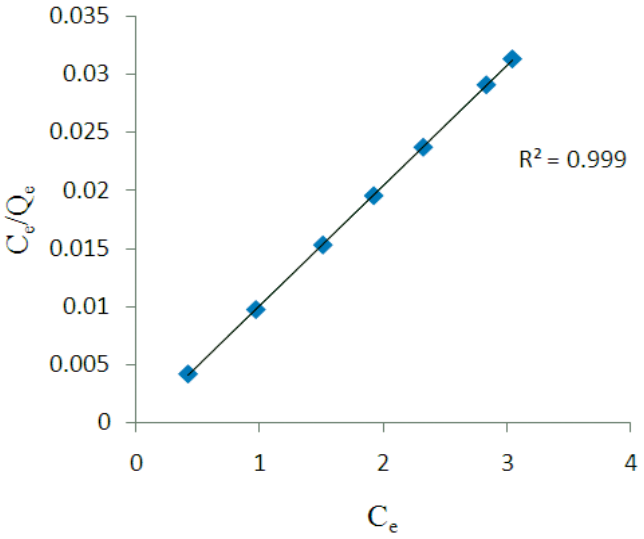

Figure 3. Langmuir isotherm model for Ni(II) adsorption.

Table 1. Langmuir and Freundlich equilibrium constants

Langmuir parameters				Freundlich parameters		
Qo	b	R2	RL	Kf	N	R2
89.08	0.5329	0.999	0.0362	86.08	2.276	0.975

the adsorption followed the Langmuir model well. Q_o and b were determined from the slope and intercept of the plot and are presented in Table.1

The essential characteristics of Langmuir isotherm model can be explained in term of a dimensionless constants separation factor or equilibrium parameter R_L, which is defined by

$$R_L = 1/(1+bC_o) \quad (3)$$

Where $\boldsymbol{b}$ is the Langmuir constant, $\mathbf{C}_o$ is the initial concentration of metal ion. According to Hall et al, it has been shown using mathematical calculations that the parameter R_L indicates the shape of isotherm as follows:

R_L value	Type of isotherm
$R_L > 1$	Unfavourable
$R_L = 1$	Linear
$0 < R_L < 1$	Favourable
$R_L = 0$	Irreversible

The Freundlich isotherm is represented by equation,

$$Q_e = K_f C_e^{1/n} \quad (4)$$

Where $\mathbf{C}_e$ is the equilibrium concentration (mg/L), $\mathbf{Q}_e$ is the amount adsorbed at equilibrium, $\mathbf{K}_f$ and $\boldsymbol{n}$ are constants incorporating all factors affecting the adsorption process such as adsorption capacity and intensity. Linear plots of In q_e versus In C_e showed in Fig.4. K_f and n were calculated from the intercept and slope of the plots. The constants were presented in Table.2

3.4 Effect of time

The adsorption of nickel increased with time and at certain point of time, it increased a constant value beyond which no

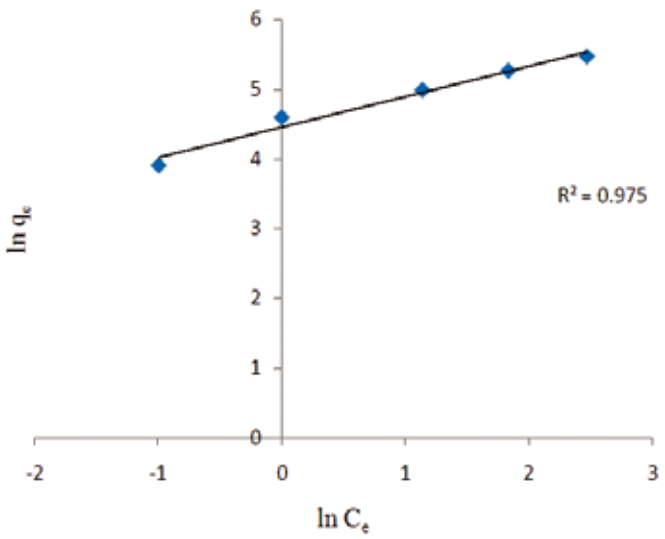

Figure 4. Freundlich isotherm model for Ni(II) adsorption.

Table 2. Kinetic constants of Ni(II) adsorption onto biomass

Ni(II) concentration (mg/l)	K_{ad} (min^{-1}) × 10^{-2}	R^2	q_e
50	1.15	0.997	58.0
100	2.29	0.990	100.5
150	3.44	0.995	166.2
200	4.54	0.993	181.8
250	5.61	0.919	231.9

more Nickel was further removed from solution. Maximum adsorption took place within the first 60 min to 80 min (Fig.5) The amount of Nickel adsorbed at the equilibrium time was the maximum adsorption uptake by the adsorbent. The results revealed that the nickel adsorption was fast at initial stage of the contact period and then become slower near the equilibrium. This phenomenon was due to the fact that a large number of vacant surface site were available for adsorption during the initial stage (Gupta and Mohapatra, 2003). Near the equilibrium the remaining vacant surface sites were different to be occupied due to repulsive forces between the solute molecules on the solid and bulk phase (Baes and Mesmer, 1976).

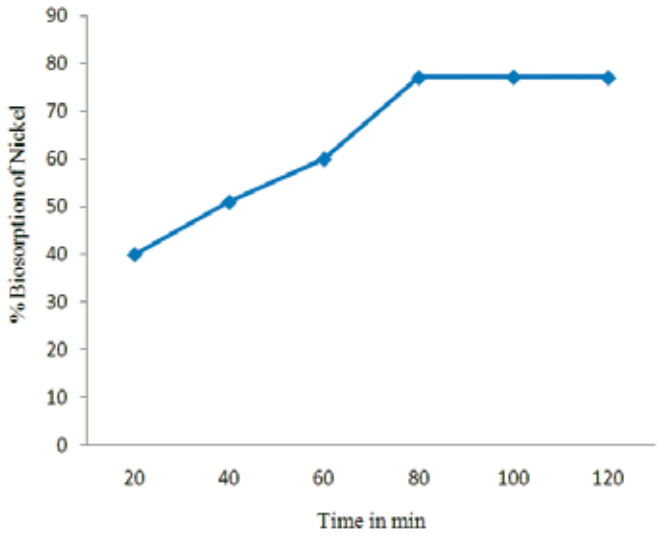

Figure 5. Effect of time on the percent removal of Nickel.

3.5 Kinetic studies

The kinetic study for the adsorption of Ni(II) was conducted at optimum pH where only adsorption takes place. The adsorption equilibrium was reached at 80, 105, 115, 130 and 140 min for 50, 100, 150, 200 and 250 mg/L, respectively. The contact time required for all the concentrations of Ni(II) removal was very short. According to these result, the stirring time was fixed at 4 hr for the further experiments to make sure to reach adsorption equilibrium. The adsorption rate constants were calculated by using the following equation given by Lagergren (Lagergren, 1898):

$$log\ (q_e\text{-}q) = log\ q_e\text{-}(K_{ad}/2.303)\ t \qquad (5)$$

Where $\mathbf{q}_e$ and $\mathbf{q}$ are the adsorption capacities at time (t) and equilibrium time, respectively. $\mathbf{K}_{ad}$ is adsorption rate constant. The K_{ad} values were calculated from slope the respective linear plots of log (q_e-q) vs t (fig.6). The results show that the removal of Ni(II) follows first order reaction. The K_{ad} values were comparable with previous reports for adsorption of Ni(II) onto bacterial biomass.

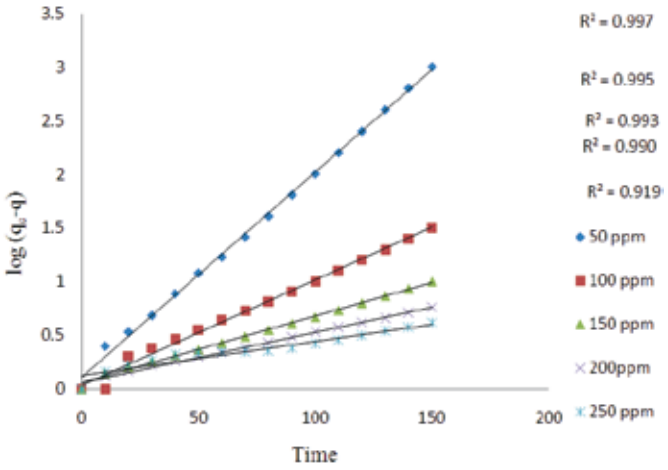

Figure 6. Pseudo-first order kinetic adsorption modal of Ni(II) onto bacterial biomass.

3.6 Mechanism of biosorption

The understanding of the mechanism by which microorganism accumulate metals is crucial to the developmental of microbial processes for concentration, removal and recovery of metals from aqueous solution. Metabolism-independent metal binding to the cell walls and external surfaces is the only mechanism present in the case of non-living biomass (Padilha et al., 2005).Metabolism-independent uptake essentially involves adsorption process such as ionic, chemical and physical adsorption. A variety of ligands located on the bacterial walls are known to be involved in metal chelation. These include carboxyl, amine, hydroxyl, phosphate and sulfhydryl groups. Metal ions could be absorbed by complexing with negatively charged reaction sites on the cell surface (Gupta et al., 2000). Microbial cell wall is rich in polysaccharide and glycoprotein such as glucans, chitin, mannans and phosphor-mannans. These polymers form abundant source of metal binding ligands (Sharma et al., 2006). In general bacteria may uptake and accumulate a significant amount of metal ions, resulting in the transfer of metals to a contaminated matrix of biomass (Garg et al., 2004). When suspension of dead biomass was mixed with metal solution, the biosorpion of nickel cations occurred due to negatively charged sites on bacterial cell wall (Kobya et al., 2005). Nickel biosorption capacity of *Pseudomonas aeruginosa* and *Bacillus subtilis* biomass was studied in batch mode. The optimum absorption pH value of Ni(II) were 5.0. Experimental showed the influence of initial metal concentration on the metal uptake for dried biomass. Both the Langmuir and Freundlich adsorption were suitable for biosorption of nickel.

3.7 Biosorbent Analysis

Scanning electron microscopy has been used as a tool for biosorbent characterisation and elucidation of probable

mechanism involved in sorption process. Electron micrographs of native bacterial biomass Fig.7 show that its surface before adsorption is irregular and porous and there can be good probability for nickel ions to be trapped and adsorbed into these pores. It is evident from SEM of nickel loaded biomass Fig.8 that its surface has become shiny due to deposition of nickel ions after adsorption.

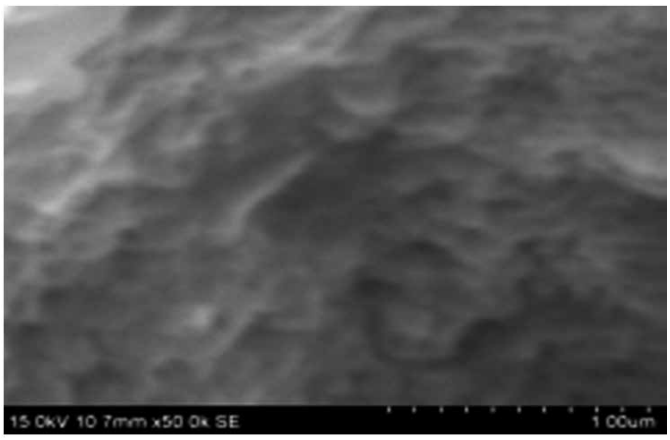

Figure 7. SEM Micrograph of before Ni(II) adsorption.

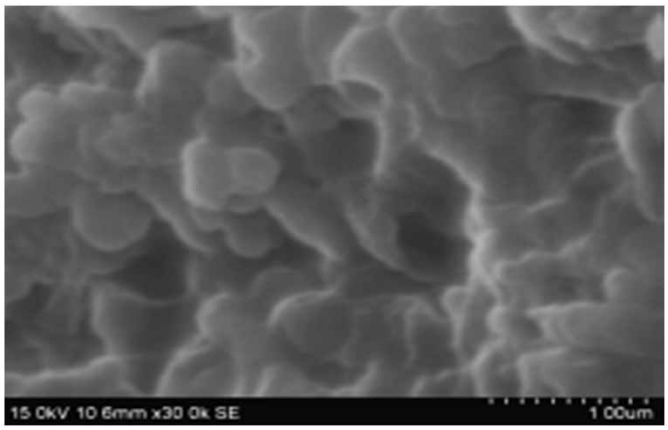

Figure 8. SEM Micrograph of after Ni(II) adsorption.

4. CONCLUSION

The present study has demonstrated that bacterial biomass possesses the adsorption capacity to remove heavy metal ions.

- Experimental conditions, such as pH, biomass concentration and reaction time have been optimised to utilize biomass as adsorbent for the removal of Ni (II) from aqueous solution. Adsorption capacity increases with metal ion concentration and also when increases the pH. Adsorption followed both Langmuir and Freundlich adsorption isotherms. The adsorption capacity (Q_o) was 89.08 mg of Ni(II).
- The kinetic results show that the removal of Ni(II) follows first order rate reaction given by Lagergren. Thus our study proves that of mixed biomass of *Bacillus subtilis* and *Pseudomonas aeruginosa* can be used for effective adsorption of Ni (II) from the contaminated sources.

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Dr K.Kadirvelu has received his PhD from Bharathiar University, in 1998 and he has completed Post doctoral research at France, in 2000. Currently he is working as Scientist 'F' at DRDO-BU Center for Life Sciences . His current area of research interests includes Heavy metal removal, environmental remediation, nanomaterials, activated carbon, activated carbon nanofiber, polmer based nanofibers, quantum dots, bacterial cellulose, chemical sensing and biosensing.
He has been a supervisor for this work and has chosen an appropriate research problem which is to be addressed. His contribution also includes the guidance throughout the work and the correction of manuscript.