

Immobilization of *Saccharomyces cerevisiae* on low-cost lignocellulosic substrate for the removal of Cd²⁺ from aquatic systems

Vasileios A. Anagnostopoulos*, Athina Vlachou, Basil D. Symeopoulos

Radiochemistry Lab, Department of Chemistry, University of Patras, Patras 26500, Greece.

ORIGINAL RESEARCH ARTICLE

ABSTRACT

Cadmium in aquatic system poses severe ecological and health threats. Bioremediation techniques can offer an efficient strategy for the removal of cadmium from aquatic systems. The present study explores the potential of a common yeast (*Saccharomyces cerevisiae*) immobilized on delignified cellulosic substrate (beech sawdust) for removal of cadmium from acidic effluents. The proposed biosorbent exhibits satisfactory Cd²⁺ removal over a wide pH range. At the initial cadmium concentration of 4 - 100 mg/L, the Cd(II) removal efficiency varied from 28 to 18%. The Langmuir, Freundlich and Linear isotherm models were tested for the present system. Of these models, the Linear and Freundlich models provided the highest correlation with the experimental data, whereas Langmuir model provided least satisfactory fit.

KEYWORDS

biosorption, cadmium, immobilization, *Saccharomyces cerevisiae*, sawdust

1. INTRODUCTION

The increase of industrial activities over the past decades has resulted in the release of significant amount of pollutants in the environment. Heavy metals are persistent contaminants that cause severe deterioration to ecosystems. In particular, cadmium is a heavy metal which is widely used in the manufacture of various products such as paint pigments, plastics, alloys, batteries, ceramics and glass (Lodeiro et al. 2005; Chen et al. 2014). Cadmium is not degraded in aqueous systems and poses a severe ecological and health threat, since it can accumulate throughout the food chain. If cadmium enters the human body, it can bind to essential respiratory enzymes leading to oxidative stress and cancer (Banjerdkiy et al. 2005).

Removal and recovery of cadmium from aqueous streams is very important with respect to both environmental and economic aspects. Several conventional physicochemical techniques, such as

electrochemical treatment, ion exchange, precipitation and reverse osmosis have been practiced for decades. These techniques exhibit high operational cost and low efficiency especially in low metal concentrations. In the case of precipitation, formation of secondary toxic sludge necessitates additional treatment and handling steps (Rajesh et al., 2014).

Bioremediation techniques can provide an alternative and efficient strategy for environmental cleanup. More specifically, biosorption is a metabolism-independent process, where metal ions are accumulated onto the surface of biological materials. Biosorption is an environmental friendly process, which uses low-cost waste materials as sorbents, and does not require high capital investment. The process efficiently removes heavy metal ions from aquatic systems, even in low concentrations. Microorganisms have shown ability to sequester metal ions due to the diversity of functional groups that are present in their cell walls, such as carboxyl, hydroxyl, amine and phosphate groups, as well as fatty acids (Choi and Yun,

Corresponding author: V.A. Anagnostopoulos

Tel: +17864449736
E. mail: vanagnos@fiu.edu

Received: 27-05-2015
Revised: 17-08-2015
Accepted: 27-08-2015
Available online: 15-09-2015

2006; Anagnostopoulos and Symeopoulos, 2014). The binding of metal ions on these groups takes place through multiple mechanisms, such as electrostatic interactions, complexation, ion-exchange and micro-precipitation (Montazer-Rahmati et al., 2011).

Saccharomyces cerevisiae is a common yeast used in many industrial applications, such as breweries, wineries and bakeries (Anagnostopoulos et al., 2010). Thus, the biomass can be effectively used as a low-cost biosorbent. Despite the promising properties of *Saccharomyces cerevisiae*, the application of the yeast biomass in industrial scale wastewater treatment is practically limited due to its fragile nature. Through immobilization technique, the yeast biomass could achieve desirable properties such as high mechanical strength and stability that would allow the biomass to be reused and easier handling (Cabuk and Ilhan, 2003).

Thus, the present work examines the potential of immobilized *Saccharomyces cerevisiae* cells on beech sawdust (*Fagus sylvatica*) for the removal of cadmium ions from aquatic systems. Sawdust is a wood treatment by-product and as a lignocellulosic material exhibits heavy metal sorption capacity (Anagnostopoulos and Symeopoulos, 2013; Anagnostopoulos et al., 2015).

2. MATERIALS AND METHODS

2.1. Substrate preparation

Beech sawdust was obtained from a local carpentry. It was sieved and the fraction $0.18 < d < 1$ mm was retained for all experiments. A part of this fraction was treated with 1% NaOH at 80 °C for 3 h under continuous stirring. After the end of the treatment, the material was washed thoroughly with deionized water in order to remove excess NaOH (Nghah and Hanafiah, 2008). The NaOH treated samples will be referred as Delignified Cellulosic Material (DCM) in this paper. DCM was autoclaved ($T = 394.15$ K, $P = 1.1$ atm, 15 min) in order to be used for microorganism immobilization (Koutinas et al., 2012).

2.2. Biomass preparation

Saccharomyces cerevisiae cells were grown using a commercial product of Baker's yeast ("L' hironnelle") by dispersing 0.5 g of commercial product into 250 mL of sterilized nutrient medium with the aid of vortex. The sterilized ($T = 121$ °C, pressure = 1.1 atm for 15 min in autoclave) nutrient medium consisted of 40 g/L

glucose, 4 g/L yeast extract (Merck), 1 g/L KH_2PO_4 , 1 g/L $(\text{NH}_4)_2\text{SO}_4$ and 10 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. Sterile air was provided by passing the air through a filter (Millex-FG, 0.2 μm , PTFE, Millipore) (Anagnostopoulos et al. 2011). Suspension was left for incubation at 30 °C for 24 h.

2.3. Biomass immobilization

Biomass was collected by centrifugation and was suspended with the aid of vortex in autoclaved nutrient medium consisting of 140 g/L of glucose. The suspension was introduced in the bioreactor, already containing sterilized DCM, and was incubated at 30 °C for 24 h in order to achieve immobilization. After 24 h, immobilized biomass on DCM was autoclaved again ($T = 121$ °C, pressure = 1.1 atm for 15 min) to metabolically inactivate the biomass.

2.4. Sorption experiments

In sorption experiments, 0.2 g of biomass was brought in contact with 5 mL of Cd^{2+} solution and ionic strength was adjusted using 0.1 M NaCl. Samples were equilibrated at 25 °C for 24 h on a rotary shaker (10 rpm). After 24 h, residual Cd^{2+} concentration in the supernatant was determined using the Flame Atomic Absorption Spectrometry (FAAS, Shimadzu 6500). To study the effect of pH, the initial metal concentration was fixed at 100 mg/L, whereas the pH varied from 2.2 to 6.2. The desired pH value was attained by adding HCl or NaOH solution. All experiments were performed at 25 °C. The metal uptake q (mg/g) was calculated from the difference between the initial (C_{init} , mg/L) and the final (C_{fin} , mg/L) metal concentration using the below equation,

$$q = (C_{\text{init}} - C_{\text{fin}}) \times V / m \quad (1)$$

where, m (g) and V (L) are the dry weight of biomass and the volume of solution, respectively.

3. RESULTS AND DISCUSSION

The microorganism was chosen to be metabolically inactive in sorption experiments because dead cells exhibit several desired characteristics over live cells, which include: (a) they remain unaffected by metal toxicity, (b) they can be stored for longer time intervals without morphological alterations and (c) they do not release soluble extracellular proteins that can complex

with metal ions and thereby hinder sorption (Huang et al., 1990). Even though metal uptake by dead cells has been documented to be a rapid process (Wilhelmi and Duncan, 1995; Göksungur et al., 2005), equilibration time was chosen as 24 h for the present study.

3.1. Effect of pH

The effect of initial pH on metal uptake is shown in Figure 1. The results indicated that cadmium uptake progressively increased with increase in pH for DCM. More specifically, Cd²⁺ percent removal increased from 42% at pH 2.2 to 68% at pH values of 4 and 6.2. On the other hand, the performance of raw sawdust over Cd²⁺ removal was comparatively less (31 - 40%) over the pH values tested (Figure 1). In Figure 2, speciation of cadmium is presented under the conditions studied, and it can be noted that cadmium remains in the form of Cd²⁺ for pH values ranging from 2.2 to 6.2. Hence, the different pattern of sorption behavior is speciation independent. A possible explanation could be based on the nature of lignocellulosic materials. Lignocellulosic materials have been documented to have carboxyl (-COOH), carbonyl (-C=O) and phenolic/hydroxyl groups (-OH) on their surface (Taty-Costodes et al. 2003). These functional groups were mainly responsible for the uptake of metal cations, such as Cd²⁺. At low pH values, carboxyl and hydroxyl groups were protonated and undissociated thereby having positive charge or no charge. Therefore, the approach of positively charged metallic species was not favored by electrostatic attraction. However, on increasing the solution pH, a negative charge density develops due to deprotonation of carboxyl groups. Thus, the interaction between the positively charged metallic species and the negatively charged sites (-COO⁻) of the sorbent was favorable which results in higher metal binding. On the other hand, oxygen atoms of carbonyl and hydroxyl groups act as strong Lewis bases and tend to form bonds with entities that lack electrons, such as metal cations (Taty-Costodes et al., 2003).

DCM exhibited higher metal loading capacity at examined pH values compared to raw sawdust. This result was in accordance with other research reports where modified biomass performed better than raw biomass such as sorption of Cd, Cu, Pb, Ni and Zn by alkali treated plant matter (Nghah and Hanafiah, 2008), uptake of cadmium and lead by modified spent grain (Low et al. 2000) and cadmium removal from sawdust (Memon et al., 2007). Several factors may explain the higher uptake of metal cations by alkali treated lignocellulosic materials such as, (a) the swelling of

the material increases surface area (Min et al., 2004) and (b) the removal of lignin allows new binding groups to emerge on the surface of the material and thereby available for metal sequestration (Park et al., 2005). It was also observed that sorption potential of raw sawdust at all pH values and DCM at pH values of 4 and 6.2 remained the same. This observation may be attributed to coverage of all binding sites by the sorbate.

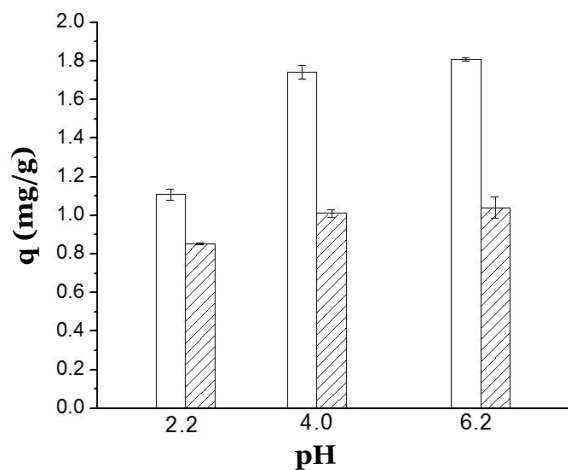


Figure 1. Cadmium uptake by raw sawdust (striped bars) and DCM (white bars) as a function of pH. Error bars represent standard deviation from triplicate experiments

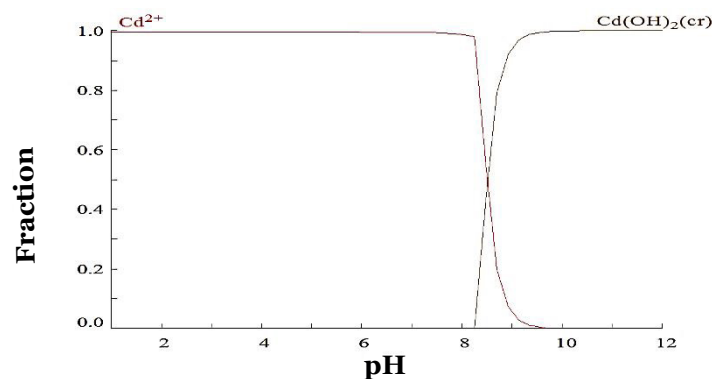


Figure 2. Cadmium speciation obtained by means of Hydra-Medusa speciation software for the conditions studied.

3.2. Effect of Solid/Solution ratio

The effect of solid/solution ratio was studied using three different adsorption suspension ratios (10, 20 and 40 mg/mL) at pH 6.2 and initial Cd²⁺ concentration of 100 mg/L. From the plot shown in Figure 3, it can be clearly seen that for all solid/solution ratios studied,

DCM showed higher cadmium sorption compared to raw sawdust. Raw sawdust exhibited constant Cd^{2+} removal efficiency of 13% at all the ratios studied. This implies that saturation of the sorbent surface has been achieved as the uptake remained unaffected even with 4-fold increase of the sorbent mass. On the other hand, cadmium uptake decreased with the increase of DCM suspension ratio. More specifically the percent removal of Cd^{2+} was determined as 70%, 47% and 28% for ratios of 10, 20 and 40 mg/mL respectively. This trend was due to agglomeration of the biomass particles leading to loss of the available surface area which decreases the available functional groups that interact with Cd^{2+} . Moreover, an increase in biomass quantity may incur stereochemical and/or electrostatic interactions among neighboring active sites, thus hindering metal sorption (Ucun et al., 2009).

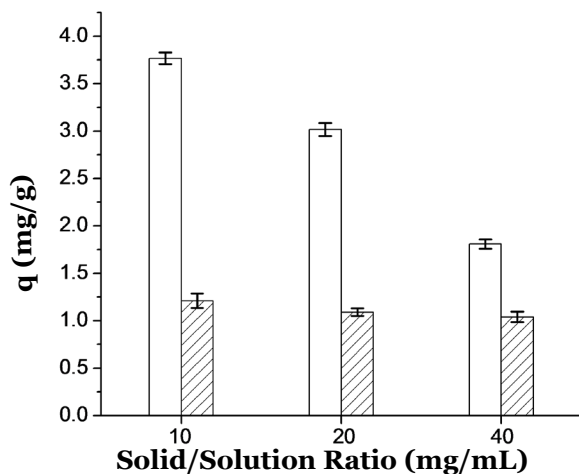


Figure 3. Cadmium uptake by raw sawdust (striped bars) and DCM (white bars) as a function of solid/solution ratio, $C_{\text{init}}=100$ mg/L, pH 6.2, temperature 25 °C, equilibration time 24 h.

3.3. Immobilization of *Saccharomyces cerevisiae* on DCM

On comparing the two sorbents (raw sawdust and DCM), based on the experimental results as presented in Sections 3.1 and 3.2, DCM showed significantly higher cadmium loading capacity than raw sawdust and therefore, it was selected as an immobilization substrate for *Saccharomyces cerevisiae*. The immobilization of *Saccharomyces cerevisiae* onto DCM was examined using scanning electron microscopy (SEM). As illustrated in Figure 4, cells of *Saccharomyces cerevisiae* can be clearly seen on the DCM surface.

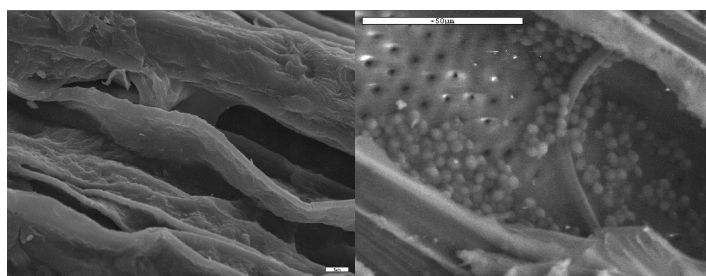


Figure 4. Scanning electron microscopy of DCM (left) and of cells of *Saccharomyces cerevisiae* immobilized on DCM (right).

3.4. Effect of Cd^{2+} initial concentration on immobilized *Saccharomyces cerevisiae* cells onto DCM

The effect of the initial Cd^{2+} concentration on sorption potential of DCM was examined by varying Cd^{2+} concentrations in the range 8–100 mg/L at fixed pH (6.2), temperature (25 °C), equilibration time (24 h) and solid/solution ratio (20 mg/mL) (Figure 5). Cadmium uptake increased with increasing initial cadmium concentration as shown in Figure 5. This confirms that initial solute concentration act as the driving force for the sorption process (Hadi et al., 2003). Furthermore, the experimental isotherm resembles that of C-type isotherm, which represents a line of zero origin indicating that the ratio of sorbate concentration remaining in the solution and those bound on the substrate remains the same (Limousin et al., 2007).

The sorption isotherm was analyzed using the Langmuir, Freundlich and Linear isotherm models. The Langmuir adsorption isotherm can be represented as shown in Eq. (2):

$$C_{\text{fin}}/q = 1/q_{\text{max}} \cdot K_L + C_{\text{fin}}/q_{\text{max}} \quad (2)$$

where q_{max} (mg/g) is the maximum metal uptake and K_L (L/mg) is a Langmuir constant.

The Langmuir isotherm model assumes monolayer sorption at specific homogeneous sites within the adsorbent and that sorption is same across the sorbent binding sites and independent of the presence of sorbate molecules in neighboring binding sites (Rahchamani et al., 2011).

On the other hand, the Freundlich model is an empirical model based on heterogeneous multi-layer sorption across sorbent binding sites and predicts exponential increase in binding with increase in initial metal concentration (Amini et al., 2009). The

Freundlich adsorption isotherm can be represented as shown in Eq. (3):

$$\ln q = \ln K_f + 1/n \cdot \ln C_{fin} \quad (3)$$

where K_f is the Freundlich constant, indicative of sorption capacity; and n is the Freundlich exponent indicative of the distribution of the active sites on the adsorbent. Generally, the higher the n value, the stronger the sorption intensity (Anayurt et al., 2009).

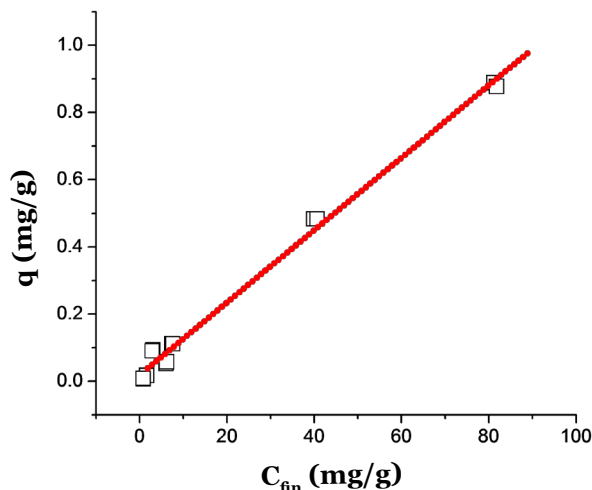


Figure 5. Adsorption isotherm for Cd^{2+} sorption onto immobilized *Saccharomyces cerevisiae* on DCM at 25 °C, pH=6.2, equilibration time 24 h, solid/solution ration 20 mg/mL. Open black boxes represent experimental data and dotted red line represents theoretical isotherm based on Linear model.

The Linear model is a simple empirical model that can describe sorption phenomena on complex substrates, but does not predict maximum uptake (Voudrias et al., 2002). The Linear model can be represented as shown in Eq. (4):

$$q = K_d \cdot C_{fin} \quad (4)$$

where K_d (L/g) is the distribution coefficient of sorbate between liquid and solid phase.

The Langmuir model failed to interpret experimental data (Table 1) with low correlation coefficient (R^2) value. A possible explanation for this result may be the actual assumptions of the Langmuir model. The model assumes monolayer sorption at specific homogeneous sites, while the sorbent used in our experiment was rather complex and heterogeneous

nature. On the other hand, both the Linear and Freundlich models described sorption isotherm with high correlation coefficients. Although the linear model has the disadvantage of being an empirical one, it has been reported to successfully describe sorption onto complex substrates (Cantrell et al., 2002). The Freundlich model reduces to the linear model when $n = 1$. Under this scenario, $K_f = K_d$ and as shown in Table 1, n was found equal to 1 and the model constants K_f and K_d values from the Freundlich and Linear models, respectively, were in good agreement.

Table 1. Regression parameters for isotherm models tested for sorption of Cd^{2+} onto immobilized *Saccharomyces cerevisiae* on DCM, pH 6.2, solid/solution ration 20 mg/mL.

Freundlich	
K_f	0.012
n	1.0
R^2	0.96
Langmuir	
K_L (L/mg)	1707
q_{max} (mg/g)	20
R^2	0.08
Linear	
K_d (L/g)	0.011
R^2	0.99

Table 2. Cd^{2+} percent removal as a function of Cd^{2+} initial concentration by immobilized *Saccharomyces cerevisiae* on DCM, pH 6.2, solid/solution ration 20 mg/mL.

Cd^{2+} C_{init} (mg/L)	% Cd^{2+} Removal
100	18
50	20
10	22
8	24
4	28

Table 2 illustrates the percent Cd^{2+} removal by immobilized *Saccharomyces cerevisiae* on DCM at different initial Cd^{2+} concentration. The results indicated that the percent Cd^{2+} removal decreased from 28 to 18% on increasing initial Cd^{2+} concentration from 4 to 100 mg/L.

4. CONCLUSIONS

The proposed biosorbent exhibits a satisfactory cadmium uptake from aqueous acidic cadmium solutions at wide range of cadmium concentration. Further experiments are required to assess the efficiency of the technology proposed under different conditions. The effect of acidic pH on the morphology of the biosorbent, which might affect the re-use potential of the biosorbent, also need to be investigated. Finally, the proposed biosorbent is required to be tested in multi-component systems.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Professors A.A. Koutinas, M. Kanellaki and A. Bekatorou for providing the knowledge of the biomass preparation. Special thanks to Dr. L. Bosnea for useful discussions on the field of fermentation and yeast technology. We also thank the European Social Fund (ESF), Operational Program for Educational and Vocational Training II (EPEAEK II), and particularly the Program PYTHAGORAS II, for funding the above work.

REFERENCES

- Amini, M., Younesi, H., Bahramifar, N. (2009) Biosorption of nickel(II) from aqueous solution by *Aspergillus niger*: Response surface methodology and isotherm study. *Chemosphere*, 75, 1483-1491.
- Anagnostopoulos, V., Bekatorou, A. and Symeopoulos, B. (2011) Contribution to interpretation of metal uptake dependence upon the growth phase of microorganisms. The case of uranium (VI) uptake by common yeasts, cultivated at different temperatures, with or without aeration. *Journal of Radioanalytical and Nuclear Chemistry*, 287, 665-671.
- Anagnostopoulos, V., Koutsoukos, P. and Symeopoulos, B. (2015) Removal of U(VI) from aquatic systems, using winery by-products as biosorbents: equilibrium, kinetic, and speciation studies. *Water Air and Soil Pollution*, 226, 1-14.
- Anagnostopoulos, V.A. and Symeopoulos, B.D. (2013) Sorption of europium by malt spent rootlets, a low cost biosorbent: effect of pH, kinetics and equilibrium studies. *Journal of Radioanalytical and Nuclear Chemistry*, 295, 7-13.
- Anagnostopoulos, V.A. and Symeopoulos, B.D. (2014) Significance of age, temperature, and aeration of yeast cell culture for the biosorption of europium from aquatic systems. *Desalination and Water Treatment*, In Press, doi:10.1080/19443994.2014.987177.
- Anagnostopoulos, V.A., Symeopoulos, B.D. and Soupioni, M.J. (2010) Effect of growth conditions on biosorption of cadmium and copper by yeast cells. *Global NEST Journal*, 12, 288-295.
- Anayurt, R.A., Sari, A. and Tuzen, M. (2009) Equilibrium, thermodynamic and kinetic studies on biosorption of Pb(II) and Cd(II) from aqueous solution by macrofungus (*Lactarius scrobiculatus*) biomass. *Chemical Engineering Journal*, 151, 255-261.
- Banjerdkij, P., Vattanaviboon, P. and Mongkolsuk, S. (2005) Exposure to cadmium elevates expression of genes in the OxyR and OhrR regulons and induces cross-resistance to peroxide killing treatment in *Xanthomonas campestris*. *Applied and Environmental Microbiology*, 71, 1843-1849.
- Cabuk, A. and Ilhan, S. (2003) Cu²⁺ removal in a biosorption column by immobilized bacterial biomass. *The European Journal of Mineral Processing and Environmental Protection*, 3, 67-74.
- Cantrell, K.J., Serne, R.J. and Last, G.V. (2002) Applicability of the linear sorption isotherm model to represent contaminant transport Processes in site-wide performance assessments. U.S. Department of Energy, Springfield, VA.
- Chen, B.-Y., Chen, C.-Y., Guo, W.-Q., Chang, H.-W., Chen, W.-M., Lee, D.-J., Huang, C.-C., Ren, N.-Q. and Chang, J.-S. (2014) Fixed-bed biosorption of cadmium using immobilized *Scenedesmus obliquus* CNW-N cells on loofa (*Luffa cylindrica*) sponge. *Bioresource Technology*, 160, 175-181.
- Choi, S.B. and Yun, Y.-S. (2006) Biosorption of cadmium by various types of dried sludge: An equilibrium study and investigation of mechanisms. *Journal of Hazardous Materials*, 138, 378-383.
- Göksungur, Y., Üren, S. and Güvenç, U. (2005) Biosorption of cadmium and lead ions by ethanol treated waste baker's yeast biomass. *Bioresource Technology*, 96, 103-109.
- Hadi, B., Margaritis, A., Berruti, F. and Bergougnou, M. (2003) Kinetics and equilibrium of cadmium biosorption by yeast cells *S. cerevisiae* and *K. fragilis*. *International Journal of Chemical Reactor Engineering*, 3, A47.
- Huang, C.-P., Huang, C.-P. and Morehart, A.L. (1990) The removal of Cu(II) from dilute aqueous solutions by *Saccharomyces cerevisiae*. *Water Research*, 24, 433-439.
- Koutinas, A.A., Sypsas, V., Kandyli, P., Michelis, A., Bekatorou, A., Kourkoutas, Y., Kordulis, C., Lycourghiotis, A., Banat, I.M., Nigam, P., Marchant, R., Giannouli, M. and Yianoulis, P. (2012) Nano-tubular cellulose for bioprocess technology development. *PLoS ONE*, 7(4), e34350.
- Limousin, G., Gaudet, J.P., Charlet, L., Szenknect, S., Barthès, V. and Krimissa, M. (2007) Sorption isotherms: A review on physical bases, modeling and measurement. *Applied Geochemistry*, 22, 249-275.
- Lodeiro, P., Cordero, B., Barriada, J.L., Herrero, R. and Sastre De Vicente, M.E. (2005) Biosorption of cadmium by biomass of brown marine macroalgae. *Bioresource Technology*, 96, 1796-1803.
- Low, K.S., Lee, C.K. and Liew, S.C. (2000) Sorption of cadmium and lead from aqueous solutions by spent grain. *Process Biochemistry*, 36, 59-64.
- Rajesh M.V., Kumar, S.K.A. and Rajesh, N. (2014) Biosorption of cadmium using a novel bacterium isolated from an electronic industry effluent. *Chemical Engineering Journal*, 235, 176-185.
- Memon, S.Q., Memon, N., Shah, S.W., Khuhawar, M.Y. and Bhangar, M.I. (2007) Sawdust—A green and economical sorbent for the removal of cadmium (II) ions. *Journal of Hazardous Materials*, 139, 116-121.
- Min, S.H., Han, J.S., Shin, E.W. and Park, J.K. (2004) Improvement of cadmium ion removal by base treatment of juniper fiber. *Water Research*, 38, 1289-1295.
- Montazer-Rahmati, M.M., Rabbani, P., Abdolali, A. and Keshtkar, A.R. (2011) Kinetics and equilibrium studies on biosorption of cadmium, lead, and nickel ions from aqueous solutions by intact and chemically modified brown algae. *Journal of Hazardous Materials*, 185, 401-407.
- Park, D., Yun, Y.-S. and Park, J.M. (2005) Studies on hexavalent chromium biosorption by chemically-treated biomass of *Ecklonia* sp. *Chemosphere*, 60, 1356-1364.

- Rahchamani, J., Mousavi, H.Z. and Behzad, M. (2011) Adsorption of methyl violet from aqueous solution by polyacrylamide as an adsorbent: Isotherm and kinetic studies. *Desalination*, 267, 256-260.
- Taty-Costodes, V.C., Fauduet, H., Porte, C. and Delacroix, A. (2003) Removal of Cd(II) and Pb(II) ions, from aqueous solutions, by adsorption onto sawdust of *Pinus sylvestris*. *Journal of Hazardous Materials*, 105, 121-142.
- Ucun, H., Aksakal, O. and Yildiz, E. (2009) Copper(II) and zinc(II) biosorption on *Pinus sylvestris* L. *Journal of Hazardous Materials*, 161, 1040-1045.
- Voudrias, E., Fytianos, K. and Bozani, E. (2002) Sorption - Desorption isotherms of dyes from aqueous solutions and wastewaters with different sorbent materials. *Global Nest Journal*, 4, 75-83.
- Wan Ngah, W.S. and Hanafiah, M.A.K.M. (2008) Removal of heavy metal ions from wastewater by chemically modified plant wastes as adsorbents: A review. *Bioresource Technology*, 99, 3935-3948.
- Wilhelmi, B.S. and Duncan, J.R. (1995) Metal recovery from *Saccharomyces cerevisiae* biosorption columns. *Biotechnology Letters*, 17, 1007-1012.