REMOVAL OF COPPER(II) IONS FROM AQUEOUS SOLUTION BY A LACTIC ACID BACTERIUM

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Abstract - *Enterococcus faecium*, a lactic acid bacterium (LAB), was evaluated for its ability to remove copper(II) ions from water. The effects of the pH, contact time, initial concentration of copper(II) ions, and temperature on the biosorption rate and capacity were studied. The initial concentrations of copper(II) ions used to determine the maximum amount of biosorbed copper(II) ions onto lyophilised lactic acid bacterium varied from 25 mg L^{-1} to 500 mg L^{-1}. Maximum biosorption capacities were attained at pH 5.0 and 6.0. Temperature variation between 20°C and 40°C did not affect the biosorption capacity of the bacterial biomass. The highest copper(II) ion removal capacity was 106.4 mg per g dry biomass. The correlation regression coefficients show that the biosorption process can be well defined by the Freundlich equation. The change in biosorption capacity with time was found to fit a pseudo-second-order equation.
Keywords: Biosorption; Copper(II); *Enterococcus faecium*; Lactic acid bacterium.

INTRODUCTION

Copper has been one of the most widely used metals for centuries and is mainly employed in electrical and electroplating industries. Because of its toxicity to living organisms, its presence in the environment causes serious toxicological concerns. Copper is known to deposit usually in the brain, skin, liver, pancreas and myocardium (Davis et al., 2000). Wastewaters of especially the electrical and electroplating industries contain high levels of Cu^{2+} ions and treatment of such waters to remove Cu^{2+} ions is needed before disposal.

A great number of studies have been carried out to develop cost-effective heavy metal removal techniques. Physicochemical methods such as chemical precipitation, solvent extraction, chemical oxidation, biological treatments and ion exchange processes have been employed for heavy metal removal from industrial wastewaters (Neamtu et al., 2004; Wahi et al., 2005; Rao and Rao, 2006; Chen and Zhao, 2009; Calero et al., 2009). These methods are sometimes expensive, are not effective at low metal concentrations, and produce sludge to be disposed of, although sludges can be good materials to remove heavy metals (Barros et al., 2006). Recently, research efforts have been directed towards bioremediation of heavy metal pollution and many biological materials, such as *Gelidium*, tree fern, *Bacillus* spp., *Rosa centifolia*, *Sargassum* sp., *Aspergillus niger*, *Rhizopus arrhizus*, *Cladophora fascicularis*, *Pseudomonas aeruginosa*, *Sphaerotilus natans*, *Cephalosporium aphidicola*, *Mucor rouxii*, Marine algae, *Phragmites australis*, *Pinus sylvestris*, *Aspergillus niger*, *Phellinus badius*, *Botrytis cinerea*, *Sporotricum sp.,* and *Crab and Arca* shell, have been proposed to be efficient and economical biomaterials for heavy metal removal from wastewaters (Valdman

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and Leite, 2000; Silva et al., 2003; Loukidio et al., 2004; Halttunen et al., 2007; Kim et al., 2007; Nasir et al., 2007; Deng et al., 2007; Dahiya et al., 2008; Tunali et al., 2009).

Because of their nature and membrane compositions, bacterial biomasses are natural adsorbents for metals. The heavy metal uptakes involve the passage of the metal species into the cell across the cell membrane during the cell metabolic cycle. This mode of metal capture is referred to as active uptake (Kapoor et al., 1999). If one considers that nonviable biomass is not biologically active, its metal uptake can be regarded as a passive adsorption process and, thus, be correlated with mathematical sorption models as the Langmuir and Freundlich equations (Cruz et al., 2004). Furthermore the bacterial surfaces contain polarizable groups (sites) such as phosphate, carboxyl, hydroxyl and amino groups, which are capable of interacting with cations. These sites may also contribute to the reversible metal binding capacity of the biomass (Liu et al., 2003). Therefore, biosorption is a process that uses any biomass to sorb ions from aqueous solutions.

In the present work, the biosorption of copper(II) ions onto a lactic acid bacterium, namely *Enterococcus faecium*, was studied by investigating the influences of different experimental parameters such as pH, contact time, initial concentration of copper(II) ions, and temperature on the rate and capacity of the biosorption.

MATERIALS AND METHODS

Growth Media and Culture Conditions

The strain used in this study was *Enterococcus faecium*, a lactic acid bacterium, isolated from meat and identified using the RiboPrinter® Microbial Characterization System (DuPont). Bacterial isolate was inoculated into MRS-broth for 48 h at 37°C. Then the biomass was centrifuged (8000×g, 15 min), washed twice with ultra-pure water (Milli-Q), lyophilized and stored at −20°C.

Copper(II) Ion Solutions

A stock solution of Cu^{2+} ions used in this study was prepared by dissolving an accurate quantity of copper(II) nitrate in deionized water. Other concentrations prepared from this stock solution by

dilution varied from 25 mg L^{-1} and 500 mg L^{-1} and the pH of the working solutions was adjusted to the desired values with 0.1 M HCl or 0.1 M NaOH. Fresh dilutions were used for each experiment. All the chemicals used were of analytical grade.

Biosorption Experiments

The biosorption of Cu^{2+} ions onto the lyophilized biomass from aqueous solutions was investigated in batch experiments. The experiments were carried out with 50 mg of the biosorbent in 100 mL of a Cu^{2+} ion solution in 250 mL bottles at 30°C on an orbital shaker operating at 150 rpm. The effect of pH on the biosorption rate was investigated in the pH range of 1.0-6.0. The pH values of the medium were adjusted to the desired value by adding HCl or NaOH at the beginning of the experiments and were not controlled further. The initial concentrations of Cu^{2+} ion solutions varied from 25 mg L^{-1} to 500 mg L^{-1}. The effect of temperature (20-40°C) on the biosorption capacities of the biosorbent was determined at pH 5. After the contact time, the solutions were centrifuged at 5000 rpm for 5 min and the supernatants were used to determine the concentration of nonadsorbed Cu^{2+} ions.

The concentrations of the nonadsorbed Cu^{2+} ions in the supernatants were determined by using a flame atomic absorption spectrophotometer (Perkin Elmer A. Analyst 800 Model) with an air-acetylene flame. The instrument response was periodically checked using a standard Cu^{2+} ion solution.

The equilibrium sorption capacity of the *E. faecium* biomass at the corresponding equilibrium conditions was determined using the following mass balance equation:

$$q_e = \frac{(C_o - C_e)\, V}{m} \tag{1}$$

where q_e is the amount of Cu^{2+} ions adsorbed onto the biomass (mg g^{-1}), C_o is the initial Cu^{2+} ion concentration in solution (mg L^{-1}), C_e is the final metal ion concentration in solution (mg L^{-1}), V is the volume of the medium (L) and m is the amount of the biomass used in the adsorption process (g).

RESULTS AND DISCUSSION

Figure 1 shows the effects of contact time for the biosorption of Cu^{2+} ions onto the lyophilized

biomass of *E. faecium*. The experimental conditions are indicated in the figure legend. A rapid sorption rate was observed within the first 30 min of the biosorption process and then a considerable increase in the uptake capacity of the biosorbent for Cu^{2+} ions continued up to 600 min. Thereafter, it remained nearly constant. This rapid initial uptake is similar to previous reports on the biosorption of heavy metals by different biosorbents (Puranik and Paknikar, 1997; Yin et al., 1999). The subsequent biosorption experiments using the lyophilized biomass of *E. faecium* were performed with a contact time of 24 h.

Earlier studies on heavy metal biosorptions have shown that pH was the single most important parameter affecting the biosorption processes because pH influences both cell surfaces (metal binding sites) and metal chemistry in the processes. Several researches have investigated the effect of pH on biosorption of heavy metals using different kinds of microbial biomass and found that maximum biosorption occurred around neutral pH (Feng and Aldrich, 2004; Xue et al., 1988).

To find the optimal pH for the effective biosorption of Cu^{2+} ions onto *E. faecium*, we performed experiments at different initial pH values varying from 1.0 to 6.0 (Figure 2). As expected, the Cu^{2+} ion removal process was a strongly pH-dependent process and the two highest uptake values were obtained at pH 5 and 6. These findings are similar to those for various biosorption processes reported in the literature. The Cu^{2+} ion removal

efficiency of *E. faecium* dropped sharply at pH≤4. To avoid precipitation of Cu^{2+} ion as its hydroxide, pH values higher than 6 were not tested for the biosorption. Based on these results, a pH value of 5.0 was used in further experiments.

The temperature of the adsorption medium could be important parameter for energy-dependent mechanisms of metal adsorption by microbial cells. Equilibrium uptakes of Cu^{2+} ions onto the *E. faecium* biomass at different temperatures (20-40°C) are shown in Figure 3. The results showed that the temperature did not alter significantly the Cu^{2+} ion uptake capacity of the biosorbent. Nearly constant values of q_e were obtained at the temperatures applied. Thus, 30°C was chosen as the temperature for further experiments.

In Figure 4, the Cu^{2+} ion adsorption capacities of the lyophilized biomass of *E. faecium* were presented as a function of the initial concentration of metal ions within the adsorption medium. Up to 250 mg L^{-1} initial concentration, the amount of adsorbed Cu^{2+} ions per unit mass of the biomass increased. This is a reasonable observation because there are many binding sites available on the biosorbent for Cu^{2+} ions at low concentration. Beyond the concentration of 250 mg L^{-1}, the adsorption capacity of the biosorbent remained relatively constant. This was possibly due to saturation of metal binding sites on the biosorbent at concentrations higher than 250 mg L^{-1}. Further experiments were carried out at 250 mg L^{-1} initial concentration of copper ions.
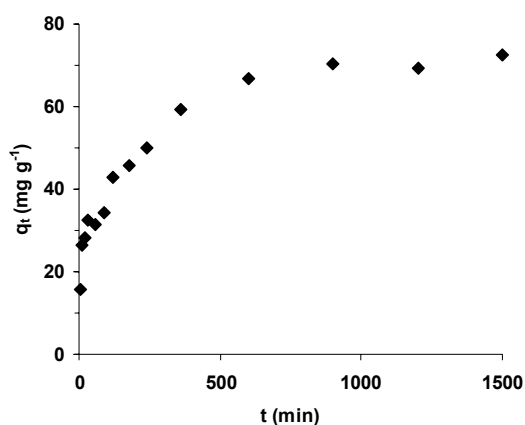

Figure 1: Effect of contact time on Cu^{2+} ion biosorption capacity of *E. faecium*. Biosorption conditions: C_o = 250 mg L^{-1}; m = 50 mg; pH = 5.0; V = 100 mL; T =30 ºC; agitation rate 150 rpm.

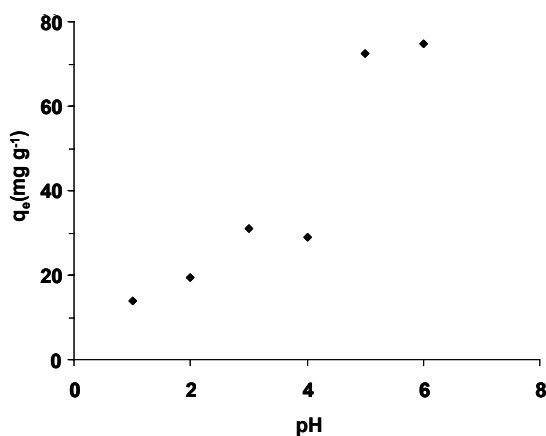**Figure 2:** Effect of the pH on Cu^{2+} ion biosorption capacity of *E. faecium*. Biosorption conditions: C_o = 250 mg L^{-1}; m = 50 mg; V = 100 mL; T =30 ºC; contact time 24 h; agitation rate 150 rpm.

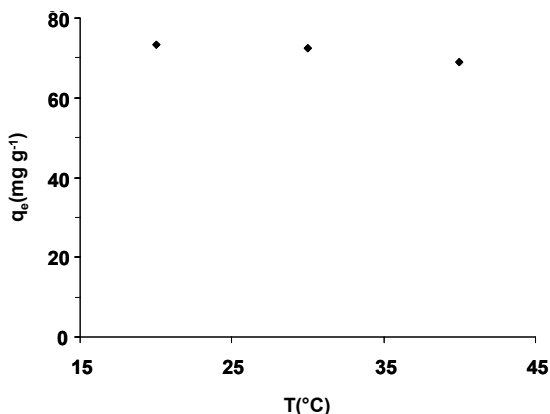**Figure 3:** Effect of temperature on Cu^{2+} ion biosorption capacity by *E. faecium*. Biosorption conditions: $C_o = 250$ mg L^{-1}; m = 50 mg; V = 100 mL; pH 5.0; agitation rate 150 rpm; contact time 24 h.

Figure 4: Effect of initial metal ion concentration on Cu^{2+} ion biosorption capacity onto *E. faecium*. Biosorption conditions: $C_o = 250$ mg L^{-1}; m = 50 mg; V = 100 mL; pH 5.0; agitation rate 150 rpm; contact time 24 h.

Langmuir and Freundlich isotherms were used to analyze the biosorption data (Langmuir, 1918; Freundlich, 1906). The Langmuir isotherm model is valid for monolayer adsorption on to surface containing a given number of identical sorption sites. The Langmuir equation is commonly expressed as:

$$\frac{C_e}{q_e} = \frac{1}{q_{max}K_L} + \frac{C_e}{q_{max}} \qquad (2)$$

where q_e and q_{max} are the amount of metal ion removed and maximum uptake capacity (mg g^{-1}), respectively, C_e is the equilibrium concentration (mg L^{-1}), and K_L is the Langmuir constant.

The Freundlich equation assumes the sorption on a heterogeneous surface and is described by the following equation:

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \qquad (3)$$

where K_F and n are Freundlich constants.

The Langmuir and Freundlich isotherm parameters calculated from the corresponding linear plots are given in Table 1. It appears that the Freundlich isotherm gives a better fit than the Langmuir isotherm. The regression coefficient value of the Freundlich equation (0.985) is better than that of the Langmuir equation (0.966). The plot of the observed equilibrium adsorption and the Langmuir and Freundlich isotherms is given in Figure 5.
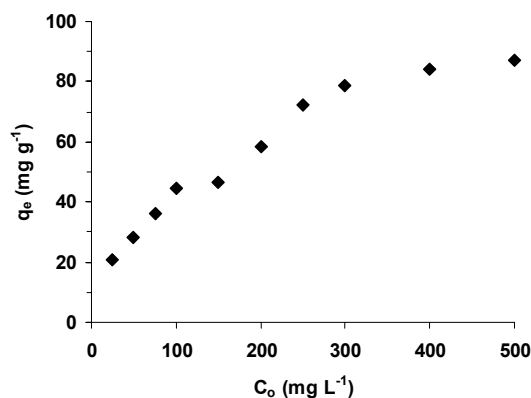
Table 1: Langmuir and Freundlich isotherm parameters for the biosorption of Cu^{2+} ions onto *E. faecium* biomass

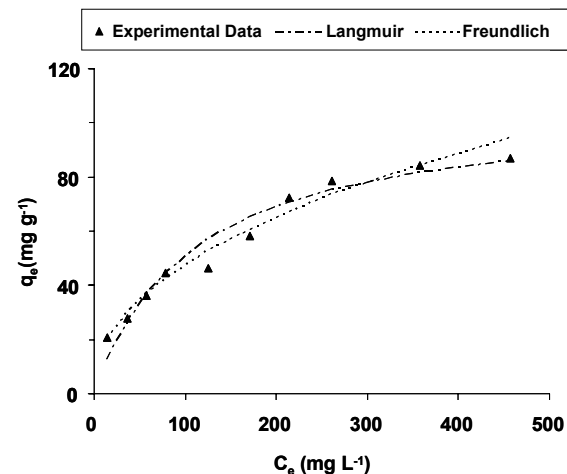| Langmuir		Freundlich	
q_{max} (mg g^{-1})	106.38	n	2.224
K_L (L mg^{-1})	0.0093	K_F (L g^{-1})	5.99
r_L^2	0.966	r_F^2	0.985

Figure 5: Comparison of experimental equilibrium data with the theoretical equilibrium data obtained from the non-linearized equations of Langmuir and Freundlich isotherms.

Having carried out the studies to determine the efficiency of Cu^{2+} biosorption on *E. faecium*, the

pseudo-first-order and pseudo-second-order kinetic models were applied to the experimental data to elucidate the biosorption mechanism.

The pseudo-first-order kinetic model equation (Lagergren, 1898; Calero et al., 2009) is:

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303} t \qquad (4)$$

where q_e and q_t are the amounts of the Cu^{2+} ions absorbed at equilibrium and at time t (mg g^{-1}) respectively, and k_1 is the pseudo first-order rate constant (min^{-1}). Values of k_1 and q_1 were calculated from the plot of log (q_e-q_t) against t.

The pseudo-second-order kinetic model (Calero et al., 2009; Ho and McKay, 1998) is expressed as:

$$\frac{t}{q_t} = \frac{1}{k_2 q_2^2} + \frac{1}{q_2} t \qquad (5)$$

where q_2 is the maximum adsorption capacity of the biomass (mg g^{-1}) for pseudo-second-order adsorption, k_2 is the rate constant for the pseudo-second-order adsorption (g mg^{-1} min^{-1}). Values of k_2 and q_2 were calculated from the plot of t/q_t against t (Figure 6). It was found that the pseudo-second-order kinetic model fit better to the experimentally obtained data then the pseudo-first-order kinetic model. The pseudo-second-order kinetic model parameters for the adsorption of Cu^{2+} ions onto the biosorbent are given in Table 2.
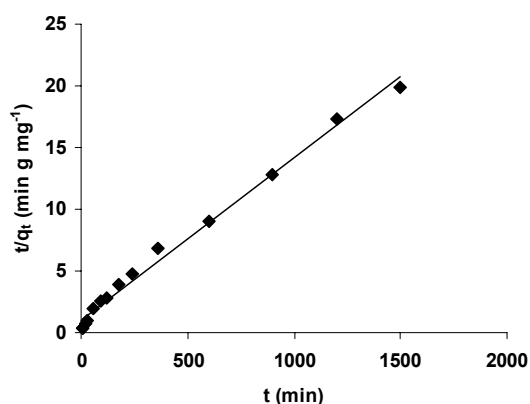

Figure 6: Pseudo-second-order kinetic plot for the biosorption of Cu^{2+}ion onto *E. faecium* biomass. Biosorption conditions: C_o = 250 mg L^{-1}; m = 50 mg; V = 100 mL; T =303 ºC; agitation rate 150 rpm.

Table 2: The pseudo-second-order kinetic model parameters for the biosorption of Cu^{2+}ions onto *E. faecium* biomass.

k_2 (g /mg/min)	17.1e-5
q_2 (mg g^{-1})	75.76
r_2	0.9911

CONCLUSION

In this study, the biosorption of Cu^{2+} ions from aqueous solutions onto a lyophilized lactic acid bacterium, *Enterococcus faecium*, was investigated. Experiments were performed by varying the pH, initial metal ion concentration, contact time, and temperature. The maximum adsorption capacity of biosorbent was found to be 106.38 mg g^{-1} under conditions of pH 5.0, contact time of 24 h, biosorbent mass of 50 mg and initial metal ion concentration of 250 mg L^{-1}. For the biosorption process, the results indicated that the adsorption equilibrium data fitted with the Freundlich isotherm model. The biosorption process was found to conform to a pseudo-second-order kinetic equation. It can be concluded that the lyophilized lactic acid bacterium, *Enterococcus faecium*, has a potential to be used as an alternative biosorbent material for the removal of Cu^{2+} ions from aqueous solution.

REFERENCES

Barros, A. J. M., Prasad, S., Leite, V. D., Souza, A. G., The process of biosorption of heavy metals in bioreactors loaded with sanitary sewage sludge. Brazilian Journal of Chemical Engineering, 23, 153-162 (2006).

Calero, M., Hernainz, F., Blazquez, G., Martin-Lara, M. A., Tenorio, G., Biosorption kinetics of Cd(II), Cr(III) and Pb(II) in aqueous solutions by olive stone. Brazilian Journal of Chemical Engineering, 26, 265-273 (2009).

Chen, H., Zhao, J., Adsorption study for removal of Congo red anionic dye using organo-attapulgite. Adsorption, 15, 381-389 (2009).

Cruz, C. C. V., Costa, C. A., Henriques, A., Luna, A. S., Kinetic modeling and equilibrium studies during cadmium biosorption by dead *Sargassum* sp. Biomass. Bioresource Technology, 91, 249-257 (2004).

Dahiya, S., Tripathi, R. M., Hegde, A. G., Biosorption of lead and copper from aqueous

solutions by pre-treated crab and arca shell biomass. Bioresource Technology, 99, 179-187 (2008).

Davis, T. A., Volesky, B., Vieira, R. H. S. F., Sargassum seaweed as biosorbent for heavy metals. Water Research, 34, 4270-4278 (2000).

Deng, L., Su, Y., Su, H., Wang, X., Zhu, X., Sorption and desorption of lead(II) from wastewater by green algae *Cladophora fascicularis*. Journal of Hazardous Materials, 143, 220-225 (2007).

Feng, D., Aldrich, C., Adsorption of heavy metals by biomaterials derived from the marine alga *Ecklonia maxima*. Hydrometallurgy, 73, 1-10 (2004).

Freundlich, H. M. F., Über die adsorption in lösungen. Z. Phys. Chem., 57, 385-470 (1906).

Halttunen, T., Salminen S., Tahvonen, R., Rapid removal of lead and cadmium from water by specific lactic acid bacteria. International Journal of Food Microbiology, 114, 30-35 (2007).

Ho, Y. S., McKay, G., Kinetic models for the sorption of dye from aqueous solution by wood. Process Safety Environmental Protection, 76, 183-191 (1998).

Kapoor, A., Viraraghavan, T., Cullimore, D. R., Removal of heavy metals using the fungus *Aspergillus niger*. Bioresource Technology, 70, 95-104 (1999).

Kim, S. U., Cheong, Y. H., Seo, D. C., Hur, J. S., Heo, J. S., Cho, J. S., Characterisation of heavy metal tolerance and biosorption capacity of bacterium strain CPB4 (*Bacillus* spp.). Water Science and Technology, 55, 105-111 (2007).

Lagergren, S., About the theory of so-called adsorption of soluble substances. K. Sven Vetenskapsakad. Handl. 24, 1-39 (1898).

Langmuir, I., The adsorption of gases on plane surfaces of glassi mica and platinum. Journal of American Chemical Society, 40, 1361-1403, (1918).

Liu, Y., Yang, S. F., Xu, H., Woon, K. H., Lin, Y. M., Tay, J. H., Biosorption kinetics of cadmium(II) on aerobic granular sludge. Process Biochemistry, 38, 997-1001 (2003).

Loukidio, M. X., Zouboulis, A. I., Karapantsios, T. D., Matis, K. A., Equilibrium and kinetic modeling of chromium(VI) biosorption by

Aeromonas caviae. Colloids and Surfaces A 242, 93-104 (2004).

Nasir, M. H., Nadeem, R., Akhtar, K., Hanif, M. A., Khalid, A. M., Efficacy of modified distillation sludge of rose (*Rosa centifolia*) petals for lead(II) and zinc(II) removal from aqueous solutions. Journal of Hazardous Materials, 147, 1006-1014 (2007).

Neamtu, M., Yediler, A., Siminiceanu, I., Macoveanu, M., Kettrup, A., Decolorization of Disperse Red 354 azo dye in water by several oxidation processes − a comparative study. Dyes and Pigments, 60, 61-68 (2004).

Puranik, P. R., Paknikar, K. M., Biosorption of lead and zinc from solutions using *Streptoverticillium cinnamoneum* waste biomass. Journal of Biotechnology, 55, 113-124 (1997).

Rao, V. V. B., Rao, S. R. M., Adsorption studies on treatment of textile dyeing industrial effluent, by flyash. Chemical Engineering Journal, 116, 77-84 (2006).

Silva, E. A., Cossich, E. S., Tavares, C. G., Cardozo, L., Guirardello, R., Biosorption of binary mixtures of Cr(III) and Cu(II) ions by *Sargassum* sp. Brazilian Journal of Chemical Engineering, 20, 213-227 (2003).

Tunali, Y., Karaca, H., Tay, T., Kivanç, M., Bayramoglu, G., Biosorption of Pb(II) from aqueous solutions by a fungal biomass in a batch system: Equilibrium and kinetic studies. Asian Journal of Chemistry, 21, 6015-6028 (2009).

Valdman, E., Leite, S. G. F., Biosorption of Cd, Zn, and Cu by *Saragssum* sp. waste Biomass. Bioprocess and Biosystems Engineering, 22, 171-173 (2000).

Wahi, R. K., Yu, W. W., Liu, Y. P., Meija, M. L., Falkner, J. C., Nolte, W., Colvin, V. L., Photodegradation of congo red catalyzed by nanosized TiO_2. Journal of Molecular Catalysis A: Chemical, 242, 48-56 (2005).

Xue, H. B., Stumm, V., Sing, L., The binding of heavy metal to algal surface. Water Research, 22, 917-922 (1988).

Yin, P., Qiming, Y., Jin, B., Ling, Z., Biosorption removal of cadmium from aqueous solution by using pretreated fungal biomass cultured from starch wastewater. Water Research 33, 1960-1963 (1999).