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COPPER(II) BIOSORPTION CHARACTERISTICS OF LYOPHILIZED AND THERMALLY TREATED *Pseudomonas* CELLS

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Abstract

Biosorption of copper(II) by lyophilized and thermally treated bacterial biomass of *Pseudomonas aeruginosa* PAO1 and *Pseudomonas fluorescens* BME in aqueous suspension was studied. The cell surface properties were characterized and the experimental conditions, *e.g.*, pH, adsorption time, and initial metal concentration were optimized for efficient biosorption. The surface charge was negative at pH above 2.5 for *P. aeruginosa*, and above pH 4 for *P. fluorescens*. The highest copper(II) uptake was observed at pHs 5 to 6 for both bacteria with a maximum uptake capacity of 60.3 and 56.5 mg copper(II)/g biomass for *P. aeruginosa* and 56 and 29 mg/g for *P. fluorescens* by the lyophilized and thermally treated cells, respectively. Both, the Freundlich and the Langmuir model, using non-linear least-squares estimation, gave a good prediction to the experimental data of copper(II) biosorption equilibrium. For the biosorption kinetic study only the pseudo second-order kinetic model could be applied at various temperatures. Temperature has only a minor effect on the adsorbed amounts in the experimental conditions studied. The laboratory bacterial strain *P. aeruginosa* PAO1 is more efficient adsorbent for copper(II) than *P. fluorescens* BME in lyophilized and even in thermally inactivated form.

Keywords: biosorption, copper, isotherm, kinetics, *Pseudomonas* sp., temperature

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1. Introduction

There are only a few percent of essential micro-nutrients or trace elements in the living organisms. In the absence of these elements the cells are unable to build up important compounds. One of the most important elements is copper, which plays an important role in normal life functions. Copper is a cofactor of the superoxide dismutase in the red blood cells: it plays a significant role in hematopoiesis, in the regulation of cellular respiration and in the enzyme-household. In an adult human there is about 100 mg copper, and a replacement of about 2-3 mg is necessary upon the daily loss (Fonyó, 2002). The drinking water may contain copper below 2 mg/L level (Hungarian Standard, MSz 201/2001), but high copper content in the water can be dangerous. Copper poisoning may cause hemolytic anemia, vomiting, and diarrhea. The *Wilson's disease* is associated with specific symptoms of copper poisoning in addition to the hepato-renal syndrome and central nervous system disorders (Fonyó, 2002).

Copper contamination in water may arise from industrial and agricultural activities, which enter the aquatic ecosystems directly or through the soil by, e.g. surface treatment of metal objects; plating, paint industry, spent catalysts, brake pads, friction surfaces, organic fertilizers and pesticides with heavy metal content (Espinosa and Oliva, 2006). The self-purification processes in living water can reduce the

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copper content entering the ecosystem, but plants and microorganisms cannot manage the increased amounts of impurities (Cukrov et al., 2008).

The removal of copper from industrial or agricultural wastewater is possible with the help of activated carbon produced from different materials. Under pH ranged 4-7, an amount of 0.1 g activated carbon adsorbent can purify about 25 mg Cu(II) (Kazemipour et al., 2008). Other possibility for metal removal from contaminated environment is by using microorganisms.

The use of biosorbents has emerged in recent years as one of the most promising alternatives to conventional heavy metal management strategies (Hashim et al., 2011; Wang and Chen, 2009). Heavy metal sequestering property of *Pseudomonas* genus is extensively studied. Free and immobilised cells of heavy metal tolerant *P. veronii* strains can adsorb cadmium(II), zinc(II) and copper(II) (Vullo et al., 2008). Free and living cells of *P. aeruginosa* AT18, isolated from petroleum contaminated soil, has a copper(II) sorption capacity of 87 mg/g at pH 6.25 (Silva et al., 2009).

The maximum adsorption capacity of resting and thermally inactivated cells of *P. aeruginosa* PU21 isolated from hospital sewage was between 19-23 mg/g at pH 5 (Chang et al., 1997). *P. fluorescens* was tested as biosorptive material by different heavy metals. *P. fluorescens* showed high adsorption capacity for nickel(II) and chromium(VI) (Uzel and Ozdemir, 2009), cadmium(II) (Mao et al., 2010b) and copper(II) (Mao et al., 2010a). The maximum copper(II) adsorption capacity was 78.99 mg/g at pH 5 at 301 K (Mao et al., 2010b).

Pseudomonas strains, with well-characterized biochemical and genetic characteristics play an important role in biosorption studies. Many studies use *Pseudomonas* strains isolated from contaminated soil and water (Juwarkar et al., 2007; Mathivanan and Rajaram, 2014; Oyetibo et al., 2014). Since many *Pseudomonas* bacteria are pathogenic, care must be taken when handling these microorganisms. Moreover, the resistance of many strains against antibiotics is well described (Ramos et al., 1994). In the case of living cells the risk for the development of multiresistant cultures is high (Kaszab et al., 2011). Therefore, these bacteria should be applied in inactivated form, which can minimize the environmental risk when using them as biosorbents.

In this study the copper(II) [Cu(II)] biosorption by lyophilized and thermally inactivated cells of *Pseudomonas aeruginosa* PAO1, which is a laboratory bacterial strain, is evaluated and compared to the biosorption by *Pseudomonas fluorescens* BME, which is isolated from an urban environment. The novelty of this work is the comparison of biosorption by laboratory and environmental strains, the study of heat treatment effect on cell adsorption properties and temperature effect on biosorption kinetics.

2. Material and methods

2.1. Bacterial strains

The bacterial strains, *P. aeruginosa* PAO1 and *P. fluorescens* BME, were obtained from and cultivated at the Institute of Medical Microbiology and Immunology (Faculty of Medicine, University of Pécs, Pécs, Hungary). The strains were cultivated at their optimal growing temperature in Mueller-Hinton broth (Difco, New Jersey, USA) at 310 K (*P. aeruginosa*) and 303 K (*P. fluorescens*) using shaken flasks with 220 rpm (BIOSAN ES-20, Biosan, Riga, Latvia). *P. fluorescens* cannot multiplicate, if the temperature is over 303 K. The reproduction curves were followed by the OD_{600} values (Spectronic, Genesys 5, Milton Roy Company, USA).

The minimum inhibitory concentration (MIC, the lowest concentration of the substance that will inhibit the visible growth of a microorganism after overnight incubation) was determined using broth dilution method (Wiegand *et al.*, 2008). For broth dilution, systems were made by applying different amounts of Cu(II) to the culture medium to the final concentration of 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 mmol/L Cu(II). Since copper(II) undergoes complexation and precipitation upon the addition to the culture medium (Kumar *et al*., 2013; Teitzel and Parsek, 2003), the residual Cu(II) concentration was determined by measuring the Cu(II) content in the supernatant using AAS analysis after the heavy metal addition. The lyophilized cells were grown in this medium, and then the MIC value was determined with solid plate cultivation and the colony formation. The control culture was grown in the absence of Cu(II) ions (Wiegand *et al.*, 2008). Mann-Whitney test was applied to compare the MIC values of *P. aeruginosa* and *P. fluorescens* cells.

2.2. Preparation of biosorbents

The cells were harvested by centrifugation (10000 rpm, 30 min) after 38-hour-incubation, rinsed twice with physiological salt solution, centrifuged again (10000 rpm, 15 min) and lyophilized at 233 K in a freeze drier (HETO, Dry Winner, Allerod, Denmark). Thermal treatment of lyophilized cells (1 g/L suspension) were made in a Digital Heatblock (VWR, Budapest, Hungary) at 373 K for 15 min.

2.3. Preparation of copper solutions

The test solutions containing Cu(II) ions were prepared from $CuCl_2$ (Fluka, Munich**,** Germany) in the concentration range of 5 − 250 mg/L.

2.4. Analysis of copper content

The Cu(II) concentration was determined by atomic absorption spectrometry (AAS, Perkin – Elmer 2380, Great Britain) of diluted solutions at 324.8 nm.

The calibration was made using dilutions of a standard 1000 mg/L Cu(II) solution (Scharlau, Barcelona, Spain) in the concentration range of 0 – 5 mg/L.

2.5. Effect of pH on biosorption

The effect of pH on the Cu(II) adsorption by *P. aeruginosa* and *P. fluorescens* biomass was studied in aqueous suspensions containing 1 g/L biosorbents. The adsorbed copper amount was determined in 25 and 50 mg/L Cu(II) solutions under the pH range of 3.0 – 6.0 at 295 K. The adsorption systems were agitated at 250 rpm in shaken flasks. After 24 hours the samples were spin-dried at 10000 rpm for 10 minutes and the Cu(II) content of the supernatant was determined with AAS after dilution.

2.6. Determination of surface charge

Lyophilized *P. aeruginosa* and *P. fluorescens* cells (1 g/L each) were suspended in 25 mL distilled water in the pH range 2–11 (the appropriate pH was obtained by adjustment with 0.1 M NaOH or 0.1 M HCl). The zeta potential of the cell surface was determined with a Zetasizer Nano-Z (Malvern Instruments, Worcestershire, UK).

2.7. Effect of biosorbent dose

The influence of the biosorbent concentration on Cu(II) adsorption was examined with aqueous suspensions (0.25 to 2 g/L) of lyophilized *P.aeruginosa* and *P. fluorescens* cells containing 50 mg/L copper(II) initial concentration. The efficiency of Cu(II) removal (in percentage) from the suspensions was calculated with Eq (1) (Nadeem et al., 2009):

$$\text{Removal}\,(\%) = \frac{(c_0 - c_e)\cdot 100}{c_0} \qquad (1)$$

where, c_0 is the initial Cu(II) concentration (mg/L), and c_e is the Cu(II) concentration at equilibrium (mg/L). The metal uptake was calculated with Eq (2) (Nadeem et al., 2009):

$$q = \frac{(c_0 - c_e)\cdot V}{m} \qquad (2)$$

where, q is the adsorbed amount of Cu(II) by 1 g adsorbent (mg/g), V is the volume of the suspension (L), and m is the mass of the biosorbent (g).

2.8. Kinetic study

Kinetics of the adsorption by *P. aeruginosa* and *P. fluorescens* in 1 g/L aqueous suspensions, containing 50 mg/L Cu(II), was evaluated by measuring the residual metal content of the samples taken at desired time intervals at 285, 290 and 295 K temperatures. The supernatants were obtained by centrifugation (10000 rpm, 5 min) and the Cu(II) content was measured using AAS analysis. Mann-Whitney test was applied to compare the results obtained for lyophilized and thermally treated cells.

2.9. Determination of biosorption isotherms

In order to determine the isotherms of the bisorption, the lyophilized and thermally inactivated cells of *P. aeruginosa* and *P. fluorescens* (1 g/L) were suspended in glass containers, and gently agitated at room temperature. The copper(II) content of the suspensions varied from 5-250 mg/L. Samples were taken from the suspensions after 24 hour incubation and the biomass was separated with centrifugation (10000 rpm, 5 min). The heavy metal content of the supernatants was measured by AAS. The experiments were made in triplicate. Mann-Whitney test was applied to compare the results obtained for lyophilized and thermally treated cells.

3. Results and discussion

3.1. Characterization of the bacteria

The optical density of bacterial cell suspensions was followed to obtain the growth curve of *P. aeruginosa* and *P. fluorescens* cultivated at their optimal cultivation temperature. The lyophilized biomass was obtained at the early stationary phase, after 38 hours incubation (Fig.1).

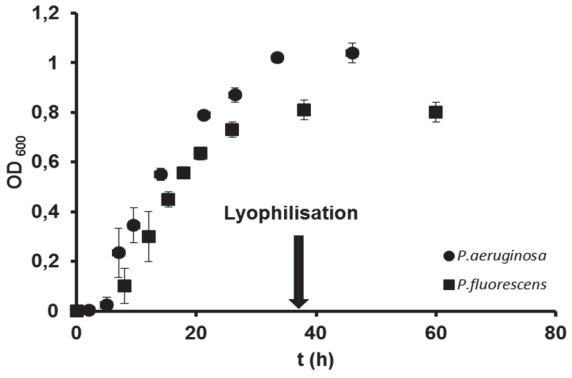

Fig. 1. Growth curves of *P. aeruginosa* (T = 310 K) and *P. fluorescens* (303 K) (bacterial samples were obtained by lyophilization at 38-hour-cultivation)

The zeta potential values of lyophilized bacterial cells in suspensions determined at pH ranged 2 and 11, showed that *P. aeruginosa* and *P. fluorescens* cells surfaces were negatively charged at pH above 2.5 and 4, respectively (Fig. 2). Above these pH values the cells were able to adsorb the positively charged Cu(II) ions.

The FT-IR spectrum of *P. aeruginosa* can be seen in Fig. 3. The IR spectrum shows the presence of carboxyl, phosphate, hydroxyl, amino groups, among them the most important is the occurrence of the carboxyl groups. Similar results have been found by other authors (Gabr et al., 2008; Sar et al., 1999). The qualitative analysis caused no visible changes in the spectrum after treatment with 100 mg/L of Cu(II) ions.

The minimum inhibitory concentration of Cu(II) (when no visible growth of the bacteria can be seen) for the *P. aeruginosa* and *P. fluorescens* cells cultivated in Mueller-Hinton broth was determined as described in the Materials and Methods. Care was taken for the determination of the Cu(II) concentration after the hydrolytic and complexation processes, which occur in the cultivation media. The MIC values were 4.15 mmol/L (260 mg/L) and 2.07 mmol/L (130 mg/L) Cu(II) for *P. aeruginosa* and *P. fluorescens,* respectively. There is a significant difference between the MIC values of the strains. These values are close to the previously reported 3.15 mmol/L (200 mg/L) for *Pseudomonas* sp.

3.2. The effect of pH on copper(II) biosorption

The affinity of cationic species towards the functional groups on the cell surfaces is strongly dependent on pH (Volesky and Holan, 1995). Fig. 4 summarizes the results of Cu(II) adsorption by *P. aeruginosa* and *P. fluorescens* bacterial cells as a function of pH having 25 or 50 mg/L Cu(II) initial concentration in the aqueous biomass suspension. The pH dependencies were similar for the two bacteria strains, and applying 50 mg/L initial Cu(II) concentration the adsorbed amount increases by pH from 25−28 mg/g biomass up to *ca.* 35 mg/g biomass (cca. 0.55 mmol/g).

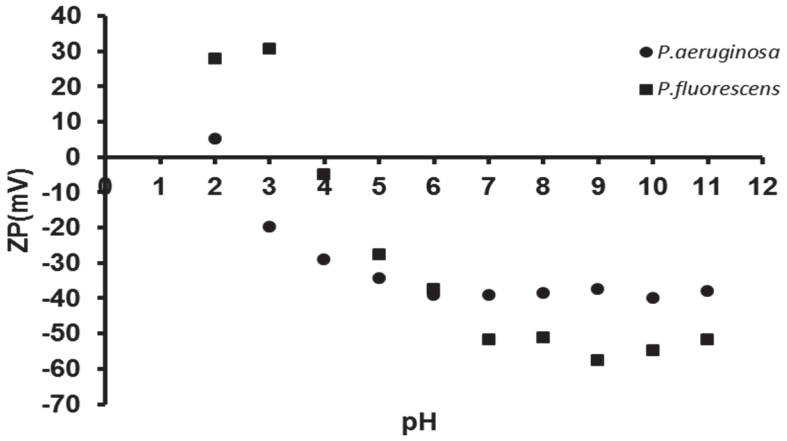

Fig. 2. Zeta potential values of *P.aeruginosa* and *P. fluorescens* bacterial cells in the pH range 2 to 11

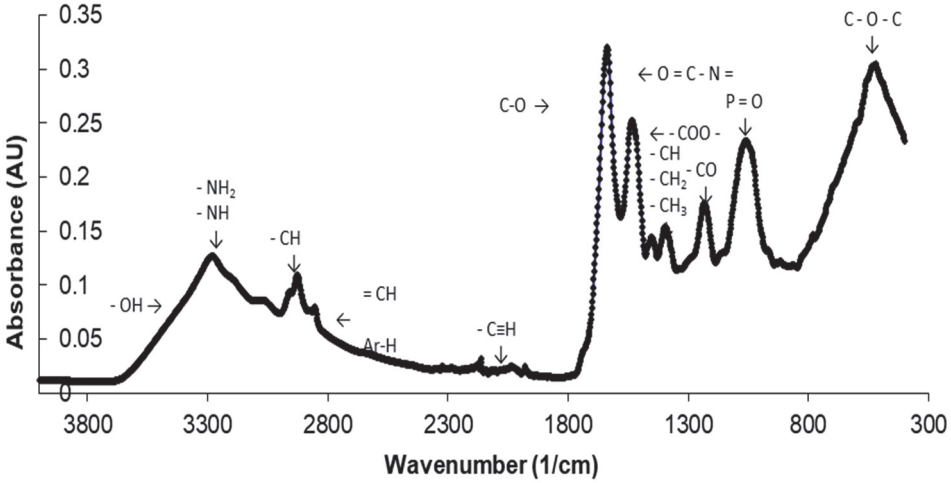

Fig. 3. FT-IR spectra of *P. aeruginosa*

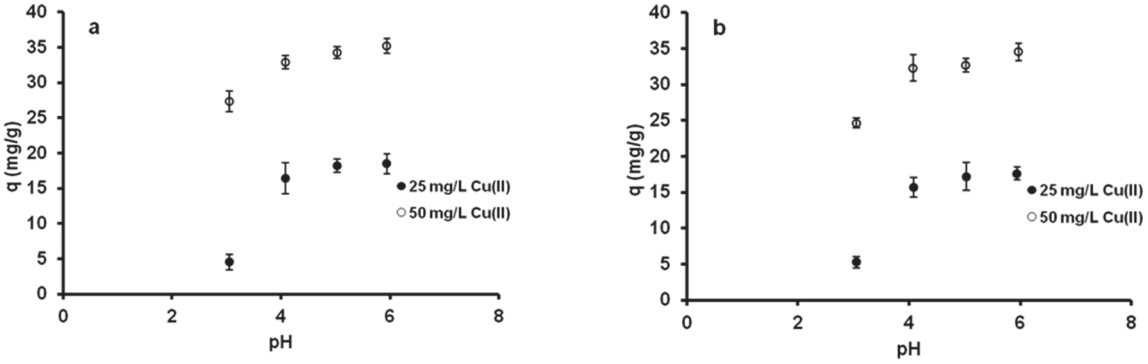

Fig. 4. Effect of pH on the adsorbed Cu(II) amount (q) on: (a) *P.aeruginosa* and (b) *P. fluorescens* biomass

The bacterial cell wall contains negatively charged functional groups such as carboxyl, phosphate, hydroxyl, imidazole and amino groups. These negatively charged groups can play role in the metal ion binding on the cell wall of Gram-negative bacteria (Fein at al., 1997; Pardo et al., 2003). The relationship between surface charge and pH indicates that the main process can be the ion exchange. Other mechanisms may also be responsible for the surface binding such as complexation, chelation or microprecipitation. Increasing pH increases the negative charge on the cell surface (Fig 2.), which favors the adsorption of the heavy metal cations. Strong acidic pH range (pH < 3) is not appropriate for adsorption due to protonation. Metal ions undergo hydrolysis as the pH increases, so high alkaline pH (pH > 6) results in metal precipitation (Chang et al., 1997, Chen et al., 2005). The effect of pH was determined in the pH range of 3.0 – 6.0. The highest Cu(II) uptake was observed at pHs 5 to 6 for both bacteria. During the process a significant decrease of solution pH can be indicated due to ion exchange mechanism. Vullo et al. (2008) reported that the optimum pH for Cu(II) biosorption by *P. veronii* cells was 5.5, while for *P. aeruginosa* PU21 the optimal pH was 5.0 (Chang et al., 1997). Silva *et al.* (2009) found that the optimal pH value for Cu(II) biosorption was 6.25 using *P. aeruginosa* AT18. According to Pardo et al. (2003) the optimal pH value was 5.0 − 6.0 for Cu(II) and more than 80% of metal ions could be removed under this condition.

3.3. Effect of contact time and temperature

The time-course profiles measured at 285, 290 and 295 K for the adsorption of Cu(II) by lyophilized bacterial cells of *P. aeruginosa* and *P. fluorescens* are shown in Fig. 5. The adsorbed Cu(II) amount per 1 g biomass is presented in the function of contact time. The major uptake of heavy metal occured within the first 10 minutes, and further significant increase in the adsorbed Cu(II) content was not observed. The Cu(II) adsorption capacities by the lyophilized cells of *P. aeruginosa* and *P. fluorescens* were 41 mg/g (82%) and 38 mg/g (76%), respectively, at initial 50 mg/L copper concentration at 295 K. There was no considerable difference between the adsorbed Cu(II) amounts neither by these *Pseudomonas* strains nor at various temperatures.

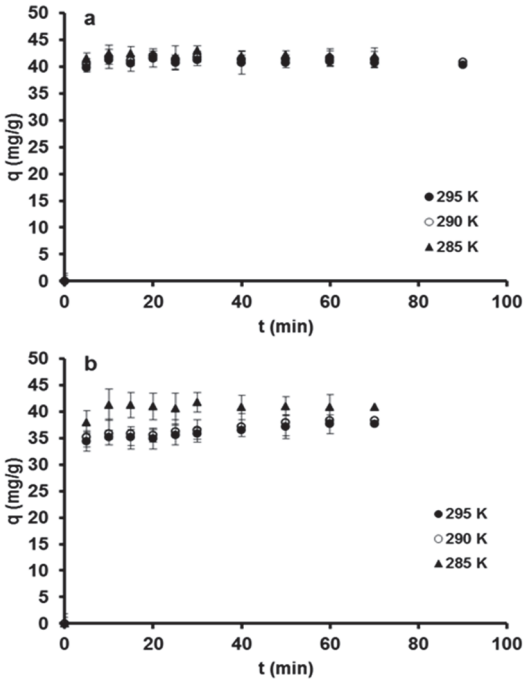

Fig. 5. Biosorption of Cu(II) by lyophilized cells of (a) *P. aeruginosa* and (b) *P. fluorescens* as a function of time at 285, 290 and 295 K temperatures

The actual adsorption of solute (for example copper ions) on the active sites of the biomass is generally considered very rapid (Veglio and Beolchini, 1997). The cells can accumulate ions by their surface and intracellular binding sites. A rapid adsorption feature is in agreement with the results of Chang et al. (1997). The metal concentration decreased rapidly during the first 30 min and remained nearly constant after 2 h of adsorption, suggesting that the biosorption was fast and reached saturation within 2 h (Chang et al., 1997). For the effect of temperature on Cu(II) biosorption processes by these strains there is no available information in the literature. The kinetics of hexavalent chromium ions adsorption by treated sawdust was examined at different

temperatures by Baral et al. (2006). The rate constant has been calculated at 303, 308, 313 and 318 K and the activation energy (E_a) was calculated using the Arrhenius equation. The ΔG^o and ΔH_f values for Cr(VI) adsorption on the sawdust showed the process to be exothermic in nature. The decrease in percentage of adsorption with rise in temperature may be due to desorption caused by an increase in the available thermal energy. Higher temperature induces higher mobility of the adsorbed ions causing desorption.

3.4. Effect of biosorbent dose

The determination of optimal biosorbent concentration is an important part in performing efficient adsorption processes. The metal removal in percentage (%) against the biosorbent doses are plotted in Fig. 6. The copper removal by 2 g/L *P. aeruginosa* biomass was 96.2%, which was the result with a metal uptake capacity of 24.1 mg/g, while applying 1 g/L of biomass the copper removal was 78.2%, but the metal uptake capacity was 40 mg/g. Similar results were obtained for *P. fluorescens*, where the 2 g/L biomass resulted in 93.6% copper removal with the metal uptake capacity of 23.4 mg/g, while 1 g/L biosorbent dosage resulted in 71.6% copper removal with the 35.8 mg/g metal uptake capacity. These results showed that a lower biomass concentration can be used for efficient copper removal. For further studies 1 g/L biomass concentration was selected as an optimal biosorbent dosage for both microorganisms.

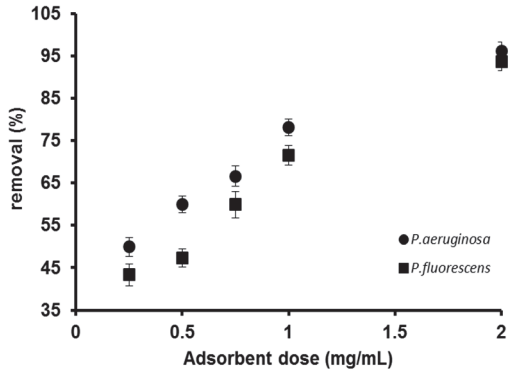

Fig. 6. Effect of the biosorbent dose on the Cu(II) uptake by *P. aeruginosa* and *P. fluorescens* biomass.

3.5. Kinetic modeling

With the help of the kinetic models the most suitable conditions of metal uptake can be selected for the removal process. Several kinetic models can be applied for evaluation of heavy metal adsorption (Aksu, 2005, Febrianto et al., 2009, Pernyeszi et al., 2009, Rao and Viraraghavan, 2002, Yu et al., 2011). Pseudo second-order model was used to fit the experimental data determined by lyophilized bacterial cells at 285, 290 and 295°K. The pseudo first-order model could not be used for modeling the biosorption kinetics.

The pseudo second-order kinetic rate equation can be written by Eq (3):

$$\frac{dq}{dt} = k_{2,ad}\,(q_{eq} - q)^2 \qquad (3)$$

The integrated linear form of pseudo second-order equation is given in Eq (4):

$$\frac{t}{q} = \frac{1}{k_{2,ad}\,q_{eq}^{\ 2}} + \frac{t}{q_{eq}} \qquad (4)$$

where: q is the mass of the adsorbed metal at time t (min), $k_{2,ad}$ is the rate constant of second order biosorption (g/mg min), q_{eq} is the mass of adsorbed metal at equilibrium (mg/g).

By plotting t/q against t the second-order rate constants, $k_{2,ad}$ and the theoretical adsorption capacities q_{eq}, can be calculated from the slope and intercept of the plots (Fig. 7). Table 1 shows that the rate constant $k_{2,ad}$ varied in the case of *P. aeruginosa* within the range of 0.19 to 0.5 g/mg·min and in the case of *P. fluorescens* within the range of 0.019 to 0.75 g/mg·min. The calculated adsorption capacities based on second-order equation are in good correspondence to the experimental data. An increase in the adsorbed amount of Cu(II) ions can be observed with increasing temperature from 285 K to 295 K, but this increase was not significant according to the results from Mann-Whitney test. So, the temperature has only a minor effect on the biosorption rate in this range. The correlation coefficients for the second-order kinetic model were close to 1.0 for all cases. This suggests that the sorption of heavy metal by the bacterial biomass follows the second-order kinetics. It is based on the sorption capacity of the solid phase and shows that the chemisorption mechanism can be a rate-limiting step (McKay et al., 1999).

Table 1. The pseudo second-order rate constants ($k_{2,ad}$) with the calculated ($q_{eq,cal}$) and experimental ($q_{eq,exp}$) equilibrium adsorption capacities by *P. aeruginosa* and *P. fluorescens* biomass at different temperatures

	$k_{2,ad}$ g/mg·min	$q_{eq,cal}$ mg/g	R^2	$q_{eq,exp}$ mg/g
P. aeruginosa				
Cu(II) 295 K	0.50	40.8	0.99	41.5
290 K	0.49	41.3	0.99	42.1
285 K	0.19	41.8	0.99	42.9
P.fluorescens				
Cu (II) 295 K	0.02	38.2	0.99	37.9
290 K	0.02	38.9	0.99	38.6
285 K	0.75	40.8	0.99	41.7

Generally, the metal biosorption follows pseudo second-order kinetic model (Joo et al., 2010). For Cu(II) adsorption process by *P. aeruginosa* Kong et al. (2009) carried out kinetic and equilibrium studies using wave anodic stripping voltammetry method. The parameters were obtained by fitting the

electrochemical experimental data to the pseudo second-order kinetic model.

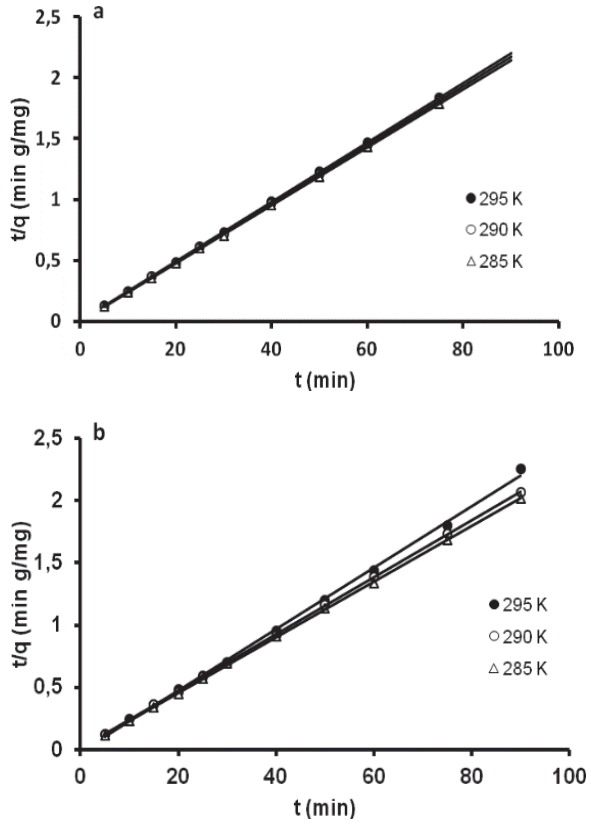

Fig. 7. The best fit of the pseudo-second order kinetic equation (Eq. (4)) for Cu(II) adsorption by lyophilized cells of (a) *P. aeruginosa* and (b) *P. fluorescens* at temperatures 285, 290 and 295 K

3.6. Biosorption isotherms

Equilibrium measurements were performed by applying lyophilized and thermally inactivated bacterial cells of *P. aeruginosa* and *P. fluorescens*, with initial Cu(II) concentration of 5 – 250 mg/L at pH 5.4. Biosorption isotherm, the plot of uptake (q) versus equilibrium concentration of the solute in heavy metal solution (c_e), is often used to evaluate the biosorption. In the literature, the Langmuir and Freundlich models have been used with a high rate of success for the description of biosorption processes. The equilibrium biosorption isotherms, determined for both bacterial cells using batch technique, showed that metal uptake by bacterial biomass was a chemically equilibrated and saturable mechanism (Fig. 8). An increase in the metal uptake was observed to the free binding sites. Preferential adsorption mechanism cannot be observed. Experimental data were applied to the adsorption by both, the Langmuir and Freundlich models, and the adsorption constants were estimated using non-linear least-squares mathematical method.

3.6.1. Langmuir isotherm

The Langmuir isotherm is valid for monolayer adsorption onto a surface with a finite number of identical sites. It is given as Eq (5):

$$q_e = \frac{q_{\max} b c_e}{1 + b c_e} \tag{5}$$

where: b is the equilibrium constant including the affinity of binding sites (L/mg), c_e is the concentration of metal ions in solution and q_e is the amount of adsorbed metal ions on the biosorbent at equilibrium. The q_{max} is the maximum amount of metal ion per unit mass of adsorbent, which forms a complex monolayer on the surface (mg/g) (Gabr et al., 2008).

3.6.2. Freundlich isotherm

The Freundlich equation based on sorption on a heterogeneous surface is given below as Eq (6):

$$q_e = k_F c_e^{1/n} \tag{6}$$

where: k_F and n are the Freundlich constants, whereas, k_F and n are the indicators of adsorption capacity and adsorption intensity of the sorbents, respectively, c_e is the concentration of metal ions in solution and q_e is the amount of adsorbed metal ions on the biosorbent at equilibrium. The Freundlich isotherm provides no information on the monolayer adsorption capacity in comparison to the Langmuir model (Joo et al., 2010).

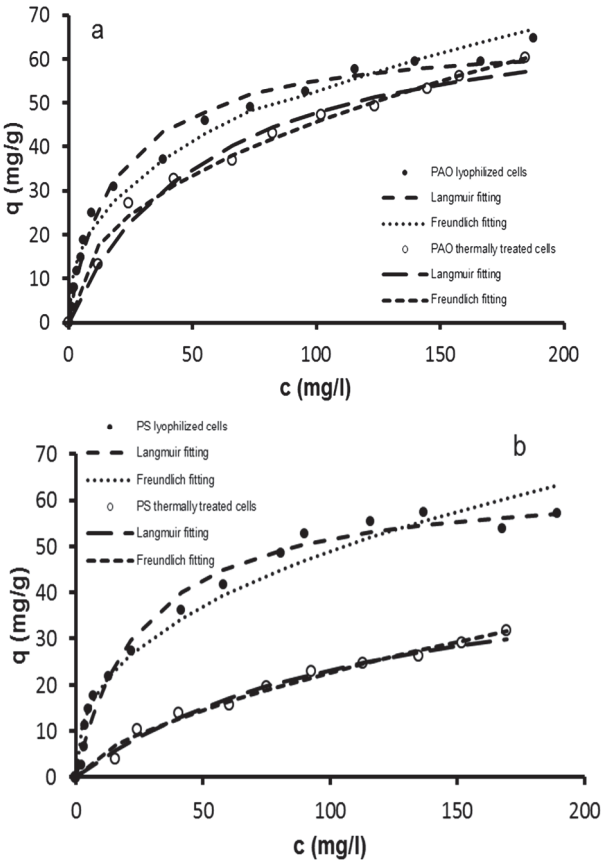

Fig. 8. Bioadsorption isotherms of lyophilized and thermally inactivated cells of (a) *P. aeruginosa* (PAO) and (b) *P.fluorescens* (PS) for Cu(II) ions in the initial Cu(II) concentration range of 5 – 250 mg/L (biomass concentration: 1 g/L, pH = 5.4, temperature: 285 K)

Chang et al. (1997) showed by using Langmuir isotherm to describe the adsorption, that the resting cells of *P. aeruginosa* PU21 were able to adsorb 23 mg Cu(II)/g at pH 5.5, and the inactivated cells adsorb 19 mg Cd(II)/g dry cell. According to Pardo et al. (2003) only the Langmuir model showed a good fit to the experimental data using the linearized isotherm equation. The highest value of adsorbed amount was found to be 6.6 mg/g at pH 6.0 and 6.3 mg/g at pH 4.5.

The non-linear fitted curves of Langmuir and Freundlich adsorption isotherms for *P. aeruginosa* and *P. fluorescens* biomasses are shown in Fig. 8. The calculated values of constants from Langmuir isotherm: q_{max} and b are summarized in Table 2, which give a good estimation of the experimental data. The experimental q_{max} value for lyophilized *P. aeruginosa* was 60.3 mg/g, while it was 56.0 mg/g for *P. fluorescens*. The thermally inactivated cells have slightly lower adsorption capacity in the case of *P. aeruginosa* (56.5 mg/g), but significantly lower capacity in the case of *P. fluorescens* (29 mg/g). The equilibrium constants (b) of the lyophilized and thermally inactivated cells of both microorganisms were obtained to be 0.06 and 0.02 L/mg for the *P. aeruginosa* and 0.04 and 0.008 L/mg for the *P. fluorescens* bacteria, respectively.

Table 2. The Freundlich and Langmuir isotherm constants of Cu(II) biosorption by lyophilized and thermally treated *P. aeruginosa* and *P. fluorescens* cells at 285 K

	Freundlich isotherm model			***Langmuir isotherm model***		
	k_F ***[(mg/g) (mg/L)^n]***	***n (L/mg)***	R^2	q_{max} ***(mg/g)***	***b (L/mg)***	R^2
P. aeruginosa						
lyophilized	8.7	2.50	0.98	62.5	0.06	0.98
thermally treated	5.8	2.23	0.98	74.1	0.02	0.97
P. fluorescens						
lyophilized	7.7	2.50	0.96	64.8	0.04	0.98
thermally treated	1.2	1.57	0.98	51.0	0.01	0.98

The free enthalpy change can be calculated based on the Langmuir equilibrium constant. For lyophilized *P. aeruginosa* ΔG = -20.3 kJ/mol; for its thermally treated cells ΔG = -17.6 kJ/mol; for lyophilized *P. fluorescens* ΔG = -19.3 kJ/mol; for its thermally treated cells ΔG = -15.4 kJ/mol. The values show that in each case the free enthalpy change is negative, which indicates that the processes are spontaneous. The estimated values of Freundlich isotherm: k_F and n are also given in Table 2 along with the regression correlation coefficients, showing that not only the Langmuir but also the Freundlich model can describe the experimental data.

The parameter k_F related to the sorption capacity was 2.5 (mg/g)(mg/L)^n for Cu(II) biosorption in both cases by lyophilized cells, 2.23 and 1.57 (mg/g)(mg/L)^n for thermally inactivated cells for *P. aeruginosa* and *P. fluorescens*. Table 2 shows that n was greater than unity for Cu(II) biosorption in all cases, indicating that heavy metal ions are favorably adsorbed by the bacterial cells. The regression correlation coefficients of both, the Langmuir and Freundlich models showed a good fit (R^2=0.96–0.98) for Cu(II) biosorption.

The difference between the adsorbed amounts by the lyophilized and thermally inactivated biomass of *P. fluorescens,* and also by *P. aeruginosa* were statistically significant. The thermally inactivated cells by *P. fluorescens* can only adsorb half amount of one by the lyophilized cells. The temperature optimum of this strain was 303 K (30ºC), and it does not replicate at higher temperature, thus probably more sensitive to changes in temperature. On the basis of the experimental data one can assume that the heat treatment destroyed the surface structure responsible for adsorption.

The MIC values by *P. aeruginosa* were higher than the applied concentration range. In case of *P. fluorescens* the applied Cu(II) concentrations are higher than the MIC values (130 mg/L). The use of these concentrations did not affect the adsorbed amounts.

4. Conclusions

The potential of lyophilized and thermally treated bacterial cells of *Pseudomonas aeruginosa* PAO1 and *Pseudomonas fluorescens* BME to adsorb copper(II) ions from aqueous solution was demonstrated. The highest copper(II) uptake was observed at pHs 5 to 6 for both bacteria and a biomass concentration of 1 g/L could be used for efficient copper(II) removal.

The rapid copper(II) ion biosorption followed pseudo second-order kinetics. A slightly temperature effect on biosorption can be observed in the studied temperature range. The Langmuir and Freundlich models exhibited a good fit to the experimental data using non-linear fitting. The thermal treatment did not influence the biosorption properties of the *Pseudomonas aeruginosa* cells, while the adsorption capacity of *P. fluorescens* significantly decreased. Both, the laboratory and the environmental strains, are promising biosorbents for metal ion removal. The biosorption capacity of the laboratory strain is higher than its environmental strain. As a consequence, there is no need to select the environmental strain in practical application.

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