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Biosorption of Pb(II) by industrial strain of *Saccharomyces cerevisiae* immobilized on the biomatrix of cone biomass of *Pinus nigra*: Equilibrium and mechanism analysis

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Abstract

The Pb(II) biosorption properties of industrial strain of *Saccharomyces cerevisiae* immobilized on cone biomass of *Pinus nigra* were investigated in a batch biosorption system. The effect of initial pH, contact time and biosorbent dosage on the biosorption process was systematically investigated. The biosorption equilibrium was attained within 30 min. Experimental data were modeled by Freundlich, Langmuir and Dubinin–Radushkevich (D–R) isotherms. The maximum monolayer biosorption capacity for immobilized biomass was determined as 1.45×10^{-4} mol g^{-1} at pH 5.0 with 2.0 g l^{-1} of biosorbent dosage. The Pb(II) loading ability of the low-cost biosorbent system was also tested for real industrial wastewater. The mechanism of the process was evaluated by FT-IR and EDAX analysis. The results revealed that this new biosorbent system was a promising candidate for eliminating Pb(II) from contaminated aquatic environment.

Keywords: Biosorption; Immobilization; Pb(II); *Pinus nigra*; *Saccharomyces cerevisiae*

1. Introduction

There has been an increasing concern over dangerous levels of heavy metals contaminating the aquatic environment and the source of drinking water. The specific problem associated with heavy metals in the environment is their accumulation in the food chain and their persistence in nature. Heavy metals such as lead, mercury, cadmium and chromium are very toxic for living thinks, even if small quantities [1]. Elevated levels of Pb(II) can be traced to industrial discharges from variety of sources, such as electric battery manufacturing, lead smelting and mining activities. The presence of Pb(II) in drinkable water is known to cause various types of serious health problems. Although the inorganic form of lead is a general metabolic poison and enzyme inhibitor, organic forms are even more poisonous [2,3]. Therefore, treatment of Pb(II) contaminated effluents is essential before discharging into receiving bodies of water.

Conventional treatment processes for industrial effluents are neither effective nor economical. Biosorption, is a considerable alternative process because of low cost and good performance, which utilizes the ability of biological materials to bind and sequester heavy metals from aqueous solutions [2,4,5]. Living, dead and immobilized forms of several biosorbent materials such as bacteria, algae, fungi and yeast can be utilize in this manner [3,6–12]. Although freely suspended biomass may have better contact with the adsorbate during the adsorption, the biomass suspension is normally not the practical form for the large-scale applications in biosorption processes. The microbial biomass is often immobilized to enhance its stability, mechanical strength, reusability and the ease of handling. Although a number of studies are available on the use of many different synthetic polymeric agent as a supporting material for the immobilization of microbial biosorbents [13–22], a limited number of studies have been focused on the use of natural carrier for the biosorbent immobilization so far [22,23].

Cone biomass, a natural and readily available biosorbent, has good biosorptive properties due to mature cones are composed of epidermal and sclerenchima cells which contain cellulose, hemicellulose, lignin, rosin and tannins in their cell walls [24].

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Earlier investigations have shown that the biosorption of nickel [25] and chromium(IV) [24] ions by *Pinus sylvestris* and copper ions by *Thuja orientalis* cone biomass [26].

The economically available and easily cultivable yeast *Saccharomyces cerevisiae* immobilized on cone biomass of *Pinus nigra* was chosen as a biosorbent material due to a lack of information on its Pb(II) biosorption ability. We think that, cone may be useful both as a matrix of immobilization for the microbial cells and has a good biosorptive properties. In the present study, *S. cerevisiae* cells were immobilized on the cone biomass of *P. nigra*. The Pb(II) removal potential of immobilized biomass was investigated as a function of initial pH, contact time, biomass and initial Pb(II) ion concentration. The biosorption data have been analyzed by Langmuir, Freundlich and Dubinin–Radushkevich (D–R) isotherm models. The interactions between biosorbent system and Pb(II) ions were also investigated by FT-IR, SEM and EDAX analysis. The real wastewater was also treated with immobilized biosorbent under the determined optimum conditions in order to evaluate the applicability of proposed method.

2. Material and methods

2.1. Microorganism, culture conditions and immobilization

S. cerevisiae was obtained from Eskişehir Sugar Factory, Alcohol Unit. The microorganism was maintained on malt extract agar (Merck) slants at +4 °C. The *S. cerevisiae* cells were first grown on agar slants using malt extract agar and was subcultured and incubated at 30 °C for 48 h. Cell suspension of stock cultures was used as an inoculum source. One milliliter of inoculant prepared by suspending the stock culture was added into the 250 ml Erlenmeyer flask containing 100 ml of malt extract broth (Merck). Furthermore, cone biomass of *P. nigra* was washed with distilled water, dried in an incubator and sterilized by the autoclave (121 °C, 15 min). Cone particles (200 μm) were added to flasks containing of malt extract broth, inoculated with *S. cerevisiae* and then incubated at 30 °C in an incubator shaker rotating at 150 rpm up to 48 h. After the incubation period, supernatant and biomass were separated by filtration.

2.2. Preparation of metal solutions

Pb(II) solution used in this study was prepared by dilution of 1000 mg l^{-1} stock solution obtained by dissolving $Pb(NO_3)_2$ in deionized water. Fresh dilutions were used in each experiment.

2.3. Biosorption experiments

To determine the optimum Pb(II) biosorption conditions, the batch biosorption experiments were conducted with 2.0 g l^{-1} of immobilized biosorbent at 20 °C in 250 ml Erlenmeyer flasks. The effect of pH on the biosorption capacity of the immobilized biosorbent for Pb(II) was investigated at several pH values (1.0, 2.0, 3.0, 4.0 and 5.0) by using known volume of 100 mg l^{-1} Pb(II) solutions. The pH of the solutions was adjusted to required values using 0.1 N HCl and 0.1 N NaOH. Biosorbent was added to medium and the reaction mixture was shaken on an orbital shaker at 125 rpm for 60 min. In the latter experiments the pH of the Pb(II) solutions were adjusted to optimum pH value (5.0 ± 0.1). The effect of contact time on the biosorption was investigated in the time range of 5–120 min. Also, the effect of biosorbent concentration was studied in the concentration range of 0.4–3.0 g l^{-1}. Similarly above, Pb(II) solutions with the concentration range of 75–350 mg l^{-1} were used to assess the effect of initial Pb(II) ion concentration. At the end of the biosorption process biosorbent was separated from the solution and supernatant was analyzed for residual Pb(II) concentration by AAS. Three replicates were used for each Pb(II) biosorption experiments and the results given are the average values. Pb(II) biosorption capacity of immobilized biomass was calculated using the general equation:

$$q_e = \frac{(C_i - C_e)V}{M} \quad (1)$$

where q_e is the amount of Pb(II) ions biosorbed on the biomass (mg g^{-1}), C_i the initial Pb(II) ion concentration in solution (mg l^{-1}), C_e the final Pb(II) ion concentration in solution (mg l^{-1}), V the volume of the medium (l) and M is the amount of the biomass used in the reaction mixture (g).

2.4. Analysis of metal ions

The concentration of residual Pb(II) ions in the supernatant was determined by using an atomic absorption spectrophotometer (Hitachi 180-70, Japan) with an air–acetylene flame. Deuterium background correction was used. The spectral slit width and working currents/wavelengths were 1.3 nm and 7.5 mA/283.3 nm, respectively. Calibration solutions prepared from atomic absorption stock solution (998 ± 2 mg Pb(II) l^{-1} in HNO_3 0.5 mol l^{-1}, Sigma) were used for checking the instrument response for every 10 readings.

2.5. Wastewater

The industrial wastewater was collected from the main drain of the casting unit of metal processing industry from Eskişehir, Turkey. Wastewater sample was placed into a sterile container and transferred to laboratory and stored at 5 °C for until the use. The various characteristics of wastewater were presented in Table 1. Furthermore, real wastewater sample was also spiked with Pb(II) and the proposed biosorption method was applied for with and without spiked samples.

2.6. Zeta potential measurements

The zeta potential of the immobilized biomass was measured with a Zetameter equipment (Malvern Zetasizer nano ZS). Known amount of immobilized biosorbent was suspended in 50 ml of deionized water and solution pH was adjusted to between 2.0 and 7.0 using either 0.1 M HNO_3 or 0.1 M NaOH. After pH adjustment mixtures were equilibrated in a magnetic stirrer for 30 min and the zeta potential was measured.

2.7. Biosorption mechanism

Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDAX) were employed to examine the interactions between the Pb(II) ions and immobilized biomass. The FT-IR spectra were recorded in a Bruker Tensor 27 Fourier transform infrared spectrometer with the samples prepared as KBr discs. SEM and EDAX microscopic analysis were carried out with a scanning electron microscope (Cam Scan Oxford Link SEM). The acceleration voltage was constant at 20 kV and the microprobe was focused at a magnification of 190×, 230× and 250×. Biomass sample was coated with a thin layer of gold and palladium under vacuum by using a coater (Agar Sputer) to increase the electron conduction and to improve the quality of the micrographs.

3. Results and discussion

3.1. Effect of pH on the biosorption

Previous studies on heavy metal biosorption showed that pH value of the solution was an important factor for both solution chemistry of metals and surface characteristics of biosorbent [1,23,27]. The effect of initial pH on the biosorption of Pb(II) by immobilized biomass is presented in Fig. 1. The Pb(II) biosorption capacity of biosorbent increased with increasing pH from 1.0 to 5.0. It is well known that, at low pH values, cell wall ligands were closely associated with the hydronium ions and restricted the approach of positively charged metal ions as a result of the repulsive force. As the pH increased, more ligands would be exposed and carried negative charges, with subsequent attraction of metal cations and biosorption onto the binding sites on the cell surface [7]. Fig. 2 shows the surface charge of the biomass at different pH conditions. The zeta potential values of the biomass was measured as positive at the pH 2.0 and the overall surface of the biomass was negatively charged at the pH values between 3.0 and 7.0. The minimum negative zeta potential value (zP = −24.03 mV) was observed at pH 5.0, which corresponded to the maximum biosorption efficiency of Pb(II). At the pH values of greater than 5.5 the Pb(II) ions became precipitate as $Pb(OH)_2$ due to increasing concentration of OH^- ions in the solution. For this reason, the experiments were not conducted

Table 1
The chemical characteristics of wastewater sample

Parameters	Effluent quality
pH	3.16
Temperature (°C)	27
Suspended solid (mg l^{-1})	26
Lead (mg l^{-1})	1.9
Copper (mg l^{-1})	137.3
Nickel (mg l^{-1})	22.3
Cadmium (mg l^{-1})	5.8
Sodium (mg l^{-1})	66.2
Potassium (mg l^{-1})	7.0
Calcium (mg l^{-1})	243.0
Magnesium (mg l^{-1})	112.0

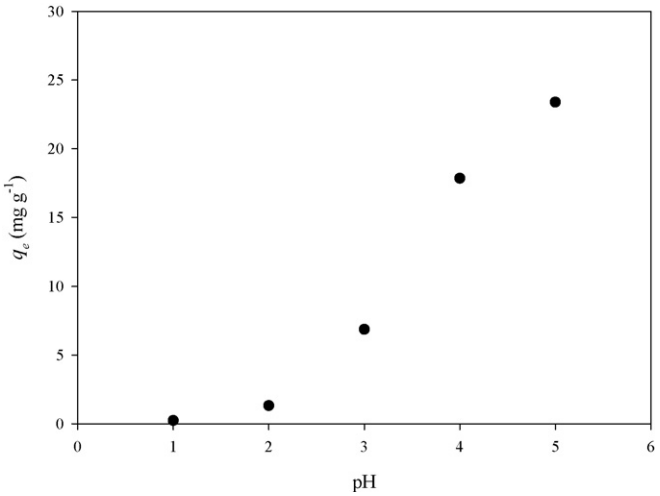

Fig. 1. Effect of pH on the Pb(II) biosorption onto immobilized *Saccharomyces cerevisiae*.

with higher pH values. Several researchers have also investigated the effect of pH on the biosorption of Pb(II) by using different microbial biomass and similar results were reported. For instance, Yan and Viraraghavan found that the Pb(II) biosorption capacity of *Mucor rouxii* cells increased with increasing pH of the solution and reached a constant value at pH 5.0 [28]. Gong et al., reported that, the optimum pH value for the biosorption of Pb(II) by *Spirulina maxima* cells was 5.5 [29].

3.2. Effect of contact time

It has been determined that rapid biosorption of Pb(II) ions by immobilized biomass was observed in first 30 min and then the Pb(II) biosorption capacity of biomass did not significantly change up to 120 min (Fig. 3). The results demonstrate that the maximum biosorption level occurred in a short time. The rapid biosorption is a significant parameter for large-scale application in industrial processes. Similar fast heavy metal uptake trend by papaya woods was reported in a previous study and this

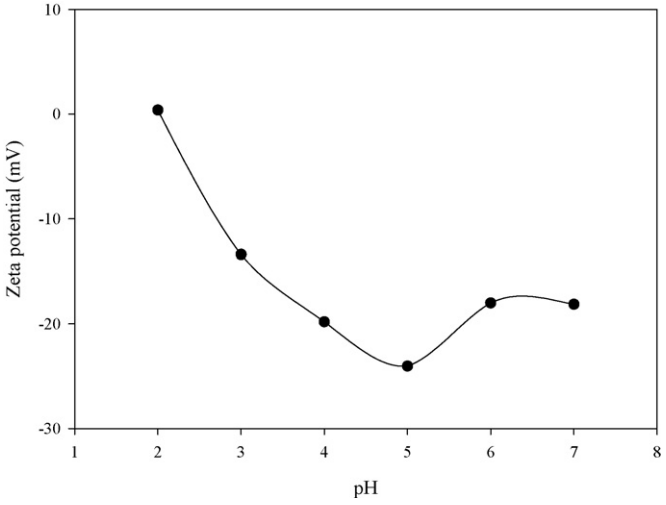

Fig. 2. Zeta potential of the immobilized biosorbent at various pH values.

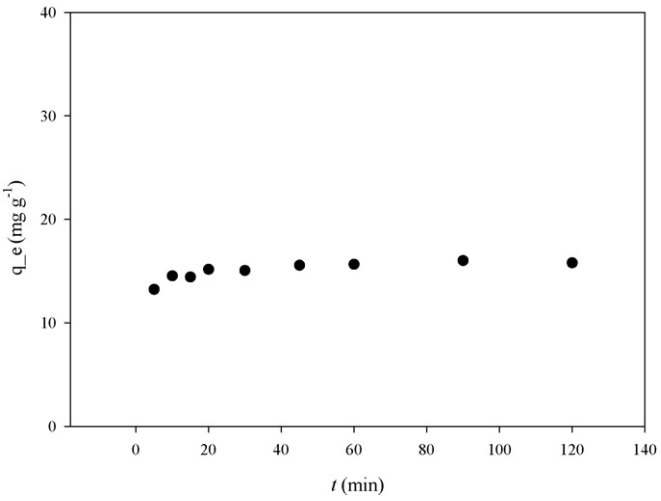

Fig. 3. Effect of contact time on the Pb(II) biosorption onto immobilized *S. cerevisiae*.

finding was attributed to highly porous and meshes structure of biosorbent, which provides ready access and large surface area for the sorption of metals on the binding sites [30]. As it can be seen from Fig. 4, the porous structure of the immobilized biomass eliminates the problem of diffusion limitation as would be expected to occur for biosorption of Pb(II) on immobilized biomass.

3.3. Effect of initial Pb(II) ion concentration

The experiments were carried out using various concentrations of Pb(II) solution under the determined optimum pH values and contact time. The effect of initial Pb(II) ion concentration was investigated in the range of 75–350 mg l^{-1}. The results are presented in Fig. 5. The Pb(II) biosorption capacity of the immobilized biomass firstly increased with increasing of the initial concentration of Pb(II) and then reached a saturation value at about 250 mg l^{-1}. Then the value did not significantly change with the initial Pb(II) ion concentration. This indicates particular suitability of *S. cerevisiae* immobilized on cone biomass for the treatment of effluents at concentrations of <250 mg l^{-1}.

3.4. Effect of biosorbent dosage

To determine the effect of the biosorbent dosage on the biosorption capacity of immobilized biomass the amounts of biosorbent added into metal solution were varied from 0.4 to 3.0 g l^{-1} and the results are presented in Fig. 6. The amount of Pb(II) ion biosorbed per unit mass of immobilized *S. cerevisiae* increased with increasing of the biomass concentration as expected. With increase in the biosorbent dosage, from 0.4 to 3.0 g l^{-1} the biosorption yield was changed from 5.34 to 30.10%. Similar results has been also reported by the other researchers for Pb(II) biosorption on *Spirulina maxima* [29] and Cr(VI) biosorption on *Mucor hiemalis* [1]. It is well known that, increasing the biomass dosage, the total removal of Pb(II) from the solution increased because the availability of active sites for metal binding increased.

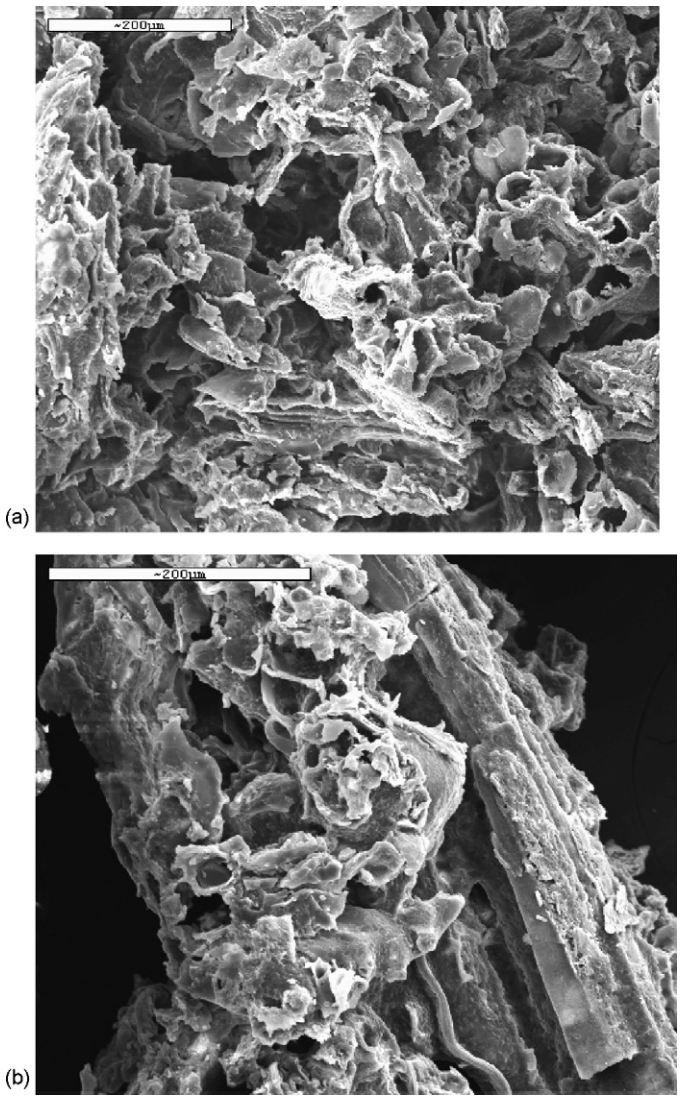

Fig. 4. SEM microscopic pictures of immobilized biosorbent with magnifications of 190× (a) and 250× (b).

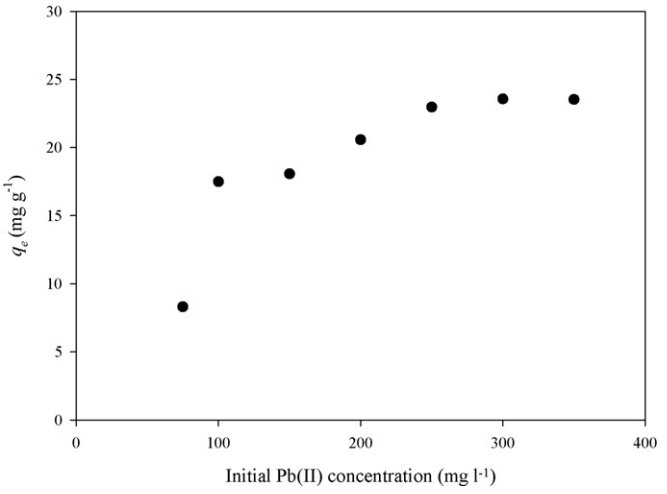

Fig. 5. Effect of initial metal ion concentration onto biosorption of Pb(II) immobilized *S. cerevisiae*.

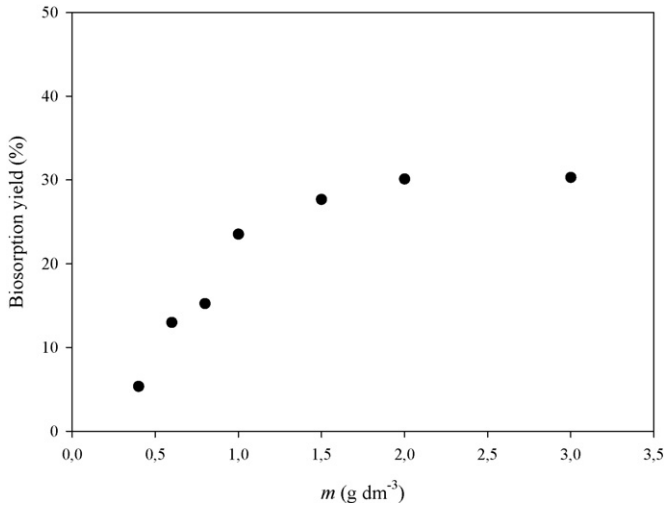

Fig. 6. Effect of biosorbent dosage on the Pb(II) biosorption onto immobilized *S. cerevisiae*.

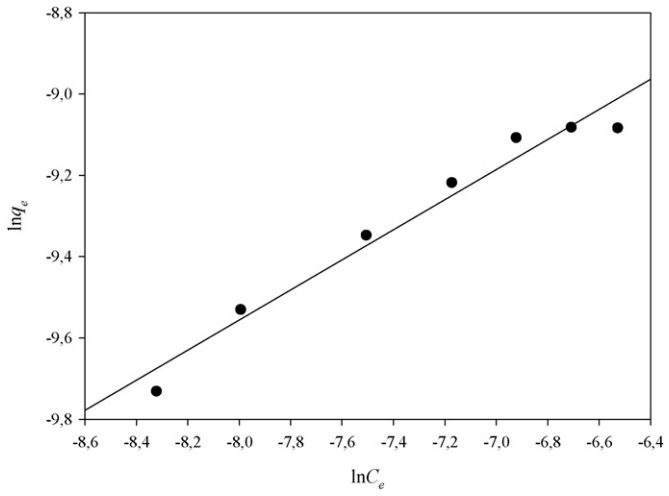

Fig. 8. The Freundlich isotherm plot for the Pb(II) biosorption onto immobilized *S. cerevisiae* at 20 °C.

3.5. Biosorption isotherms

The equilibrium biosorption isotherms are one of the most important data to understand the mechanism of the biosorption. Langmuir, Freundlich and Dubinin–Radushkevich (D–R) isotherms were used for the modeling of the experimental biosorption data obtained from the batch system. The biosorption isotherm plots for the Pb(II) biosorption onto immobilized biosorbent at 20 °C were presented in Figs. 7–9.

The Langmuir isotherm model assumes a monolayer sorption, which takes place at specific homogeneous sites within the biosorbent. The linear Langmuir isotherm equation is represented by the following equation [31]:

$$\frac{1}{q_e} = \frac{1}{q_{max}} + \left(\frac{1}{q_{max} K_L}\right) \frac{1}{C_e}, \quad (2)$$

where q_e is the equilibrium Pb(II) concentration on the biosorbent (mol g^{-1}), C_e the equilibrium Pb(II) concentration in the solution (mol l^{-1}), q_{max} the monolayer biosorption capacity of the biosorbent (mol g^{-1}), and K_L is the Langmuir adsorption constant (l mol^{-1}) and is related to the free energy of adsorption. The plot of $1/q_e$ versus $1/C_e$ for the adsorption gives a straight line of slope $1/q_{max}K_L$ and intercepts $1/q_{max}$.

The Freundlich isotherm is an empirical equation employed to describe heterogeneous systems. The linear form of Freundlich equation is [32]:

$$\ln q_e = \ln K_F + \frac{1}{n} \ln C_e, \quad (3)$$

where K_F (l g^{-1}) and n are Freundlich isotherm constants, being indicative of the extent of the biosorption and the degree of non-linearity between solution concentration and adsorption, respectively. The plot of $\ln q_e$ versus $\ln C_e$ for the biosorption was employed to generate K_F and n from the intercept and the slope values, respectively. The Dubinin–Radushkevich (D–R) isotherm is more general than the Langmuir isotherm. It was applied to distinguish the nature of biosorption as physical or chemical [33]. The linear form of D–R isotherm equation is

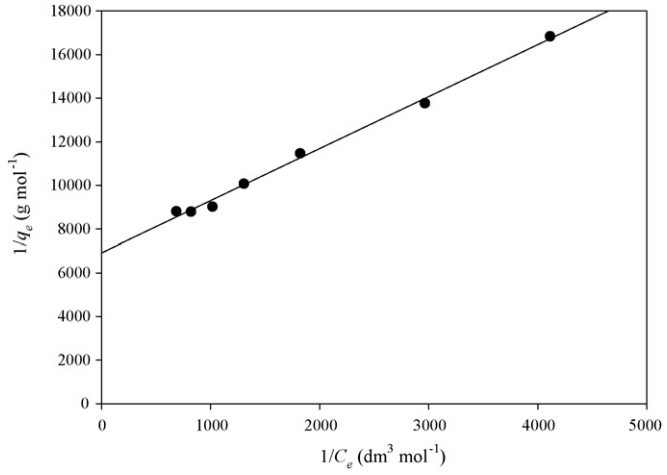

Fig. 7. The Langmuir isotherm plot for the Pb(II) biosorption onto immobilized *S. cerevisiae* at 20 °C.

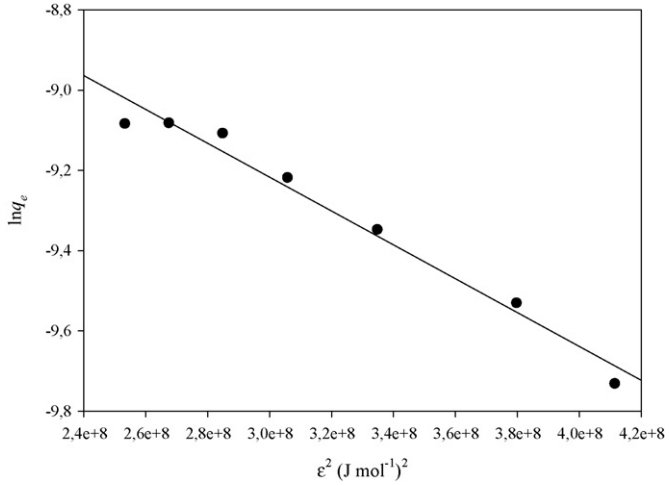

Fig. 9. The D–R isotherm plot for the Pb(II) biosorption onto immobilized *S. cerevisiae* at 20 °C.

[34]:

$$\ln q_e = \ln q_m - \beta\varepsilon^2, \quad (4)$$

where β is a constant related to the mean free energy of biosorption ($mol^2\,J^{-2}$), q_m the theoretical saturation capacity, and ε is the Polanyi potential, which is equal to $RT\ln(1+(1/C_e))$, where R ($J\,mol^{-1}\,K^{-1}$) is the gas constant and T (K) is the absolute temperature. Hence by plotting $\ln q_e$ against ε^2 it is possible to generate the value of q_m ($mol\,g^{-1}$) from the intercept, and the value of β from the slope.

The constant β gives an idea about the mean free energy E ($kJ\,mol^{-1}$) of biosorption can be calculated using the relationship [35–37]:

$$E = \frac{1}{(2\beta)^{1/2}}, \quad (5)$$

E values give information about biosorption mechanism as chemical ion-exchange or physical adsorption. The numerical value of the mean free energy of biosorption is $10.89\,kJ\,mol^{-1}$ may correspond to a chemical ion-exchange mechanism.

The Langmuir, Freundlich and D–R parameters for the biosorption of Pb(II) onto immobilized biomass are listed in Table 2. It is indicated that all of the isotherm models fit very well when the r^2-values are compared.

The essential feature of the Langmuir isotherm can be expressed by means of a separation factor or equilibrium parameter, R_L, is calculated using the following equation:

$$R_L = \frac{1}{1 + K_L C_o}, \quad (6)$$

where C_o is the highest Pb(II) concentration ($mol\,l^{-1}$). As the R_L values lie between 0 and 1, the biosorption process is favorable [38,39]. The R_L value for this study was 0.170, therefore, biosorption of Pb(II) was favorable.

The Freundlich constants K_F and n indicate the biosorption capacity of the biosorbent and a measure of the deviation from linearity of the biosorption, respectively. The values of K_F and n at equilibrium were $1.37 \times 10^{-3}\,l\,g^{-1}$ and 2.70, respectively.

3.6. Application of immobilized biosorbent to real wastewater

In order to evaluate the potential performance of immobilized biosorbent for the biosorption of Pb(II) ions from real wastewater, optimized biosorption procedure was tested with model wastewater samples with and without spikes at described optimum experimental conditions. The results are shown in Table 3. As can be seen from the table the Pb(II) biosorption yields of immobilized biomass were varied from 54.50 to 97.34% for real and spiked wastewater samples, respectively. The results showed that the proposed method could be successively applied for the treatment of real wastewater for the removal of Pb(II) ions.

Table 3
The application of the proposed method in wastewater sample

Sample	Concentration of added lead(II) ($mg\,l^{-1}$)	q_e ($mg\,g^{-1}$)	Biosorption yield (%)
Real wastewater	–	0.27 ± 0.07	54.50
Spiked sample	4.0	1.40 ± 0.86	94.01
Spiked sample	8.0	2.42 ± 0.18	97.34

3.7. Biosorption mechanism

The nature of the possible biosorbent–Pb(II) ions interactions was elucidated on the basis of FT-IR and EDAX analyses. The FT-IR spectra of unloaded and metal loaded forms of immobilized biosorbent in the range of 400–4000 cm^{-1} were taken and presented in Fig. 10. The FT-IR spectrum of unloaded immobilized biomass showed several distinct and sharp absorptions at 3379 cm^{-1} (indicative of −OH and $-NH_2$ groups), 2930 cm^{-1} (indicative of C–H group), 1633 cm^{-1} (indicative of amide I band of amide bond in *N*-acetyl glucosamine polymer or of the protein peptide bond), 1452 cm^{-1} (indicative of the bending of CH_3) and the band at 1035 cm^{-1} (indicative of C–N stretching vibrations) [40]. The FT-IR spectra of immobilized biomass exposed to Pb(II) ions (Fig. 10(b)) indicated no shifts or change in any of the characteristic absorbance bands present in Fig. 10(a) with the exception of a peak shift at 1633 cm^{-1}. This current results implied not only involvement of amide groups in biosorption of Pb(II) ions, but also the possibility that biosorption could be taken place through an ion-exchange process rather

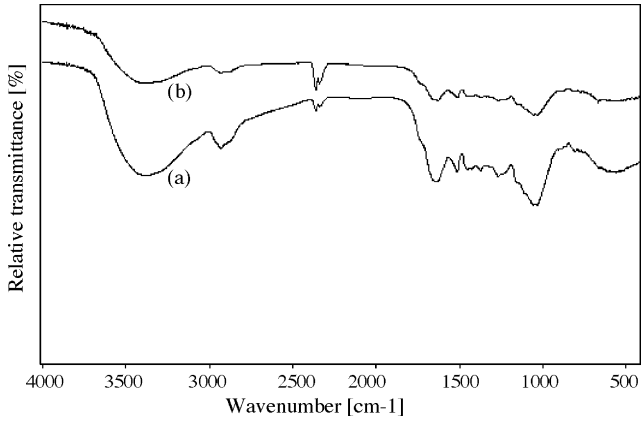

Fig. 10. The FT-IR spectrum patterns of immobilized *S. cerevisiae* before (a) and after (b) Pb(II) biosorption.

Table 2
Biosorption isotherm constants for the biosorption of Pb(II) onto immobilized *Saccharomyces cerevisiae* at 20 °C

Langmuir isotherm	$q_{max} = 1.45 \times 10^{-4}\,mol\,g^{-1}$	$K_L = 2.90 \times 10^3\,l\,mol^{-1}$	$r_L^2 = 0.995$	$R_L = 0.170$
Freundlich isotherm	$n = 2.70$	$K_F = 1.37 \times 10^{-3}\,l\,g^{-1}$	$r_F^2 = 0.965$	
D–R isotherm	$q_{max} = 3.52 \times 10^{-4}\,mol\,g^{-1}$	$\beta = 4.22 \times 10^{-3}\,mol^2\,kJ^{-2}$	$r_{D-R}^2 = 0.975$	$E = 10.89\,kJ\,mol^{-1}$

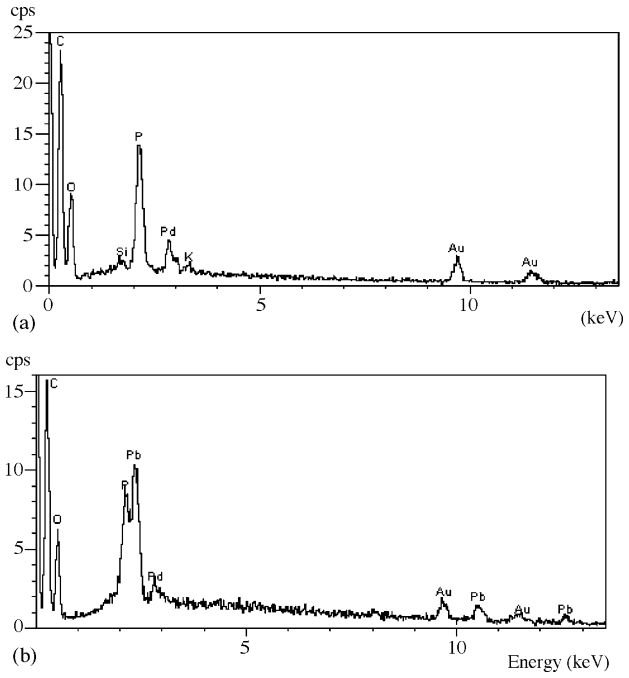

Fig. 11. Typical EDAX spectra of immobilized *S. cerevisiae* before (a) and after (b) Pb(II) biosorption.

than complexation. When compared the typical EDAX spectra of the Pb(II) loaded biomass (Fig. 11(b)) with that of unloaded biomass (Fig. 11 (a)) it was observed that the intensity of the phosphorus signal at about 2.1 keV was considerably reduced after the Pb(II) biosorption. This could be indicative of the complexation of Pb(II) ions with phosphate groups on the biomass surface. Also the appearance of Pb(II) signal at about 2.2, 10.6 and 12.7 keV and the disappearance of K^+ signal at about 3.4 keV were observed after Pb(II) biosorption. These findings indicated that, biosorption process also included ion-exchange mechanism for the removal of Pb(II) ions by this immobilized biosorbent which is confirmed by the *E*-value obtained from D–R isotherm.

4. Conclusion

The present investigation suggested that the *S. cerevisiae* immobilized on cone biomass of *P. nigra* appears as a low cost possible biosorbent and to be used for treatment of Pb(II) bearing solutions. The biosorption characteristic of Pb(II) ions onto immobilized biomass has been examined with the variations in the parameters of pH, biosorbent dosage and contact time. The maximum biosorption capacity was determined to be 30.04 mg g^{-1} at pH 5.0, 2.0 g l^{-1} biosorbent dosage and 30 min. The experimental data were evaluated by Langmuir, Freundlich and Dubinin–Radushkevich isotherms and fitted well to all of the isotherm models with good regression coefficients. The interactions between metal ions and the functional groups on the biosorbent surface of the immobilized system were examined by FT-IR and EDAX analysis. The immobilized biomass can obtain much better treatment efficiency for Pb(II) removal from real wastewater.

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