

The metal binding potential of a dairy isolate

K. Ramyakrishna and M. Sudhamani

ABSTRACT

Excess iron in water resources can lead to health hazards and problems. The ability of lactic acid bacteria to bind iron has not yet been widely studied. In the present study, sorption of iron ions from aqueous solutions onto lactic acid bacterium was determined. Elemental analyses were carried out by inductively coupled plasma optical emission spectrometry. The kinetics of Fe(III) biosorption was investigated at different initial concentrations of metal ion. The highest uptake capacity was found to be 16 mg of Fe(III) per gram of adsorbent with a contact time of 24 hr and at initial metal ion concentration of 34 mg/L. The uptake capacity of Fe(III) ion varied from 83.2 to 46.7% across the range of initial metal ion concentrations. The equilibrium data were evaluated by Langmuir and Freundlich isotherms, and were found to fit better with the latter ($R^2 = 0.9999$). The surface morphology of the biomass and percentage of metal was characterized by using a scanning electron microscope equipped with energy dispersive X-ray spectroscopy. The functional groups on the cell wall surface of biomass involved in biosorption of heavy metals were studied by Fourier transform infrared spectroscopy spectrum.

Key words | adsorbate, biosorption, heavy metals, iron, isotherms, lactic acid bacteria

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INTRODUCTION

Iron is one of the most abundant elements on earth. It is also found in drinking water. The drinking water standard for iron is 0.3 milligrams per liter (mg/L). High levels of iron can be fatal and can cause ill-health, besides resulting in bad taste and discoloration (Aryal & Liakopoulou-Kyriakides 2013). Overdose 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 (Aryal & Liakopoulou-Kyriakides 2013). The presence of iron in water beyond the WHO limit affects taste/appearance and has adverse effects on domestic use and water supply structures, and promotes iron bacterial growth (Chaurasia *et al.* 2012). Slime causes clogging of pipe lines. Iron may react with tannins present in coffee, tea, and some alcoholic beverages to produce

sludge, affecting both the taste and appearance (Dvorak *et al.* 2014). Wastewater containing large quantities of iron is produced by coatings, and motor, aeronautic and steel industries. In this context, removal of excess iron from various water resources or wastewater is a necessity.

Conventional approaches like precipitation, flocculation, ion exchange and membrane filtration have been attempted for the removal of heavy metals from water. Although these methods are found to be effective, in some cases, they are expensive, disruptive, and not effective at low metal concentrations, produce sludge for disposal and are less practical in normal environmental conditions. Use of low cost adsorbents (biomasses of algae, fungi, and bacteria) for binding of metals is an alternative (Das *et al.* 2008). Metal ion binding by microorganisms is a complex process involving two basic mechanisms, i.e., biosorption – the passive non-metabolically mediated binding process, and bioaccumulation – the process associated with metabolism (Mrvčić *et al.* 2012). Many studies have been carried out using microorganisms as natural biosorbent in the

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removal of metal ions (Pan *et al.* 2007; Kazy *et al.* 2009; Talos *et al.* 2009; Lima *et al.* 2013; Shivakumar *et al.* 2014).

In particular, biomass of different microorganisms has been employed for iron binding (Moppert *et al.* 2009; Quintelas *et al.* 2009). Lactic acid bacteria (LAB) are a diverse group (*Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Bifidobacter*, *Streptococcus*, *Enterococcus*) and the individual species may have different cell surface components that can influence the iron binding. *Lactobacillus* sp. produces surface layers while some LAB are also known to produce capsules and exopolysaccharides (Boot *et al.* 1993; Ruas-Madiedo & De Los Reyes-Gavilán 2005). The potential of LAB to accumulate selenium, chromium, cadmium, and lead has been evaluated (Halttunen *et al.* 2007; Pieniz *et al.* 2014). On the other hand, the principles of iron binding by LAB species have not been widely studied. In the present study, biosorption of iron by LAB biomass has been investigated.

Accordingly, the aim of the present study is to investigate the biosorption behavior of LAB for the removal of iron from various contaminated water resources.

MATERIALS AND METHODS

Isolation and screening of LAB

LAB were isolated from fermented milk samples collected from various sources and places in Vijayawada, Andhra Pradesh, India. The samples were serially diluted and were plated onto a medium containing 11% skim milk, 0.35% yeast extract, 1% lactose, and 1.5% agar which was adjusted to pH 7. The plates were incubated at 30 °C for 48 hr for isolation of LAB. Based on Gram staining and catalase tests, LAB isolates were selected. The isolates were purified by streaking and preserved in MRS–lactose broth (Paulo *et al.* 2012) supplemented with 50% (v/v) sterile glycerol, at –80 °C for long-term storage use.

Identification of the isolates

The isolated, purified LAB strains were identified by phenotypic and molecular methods. Colony morphology and Gram staining character were examined. To confirm the identity of screened strains, molecular methods were used.

Genomic DNA was extracted from the isolates and amplified by primers derived from 16S rRNA gene (Heilig *et al.* 2002). The polymerase chain reaction (PCR) amplification was carried out with a reference strain – *Lactobacillus plantarum* NCDC 400 obtained from the National Collection of Dairy Cultures, Karnal, as a positive control. The amplified PCR products were checked for the presence of desired bands on 1% agarose gel.

Preparation of biomass

The growth medium used for the preparation of LAB biomass was de Man Rogosa and Sharpe (MRS) medium (Adebayotayo & Onilude 2008) with lactose. The bacterial isolate was inoculated in MRS–lactose broth and incubated for 18 hr at 30 °C. Cells were harvested by centrifugation at 8,000 rpm for 15 min. The cell pellets were washed twice with distilled water before being used for biosorption studies.

Metal solutions

The selected metal for binding assays used in this study was iron. Stock metal ion solutions of Fe(II) and Fe(III) were prepared by dissolving accurate quantities of FeSO₄·7H₂O (iron(II) sulfate heptahydrate) and FeCl₃ (iron(III) chloride anhydrous), respectively, in distilled water. The dilutions with varied concentrations (working standards) were prepared by diluting the stock solution of 5 g/L just before use. All the chemicals used were of commercially available analytical grade. To remove the possible interference of other metals in sorption experiments, the glassware used was washed with 10% nitric acid and rinsed with distilled water prior to use.

Biosorption experiments

The biosorption of Fe(II) and Fe(III) ions onto the biomass from aqueous solutions was investigated (Quintelas *et al.* 2009; Tapia *et al.* 2011). The experiments were carried out in 250 mL Erlenmeyer flasks. The initial concentration of metal (FeCl₃) varied from 10 mg/L to 100 mg/L with a biomass concentration of 100 mg dissolved in 100 mL distilled water. The pH of the experimental solutions was adjusted to the desired value of pH 3 for FeCl₃ with 1 N NaOH or 1 N

HCl (Quintelas *et al.* 2009) and at pH 4.5 for FeSO₄·7H₂O with 1 N NaOH or 98% H₂SO₄ (Moppert *et al.* 2009). Then, the flasks were sealed and agitated on an orbital shaker with a speed of 150 rpm at 30 °C until equilibrium sorption was reached (24 hr). Negative controls and appropriate blanks were maintained throughout the experiment at the same conditions by dissolving equal amounts of bio-sorbent in distilled water and to ensure the absence of glassware sorption of metals.

Analysis of iron content

After incubation, samples were taken from the suspension. The solutions were centrifuged for 10 min at 7,500 rpm to collect the biomass. The supernatant fractions were used for analysis to determine the concentration of the unbound Fe(II) and Fe(III) ions. The resultant metal ion concentration was determined by inductive coupled plasma optical emission spectrometer (Perkin Elmer Optima 5300 DV ICP-OES) equipped with ultrasonic nebulizer at Shiva Analytical Research Center and BARC, Bangalore.

The equilibrium sorption capacity of the biomass at the corresponding equilibrium conditions was determined using the following mass balance equation (Bishnoi *et al.* 2007):

$$qe = \frac{(C_i - C_e) V}{m}$$

where q_e is the amount of adsorbed metal ion per unit weight of the adsorbent (mg/g), C_i is the initial concentration of metal ion in solution (mg/L), C_e is the equilibrium concentration of metal ion in solution (mg/L), V is the volume of the medium (L), and m is the amount of the biomass used in the adsorption process (g).

The percent biosorption ($R\%$) of metal was evaluated from the following equation:

$$R\% = \frac{C_i - C_e}{C_i} \times 100$$

Isotherm modeling

To examine the relationship between the biosorption capacity of the biomass and adsorbent concentration, two different adsorption isotherm models were used.

For the Langmuir isotherm model, the experimental data were represented in a nonlinear form as (Panday & Banerjee 2012; Lima *et al.* 2013):

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

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

The affinity of the adsorbent to the adsorbate can be measured by R_L (Hall isolation factor) using the following equation (Galedar & Younesi 2013):

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

where C_i is the highest initial concentration of the adsorbate (mg/L).

For the Freundlich isotherm model, the experimental data were analyzed based on the following equation (Yilmaz *et al.* 2010):

$$q_e = K_F C_e^{1/n}$$

where K_F is a constant between biosorption capacities and n is an experimental parameter between intensity of biosorption which can be evaluated from the linear plot of $\log q_e$ versus $\log C_e$.

Scanning electron microscopy and energy dispersive X-ray microanalysis

The surface morphology of the biomass before and after biosorption were investigated using a scanning electron microscope (SEM) (Carl Zeiss Supra 55 Gemin; German Technology, Jena, Germany). The biomass samples to be analyzed were placed on the sample holder (stub) with carbon tape. In order to increase the electron conduction and to improve the quality of micrographs, a conductive

layer of gold palladium was made with portable SC7620 'Mini' sputter coater/glow discharge system (Quorum Technologies Ltd, Laughton, UK) (Michalak *et al.* 2014). Thereafter, the samples were placed in a sample holding vacuum chamber and a voltage of 5 kV was applied. Images were captured by signal SE2 detectors with a working distance of 6.8 mm. The spot sizes varied from 2 μm to 200 nm depending on the applied magnifications. Elemental composition of the biomass before and after metal sorption was determined by energy dispersive X-ray analyzer (EDX, Oxford Instruments) equipped with SEM. The X-ray spectrum of the elements was obtained by the applied acceleration voltage of 16 keV.

Fourier transform infrared spectroscopy

Analysis of functional groups was performed with a Fourier transform infrared spectroscopy (FTIR) spectrophotometer (Perkin Elmer Spectrum Two™ equipped with LiTaO₃ (lithium tantalite) pyroelectric infrared detector. Infrared spectra of the unloaded and the metal loaded biomass were obtained by grinding a mixture of 2 mg dry biomass with 200 mg dry KBr powder (1:100) in an agate mortar. The mixture was then pressed into a 16 mm diameter mold to prepare translucent sample disks using pressure bench press. The discs were then immediately analyzed within the range of 450–4,000 cm^{-1} . The influence of atmospheric water and CO₂ was always subtracted (by analyzing the disks immediately). The obtained FTIR spectra were analyzed using Spectrum 10 version 10.3.06 software.

Statistical analysis

All the experiments were carried out in triplicate. Data from each experiment were analyzed statistically by applying one-way analysis of variance, and differences among the treatments were determined by T-test at $p < 0.05$.

RESULTS AND DISCUSSION

Isolation and identification of LAB

Six isolates which were Gram positive, catalase negative, and curdled milk were subject to LAB group specific PCR.

Isolates showing amplification of 700 bp (Figure 1(a)) were considered to be LAB. One of the LAB isolates was selected for metal sorption studies. It was also found to have capsules (Figure 1(b)).

Biosorption of metal ions by LAB

Biosorption or bioaccumulation of metal ions by various fungi and algae has been studied (Talos *et al.* 2009; Tamilselvan *et al.* 2011; Khowala 2012). The metal uptake capacity of different species of bacteria (e.g., *Bacillus*, *Pseudomonas*, *Streptomyces*, etc.) has also been tested. However, the ability of LAB for sorption of essential metal ions has been explored only in recent years. Specific LAB, namely, *Lactobacillus fermentum* and *Bifidobacterium lactis*, are involved in the rapid removal of toxic metal ions of lead and cadmium (Halttunen *et al.* 2007). *Enterococcus faecium*, a lactic acid bacterium, was evaluated for its ability to

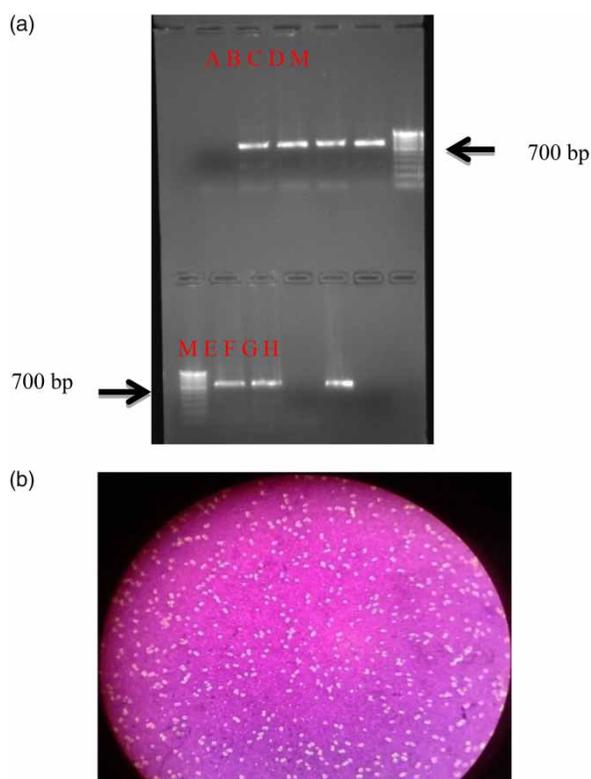


Figure 1 | (a) Molecular identification of isolates. M: GeNei™ Stepup™ 100 bp DNA ladder. Lanes A to F: amplifications with DNA from various isolates LAB1, LAB2, LAB3, LAB4 (selected isolate for metal binding); E-LAB5; F-LAB6. Lane G: negative control (distilled water). Lane H: positive control. (b) Capsule staining – selected isolate of LAB showing capsules.

Table 1 | Amount of metal biosorbed (uptake capacity) and percentage of bound Fe(III) by LAB at different initial concentrations of metal under given conditions with 1 g/L of biomass

S. no.	Initial concentration of FeCl ₃ (mg/L)	Initial metal ion Fe(III) concentration C _i (mg/L)	Final metal ion Fe(III) concentration C _e (mg/L) [mean]	Amount of metal ion Fe(III) biosorbed q _e (mg/g)	% of bound metal ion Fe(III)
1	10	3.4	0.57	2.83	83.2
2	20	6.8	1.73	5.07 ^a	74.5
3	50	17	6.7	10.3 ^{a,b}	60.5
4	100	34	18.1	15.9 ^{a,b,c}	46.7

^{a,b,c}means with the same letter among the concentrations are significantly different ($p < 0.05$).

remove copper ions from contaminated water (Yilmaz *et al.* 2010). There is only scant information (Sofu *et al.* 2015) on the ability and mechanism of iron biosorption by LAB. Hence, the experimental conditions for sorption of iron with LAB biomass (pH, temperature, contact time, concentration of metal, and biomass) were chosen based on the studies done with other groups of microorganisms (Quintelas *et al.* 2009; Sofu *et al.* 2015).

The bacterial cells are efficient metal chelating agents due to the nature and composition of the cell walls and cells. A great deal of variation is prevalent in heavy metal uptake capacity among different bacterial genera. However, the key factors which control and characterize these mechanisms are: type of biological ligand and biosorbent; chemical, stereo-chemical and co-ordination characteristics of the target metal; and characteristics of metal solution (Mrvčić *et al.* 2012).

The biosorption of Fe(III) ions by LAB was investigated by inductively coupled plasma optical emission spectrometry (ICP-OES). Results (Table 1, Figure 2(a)) demonstrate that there was significant increase in the amount of metal adsorbed onto the biomass with the increase in initial metal ion concentration. This trend is reflected in other studies. The amount of metal ion biosorbed increases with the increase in initial metal ion concentration from 1.0 mg/L to 10 mg/L (Moppert *et al.* 2009; Sofu *et al.* 2015). Based on these results, comparison can be done with other biosorbents on weight basis also. For example, the amount of iron biosorbed by LAB (16 mg/g) in the present study is comparable with other biomasses, namely, *Escherichia coli* (16.5 mg/g) (Quintelas *et al.* 2009) and *Polyporus squamosus* (16 mg/g) (Razmovski & Šciban 2008) with the initial salt concentration of 100 mg/L.

Environmental/physiochemical parameters greatly influence the metal sorption capacity of the microorganisms. Hence, sorption mechanism was investigated initially as a function of pH and biomass concentration (Mitic-Stojanovic *et al.* 2011; Tamilselvan *et al.* 2011; Oves *et al.* 2013).

Metal ion biosorption is greatly influenced by the pH of the solution due to its influence on the ionization states of the surface functional groups of the biosorbent, and solubility of the metal ions. An increase in pH resulted in an increase in Fe(II) biosorption. At pH 4.5, 100 mg of biomass biosorbed about 42% of Fe(II) ions compared to 14% of ions at pH 3. The effect of pH in the case of Fe(III) ions is different. At an initial pH of 4.5 for Fe(III) ions sorption, a slight deposit of iron oxyhydroxyde (FeOOH) was observed in the medium at the end of the experiments. Hence, the initial pH of 3 was selected for all experiments to prevent the formation of such deposits (Figure 2(b)). These observations support the findings of other studies (Moppert *et al.* 2009; Quintelas *et al.* 2009; Sofu *et al.* 2015).

The effect of concentration of bacterial biomass on biosorption of Fe(III) ion was studied (Figure 2(c)). Based on a previous study (Sofu *et al.* 2015), a biomass concentration of 1 g/L was selected in the present study. At the initial metal concentration of 34 mg/L (100 mg/L of FeCl₃), the amount of Fe(III) ion bound was found to be 16 mg/g of biomass. With the further increase of biomass concentration keeping the volume and concentration of the metal solution constant, it was observed that there was no significant increase in the uptake capacity of the biomass. This may be attributed to unsaturation of active sites or due to reduction of the total area of biosorbent (due to aggregation of biomass) during the biosorption process. These results are in accordance with other studies (Bishnoi *et al.* 2007; Mrvcic *et al.* 2009; Galedar & Younesi 2013).

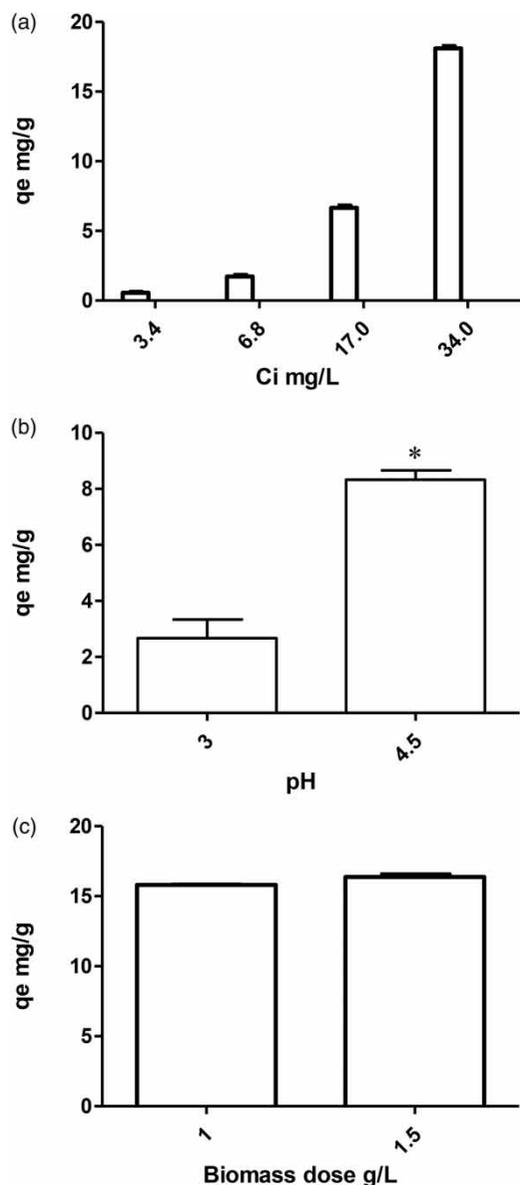


Figure 2 | (a) Effect of initial concentration of metal ion (C_i) on biosorption (q_e , uptake capacity) of Fe(III) at biomass dose of 1 g/L and at pH 3. (b) Influence of pH of aqueous solution of Fe(II) on biosorption by LAB biomass under given conditions (biomass at 1 g/L and at initial metal ion concentration of 20 mg/L Fe(II)). * means among the treatments there was significant difference ($p < 0.05$). (c) Influence of dosage of LAB biomass on biosorption (q_e , uptake capacity) of Fe(III) at initial metal ion concentration of 34 mg/L at pH 3.

Isotherm modeling

Equilibrium sorption isotherms represent the distribution of metal ions between the aqueous and the gel phase versus metal concentration. Modeling of equilibrium data is important to characterize biosorbents under various operational

conditions and also to evaluate the relationship between adsorbate and adsorbent. A rapid equilibrium is established between unadsorbed metal ions in solution (C_e) and the metal ions adsorbed on the bacterial cell (q_e). Experimental results fitted to a theoretical model enables one to calculate specific descriptive parameters. In the present study, the biosorption potential of LAB biomass was evaluated using two sorption isotherms: Langmuir isotherm and Freundlich isotherm.

The former model is most frequently used for the biosorption process of heavy metal ions. It is valid for monolayer adsorption on to the surface with a hypothesis that at particular homogenous sites, the biosorption process occurs in the adsorbent. It also compares the performance of different biosorbents. The hypothesis of Freundlich model is heterogeneous adsorption.

The constants of both isotherms (q_{max} , K_L , K_F , n) were evaluated from corresponding linear plots (Figure 3(a) and 3(b), and Table 2). The maximum adsorption capacity (q_{max}) for Fe(III) ions obtained by the model was 5.68 mg/g of biomass. At the maximum initial metal ion concentration ($C_i = 34$ mg/L; $FeCl_3$ at 100 mg/L), the value of R_L was measured and found to be 0.0123. Referring to the regression coefficient value (R^2), the Freundlich model (0.999) was found to be a better fit with the adsorption process by LAB biomass than the Langmuir model (0.972). Some studies show experimental data fitted better for the Freundlich isotherm (Najim *et al.* 2009; Talos *et al.* 2009; Yilmaz *et al.* 2010; Tapia *et al.* 2011) while others show the Langmuir isotherm (Loaëc *et al.* 1997; Mrvčić *et al.* 2009; Galedar & Younesi 2013).

SEM-EDX analysis

The surface morphology of the biomass before and after metal biosorption, was investigated using SEM. SEM micrographs (Figure 4(a)–4(f)) revealed significant changes in the cell surface morphology of LAB biomass in the presence of metal. Formation of depressions, and slight shrinkage of the cell surface compared to control (untreated biomass), indicated that the metal is biosorbed on to the biomass when treated with either Fe(II) or Fe(III) under experimental conditions. Similar depressions were observed by Sofu *et al.* (2015) in the cell surface of *Streptococcus thermophilus*. The presence of jelly like matrix in

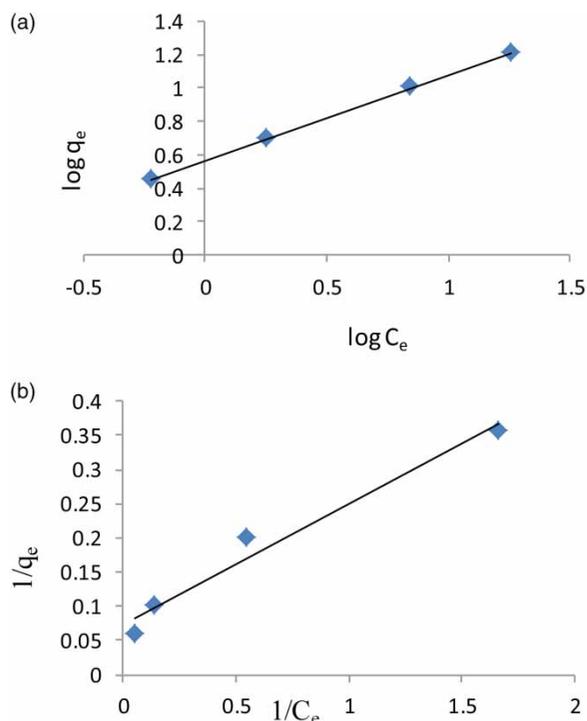


Figure 3 | (a) The linearized Freundlich adsorption isotherm for biosorption of Fe(III) ion by LAB biomass at a biomass concentration of 1 g/L. (b) The linearized Langmuir adsorption isotherm for biosorption of Fe(III) ion by LAB biomass at a biomass concentration of 1 g/L.

Table 2 | Constants of Langmuir and Freundlich isotherm for the biosorption of Fe(III) ions onto LAB biomass

Langmuir		Freundlich	
q_{\max} (mg/g)	5.68	n	1.96
K_L (L/mg)	2.39	K_F (L/mg)	3.63
R^2	0.972	R^2	0.999
R_L	0.0123		

the SEM picture is, possibly, due to the secretion of exopolysaccharide by the LAB. It may be noted that the isolate is a capsule producer (Figure 1(b)).

Metal quantification of the LAB biomass was performed by EDX analysis. The spectra of biosorbed biomass showed significant difference with the spectrum of the control (untreated biomass). Biomass treated with metals displayed a peak for treated metal (Fe) when compared with the control. As the initial metal concentration was increased, there was an increase in the percentage composition of Fe(III) in the treated biomass (Figure 4(b)–4(f)). Maximum concentration of

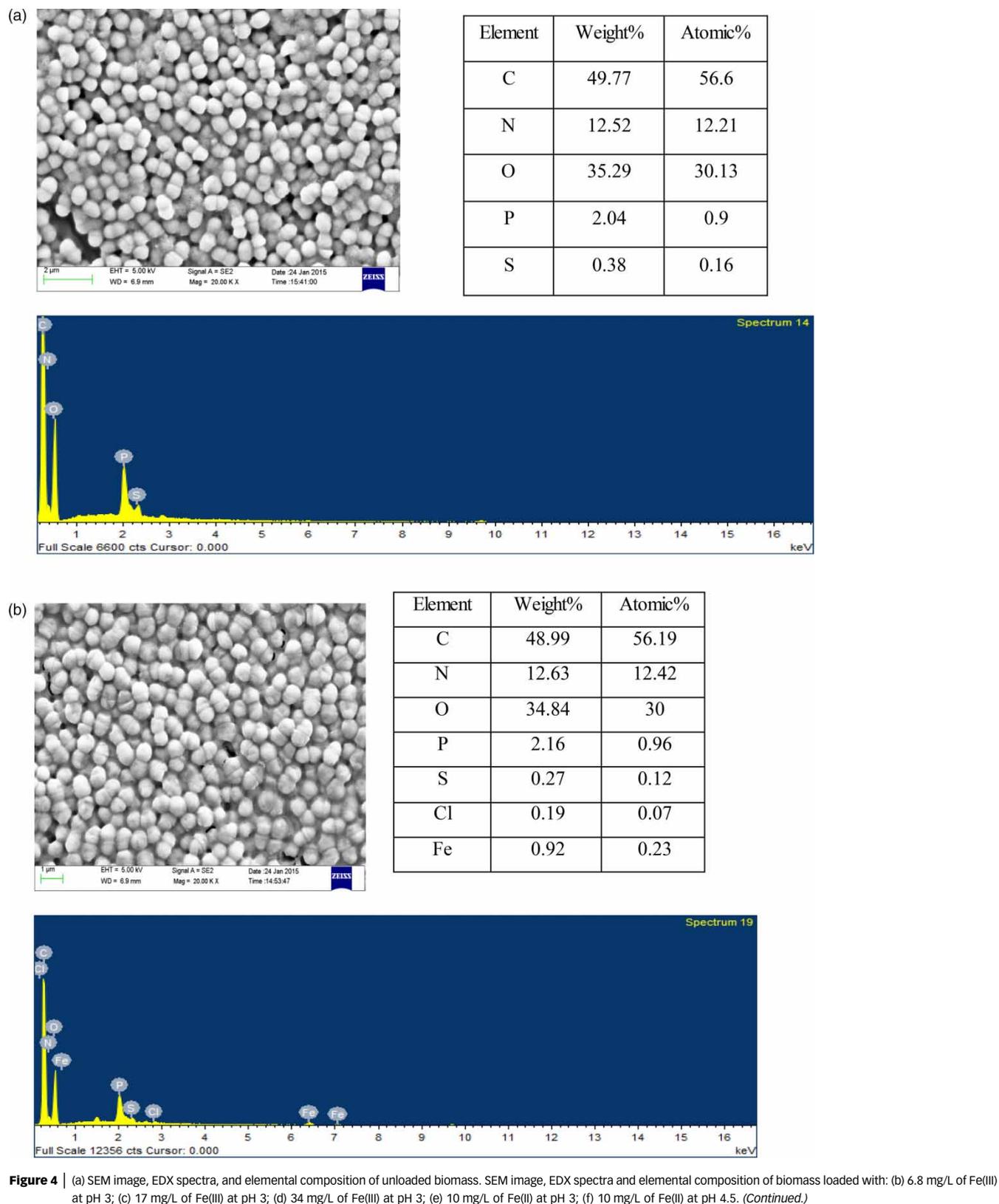
metal in the biomass was achieved with the highest initial metal ion concentration (100 mg of FeCl_3) (Figure 4(d)). On the other hand, as described above, the percent composition of the Fe(II) was higher at pH 4.5 than at pH 3 (Figure 4(e) and 4(f)). These results are in agreement with those obtained by ICP-OES. A peak for chlorine (Cl) in the spectrum of biomass treated with FeCl_3 was detected (Figure 4(b)–4(d)). Similarly, a high percentage of sulfur (S) in the spectrum of biomass treated with $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ was noted (Figure 4(e) and 4(f)). The technique of EDX has been applied successfully to analyze biosorption of various heavy metals by microbial biomass (Michalak *et al.* 2011, 2014). Some studies show ion exchange mechanism as a dominant process of biosorption (Halttunen 2008; Vijayaraghavan *et al.* 2009; Mitic-Stojanovic *et al.* 2011; Lima *et al.* 2013; Shivakumar *et al.* 2014). The EDX spectra of the biosorbent of *Aspergillus aculeatus* before and after metal uptake revealed that the main mechanism of adsorption was ion exchange where K^+ was replaced by Cd^{2+} (Panday & Banerjee 2012). Hence, EDX analysis can be used as a powerful tool for elemental mapping of the sample. The percentage composition and atomic weight percent of unloaded biomass and metal loaded biomass was compared (Figure 4(a)–4(f)). However, no mechanism of ion exchange for Fe, Cl, or S could be inferred.

Overall the results of EDX analysis imply that iron was biosorbed by the LAB biomass, and support the findings of ICP-OES, and SEM analysis.

FTIR analysis

Analysis of functional groups involved in metal biosorption was performed by FTIR spectroscopy. Infrared spectroscopy has proven to be a powerful tool for studying biological molecules and for obtaining information about metal biomass binding. Adsorption behavior of LAB biomass towards metal ions is a function of the chemical groups present on the active sites of the biomass. The FTIR analysis indicates the possible functional groups that play a role in the biosorption of metal ions (Durve & Chandra 2014). The cell wall constituents play a primary role in metal ion binding. The presence of functional groups with various charge distributions helps in selective binding of metal ions.

The FTIR spectra within the wavelength range of 450–4,000 cm^{-1} before and after biosorption of Fe(II) or Fe(III)



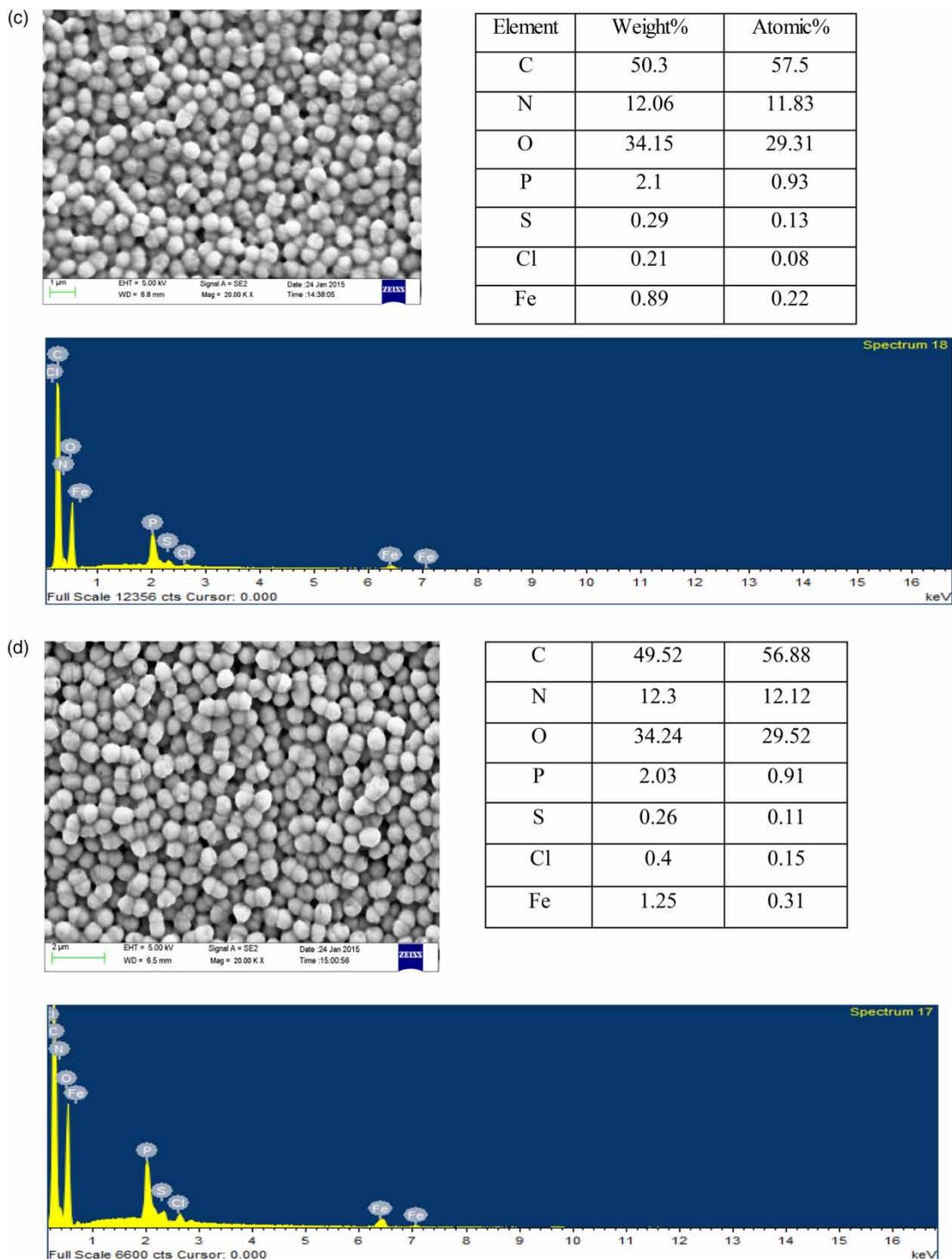
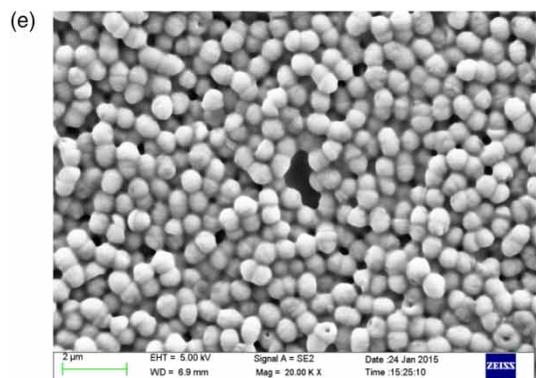


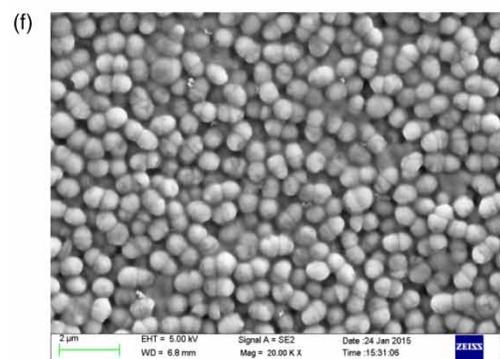
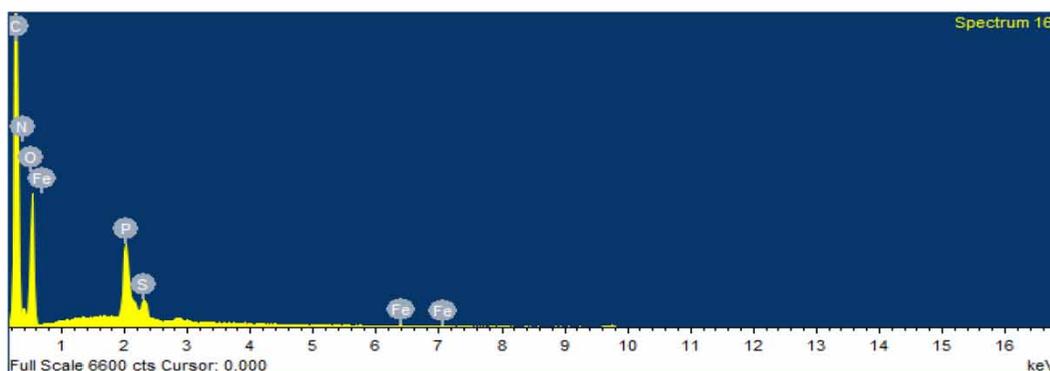
Figure 4 | Continued.

ions onto the biomass (Figure 5(a) and 5(b)) was carried out to ascertain the presence of functional groups that could possibly be involved in the biosorption process.

Analysis of the FTIR spectrum of the unloaded biomass showed peaks including for hydroxyl group at $3,289\text{ cm}^{-1}$, N-H group at $2,927\text{ cm}^{-1}$, C-C at $1,668\text{ cm}^{-1}$, C-H group at



Element	Weight%	Atomic%
C	50.58	57.61
N	11.66	11.39
O	34.74	29.7
P	2.36	1.04
S	0.57	0.24
Fe	0.09	0.02



Element	Weight%	Atomic%
C	49.81	56.99
N	11.97	11.75
O	34.84	29.93
P	2.27	1.01
S	0.31	0.13
Fe	0.79	0.2

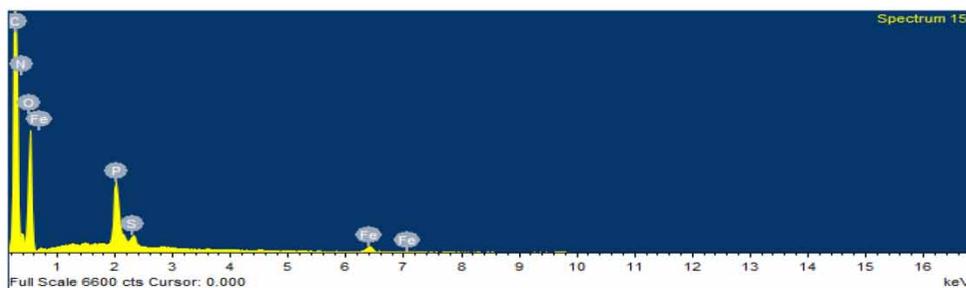


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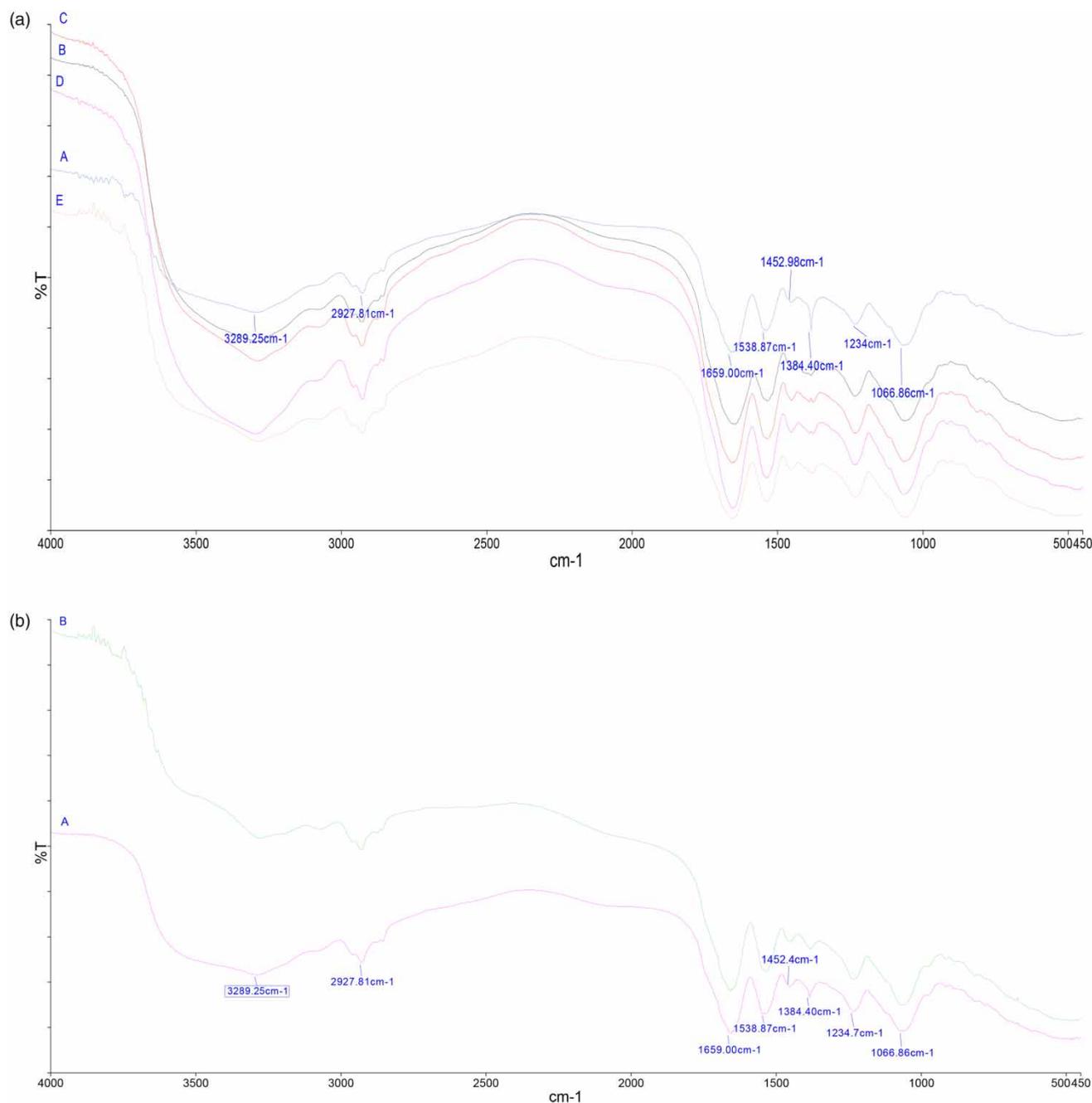


Figure 5 | (a) Comparison of FTIR spectra of LAB biomass before (curve A) and after (curves B, C, D, E) biosorption at pH 3 different initial metal concentrations. Curve B: biosorption at 6.8 mg/L Fe(III)/20 mg FeCl₃; C: biosorption at 17 mg/L Fe(III)/50 mg FeCl₃; D: biosorption at 34 mg/L Fe(III)/100 mg FeCl₃; E: biosorption at 10 mg/L Fe(II)/50 mg FeSO₄. (b) Comparison of FTIR spectra of LAB biomass before (curve A) and after (curve B) biosorption of Fe(II) at pH 4.5.

1,384 cm⁻¹ and the C-O bond of the aromatic and carboxyl groups at 1,234 and 1,066 cm⁻¹. The IR spectra of the metal loaded biomass showed a shift in the peaks in the regions, i.e., 1,660 and 1,066 cm⁻¹, which can be attributed to the interaction of the metal ions causing significant stretching

of the C-C and C-O bonds. Interestingly, the peak at 1,382 cm⁻¹ corresponding to the C-H group disappeared, suggesting that it is involved in the biosorption process both with Fe(II) and Fe(III) ions. However, the adsorption peaks around 3,289, 2,927, 1,538, 1,234 cm⁻¹ remain

unchanged, indicating that the functional groups are not involved in the biosorption process. The significant changes in the vibrational frequencies indicate that C-C, C-H, C-O groups interacted with the metal ions through complexation reactions. It can be hypothesized that C-H groups are probably involved in the chelation of iron by biomass as hydrogen atoms from these groups could be weak donors. Likewise, the stretching of the band at 2,900–3,000 cm^{-1} of the C-H bond of the $-\text{CH}_2$ group was reported (Quintelas *et al.* 2009). The shift in the spectrum due to binding of iron to the exopolysaccharide was also reported (Tapia *et al.* 2011). Similar changes in the peak intensities have been reported (Moppert *et al.* 2009; Ova & Ovez 2013; Sofu *et al.* 2015).

CONCLUSIONS

It has been demonstrated that this LAB biomass offers interesting possibilities as a biosorbent (for Fe(II) or Fe(III)). The biosorption of metal ion by the biomass was affected by initial metal ion concentration, pH, and biomass dosage. ICP-OES, SEM-EDAX analysis of the solution showed that metal ions were bound to the biomass. Changes in the intensity of the peaks in the FTIR spectrum indicate that those functional groups are involved in the biosorption. Hence, LAB can be used as an effective biosorbent for the removal of iron from contaminated water resources.

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