Bioadsorption of Pb(II) and Co(II) ions by the yeast *Saccharomyces cerevisiae*

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Abstract

Present study deals with the biosorption of Pb(II) and Co(II) ions from aqueous solution by the NaOH-treated biomass of *S. cerevisiae*. The optimum biosorption conditions were studied for each metal separately and determined by investigating pH, time course, initial metal concentration, agitation speed, biomass dose, biomass age, temperature, and co-ions. The maximum biosorption of metal ions were observed at pH 4 and 7 for Pb(II) and Co(II) ions, respectively. The metal biosorption capacities were 69.19 ± 0.69 and 11.69 ± 0.34 mg g^{-1} for Pb(II) and Co(II), respectively. No significant effect on the biosorption of Pb(II) ions by co-existing cations and anions was observed, except PO_3^{4-}. The Langmuir and Freundlich equilibrium models represent well the experimental data. The second-order kinetic model described the biosorption kinetics accurately for each metal ion. The calculated thermodynamic parameters, ΔG^o, ΔH^o, and ΔS^o showed that the biosorption of Pb(II) and Co(II) ions by NaOH-treated *S. cerevisiae* was feasible, spontaneous, and endothermic for Pb(II) and exothermic for Co(II). Infrared spectral analysis revealed that carboxyl, amine, and hydroxyl groups on the biomass surface were involved in the biosorption of Pb(II) and Co(II) ions.

Keywords: *Biosorption, heavy metals, Pb(II), Co(II), Saccharomyces cerevisiae*

1. Introduction

Our environment has been strongly exposed to the effect of different harmful pollutants, especially heavy metals which has become one of the most serious environmental problems (**Wang and Chen, 2006**). Pollution caused by heavy metals is now a worldwide phenomenon (**Landis and Yu, 2005**), and discharge of these metals from metal processing industries is known to have adverse effects on the environment (**Ahluwalia and Goyal, 2007**)**.** Heavy metals are not biodegradable and tend to accumulate in living organisms, leading to various diseases and disorders (**Liao *et al*., 2008**). The main treatment technologies include ion-exchange, precipitation and coagulation, membrane processes and electrolytic technologies. However conventional treatment technologies like precipitation and coagulation become less effective and more expensive when situations involving high volumes and low metal concentrations are encountered (**Kapoor and Viraraghavan, 1995**). The removal of heavy metals from the environment especially wastewater is now shifting from the use of conventional adsorbents to the use of biosorbents in a process known as biosorption (**Bahadir *et al*., 2007; Igwe and Abia, 2006**). Biosorption is an innovative and low cost effective method for the removal of toxic substances from wastewaters (**Silva *et al*., 2009**). The potential of fungi for biosorption a range of heavy metals from aqueous solutions is well known (**Kapoor and Viraraghavan, 1995; Sarı and Tuzen, 2009 a, b; Tobin, 2001; Wang and Chen, 2006**). Beside *S. cerevisiae* is easily cultivated using cheap media, it is found in large quantities as a by-product of the alcoholic fermentations (**Wang and Chen, 2006**). In this term, *S. cerevisiae* may be used as a low-cost biosorbent for the removal of toxic heavy metals as a biotechnological application for environmental protection. Isotherm, kinetic, and thermodynamic studies, as well as a lot of parameters influencing the biosorption of Pb(II) and Co(II) by *S. cerevisiae* are lessly performed in the same study. Therefore, this study was carried out to determine the potential of the dead cells of *S. cerevisiae* to adsorb Pb(II) and Co(II) ions in a batch mode and function of many environmental factors. These factors are pH, time course, initial metal ions concentration, temperature, biomass dose, biomass age, agitation speed and co-ions.

2. Materials and methods

2.1. Chemicals, reagents, and equipments

The metal salts $Pb(NO_3)_2$ and $CoCl_2.6H_2O$ were of analytical grade and obtained from Oxford (Mumbai, India) and Hayashi (Japan), respectively. Double distilled water (ddH$_2$O) was used throughout this study. For the quality control purpose, all glassware and sample bottles were soaked with 1M HNO$_3$ for at least 24 h, rinsed several times with tap water, and finally rinsed with ddH$_2$O three times. The cleaned glassware was dried prior to use in

experiments. Other chemicals and equipment used in this study were mentioned elsewhere in this paper.

2.1 Microorganism, medium and cultivation

A baker's yeast *Saccharomyces cerevisiae* used in this study was obtained from a supermarket in the form of lyophilized cells, and then cultured in sterilized Potato Dextrose (PD) agar at 28 ℃ and then routinely maintained at 4 ℃ on PD agar.

2.2 Production of the biomass

For production of yeast biomass, PD medium was used. Cultivation was carried out in 500 ml Erlenmeyer flasks with 250 ml growth medium. Using 1N HNO_3 and 1N NaOH, the pH of the growth medium was adjusted to 5.5 using a pH meter (Hanna HI 8519 pH meter). After autoclaving and cooling the medium to ~ 30 ℃, 2.5 ml cell suspension was used as inoculum to a flask, and then incubated on an orbital rotary shaker (Jeio Tech SI-900 R, Korea) at 125 rpm and 28 ℃. After 48 h of incubation, the biomass was harvested from the growth medium by centrifugation for 5 min at 10000 rpm. The biomass collected was washed with generous amounts of ddH_2O to remove residual growth medium, and the rinsed yeast was again centrifuged.

2.3 Pretreatment of the biomass

According to previous screening in order to choice the best chemical or physical pretreatment method leading to highest biosorption of Pb(II) and Co(II) ions, pretreatment with NaOH solution was the best for metal biosorption enhancement (**Kapoor, 1998**). Therefore, this treatment was adopted and carried out by boiling 4 g wet weight of live harvested biomass in 100 ml of 0.5 N NaOH solution for 15 min. After pretreatment, the biomass was washed with generous ddH_2O until the pH of the washing solution was close to neutral range (6.8~7.2). Before being contacted with the metal bearing solution, the biomass was dried in an oven for 24 h at 60 ℃. Then, the dried biomass was powdered in a mortar with a pestle, sieved through a sieve with 125 μm openings and then stored in a desiccator until use.

2.4 Preparation of the metal stock solutions

The stock solutions with the concentration 1000 mg L^{-1} of Pb(II) and Co(II) ions were prepared by dissolving 1.6 g of $Pb(NO_3)_2$ and 4.04 g of $CoCl_2.6H_2O$, respectively in one liter of ddH_2O, separately. Calculated quantities of these stock solutions were measured and used for experimental solution preparation. Fresh dilutions were used for each experiment. pH was controlled by adding 1M HNO_3 or 1M NaOH.

2.5 Biosorption studies

A batch agitated method was used to determine biosorption of Pb(II) and Co(II) from aqueous solutions by NaOH-pretreated *S. cerevisiae*, and carried out in 100 ml Erlenmeyer flasks containing 20 ml solution on a rotary shaker. The effect of solution pH on the biosorption capacity was investigated in the range of 1–8. Then, 0.04 g of dried biomass was added to the metal solution and the reaction mixture was shaken on an orbital shaker at 125 rpm and 25 ℃ for 2 h. Similarly, the effect of time course (5–90 min), initial metal ion concentration (50–500 mg l^{-1}), agitation speed (0-200 rpm), biomass dose (0.01–0.08 g), biomass age (12-60 h) and temperature (15–40 ℃) on the biosorption of both metals were performed. Experiments were also conducted to evaluate the effect of other heavy metal cations (Cd^{2+}, Cu^{2+} and Zn^{2+}), light metal cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) and metal anions as ammonium salts (chloride, nitrate, phosphate and sulphate) in the concentration 50 mg L^{-1}. In the biosorption experiments, unless otherwise conditions stated, the initial metal concentration, temperature, and biomass dose were kept constant at 50 mg L^{-1}, 25 ℃, and 0.04 g, respectively. Samples taken after the desired incubation period were centrifuged (for 5 min at 10000 rpm) and the supernatant samples were analyzed for concentration of Pb(II) and Co(II) ions using an atomic absorption spectrophotometer (AAS; UNICAM-929, Philips Company, UK) with an air-acetylene flame. For each experiment, a blank containing 20 ml of only the Pb(II) or Co(II) solution without any biosorbent was shaken simultaneously to determine any adsorption of the metal onto the walls of the conical flasks. A control, with 20 ml of ddH$_2$O (no metal ion added) and 0.04 g of biosorbent was also shaken to determine any leaching of metal from *S. cerevisiae*.

2.6 Biosorbent characterization

2.6.1 Surface area analysis

The specific surface areas of the untreated and NaOH-treated fungal biomasses (as free) samples were measured by the Brunauer, Emmett and Teller (BET) technique using a BET surface area analyzer at Central Metallurgical Research and Development Institute, Helwan, Egypt. The microstructure of the biosorbent was characterized using physical adsorption/desorption of nitrogen at −196 ℃. The samples were analyzed with an elemental analyzer, and on the basis of difference in the nitrogen content. Nitrogen isotherms were measured at 77.40 K. Samples were previously degassed under vacuum at 100 ℃.

2.6.2 Fourier transforms infrared spectroscopy (FTIR) analysis

In order to characterization of the surface functional groups, Fourier transform infrared (FTIR) spectra of NaOH-pretreated fungal biomasses before and after metal biosorption were carried out. The samples were dried

overnight to remove any water retained which could interfere with observation of hydroxyl groups on the surface. This was followed by encapsulation into dry potassium bromide (KBr) discs. The discs were then scanned into transmission mode using a Fourier transform infrared (FTIR) spectrometer (Nicolet Nexus 670, Thermo Nicolet Corporation, USA) at National Research Center, Cairo, Egypt, through a wavelength range from 4000 to 400 cm^{-1}. IR spectra of control and metal loaded biomasses were recorded.

2.7 Data evaluation

2.7.1 Metal uptake and biosorption percent efficiency

The amount of metal ion adsorbed per unit biomass was obtained by using the following equation (**Kapoor, 1998**):

$$q = \frac{V(C_i - C_f)}{M} \qquad (1)$$

Where V is the volume of metal solution (L), C_i is the initial metal concentration (mg L^{-1}), C_f is the final/residual concentration (mg L^{-1}) and M is the amount of biomass (g).

The percent biosorption of metal ion was calculated as follows:

$$\text{Biosorption (\%)} = \left(\frac{C_i - C_f}{C_i}\right) \times 100 \qquad (2)$$

2.7.2 Biosorption isotherms

Several mathematical models have been developed to quantitatively express the relationship between the extent of adsorption and the residual solute concentration. The most widely used models are the Langmuir and Freundlich adsorption isotherm models (**Rathinam et al., 2010**). To determine the adsorptive capacity *S. cerevisiae* for Pb(II) and Co(II) ions, the initial metal concentration varied from 50-500 mg L^{-1}; while the biosorbent was constant at 0.04 g 20 ml^{-1}. A Langmuir isotherm was then obtained by plotting the values of biosorption capacity (q) versus the residual metal concentration (C_f) in solution.

The classical Langmuir equation is given as follows (**Foo and Hameed, 2010**):

$$q_e = \frac{q_{max}bC_f}{1 + bC_f} \qquad (3)$$

Where,

q_e = metal adsorbed on the biosorbent (mg g^{-1}) at equilibrium;

q_{max} = maximum possible amount of metal adsorbed per unit weight of biosorbent;

C_f = residual concentration of metal (mg L^{-1}) in the solution;

b = equilibrium constant related to the affinity of the binding sites for the metals.

Equation (3) can be linearized as follows:

$$\frac{1}{q_e} = \left(\frac{1}{q_{max}b}\right)\left(\frac{1}{C_f}\right) + \left(\frac{1}{q_{max}}\right) \tag{4}$$

When $1/q_e$ is plotted against $1/C_f$, a straight line with slope $1/q_{max}\, b$ is obtained and the intercept is corresponding to $1/q_{max}$. Also, q_{max} and b were determined.

The classical Freundlich equation is given as follows (**Foo and Hameed, 2010**):

$$q_e = K_f\, C_f^{\frac{1}{n}} \tag{5}$$

Where,

q_e = metal adsorbed on the biosorbent (mg g^{-1} d.wt) at equilibrium;

C_f = residual concentration of metal (mg L^{-1}) in the solution;

K_f = an empirical constant that provides an indication of the intensity of adsorption.

n = Freundlich adsorption constant

This equation can be linearized by taking natural logarithm of both sides of the equation, which can be given as follows:

$$\log q_e = (1/n) \log C_f + \log K_f \tag{6}$$

When the values of *Log C_f* are plotted against the values of *Log q_e*, the adsorption constants (K_f and n) were obtained.

2.7.3 Biosorption kinetics

In order to analyze the biosorption kinetics of Pb(II) and Co(II) ions, the pseudo-first-order and the pseudo-second-order kinetic models (**Ho and McKay, 1999; Wang and Chen, 2009**) were applied to data.

The pseudo-first-order equation is expressed as:

$$\frac{dq_t}{d_t} = K_1 (q_e - q_t) \tag{7}$$

Where,

q_e = biosorption capacity (mg g^{-1} d.wt) at equilibrium;

q_t = metal adsorbed on the biosorbent (mg g^{-1} d.wt) at time t;

K_1 = pseudo-first-order model constant (min^{-1});

Equation (7) can be linearized as follows:

$$\log(q_e - q_t) = \log(q_e) - \frac{K_1}{2.303}t \qquad (8)$$

When log (q_e-q_t) is plotted against t, a straight line with slope $K_1/2.303$ is obtained and the intercept is corresponding to log (q_e). Also, q_e and K_1 were determined.

The classical pseudo-second-order equation is given as follows:

$$\frac{dq_t}{dt} = K_2(q_e - q_t)^2 \qquad (9)$$

Where,

q_e = biosorption capacity (mg g^{-1} d.wt) at equilibrium;

q_t = metal adsorbed on the biosorbent (mg g^{-1} d.wt) at time t;

K_2 = pseudo-second-order model constant (g mg^{-1} min^{-1});

Equation (9) can be linearized as follows:

$$\frac{1}{q_t} = \frac{1}{K_2 q_e t} + \frac{1}{q_e} \qquad (10)$$

When $1/q_t$ is plotted against $1/t$, a straight line with slope $1/K_2\,q_e$ is obtained and the intercept is corresponding to $1/q_e$. Also, q_e and K_2 were determined.

2.7.4 Biosorption thermodynamics

Thermodynamic parameters including Gibbs free energy change (ΔG°), entropy (ΔS°), and enthalpy (ΔH°) change were used to describe thermodynamic behavior of the biosorption of Pb(II) and Cd(II) by *S. cerevisiae* biomass. These parameters were determined by the following equations (**Majumdar *et al*., 2010**):

$$\Delta G^\circ = -RT \ln b \qquad (11)$$

$$\ln(b) = \left(\frac{\Delta S^\circ}{R}\right) - \left(\frac{\Delta H^\circ}{RT}\right) \qquad (12)$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \qquad (13)$$

Where,

R = universal gas constant (8.314 J mol^{-1} K^{-1});

T = solution temperature in kelvin (K);

b = equilibrium constant obtained from Langmuir isotherm.

When $\ln(b)$ is plotted against $1/T$, a straight line with slope $-(\Delta H°/R)$ is obtained and the intercept is corresponding to $(\Delta S°/R)$. Also, $\Delta H°$ and $\Delta S°$ were determined.

2.8 Statistical analysis

The values presented in the study were means of three replicates. The SPSS (Statistical Package for Social Sciences) 9.05 version was employed to determine the standard error and correlation coefficients.

3. Results and discussion

3.1. Influence of pH

pH plays a major role in the biosorption of metals onto fungal biomasses. It strongly influences site dissociation of the fungal biomass and the chemistry of heavy metals as well as the competition of metallic ions for the binding site (**Wang and Chen, 2006**). Fig. 1a illustrates the effect of pH versus the amount of metal adsorbed (mg g^{-1}) of both metals. It can be seen that metal biosorption increases with increasing pH in the range of pH 1-4 for Pb(II) and pH 1-7 for Co(II), and maximum Pb(II) and Co(II) biosorption capacities were 23.77 ± 0.25 mg g^{-1} and 6.20 ± 0.32 mg g^{-1} at pH 4 and 7, respectively. The same finding was also observed by **Gharieb *et al.* (2014)** in the biosorption of Pb(II) and Co(II) by NaOH-treated *R. oryzae*. They found that the optimum pH values for Pb(II) and Co(II) biosorption were 4 and 7, respectively. **Göksungur *et al.* (2005)** studied the biosorption of Cd(II) and Pb(II) by using NaOH pretreated baker's yeast (*S. cerevisiae*) and found that an increase or decrease in the pH from these optimum pH values resulted in a reduction in the biosorption of these metal ions. Therefore these optimum pH values were used for further study. According to **Fan *et al.* (2008)**, low biosorption at pH 1 could be ascribed to hydrogen ions (H^+) competing with metal ions for binding sites. This means that as the pH increases, more negatively charged cell surface becomes available thus facilitating greater metal biosorption. However, metal complexes are formed at high pH (>7) which rejected by negatively charged biomass. Several researchers have also investigated the effect of pH on biosorption of heavy metals by using different fungal biomasses and found similar results with this study. **Dursun, (2006)** and **Huang *et al.* (2009)** investigated the biosorption of Pb(II) ions by *Aspergillus niger* and *Agaricus bisporus*, respectively, and determined the optimum pH for biosorption as 4. **Baysal *et al.* (2009)** found that the biosorption of Pb(II) by *Candida albicans* was maximally obtained at pH 5. **Pal *et al.* (2006)** used *Mortierella* SPS 403 for Co(II) biosorption and found that metal uptake increased with increase in medium pH and had a maximum value at pH 7.

3.2. Influence of contact time

Contact time is another important variable during metal biosorption. Fig. 1b shows the effect of time course profiles for the biosorption of Pb(II) and Co(II) by *S. cerevisiae* biomass. In order to determine the effect of contact time on the biosorption of both metal ions, the contact time was varied from 5 to 90 min. From the results it was observed that Pb(II) and Co(II) biosorption by *S. cerevisiae* biomass was relatively fast and equilibrium was achieved in about 60 and 30 min for Pb(II) and Co(II), respectively. The biosorption capacity values were 23.67±0.19 and 6.41±0.31 at the same order. The rapid initial biosorption is probably due to the abundant of availability of active metal binding sites on the biosorbent surface and the gradual occupancy of those sites. This rapid initial biosorption is consistent with the finding of **Gharieb *et al.*** (**2014**) in the biosorption of Pb(II) and Co(II) by NaOH-treated *R. oryzae*, and **Li *et al.*** (**2008**) in biosorption of Pb(II) and Cu(II) ions by *Penicillium simplicissimum*, and equilibrium values were attained at 60 min. Mn(II) biosorption equilibrium time for *A. niger* and *S. cerevisiae* were found to be 60 and 20 min, respectively (**Parvathi *et al.*, 2007**). About 80% of Pb(II) ions were biosorbed by *Rhodotorula glutinis* in the first 10 min of contact time (**Cho and Kim, 2003**). These observations suggest very active interaction of these metals with functional groups located on the surface of the fungal biomass, which has significant practical importance in biosorption of heavy metals on large scale as it will facilitate smaller reactor volumes ensuring efficiency and economy (**Herrero et al., 2005**).
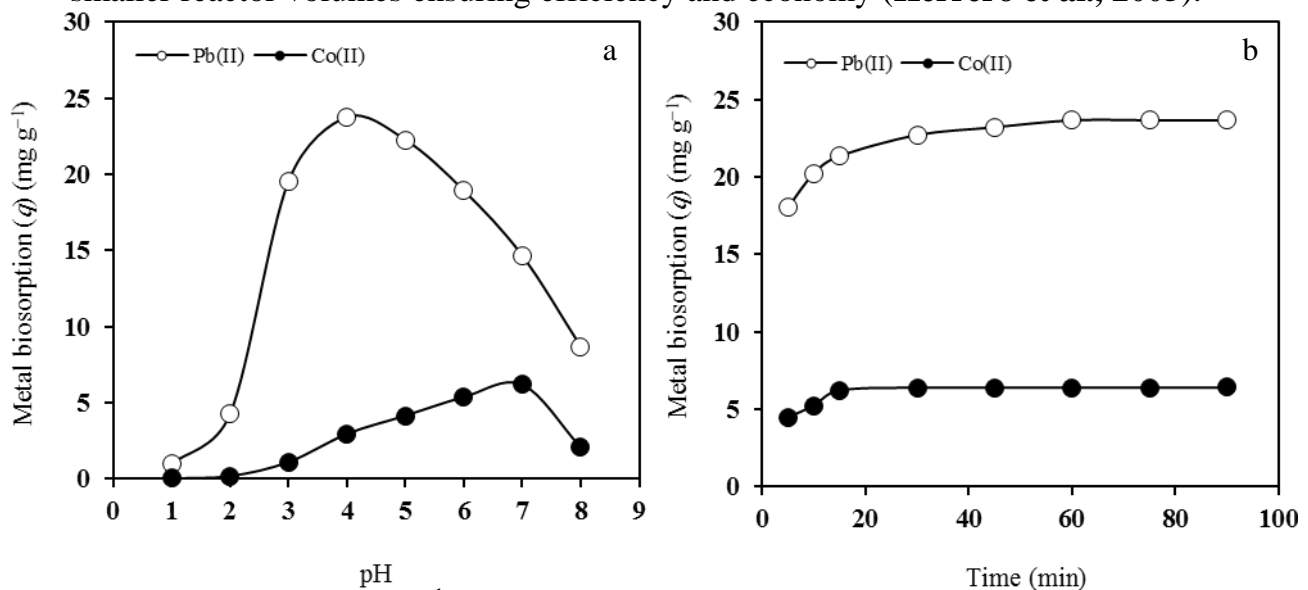

Fig.1: Biosorption (mg g^{-1}) of Pb(II) and Co(II) by *S. cerevisiae* at (a) different pH values and (b) different time intervals. The data are the mean values of 3 replicates, and the bars indicate the standard error of the mean (when not shown it is smaller than the scale).

3.3. Influence of initial metal concentration

Initial metal concentration remarkably influences the adsorption of metal to the biomass surface. The results presented in Fig. 2a indicate that metal biosorption increased with increasing metal concentration and then reached a saturation values at about 450 mg/l and 350 mg/l of Pb(II) and Co(II), respectively. At these concentrations, the biosorbed Pb(II) and Co(II) reached 69.19±0.69 and 11.69±0.34

mg g^{-1} at the same order. Then there was no significant increase in biosorbed metal. This increase in uptake capacity of the biosorbent with the increase in initial metal concentrations is due to higher availability of metal ions for the biosorption. Moreover, higher initial concentration provides increased driving force to overcome all mass transfer resistance of metal ions between the aqueous and solid phase resulting in higher probability of collision between metal ions and biosorbent leading to higher metal uptake (**Tewari *et al.*, 2005**).

3.4. Influence of agitation speed

The effect of shaker agitation speed (0-200 rpm) on Pb(II) and Co(II) biosorption by *S. cerevisiae* was studied. From the results illustrated in Fig. 2b, it was noticed that optimum values of Pb(II) and Co(II) biosorption were 23.39±0.28 and 7.55±0.43 mg g^{-1}, respectively and achieved at the agitation speed of 150 rpm. Beyond which biosorption of both metals is decreased. These results indicate that the agitation rate assures all the yeast cell wall binding sites are available for metal biosorption. At high agitation speed, vortex phenomenon occurs and the suspension is no longer homogenous which makes the adsorption metals difficult (**Guo *et al.*, 2006**). Similar results were reported for the biosorption of Pb(II) and Co(II) by NaOH-treated *Rhizopus oryzae* (**Gharieb *et al.*, 2014**) and biosorption of inorganic mercury onto marine alga *Sargassum tenerrimum* (**Rajamohan and Sivaprakash 2010**). They found that the optimum agitation speed values were 150 rpm. In the literature, the optimal values of Cd(II) biosorption capacity by *A. niger* (**Guo *et al.*, 2006**) and Cr(VI) by *R. nigricans* (**Bai and Abraham, 2001**) were obtained at agitation speed of 120 rpm. Opposite result was reported by **Selatnia *et al.* (2004**). They found that the optimum agitation speed for Pb(II) biosorption by a bacterial dead *Streptomyces rimosus* biomass was 250 rpm.
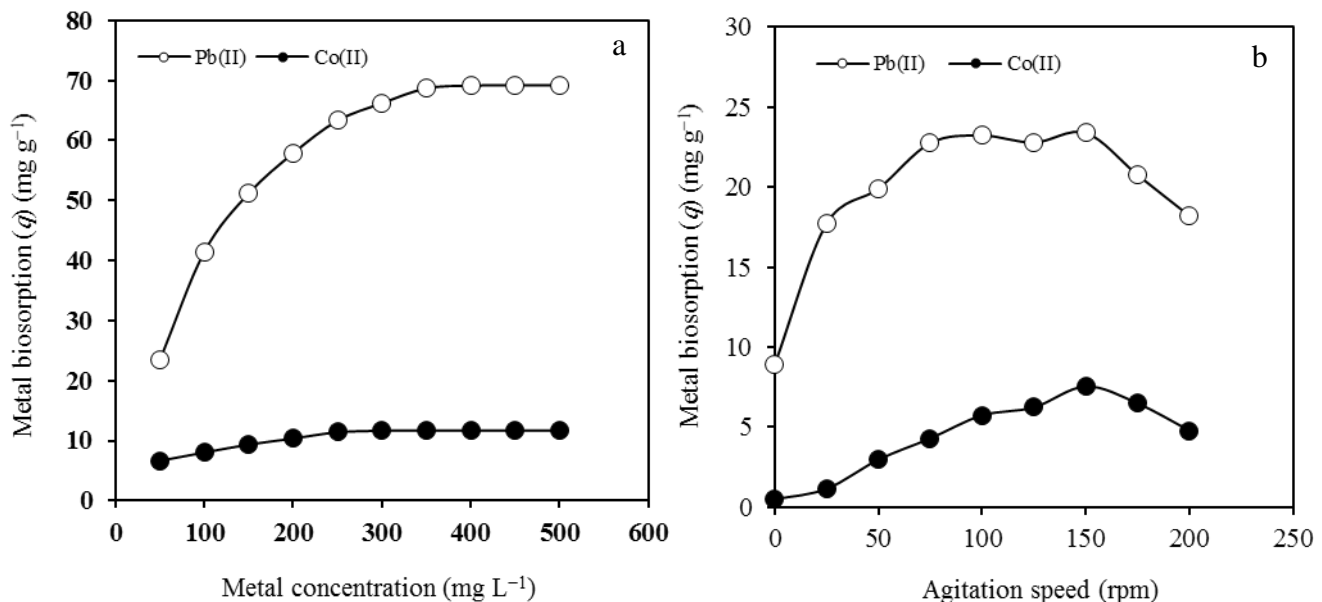

Fig. 2: Biosorption (mg g−1) of Pb(II) and Co(II) by S. cerevisiae at (a) different metal concentrations and (b) different agitation speed. The data are the mean values of 3 replicates.

3.5. Influence of biomass dose

The quantity of biosorbent is a significant factor to be considered for effective biosorption. Biosorption of Pb(II) and Co(II) by *S. cerevisiae* was studied at different biomass weights in the level (0.5, 1, 2, 3, 4, and 5 g L^{-1}). Biosorption of Pb(II) and Co(II) decreased significantly as the amount of biosorbent added increased (Table 1). The biosorbed metal decreased from 39.57±2.39 to 8.69±0.17 mg g^{-1} for Pb(II) and from 8.90±0.83 to 1.30±0.17 mg g^{-1} for Co(II) due to ten-fold increase in biosorbent. This phenomenon is expected because as the dose of biosorbent increased, there was increase in the available exchangeable sites for metal ions. On the other hand, biosorbent dose of 4 and 3 g L^{-1} were required to remove 96.40% and 24.30% of Pb(II) and Co(II) ions, respectively from aqueous solution. Similar results were obtained with other fungal biosorbents including NaOH-treated *R. oryzae* in the biosorption of Pb(II) and Co(II) (**Gharieb *et al*., 2014**), *Pycnoporus sanguineus* in the biosorption of Pb(II) (**Azila *et al*., 2008**) and *Mortierella* SPS 403 in the biosorption of Co(II) (**Pal *et al*., 2006**). Reductions in biomass dose in the biosorption medium at a given metal concentration enhanced the metal/biosorbent ratio and thus increased the metal uptake per unit weight of biosorbent as long as the later is not saturated (**Anayurt *et al*., 2009; Romera *et al*., 2007**). There are many reasons have been suggested to explain the decreased biosorption capacity at increasing biosorbent dose including electrostatic interactions and interference between binding sites (**Tunali *et al*., 2006**) and a partial aggregation of biomass at higher biomass doses, which in turn results in a decrease in effective surface area available for the biosorption (**Karthikeyan *et al*., 2007; Selatnia *et al*., 2004**).

Table 1 Biosorption (mg g^{-1}) of Pb(II) and Co(II) at different biomass doses (g L^{-1}), biomass ages(h) and temperatures (ºC). The data are the mean values of 3 replicates ± standard error of the mean.

Biomass dose	Metal biosorption (mg g^{-1})	
g L^{-1}	Pb(II)	Co(II)
0.5	39.57 ± 0.69	8.9 ± 0.83
1	35.59 ± 0.9	7.06 ± 0.67
2	22.97 ± 0.31	5.85 ± 0.48
3	15.91 ± 0.18	4.05 ± 0.12
4	12.06 ± 0.07	2.49 ± 0.12
5	8.09 ± 0.17	1.3 ± 0.17
Biomass age (h)		
12	24.97 ± 0.03	11.68 ± 0.91
24	24.99 ± 0.01	9.03 ± 0.41

36	24.76 ± 0.16	8.47 ± 0.28
48	23.24 ± 0.35	6.11 ± 0.47
60	22.83 ± 0.32	6.04 ± 0.19
Temperature (ºC)		
15	15.41 ± 0.32	5.48 ± 0.17
20	19.36 ± 0.17	6.21 ± 0.19
25	22.97 ± 0.05	6.57 ± 0.04
30	23.24 ± 0.16	6.54 ± 0.1
35	20.5 ± 0.16	6.48 ± 0.04
40	19.37 ± 0.18	6.42 ± 0.07

3.6. Influence of biomass age

The effect of biomass age of *S. cerevisiae* on Pb(II) and Co(II) biosorption was also investigated. In order to carry out this experiment, *S. cerevisiae* was grown to different times of growth (12-60 h) in PD medium. The results indicate that no big difference was found in Pb(II) biosorption for cell incubation periods ranging from 12 to 60 h, and adsorbed metal values were 24.97±0.03 and 22.83±0.32 mg g^{-1} of Pb(II) by 12-old- and 60-old-biomasses, respectively (Table 1). On the other hand, the younger biomass (12 h) in its logarithmic phase removed 2 times more Co(II) than the older one (60 h), and the adsorbed Co(II) values were 11.68±0.91 and 6.04±0.19 mg g^{-1} by 12-old- and 60-old-biomasses, respectively. Usually, the cells at lag phase or early stages of growth have a higher biosorptive capacity for metal ions than that of stationary phase (**Kapoor and Viraraghavam, 1997**). These results are in agreement with **Volesky and Phillips** (**1995**) in biosorption of uranyl ions from aqueous solution. They found that older biomasses of baker's yeast showed a decrease in metal biosorption capacity.

3.7. Influence of temperature

Results of metal biosorption experiments carried out at different temperatures ranging from 15 to 40 ºC are shown in Table 1. The results indicate that the change of temperature influences the biosorption capacity of Pb(II) by *S. cerevisiae.* Maximum biosorption of Pb(II) is obtained at 30 ºC and the biosorbed Pb(II) was 23.24±0.16 mg g^{-1}. Similar result was obtained by **Bahadir *et al*.** (**2007**) in Pb(II) biosorption by *R. arrhizus*. They found that the optimum temperature was 30 ºC. Increasing temperature is known to increase the diffusion rate of adsorbate molecules within pores as a result of decreasing solution viscosity and will also modify the equilibrium capacity of the biosorbent for a particular adsorbate (**Khezami and Capart, 2005**). On the

other hand, data show no significant difference in Co(II) biosorption for *S. cerevisiae* at different temperatures in the range 20-40 ºC, indicating that Co(II) biosorption by *S. cerevisiae* is temperature-independent biosorption. Temperature-independent biosorption has been reported by other researchers on different metals (**Ahmadpour** *et al.*, **2009; Arica** *et al.*, **2004; Bayramoğlu** *et al.*, **2006; Gharieb** *et al.*, **2014; Kahraman** *et al.*, **2005**).

3.8. Influence of interfering ions

The influence of other ions on the biosorption of Pb(II) and Co(II) ions is very important because industrial effluents have ions other than the targeted ions which greatly affect the biosorption of the required ion in aqueous medium. To investigate possible interference caused by the presence of other metal cations or anions at concentration 50 mg L^{-1} were added to the Pb(II) or Co(II) solution in a batch system. From the results presented in Table 2, it is noticed that there is no antagonistic effect on Pb(II) biosorption in binary solutions Pb-Cd, Pb-Cu, and Pb-Zn, or multi-metal solution Co-Cd-Cu-Zn. In many multi-metal biosorption studies, Pb(II) generally showed high biosorptive capacity by various fungal biomasses. **Kogej and Pavko** (**2001**) found that the biosorption binding constant determined for Pb(II) ion is substantially higher than those of other metal ions, which indicates a very favorable biosorption of Pb(II) by *R. nigricans*. However, multi-metal solution Pb-Cd-Cu-Zn had a significant effect on Co(II) biosorption and reduced the Co(II) biosorption capacity from 6.38±0.20 to 4.16±0.11 mg g^{-1}. Similar results were observed by **Pal** *et al.* (**2006**) in the biosorption of Co(II) by the fungus *Mortierella* SPS 403. They found that the presence of additional metal cations such as Pb(II), Cu(II), Cd(II), Zn(II) and Ni(II) had inhibitory effect on Co(II) biosorption. This might be due to competition of divalent metal cations for complexation with the active binding sites of fungal biomass leading to decrease in Co(II) biosorption.

The effect of the presence of light metal ions such as Na^+, K^+, Ca^{2+} and Mg^{2+} on Pb(II) and Co(II) biosorption capacity of *S. cerevisiae* is shown in Table 2. The results revealed that Pb(II) biosorption was unaffected in the presence of Na^+, K^+, Ca^{2+}, or Mg^{2+}. In the literature, similar results were reported for biosorption of Pb(II) and Co(II) (**Gharieb** *et al.*, **2014**) and Pb(II) biosorption by *Rhodotorula glutinis* (**Cho and Kim, 2003**) and *Phellinus badius* (**Matheickal and Yu, 1997**) in which Pb(II) biosorption was not affected by the presence of Na^+, K^+, Ca^{2+}, or Mg^{2+} ions. On the other hand, Co(II) biosorption decreased from 6.38±0.20 to 6.11±0.15, 6.08±0.16, 3.78±0.13, and 4.09±0.20 mg g^{-1} in the presence of 50 mg L^{-1} Na^+, K^+, Ca^{2+}, and Mg^{2+}, respectively.

The influence of anions was investigated as well, including Cl^-, NO_3^-, PO_4^{3-}, and SO_4^{2-}. From the results presented in Table 2, it was observed that

Cl^-, NO_3^-, and SO_4^{2-} did not affect the biosorption of Pb(II). On the other hand, Pb(II) biosorption decreased from 23.68±0.30 mg g^{-1} to 13.31±0.23 mg g^{-1} when 50 mg L^{-1} of PO_4^{3-} was added to the biosorption medium. Moreover, Co(II) biosorption was decreased from 6.38±0.20 mg g^{-1} to 4.20±0.08, and 5.79±0.10 mg g^{-1} in the presence of PO_4^{3-}, and SO_4^{3-}, respectively. Nearly, the same results were observed by **Das *et al*.** (**2002**) in the biosorption of Pu^{4+} by *S. cerevisiae*. They found that Cl^-, NO^{3-}, SO_4^{2-}, $C_2O_4^{2-}$, and CH_3COO^- do not have any adverse influence on Pu^{4+} biosorption, while the presence of PO_3^{4-} reduces the biosorption of this metal.

Table 2: Pb(II) and Co(II) biosorption (mg g^{-1}) with or without other Co-ions at a concentration of 50 mg L^{-1}. The data are the mean values of 3 replicates ± standard error of the mean.

Competitive ion (mg L^{-1})	Metal biosorption (mg g^{-1})	
	Pb(II)	Co(II)
Non (control)	23.68±0.30	6.38±0.20
Heavy metal cation		
Cd	23.63±0.24	5.11±0.15
Cu	23.66±0.19	5.21±0.17
Zn	23.61±0.22	5.29±0.18
Co+Cd+Cu+Zn	23.13±0.18	−
Pb+Cd+Cu+Zn	−	4.16±0.11
Light metal cation		
Na$^+$	23.62±0.13	6.11±0.15
K$^+$	23.63±0.11	6.08±0.16
Ca^{2+}	23.60±0.19	3.78±0.13
Mg^{2+}	23.67±0.18	4.09±0.20
Anion		
Cl$^-$	23.72±0.11	6.21±0.13
NO$_3^-$	23.74±0.15	6.18±0.12
PO$_3^{4-}$	13.31±0.23	4.20±0.08
SO$_4^{2-}$	23.51±0.20	5.79±0.10

3.9. Biosorption isotherms

Biosorption isotherm provides a relationship between the concentration of metal in solution and the amount of metal on biosorbent when both the phases are at equilibrium. Modeling of the Pb(II) and Co(II) biosorption on both fungal biomasses were realized by applying Langmuir and Freundlich

adsorption isotherms, which are widely used to analyze data for water and wastewater treatment applications. The linearized forms of the Langmuir (Eq. 4) and Freundlich (Eq. 6) isotherm models were used to analyze and fit the data to these models. Figs. 3 show the isotherm plots for Pb(II) and Co(II) by *S. cerevisiae*. The Langmuir constants (q_{max} and *b*) with correlation coefficients (R^2) were calculated from the plots in Figs. 4 and the results are presented in Table 3. Also, Freundlich constants (K_f and *n*) with correlation coefficients (R^2) were calculated from the plots in Figs. 5, and presented in Table 4. The fit of experimental data to these models was evaluated by the correlation coefficients (R^2). From the final results and based on the values of correlation coefficients (R^2), Langmuir and Freundlich models best described the experimental data for biosorption of Pb(II) and Co(II) at different temperatures. In view of the Langmuir constant (q_{max}) values, the q_{max} values at 25 ℃ are close to the experimental q_{max} values. The parameter q_{max} reflects the metal affinity to the sites of the yeast biomass. The Langmuir adsorption constant (*b*) is related to the affinity of the biosorbent towards the metal ions (**Singh *et al*., 2010**). The favorable biosorption is indicating by higher than 1 values of Freundlich sorption constant *n*. The values of *n* obtained greater than one for both metals indicated that physical and multilayer adsorption takes place. The value of (*n*) falling in the range 1-10 indicates favorable biosorption (**Aksu and Kutsal, 1991**). The small (K_f) values for Co(II) indicate a lower extent biosorption, while more biosorption was observed for Pb(II) ions because of its larger (K_f) values. Similar results were also reported in the literature (**Akar *et al*., 2007; Gharieb *et al*., 2014; Gopal *et al*., 2002; Jonglertjunya, 2008; Oh *et al*., 2009; Singh *et al*., 2010**).
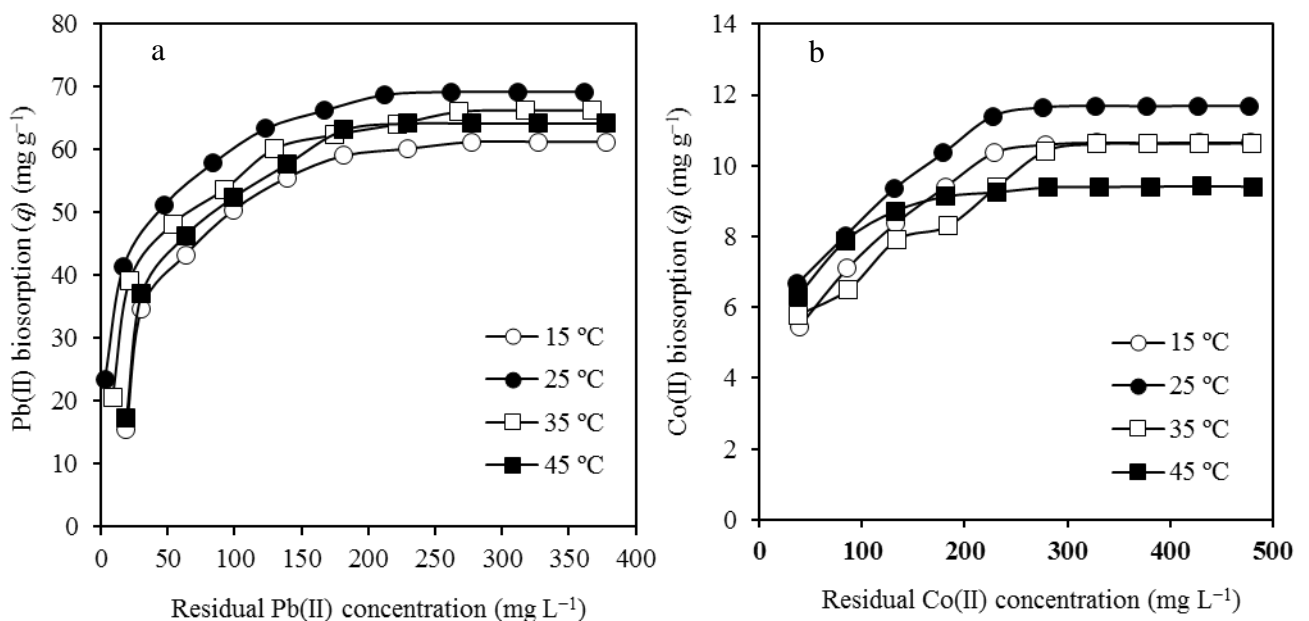

Fig. 3: Biosorption isotherm plots for (a) Pb(II) and (b) Co(II) ions. Amount of dried biomass: 0.04 g; initial pH: 4; suspension volume: 20 ml; agitation rate: 150 rpm.
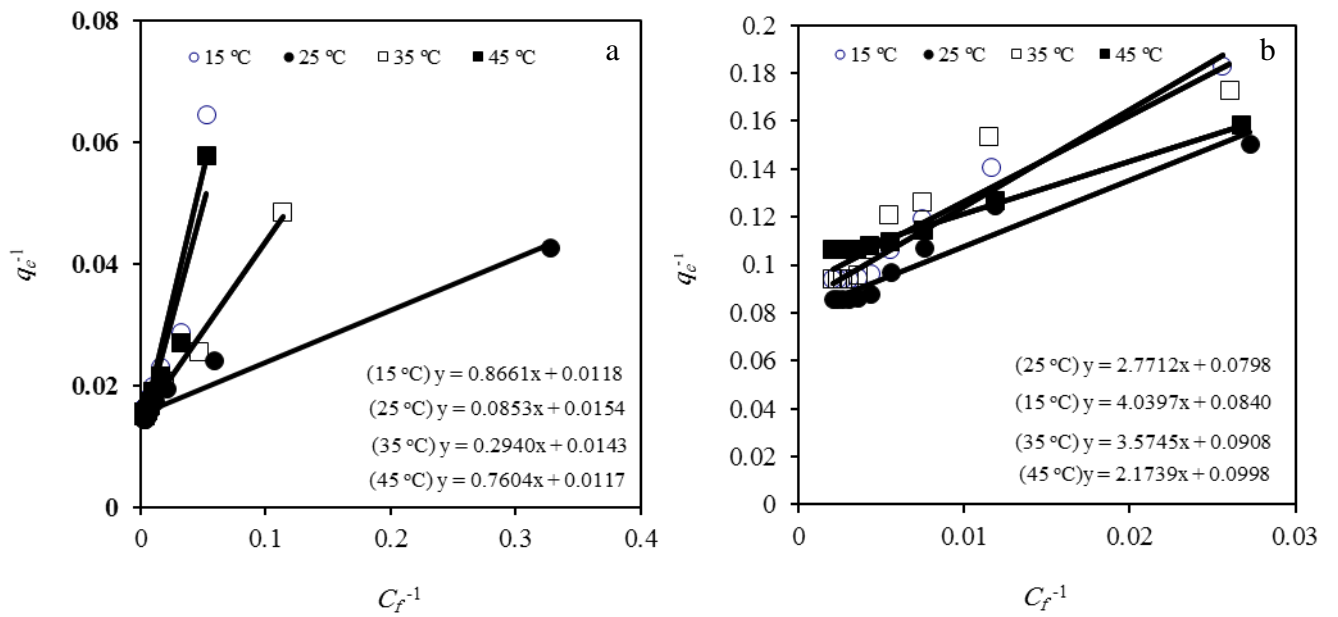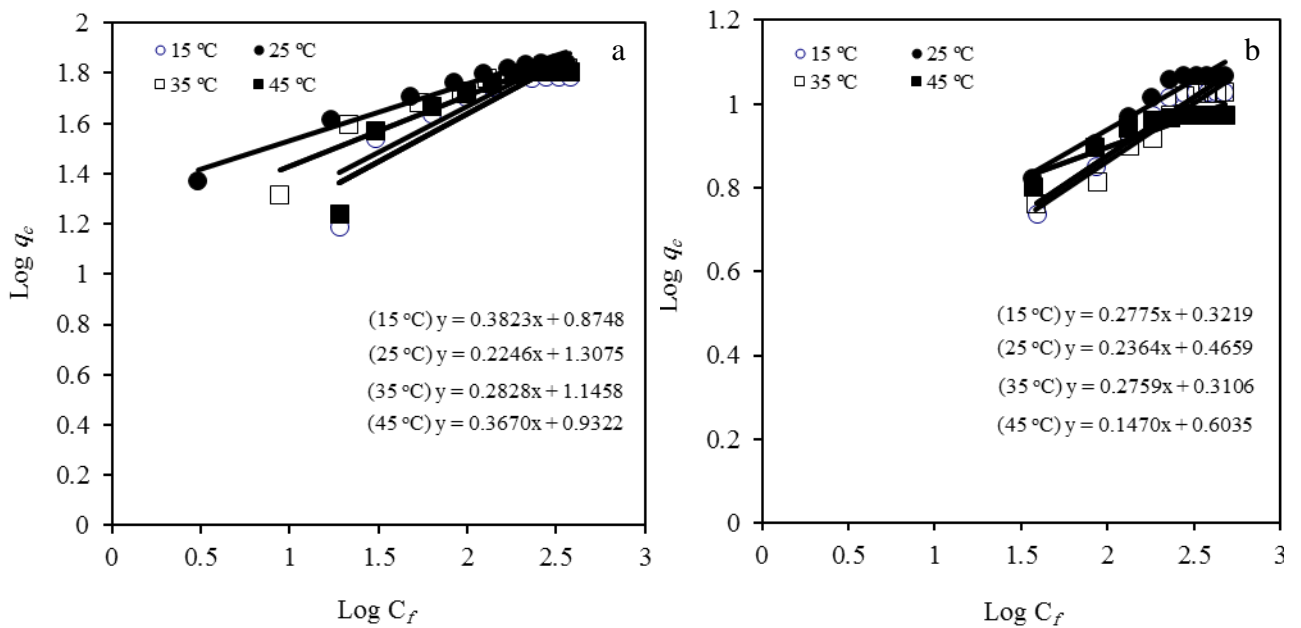
Fig. 4: Application of Langmuir isotherm model for (a) Pb(II) and (b) Co(II) biosorption.

Table 3
Langmuir isotherm model parameters of Pb(II) and Co(II) biosorption by *S. cerevisiae*.

Metal ion	Temperature °C	Freundlich isotherm parameters			
		K_f (L g^{-1})	n	$1/n$	R^2
Pb(II)	15	7.50	2.62	0.38	0.898
	25	20.30	4.45	0.22	0.980
	35	13.99	3.54	0.28	0.948
	45	8.55	2.72	0.37	0.901
Co(II)	15	2.10	3.60	0.28	0.966
	25	2.92	4.23	0.24	0.970
	35	2.04	3.62	0.28	0.977
	45	4.01	6.80	0.15	0.929

Fig. 5: Application of Freundlich isotherm model for (a) Pb(II) and (b) Co(II) biosorption.

Table 4
Freundlich isotherm model parameters of Pb(II) and Co(II) biosorption by *S. cerevisiae*.

Metal ion	Temperature °C	Experimental q_{max} (mg g^{-1})	Langmuir isotherm parameters		
			q_{max} (mg g^{-1})	b (L mg^{-1})	R^2
Pb(II)	15	61.23	84.75	0.013	0.948
	25	69.19	64.94	0.180	0.980
	35	66.23	69.93	0.048	0.995
	45	64.14	85.47	0.015	0.952
Co(II)	15	10.65	11.90	0.020	0.987
	25	11.69	12.53	0.028	0.971
	35	10.63	11.01	0.025	0.929
	45	9.42	10.02	0.045	0.996

3.10. Biosorption kinetics

In order to examine the controlling mechanism of the biosorption process such as mass transfer and chemical reaction, kinetic models such as pseudo-first-order and pseudo-second-order were used to test the experimental data. The linearized forms of the pseudo-first-order model (Eq. 8) and pseudo-second-order model (Eq. 10) were used to analyze and fit the experimental data to these models. Fig. 6 shows the time profile for Pb(II) and Co(II) biosorption by *S. cerevisiae*. The constants (q_e and K_1) and (q_e and K_2) of pseudo-first-order and pseudo-second-order models, respectively with correlation coefficients (R^2) were calculated from the plots in Fig. 7 and the results are presented in Table 5. The values of correlation coefficient R^2 for the pseudo-second-order model are relatively high (0.964-0.996) and the biosorption capacities calculated by the model are also close to those determined by experiments. However, the values of R^2 for the pseudo-first-order are found to be largely lower and ranged from -0.691 to -0.917 (negative weak correlation).

Therefore, it has been concluded that biosorption of Pb(II) and Co(II) by *S. cerevisiae* is well described by the pseudo-second-order model. **Aksu and Tezer (2000)** suggested that the pseudo-first-order model does not fit well during the entire adsorption period and is generally applicable in the initial 20-30 min of the sorption process. In contrast, the pseudo-second-order model predicts the behavior of biosorption over the whole adsorption time and is in agreement with adsorption mechanism being the rate-controlling step (**Guo *et al.,* 2006**). In the literature, similar results were also reported in the biosorption of Pb(II) and Co(II) (**Gharieb *et al.,* 2014**), Pb(II) by *Mucor rouxii* (**Majumdar *et al.,* 2010**) and Cu(II) by *Pycnoporus sanguineus* (**Yahaya *et al.,* 2009**).
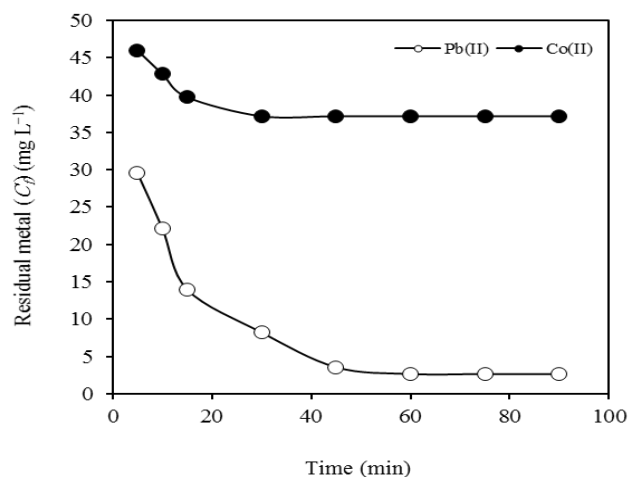

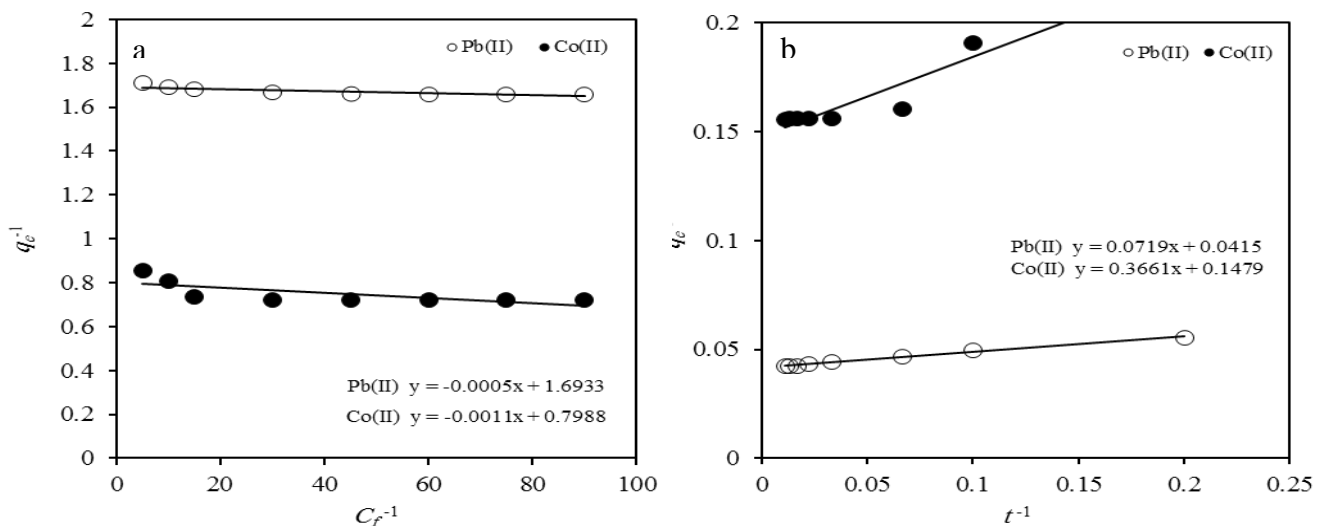Fig. 6: Time profile of Pb(II) and Co(II) biosorption by *S. cerevisiae*.

Fig. 7: Linearized (a) pseudo-first-order and (b) pseudo-second-order plots for the biosorption of Pb(II) Co(II) by *S. cerevisiae*.

Table 5

The parameters of pseudo-first-order and pseudo-second-order models for the kinetic study of Pb(II) and Co(II) biosorption by *S. cerevisiae*.

Pseudo-first-order				
Metal ion	Experimental q_e (mg g^{-1})	q_e (mg g^{-1})	$K_1 \times 10^{-3}$ (min^{-1})	R^2
Pb(II)	23.67	49.35	1.2	-0.845
Co(II)	6.42	6.29	2.5	-0.691

Pseudo-second-order				
Metal ion	Experimental q_e (mg g^{-1})	q_e (mg g^{-1})	K_2 (g mg^{-1} min^{-1})	R^2
Pb(II)	23.67	24.10	0.58	0.996
Co(II)	6.42	6.76	0.40	0.972

3.11. Biosorption thermodynamics

Thermodynamic parameters including the Gibbs free energy change (ΔG^o), entropy (ΔS^o), and enthalpy (ΔH^o) were used to describe thermodynamic behavior of the biosorption process. The values of these parameters were calculated (using Eqs. 11, 12, and 13) and presented in Table 6. The negative values of ΔG^o confirm the feasibility and spontaneous nature of the biosorption process. The decrease in ΔG^o values shows a decline in feasibility of biosorption as temperature is increased. Similar findings were also reported in the literature (**Dursun, 2006; Sarı and Tuzen, 2008; Anayurt *et al.*, 2009; Singh *et al.*, 2010**). According to **Yu *et al.* (2001)**, the absolute magnitude of the ΔG^o for physisorption is between −20 and 0 kJ mol^{-1} and chemisorption has a range of −80 to −400 kJ mol^{-1}. The ΔS^o and ΔH^o values (Table 7) were calculated from the intercept and slope of the plot, respectively (using Eq. 12). The ΔS^o was calculated to be 58 J mol^{-1} K^{-1} for Pb(II) and 120 J mol^{-1} K^{-1} for Co(II) biosorption. The positive value of ΔS^o reveals the increased randomness at the solids/solution interface during the fixation of metal ions on the active sites of the biosorbent. (**Bayramoğlu *et al.*, 2003; Mashitah *et al.*, 2008; Singh *et al.*, 2010**). The ΔH^o values for Pb(II) and Co(II) were found to be 4.95 and −17.55 kJ mol^{-1}, respectively. The positive ΔH^o values of Pb(II) biosorption is indicator of endothermic nature of this biosorption. In the literature, the endothermic nature was also reported for biosorption of Pb(II) and Co(II) by NaOH-treated *R. oryzae* (**Gharieb *et al.*, 2014**), Pb(II) by brewery yeast (**Kim *et al.*, 2005**) and *Penicillium simplicissimum* (**Fan *et al.*, 2008**). In contrast, the exothermic nature was also reported for Pb(II) biosorption by *Mucor rouxii* (**Majumdar *et al.*, 2010**). The negative ΔH^o values of Co(II) biosorption in this study show that the biosorption is exothermic in nature, which in agreement with previous reports (**Bhatnagar *et al.* 2010; Gharieb *et al.*, 2014**).

Table 6

Values of ΔG^o of thermodynamic parameters of Pb(II) and Co(II) biosorption at different temperatures.

Metal	Temperature (K)	b (L mg^{-1})	ΔG^o (kJ mol^{-1})
Pb(II)	288	0.013	-18.91
	298	0.180	-26.80
	308	0.048	-23.57
	318	0.015	-21.26
Co(II)	288	0.020	-16.93
	298	0.028	-18.36
	308	0.025	-18.68
	318	0.045	-20.84

Metal	Equation	ΔS^o (J mol^{-1} K^{-1})	ΔH^o (KJ mol^{-1})
Pb(II)	y = 594.91x + 6.9518	58	4.95
Co(II)	y = −2111.4x + 14.393	120	-17.55

Table 7
Values of ΔS^o and ΔH^o of thermodynamic parameters of Pb(II) and Co(II) biosorption.

3.12. Characterization of the biosorbent

3.12.1. Surface area estimation

The specific surface areas of the untreated and NaOH-treated fungal biomass preparations were measured by the Brunauer, Emmett, and Teller method (BET method). From the obtained results, it can be seen that when NaOH was applied to the biomass, the surface areas of the fungal biomasses increased from 3.83 to 4.55 m^2 g^{-1}. According to these results, it should be noted that NaOH treatment appears to provide more surface area for the biosorbents leading to higher biosorption capacity for Pb(II) and Co(II) ions due to the increase in the surface area of the modified fungal mycelia.

3.12.2. FTIR spectral analysis

The mechanism of Pb(II) and Co(II) biosorpiton by *S. cerevisiae* biosorbent was elucidated on the basis of FTIR. The FTIR spectra (in the range 4000–400 cm^{-1}) of *S. cerevisiae* before and after metal biosorption were taken. As shown in Fig. 8, the broad adsorption bands at 3384.46 cm^{-1}, representing –OH groups of the glucose and the –NH stretching of the protein and chitosan (**Xiangliang *et al*., 2005**). This band shifted only to 3408.56 and 3405.67 cm^{-1} after Pb(II) and Co(II) biosorption, respectively indicating that these groups were involved in biosorption of both

metals. The band observed at 2926.45 cm^{-1} can be assigned to C–H stretching (**Bueno *et al*., 2008**). This band does not changed after Pb(II) and Co(II) biosorption indicating that this group did not participate in biosorption of these metals. The peaks at 1656.55 and 1548.56 cm^{-1} can be assigned to a carbonyl group (C=O) stretching in carboxyl or amide groups. A slight shifting was occurred from 1656.55 cm^{-1} to 1658.48 and 1657.52 cm^{-1} after Pb(II) and Co(II) biosorption, respectively. The band at 1405.85 cm^{-1} in the spectra of unloaded biomass can be attributed to vibrations of –CH and –OH bending and –COO^{-} groups. Further, the band at 1044.29 cm^{-1} is indicative to P–O group (**Khambhaty *et al*., 2008**). This band shifted to 1049.09 cm^{-1} either after Pb(II) or Co(II) biosorption. The bands <700 represents C–N–C scissoring found only in protein structures (**Bayramoğlu *et al*., 2006**), which disappeared after Pb(II) and Co(II) biosorption. Finally, we can concluded that functional groups which can biosorb Pb(II) and Co(II) ions are of the type –OH, –NH, COO^{-} and –P–O. Nearly, the same finding was observed by **Tunali *et al*. (2006)** in biosorption of Pb(II) by *Cephalosporium aphidicola*.
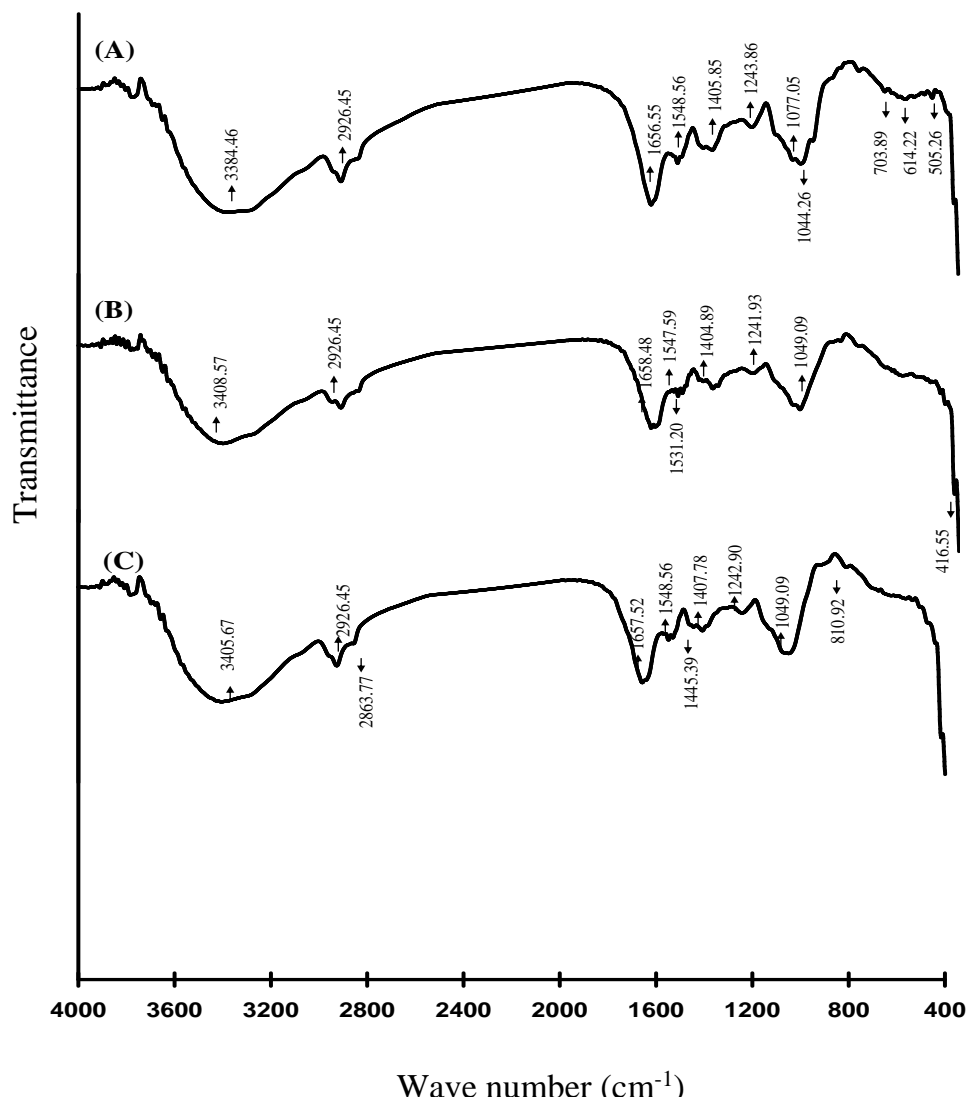

Fig. 8: FTIR spectra of (A) dried unloaded, (B) Pb(II)-loaded and (C) Co(II)-loaded *S. cerevisiae* biomass.

Conclusion

The present study evaluates the biosorption of Pb(II) and Co(II) by *S. cerevisiae* biomass. The biosorption process depends significantly on the pH of the solution and is favored at around pH value of 4 and 7 for Pb(II) and Co(II), respectively. Biosorption of Pb(II) and Co(II) onto the yeast is better described by the pseudo-second-order kinetic model. Thermodynamic results indicated that metal biosorption by is feasible, spontaneous, endothermic in nature for Pb(II) and exothermic for Co(II). In this study, we presented an assessment of the *S. cerevisiae* biosorbent for the application in the field of bioremediation of wastewaters or industrial effluents containing high concentrations of Pb(II) and Co(II) ions.

Acknowledgement

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