BIOSORPTION OF IRON BY HEAVY-METAL TOLERANT *Micrococcus* sp.

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ABSTRACT
The presence of metal ions in aqueous solutions represents, nowadays a major environmental problem. The present study investigates the potential adsorption capacity of Micrococcus sp. for the removal of iron (III) ions from aqueous solution. The adsorbent was characterized using FTIR analysis, in order to reveal the presence of functional groups. Batch adsorption experiments were carried out to evaluate the effect of process parameters such as pH, contact time, initial iron concentration, temperature and adsorbent dosage on removal capacity. The optimum removal of iron (III) ions was obtained at pH 6 with 94.2%, adsorbent dose of 0.5g with 92.5% and equilibrium time of 30 min with 88.6%. Kinetic data were successfully described with pseudo-first order, pseudo-second order and elovich models. The results show that the biosorption process of Fe (III) followed pseudo second order with $R^2 = 0.999$. The equilibrium data was further tested using Langmuir, Freundlich and Temkin models, and was found to follow the Langmuir isotherm ($R^2 = 0.978$).Thermodynamic parameters such as ΔG^o, ΔH^o and ΔS^o were evaluated and it has been found that the biosorption process was feasible, spontaneous and exothermic in nature.
Keywords: Bacteria, Biosorption, Iron, Isotherm, Kinetic

INTRODUCTION
Heavy metal pollution occurs directly by effluent outfall from industries, refineries and waste treatment plants and indirectly by the contaminants that enter the water supply from soil/ground water systems and from the atmosphere via rain water. Among toxic substance reaching hazardous levels are heavy metals (Vieira & Volesky, 2000). Heavy metals are the group of contaminants of concern, which comes under the inorganic division. Exposure to heavy metals has been linked with developmental retardation, various cancers, kidney damage, and even death in some instances of exposure to very high concentrations (Ahmaruzzaman, 2011; Gawali *et al.*, 2014). Heavy metals do not degrade into harmless end products and are generally accumulated along the food chain (water–plants–animals–humans), thus, posing a great threat to living organisms (Benaisa *et al.*, 2016).

Metals play an integral role in the life processes of living organisms. Heavy metals are metals with densities higher than 5 g/cm^3. Some metals (Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni and Zn) are essential, they serve as micronutrients and are used for redox-processes, to stabilize molecules through electrostatic interactions; as components of various enzymes; and regulation of osmotic pressure. While many other metals (e.g., Ag, Al, Cd, Au, Pb, and Hg) have no biological role and they are nonessential. They are potentially toxic to living organisms, especially microorganisms. Toxicity of nonessential metals occurs through the displacement of essential metals from their native binding sites or through ligand interactions. Heavy metals in wastewater come from industries and municipal sewage, and they are one of the main causes of water and soil pollution (Ashok *et al.*, 2010). These practices leave persistent toxic heavy metals like chromium, nickel, lead, zinc,

cadmium and copper, which tend to accumulate and deteriorate the environment (Abbas *et al.*, 2010). Contamination of heavy metals in the environment is a major concern because of their toxicity and threat to human life and environment (Rajendran & Gunasekaran, 2007). These heavy metal ions are toxic to both human beings and animals. The toxic metals cause physical discomfort and sometimes life threatening illness and irreversible damage to vital body system (Malik, 2004).

In another different approach, Zully (2012), studied biosorption of Lead and Copper by heavy metal tolerant *Micrococcus luteus*DE2008. They find out that Lead had no effect on the biomass expressed as mg carbon cm^3 of *Micrococcus luteus* DE2008, but in the case of Copper, the minimum metal concentration that affects the biomass was 0.1mM Cu (II). According to these result this microorganisms shows a greater tolerance for Lead.

Abioye *et al*. (2015) study biosorption of zinc and manganese by *micrococcus variance* and *staplylococcus Aureus* isolated from agricultural soil. In their studies, they investigated that the metal concentrations were determined using an Atomic Absorption spectrophotometric (AAS).

Iron as a common contaminant enters the water bodies through effluents of industries such as electroplating, mining and steel processing among others (Pavani & Kumar, 2013).The presence of Fe in water results in undesirable colour, odour and taste which makes water unfit for industrial and domestic consumption. Exceeding iron concentration greater than 0.3 mg/L causes water staining that adversely affect plumbing fixtures, dishware and clothes and produce a yellow to reddish appearance in water. These levels may also impart taste and odor to drinking water (Shokoohi *et al.*, 2009).

Many Methods exist for removal of heavy metals from industrial wastewater, these methods includes: Physico-chemical method, Chemical Precipitation, Coagulation and Flocculation, Electrochemical Treatments, Ion Exchange, Membrane Filtration, Biological Methods, Electrodialysis(Gunatilake, 2015). The aim of the present work is to investigate iron biosorption using dead *Micrococcus sp.*

MATERIALS AND METHODS
Sample Collection and Isolation of the Bacteria
The effluent sample was collected from Jana Plastic Industry Sharada phase II. The collected sample was transferred to sterile glass bottle container and immediately taken to the laboratory for isolation, in which 1ml of effluent was serially diluted using 10ml of distilled water and spread over nutrient agar solid plates. The plates were then incubated at 35^oC for 24hrs.

Selection of Heavy Metal Tolerant Bacterial Strain
Iron resistant bacterial strain was isolated from the effluent using bacterial nutrient agar and broth medium, which was amended with 0ppm, 50ppm, 100ppm and 200ppm of Fe and standard spread plate method was performed. The inoculated plate was incubated at 35^oC for 24hrs. After 24hrs incubation larger identical colonies from each plate were isolated. These isolates were characterized and further employed for the iron removal. Morphological, physiological and biochemical characteristics of the isolated bacteria species were carried out (Christopher *et al.,* 2014).

Bacterial Biomass Production
Selected bacterial strain was maintained on nutrient agar medium. Biomass of bacteria was produced by growing bacterial culture in nutrient broth in a conical flask at 35^oC for 24hrs. Cells were harvested by centrifugation at 7500rpm for 20min. Cell pellet were washed three times with distilled water, autoclaved at 121^oC for 15min to kill the bacteria, After that the cell was dried at 100^oC for 48hrs in an oven (Mohammed *et al.,* 2013).

FTIR Analysis
FTIR Analysis was used to identify the chemical functional groups present in the fresh biomass and Fe-loaded biomass using 650 -4000cm^{-1} Fourier transform infrared spectroscopy (Cary 630; Agilent Technologies).

Batch Biosorption Experiments
The biosorption of iron (III) on the obtained biomass was performed in a batch system. In the present work several effects on the biosorption of iron were studied, such as the initial pH, the contact time and kinetics, equilibrium parameters and thermodynamics. The effect of pH was studied by performing series of experiments at a pH ranging from 2.0 to 10, solutions of 0.1M HCl and 0.1 M NaOH were used to adjust the adsorbate pH at room temperature. The kinetic study of the biosorption of Fe (III) was carried out in glass bottles containing 100 ml of Fe (III) with a concentration of 10 mg /L mixed with 0.5 g of dried biomass. The pH was adjusted to the optimum value and the bottles remain in agitation at 150 rpm for 30min at 30 °C. Aliquots of 2 ml were taken every 5 min for the first 20 minutes then every 10 min, and then every 20 min for the rest of the experiment. Next the samples were filtered and analyzed by measuring the absorbance using AAS in order determine the residual concentration of iron. The equilibrium study of Fe (III) removal was performed in glass bottles containing different concentrations of iron (10, 20, 30,40 and 100 mg/L) and 0.5 g of dried biomass. The bottles are placed in agitation (150 rpm) for 30min at room temperature. The initial pH is adjusted as described above. The thermodynamic of Fe (III) removal was performed in glass bottlesshaken at 25˚C, 30˚C, 35˚C and 40˚C, and 150rpm on orbital shaker. Centrifugation was carried out after the defined contact time and supernatant was carried for analysis using Atomic adsorption spectrophotometer AAS (Perkin Elmer, Analyst 200).

RESULTS AND DISCUSSION
FTIR spectral analysis
Biosorption in bacteria may mainly be attributed to the cell wall structure containing functional groups such as amino, hydroxyl, and organic phosphate group ((Wang, 2007; Magdalena & Małgorzata, 2014)). The FT-IRanalysis was performed to obtain information about the interaction between the functional groups of biomass and Fe (III) ions. The FT-IR spectra of the bacterial biomass, before and after iron treatment are shown in Figure 1 and 2. The vibration around 3342cm^{-1} - 3316cm^{-1}is indicative of the presence of the −OH group on the bacterial biomass. The peak around 2959 cm^{-1} could be assigned to the C-H stretch. By comparing iron loaded biomass with FTIR spectra of pure biomass significant the changes in some peaks were observed. As can be seen in Figure.2, the peak at 3335cm^{-1} - suggests the major role of -OH and 1598cm^{-1} -NH group for Fe (III) biosorption onto *Micrococcus sp.*.These observations indicate that (hydroxyl and amine) functional groups on the surface of the biosorbent are responsible for the iron binding which is a strong bond.
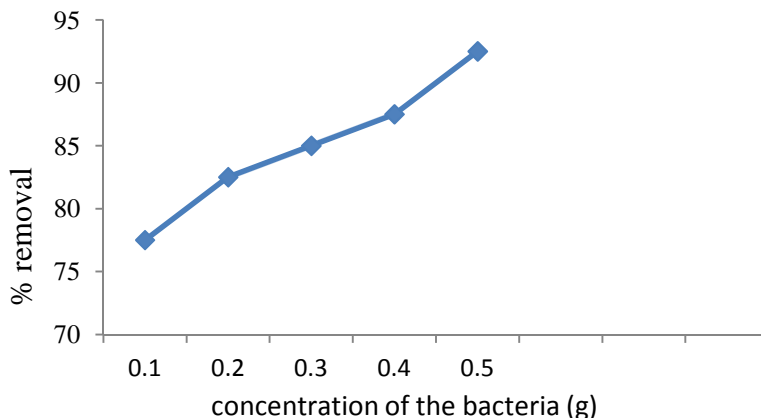
Figure 1: FT-IR Spectra of Dead Bacterial Cells**.**

Figure 2: FT-IR Spectra of Dead Bacterial Cells after Adsorption of Iron**.**

Effect of contact time

The removal percentage of both metals increased with the increasing of contact time as shown in Figure (3). The kinetics of iron and cadmium biosorption was very fast initially for a period of 5 and 15minutes with 40% and 60% respectively. This can be due to the availability of active sites on the surface of the biomass and that the solution concentration is higher at the beginning of the experiment. The biosorption equilibrium was reached at 30mins with percentage removal of 88.6%. This is in agreement with result obtained by (Mohammad *et al.*, 2013), during the biosorption of heavy metals by Bacillus thuringiensis strain OSM29 originating from industrial effluent contaminated north Indian soil. They observed that the biosorption of cadmium and copper increases with increasing contact time and reached equilibrium within the first 30minutes.

Figure 3: Effect of Contact Time on % Removal

Effect of dosage

To investigate the effect of the biosorbent dose on the elimination of Fe, an amount from 1.0g to 5.0g of bacterial biomass was mixed with 100ml of Fe solution a concentration of 10ppm. The biosorption percentage removal of Fe versus the amount of bacterial biomass is plotted in Figure (4). According to the result plotted in this figure, an increase of the quantity from 0.1g to 0.5g causes an improvement of Iron removal efficiency from 77.5% to 92.5% of Iron. This is due to external surface and the availability of active biosorption sites. This is in consistence with results obtained by Christopher *et al.*, (2014) and Benaisa *et al.*, 2016, during the biosorption of copper(II) by live biomasses of two indigenous bacteria isolated from copper- contaminated water. They observed that the biosorption of copper(II) increases with increasing in amount of biomass.

Figure 4: Effect of Biosorbent Dosage on % Removal

Effect of Initial Concentration
The biosorption experiment was conducted at different initial concentrations (10, 20, 30, 40 and 100mg/L) using an optimum time of 30min and a close of 0.5g of the bacterial cell. The percentage removal of iron from aqueous solution decrease with increase in the initial concentration from 92.5% to 40%while amount absorbed qe values increases with increase in the initial concentration. This variation is represented in Figure (5), which data revealed that, the biosorption potential was higher at lower initial concentration of iron added to aqueous solution of the bacterial cell. These characteristics indicated that the surface saturation of biosorbent depends on the initial concentration. The greater uptake of metal by the biosorbent materials at the lowest concentration could probably be due to a rapid metal absorbing ability of bacterial cell. The results are in good agreement with that of Christopher *et al.*, (2014) and Mohammad *et al.*, (2013) during the biosorption of heavy metals by Bacillu thuringiensis strain OSM29 originating from industrial effluent contaiminated north indian soil. Similarly this is in agreement with study of Harikrishna & Nanganuru (2012) during the biosorption of cadmium by *pseudomonas putida*, who mentioned that the maximum initial metal concentration of Cd was obtained at $10mg/L$.
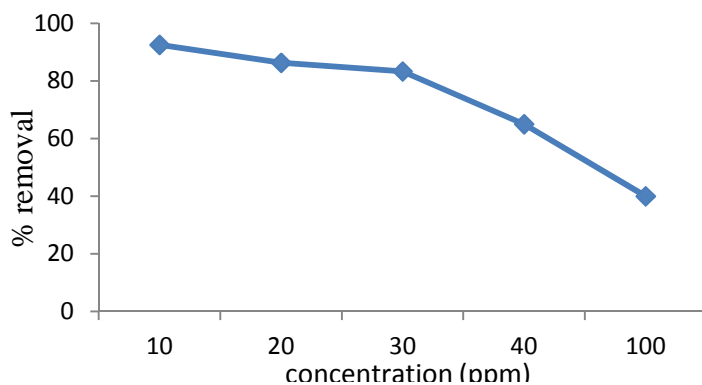

Figure 5: Effect of Initial Concentration on % Removal

Effect of pH
pH is an important parameter influencing heavy metal biosorption from aqueous solutions. It influences not only the surface charge of the adsorbent, the degree of ionization of material present in the solution, but also the dissociation of functional groups on the active sites of the adsorbent (Panida *et al.*,2014). The effect of pH was studied in range 2,4,6,8 and 10 as shown in Figure (6).The percentage removal of Fe was observed to depend on the pH, the percentage removal increases with pH from 2.0 to 6.0 for both dead and living bacterial cells, and above these levels, a further increase in pH decreases the percentage removal. At low pHvalues, there is a competition between the protons Fe ions on the sorption sites. At higher pH, in soluble iron hydroxide starts to precipitate from the solutions. The maximum removal efficiency was 94.2 %and 90.4 % at pH 6.0.This result agrees with the study of Mohammad *et al.*,(2013) in which the optimum biosorption pH value of Cd^{2+} by Bacillus thuringiensis strain OSM29 was 6.0.similarly this result is in agreement with the result obtained by Guven *et al.*,(2004) in which the optimum adsorption pH values of cadmium(II) was determined as 6.0.
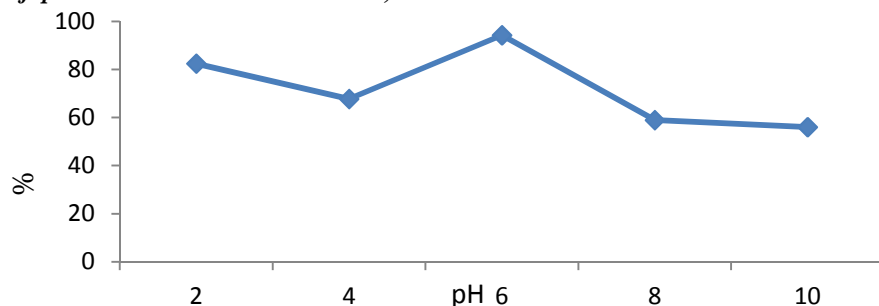
Figure 6: Effect of pH on % Removal

Effect of Temperature

In this research, biosorption of Iron by *Micrococcus* sp. appears to be temperature dependent. Maximum removal of iron was 30^0C with the percentage removal of 93.1% as shown in Figure (7), which may depend on cell metabolism that are mostly likely to be inhibited by low temperature, the higher temperature also affect the integrity of the cell membrane and also affect the cell wall configuration. This is in agreement with the result obtained by Ritixa & Monika (2013) in which the optimum temperature for Fe was 30 ℃ with percentage removal of 92% during the study of the effect of pH and temperature on the biosorption of heavy metals by *Bacillus licheniformis* and similarly this is in agreement with study of Abd El-Raheem*et al.,*(2013) who mentioned that the maximum biosorption rate for Cd, Co, Zn and Pb by *Enterobacter* sp. Was obtained at 30ºC.
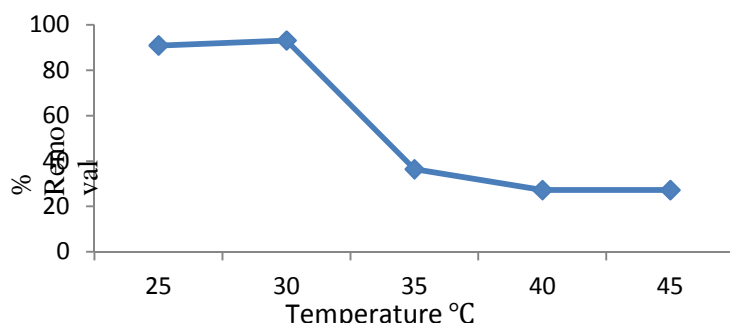

Figure 7: Effect of Temperature

Biosorption Kinetics

Different kinetic models including pseudo-first-order, pseudo-second-order and Elovich model were tested, in order to study the mechanism through which the biosorption process.

Pseudo-first-order equation

Pseudo first order integrated rate equation can be represented by the linear equation:

$$\log(q_e - q_t) = \log q_e - \left(\frac{K1}{2.303}\right)t$$

(1)

The experimental results of the first order rate constants are presented in Table 1. The biosorption data have a lowregression coefficient which suggests that the adsorption of iron on the biosorbent does not follows a pseudo-first order adsorption kinetics.

Pseudo-second order equations

Pseudo second order integrated rate equation is defined by the relation:

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{1}{q_e}t \quad (2)$$

Values of k_2 and equilibrium adsorption capacity (q_e) were calculated from the intercept and slope of the plots of $\frac{t}{qt}$ vs. t, respectively, the values were presented in Table 1, From which , the correlation coefficient of $Fe^{3+}$was 0.995 which is better than that of both the pseudo- first and Elovich models. This result agreed with that reported by Arezoo *et al.,* 2015, in which the same conclusion indicated that the second-order equation correlates well with the adsorption studies.

Elovich equation

The Elovich equation based on the adsorption capacity and the rate expression can be presented in a linear form as:

$$q_t = \frac{1}{\beta}\ln(\alpha\,\beta) + \frac{1}{\beta}\ln(t)$$

(3)

The constants α and β were obtained from the slope and intercept of the linear plot of qt versus ln$t$ as shown in Table 1. The data did not show a good correlation with the Elovich equation. The correlation coefficient for the linear plot, R^2, suggests a poor relationship between the parameters and also explained that the process of biosorption do not fit Elovich model.

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Table 1: Kinetics parameters for biosorption of Iron

Metal	Pseudo first order			Pseudo second order			Elovich equation		
	$k_1(min^{-1})$	$q_e(mg/g)$	R^2	k_2	q_e	R^2	α	β	R^2
Fe	-0.034	0.904	0.189	0.117	9.183	0.999	1.188	2.426	0.864

Langmuir Isotherm

The Langmuir isotherm model is based on the assumption that there is a finite number of active sites which are homogeneously distributed over the surface of the adsorbent These active sites have the same affinity for adsorption at a mono molecular layer level and there is no interaction between adsorbed molecules. A well-known linear form of the Langmuir equation can be expressed as in eqn (4):

$$\frac{C_e}{q_e} = \frac{1}{K_L Q_m} + \frac{C_e}{Q_m}$$ (4)

where: K_L is the Langmuir adsorption constant (Lmg^{-1}), and Q_m is the theoretical maximum (monolayer) adsorption capacity (mgL^{-1}) (Boparai , 2011). A plot of C_e/q_e against C_e is a straight line graph with $1/Q_m$ as the slope and $\frac{1}{K_L Q_m}$ as the intercept. The values of Q_m and K_L constants with the correlation coefficients for Langmuir isotherm are presented in Table 2. The maximum monolayer capacity (Q_m) was found to be $6.36 mg g^{-1}$. The correlation coefficient was extremely high (> 0.9). The biosorption process is found to be favorable since the R_L values lies between 0 and 1.

Freundlich Isotherm

  The Freundlich isotherm model can be applied to a adsorption on a heterogeneous surface between the adsorbed. The Freundlich isotherm is given in eqn 5:

$$logq_e = logK_f + \frac{1}{nf} logC_e$$ (5)

Where K_f is the Freundlich constant and $(1/n_f)$ is the heterogeneity factor (Boparai , 2011). Table 2 shows the Freundlich adsorption isotherm constants and the respective correlation coefficients $\frac{1}{nf}$ and K_f constants were determined from the slope and intercept of the plot of $logq_e$ against $logC_e$. The value of $1/n_f$ (heterogeneity factor) was between 0 and 1. For heterogeneous surface, the $\frac{1}{nf}$ value is close to zero.

The data in table 2 shows that for both the metals, $1/n_{f,}$ values were less than one which shows that n_f is greater than one, indicating that the biosorption of iron onto bacterial cells are favorable physisorption (multilayer) biosorption process.

Temkin Isotherm

The Temkins isotherm model was also applied to the experimental data, unlike the Langmuir and Freundlich isotherm models; this isotherm takes into account the interactions between adsorbents and adsorbate and is based on the fact that the free energy of adsorption is simply a function of surface coverage (Surchi, 2011; Tan, *et al.,*2008). The linear form of the Temkins isotherm model equation is given in eqn 6

$$q_e = B_T lnK_T + B_T lnC_e$$ (6)
$$B_T = \frac{RT}{b_T}$$

T is the absolute temperature in Kelvin, R is the universal gas constant (8.314Jmol^{-1}K^{-1}), and B_T is related to heat of adsorption, while K_T is the equilibrium binding constant (Lmg^{-1}). This isotherm was applied by a linear plot of q_e against $\ln C_e$, the constants B_T and K_T were calculated from the slope and intercept respectively,and are presented in Table 2 with the R^2 value . Again, looking at the regression R^2 obtained (0.726), it is seem that this isotherm is not applicable for the description of equilibrium data. However, comparing the isotherms applied, the Langmuir isotherms gave the best fit, followed by the Freudlich isotherm and then the least fit was obtain with the Temkin isotherms.

Table 2: Biosorption Isotherm parameters

Metal	Langmuir	isotherm			Freundlich isotherm			Temkin Isotherm		
	Q_m	K_L	R_L	R^2	K_f	$1/n$	R^2	K_T	B_T	R^2
Fe	6.361	0.164	0.058	0.979	2.013	0.252	0.970	8.443	0.869	0.726

Biosorption thermodynamics

Thermodynamic parameters such as the freeenergy standard free energy change (ΔG^o), enthalpy change

$$K = \frac{C_{ads}}{C_e}$$
$$\Delta G^0 = -RTlnK$$ (7)
$$lnK = \frac{\Delta S^O}{R} - \frac{\Delta H}{RT}$$

(ΔH^o) and entropy change (ΔS^o)changes during adsorption can be evaluated from the following Equations (7).

(7)   and

Where K is the equilibrium constant, T is the absolute temperature in Kelvin, and R is the universal gas constant ($8.314 Jmol^{-1}K^{-1}$). The ΔH^o and ΔS^o values were obtained from the slope and intercept of the Vant Hoff's plots (lnK vs. 1/T) for the metals adsorption from aqueous solution onto the microbial cells at various temperatures. The values obtained are tabulated in Table 3. The values of the enthalpy change (ΔH^o) and the entropy change (ΔS^o) found in this work were -152.3kJ/mol and -0.485kJ/mol/K,

respectively. The negative ΔG^o value indicates that the process is feasible and spontaneous in nature. The negative ΔH^o value indicates the exothermic nature of biosorption and the negative value ΔS^o corresponds to a decrease in degree of freedom of the adsorbed species. This is in agreement with result obtained by Senthil *et al* (2010) in which ΔG^o, ΔH^o and ΔS^o are all negative during the thermodynamic and kinetic studies of cadmium adsorption from aqueous solution onto rice husk.

Table 3: Thermodynamic parameters

Metal	Temperature (k)	ΔG^O (KJ/mol)	ΔH^O (KJ/mol)	ΔS^O(kJ/mol.K)
Fe	298	-7.90		
	303	-5.48	-152.3	-0.485
	308	-3.06		
	313	-0.63		

CONCLUSION

From the present study it could be concluded that bacteria play a very important role in the removal of heavy metals from waste water. The bacteria (*Micrococcus* sp.) were isolated from industrial effluent collected from Jana plastic industry Sharada using nutrient agar (NA) and broth media,Langmuir isotherm fitted well with the biosorption process. Biosorption is moreover influenced by various parameters such as time, dosage, initial metal concentration, pH, and temperature.The kinetic data were analyzed using pseudo-first order, pseudo-second order and elovich equation. From the linear regression coefficient values the data were found to be best fitted to pseudo second order kinetics.The thermodynamic parameters illustrated the biosorption process is feasible, spontaneous and exothermic

Conflict of interest
There is no conflict of interest.

Contribution of individual authors
Ladifa Hamisu Muhammad was the student who performed the experiments. Prof. Muhammad B. Ibrahim was her thesis supervisor.

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