

Biosorption of Pb(II) and Co(II) Ions from Aqueous Solutions Using Pretreated *Rhizopus oryzae* (Bread Mold)

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Abstract Biosorption of Pb(II) and Co(II) ions from aqueous solutions was studied in a batch mode by using NaOH-pretreated *Rhizopus oryzae*. The optimum biosorption conditions were studied and determined by investigating pH, time course, initial metal concentration, temperature, co-ions and others. Optimum experimental parameters were determined to be pH (4 for Pb(II) and 7 for Co(II)), contact time 60 min, biomass dose 2 g L⁻¹ of solution, and temperature 25 °C. Metal biosorption reached a saturation value at about 200 mg L⁻¹. At this concentration, the biosorbed Pb(II) and Co(II) reached 69.73 ± 1.48 and 13.56 ± 0.37 mg g⁻¹, respectively. Contrary to Co(II) biosorption, Pb(II) biosorption was unaffected by the presence of many competing ions, but significantly decreased when PO₄³⁻ was added. The Langmuir constant (q_{max}) values are close to the experimental, indicating the ability of this model to describe biosorption process. According to q_e values obtained from pseudo-second-order model and values of R^2 (0.964 for Pb(II) and 0.992 for Co(II)), the kinetic studies indicated that the biosorption process for both metals followed well pseudo-second order model. Regarding thermodynamic parameters, the values of ΔG° at 25 °C were -26.58 kJ mol⁻¹ for Pb(II)

and 20.70 kJ mol⁻¹ for Co(II), ΔS° (69 J mol⁻¹ K⁻¹ for Pb(II) and Co(II)), and ΔH° (2.82 kJ mol⁻¹ for Pb(II) and -0.47 kJ mol⁻¹ for Co(II)). The results showed that metal biosorption by *R. oryzae* is feasible, spontaneous, endothermic in nature for Pb(II) and exothermic for Co(II). FTIR spectral analysis indicated the involvement of -COOH, -OH and -NH groups in the biosorption process.

Keywords Biosorption · *Rhizopus oryzae* · Lead(II) · Cobalt(II)

الخلاصة

تمت دراسة الامتزاز الحيوي لأيونات الرصاص والكوبالت من المحاليل المائية باستخدام فطر عفن الخبز المعالج بهيدروكسيد الصوديوم. وقد تمت الدراسة في نظام مغلق تحت ظروف مختلفة من درجة الحموضة، وزمن الامتزاز، وتركيز الأيون، ودرجة الحرارة، وكذلك الامتزاز في وجود عناصر أخرى موجبة وسالبة التآين. وتبين من خلال النتائج أن الظروف الأفضل لعملية الامتزاز كانت على النحو الآتي: درجة الحموضة = 4 بالنسبة لأيونات الرصاص و 7 بالنسبة لأيونات الكوبالت، وزمن الامتزاز = 60 دقيقة، وتركيز عامل الامتزاز = 2 جم لتر⁻¹، ودرجة الحرارة = 25 °م. ووصلت القدرة الامتزازية لأيونات الرصاص والكوبالت إلى حوالي 200 ملجم لتر⁻¹ و 69.73 ± 1.48 و 13.56 ± 0.37 ملجم جم⁻¹ على التوالي عند تركيز 200 ملجم لتر⁻¹. كما بينت النتائج تأثير امتزاز أيونات الرصاص بإضافة الفوسفات إلى وسط الامتزاز وعدم تأثيره بوجود عناصر أخرى منافسة. وقد وجد أن نموذجي لانجمير وفريندلخ قادران على توصيف الامتزاز الحيوي لكلا الأيونين المدروسين. وتبين أيضاً أن نموذج الامتزاز (pseudo-second-order) هو الأكثر ملاءمة في توصيف حركية الامتزاز اعتماداً على قيم معاملات الارتباط (0.964 لأيونات الرصاص و 0.992 لأيونات الكوبالت). وأشارت النتائج - في دراسات الديناميكا الحرارية- إلى تلقائية التفاعل لكلا الأيونين، وأن تفاعل امتزاز أيونات الرصاص هو تفاعل ماص للحرارة وأن تفاعل امتزاز أيونات الكوبالت هو تفاعل طارد للحرارة اعتماداً على القيم الموجبة والسالبة للمحتوى الحراري. وباستخدام تحاليل الأشعة تحت الحمراء تم الاستدلال بأن مجاميع الكاربوكسيل، والأمين، وكذلك الهيدروكسيل هي مجاميع نشطة قادرة على الارتباط بأيونات الرصاص والكوبالت.

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1 Introduction

Heavy metal pollution has become one of the most serious problems threatening ecosystem. Unlike organic pollutants, heavy metals are non-biodegradable and so the removal of them is extremely important in terms of health of livings [1]. Lead (Pb(II)) pollution that created by battery manufacturing, textiles, paints, pigments, petrol, photographic material, explosive manufacturing, mining wastes, automobiles and metal finishing [2] is a serious environmental problem caused by some industrial activities and poses a significant threat to the environment and public health [3]. Although cobalt (Co(II)) element has beneficial effects on human health, it may cause several problems at high levels [4]. Various processes are available for the removal of heavy metal ions from industrial effluents. These processes include chemical precipitation and sludge separation, reverse osmosis, filtration, adsorption using activated carbon, electrochemical treatment and evaporative recovery [5]. Biomass-based technology for removing of heavy metals from industrial wastewater, termed as biosorption, has received more attention recently by many researchers. Various types of microbial biomass including algae and bacteria [6] have been investigated as biosorbents. Among the microbial biosorbents, fungi show excellent metal-binding properties due to a high percentage of their cell wall material that offer a wide range of chemical groups attracting and sequestering the metals on biomass [7]. Fungi are unique in metal biosorption, and this process is known as mycosorption. The fungal biomass used in mycosorption is termed mycosorbent. Mycosorption is a topic of great interest for researchers all over the world [8–11]. Fungi can be of interest for biosorption because they are able to remove heavy metals from aqueous solutions in rather substantial quantities. In certain instances, the removal of heavy metals by fungal biomass has been observed to be more than that by other adsorbents [12]. *Rhizopus* such as *Rhizopus arrhizus* [13], *Rhizopus nigricans* [14], *Rhizopus oligosporus* [15], and *Rhizopus cohnii* [16] have already been studied as potential biomass for removal of heavy metals from aqueous solution. In the literature, few studies have focused on biosorption of Co(II) by microorganisms. Biosorption processes are applicable to treat effluents containing low concentrations of heavy metals. This aspect makes biosorption even more attractive for treatment of dilute effluent that originates either from an industrial plant or from the primary wastewater treatment facility [17]. Therefore, this study aimed to determine the potential of the biomass of *Rhizopus oryzae* to adsorb Pb(II) and Co(II) ions in a batch mode which have a potentiality in biotechnological context in bioremediation of industrial effluents such as battery manufacturing effluents (rich with Pb(II)) and painting manufacturing wastewaters (rich with Co(II)). The biosorption process was studied with regard to the effects of pH,

time course, initial metal ions concentration, temperature, biomass dose, biomass age, agitation speed and co-ions. In addition to this, the biosorption equilibrium was evaluated using the Langmuir and Freundlich isotherm models. Kinetics and thermodynamics of the biosorption at various temperatures were also evaluated.

2 Materials and Methods

2.1 Chemicals and Equipment

All chemicals used in this study were of analytical reagent grade and were used without further purification. Double-distilled water (ddH₂O) was used in all solutions. Adjustment of pH was carried out by 0.1 N HCl (34–37 %, Fisher Scientific, UK) and 0.1 N NaOH (97 %, Sigma-Aldrich Company, England). The pH of solutions was measured by a pH meter (Hanna HI 8519 pH meter). All equipments (glassware and plastics) were cleaned by soaking in 1 N HCl for at least 2 h, followed by rinsing three times in ddH₂O. Other chemicals and equipment used in this study were mentioned elsewhere in this paper.

2.2 Fungus and Growth Conditions

A filamentous fungus *Rhizopus oryzae* used in this study was isolated from a street soil near car parking (Shebein El-kom, Menoufia Governorate, Egypt) and identified by Regional Center for Mycology and Biotechnology (Al-Azhar University, Cairo, Egypt). This fungus was cultivated on Potato Dextrose (PD) agar (Oxoid, England) at 28 °C and then routinely maintained at 4 °C on PDA.

2.3 Preparation of Biosorbent

For production of the biosorbent, the fungus was cultivated on a rotary shaker (Jeio Tech SI-900 R, Korea) at 125 rpm and 28 °C for 7 days (7 d) in 500 mL Erlenmeyer flask containing 250 mL of PD medium. The harvested biomass was washed with generous amounts of ddH₂O to remove residual growth medium and drained to remove excess water by gently pressing through filter paper. For the treatment of biomass, live harvested biomass (20 g wet weight) was boiled for 15 min in 500 mL of 0.5 N NaOH solution. After pretreatment, the biomass was washed with ddH₂O until the pH of the washing solution was close to neutral range (6.8–7.2). The biomass was then dried in an oven for 24 h at 60 °C. 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 for future use.

2.4 Preparation of Metal Ion Solutions

For preparation of stock lead (Pb(II)) and cobalt (Co(II)) solutions with the concentration $1,000 \text{ mg L}^{-1}$, 1.6 g of $\text{Pb}(\text{NO}_3)_2$ (99 %, Oxford, Mumbai, India) and 4.04 g of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (99.8 %, Hayashi, Japan) were dissolved in 1 L 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.

2.5 Biosorption Experiments

Batch biosorption experiments were conducted in 100 mL Erlenmeyer flasks containing 20 mL solution on a rotary shaker. The effect of pH on the biosorption capacity was evaluated in the pH range of 1–8, in increments of 1. The initial pH of each metal solution was adjusted to the required pH value. Then, 0.04 g of dried biosorbent was added to the metal solution and the reaction mixture was shaken on an orbital shaker at 125 rpm and 25°C for 2 h. Similarly, the effect of time course (5–90 min), initial metal ion concentration ($50\text{--}500 \text{ mg L}^{-1}$), temperature ($15\text{--}40^\circ\text{C}$), biomass dose ($0.01\text{--}0.08 \text{ g}/20 \text{ mL}$), biomass age (3–7 days) and agitation speed ($0\text{--}200 \text{ rpm}$) on the metal biosorption were performed. Experiments were also carried out to evaluate the effect of other heavy metal cations (Cd, Cu and Zn), light metal cations (Na, K, Ca and Mg) and metal anions as ammonium salts (chloride, nitrate, phosphate and sulfate) in the concentration 50 mg L^{-1} . After the end of each experiment, the mixtures were centrifuged (for 5 min at 10,000 rpm) and metal ion concentrations in the supernatant were determined. All biosorption experiments were done in triplicate and mean values reported. The amount of adsorbed metal ions is estimated as the amount of metal (mg) per unit of mycelium dry weight (g) using the following equation:

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

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:

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

2.6 Analytical Procedure

The concentration of unadsorbed Pb(II) and Co(II) ions in the supernatant of biosorption medium was determined by using an atomic absorption spectrophotometer (AAS) (Unicam 929, Philips Company, UK) with an air-acetylene flame. The working wavelengths for Pb(II) and Co(II) were 283.3 and 240.7 nm, respectively. The instrument calibration

was checked periodically by using standard metal solutions. Before measured by AAS, the metal solutions were appropriately diluted with ddH_2O to ensure the metal concentration in the sample was linearly dependent on the absorbance detected. Infrared (IR) spectra of free and metal-loaded *R. oryzae* biomass were recorded in a Fourier-transform infrared (FTIR) spectrometer (Nicolet Nexus 670, Thermo Nicolet Corporation, USA) in potassium bromide (KBr) discs.

2.7 Statistical Analysis

All the experiments were carried out in triplicate and the data shown are mean from three separate experiments. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) 9.05 for Windows, where it was possible to evaluate whether the effect and the interaction among the investigated factors were significant with respect to the standard error.

3 Results and Discussion

Depending on previous screening for choice, the best chemical or physical pretreatment method leading to highest uptake of metals, pretreatment with NaOH solution was the best for metal biosorption enhancement (unpublished data).

3.1 Effect of pH

In the biosorption phenomenon, pH is a key parameter. It affects the chemistry of metals and the activity of functional groups on the fungal cell wall. The variation of metal biosorption with initial pH is given in Fig. 1. From the results, it was found that the biosorption capacities of Pb(II) and Co(II) ions were lower at low pH and started to increase as the pH solution increased. The optimum pH value for Pb(II) and Co(II) biosorption were 4 and 7 at which the Pb(II) and Co(II) biosorption values were 24.86 ± 0.44 and

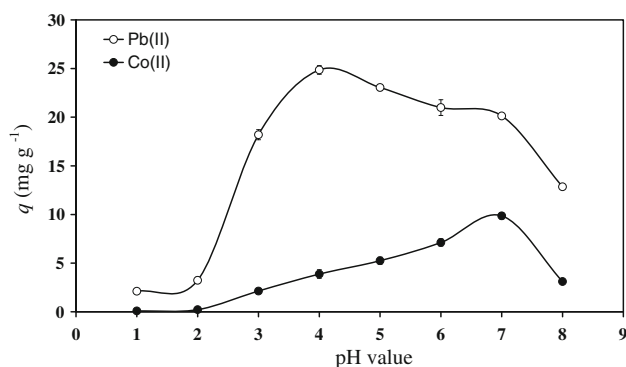


Fig. 1 Pb(II) and Co(II) biosorption (mg g^{-1}) by *R. oryzae* at different pH values

$9.86 \pm 0.33 \text{ mg g}^{-1}$, respectively. An increase or decrease in the pH from these optimum pH values resulted in a reduction in the biosorption of these metal ions. This observation suggests that pH has to be closely monitored and maintained at least above 3.0 and 5.0 for effective Pb(II) and Co(II) removal, respectively by *R. oryzae*. This is in agreement with the character of metal cations biosorption. Successful biosorption of base metal cations usually takes place in the range of pH 3–7 and is extremely pH dependent [18]. Initial pH of the biosorption medium is considered one of the most important environmental factors affecting the metal biosorption by biosorbents as it could change biosorbent surface charge, degree of ionization and availability of the functional groups such as hydroxyl (R–OH), carboxyl (R–COOH), amino (–NH₂) and sulfhydryl (–SH) groups [19]. The optimum pH values for Pb(II) and Co(II) biosorption were taken for further batch experiments.

3.2 Effect of Time Course

Contact time is one of the important parameters for successful biosorption application. To determine the equilibrium time for adsorption of Pb(II) and Co(II) ions by biomass of *R. oryzae*, batch kinetic studies were conducted at optimum pH. Figure 2 illustrates the effect of contact time (min) versus the amount of metal adsorbed (mg g^{-1}) of both metals. The results indicate that the amount of adsorbed Pb(II) ions was very high at the beginning of biosorption (more than 70 % Pb(II) was adsorbed within the initial 5 min) and the saturation level was gradually reached at about 60 min where the Pb(II) biosorption value reached $23.85 \pm 0.28 \text{ mg g}^{-1}$. The same pattern was found for Co(II) biosorption and equilibrium time was attained in 60 min and Co(II) biosorption value was $10.19 \pm 0.20 \text{ mg g}^{-1}$. This observed rapid kinetics has significant practical importance in biosorption of heavy metals on large scale as it will facilitate smaller reactor volumes ensuring efficiency and economy [20]. After these equilibrium periods, either in Pb(II) or Co(II) biosorption, the

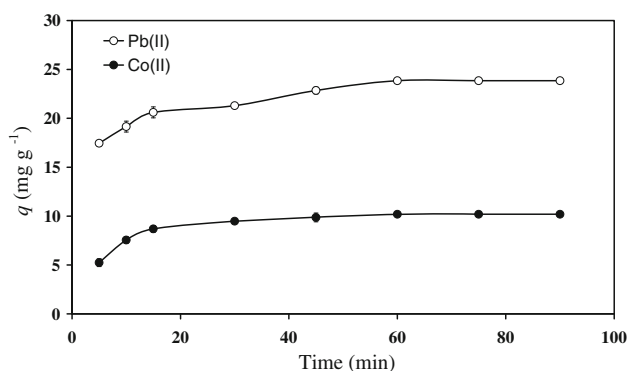


Fig. 2 Pb(II) and Co(II) biosorption (mg g^{-1}) by *R. oryzae* at different time intervals

amount of adsorbed metal ions did not significantly change with time. Data on the biosorption rates of heavy metal ions by various biosorbents have shown a wide range of biosorption time. About 80 % of Pb(II) ions were biosorbed by *Rhodotorula glutinis* in the first 10 min of contact time [21]. High biosorption efficiencies of Co(II) by almond green hull biosorbent were occurred in the first 1 min of contact time [22]. In the case of uranium biosorption, equilibrium was attained in 90 min by *Rhizopus arrhizus* [13].

3.3 Effect of Initial Metal Concentration

Another important parameter in biosorption is the initial metal concentration which influences the adsorption of metal to the biomass surface. The results presented in Fig. 3 indicate that metal biosorption increased with increasing metal concentration and then reached a saturation values at about 200 mg L^{-1} . At this concentration, the biosorbed Pb(II) and Co(II) reached 69.73 ± 1.48 and $13.56 \pm 0.37 \text{ mg g}^{-1}$, respectively. Then there was no significant increase in biosorbed metal. In addition, while the highest biosorption efficiency for Pb(II) removal was 95.6 %, the highest biosorption efficiency for Co(II) was 39 % and attained at concentration 50 mg L^{-1} . The slow metal adsorption beyond these concentrations would be ascribed to the saturation of binding sites. Initial metal concentration provides a driving force to overcome mass transfer resistances between the biosorbent and biosorption medium [23]. So that higher biosorption capacities were obtained at higher initial concentrations for the two metal ions.

3.4 Effect of Temperature

The temperature of biosorption medium may affect the removal efficiency of the heavy metals from aqueous solutions [24]. The results of metal biosorption experiments that carried out at different temperatures ranging from 15–40 °C are presented in Table 1. From the results, data show

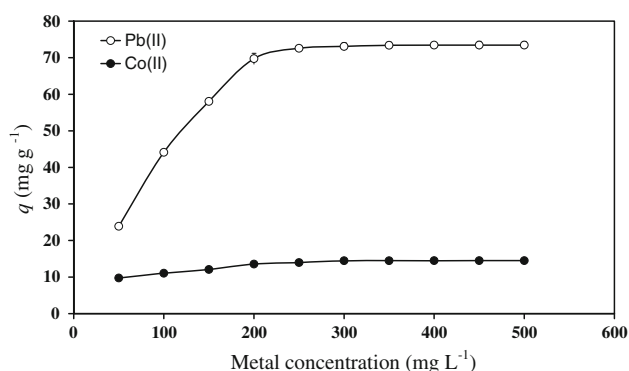


Fig. 3 Pb(II) and Co(II) biosorption (mg g^{-1}) by *R. oryzae* at different metal concentrations

Table 1 Pb(II) and Co(II) biosorption (mg g^{-1}) at different temperatures ($^{\circ}\text{C}$), biomass doses and g L^{-1} biomass ages (days)

	Metal biosorption (mg g^{-1})	
	Pb(II)	Co(II)
Temperature ($^{\circ}\text{C}$)		
15	17.33 ± 0.25	9.19 ± 0.12
20	20.21 ± 0.02	9.39 ± 0.04
25	23.31 ± 0.06	10.14 ± 0.09
30	23.29 ± 0.03	10.11 ± 0.03
35	21.06 ± 0.02	10.08 ± 0.12
40	18.12 ± 0.02	9.89 ± 0.31
Biomass dose g L^{-1}		
0.5	56.09 ± 2.95	14.94 ± 1.88
1	39.76 ± 0.57	13.63 ± 1.25
2	24.21 ± 0.20	8.80 ± 0.44
3	16.36 ± 0.06	5.47 ± 0.16
4	10.57 ± 0.10	3.75 ± 0.15
5	7.48 ± 0.17	1.70 ± 0.28
Biomass age (days)		
3	24.27 ± 0.19	11.41 ± 0.83
4	24.55 ± 0.09	11.14 ± 1.06
5	24.98 ± 0.01	11.03 ± 0.16
6	24.17 ± 0.01	10.23 ± 0.18
7	23.26 ± 0.44	9.50 ± 0.61

The data are the mean values of 3 replicates \pm standard error of the mean

that change of temperature influences the biosorption rates of Pb(II) ions and the optimum biosorption of Pb(II) is obtained at 25°C . The maximum Pb(II) biosorption value was $23.31 \pm 0.06 \text{ mg g}^{-1}$. These results are in agreement with Guo et al. [25] in biosorption of Cd(II) and Zn(II) ions by *Aspergillus niger*. They found that the best temperature was in the range $25\text{--}30^{\circ}\text{C}$. Bahadir et al. [26] reported that optimum temperature for Pb(II) biosorption by *Rhizopus arrhizus* was 30°C . On the other hand, data show no significant differences in Co(II) biosorption by this fungus and the biosorption seemed as temperature-independent process. Cho and Kim [21] observed that the temperature change between 15 and 45°C did not affect the biosorption capacity of Pb(II) by *Rhodotorula glutinis*.

3.5 Effect of Biomass Dose

Amount of biosorbent used for biosorption studies determines the potential biosorbent to remove a metal species at a given initial dose. This experiment was carried out at different biomass weights in the level ($0.5, 1, 2, 3, 4$ and 5 g L^{-1}). Table 1 shows a significant decrease in biosorbed Pb(II) and Co(II) with increasing biosorbent mass. The biosorbed

metal decreased from 56.09 ± 2.95 to $7.48 \pm 0.17 \text{ mg g}^{-1}$ for Pb(II) and from 14.94 ± 1.88 to $1.70 \pm 0.28 \text{ mg g}^{-1}$ for Co(II) due to ten fold increase in biomass. The maximum Pb(II) biosorption efficiency reached 98.2% at biomass weight 3 g L^{-1} , whereas the maximum Co(II) biosorption efficiency reached 35.2% at biomass weight 2 g L^{-1} . Because biomass weights of both fungi over 2 g L^{-1} did not lead to significant improvement in biosorption efficiency of both metals, the optimum biomass weight was taken as 2 g L^{-1} for further batch experiments. 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 latter is not saturated [1,27]. Various reasons have been suggested to explain the decreased biosorption capacity at increasing biosorbent dose including electrostatic interactions and interference between binding sites [28,29], 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 [30,31].

3.6 Effect of Biomass Age

Biomass age has also been found to affect the biosorption of metal ions [24]. To investigate the effect of biomass age on biosorption of Pb(II) and Co(II), *R. oryzae* was grown at different times of growth (3, 4, 5, 6, and 7 days) in PD medium. The results indicate that no big difference was found in biosorbent metal affinity for biomass incubation periods ranging from 3 to 5 days. The adsorbed metal values were 24.27 ± 0.19 and $24.98 \pm 0.01 \text{ mg g}^{-1}$ for Pb(II) and 11.41 ± 0.83 and $11.03 \pm 0.16 \text{ mg g}^{-1}$ for Co(II) by 3-old- and 5-old-biomasses, respectively (Table 1). On the other hand, the biosorbent obtained from older cultures (6 and 7 days) showed a slow decrease in metal biosorption capacity and the adsorbed metal values were 24.17 ± 0.01 and $23.26 \pm 0.44 \text{ mg g}^{-1}$ for Pb(II) and 10.23 ± 0.18 and $9.50 \pm 0.61 \text{ mg g}^{-1}$ for Co(II) by 6-old- and 7-old-biomasses, respectively. These results are in agreement with Delgado et al. [32] in biosorption of Cu(II), Cd(II) and Ni(II). They found that older cultures of *Fusarium flocciferum* showed a decrease in metal biosorption capacity. Usually, the cells at lag phase or early stages of growth have a higher biosorptive capacity for metal ions than that of stationary phase [33].

3.7 Effect of Agitation Speed

The effect of shaker agitation speed on biosorption of Pb(II) and Co(II) by pretreated *R. oryzae* was investigated by varying the agitation speed of the biosorption media from 0 (no agitation; control), 25, 50, 75, 100, 125, 150, 175 to 200 rpm. Figure 4 illustrates the effect of agitation speed (rpm) versus the amount of metal adsorbed (mg g^{-1}) of Pb(II) and

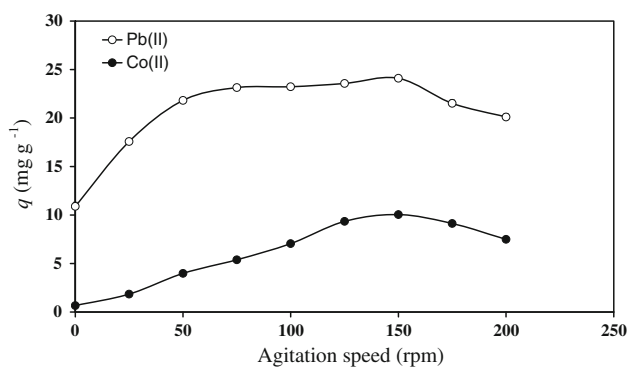


Fig. 4 Pb(II) and Co(II) biosorption (mg g^{-1}) at different agitation speed

Co(II) ions. The results indicate that there was a significant increase in biosorption of both metals with increasing the agitation speed up to 150 rpm, beyond which there was a detectable decrease in biosorbed metal. At this agitation speed, the biosorbed Pb(II) and Co(II) reached 24.10 ± 0.17 and $10.04 \pm 0.79 \text{ mg g}^{-1}$, respectively. These results are in agreement with Bai and Abraham [14] in biosorption of Cr(VI) by *Rhizopus nigricans*. They found that the optimal value of Cr(VI) adsorption capacity was achieved at agitation speed of 120 rpm. Opposite result was reported by Selatnia et al. [31] for biosorption of Pb(II) by a bacterial dead *Streptomyces rimosus* biomass. They found that the optimum agitation speed for biosorption of this metal is 250 rpm. The biosorption of metal at 0 rpm (no agitation) exhibited low biosorption values and were 10.89 ± 0.52 and $0.66 \pm 0.20 \text{ mg g}^{-1}$ for Pb(II) and Co(II), respectively.

3.8 Effect of Co-Ions

Competing ions may interfere with removal of the test metal. Accordingly, the effect on Pb(II) and Co(II) biosorption of various cations and anions was studied. From the results presented in Table 2, it is noticed that there is no significant effect on Pb(II) biosorption in binary solutions Pb-Cd, Pb-Cu, and Pb-Zn, or multi-metal solution Co-Cd-Cu-Zn. On the other hand, multi-metal solution Pb-Cd-Cu-Zn reduced the Co(II) biosorption capacity from 10.06 ± 0.12 to $7.11 \pm 0.17 \text{ mg g}^{-1}$. In the literature, *Penicillium purpurogenum* exhibits selectivity for Pb(II) over metals such as Cd(II), Hg(II) and As(III). The biosorptive capacity for these metals decreased in the following order: Pb(II) > Cd(II) > Hg(II) > As(III) [34]. Biosorptive capacity series for *Rhizopus arrhizus* was U > Pb > Cd > Zn > Cu [35]. The lower effect of Cd(II), Cu(II), and Zn(II) on Pb(II) biosorption could be due to the greater atomic weight and electronegativity of the Pb(II) as discussed by Wong et al. [36].

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}

Competitive ion (mg L^{-1})	Metal biosorption (mg g^{-1})	
	Pb(II)	Co(II)
Non (control)	23.82 ± 0.22	10.06 ± 0.12
Heavy metal cation		
Cd ²⁺	23.81 ± 0.33	9.59 ± 0.14
Cu ²⁺	23.78 ± 0.25	8.61 ± 0.13
Zn ²⁺	23.83 ± 0.17	9.29 ± 0.24
Co ²⁺ + Cd ²⁺ + Cu ²⁺ + Zn ²⁺	23.74 ± 0.22	–
Pb ²⁺ + Cd ²⁺ + Cu ²⁺ + Zn ²⁺	–	7.11 ± 0.17
Light metal cation		
Na ⁺	23.83 ± 0.30	8.12 ± 0.10
K ⁺	23.84 ± 0.16	8.33 ± 0.13
Ca ²⁺	23.82 ± 0.20	6.19 ± 0.20
Mg ²⁺	23.84 ± 0.18	8.03 ± 0.17
Anion		
Cl ⁻	23.83 ± 0.14	8.88 ± 0.11
NO ₃ ³⁻	23.84 ± 0.19	8.78 ± 0.10
PO ₃ ⁴⁻	10.56 ± 0.25	7.17 ± 0.13
SO ₄ ²⁻	23.84 ± 0.19	8.09 ± 0.12

The results obtained from the effect of light cations such as Na⁺, K⁺, Ca²⁺ and Mg²⁺ revealed that Pb(II) biosorption was unaffected in the presence of these ions, but Co(II) biosorption decreased from $10.06 \pm 0.12 \text{ mg g}^{-1}$ to 8.12 ± 0.10 , 8.33 ± 0.13 , 6.19 ± 0.20 and $8.03 \pm 0.17 \text{ mg g}^{-1}$ in the presence of 50 mg L^{-1} Na⁺, K⁺, Ca²⁺ and Mg²⁺, respectively. Similar results were reported in the literature in which Pb(II) biosorption was not affected by the presence of Na⁺, K⁺, Ca²⁺ or Mg²⁺ in case of *Phellinus badius* [37] and *Rhodotorula glutinis* [21].

The effect of anions such as Cl⁻, NO₃³⁻, PO₃⁴⁻, and SO₄²⁻ on the biosorption of Pb(II) and Co(II) ions by *R. oryzae* was also investigated. From the results, it was observed that Cl⁻, NO₃³⁻, and SO₄²⁻ did not affect the biosorption of Pb(II), but Pb(II) biosorption decreased from 23.82 ± 0.22 to $10.56 \pm 0.25 \text{ mg g}^{-1}$ when 50 mg L^{-1} of PO₃⁴⁻ was added to the biosorption medium. On the other hand, Co(II) biosorption was decreased from $10.06 \pm 0.12 \text{ mg g}^{-1}$ to 8.88 ± 0.11 , 8.78 ± 0.10 , 7.17 ± 0.13 , and $8.09 \pm 0.12 \text{ mg g}^{-1}$ in the presence of Cl⁻, NO₃³⁻, PO₃⁴⁻ and SO₄²⁻, respectively. Das et al. [38] found that anionic impurities such as Cl⁻, NO₃³⁻, SO₄²⁻, C₂O₄²⁻, and CH₃COO⁻ do not have any adverse influence on Pu⁴⁺ biosorption, while the presence of PO₃⁴⁻ reduces the biosorption by the yeast *S. cerevisiae*. It is well known that metal ions in water undergo hydrolysis and complexation reactions with some anions, which may influence the binding of the metals to solid surfaces. Also, increased amount of electrolyte can swamp the biosor-

bent surface, decreasing metal ion access to the surface for biosorption and therefore the metal biosorbed may be significantly decreased [39].

3.9 FTIR Spectral Analysis

The FTIR spectra of unloaded and metal-loaded *R. oryzae* biomass in the range 400–4,000 cm^{-1} (Fig. 5) were taken and compared with each other to obtain information on the nature of the possible biosorbent–metal ions interactions. Band positions for each sample are also listed in Table 3. As shown in Fig. 5 which summarized in Table 3, the FTIR spectrum of unloaded *R. oryzae* biomass displays a number of absorption peaks which reflect the complex nature of examined biomass. The broad stretching absorption peak at 3,406.6 cm^{-1} is assigned to hydroxyl (–OH) or amine (–NH) groups. The bands observed at 2,923.6 and 2,853.2 cm^{-1} could be assigned to symmetric and asymmetric stretching vibrations of the –CH₃ and –CH₂ groups, and its bending vibration is 1,410.7 cm^{-1} . The peaks in the region 1,600–1,700 cm^{-1} were attributed to stretching carbonyl group (C=O) from carboxylate (–COO[–]) or ketone and aldehyde. The peaks at 1,155.2 and 1,156.1 cm^{-1} of metal-loaded biomass corre-

spond to stretching P=O. Further, the peaks at 1,077.1 and 1,032.7 cm^{-1} are indicative of P–OH stretching vibrations or C–O stretching of alcohols and carboxylic acids.

The broad peak at 3,406.6 cm^{-1} of unloaded biomass was shifted to 3,422.1 and 3,421.1 cm^{-1} after the biosorption of Pb(II) and Co(II), respectively. This shifting indicates the involvement of –OH and –NH groups. The carbonyl group (in carboxylate) at 1,637.3 cm^{-1} was observed at 1,651.7 and 1,634.4 cm^{-1} after the biosorption of Pb(II) and Co(II), respectively. The peak of P–OH and/or C–O group was shifted from 1,032.7 to 1,024 cm^{-1} after Pb(II) biosorption and 1,027.9 cm^{-1} after Co(II) biosorption. These results reflect the interaction between these groups and the metal ions. The similar FTIR results were reported for the biosorption Pb(II) by *Cephalosporium aphidicola* fungal biomass [28]. Finally, the peaks under 700 cm^{-1} could be attributed to an interaction between metal ions and N-containing bioligands [40, 28].

3.10 Biosorption Isotherms

The capacity of a biomass can be described by equilibrium sorption isotherms which express the surface properties and

Fig. 5 FTIR spectra of (a) dried unloaded, (b) Pb(II)-loaded and (c) Co(II)-loaded *R. oryzae*

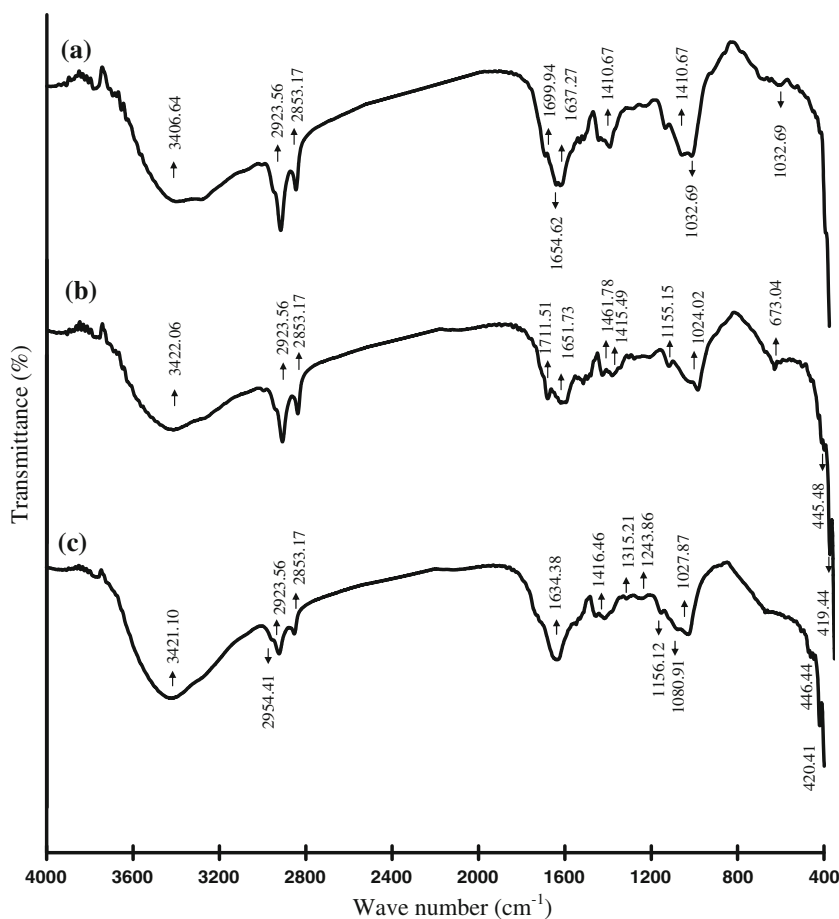


Table 3 Band positions from IR spectra before and after Pb(II) and Co(II) biosorption

Suggested assignment	Band positions (cm ⁻¹)		
	Unloaded biomass	Pb(II)-loaded biomass	Co(II)-loaded biomass
–OH and/or –NH ₂ stretching ^a	3,406.6	3,422.1	3,421.1
–CH ₂ symmetric stretching	2,923.6	2,923.6	2,923.6
–CH ₂ symmetric stretching	2,853.2	2,853.2	2,853.2
–C=O stretching ^a	1,699.9	1,711	–
–C=O stretching ^a	1,637.3	1,651.7	1,634.4
–CH ₂ bending vibrations ^a	1,410.7	1,415.7	1,416.7
–P=O stretching	–	1,155.2	1,156.1
–P–OH stretching and/or –C–O ^a	1,032.7	1,024	1,027.9
–N-containing bioligands	–	<700	<700

^a Denotes band shifting after Pb(II) or Co(II) biosorption

affinity of the biomass. In this study, Langmuir and Freundlich isotherms were applied. To determine the biosorptive capacity of *R. oryzae* for Pb(II) and Co(II) ions, the initial metal concentration varied from 50 to 500 mg L⁻¹; while the biosorbent was constant at 0.04 g/20 mL. A Langmuir isotherm was then obtained by plotting the values of biosorption capacity (q_e) versus the residual metal concentration (C_f) in solution.

The classical Langmuir equation is given as follows [41]:

$$q_e = \frac{q_{\max} b C_f}{1 + b C_f} \quad (3)$$

where, q_e is the metal adsorbed on the biosorbent (mg g⁻¹), q_{\max} is the maximum possible amount of metal adsorbed per unit weight of biosorbent, C_f is the residual concentration of metal (mg L⁻¹) in the solution and b is the equilibrium constant related to the affinity of the binding sites for the metals. Equation (3) can be linearized as follows:

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

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 Langmuir constants (q_{\max} and b) with correlation coefficients (R^2) were calculated from the plots in Fig. 6 for biosorption of Pb(II) and Co(II) on the fungal biomass and the results are presented in Table 4.

The classical Freundlich equation is given as follows [42]:

$$q_e = K_f C_f^{\frac{1}{n}} \quad (5)$$

where K_f is the empirical constant that provides an indication of the intensity of adsorption. Equation (5) can be linearized by taking natural logarithm of both sides of the equation, which can be given as follows:

$$\log q_e = \left(\frac{1}{n} \right) \log C_f + \log K_f \quad (6)$$

Freundlich constants (K_f and n) with correlation coefficients (R^2) were calculated from the plots in Fig. 7 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 at different temperatures, but biosorption of Pb(II) is more described by Langmuir model. In view of the Langmuir constant (q_{\max}) values, the q_{\max} values at 25 °C are close to the experimental q_{\max} values (Table 4). The increasing values of b for the metal ion indicate the increase in the biosorption. The favorable biosorption is indicated by higher than 1 value of Freundlich sorption constant n for fungal biomass. The values of n obtained greater than one for Pb(II) and Co(II) indicated that physical and multilayer adsorption takes place for both metal ions. 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. Generally, the higher values of Freundlich constants (K_f and n) and the lower value of Langmuir constant (b) indicate the higher affinity of the biomass [43,44]. In the literature, the biomass of *Rhizopus nigricans* was used for adsorption of Pb(II) and the biosorption process followed Langmuir and Freundlich adsorption isotherms [45]. Similar results were also reported for biosorption of Pb(II) by *Aspergillus parasiticus* [46] and Pb(II), Cu(II) and Cd(II) by *Phanerochaete chrysosporium* [47] and *Trichoderma viride* [40].

3.11 Biosorption Kinetics

Numerous kinetic models have been suggested to describe the reaction order of adsorption systems. The pseudo-first-order and pseudo-second-order models were used to analyze

Fig. 6 Application of Langmuir isotherm model for **a** Pb(II) and **b** Co(II) biosorption by *R. oryzae* at different temperatures

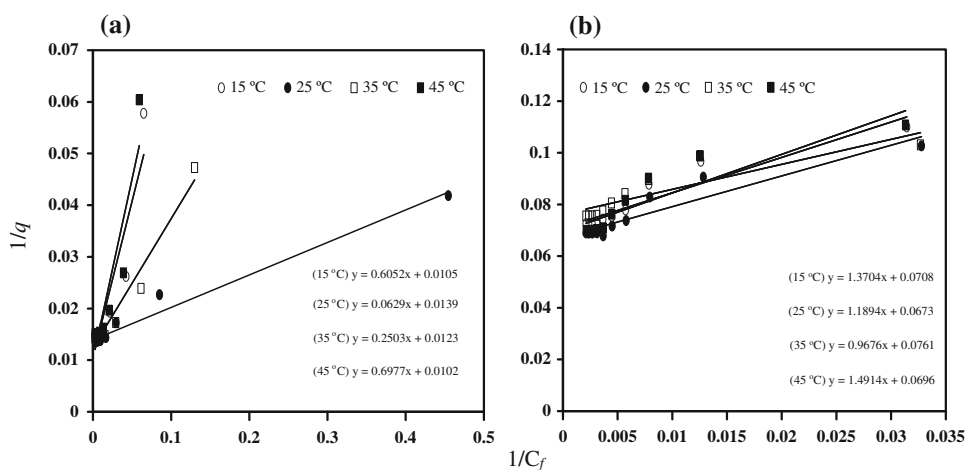
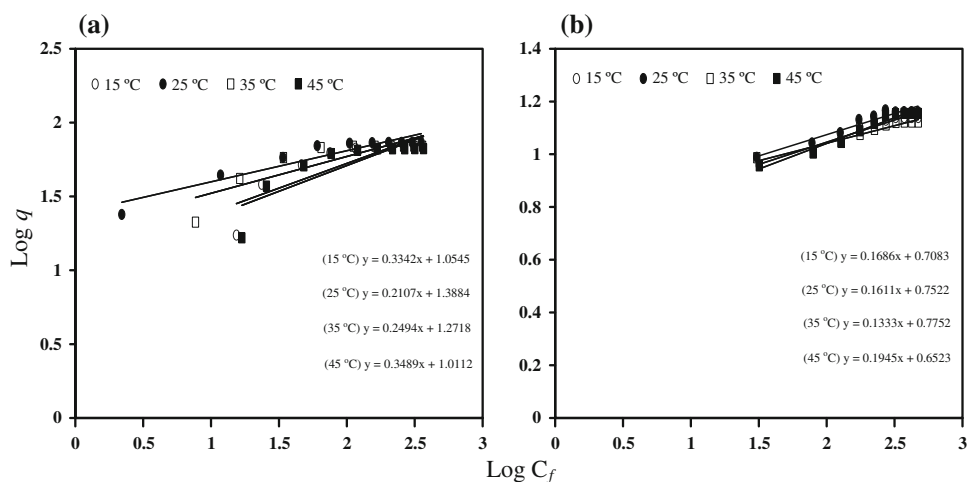


Table 4 Isotherm parameters of two models for Pb(II) and Co(II) biosorption by *R. oryzae* at different temperatures

Metal	T (K)	Langmuir			Freundlich		
		q_{max} (mg g ⁻¹)	b (L mg ⁻¹)	R^2	K_f	n	R^2
Pb(II)	15	95.24	0.017	0.934	11.34	2.99	0.845
	25	71.94	0.220	0.988	24.46	4.75	0.941
	35	81.30	0.195	0.982	18.70	4.01	0.858
	45	98.03	0.014	0.928	10.26	2.87	0.842
Co(II)	15	14.12	0.051	0.949	5.11	5.93	0.974
	25	14.51	0.056	0.943	5.65	6.21	0.968
	35	13.24	0.078	0.880	5.96	7.50	0.974
	45	14.30	0.046	0.917	4.49	5.14	0.980

Fig. 7 Application of Freundlich isotherm model for **a** Pb(II) and **b** Co(II) biosorption by *R. oryzae* at different temperatures



and fit the data to these models [48]. The pseudo-first-order equation is expressed as:

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

where q_t is the metal adsorbed on the biosorbent (mg g⁻¹) at time t and K_1 is the pseudo-first-order model constant.

Equation (7) can be linearized as follows:

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

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.

Table 5 The first-order and second-order kinetics for biosorption of Pb(II) and Co(II) biosorption by *R. oryzae* at $25 \pm 1^\circ\text{C}$

Metal	Experimental q_e (mg g ⁻¹)	Pseudo-first-order		
		q_e (mg g ⁻¹)	$K_1 \times 10^{-3}$ (min ⁻¹)	R^2
Pb(II)	23.85	54.74	1.4	-0.917
Co(II)	10.19	7.31	7.4	-0.844
Metal	Experimental q_e (mg g ⁻¹)	Pseudo-second-order		
		q_e (mg g ⁻¹)	K_2 (g mg ⁻¹ min ⁻¹)	R^2
Pb(II)	23.85	23.81	0.50	0.964
Co(II)	10.19	11.20	0.18	0.992

Table 6 The thermodynamic parameters of Pb(II) and Co(II) biosorption by *R. oryzae* at different temperatures

Metal	T (K)	$-\Delta G^\circ$ (kJ mol ⁻¹)	ΔS° (kJ mol ⁻¹ K ⁻¹)	ΔH° (kJ mol ⁻¹)
Pb(II)	15	19.56	69	2.82
	25	26.58		
	35	27.16		
	45	21.80		
Co(II)	15	19.18	69	-0.47
	25	20.70		
	35	21.59		
	45	20.90		

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

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

where K_2 is the pseudo-second-order model constant. Equation (9) can be linearized as follows:

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

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.

The pseudo-first-order constants (q_e and K_2) and pseudo-second-order constants (q_e and K_2) with correlation coefficients (R^2) were calculated and presented in Table 5. The values of q_e estimated from the pseudo-first-order model give significantly different values compared with the experimental biosorption capacities, and the correlation coefficients (R^2) are also found to be largely lower and ranged from -0.691 to -0.917 (negative weak correlation). On the other hand, the values of q_e obtained from pseudo-second-order model were close to those determined by experiments (Table 5). Also, correlation coefficients (R^2) were in the range 0.964–0.996, indicating that the biosorption of Pb(II) and Co(II) by *R. oryzae* is well described by the pseudo-second-order model, based on the assumption that the rate limiting step may be

chemisorption involving valency forces through sharing or exchange of electrons between biosorbent and sorbate. In most cases, 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 [49]. Similar results were also reported in the biosorption of Pb(II) by *Mucor rouxii* [50], Cu(II) by *Pycnoporus sanguineus* [51], and Sr(II) by *Aspergillus niger* [52].

3.12 Biosorption Thermodynamics

To determine the thermodynamic parameters, experiments were carried out at different temperatures (288, 298, 308, and 318 K). The thermodynamic parameters include Gibbs free energy change (ΔG°), entropy (ΔS°) and enthalpy (ΔH°) change were used to describe the thermodynamic behavior of the biosorption of Pb(II) and Co(II) ions by *R. oryzae* biomass. These parameters were determined by the following equations:

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

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

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

where R is the universal gas constant (8.314 J mol⁻¹ K⁻¹), T is the solution temperature in kelvin (K), and b is the equilibrium constant obtained from Langmuir isotherm. When $\ln(b)$ is plotted against $1/T$, a straight line with slope $-(\Delta H^\circ/R)$ is obtained and the intercept corresponds to $(\Delta S^\circ/R)$. Also, ΔH° and ΔS° were determined. From the results presented in Table 6, it can be seen that all the ΔG° values are negative which indicate the feasibility of the process and spontaneous nature of the biosorption. The decrease in ΔG° values shows a decline in feasibility of biosorption as temperature is increased. Reactions occur spontaneously at a given temperature if ΔG° is a negative quantity [39]. Generally, the absolute magnitude of the ΔG° for physisorption is between -20 and 0 kJ mol⁻¹ and chemisorption has a range of -80 to -400 kJ mol⁻¹ [53]. Hence, Pb(II) and Co(II) biosorption



onto *R. oryzae* can be considered as physisorption. Similar findings were also reported in the biosorption of Pb(II) and Cu(II) by NaOH- pretreated *Aspergillus niger* [23], Pb(II) and Cd(II) by *Lactarius scrobiculatus* [1] and Pb(II), Cu(II) and Cd(II) by *Trichoderma viride* [40].

The ΔS° and ΔH° values were calculated from the intercept and slope of the plot, respectively (using Eq. 13). The ΔS° was calculated to be $69 \text{ J mol}^{-1} \text{ K}^{-1}$ for Pb(II) and Co(II). The ΔH° values for Pb(II) and Co(II) biosorption were found to be 2.82 and $-0.47 \text{ kJ mol}^{-1}$, respectively, indicating that the Pb(II) biosorption is endothermic in nature, while Co(II) biosorption is exothermic. The positive values of ΔS° suggest increased randomness at the solids–solution interface during the biosorption of metal ions onto biosorbent [54,55,40]. The endothermic nature of metal ion biosorption by fungal biomasses has also been reported previously for biosorption of Pb(II), Cu(II), and Cd(II) by *Saccharomyces cerevisiae* [56] and Cd(II), Zn(II), and Pb(II) by *Penicillium simplicissimum* [7]. Opposite results were reported for biosorption of Pb(II) by *Mucor rouxii* [50] and Cu(II) and Ni(II) by *Zoogloea ramigera* and *Rhizopus arrhizus* [57]. The authors found that the biosorption of these metals are exothermic in nature. On the other hand, the exothermic nature of Co(II) biosorption is in agreement with Bhatnagar et al. [58] in the biosorption of Co(II) by lemon peel as the biosorbent.

4 Conclusion

The present study evaluates the potential of *R. oryzae* biomass for removal of Pb(II) and Co(II) ions. It was concluded that the biosorption is a relative process affected by many surrounding factors. According to the results of thermodynamic studies, Pb(II) and Co(II) biosorption by *R. oryzae* is feasible. The interactions between Pb(II) and Co(II) ions and the functional groups on the fungal cell wall was confirmed by FTIR analysis and the mechanism of biosorption could be a combination of ion-exchange and complexation with the functional groups present on the fungal cell wall. Generally, *R. oryzae* biomass appears to be suitable biosorbent for the treatment of wastewaters or industrial effluents with low levels of these metal ions studied.

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