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Original Research

Biosorption of Iron (II) by *Lactobacillus fermentum* from Aqueous Solutions

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Abstract

The biomass of *Lactobacillus fermentum* was used as biosorbent for the biosorption of iron (II) ions from aqueous solutions. Batch biosorption experiments were carried out with varying initial concentrations of Fe(II) ions, biosorbent dose, pH and time intervals. From the inductive coupled plasma optical emission spectroscopy (ICP-OES) result, the maximum biosorption capacity per gram of the biosorbent was found to be 7.25 mg for Fe(II) ions at a biomass concentration of 1 g/L at pH 4.5, contact time of 24 hrs, and an initial Fe(II) ion concentration of 100 mg/L. The equilibrium data was found to fit better with the Freundlich isotherm and pseudo second order kinetic model. The surface morphology and elemental composition of the biomass was characterized by using a scanning electron microscope (SEM) equipped with EDX, which confirmed that the iron was biosorbed, evident as bulky particles on the surface of the biomass, and as a peak for Fe in the EDX spectrum. Fourier transform infrared spectroscopy (FTIR) indicated that hydroxyl, amino and carboxyl functional groups are mainly involved in the biosorption of iron ions. Point zero charge of the biosorbent was found to be 3.0. X-ray diffraction (XRD) analysis revealed the amorphous nature of the biomass. The biomass of *Lb. fermentum* can be used as an efficacious and inexpensive biosorbent for the removal of Fe (II) ions from contaminated water resources.

Keywords: trace elements, isotherms, iron, biosorption, kinetics

Introduction

Heavy metal pollution has become a major concern because of its toxicity, threatening the ecological environment and human population by accumulating in the food chain. Heavy metal ions are non-biodegradable and hence their accumulation beyond a certain level is toxic [1]. Iron is both toxic and vital for many biological systems.

According to WHO, the allowable concentration of Fe in drinking water is 0.3 mg/L. Many industries such as steel, electroplating and mining discharge effluents containing high levels of iron into lakes and streams, causing adverse impact on the environment [2]. Iron accumulation in the living organisms causes severe health problems like kidney failure, cancer, metabolic acidosis and ulcer. Iron in the presence of water and oxygen corrodes, and results in the formation of hydrated oxides [2]. Hence, untreated effluents have to be remediated for the removal of iron.

Many conventional methods like the oxidation reduction process, ion exchange, membrane filtration

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and reverse osmosis have been explored for the removal of toxic heavy metals from wastewater. Although these methods are effective in removing heavy metals, they have disadvantages like high chemical and energy requirement, low efficiency at low metal concentration, expense, and production of toxic sludge for disposal [3]. Hence, there is a need for an efficient and environmentally compatible technique for removal of heavy metals.

Adsorption is an effective conventional technique for the removal of contaminants from various water resources/effluents [4, 5]. The process of adsorption is mainly defined as a physiochemical process where the solute molecules (adsorbate) accumulate on the surface of the solid (adsorbent) [6]. The most widely used adsorbent is activated carbon. However, owing to the cost of its production and regeneration, its application has been widely limited in recent years [7, 8]. Many different low-cost adsorbents like carbon slurry, bottom ash, and alumina have been used for the removal of contaminants from waste waters [9-11]. On the other hand, biosorption involves a solid phase of biological origin (biosorbent) and the liquid phase, which contains solutes like metal ions to be sorbed (biosorbate) [12]. The process of biosorption involves the removal of contaminants from solution by using biological materials (biomass) like yeast, bacteria, fungi, and algae. Biosorption may involve different mechanisms including physical adsorption or ion exchange or complexation [5, 13]. The other advantages associated with the process of biosorption are reusability, high efficiency, low operational cost and a reduced amount of sludge to be disposed of [7].

Specific micro-organisms can bind metal ions to their cell surface. Since a microbial cell is a natural adsorbent of metal ions [14], its application in metal ion binding has received growing interest in recent years [15, 16]. Hence, many studies have been carried out using micro-organisms as natural biosorbents for the biosorption of metal ions [17-19].

Lactic acid bacteria (LAB) are non-pathogenic and non-toxic (GRAS; generally regarded as safe) microorganisms routinely used in food and feed fermentations. Owing to this, they are more suitable over other microorganism for purification of water contaminants, including heavy metals. Also, their growth and production conditions are well known. Hence recently, the potential of LAB to biosorb metal ions has been evaluated in many studies. For example, *Leuconostoc mesenteroides* was evaluated for its ability to biosorb copper ions [20]. Lactobacilli have also been reported to biosorb other metal ions like lead and arsenic [21]. The biosorption potential of various microorganisms for the removal of Fe(II) was also evaluated in many studies [22, 23]. However, scanty information is available regarding the biosorption of iron ions by LAB.

In this context, the aim of the present study is to investigate the biosorption behavior of a lactic acid bacterium, namely, *Lactobacillus fermentum*, for the biosorption of iron ions from aqueous solutions.

Materials and Methods

Microorganism and Growth Conditions

The strain *Lactobacillus fermentum* NCDC400, a lactic acid bacterium, was obtained from the National Collection of Dairy Cultures, NDRI, Karnal. The stock culture was maintained on skim milk agar plates and was sub-cultured monthly. Glycerol stocks were also maintained at -80ºC. For biomass preparation, the strain was inoculated into MRS lactose broth and incubated at 37ºC with agitation (150 rpm) for 24 hrs. The bacterial cells were separated from the culture medium by centrifuge (8000 rpm, 10 min), and then the biomass was washed twice with ultra pure water before being used in the iron biosorption experiments.

Iron (II) Ion Solutions

A stock solution of Ferrous (II) sulfate heptahydrate ($FeSO_4.7H_2O$) was prepared (500 mg/100 ml) by dissolving in deionized water. Working metal solutions were prepared by diluting the stock solution freshly. Analytical-grade chemicals were used for all the experiments.

Biosorption Experiments

Biosorption potential of *Lb. fermentum* for Fe(II) ions was investigated. Batch experiments were carried out in 250 ml Erlenmeyer flasks by suspending 100 mg of biosorbent in 100 ml of Fe (II) ion solution. The flasks were incubated in an orbital shaker at 30ºC and 100 rpm for 24 hrs. The pH of the medium was adjusted by adding 1N HCl or H_2SO_4 at the beginning of the experiments. Appropriate blanks (biomass without metal ions) were maintained throughout the experiment under the same conditions. After incubation, the samples were centrifuged (8000 rpm, 8 min). The obtained supernatants were assessed by using an inductive coupled plasma optical emission spectrometer (PERKIN ELMER OPTIMA 5300 DV ICP-OES) to determine the concentration of residual Fe (II) ions. The instrument response was periodically checked using standard Fe (II) ion solutions.

The equilibrium biosorption capacity of the *Lb. fermentum* biomass at the corresponding equilibrium conditions was determined using the following mass balance equation:

$$qe = \frac{(Ci - Ce)\,V}{m}$$

…where q_e is the amount of metal ion biosorbed per gram of the biosorbent (mg g^{-1}), C_i is the initial concentration

of metal ion (mg L^{-1}), C_e is the final concentration of metal ion (mg L^{-1}), V is the volume of the medium (L) and m is the concentration of biosorbent (g).

The removal efficiency (R %) of the biosorbent was evaluated from the following equation:

$$R\% = \frac{Ci-Ce}{Ci} \times 100$$

Effect of pH

The effect of pH on biosorption was evaluated by suspending biomass at the rate of 1 g/L in metal ion solution (100 mg/L) and subjecting to pH varying between 2 and 9 and incubating at 30ºC at 100 rpm for 24 hrs. After incubation, the samples were removed and supernatants were analyzed for residual metal ion concentration.

Effect of Initial Metal Ion Concentration

To determine the effect of initial metal ion concentration, experiments were carried out with different metal concentrations of 2, 4, 10, 20, and 30 mg/L at pH 4.5 by using 1 g/L of biosorbent and incubating for 24 hrs in an orbital shaker operating at 30ºC and 100 rpm. The free metal ions present in the supernatant were evaluated using ICP-OES.

Effect of Contact Time on Biosorption

The optimum time required for biosorption was obtained by using 1 g/L of biosorbent suspended in 100 mg/L metal ion solution at pH 4.5. Metal solutions were taken at the desired time interval (1-24 hrs), and centrifuged to determine the heavy metal ion concentration in the supernatant by ICP-OES.

Effect of Biosorbent Dose

In order to determine the effect of biosorbent dose on biosorption of Fe (II) ions by *Lb. fermentum*, biomass was suspended at different rates ranging from 1 g/L to 3 g/L in a metal solution of 100 mg/L at pH 4.5. The flasks were then incubated at 30ºC for 24 hrs by shaking at 100 rpm. The residual metal ion concentration in the supernatant after incubation was determined by ICP-OES.

Isotherm Modeling

Isotherm models, namely, Langmuir, Freundlich and Temkin were used for the analysis of fit of data. Data for Fe biosorption isotherms were obtained at constant biosorbent dosage (1 g/L) and at other conditions such as time, pH, temperature, etc.

The linearized Langmuir isotherm model is represented by the equation:

$$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{K_L q_m}$$

…where q_e is the amount of metal ion biosorbed per gram of biosorbent (mg/g), C_e is the final concentration of the metal ions (mg/L), q_m is the maximum uptake capacity of the biosorbent (mg/g) and k_L is the Langmuir biosorption constant (L/mg). Based on the experimental data, the constants K_L and q_{max} were evaluated from the slope and the intercept of the linear plot of $1/q_e$ versus $1/C_e$.

The affinity (R_L, Hall isolation factor) of the biosorbent to biosorbate can be calculated by using the following equation

$$R_L = \frac{1}{1+k_L C_i}$$

…where C_i is the maximum initial concentration of the metal ion (mg/L).

The linearized Freundlich isotherm model is represented by the equation:

$$logq_e = logK_F + \frac{1}{n} \log C_e$$

…where K_F is a Freundlich biosorption constant (mg/g) and n is an experimental parameter. The values of K_F and n can be determined from the linear plot of log q_e versus log C_e.

The linearized Temkin isotherm model is represented by the equation:

$$qe = B \ln A + B \ln Ce$$

…where B = RT/b, b is the Temkin isotherm constant; A is the Temkin isotherm equilibrium binding constant (L/g); R is the universal gas constant (8.314 J/mol/K); T is the temperature at 803K; and B is constant related to the heat of sorption (J/mol). The constants A and B are obtained from the slope and intercept by plotting the quantity adsorbed (q_e) against ln Ce.

Biosorption Kinetics

In order to determine the biosorption mechanism of *Lb. fermentum,* two kinetic models, namely pseudo first and second order, were applied to the experimental data.

Pseudo first order kinetic model can be expressed in the following linear form:

$$\log(q_e - q_t) = logq_e - \frac{k_1 t}{2.303}$$

Pseudo second order kinetic model can be expressed in the following linear form:

$$\frac{t}{q_t} = \frac{1}{k_2.q_e^2} + \frac{t}{q_e}$$

…where q_e and q_t are the amount of Fe (II) ions biosorbed (mg/L) at equilibrium and at time t; k_1 is the pseudo first order equilibrium rate constant (min^{-1}); k_2 is the pseudo second order rate constant (g mg^{-1} min^{-1}); and t is contact time (min). The parameters of the two models can be calculated from the slope and intercept of linear plots of t versus log ($q_e - q_t$) and t versus t/q_t, respectively.

Scanning Electron Microscopy (SEM) and Energy Dispersive x-ray Spectrometry (EDX) Analysis

Biomass was observed under a scanning electron microscope. A drop of *Lb. fermentum* sample was dried on a clean silicon wafer and sputtered with gold nanoparticles using a gold sputter coater (SC7620 'Mini' sputter coater). Coated cells were applied with electron acceleration voltage of 5 KeV and viewed under a field-emission SEM (CARL ZEISS SUPRA 55 GEMIN-German Technology Jena, Germany). The elemental composition of *Lb. fermentum* biomass before and after metal ion biosorption was obtained by performing EDX (OXFORD EDS) analysis at 16 KeV.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The functional groups of dried *Lb. fermentum* biomass that are involved in Fe biosorption were determined by an FTIR spectrometer (Thermo Nicolet Avatar 370 FTIR, Madison, US). Dried bacterial biomass (2 mg) was mixed and ground with 200 mg of KBr in an agate mortar. The translucent disks were prepared by pressing the ground material with the aid of a pressure bench press. The tablet was then immediately analyzed using a spectrophotometer in the range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1}.

Point Zero Charge of Biosorbent (pH_{pzc})

Point zero charge is considered an important parameter for predicting the biosorption property of biosorbent. PZC was determined according to the procedure described by other researchers [24]. Biomass (0.15 g) of *Lb. fermentum* was introduced in 11 Erlenmeyer flasks (100 ml) containing 50 ml of 0.01 M NaCl solution. Initial pH of all the flasks were adjusted (pH 2.0 to pH 12) by adding a few drops of either 0.1 M HCl or 0.1 M NaOH before adding the biosorbent. The samples were allowed to equilibrate for 48 hrs in an orbital shaker at room temperature. After incubation, the final pH of all the flasks were measured and point zero charge was determined from the plot of initial pH vs. final pH.

X-ray Diffraction (XRD) Analysis

The diffraction patterns of the dried powdered biomass before and after biosorption with Fe (II) ions were recorded by using a Bruker D8-Advance XRD model equipped with monochromatic CuKα (1.5406 Aº) target radiation. Peaks for XRD were observed as a function of 2θ angle at a scan range of 3º to 80º, 40 kV voltages and 35 mA of current.

Results and Discussion

Effect of pH

According to the complexation theory, at low pH the functional groups retain their protons, thus reducing the possibility to bind to the positively charged groups like metal ions. The binding of metal ions to the biosorbent gets reduced at low pH due to increased competition between the metal ions and protons to the same binding site [25]. Quintelas et al. [26] explained that at low pH there exists competition between the metals and protons for binding to the biosorbent. Furthermore, in our experiments with NCDC400 and other strains, at low pH of 3, damage to the biomass was observed and flakes were formed. At high pH values (pH>4.5) precipitation of iron and the formation of hydroxides may occur, hindering biosorption. For example, at pH above 5, the formation of anionic complexes such as $Fe(OH_3)^-$ and $Fe(OH_4)^{2-}$ occurred [27]. Some researchers explained that at pH values higher than 6.5, biosorption studies could not be performed due to the precipitation of iron ions [28]. The reports are in conjunction with our studies, where at pH 6 yellowing and aggregate formation were observed. Hence, our study was carried out at pH 4.5 to ensure the maximum biosorption capacity of the biomass, assuming the functional groups can be deprotonated and become negatively charged to bind the positively charged metal ions.

Effect of Initial Metal Ion Concentration

The biosorption potential of *Lb. fermentum* with varied concentrations of Fe (II) ions was evaluated based on the results obtained by ICP-OES. From Fig. 1 it can be seen that as the initial metal ion concentration increased from 2 mg/L to 30 mg/L, the amount of metal biosorbed onto the biomass increased from 1.97 to 7.13 mg/g of the biosorbent (the removal efficiency was decreased from 98.5% to 23.76%). The amount of Fe biosorbed (biosorption capacity, q_e) is highest at initial metal ion concentration of 20 mg/ and stabilized thereafter. At low metal ion concentration sufficient biosorption sites are available, resulting in maximum biosorption capacity. However, at higher initial metal ion concentration, the number of metal ions will be high compared to the availability of binding sites, resulting in lower biosorption capacity. Hence, further experiments

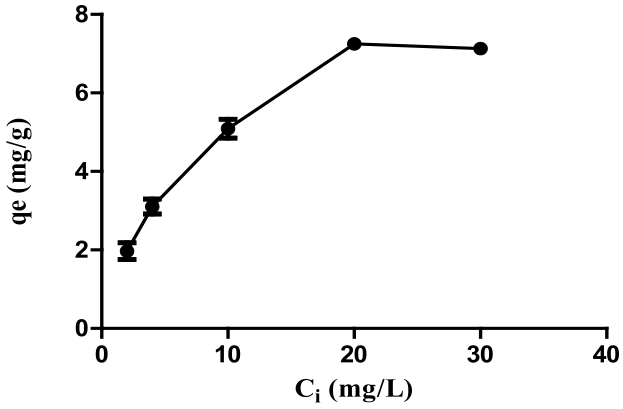


Fig. 1. Effect of initial metal ion concentration on Fe (II) biosorption by *Lb. fermentum* (m: 0.1 g, V: 100 ml, 30ºC, pH: 4.5, 24 hrs).

were carried out using 20 mg/L of initial concentration of Fe (II) ions. The result is in accordance with other studies. The biosorption capacity of *Bacillus sphaericus* for Ni (II) ions p increased from 9 mg/g to 83.01 mg/g with an increase in initial metal ion concentration from 10 mg/L to 400 mg/L [29].

Effect of Contact Time

The effect of contact time on the biosorption potential of *Lb. fermentum* for Fe (II) ions was noticed occurring in multiple phases (Fig. 2). In the first phase, biosorption was rapid and was found to be 9.71 mg/g (48.55% removal) of the biomass in the first 60 min (1 hour). Subsequently, there was a decrease followed by an increase in the amount of metal ion biosorbed during the time interval from 2 to 24 hr. During the first stage, rapid initial biosorption of metal ion occurs due to the availability of abundant binding sites. Further decreases may be due to the initial accumulation of metal ions leading to repulsion, resulting in dislodging of metal ions into solution. Desorption of metal ions at saturation was also reported previously [30]. In the second stage, penetration of metal ions to the interior of the biosorbent improves biosorption. Subsequent repulsion between accumulated metals ions causes loss of metal ions into the solution, leading to a decrease in biosorption. Hence, an incubation time of 24 hrs was considered as optimum with the metal ion biosorption of 7.25 mg/g (36.25 % removal) of the biosorbent. A similar trend of multiple phases of biosorption of Cr (VI) and Pb (II) ions with the biomasses of *Pleurotus ostreatus* and *Ficus benghalensis,* respectively, was observed [22, 31].

Effect of Biosorbent Dose

The extent of biosorption is strongly influenced by the biosorbent dose. Fig. 3 shows that a decrease in biosorption capacity of the biomass from 7.5 mg/g to 3.48 mg/g occurred with the increase in biosorbent dose from 1 g/L to 3 g/L (removal efficiency was increased from 36.25% to 52.25%). An increase in biomass concentration results in aggregation or overlapping of available binding sites, which leads to a decrease in the available total area of the biosorbent for biosorption. Hence a decrease in the biosorption capacity was observed with the increase of biosorbent dose. Another important factor for lower metal biosorption at higher biosorbent doses is due to the insufficient availability of metal ions and also due to interference caused by the available binding sites [32]. Hence, 1 g/L was taken as optimum biomass concentration and further studies were carried out. A similar decrease in biosorption capacity with an increase of biosorbent dose from 7.5 to 10 g/L was observed with *Arthrobacter* sp. for the biosorption of Pb (II) ions [33].

Isotherm Modeling

Through the modeling of equilibrium data, it is possible to predict the overall sorption behavior of biosorbents under different operational conditions. This

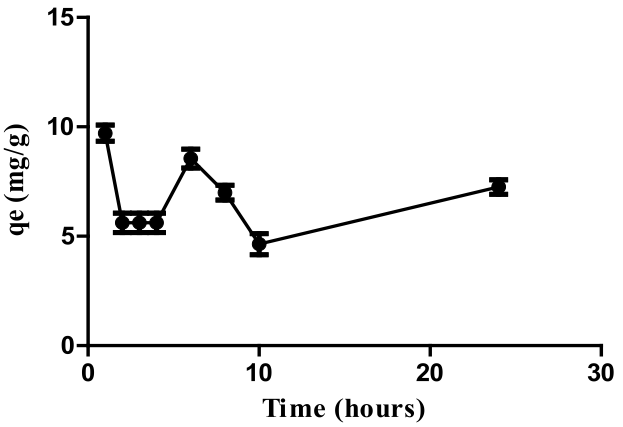


Fig. 2. Effect of contact time on Fe (II) biosorption by *Lb. fermentum* (m: 0.1 g, V: 100 ml, 30ºC, pH: 4.5, Fe (II): 10 mg/100 ml).

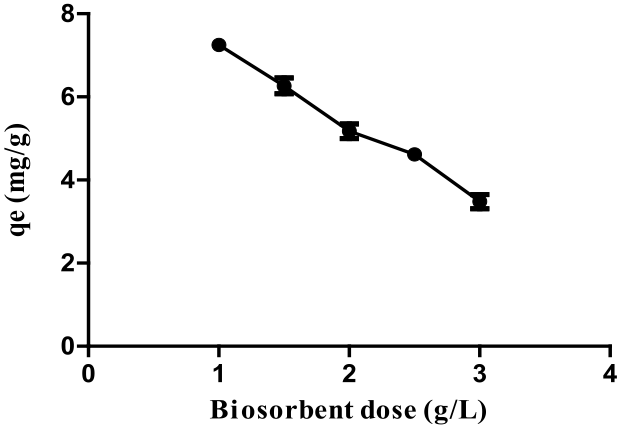


Fig. 3. Effect of biosorbent dose on Fe (II) biosorption by *Lb. fermentum* (V: 100 ml, 30ºC, pH: 4.5, Fe (II): 10 mg/100 ml, 24 hrs).

Table 1. Constants of isotherm models for the biosorption of Fe (II) ions onto *Lb. fermentum.*

Langmuir isotherm		Freundlich isotherm		Temkin isotherm	
q_{max} (mg/g)	14.32 mg/g	n	3.305	B	1.215
K_L (L/mg)	0.39 g/L	K_F (mg/g)	3.029 g/L	A (L/g)	17.48 L/g
R^2	0.8963	R^2	0.9863	R^2	0.9462
R_L	0.078				

can be useful for future industrial application [26]. It enables us to calculate specific descriptive parameters by fitting the experimental results to the theoretical model [34]. The equilibrium established between biosorbed metal ions on the biomass (q_{eq}) and metal ions remaining in the solution has been extensively described by the classical biosorption models. The biosorption potential of *Lb. fermentum* was evaluated by using three equilibrium sorption isotherm models: Langmuir, Freundlich and Temkin models.

The primary hypothesis of the Langmuir model is based on the assumption that maximum biosorption occurs when a saturated monolayer of solute molecules is present on the biosorption surface. The hypothesis of the Freundlich model is basically empirical and was developed for heterogenous surfaces. The Temkin model considers some important indirect interactions of biosorbent/biosorbate and suggests that because of these interactions the heat of sorption is linear rather than logarithmic [35].

The constants of Langmuir, Freundlich and Temkin isotherms ($q_{max,}$ $K_{L,}$ $K_{F,}$ n, A, B) were evaluated from their corresponding linear plots and presented in Table 1. The maximum biosorption capacity (q_{max}) was found to be 14.32 mg/g of the biosorbent. At the highest initial metal ion concentration (C_i = 30 mg/L), the value of R_L was 0.078. The values of A = 17.48 L/g and B = 1.215 J/mol indicate that the heat of sorption is a physical biosorption process. Based on the regression coefficient (R^2), the Freundlich isotherm model (R^2 = 0.9863) (Fig. 4) was found to be a fit better than the Langmuir (R^2 = 0.8963) and Temkin (R^2 = 0.9462) isotherm models. Various studies have reported the Freundlich [36, 37] or Langmuir [38, 39] or Temkin [40] isotherm models as the better fit in order to explain the biosorption behavior of different microorganisms.

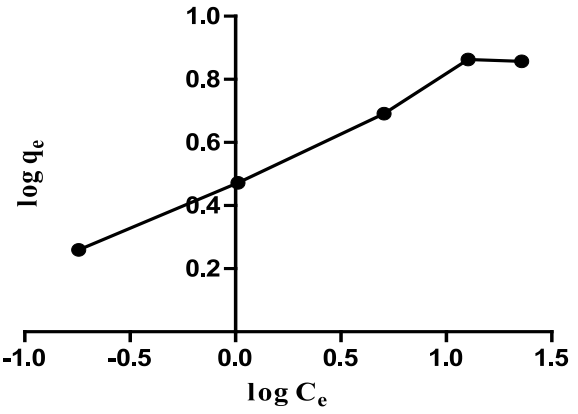


Fig. 4. Freundlich isotherm of Fe (II) biosorption by *Lb. fermentum* biomass at pH 4.5, dosage 1 g/L and contact time 24 hr.

Biosorption Kinetics

The prediction of biosorption kinetics is necessary for designing a good biosorption system. Evaluation of basic qualities of a good biosorbent can be done by measuring the rate constants and order of reaction. Table 2 shows that the sorption kinetics of Fe (II) onto *Lb. fermentum* biomass fit well with the pseudo second order kinetic model exhibiting higher correlation coefficient (R^2 = 0.9128) than with the pseudo first order kinetic model (R^2 = 0.329). This indicates that the chemisorption process occurred between the biosorbent and biosorbate by ion exchange mechanism (electron transfer), which affects the overall biosorption rate. Also, the value of theoretical q_e is almost similar to experimental q_e, thus confirming that the pseudo second order kinetic model was the better fit for explaining the biosorption behavior of *Lb. fermentum* for Fe (II) ions. Pseudo second order kinetic model was also found as the better fit for describing biosorption behavior of *Enterococcus faecium* and *Bacillus subtilis* for Cu (II) ion [41, 42].

SEM-EDX Analysis

Scanning electron microscopy (SEM) was conducted to observe possible changes in the morphology or surface characteristics of *Lb. fermentum* due to metal binding. Compared to the control (unloaded biomass), the metal loaded biomass showed the presence of bulky particles and a shrunken or distorted shape of the cell (Fig. 5a-b). Similar structural modifications occurred with the *Bacillus salmalaya* upon biosorption of chromium ions. Before biosorption the cells were thin, long and rod-shaped. After biosorption with chromium ions, the cells appeared to be plump and spongy [43]. Earlier, the deposition of Fe (II) ions was observed on the surface of lactic acid bacteria [44].

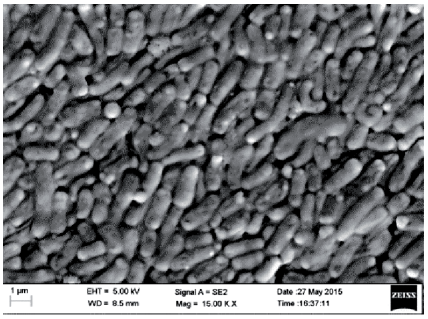
Elemental composition of the metal-treated biomass differed from that of the control. The elements potassium and oxygen, which were initially present in the control,

Table 2. Parameters of kinetic models for the biosorption of Fe (II) onto *Lb. fermentum.*

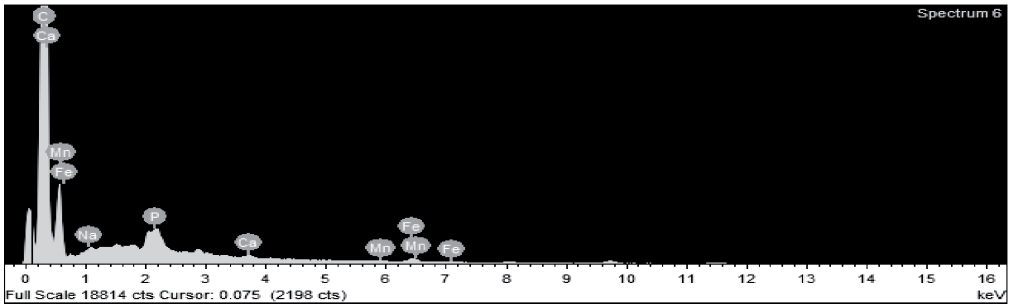
Pseudo first order kinetic model			Pseudo second order kinetic model		
q_e (mg/g)	K_1 (min^{-1})	R^2	q_e (mg/g)	K_2 (gmg^{-1} min^{-1})	R^2
1.865	0.027	0.329	7.04	0.139	0.9128

are not represented in the metal loaded biomass (Fig. 5a-b). Furthermore, the reduction in Na, P and Mn can be observed in the metal-treated biomass when compared with the control biomass. These observations suggest that Fe (II) ions replaced some of the ions initially present in the control. Halttunen showed the reduced content of Na and K when lactic acid bacteria was treated with lead and cadmium [45]. Hence, it is suggested that ion exchange plays a critical role in the biosorption of Fe (II) by *Lb. fermentum*. Earlier studies also showed that the mechanism of ion exchange was involved in the biosorption of metal ions [46].

a)



Element	Weight %	Atomic %
C	65.75	73.59
O	29.11	24.46
Na	0.28	0.16
P	2.14	0.93
K	1.97	0.68
Mn	0.76	0.19



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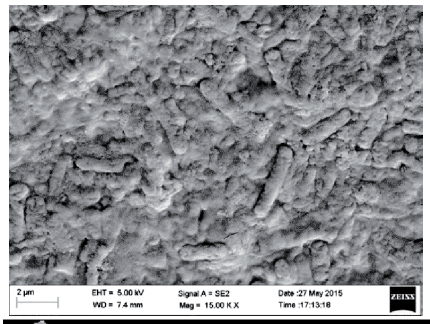


Element	Weight %	Atomic %
C	98.33	99.49
Na	0.23	0.12
P	0.36	0.14
Ca	0.17	0.05
Mn	0.20	0.04
Fe	0.70	0.15

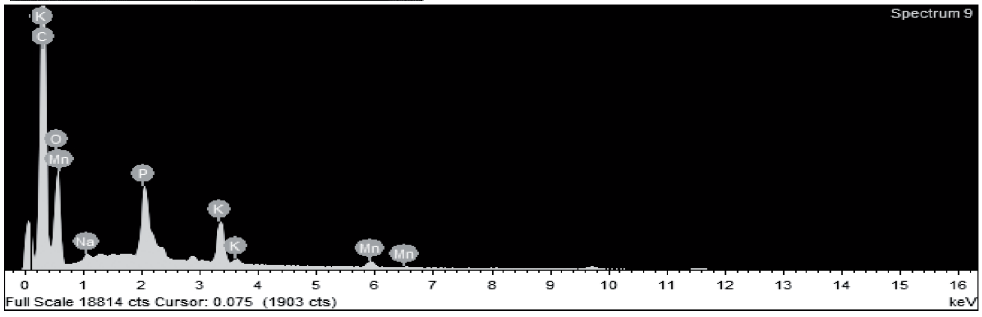
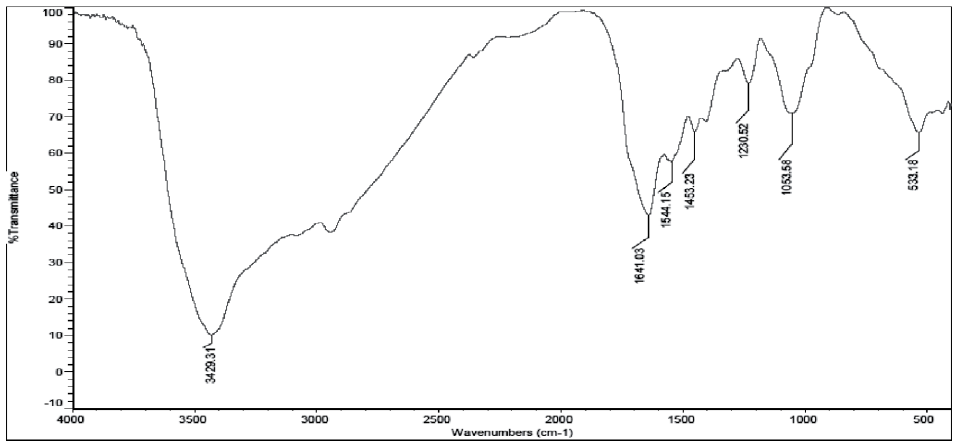


Fig. 5. a) SEM image, EDX spectrum and elemental composition of unloaded *Lb. fermentum* biomass (control); b) SEM image, EDX spectrum and elemental composition of *Lb. frementum* biomass loaded with 100 mg/L of Fe (II) at pH 4.5.

a)



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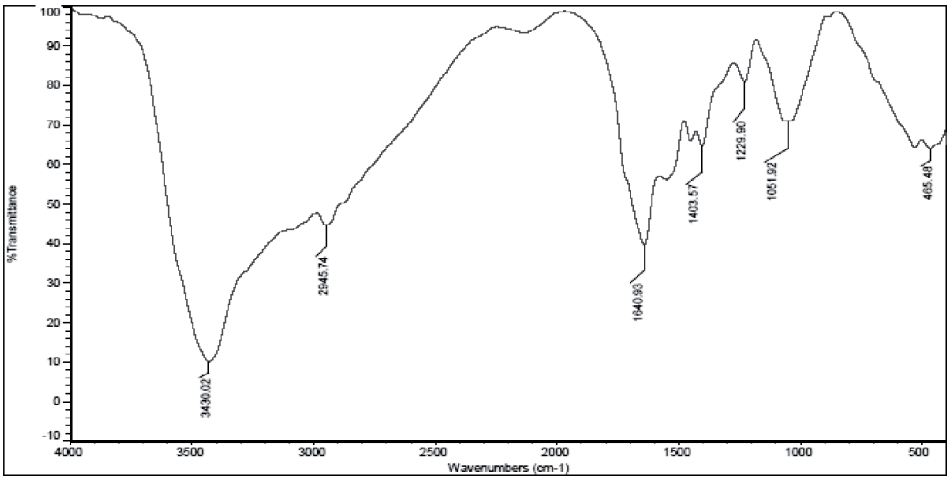

Fig. 6. a) FT-IR spectra of *Lb. fermentum* biomass at pH 4.5; b) FT-IR spectra of Fe (II) ion biosorbed *Lb. fermentum* biomass (100 mg/L) at pH 4.5.

FTIR Analysis

Biosorption of metal ions depends on the functional groups present on the surface of bacterial cells. Comparison of FTIR spectrum of control and metal-loaded biomass helps identify the characteristic peaks associated with the functional groups involved in metal biosorption. The potential of biosorption is strongly influenced from the surface structures of the biosorbents, such as the type and number of functional groups. FTIR spectroscopy is a valuable technique due to its high sensitivity in detecting changes in the functional groups.

The FTIR analysis was carried out for the biosorbent with and without the metal ion within in the wavelength range of 450-4000 cm^{-1} (Fig. 6a-b). Compared with the control, several new peaks appeared in the spectral pattern of metal-loaded biomass. The unloaded biomass displays a number of absorption peaks, which indicates the complex nature of the biomass. A peak at 3429 cm^{-1} region is due to the stretching of the hydroxyl group. The absorption peak at 1641 cm^{-1} represents the amide group. The COO^- bond of the carboxylate groups appeared at 1544 cm^{-1} and the peak located at 1453 and 1230 cm^{-1} represents C=O and C-C functional groups, whereas those located at 1053 cm^{-1} and 533 cm^{-1} represent C-O and H-O groups.

The IR spectra of the metal-loaded biomass showed significant changes in the regions 3430 cm^{-1}, 1640 cm^{-1}, 1051 cm^{-1}, 1229 cm^{-1}, indicating that those functional groups are involved in metal ion biosorption. The new peaks appeared in the region 2945 cm^{-1} is assigned to the stretching of C-H. The peak at 1403 cm^{-1} belongs to additional vibrations of amino groups and alkyl side chains in proteins. The band at 465 cm^{-1} is a fingerprint zone consisting of phosphate and sulphur functional groups. Additionally, a clear shift in the region of proteins is exhibited by showing a difference in the band intensity in the protein region (1640 cm^{-1}), indicating the role of proteins in Fe (II) ion binding. Conclusively, the changes in the vibrational frequencies implicate that these functional groups are involved in binding of the metal ions Fe (II). Mrvcic [47] reported that biosorption of zinc ions onto lactic acid bacterium also result in the shift of the spectrum. Biosorption of

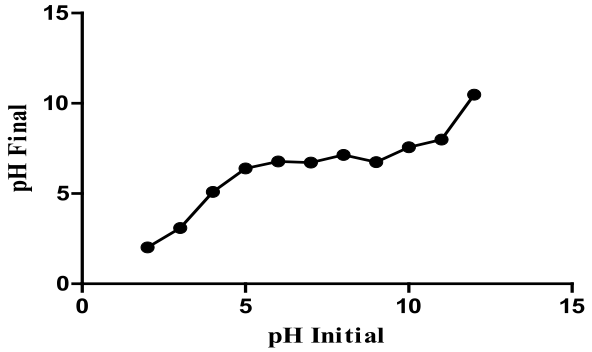

Fig. 7. Plot of initial pH versus final pH for determination of point zero charge of *Lb. fermentum.*

Fe (II) and Zn (II) onto the lactic acid bacteria also showed a similar change in the peak intensities at 3405 cm^{-1}, 1636 cm^{-1}, and 1050 cm^{-1}, which indicate that the carboxyl and hydroxyl groups are involved in biosorption, which implicates the present study [44]. Similar changes at 3423 cm^{-1}, 1648 cm^{-1}, 1053 cm^{-1}, 2935 cm^{-1} and 1453 cm^{-1} are in line with those reported by other researchers [17, 48].

Point Zero Charge of Biosorbent (pH_{pzc})

Point of zero charge is a convenient index of the propensity of a surface to become either positively or negatively charged as a function of pH. It is the value of pH required to give net zero surface charge. The pH of the solution affects the functionality of the surface and thus plays a key role in the biosorption process and affects the biosorption capacity. From Fig. 7, the pH_{pzc} of the *Lb. fermentum* was found to be 3. This indicates that at pH less than 3, the biosorbent in the solution will be positively charged and thus decreases the biosorption of divalent cations, resulting in low biosorption capacity. At

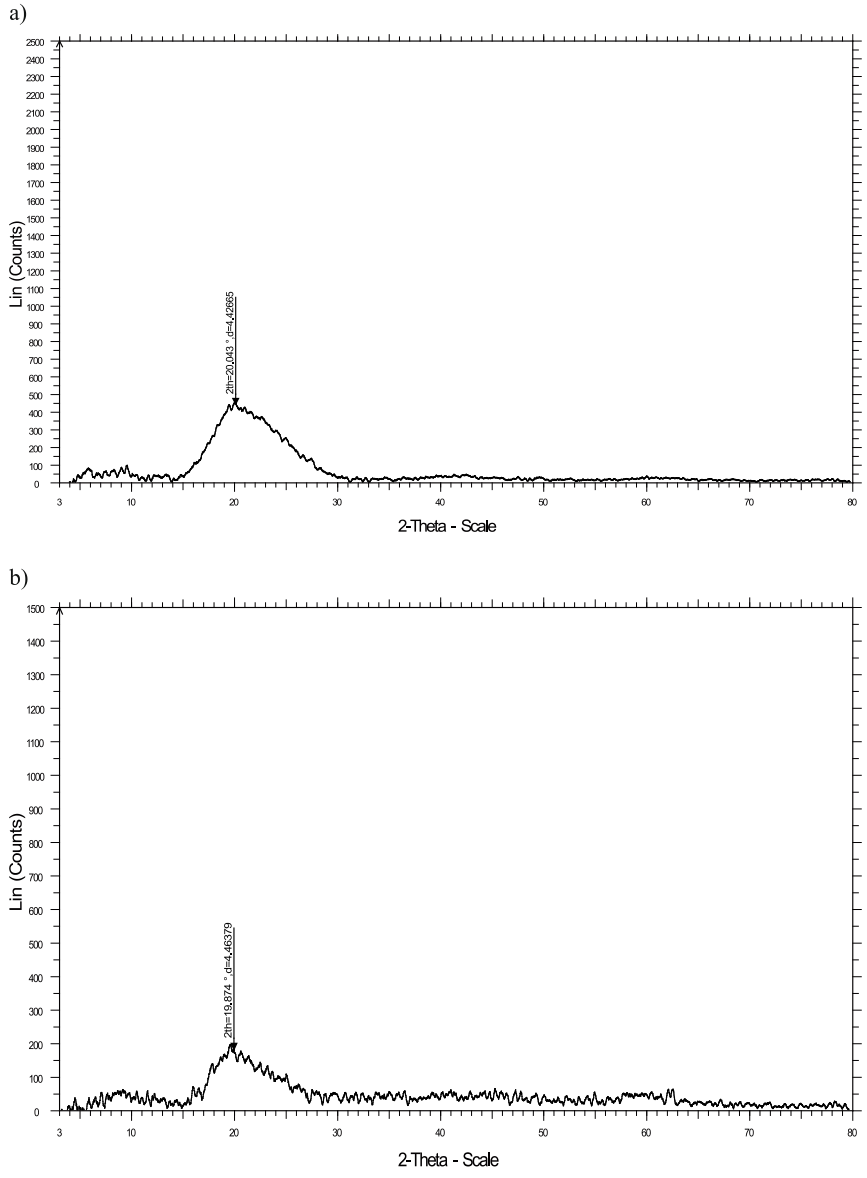

Fig. 8. a) XRD spectra of *Lb. fermentum* before biosorption with Fe (II); b) XRD spectra of *Lb. fermentum* after biosorption with Fe (II).

pH greater than 3, the surface of the biosorbent will be negatively charged, facilitating the biosorption of Fe (II) ions by electrostatic attraction. Maximum biosorption of Fe (II) ions by *Lb. fermentum* biosorbent was found to occur at pH 4.5, which is greater than pH_{pzc}. However, at higher pH (pH>6) a decrease or no biosorption capacity can be observed due to the formation of metal hydroxides [49]. Similar behavior was observed with *Polypores* and *Porphyridium cruentum* biomasses for the biosorption of lead, cadmium and mercury ions respectively [7, 50].

XRD Analysis

The crystalline or amorphous nature of the biosorbent can be assessed by using x-ray diffraction analysis. The XRD patterns of *Lb. fermentum* before and after biosorption with Fe (II) ions were depicted in Fig. 8a-b. The XRD pattern of control biomass before biosorption reveals the presence of poorly resolved peaks and a broad peak at 2θ = 20.043º with d spacing of 4.42665. This indicates a predominantly amorphous nature favorable for metal ion binding to the biosorbent [51, 52]. Biosorption of metals to the biomass results in a change of relative intensity as reported in earlier studies [53, 54]. Hence a shift in 2θ (19.8740) and d-spacing values (4.46379) indicates a change in crystallinity due to metal ion biosorption. A similar trend was also observed by *Caulerpa fastigiata* after biosorption with Cd (II) ions [55].

Conclusions

From the results we can demonstrate that the biomass of *Lb. fermentum* has the potential for biosorption of iron ions with a maximum biosorption capacity of 7.25 mg/g (36.25% removal) of the biosorbent (ICP-OES) with initial metal ion concentration of 100 mg/L, at pH 4.5 for 24 hrs at 30ºC. Biosorption data fit well with the Freundlich adsorption isotherm model and pseudo second order kinetic model. SEM-EDX analysis of the biomass showed that metal ions were biosorbed on to the biomass with an ion exchange mechanism. Changes in peak intensities in the FTIR spectrum indicate that the surface functional groups are involved in biosorption. The optimum pH value for Fe (II) was much higher than pH_{PZC} of 3.0, which indicates that the metal ion biosorption was due to the electrostatic interaction of the metal ions to the negatively charged surface of the biosorbent. The XRD study reveals the amorphous nature of the *Lb. fermentum*, which is favorable for biosorption of metal ions. These studies suggest that the biomass of *Lb. fermentum* could be used as an inexpensive (low cost) and effective (strong affinity to the bacterial cell) biosorbent for removal of metal ions from aqueous solutions.

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Conflict of Interest

The authors declare no conflict of interest.

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