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REMOVAL OF Cu(II) AND Pb(II) FROM AQUEOUS SOLUTIONS BY LACTIC ACID BACTERIA

USUWANIE Cu(II) I Pb(II) Z ROZTWORÓW WODNYCH PRZEZ BAKTERIE KWASU MLEKOWEGO

Abstract: The aim of the study was to compare the removal of Cu(II) and Pb(II) from aqueous solution by lactic acid bacteria (LAB). Effect of various process parameters, viz., initial metal ions concentration, pH, and contact time has been studied for the removal of copper and lead ions. Langmuir and Freundlich models were applied to describe the biosorption isotherm of the metal ions by LAB biomass. Langmuir model fitted the equilibrium data better than the Freundlich isotherm. The sorbent showed the maximum sorptive capacity amounting to be 11.07 and 10.51 mg $\cdot$ g^{-1} for Cu(II) and Pb(II) ions, respectively. The optimum conditions were pH 6.0 with equilibrium time of 40 min for both metal ions. The involvement of functional groups on the surface of dried biomass in biosorption process is also discussed.

Keywords: biosorption, Cu(II), Pb(II), isotherm, lactic acid bacteria

Lactic acid bacteria (LAB) are nonpathogenic, food safe bacteria that are used in the production of many fermented food products. They ferment glucose into lactic and acetic acid, ethanol and CO_2, which contributes to better quality, texture and aroma of fermented products. LAB also produces bacteriocins and other compounds that inhibit the growth of undesirable microorganisms present in food. They are a part of the microbial population of the digestive tract of healthy humans and animals, and are involved in their metabolism [1]. Microbial cell is the natural adsorbent for metal ions due to its nature and composition of the cellular membrane [2]. Application of LAB in the process of metal ions binding has started recently, although studies that examined the binding ability of metal ions by other microorganisms were carried out. Most of the studies have used readily available waste microbial biomass from the pharmaceutical or food industries, in order to remove heavy metals from industrial wastewater. However, the investigated waste microbial biomass cannot be used in the field of food technology. In drinking water and food processing only food safe microorganisms can be used. Among all of them, LAB has the added advantage because of probiotic properties of some strains. Food and water contamination by heavy metals continues to increase due to the increasing environmental pollution. Heavy metals in food, even at a very low concentration, may lead to development of adverse health effects. If they enter the body, they are not degradable and tend to form complexes and accumulate in the tissues [3]. There are various conventional physical and chemical methods for removing heavy metals from water as chemical precipitation, ion exchange, membrane technologies etc. These methods are effective, but often dependent on the metal ion concentration, expensive and not environment friendly. Binding of heavy metals on the LAB could be a promising solution for heavy metal removal from water, liquid food and from the body [1].

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The objective of the present work is to investigate the biosorption potential of LAB in the removal of Cu(II) and Pb(II) from aqueous solution. The influence of different parameters on biosorption, such as pH, initial metal concentration and contact time, were performed. Different equilibrium and kinetic models were applied to describe the biosorption process. The desorption experiments were implemented to identify the release of metal ions and recovery of biosorbents, while competitive biosorption and the Fourier transform infrared (FTIR) analysis were also used in this study to look at potential binding sites and possible functional groups of LAB.

Material and methods

The bacteria used in this work were lyophilized cultures of *Lactobacillus rhamnosus, L. casei* and *L. plantarum* (Avet PHARMA, Warszawa, Poland). The bacteria transferred to MRS plates and cultured anaerobically for 71 h at 37ºC. A colony from the plate was transferred to MRS broth and incubated under anaerobic conditions for 48 h at 37ºC. The cultured biomass was washed twice with deionized water and lyophilized.

The concentrations of Cu(II) and Pb(II) in the biosorption experiments were determined spectrophotometrically (Photolab Spectral, WTW, Germany). Before measuring the samples were passed through a Whatman filter (pore size 0.45 µm) and then diluted with deionized water. The initial and the final concentrations of heavy metals used in batch mode studies were estimated spectrophotometrically. The removal efficiency of the microorganisms was calculated from the difference between initial and final concentrations.

Experimental tests were conducted in 250 cm^3 Erlenmeyer flasks with of 50 cm^3 of metal solution at constant level of biomass (0.1 g), at 37ºC and agitation of 120 rpm. Biosorption experiments were carried out to investigate the effects of pH, contact duration and initial metal concentration. The pH values were adjusted between 2.0-8.0 by adding 0.1 M NaOH or 0.1 M HNO$_3$. The contact durations ranged from 0-120 min. The initial Cu(II) and Pb(II) concentrations varied from 8.0 to 150.5 g · dm^{-3} and 9.5 to 154.6 g · dm^{-3}, respectively. The functional groups present in the biomass, before and after interaction with Cu(II) and Pb(II) were detected using a Fourier transform infrared (FTIR) spectrometer. The spectrum was recorded in the range of 4000-500 cm^{-1}.

The metal uptake [mg metal · g^{-1} dry biomass] was calculated:

$$q_e = \frac{(C_0 - C_e) \cdot V}{M} \tag{1}$$

where C_0 and C_e are the respective initial and equilibrium metal concentrations in the solution [mg · dm^{-3}], V is the volume of the solution [dm^3], and M is the dry weight of the biomass [g]. The metal sorption ability of the biomass was determined by the above-mentioned procedure in all the following experiments unless stated otherwise.

Heavy metals biosorption isotherms were obtained at constant pH and ionic strength. To test the fit of data, Langmuir and Freundlich isotherm models were applied to this study. The Langmuir isotherm model is valid for monolayer sorption onto a surface and a finite number of identical sites, and is given by:

$$q_{eq} = \frac{q_{max} K_L C_{eq}}{1 + K_L C_{eq}} \tag{2}$$

or presented in linear form as follows:

$$\frac{1}{q_{eq}} = \frac{1}{q_{max}} + \frac{1}{K_L \cdot q_{max} \cdot C_{eq}} \tag{3}$$

where q_{max} [mg $\cdot$ g^{-1}] is the maximum amount of the metal ion per unit weight of the cell to form a complete monolayer on the surface bound at a high C_{eq} [mg $\cdot$ dm^{-3}] and K_L a constant related to the affinity of the binding sites, q_{max} represents a practical limiting adsorption capacity when the surface is fully covered with metal ions and assists in the comparison of adsorption performance, particularly in cases where the sorbent did not reach full saturation in experiments.

The empirical Freundlich isotherm model based on a heterogeneous surface is given by:

$$q_{eq} = K_F C_{eq}^{1/n} \tag{4}$$

where K_F and n are Freundlich constants characteristic of the system, K_F and n are indicators of adsorption capacity and intensity, respectively. Freundlich parameters can be determined from the linear form of the eq. (4) by plotting the ln q_{eq} versus ln C_{eq} the slope is the value of $1/n$ and the intercept is equal to ln K_F. The Freundlich isotherm is also more widely used and provides information on the monolayer adsorption capacity, in contrast to the Langmuir model. All data shown are the mean values of three replicate experiments, and error bars are indicated wherever necessary.

Results and discussion

The pH of the solution is one of the most important parameters affecting the biosorption process due to its impact on both the solubility of metal ions and the ionization states of surface functional groups of the biosorbent system [4].
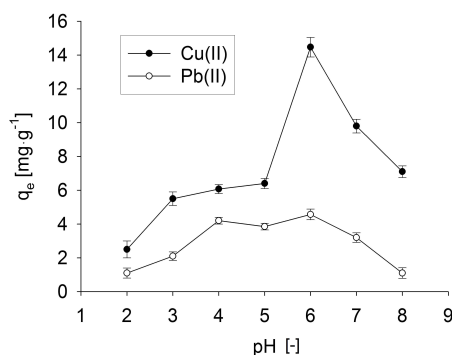

Fig. 1. Initial pH effect on Cu(II) and Pb(II) removal conditions by LAB (metals concentration 78.0 and 92.0 mg $\cdot$ dm^{-3} respectively, contact time 60 min, biomass 0.1 g, temperature 37ºC, agitation rate 120 rpm)

As depicted in Figure 1, the metal ions uptake ions was strongly affected by the pH of the medium as it increased with solution pH increase. The sharpest increase in maximum

capacity (q_e) was obtained between pH values of 2 and 6, corresponding for the latter pH value to capacities of 14.47 and 4.57 mg · g^{-1} for copper and lead, respectively. Further lead and copper biosorption studies were carried out at pH 6. At low pH values, the high concentration of H$^+$ protons competes with and decreases the adsorption of positively charged metals ions [5, 6] but an increase in the pH causes the ionization of the biosorbent surface functional groups and thus, increases the total negative charge which increases biosorption. At pH values higher than 6, precipitation of insoluble metals hydroxides may be formed and the concentration of free Cu(II) and Pb(II) ions decreases, thereby the adsorption capacity of Cu(II) and Pb(II) decreases [7].

The biosorption rates of Cu(II) and Pb(II) ions on LAB biomass were shown in Figure 2. Rapid uptake of metal species was occurred within 20 min and an equilibrium was reached in 40 min. After this equilibrium period the amount of adsorbed metal ions did not change significantly with contact time. Such a phenomenon of rapid removal of Cu(II) and Pb(II) ions has also been reported earlier for biosorbents such as *Bacillus thioparans*, green algae *Cladophora fascicularis*, *Neurospora crassa* and *Candida albicans* [8-11].
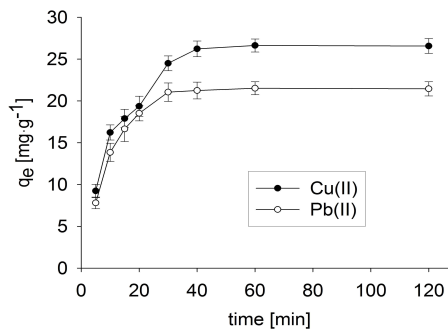

Fig. 2. Biosorption of Cu(II) and Pb(II) by LAB as a function of time (metals concentration 101.5 and 102.7 mg · dm^{-3} respectively, pH 6.0, biomass 0.1 g, temperature 37ºC, agitation rate 120 rpm)
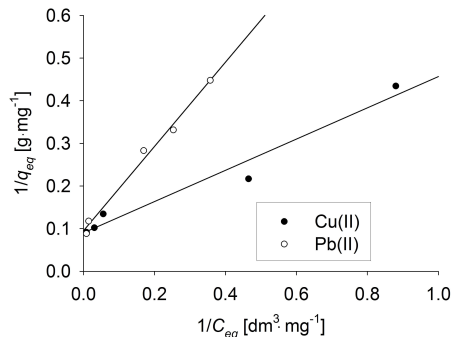

Fig. 3. Langmuir isotherms for Cu(II) and Pb(II) biosorption by LAB (initial pH 6.0, biomass 0.1 g, temperature 37ºC, agitation rate 120 rpm)
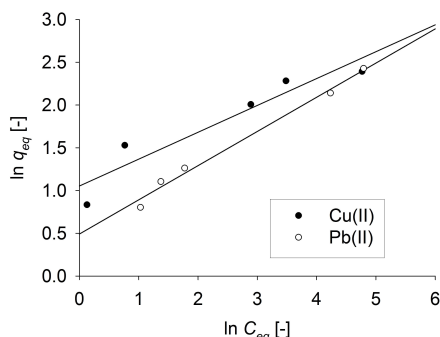
Fig. 4. Freundlich isotherms for Cu(II) and Pb(II) biosorption by LAB (initial pH 6.0, biomass 0.1 g, temperature 37ºC, agitation rate 120 rpm)

The linearized Langmuir and Freundlich isotherms of Cu(II) and Pb(II) ions are given in Figures 3 and 4. The isotherm constants and correlation coefficients are given in Table 1. Langmuir sorption model served to estimate the maximum metal uptake values where they could not be reached in the experiments. In general, for good biosorbents, high q_{max} and high K_L are desirable [12]. However, biosorbent with a low q_{max} and a high K_L could outperform a biosorbent with high q_{max} and a low K_L [13]. In this study, K_L and q_{max} value were found out 0.197 and 11.07 mg · g^{-1} for Cu(II) and 0.088 and 10.51 mg · g^{-1} for Pb(II) which indicated that LAB is an encouraging biosorbent for the tested metal ions (Table 1). The correlation coefficients (R^2) were found to be 0.96 and 0.99 for Cu(II) and Pb(II) biosorption, respectively. Mrvčić et al [1] reported a q_{max} value of 15.53 mg · g^{-1} for Cu(II) biosorption by *Lactobacillus plantarum*.

Fitting of the biosorption data of Cu(II) and Pb(II) to Langmuir isotherm showed that the binding energy on the whole surface of the LAB was uniform. Fitting adsorption data to Langmuir isotherms also showed that the adsorbed metal ions do not interact or compete with each other and they are adsorbed by forming a monolayer. This phenomenon, at the same time, indicated that chemosorption was the principal removal mechanism in biosorption [14].

Table 1

Adsorption isotherm parameters for Cu(II) and Pb(II) ions on LAB

Metal ions	Langmuir model			Freudlich model		
	K_L	q_{max} [mg · g^{-1}]	R^2	n	K_F	R^2
	$q_{eq} = q_{max}K_L C_{eq}/(1+K_L C_{eq})$			$q_{eq} = K_F C_{eq}^{1/n}$		
Cu(II)	0.197	11.07	0.96	0.73	2.87	0.90
Pb(II)	0.088	10.51	0.99	1.12	1.63	0.98

Table 1 suggests that metal biosorption is less confidentially explained by Freundlich model, on the basis of R^2, than Langmuir model.

The FTIR spectra of LAB before and after interaction with heavy metals (copper and lead) are shown in Figures 5-7. The sharp peak at 3378 cm^{-1} was characteristic of hydroxyl groups bound to alcohol, and strong CH$_2$ stretching frequency gave rise to peak

at 2926 cm^{-1}. Differences were also noted at 1634 cm^{-1} (C═O, stretching vibrations). Some differences in the spectra of the 3 samples were observed in the broad range of 1218 to 1413 cm^{-1} indicating the presence of ─OH groups, COO– anions, and C═O groups (deformation and stretching vibrations). A clear difference in signal intensity was also found at 1028 cm^{-1} (C─O groups).
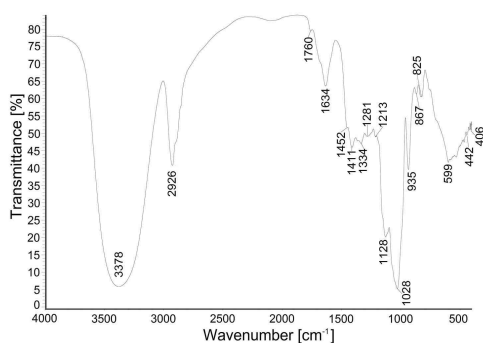

Fig. 5. FTIR spectrum of native LAB

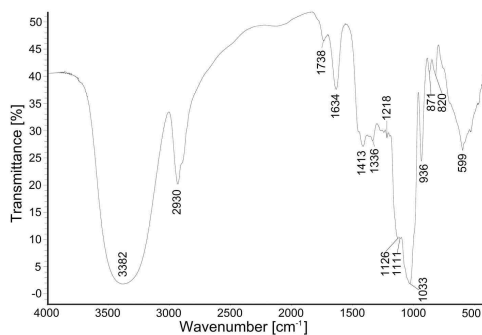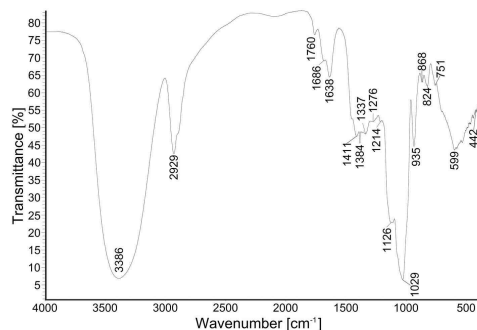Fig. 6. FTIR spectra of LAB after treatment with Cu(II)

Fig. 7. FTIR spectra of LAB after treatment with Pb(II)

The analysis of the FTIR spectrum confirmed the presence of many ionizable functional groups, which are able to react with Cu(II) and Pb(II) ions. Carboxyl or hydroxyl groups are known to be involved in metal binding by forming coordination bonds that facilitate the stability of the metal complexes [15]. The results of FTIR analysis obtained in this study demonstrated that ─OH, C═O, and COO– groups played an important role in the binding of Cu(II) and Pb(II) ions onto the LAB biosorbent.

Conclusions

In this study, the LAB were used as effective biosorbents of Cu(II) and Pb(II) from waters. The biosorption performances were strongly affected by parameters such as pH, contact duration and heavy metal concentration. The optimum pH for the biosorption of Cu(II) and Pb(II) by LAB at pH 6.0. The uptake of metals was very fast. Adsorption equilibrium was reached within 40 min of biomass addition. The batch experimental results fitted well to the Langmuir isotherm model. The maximum adsorption uptake (q_{max}) of respectively Cu(II) and Pb(II) calculated from the Langmuir equation for biosorption by LAB were 11.07 and 9.79 mg · g^{-1}. The FTIR analysis also suggests the involvement of some functional groups (─OH, C═O, and COO–) in Cu(II) and Pb(II) biosorption.

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USUWANIE Pb(II) I Cu(II) Z ROZTWORÓW WODNYCH PRZEZ BAKTERIE KWASU MLEKOWEGO

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Abstrakt: Celem pracy było porównanie zdolności usuwania Cu(II) i Pb(II) z roztworu wodnego przez bakterie kwasu mlekowego (LAB). Badano wpływ różnych parametrów, tj. stężenia jonów metali, pH i czas kontaktu, na proces usuwania jonów miedzi i ołowiu. Do opisu izoterm adsorpcji jonów metali przez biomasę LAB zastosowano modele Langmuira i Freundlicha. Uzyskane dane doświadczalne były lepiej dopasowane do modelu Langmuira niż Freundlicha. Sorbent wykazał maksymalną zdolność sorpcyjną, wynoszącą 11,07 i 10.51 mg · g^{-1} odpowiednio dla jonów Cu(II) i Pb(II). Optymalne warunki biosorpcji obu jonów metali wynosiły: pH 6,0 i czas równowagi 40 minut. Omówiono również udział grup funkcyjnych na powierzchni biomasy w procesie biosorpcji.

Słowa kluczowe: biosorpcja, Cu(II), Pb(II), izoterma, bakterie kwasu mlekowego