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Bioadsorption of lead(II) and cadmium(II) ions onto the lyophilized cell surface of *Pseudomonas aeruginosa* in aqueous suspension

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Abstract. Biosorption of Cd(II) and Pb(II) ions from aqueous solution using lyophilized *Pseudomonas aeruginosa* (PAOI) cells was studied under various experimental conditions. The effect of pH, initial metal concentration and adsorption time on bioadsorption was investigated. The optimum pH value range for Cd(II) adsorption was found to be 4.0-7.0, and for Pb(II) it was 4.0-5.0. Pb(II) and Cd(II) bioadsorption equilibrium was analyzed by using Langmuir model. The maximum uptake capacity of Pb(II) and Cd(II) was estimated to be 164 mg g^{-1} and 132 mg g^{-1}, respectively.

Keywords: heavy metals, biosorption, *Pseudomonas aeruginosa*, pH, kinetics, isothermh

1 Introduction

Heavy metal pollution is one of the most important environmental problems today. In recent years, applying biotechnology in controlling and removing metal pollution has been paid much attention, and gradually becomes hot topic in the field of metal pollution control because of its potential application. Alternative process is biosorption, which utilizes various certain natural materials of biological origin, including bacteria, fungi, yeast and algae. These biosorbents possess metal-sequestering property and can be used to decrease the concentration of heavy metal ions out of dilute complex solutions with high efficiency and quickly, therefore it is an ideal candidate for the treatment of high volume and low concentration complex wastewaters. Therefore researches on biosorption have become an active field for the removal of metal ions or organic compounds [1, 2].

The capability of some living microorganisms to accumulate metallic elements have been observed at first from toxicology point of view. However, inactive/dead microbial biomass can also passively bind metal ions via various physicochemical mechanisms. Mechanisms responsible for biosorption, although understood to a limited extent, may be one or combination of ion exchange, complexation, coordination, adsorption, electrostatic interaction, chelation and microprecipitation [1, 3]. The uptake of heavy metals by biomass is usually classified into three categories: (1) cell surface binding, (2) intracellular accumulation and (3) extracellular accumulation [1, 4]. Being metabolism-independent, the cell surface binding can occur in either living or inactivated microorganisms, whereas the intracellular and extracellular accumulation of metals are usually energy-driven processes, and thus can take place in living cells [5].

Several investigations have reported that *Pseudomonas aeruginosa* displays efficiency for metal uptake [6, 7, 8]. Chang and Chen studied the biosorption of cooper(II), lead(II) and cadmium(II) ions on *P. aeruginosa* and the multi-metal adsorption results showed that lead and cooper significantly inhibited the adsorption of cadmium, while the effects of cadmium on the adsorption of cooper and lead were limited [9]. They also reported the combined effects of two or more metal ions on inactivated *P. aeruginosa* depend on the number of the metals competing for binding sites, metal combination, levels of metal ion concentration, order of metal addition, and residence time [9], [10]. Leung et al. selected *Pseudomonas* as biosorbent for lead, cooper and nickel, among 12 bacteria isolated from activated sludge. They reported the maximum sorption capacity 271.7 and 46.8 mg g^{-1} for lead(II) and copper(II) ions, respectively

[9, 10]. Kang et al. the Cr(III) and Cr(VI) biosorption studied onto the cell surface of *P. aeruginosa*. Batch experiments were conducted with various initial concentrations of chromium ions to obtain the sorption capacity and isotherm. It was found that the sorption isotherm of *P. aeruginosa* for Cr(III) was described well by Langmuir isotherm model, while Cr(VI) appeared to fit Freundlich model. The results of FT-IR analysis suggested that the chromium binding sites on the bacterial cell surface were most likely carboxyl and amine groups. The bacterial surface of *P. aeruginosa* seemed to engage in reductive and adsorptive reactions with respect to Cr(VI) biosorption [11].

In this study the biosorption characteristics of lyophilized *Pseudomonas aeruginosa* bacterial cells for cadmium(II) and lead(II) ions in aqueous suspension are being presented. The effect of pH, contact time and initial heavy-metal concentration on biosorption were investigated for both heavy-metal ions. Bioadsorption isotherms were determined for cadmium(II) and lead(II) ions in batch systems in the initial concentration range of 5-250 mg l^{-1}.

2 Materials and Methods

Bacterial strain and cultivation

The microorganism used in this study was *Pseudomonas aeruginosa* (PAO1). The strain was cultivated in Mueller-Hinton broth (Difco) using shaken flasks. They were incubated at 37 °C and the liquid cultures were agitated at 220 rpm. The reproduction curve was performed by using OD_{600} values by spectrophotometry (Fig. 1., Spectronic, Genesys 5, Milton Roy Company, USA).

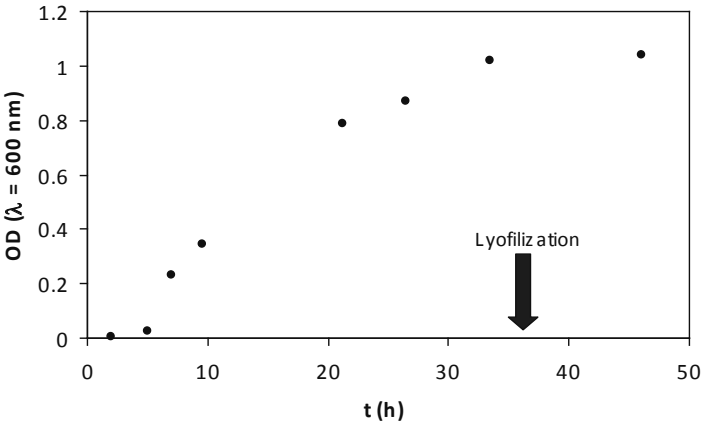


Figure 1: Time-course profiles of growth by *Pseudomonas aeruginosa* PAO1 cells.

In Fig. 1 the optical densities of bacterial cell suspension were presented against incubation time. The minimum inhibitory concentration (MIC) was determined with half dilution method by using 96 mmol l^{-1} initial heavy-metal concentration. The concentrations of heavy-metals were 96.0, 48.0, 24.0, 12.0, 6.0, 3.0 and 1.5 mmol l^{-1}. The MIC values were determined after solid plate cultivation and the cell number was compared with the control culture.

Preparation of biosorbents

Cells were harvested by centrifugation (10000 rpm, 30 min) at early-stationary phase of growth, after 38 h incubation. Cells were twice rinsed with physiological salt solution, then freeze-dried (lyophilization) after centrifugation.

Preparation of heavy-metal solution

The heavy-metal test solutions containing Pb(II) and Cd(II) ions were prepared from PbNO3 (Fluka, Germany) and $CdNO_3 \cdot H_2O$ (Fluka, Germany) in the concentration range of 5-500 mg l^{-1}. The pH values of the adsorption systems were adjusted by using 0.1 M NaOH and 0.1 M HCl solutions.

Analysis of heavy-metals

The concentration of heavy-metals in solutions was measured by Atomic Absorption Spectrometer (Perkin – Elmer 2380) at 217 nm for Pb(II) and 228 nm for Cd(II). Before measurement the heavy-metal solutions were diluted with deionized water to ensure that the heavy-metal concentration in the sample was linearly dependent on the absorbance detected. The calibration of cadmium(II) and Pb(II) was made with standard cadmium and lead solution (Scharlau) in the concentration range of 0-2.5 mg l^{-1} for Cd(II and 0-10 mg l^{-1} for Pb(II).

Study of pH effect on biosorption

The effect of pH on Cd(II) and Pb(II) adsorption was investigated by *Pseudomonas aeruginosa* biomass in aqueous suspension. To determine the optimum pH range of bioadsorption, adsorption measurements with 25 and 50 mg l^{-1} solutions were performed for both Cd(II) and Pb(II) ions in the pH range of 3.0-8.0. The suspension concentration was 1 g l^{-1}. The initial pH of the suspension was 5.6 and the expected pH was regulated with 0.1 M NaOH and 0.1 M HCl solutions, then the adsorption systems were agitated at 250 rpm. After 24 hours samples were taken from the adsorption systems and were spin-dried

at 10000 rpm till 10 minutes and diluted for analysis by atomic absorption spectrophotometry.

Kinetics study of biosorption

In the Cd(II) and Pb(II) biosorption kinetics study by *Pseudomonas aeruginosa* the concentration of Cd(II) and Pb(II) ions were 50 mg l^{-1} at suspension concentration of 1 g l^{-1}. Samples were taken from the solutions at desired intervals and the metal concentrations of the supernatants were measured after centrifugation. The supernatants were spin-dried at 5500 rpm till 10 minutes and diluted for analysis by atomic absorption spectrophotometry.

Determination of bioadsorption isotherms

The biomasses (1g l^{-1}) were suspended in heavy-metal solutions in the glass containers, which were gently agitated at room temperature. For the determination of adsorption isotherms for Pb(II) and Cd(II) solutions in the concentration range of 5-250 mg l^{-1} were used. After 24 hours incubation, samples were taken from the suspensions, and the biomass was separated from the heavy-metal solution at 10 000 rpm for 5 min, and then the heavy-metal content of the supernatant was measured by AAS. The metal uptake was calculated as follows:

$$q = \frac{(c_0 - c_e) \cdot V}{m} \tag{1}$$

q is the adsorbed amount of heavy-metals (mg g^{-1}),
c_0 is the initial heavy-metal concentration (mg l^{-1}),
c_e is the heavy-.metal concentration in the adsorption equilibrium (mg l^{-1}),
V is the volume of the solution (l), and
m is the mass of biosorbent (g).
All experiments were performed in duplicates.

3 Results and discussions

Determination of minimum inhibitory concentration for cadmium(II) and lead(II) ions

Free cells of *P. aeruginosa* were susceptible to the heavy-metals tested. The measured value of MICs for cadmium(II) was 20 mmol l^{-1} and for lead(II) was 12 mmol l^{-1} , respectively. These values were close to previously reported for *P. aeruginosa* (15 mmol l^{-1} for Pb(II)) [12]. The growth medium having

complex rich composition (Mueller-Hinton broth) was used, which resulted in a high level of complexation between the metal cations and components of the growth medium. The residual concentrations of supernatant in the heavy-metal stock solutions were determined after centrifugation. Due to precipitation the MIC value was found to be 6 mmol l^{-1} for cadmium(II) and 1.8 mmol l^{-1} for lead(II).

pH effect on cadmium(II) and lead(II) bioadsorption

It has been shown that the affinity of cationic species towards the functional groups present in the cellular surface is strongly dependent on the pH [13]. Fig. 2a and 2b summarize the results of the adsorption of Cd(II) and Pb(II) ions by *Pseudomonas aeruginosa* PAO1 bacterial cells as a function of pH at initial concentrations of 25 and 50 mg l^{-1}.

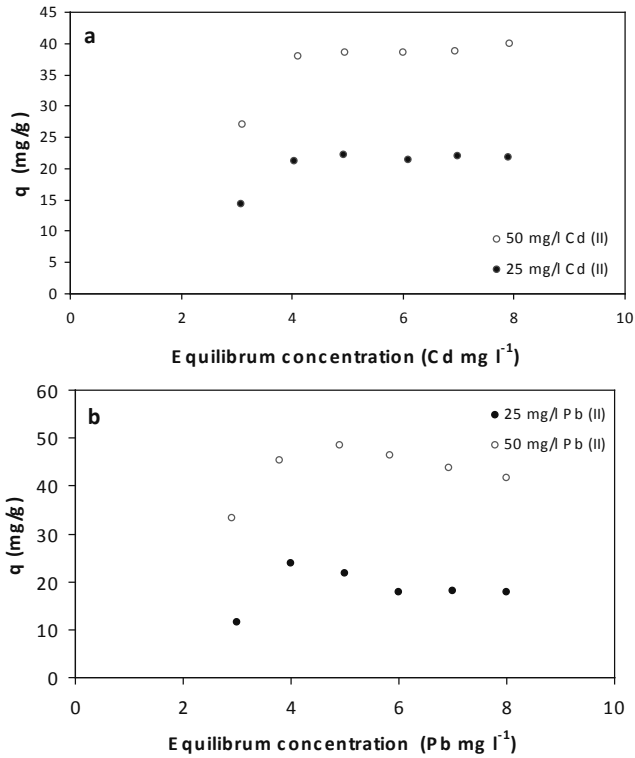
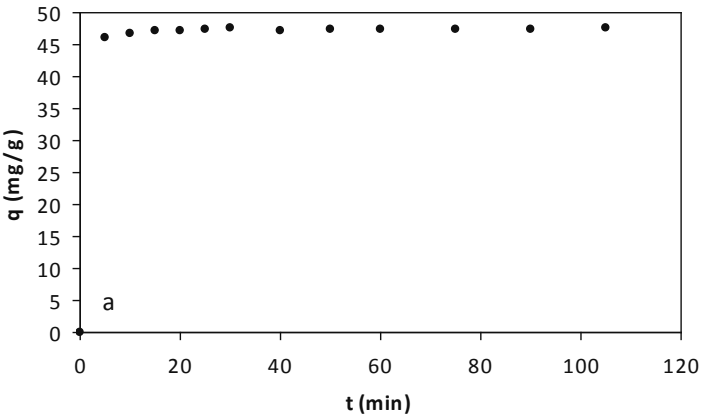


Figure 2: Effect of pH on (a) Cd(II) and (b) Pb(II) biosorption by *Pseudomonas aeruginosa* PAO1 bacterial biomass. Initial concentrations: 25 and 50 mg l^{-1}, contact time: 24 h, biomass concentration: 1 g l^{-1}, temperature: 22.5°C.

In both cases, metal uptake by the biomass increases with increasing pH till it reaches a maximum after which the metal uptake decreases. The bacterial cell wall contains negatively charged functional groups such as carboxyl, phosphate, imidazole and amino groups. They are primarily responsible for the anionic character and metal binding capacity of the cell wall by Gram-negative bacteria [14]. Increasing pH increases the negative charge on the cell surface, which favors the adsorption of the heavy-metal cations. In addition, metal ions undergo hydrolysis as the pH inceases, so strong acidic pH range (pH $<$ 3) is not appropriate for adsorption. High alkaline pH (pH $>$ 8) results metal precipitation. So the effect of pH was determined in the pH range of 3.0-8.0. Optimum pH values were found to be at 4.0-7.0 for cadmium(II) and 4.0-5.0 for lead(II) biosorption. Other investigators like Chang et al. reported that the maximum pH by inactivated and resting cells of *P. aeruginosa* PU21 was 5.5 for lead(II) and 6.0 for cadmium(II) [5, 15]. For *P. pudita* it was 6.0 [16].

Time-course of biosorption

The time-course profiles for the adsorption of Pb(II) and Cd(II) ions by freeze-dried bacterial cells are shown in Fig. 3a-b. and 4a-b. Fig. 3a and 4a represent the adsorbed amounts of Cd(II) and Pb(II) by the biomass, respectively, in the function of contact time. In Fig. 3b and 4b the adsorption efficiencies by the biomass for Cd(II) and for Pb(II) are presented against contact time. The metal concentration decreased rapidly during the first 10 minutes and remined nearly constant after 20 minutes of adsorption, suggesting that the biosorption was very fast and reached saturation within 20 minutes.



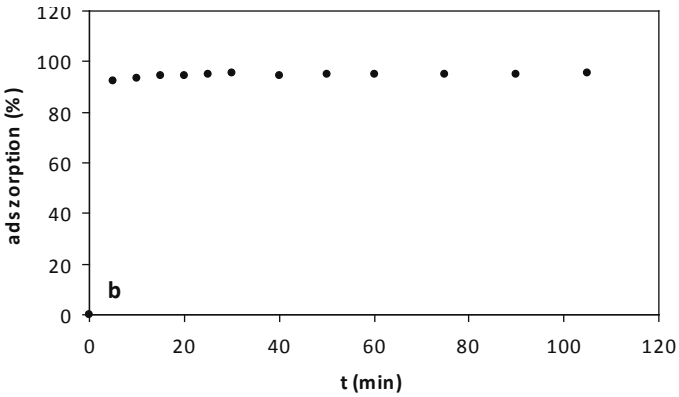


Figure 3: Biosorption of Cd(II) by lyophilized cells of *Pseudomonas aeruginosa* PAO1 (pH = 5.6) as a function of time. Biomass concentration: 1 g l^{-1}, initial concentration 50 mg l^{-1}, temperature: 22.5°C.

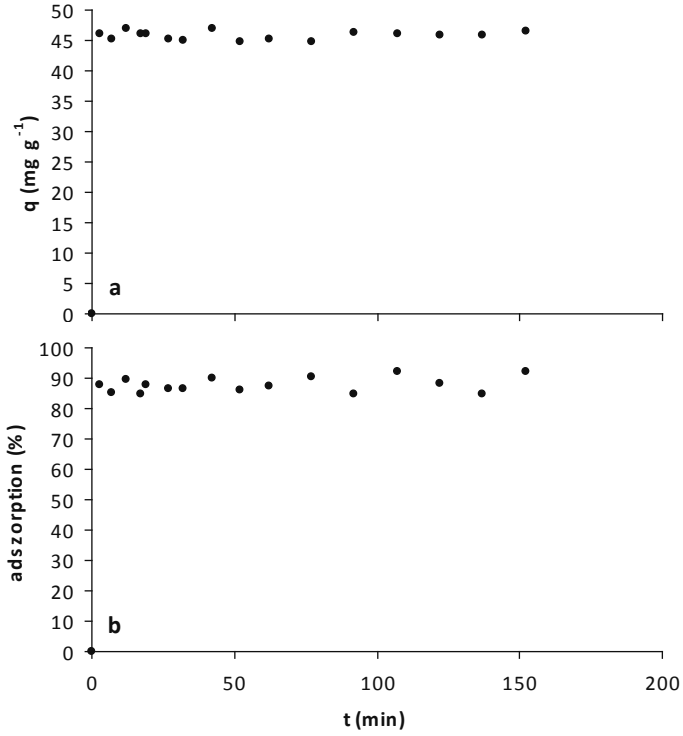


Figure 4: Biosorption of Pb(II) by lyophilized cells of *Pseudomonas aeruginosa* PAO1 (pH = 5.6) as a function of time. Biomass concentration: 1 g l^{-1}, initial concentration 50 mg l^{-1}, temperature: 22.5°C.

This finding is in agreement with earlier studies [5,15]. The cells can accumulate metal ions by their surface and intracellular binding sites. The metal adsorption capacity of lyophilized cells for Cd(II) was 47.6 mg g^{-1} (95 %) and for Pb(II) it was 46.6 mg g^{-1} (92 %), which values do not show significant difference at initial 50 mg l^{-1} metal concentration. Further examination of bioadsorption equilibrium is needed.

Bioadsorption isotherms

Cadmium(II) and lead(II) sorption performance on lyophilized bacterial cells of *P. aeruginosa* PAO1 was achieved by the biosorption equilibrium measurements at initial concentration of 5-250 mg l^{-1} for both metals at pH 5.6. Biomass concentration was 1 g l^{-1}. The equilibrium bioadsorption isotherms determined for both heavy-metals using batch technique shows that metal uptake by bacterial biomass was a chemically equilibrated and saturable mechanism (Fig. 5.) Thus, there was an increase in metal uptake as long as binding sites were free. Preferential adsorption mechanism can be observed for Pb(II) adsorption in comparison with Cd(II) adsorption process. Experimental data were applied to adsorption model given by Langmuir, where its mathematical formulas can be expressed as:

$$q_e = \frac{q_{max} b c_e}{1 + b c_e} \tag{2}$$

and its linear form is represented by the following equation:

$$\frac{c_e}{q} = \frac{1}{q_{max} \cdot b} + \frac{c_e}{q_{\min}} \tag{3}$$

where b is the adsorption equilibrium constant including the affinity of binding sites (l mg^{-1}), ce and qe are unadsorbed metal ions in solution and adsorbed metal ions on the biosorbent at equilibrium, respectively, qmax is the maximum amount of metal ion per unit weight of adsorbent to form a complex monolayer on the surface (mg g^{-1}) [1, 2, 5, 13, 15, 17].

The calculated qmax Langmuir parameter gave a correlation with the experimental value. The calculated qmax value obtained for Pb(II) was 163.9 mg g^{-1}, it was higher than that for Cd(II): 131.6 mg g^{-1}. The experimental qmax value for Pb(II) was 155 mg g^{-1}, while it was 117 mg g^{-1} for Cd(II). The values of equilibrium constant b for Pb(II) and Cd(II) were calculated to be 0.508 l mg^{-1} and 0.054 l mg^{-1}, respectively, which indicates that, lyophilized cells of *P. aeruginosa* possesses a higher adsorption affinity for Pb(II) ions as

compared to that for Cd(II) ions. Such correlation led us to conclude that the energy of adsorption is more favorable for lead (II) ions that for Cd(II) ions.

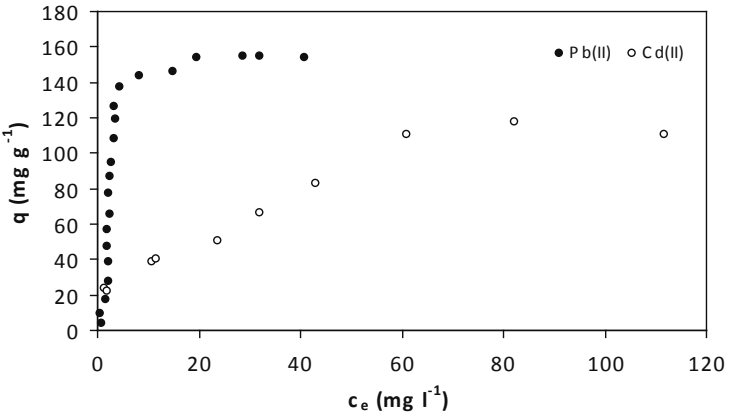


Figure 5: Bioadsorption isotherms of freeze-dried *Pseudomonas aeruginosa* PAO1 bacterial cells.for Cd(II) and Pb(II) ions in the initial heavy-metal concentration of 5-250 mg l^{-1} . Biomass concentration: 1 g l^{-1}, pH = 5.6, temperature: 22.5°C.

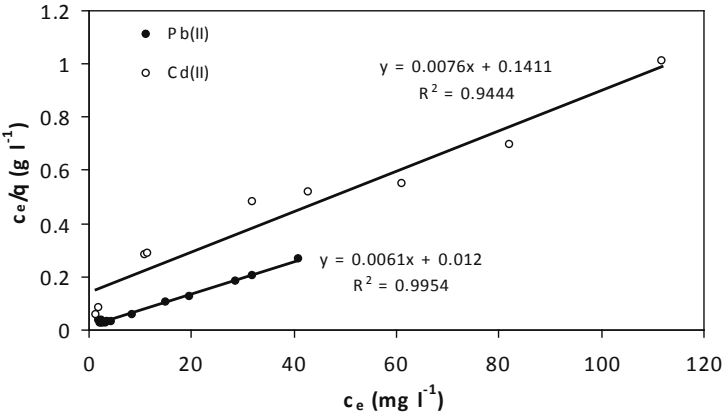


Figure 6: Bioadsorption isotherms of freeze-dried *Pseudomonas aeruginosa* PAO1 bacterial cells.for Cd(II) and Pb(II) ions in the initial heavy-metal concentration of 5-250 mg l^{-1} . Biomass concentration: 1 g l^{-1}, pH = 5.6, temperature: 22.5°C.

4 Conclusion

Lyophilized bacterial cells of *Pseudomonas aeruginosa* PAO1 was able to adsorb cadmium(II) and lead(II) ions with considerably high capacities. As the pH increased, the metal adsorption capacity increased significantly. However, with the restriction of forming insoluble metal hydroxides at high pH values, the optimal operating pH in this study was 4.0-7.0 for biosorption of Cd(II) and 4.0-5.0 for biosorption of Pb(II), respectively. At low initial concentrations of Cd(II) and Pb(II) ions there was no significant difference in adsorption capacities. The biosorption was very fast and reached saturation within 20 minutes. Adsorption data were well described by the Langmuir model. The maximum uptake capacity of Pb(II) and Cd(II) ions was estimated to be 164 mg g^{-1} and 132 mg g^{-1}, respectively. Biomass of *Pseudomonas aeruginosa* PAO1 appears to have the possibility to be an effective adsorbent for the removal of heavy-metals from polluted wastewaters.

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References

[1] Wang, J. L., Chen, C. (2006), Biosorption of heavy metals by *Saccharomyces cerevisiae*: a review. *Biotechnol. Adv.* 24, pp. 427-451.

[2] Wang, J. L., Chen, C. (2009), Biosorbents for heavy metals removal and their future. *Biotechnol. Adv.* 27, pp. 195-226.

[3] Vjayaraghavan, K.,Yun, Y. S. (2008), Bacterial biosorbents and biosorption. 26, pp. 266-291.

[4] Gadd, G. M., (1988), Accumulation of metals by microorganisms and algae. *Biotechnology*, 6b, pp. 401-430. VCH Weinheim, Germany.

[5] Chang, J-S., Law, R., Chang, Chung-Cheng, C. (1997), Biosorption of lead, cooper and cadmium by biomass of *Pseudomonas aeruginosa* PU21. *Wat. Res.* 31, pp. 1651-1658.

[6] Strandberg, G. M., Shumate, S. E., Parott, J. R. (1981), Microbial cells as biosorbents for heavy metals: accumulation of uranium by *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa. Appl. Environ. Microbiol.* 41, pp. 237-245.

[7] Texier, A. C., András, Y., Le Cloirec, P. (1999), Selective biosorption of lanthanide (La, Eu, Yb) ions by *Pseudomonas aeruginosa. Environ. Sci. Technol.* 33, pp. 489-495.

[8] Texier, A. C., András, Y., Le Cloirec, P. (2000), Characterization of lanthanide ions binding sites in the cell wall of *Pseudomonas aeruginosa.* Environ. Sci. Technol. 34, pp. 610-615.

[9] Chang, J-S., Chen, C-C. (1998) Quantative analysis and equilibrium models of selective adsorption in multimetal system using a bacterial biosorbent. *Sep. Sci. Technol.* 33, pp. 611-632.

[10] Uslu, G., Tanyol, M. (2006) Equilibrium and thermodynamic parameters of single and binary mixture biosorption of lead (II) and copper (II) ions onto *Pseudomonas putida*: Effect of temperature. *Journal of Hazardous Materials B* 135, pp. 87-93.

[11] Kang, S-Y., Lee, J-U., Kim, K-W. (2007) Biosorption of Cr(III) and Cr(VI) onto the cell surface of *Pseudomonas aeruginosa. Biochemical Engineering Journal* 36, pp. 54-58.

[12] de Vincente, A., Avile‘s, M., Codina, J. C., Borrego, J. J., Romero, P. (1990) Resistance to antibiotics and heavy metals of *Pseudomonas aeruginosa isolated from natural waters. J. Appl. Bacteriol.* 68, pp. 625-632.

[13] Volesky, B., Holan, Z. R. (1995) Biosorption of heavy metals. *Biotechnology Progress* 11, pp. 235-250.

[14] Sherbert, G. V., (1978) The biophysical characterization of the cell surface. London: Academic press

[15] Gabr, R. M., Hassan, S. H. A., Shoreit, A. A. M. (2008) Biosorption of lead and nickel by living and non-living cells of *Pseudomonas aeruginosa* ASU 6a. *International biodeterioration & Biodegradation* 61, pp. 195-203.

[16] Pardo, R., Herguedas, M., Barrado, E., Vega, M. (2003), Biosorption of cadmium, copper, lead and zinc by inactive biomass of *Pseudomonas pudita. Analytical and Bioanalytical Chemistry* 376, pp. 26-32.

[17] Uslu, G., Tanyol, M. (2006), Equilibrium and thermodynamic parameters of single and binary mixture biosorption of lead(II) and copper(II) ions onto *Pseudomonas pudita*: Effect of temperature. *Journal of Hazardous Materials* B 135, pp. 78-93.