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Binding Mechanism and Biosorption Characteristics of Fe(III) by *Pseudomonas* sp. cells

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ABSTRACT

Pseudomonas sp. biomass was used to remove Fe(III) ions from aqueous solutions. Optimum values including pH, biomass concentration and biomass-Fe(III) contact time were determined at 2.5, 1.0 g/L, and 25 min respectively. The maximum uptake capacity of *Pseudomonas* sp. biomass for Fe(III) was calculated at 86.206 mg/g using Langmuir isotherm model. The negative values of Gibbs free energy change and enthalpy change confirmed the spontaneous and exothermic nature of Fe(III) biosorption, whereas negative values of entropy change showed the decreased randomness at the solid-solution interface during Fe(III) biosorption. The sorption efficiency of Fe(III) did not affect by the presence of SO_4^{2-}, Cl^-, CO_3^{2-}, NO_3^-, Mg^{2+} and Ca^{2+} ions from 10 to 200 mg/L, whereas Fe(III) sorption was reduced with increasing the concentrations of Cr(VI), Fe^{2+}, Zn^{2+}, Mn^{2+}, Cd^{2+}, Cu^{2+} and Ni^{2+} ions above 20 mg/L. Potentiometric titration and FTIR results confirmed amine and carboxylic groups as the active binding sites for Fe(III) ions. This biomass could be effectively reused up to five cycles, making their application in wastewater detoxification.

Keywords: *Pseudomonas* sp.; Fe(III); biosorption; desorption; biomass characterization

1. INTRODUCTION

Iron is one of the most abundant transition metals of the earth's crust. The elevated iron concentration in natural water may be attributed to the dissolution of rocks and minerals. Many industries such as coatings, car, aeronautic and steel industries produce wastewater containing large quantities of iron (Sag and Kutsal, 1996; Selatnia et al., 2004). Iron exists in two inorganic forms, unstable divalent Fe(II) and stable trivalent Fe(III) ions. Iron enters into water bodies in the form of Fe(II), which can be easily oxidized to Fe(III) ions by the oxygen dissolved in water and then hydrolyse with water (Ahalya et al., 2006). Thus, iron in water is generally present in the trivalent state. Excessive amounts of iron in public water supplies cause turbidity, staining of laundry, plumbing fixtures and have unpleasant taste and odor (Namdeo and Bajpai, 2008; Ostroski et al., 2009). Iron an essential mineral for human, but over dose can cause severe health problems such as anorexia, diarrhea, diphasic shock, metabolic acidosis and death, vascular congestion of the gastrointestinal tract, brain, spleen and thymus (Namdeo and Bajpai, 2008; Yavuz et al., 2005).

The existence of iron species depends on whether the aqueous environment is aerated or polluted with organic wastes. Many of the organic substances have a tendency to reduce the soluble and insoluble ferric iron to ferrous iron. The relationship between redox potential (Eh) and pH is important for understanding the sta-

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bility of iron species in a particular environment and the effectiveness of iron treatment systems. More specifically, higher and lower potentials are responsible for oxidation and reduction of iron in aqueous environment. In acidic solutions, hydrolysis of aqueous Fe(II) yields $Fe(OH)^+$ and $Fe(OH)_2$, whereas in alkaline solutions, anionic complexes such as $Fe(OH)_3^-$ and $Fe(OH)_4^{2-}$ are formed. In the case of iron(III), aqueous Fe(III) easily hydrolyses as pH increases to $Fe(OH)^{2+}$, $Fe(OH)_2^+$, $Fe(OH)_3$, and several polynuclear species such as $Fe(OH)_2^{4+}$ and $Fe(OH)_4^{5+}$, where polynuclear hydrolysis complexes are appeared only at high concentration of dissolved iron. The solubility of $Fe(OH)_3$ increases in alkaline solutions and it is transformed into the readily soluble tetra-hydroxy complex, $Fe(OH)_4^-$. In acidic solution and higher potentials, ferrous iron oxidises to Fe^{3+} and $Fe(OH)^{2+}$. In addition, at higher potentials, $Fe(OH)_2$ also oxidises through magnetite or iron(III) hydroxide, hematite or goethite to Fe(VI), possibly $Fe(OH)_4^-$ (Beverskog and Puigdomenech, 1996).

Heavy metals can be removed from wastewaters by conventional methods including membrane separation, filtration, chemical oxidation or reduction, evaporative recovery, ion exchange or flotation and reverse osmosis, however each treatment technology has been associated with some severe limitations. These technologies are also inconvenient for treatment of industrial effluents with less than 100 mg/L of dissolved metal ions. In recent years, biosorption treatment technology has become one of the alternative treatments to remove heavy metals. Different types of biomass have been used for Fe(III) removal such as Staphylococus xylosus (Aryal et al., 2010), Streptomyces rimosus (Selatnia et al., 2004), Thiobacillus ferroxidans (Banerjee, 2007), Rhizopus arrhizus (Sag and Kutsal, 1998), Microcystis sp. (Pradhan et al., 2007), Chlorella vulgaris (Aksu and Acikel, 2000), Physalis philadelphica Lam (García-Mendieta et al., 2012), Chitin (Karthikeyan et al., 2005), Cajanus cajan (Ahalya et al., 2007), Cicer arientinum (Ahalya et al., 2006), Polyporus squamosu (Razmovski and Sciban, 2008), Olive Cake (Al-Anber and Al-Anber, 2008), Orange peel (Lugo-Lugo et al., 2012), Chicken eggshells (Yeddou and Bensmaili, 2007), maize cobs and palm fruit bunch (Nassar et al., 2004).

The main objective of this study was to investigate the potential of *Pseudomonas* sp. biomass to sorb Fe(III) from aqueous solutions. Langmuir and Freundlich isotherm models were used to describe the equilibrium data, whereas thermodynamic parameters were calculated in order to determine the Fe(III) biosorption nature. The interference and competitive phenomena of co-presence ions for sorption sites were studied. Potentiometric titration and FTIR were used to confirm the interaction of biomass surface functional groups for Fe(III) ions. In addition, desorption and regeneration studies were also carried out.

2. MATERIALS AND METHODS

2.1 Bacteria and media

Pseudomonas sp., a gram-negative bacterium was isolated from contaminated soil in a mining industry near Stratoni, Chalkidiki, Greece. According to our previous publications (Aryal et al., 2010; Aryal et al., 2011; Aryal and Liakopoulou-Kyriakides, 2011; Aryal et al., 2012), it was cultivated in Luria-Bertani broth containing 1.0% tryptone, 0.5% yeast extract and 0.5% NaCl (Scharlau Chemie S. A., Barcelona, Spain) at 30 °C, using rotary shaker of an incubator at 180 rpm (Sanyo, MIR-153, Osaka, Japan). Cells were harvested by centrifugation (Kubota 5922, Tokyo, Japan) at 2000 g for 20 min at the static phase of growth after 24 h of incubation and autoclaved (Systec, Greiz, Germany) at 121°C for 20 min before their use. Moisture content was

determined by drying a pre-weighted amount of the cells in an oven (Heraeus KT 5050, West Midlands, U. K.) at 100 °C for 10 h.

2.2 Biosorption experiments

Fe(III) stock solution at 1000 mg/L was prepared from $FeCl_3$ (Merck, Darmstadt, Germany) in distilled water containing 20 ml HCl (12M). The pH was investigated in the range of 1.0 to 2.5 at biomass concentration of 1.0 g/L, initial Fe(III) concentration of 100 mg/L and contact time 24 h. The biomass concentration was conducted from 1.0 to 6.0 g/L at pH 2.5, initial Fe(III) concentration of 100 mg/L and contact time 24 h respectively. Kinetic study of Fe(III) sorption was performed at initial Fe(III) concentration of 100 mg/L, pH 2.5, biomass concentration of 1.0 g/L up to 120 min. The effect of initial Fe(III) concentration on the retaining capacity was studied from 10 to 400 mg/L at pH 2.5, biomass concentration of 1.0 g/L and contact time 30 min respectively. Effect of temperature was also carried out in the range of 20 to 40 °C on rotary shaker of an incubator under 180 rpm.

2.3 Effect of interfering ions on Fe(III) biosorption

Reference solutions of SO_4^{2-}, Cl^-, CO_3^{2-}, NO_3^-, Mg^{2+}, Ca^{2+}, Cr(VI), Fe^{2+}, Zn^{2+}, Mn^{2+}, Cd^{2+}, Cu^{2+} and Ni^{2+} were prepared from Na_2SO_4, NaCl, Na_2CO_3, Na_2NO_3, $MgSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 2H_2O$, $K_2Cr_2O_7$, $FeSO_4 \cdot 7H_2O$, $ZnSO_4 \cdot 7H_2O$, $MnSO_4 \cdot H_2O$, $Cd(NO_3)_2 \cdot 4H_2O$, $Cu(C_2H_3O_2)_2 \cdot H_2O$ and $NiSO_4 \cdot 6H_2O$ (Merck, Darmstadt, Germany). The effect of interfering ions ranging from 10 to 200 mg/L on Fe(III) biosorption at 100 mg/L was carried out using the same procedures previously adopted in biosorption experiments.

2.4 Biomass characterization

Potentiometric titration was performed by the addition of 0.1 ml 0.1 M NaOH to 1.0 g/L of biomass in deionised water. Suspension pH was measured following titrant addition, starting from an initial value of 2.50, until equilibrium state was reached, using a Denver pH-meter with a Mettler Toledo Inlab Easy pH electrode (USA).

The surface functional groups of *Pseudomonas* sp. biomass before and after sorption of Fe(III) ions were examined using Fourier Transform Infrared Spectrophotometer (Equinox 55, AXS Bruker, USA). Potassium bromide disks were prepared by mixing 1 mg of lyophilized samples with 200 mg KBr, and the spectra were recorded at wave number ranging from 400 to 4000 cm^{-1} with a resolution number of 2 cm^{-1}.

2.5 Desorption and regeneration studies

Biosorption experiments of Fe(III) on *Pseudomonas* sp. cells with initial Fe(III) concentration of 100 mg/L at pH 2.5, biomass concentration 1.0 g/L and contact time 30 min were carried out first. Then, Fe(III)-loaded biomass was suspended using 0.3 M HNO_3, where solid to liquid ratio was kept equal to 1.0 g/L. In order to investigate the possible reusability of the biomass, the same biomass was used up to five consecutive sorption -desorption cycles.

2.6 Equilibrium modeling

The theoretical basis of Langmuir equation relies on the assumption that there are a finite number of binding sites, which are homogeneously distributed over the adsorbent surface, having the same affinity for adsorption of a single molecular layer and there is no interaction between adsorbed molecules (Langmuir, 1918). The mathematical description of this model is given by following equation:

$$Q_e = \frac{Q_{\max} \cdot b \cdot C_e}{1 + b \cdot C_e} \quad (1)$$

Where C_e is the residual metal concentration (mg/L), Q_e is the amount of metal sorbed (mg/g) at equilibrium, Q_{max} is the maximum uptake capacity corresponding to sites saturation (mg/g) and b is the biomass metal binding affinity (L/mg).

The essential characteristics of the Langmuir isotherm can be expressed by a separation or equilibrium parameter, a dimensionless constant, which is defined by equation (Hall et al., 1966):

$$K_L = \frac{1}{(1+bC_e)} \quad (2)$$

The value of K_L indicates the type of the isotherm. The biosorption is considered as unfavourable when $K_L > 1$, linear when $K_L = 1$, favourable when $0 < K_L < 1$) and irreversible when $K_L = 0$.

The Freundlich equation is the empirical relationship, whereby it is assumed that the sorption energy of a metal binding to a site on an adsorbent depends on whether the adjacent sites are already occupied or not (Freundlich, 1906). This empirical equation has the form of:

$$Q = K_f \cdot (C_e)^{1/n} \quad (3)$$

Where K_f and n are the constants describing sorption capacity (mg/g) $(L/mg)^{1/n}$ and sorption intensity respectively.

2.7 Thermodynamic modeling

Standard Gibbs free energy change (ΔG^o) indicates the degree of spontaneity of the sorption process. The value of ΔG^o can be calculated from the following mathematical expression:

$$\Delta G^o = -RT \ln K_c \quad (4)$$

Where K_c is the distribution coefficient (Q_e/C_e). The values of Q_e and C_e were obtained from equilibrium data of 100 mg/L of Fe(III) ions from 20 to 35 °C respectively.

The change in heat of sorption (ΔH^o) and entropy (ΔS^o) are related to standard Gibbs free energy change (ΔG^o), according to the following equation:

$$\Delta G^o = \Delta H^o - T\Delta S^o \quad (5)$$

Equations (4) and (5) can be combined as:

$$\ln K_c = -\frac{\Delta H^o}{RT} + \frac{\Delta S^o}{R} \quad (6)$$

Where ΔH^o and ΔS^o can be obtained from the slope and intercept of a plot of $\ln K_c$ versus $1/T$.

2.8 Determination of Fe(III) concentration

Fe(III) concentration was determined at 530 nm based on the deep purple color complex formed between Fe(III) and sodium salicylate using spectrophotometer (Shimadzu UV-160A, Kyoto, Japan) (Snell and Snell, 1959). The values reported in this study were obtained from the average of triplicate analysis for each sample.

3. RESULTS AND DISCUSSION

3.1 Effect of pH

The pH of the aqueous solution has been considered as one of the most important factors influencing the biosorption processes. It influences not only the dissociation of functional groups on the active sites of the biosorbent but also the solution metal ions chemistry. Almost all iron(III) was present as ionic form of Fe^{3+} in aqueous solution up to pH 1.0 and its concentration decreased followed to pH 3.0 (Lugo-Lugo et al., 2012), but it started to precipitate as $Fe(OH)_3$ above pH 2.5 (Aksu and Gulen, 2002). The $Fe(OH)^{2+}$ species began to form above pH 1.0 and were mostly found pH between 2.0 and 2.5 (Tapia et al., 2011; Welham

et al., 2000). On the other hand, $Fe(OH)_2^+$ species also started to form at pH above 2.0 and predominated around pH 3.0 (Lugo-Lugo et al., 2012), whereas $Fe(OH)_4^-$ was the dominant species in a strong alkaline medium (Beverskog and Puigdomenech, 1996; Welham et al., 2000). Here, pH values higher than 2.5 were not investigated due to the reduced solubility and precipitation of Fe(III) ions as insoluble hydroxides (Aksu and Gulen, 2002).

Figure 1 shows that biosorption efficiency of Fe(III) increased from 20.96 to 66.63% on *Pseudomonas* sp. biomass with increasing initial pH from 1.0 to 2.5. This increase in Fe(III) sorption efficiency may be attributed to the increase in negatively charged surface groups with subsequent increase in attraction for positively charged $Fe(OH)^{2+}$ ions. Similar pH value at 2.5 for Fe(III) biosorption on Kaolin treated Escherichia coli (Quintelas et al., 2009), Cajanus cajan (Ahalya et al., 2007), Cicer arientinum (Ahalya et al., 2006), Rhizopus arrhizus (Aksu and Gulen, 2002) and Chlorella vulgaris (Sag and Kutsal, 1998) has also been reported. Therefore, further Fe(III) biosorption experiments were carried out at pH 2.5 in order to check the precipitation of Fe(III) ions as their hydroxides.

3.2 Effect of biomass concentration

The effect of biomass concentrations on Fe(III) biosorption is depicted in Figure 2. The results indicated that the percentage removal of Fe(III) increased from 63.26% to 98.596% with increase in biomass concentration from 1.0 to 6.0 g/L. As shown in Figure 2, a lower increment of Fe(III) biosorption percentage was observed above 1.0 g/L of biomass concentration. Increase in sorption efficiency with increasing biomass concentration was probably due to the increase in the number of binding sites, whereas a lower increment of Fe(III) biosorption with an increase in biomass concentration at higher than 1.0 g/L in a fixed volume may be due to the partial cell aggregation that occurs at high biomass concentrations as well as to strong limitations of Fe(III) species mobility in the biosorption medium, leaving some binding sites unsaturated (Aryal et al., 2010).

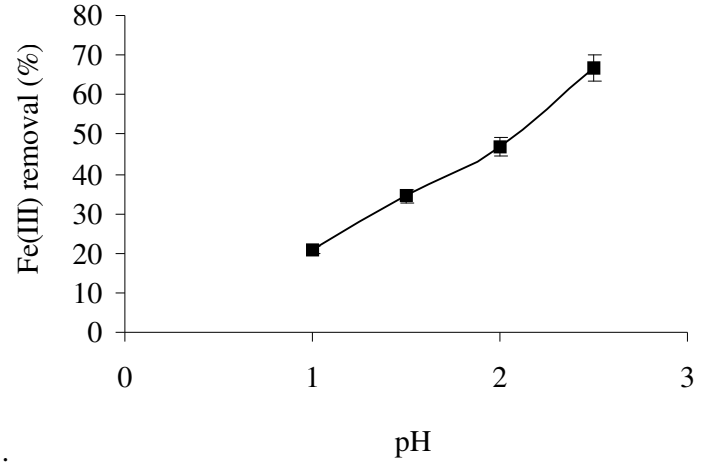

Figure 1 Effect of pH on Fe(III) biosorption using *Pseudomonas* sp. at initial Fe(III) concentration 100 mg/L, biomass concentration 1.0 g/L and contact time 24 h respectively

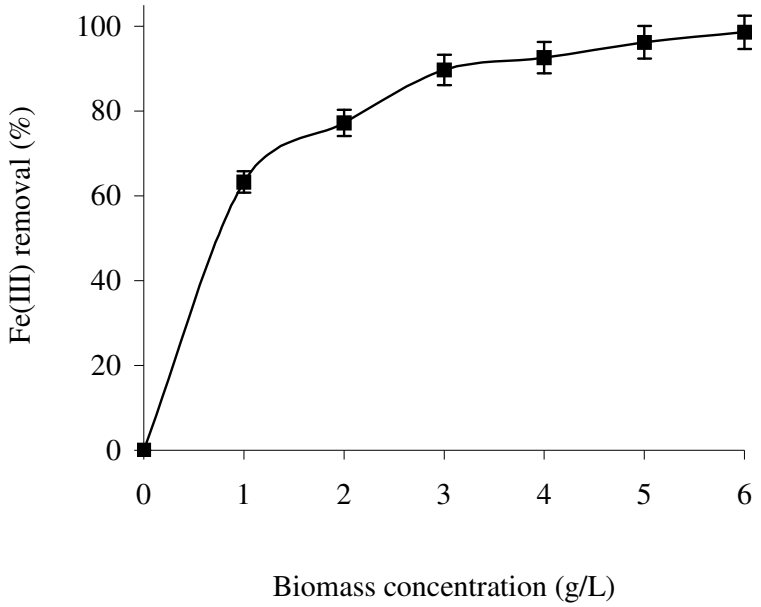

Figure 2 Effect of biomass concentration on Fe(III) biosorption using *Pseudomonas* sp. at initial Fe(III) concentration 100 mg/L, pH 2.5 and contact time 24 h respectively

3.3 Effect of contact time

The effect of contact time for Fe(III) removal by *Pseudomonas* sp. biomass is given in Figure 3. It was found that Fe(III) sorption was very fast in the initial period of 2 min and then slowed down, where equilibrium was reached at 25 min. Further increase in contact time above 25 min did not result in increase in Fe(III) sorption efficiency. The fast biosorption of Fe(III) ions on biomass surfaces may be due to the occupation of high affinity binding sites, whereas the relatively slow biosorption kinetics after initial period may reflect the relative inaccessibility of the remaining binding sites (Aryal et al., 2010).

3.4 Biosorption isotherm studies-Effect of temperature

The effect of initial Fe(III) concentrations on equilibrium uptake capacity at different temperatures is shown in Figure 4. As it can be seen from this figure, the sorbed Fe(III) per unit mass of *Pseudomonas* sp. biomass increased upon increase in initial Fe(III) concentrations. This initial Fe(III) concentration may provide the necessary driving force to overcome the resistance to the mass transfer of Fe(III) species between aqueous and solid phases (Aryal et al., 2010). The results showed that high Fe(III) concentrations may enhance the interaction between Fe(III) ions and biomass, resulting to the higher biosorption capacity of Fe(III).

The calculated isotherm constants and correlation coefficients for Fe(III) sorption at different temperatures are presented in Table 1. The high correlation coefficient (R^2) values indicated that Langmuir isotherm model well described the Fe(III) equilibrium data. In addition, K_L values were determined in the range of 0 to 1, indicating that Fe(III) sorption process is favorable (Hall et al., 1966). On the other hand, relatively low correlation coefficient values suggest that Fe(III) biosorption did not follow Freundlich isotherm model.

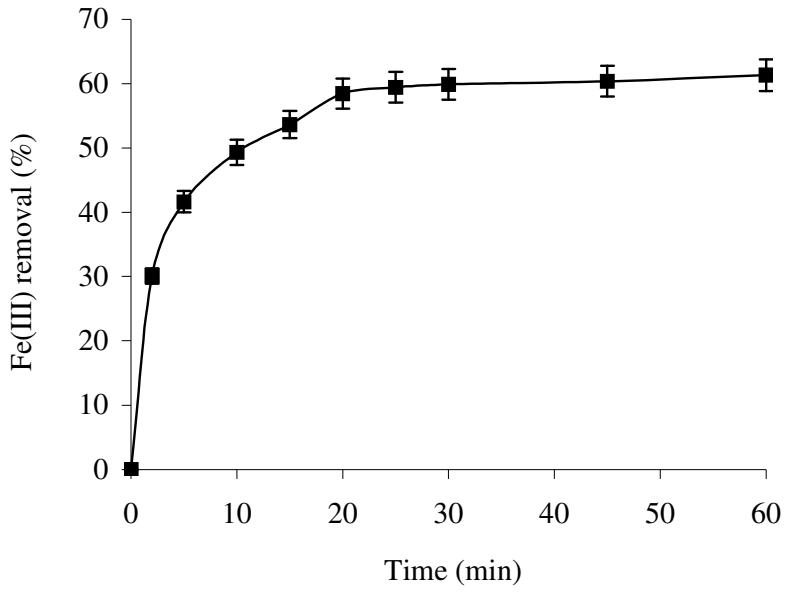

Figure 3 Effect of contact time on Fe(III) biosorption using *Pseudomonas* sp. biomass with initial Fe(III) concentration 100 mg/L, pH 2.5 and biomass concentration 1.0 g/L respectively

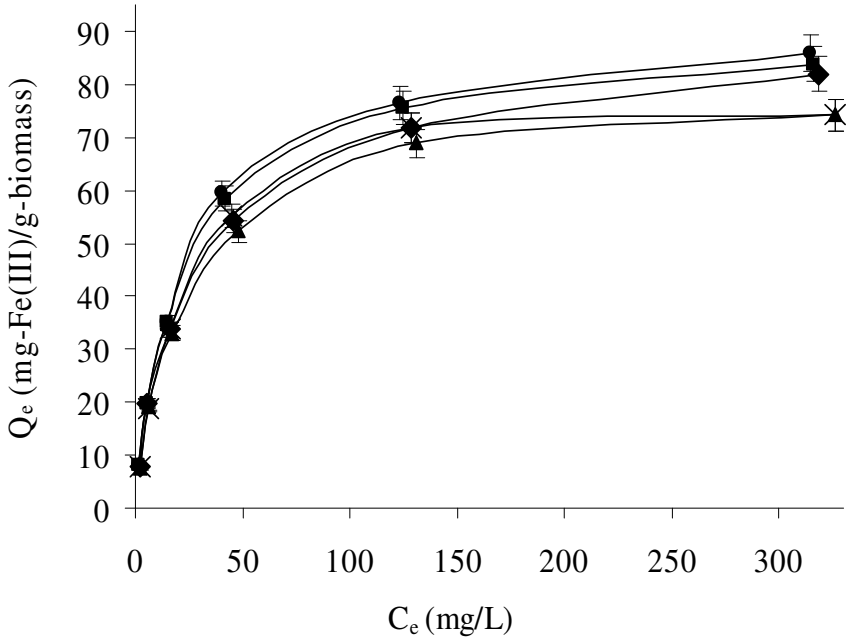

Figure 4 Effect of initial Fe(III) concentration on equilibrium uptake capacity of *Pseudomonas* sp. biomass at pH 2.5, biomass concentration 1.0 g/L and contact time 30 min at 20; (▲), 25; (♦), 30; (■), 35; (●) and 40 °C; (×) respectively

Table 1 Isotherm parameters of Fe(III) sorption onto *Pseudomonas* sp. biomass at pH 2.5, biomass concentration 1.0 g/L and contact time 25 min respectively

Model/Parameters	20 °C	25 °C	30 °C	35 °C	40 °C
Langmuir isotherm model					
Q_{max} **(mg/g)**	80	81.967	84.745	86.206	81.300
b **(L/mg)**	0.069	0.067	0.056	0.056	0.049
R^2	0.990	0.996	0.998	0.998	0.999
K_L	0.029-0.901	0.022-0.889	0.024-0.904	0.024-0.905	0.028-0.903
Freundlich isotherm model					
K_f	8.788	9.180	9.822	9.722	8.995
n	2.723	2.583	2.582	2.553	2.640
R^2	0.866	0.896	0.881	0.894	0.863

As it can be seen from Table 1, maximum uptake capacity of Fe(III) ions increased with increase in temperature from 20 to 35 °C and decreased upon further increase in temperature from 35 to 40 °C. This enhancement of sorption capacity with increasing temperature from 20 to 35 °C may be attributed to the acceleration of some originally slow sorption steps or increase in some new active sites due to bond rupture, whereas decrease in uptake capacity above 35 °C may be due to the exothermic nature of Fe(III) biosorption, resulting to the destruction of some binding sites (Aryal and Liakopoulou-Kyriakides, 2011).

A comparison of maximum uptake capacity obtained in this study with other biosorbents is presented in Table 2. The higher uptake capacity of *Pseudomonas* sp. biomass for Fe(III) compared to other biosorbents, suggested its application in wastewater detoxification. *Pseudomonas* sp. has been already used for the removal of other metal ions with very high uptake capacities at 508 mg-Ni^{2+}/g- *Pseudomonas* sp. (Gialamouidis et al., 2009) and 278 mg-Cd^{2+}/g-*Pseudomonas* sp. (Ziagova et al., 2007).

3.5 Effect of interfering co-ions

Table 3 shows the effect of co-existing ions on Fe(III) sorption using *Pseudomonas* sp. biomass. As it can be seen, in all binary mixtures, where SO_4^{2-}, Cl^-, CO_3^{2-}, NO_3^- Mg^{2+} and Ca^{2+} were present, there was great selectivity towards Fe(III) ions, possibly due to their inability to chelate the surface functional groups. On the other hand, Fe(III) sorption efficiency reduced significantly with increasing the concentration of co-presence Cr(VI), Fe^{2+}, Zn^{2+}, Mn^{2+}, Cd^{2+}, Cu^{2+} and Ni^{2+} ions above 20 mg/L, may be due to their antagonistic action for the same sorption sites on the bacterial cell wall.

3.6 Biosorption thermodynamic studies

The change in standard Gibbs free energy (ΔG^o) was calculated at -26.68, -26.65, -26.60 and -26.53 kJ/mol for Fe(III) sorption on *Pseudomonas* sp. biomass at 20, 25, 30 and 35 °C respectively. The negative values of ΔG^o at all temperatures used confirm that Fe(III) biosorption was spontaneous and thermodynamically favorable. The negative values of standard enthalpy change (ΔH^o) at -29.19 kJ/mol indicated the exothermic nature of Fe(III) sorption, whereas negative standard entropy change (ΔS^o) at -8.52 J/mol/K may be interrelated to the decreased randomness at solid-liquid interface during the Fe(III) biosorption.

Table 2 Comparison of Fe(III) uptake capacity with other biosorbents

Biosorbent	Q_{max} (mg/g)	References
EPS of *Acidiphilium* sp.	536.1	Tapia et al. (2011)
Microcystis sp.	303.03	Pradhan et al. (2007)
Streptomyces rimosus	125	Selatnia et al. (2004)
Pseudomonas sp.	86.20	This study
Cicer arientinum	72.16	Ahalya et al. (2006)
Staphylococus xylosus	69	Aryal et al. (2010)
Cajanus cajan husk	66.65	Ahalya et al. (2007)
Olive cake	58.47	Al-Anber and Al-Anber (2008)
Thiobacillus ferroxidans	34.65	Banerjee (2007)
Rhizopus arrhizus	33.99	Aksu and Gulen (2002)
Polyporus squamosus	31.2	Razmovski and Šćiban (2008)
Chlorella vulgaris	24.49	Aksu and Acikel (2000)
Physalis philadelphica Lam	19.83	García-Mendieta et al. (2011)
Orange peel	18.19	Lugo-Lugo et al. (2012)
Kaolin treated *Escherichia coli*	16.5	Quintelas et al. (2009)
Chicken eggshells	8.73	Yeddou and Bensmaili (2007)
Maize cobs	2.5	Nassar et al. (2004)
Palm fruit bunches	1.98	Nassar et al. (2004)
Chitin	1.37	Karthikeyan et al. (2005)

Table 3 Effect of interfering ions on Fe(III) biosorption at initial Fe(III) concentration of 100 mg/L, pH 2.5, biomass concentration 1.0 g/L and contact time 30 min respectively

Co-ions	Fe(III) removal (%) with different co-ions concentration				
	10 mg/L	20 mg/L	50 mg/L	100 mg/L	200 mg/L
SO_4^{2-}	56.74	51.11	48.66	57.26	55.80
Cl^-	52.88	53.10	58.00	53.29	55.42
CO_3^{2-}	58.33	60.01	61.00	55.60	57.16
NO_3^-	57.00	51.90	56.43	54.11	58.81
Cr(VI)	48.00	39.67	29.22	22.09	15.81
Mg^{2+}	55.02	58.81	60.19	60.24	59.12
Ca^{2+}	58.90	54.76	51.11	58.66	61.92
Fe^{2+}	55.98	56.30	43.12	32.19	29.03
Zn^{2+}	53.97	46.81	37.71	30.02	25.73
Mn^{2+}	54.41	43.96	33.27	28.53	22.17
Cd^{2+}	55.05	40.00	35.90	26.51	20.35
Cu^{2+}	50.79	38.12	31.99	22.87	16.93
Ni^{2+}	56.27	40.14	33.78	23.33	19.65

3.7 Potentiometric titration and FTIR spectral analysis

In order to understand the surface binding mechanism, it is important to identify the functional groups present on the biomass surface. The pK_a values of corresponding surface functional groups were determined by inflection points of the potentiometric titration curve resulting from the addition of NaOH (Figure 5). The inflection points were observed for *Pseudomonas* sp. biomass at 4.27 ± 0.2, 5.61 ± 0.1, 7.36 ± 0.3, 8.92 ± 0.2 and 10.1 ± 0.1 corresponding to pK_a values at 4.27, 5.61, 7.36, 8.92 and 10.1 respectively. The pK_a values at 4.27, 5.61, 7.36, 8.92 and 10.1 are possibly carboxyl, phosphoryl, phosphate, amine and hydroxyl groups (Aryal et al., 2012; Barkleit et al., 2009; Chojnacka et al., 2005; Yee et al., 2004) and are potential metal-binding sites for Fe(III) ions in the present biomass.

Figure 6 (a and b) shows the FTIR spectra of *Pseudomonas* sp. biomass before and after Fe(III) sorption. The strong broad bands in the region of 3500-3300 cm^{-1} correspond to the N-H stretching in primary NH_2 groups. The sorption peaks at 2940, 1660 and 1550 cm^{-1} may be due to the C-H bond of alkanes, amide I (C=O stretching) and amide II groups. Peak positions at 1460 cm^{-1} may correspond to -OH bending in carboxyl groups, whereas peaks at 1380 and 1400 cm^{-1} may be attributed to COO^- present on the biomass surface. In addition, C-O-C stretching appeared at 1250 cm^{-1} and P-O-C links of the organic phosphate groups at 1080 cm^{-1} (Lambert et al., 1998). The results showed that transmittance wave number at 3370 and 1380 cm^{-1} have been shifted to 3385 and 1400 cm^{-1} after Fe(III) sorption, suggesting the amine and carboxylic groups are mainly participated for Fe(III) ions interaction on *Pseudomonas* sp. biomass surface.

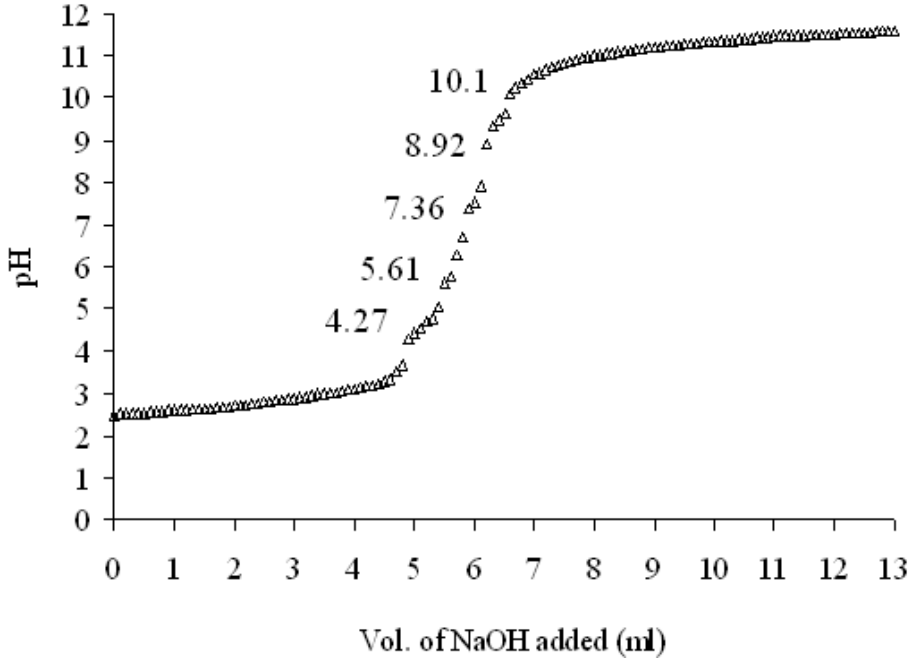

Figure 5 Potentiometric titration curve of *Pseudomonas* sp. biomass resulting from the addition of NaOH

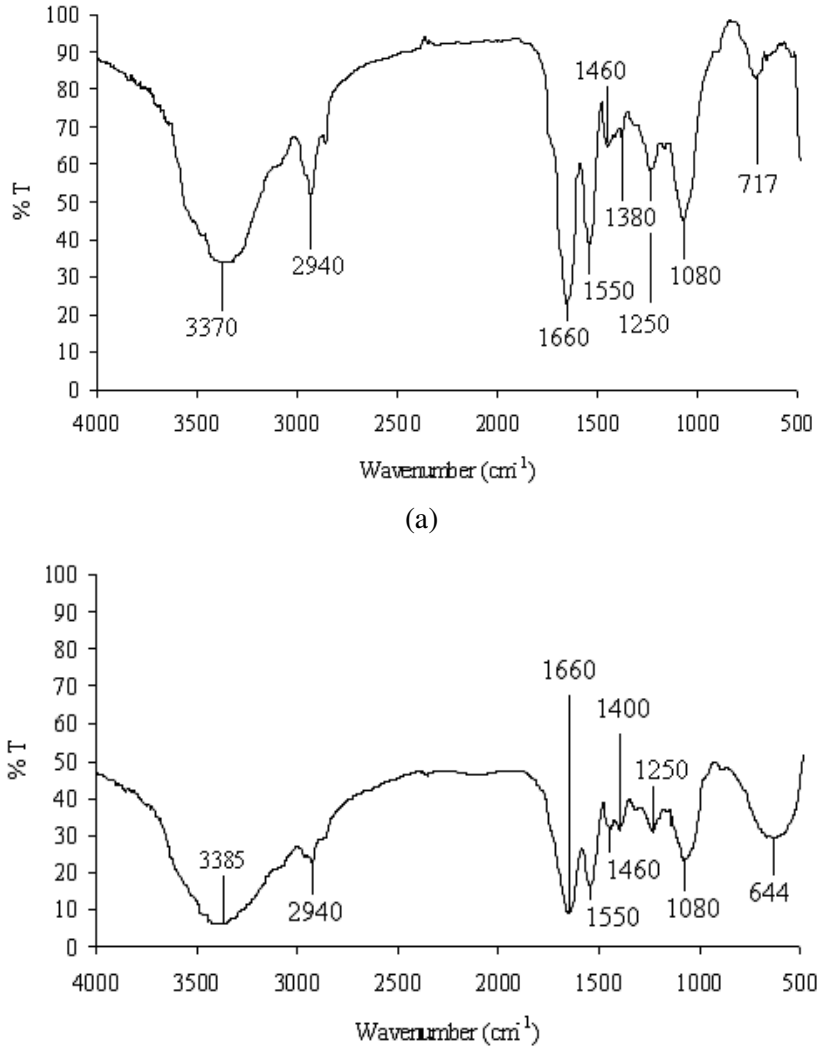

Figure 6 FT-IR spectra of *Pseudomonas* sp. (a) raw biomass and (b) after Fe(III) biosorption respectively

3.8 Binding mechanism

The existence of Fe(III) on biomass surface depended on pH of biosorption medium. Fe(III) mostly existed as $Fe(OH)^{2+}$ species around pH 2.5. FITR spectra revealed that carboxylic and amine groups were responsible for Fe(III) binding. The strong pH sorption behavior may indicate the metal binding by carboxylic sites. Carboxylic groups existed as carboxylate anions due to the deprotonation and these anions of biomass surface could be responsible for electrostatic interaction with positively charged $Fe(OH)^{2+}$ species. One mole of $Fe(OH)^{2+}$ may be formed on biomass surface after reaction of two moles of carboxylate anions (Aryal et al., 2010; Tapia et al., 2011). On the other hand, iron(III) generally acts as a harder Lewis acid, since it can accept the lone pairs of electron, whereas amine group acts as a strong Lewis base due to the tendency to donate the lone pair of electron from N-atom and this interaction of lone pair of electron from N-atom to Fe(III)-atom is considered to

be stronger than electrostatic attractions. Thus, possible mechanism of Fe(III) binding with carboxylic and amine groups on biomass surface is given by following reactions:

$$R\text{-}COOH \longleftrightarrow R\text{-}COO^- + H^+$$

$$2R\text{-}COO^- + Fe(OH)^{2+} \longleftrightarrow (R\text{-}COO^-)_2\text{-}Fe(OH)^{2+}$$

$$R\text{-}NH_2 + H^+ \longleftrightarrow R\text{-}NH_3^+$$

$$R\text{-}NH_3^+ + Fe(OH)^{2+} \longleftrightarrow [R\text{-}NH_2\text{:}Fe(OH)]^{2+} + H^+$$

Fe(III) atom can coordinate three to eight ligands and often exhibits an octahedral coordination because of free d-orbitals presence in its electronic structure ($[Ar]4S^0 3d^5$). Gram-negative cell walls contain a thin layer of peptidoglycan compared to gram-positive cells and have a complex outer membrane but they do not include teichoic and teichuronic acid constituents. The outer membrane contains phospholipids, lipoproteins, lipopolysaccharides, and various proteins. The three- dimensional network structure of peptidogly can may responsible for metal ions binding. Fe(III)-PG complex may be formed by two carboxylic groups with additional amine coordination (Texier et al., 2000). Therefore, the possible structure of the compound obtained by the interaction between $Fe(OH)^{2+}$ ion and biomass surface functional groups may be represented by following scheme:

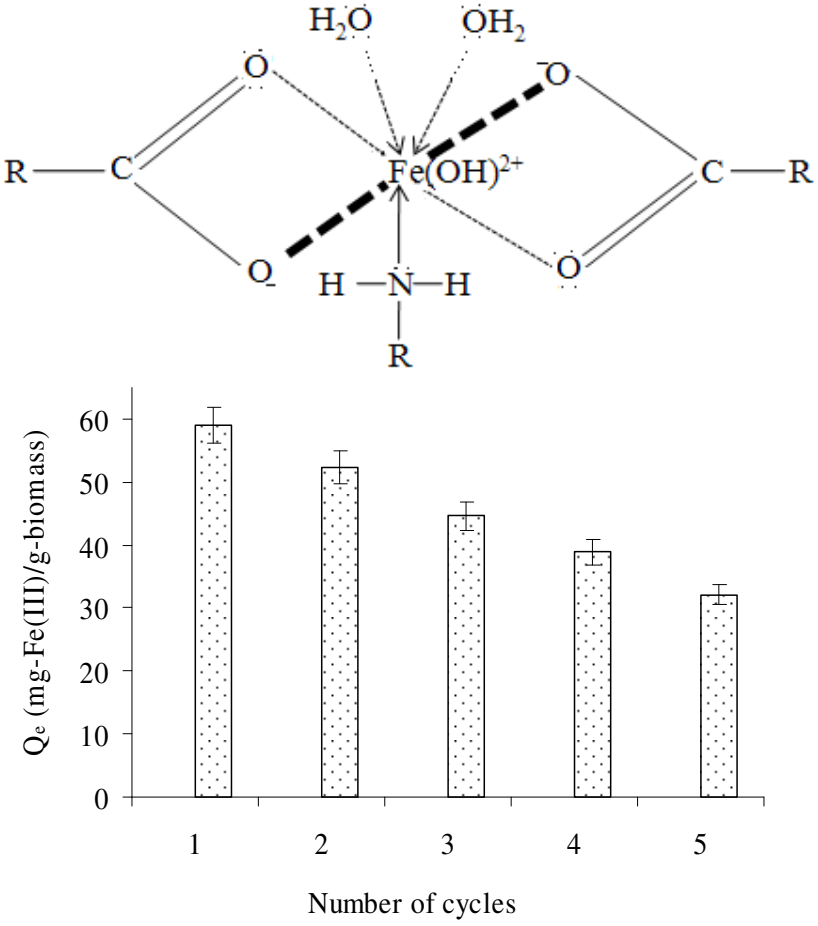

Figure 7 Effect of subsequent adsorption-desorption cycles using *Pseudomonas* sp. with 0.3 M HNO_3 at initial Fe(III) concentration 100 mg/L, pH 2.5 and biomass concentration 1.0 g/L respectively

3.9 Fe(III) desorption and regeneration of biomass

The effect of subsequent sorption-desorption cycles with each regenerating system is depicted in Figure 7. The first desorption of Fe(III) ions from Fe(III)-loaded *Pseudomonas* sp. biomass was determined at 59.03 mg/g. The observed loss of biomass binding capacity after the first cycle may be attributed to the biomass deterioration caused by subsequent acid treatments and also weight lost by subsequent adsorption-desorption cycles (Aryal et al., 2010).

CONCLUSIONS

Pseudomonas sp. biomass showed higher uptake capacity for Fe(III) ions from aqueous solutions compared to other biosorbents reported so far in the literature, suggesting its application in wastewater detoxification. Fe(III) equilibrium sorption data were well followed by Langmuir isotherm model than Freundlich isotherm model. The negative ΔG^{o} and ΔH^{o} values showed the spontaneous and exothermic sorption process, whereas negative ΔS^{o} values indicated the decreased randomness at solid/solution interface during Fe(III) sorption. Competitive biosorption experiments indicated that *Pseudomonas* sp. biomass can be effectively used for Fe(III) removal from municipal and industrial wastewater. Potentiometric titration and FTIR spectroscopy results showed that amine and carboxylic groups are responsible for Fe(III) ions binding. This biomass can be used up to five cycles as efficient and economic biosorbent for removal and recovery of the Fe(III) from polluted waters.

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