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ORIGINAL ARTICLE

Biosorption of Heavy Metal Ions from Petrochemical Wastewaters by *Pseudomonas sp* Bacterium

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ABSTRACT
In this study we aim to investigate the biosorption of heavy metals ions, Pb (ii) and Ni (ii) from petrochemical wastewaters. In this study, Pseudomonas sp bacterium was used as a biosorbent Pb and Ni samples were obtained from wastewaters of petrochemical industry site in Abadan, Iran. Sorption rate, equilibrium time, pH value, and desorption rate were measured. Biosorption ability of Pb and Ni were tested by atomic adsorption spectrophotometer. According to our results, maximum sorption of Pb and Ni were about 6.35 and 6.44 mg/g dry weight cell, respectively, and best sorption time at equilibrium and pH value was 5 min, and 5, respectively. Also, we found out that nitric acid play significant role in desorption of two heavy metal ions from Pseudomonas sp. We concluded that pseudomonas sp can be a suitable biosorbent for the removal of heavy metals from petrochemical wastewaters.
Keywords: biosorption, bacteria, heavy metals, *Pseudomonas sp*, petrochemical wastewaters.

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INTRODUCTION

Increasing development of industries in the last decade has been the most important factor of environmental pollution and contaminated wastewaters of different industries such as leather making, mining and petrochemical can causes large amounts of heavy metals such as chromium, cadmium, nickel, cobalt, zinc and lead in the environment. Toxic effects of metals and their accumulation throughout the food chain can lead to serious ecological and health problems and can disturb the equilibrium and ecosystem order. Metals and chemicals widely used in industrial processes generate waste water with high percentages of metal ions. The mining and metallurgical extraction operations also impose a lot of toxic waste into the environment in liquid phase. As a result, due to the removal of pollutants from waste water, is of particular importance [1]. On the other hand, heavy metals, because of high stability in the environment and the presence of large amounts of them in plant effluents, are the most important environmental pollutants, so their filtration is essential before recovering them. Heavy metals, even in low amounts, can have adverse effects on the environment. Hence, investigating the methods of eliminating these pollutants is essential. Studies have shown that the most common physico-chemical processes such as oxidation and reduction, chemical precipitation, filtration, electrochemical treatment, evaporation, ion-exchange and reverse osmosis processes are limited and are not cost-effective; Metal remediation through these techniques is expensive and unsuitable in case of voluminous effluents containing complexing organic matter and low metal contamination.. It seems that biologic filtration can be a good alternative or at least a suitable complement for other methods, and in this regard, many microorganisms can be useful for the removal of heavy metals from wastewater systems [2].
Biological treatment with biological agents such as fungi, yeast, bacteria and algae, because of having mechanisms of adsorption, complication with the cell surface, ion exchange, and deposition as well as benefits such as cost effectiveness, low volume of biological and chemical waste sludge, high efficiency, the ability to recovery biomaterials, and processing of heavy metals have received more attention. The biosorption or ''passive uptake'' [2] is a combination of two processes, adsorption and absorption which is done by biomasses [3]. Gadd [4] and Brierley [5] have described the many ways in which bacteria, fungi

and algae can take up toxic metal ions. Recent studies shows that the strains (bacteria, yeast and fungi) isolated from contaminated sites possess excellent capability of metal scavenging. Some bacterial strains possess high tolerance to various metals and may be potential candidates for their simultaneous removal from wastes. Biosorption of heavy metals have been observed in bacterial species such as *Citrobacter sp* [6], *Saccharomyces cervisiae* [7], *Pseudomonas aeruginosa* [8, 9], *Bacillus sp* [10-12] , and some other species. Leusch et al [13] investigated biosorption of heavy metals (Cd, Cu, Ni, Pb, Zn) by two marine algae, *Sargassum fluitans* and *Ascophyllum nodosum*, and found out that *S. fluitans* is a better sorbent material for a given metal, size and modification. Different studies have been conducted on the removal of heavy metals by bacteria. For example, Cooksey and Azad [14] employed copper-resistant *Pseudomonas syringae* for accumulation of copper. Soltan [15] used *Pseudomonas aeruginosa* for the removal of heavy metals of lead, cadmium, mercury, zinc, silver, and molybdenum. Malekzadeh et al [16] studied the isolation of heavy metals from electroplating effluent by *Pseudomonas* MGF-48 bacterium and concluded that the bacterium was capable of accumulating several metals especially uranium. Some scholars have used the bacteria for biosorbing heavy metals of Pb and Ni [17, 18]. Considering previous studies, the main purpose of this study is to investigate the biosorption of Pb and Ni by *Pseudomonas sp* bacterium from petrochemical wastewaters.

MATERIALS AND METHODS

Sample collection

Studied samples were collected from wastewaters of petrochemical industry site in Abadan, Iran containing Pb and Ni ions. For colleting samples, sterile glass tubes were used.

Biosorbent preparation

For this study, *Pseudomonas sp* bacterium was used as a biosorbent. *Pseudomonas sp* cultured for 24 h, and then washed by ion-free distilled water. Biochemical and morphological characteristics of this bacterium is presented in table 1.

Spectrophotometery

Calculation and analysis of Pb and Ni metal ions before and after each test was measured using atomic adsorption spectrophotometer (AAS).

Adsorption kinetic

After cultivation of the biomass, 0.6 gram of it was mixed with Pb and Ni solution in flask, and adsorption rate of the heavy metals was analyzed at 0, 5, 10, 150, 250, 1200 and 1400min by AAS. In this study, the adsorption percentage of metal ions was calculated using equation (1) and the adsorption capacity was calculated using equation (2).

$$\text{Adsorption (\%)} = \frac{C_0 - C_e}{C_0} \times 100 \quad (1)$$

$$q_e = \frac{(C_0 - C_e)V}{m} \quad (2)$$

Where m is the amount of adsorbent (mg), q_e= adsorption capacity at equilibrium (mM^{-1}), V= volume of metal ion solution (L^3), C_0=initial concentration of the solution (mL^{-3}), and C_e = equilibrium concentration of the solution (mL^{-3}). [9]

For analyzing adsorption kinetics we used Langmuir-Hinshelwood (LH) equation:

$$\frac{dC}{dt} = K_{app}C,\ K_{app} = k_r k_e \quad (3)$$

$$In\left(\frac{C_o}{C}\right) = K_{app}t \quad (4)$$

where C is the concentration of bacterial cell mass (mgL^{-1}), K_{app} represents the apparent rate constant of first order reaction (min^{-1}) , k_r is the first-order reaction rate constant (mg L^{-1} min^{-1}), k_e is the equilibrium constant for the adsorption of metals at the reaction temperature (L mg^{-1}), and C_0 is the initial concentration of adsorbed metals by the bacterium (mM), and t represents adsorption time.

Adsorption Isotherm

For conducting surface adsorption isotherm, initial concentration of pb and Ni ranged from 0.01 to 1 mM/L were used. Bacterial biomass was mixed with this amount of metals. The used biomass for adsorption of the two heavy metals in this test was equal to 0.8g which included 4% of total concentration (solution volume=20 mL). Bacterial biomass was dried at the temperature of 37 and 70°C, and then was poured into suspension solution (the solution was contained about 10 mL distilled water, pH=5, under 300 rpm agitation speed for 2h). Next, 10 mL of the solution containing Pb and Ni was added to this suspension, and mixed for 3h. After this step, the whole solution was agitated for 10 min and a speed of 200 rpm using centrifuge. Then, 10 mL of the final solution, after passing from the syringe nanometer filter, was calculated in terms of Pb and Ni amount.

To study adsorption isotherm of Pb and Ni ion by *Pseudomonas sp* bacterium we used Langmuir equation [20]:

$$q_e = \frac{q_m b_L C_e}{1+b_L C_e} \qquad (5)$$

where q_m is the maximum sorption capacity (mg/g), and b_L represents the equilibrium constant related to the energy of sorption (L/g).

Desorption experiments

In this study we used desorption agent of Nitric acid (HNO3) ; first 0.8g of *pseudomonas sp* bacterium was exposed to 6 mmol of Pb and Ni solutions for 30 min; then, it bacterium was exposed to 0.1 mol HNO3 with pH=5. Again, we used the firstly used biomass for adsorbing Pb and Ni. After adsorption, again Pb and Ni was desorbed from the biomass, and for the third time, the bacterium was used to adsorb the two metals. The efficiency of adsorption and desorption was measured by AAS.

Assessing the effect of pH value on adsorption

Another parameter that can affect adsorption rate of heavy metals is pH ratio of the metal ion solution. To evaluate its effect, samples containing Pb and Ni solution with different pH ratios were prepared. The exposure time of bacterium and the solution was 2h; the flask containing bacterial biomass and two metals was incubated with a temperature of 23±3°C and shaker of 150 rpm. During this time, the solutions containing bacterium was centrifuged with a speed of 1200 rpm for 10 min. at the end, adsorption efficiency and adsorption capacity of Pb and Ni was measured by AAS in different pH ratios.

RESULTS AND DISCUSSION

Adsorption kinetics

Adsorption percentages of two metal ions are presented in table 2. Figures 1 and 2 depict adsorption kinetics of Pb and Ni, respectively. The adsorption kinetic is so fast and noticeable, and it only needs 5 min for isolating 50% of Pb and Ni from the solution. Based on Langmuir-Hinshelwood equation (Eq. 3 and 4), it was found that first-order constant of K_{app} is about 4.24×10^{-1} (min^{-1}). Adsorption time of Pb and Ni by the bacterium at equilibrium showed that the required time for the bacterium to remove the metal from aqueous solution was reasonable and is valuable in industrial uses.

Adsorption isotherm

Adsorption isotherms of the study metal ions are shown in figure 3 and 4. As can be seen, the increase of ambient concentration of metal ions can increase its adsorption because a more amount of metal ions are exposed to surface bacterial groups. Due to the constant amount of bacterium and adsorption positions on the bacterial biomass, adsorption rate is unchanged. The maximum adsorption rate of pb and Ni is respectively about 6.35 and 6.44 mg/g dry weight bacterial cell.

pH effect of metal ion solution on adsorption of Pb and Ni

In this section we examined the pH effect of metal ion solutions on adsorption of two heavy metals. Results are shown in figure 5 and 6. As can be seen, the highest adsorption value of pb and Ni by *pseudomonas sp* is occurred in pH=5, which is respectively about 6.35 and 6.44 mg/g. In pH ratios higher than 6, due to deposition of Pb and Ni, adsorption rate has decreased significantly.

Desorption results

In process of studying desorption of Pb and Ni from *pseudomonas sp* by nitric acid, our results presented in figures 7 and 8 showed that the highest desorption percent by nitric acid was observed in the first biosorption-desorption cycle which were 43.4 and 44% for Pb and Ni, respectively. In this test, the maximum adsorption rate for Pb and Ni were to 6.35 and 6.44 mg/g dry weight cell. The results have been compared to these values. As can be seen, the value has decreased in next cycles which indicate that after 3 or 4 times repetition of biosorption-desorption cycle in nitric acid solution, adsorption rate by the bacterium decrease which is because of the effect of nitric acid on adsorption metabolism of the bacterium.

Table 1. Characteristics of *Pseudomonas sp*

Tests	Result
Gram test	-
Spore	-
Flagella	+
Pigment production	+
Dextrose fermentation	-
Lactose fermentation	-
Maltose fermentation	-
Mannitol fermentation	+
Xylose fermentation	-

Starch hydrolysis	-
Indole production	+
Methyl red	-
Voges-Proskauer	-
Citrate	+
Nitrate reduction	+
Oxidase	+
Gelatin hydrolysis	+
Motility	+
Catalase	+
L. Arginine	+
B-Alanine	+
Shape	Rod-shaped

Note: - negative; + positive

Table 2. Values related to Adsorption kinetics of Pb and Ni (Wet cell mass)

Time (min)	Adsorption ($In\left(\frac{C_o}{C}\right)$)	
	Pb	Ni
0	0	0
5	6.44	6.45
80	6.01	6.03
150	5.91	6.08
250	5.82	5.98
1200	4.56	4.61
1400	2.13	2.22

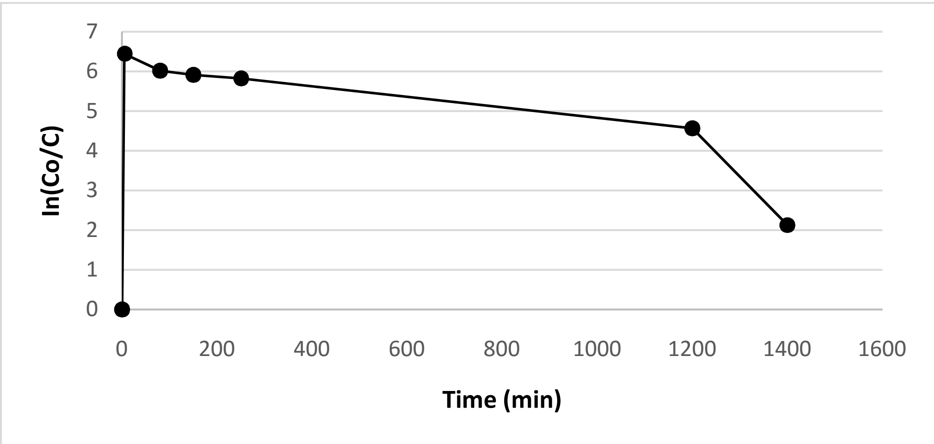

Figure 1. Adsorption kinetic of Pb by *Pseudomonas sp*; Wet cell mass 4% =0.6 g, C_0 =4 mM, agitation speed= 300 rpm, temperature = 23±3°C.

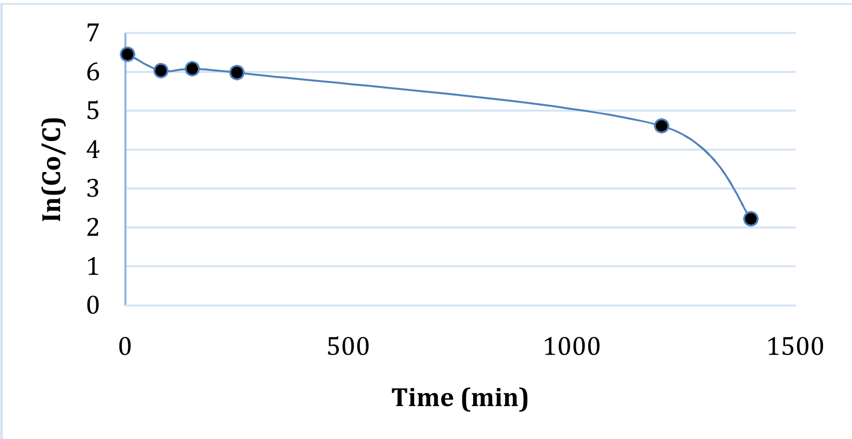

Figure 2. Adsorption kinetic of Ni by *pseudomonas sp;* Wet cell mss 4% =0.6 g, C_0 =4 mM, agitation speed= 300 rpm, temperature = 23±3°C.

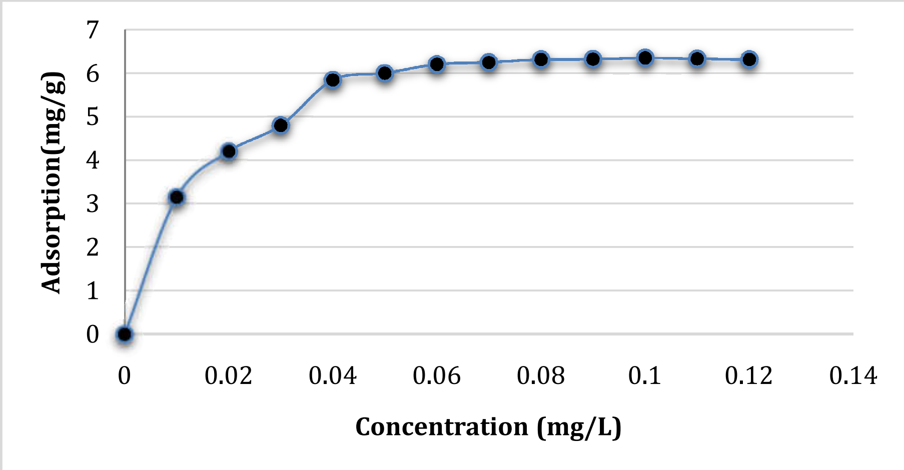

Figure 3. Adsorption isotherm of Pb by *pseudomonas sp;* C_0= 0.01- 1(mmol/L), wet biomass 4%, amount: 20 ml, pH=5, agitation speed: 300 rpm, and temperature: 23±3°C.

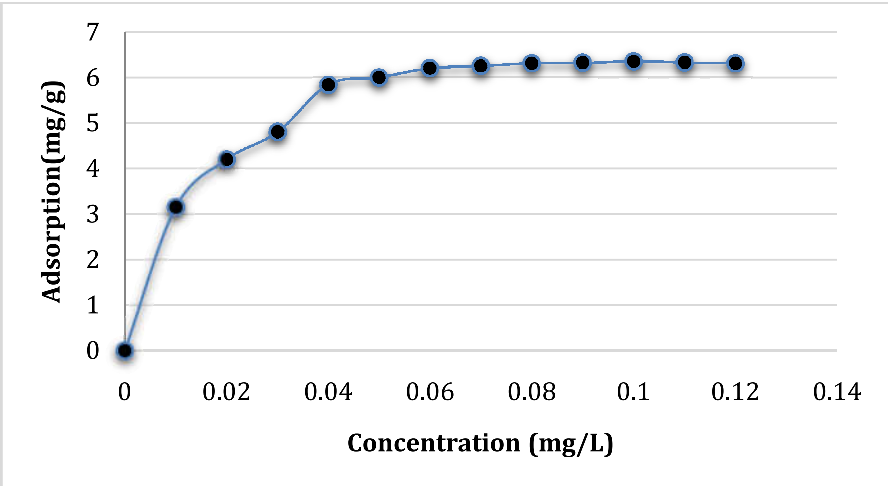

Figure 4. Adsorption isotherm of Ni by *pseudomonas sp*; C_0= 0.01- 1(mmol/L), wet biomass 4%, amount: 20 ml, pH=5, agitation speed: 300 rpm, and temperature: 23±3°C.

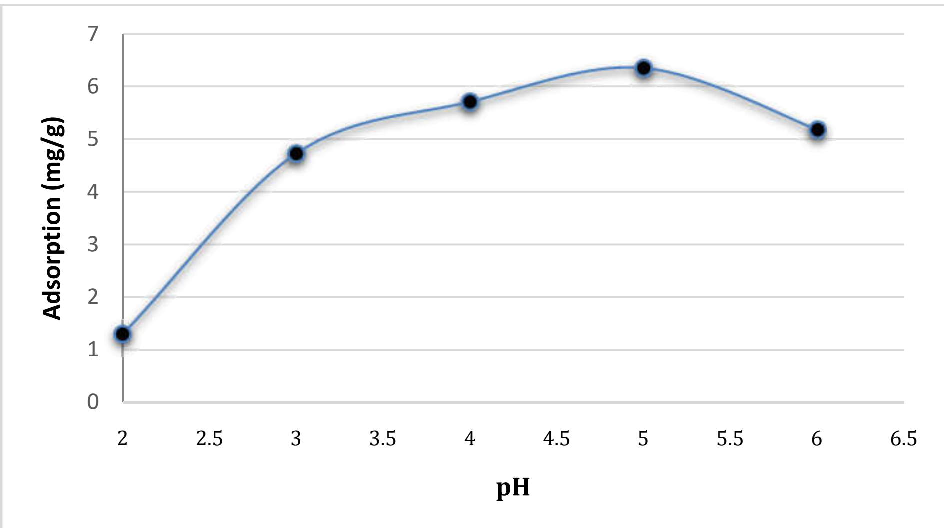

Figure 5. Effect of pH on adsorption of Pb by *pseudomonas sp*

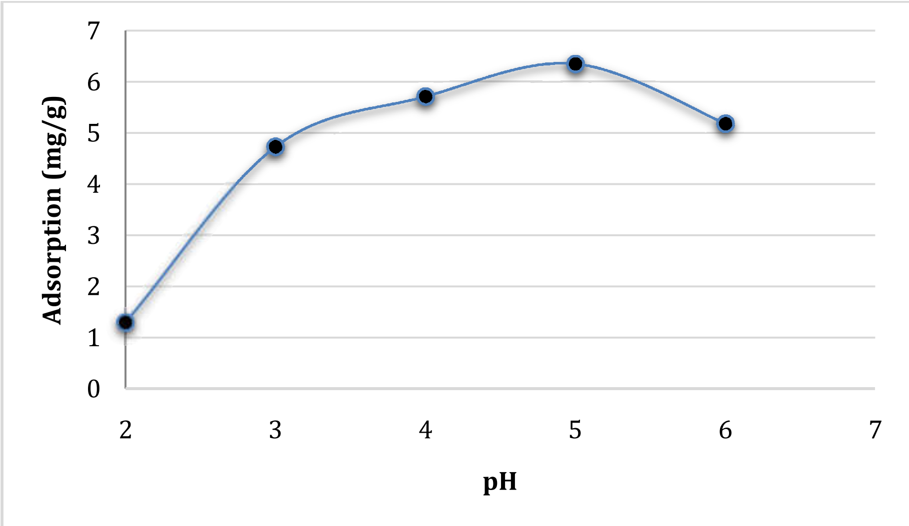

Figure 6. Effect of pH on adsorption of Ni by *pseudomonas sp*

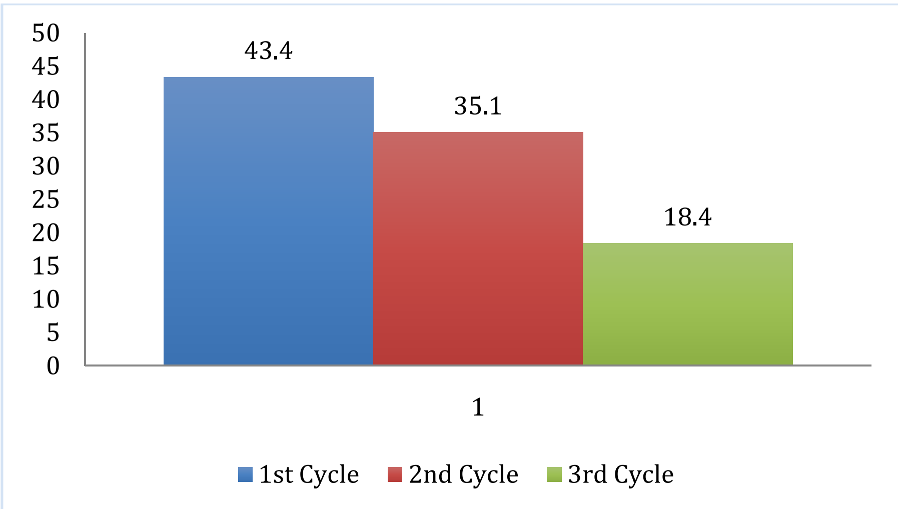

Figure 7. Desorption of Pb ion from *pseudomonas sp* by nitric acid

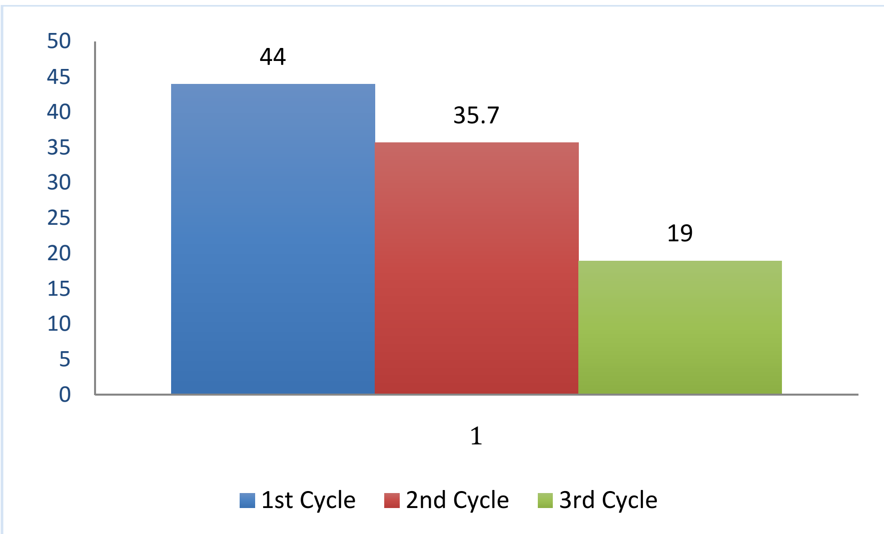

Figure 8. Desorption of Ni ion from *pseudomonas sp* by nitric acid

CONCLUSION

Biosorption technology is cheaper and more efficient and better method. In this study we tried to investigate the biosorption ability of *pseudomonas sp* bacterium in removal of heavy metals Pb and Ni from petrochemical wastewaters. This biosorbent was able to adsorb Pb and Ni with a capacity of 6.35 and 6.44 mg/g dry cell, respectively. According to model simulations of adsorption kinetics and isotherms, cell-surface binding and intracellular accumulation are involved in adsorption of Lead and nickel. the highest adsorption value of pb and Ni by *pseudomonas sp* is occurred in pH=5; In pH ratios higher than 6, due to deposition of Pb and Ni, adsorption rate was decreased. The regenerated biosorbent can achieve its original adsorption capacity after repeated operations for one time. The biosorption-desorption cycle using nitric acid solution were 43.4 and 44% for Pb and Ni. After 3 or 4 times repetition of the cycle, adsorption rate by the bacterium decrease. Hence, the biosorbent seems not to have the potential to be repeatedly used for practical applications. Overall, we can say that *pseudomonas sp* bacterium appears to have the potential to become an effective adsorbent for the removal and recovery of heavy-metals from polluted wastewater. Industrial application of these bacteria is seen as an advantage because in this way there will be no restrictions on the use of living cells. Recovery ability of *pseudomonas sp* can be important for industrial applications in continuous reactors.

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