Asian Journal of Chemistry Vol. 22, No. 2 (2010), 1394-1402

Biosorption of Crystal Violet onto *Aspergillus wentii* From Aqueous Solution

BILAL ACEMIOGLU*, METIN KERTMEN†, METIN DIGRAK†,
M. HAKKI ALMA‡ and FAZILET TEMIZ†
Department of Chemistry, Faculty of Science and Arts, Kilis 7 Aralik University, 79000 Kilis, Turkey
Fax: (90)(348)8222351; E-mail: acemioglu@kilis.edu.tr

In this study *Aspergillus wentii*, a fungal biomass was utilized as a biosorbent for adsorption of crystal violet (a cationic dye) from aqueous solution. Biosorption experiments were performed as a function of various parameters, such as contact time, initial dye concentration, temperature, biosorbent dose and solution pH. Required time for maximum dye biosorption was 45 min. Biosorption was in consistent with Freundlich isotherm. It was seen that the amounts of the dye biosorbed onto *Aspergillus wentii* increased with increasing contact time, initial dye concentration, temperature, biosorbent dose and solution pH. Kinetic studies also showed that the bisorption process obeyed well the pseudo-second order kinetic model.

Key Words: *Aspergillus wentii*, Crystal violet, Dye, Biosorption, Isotherm, Kinetics.

INTRODUCTION

Wastewaters from textile, cosmetics, printing, dying, food colouring, paper-making industries are polluted by dyes. Dyestuffs from various factories are known to be carcinogenic and highly toxic to living beings. Therefore, it is required to remove the undesirable dye pollutions from wastewaters. For this purpose, many methods such as activated carbon adsorption, chemical oxidation, coagulation, ultra-filtration, electrochemical, *etc.* have been developed for treating dye-containing wastewaters[1]. Of these methods, activated carbon adsorption is outstanding to be an effective method for the water and wastewater treatment[2]. However, the use of activated carbon is not suitable for developing countries due to its high cost. Therefore, many investigators have studied the usage of low-cost materials such as waste orange peel[3], fly ash[4], perlite[5,6], peat[7], sawdust[8,9], as an adsorbent for the removal of various dyes from wastewaters. Some researchers have also extensively studied the removal of dyes by various biological biomasses such as *Aspergillus niger*[10], *Aspergillus foetidus*[11], *Phanerochaete chrysosporium*[12], *Rhizopus arrhizus*[13] and *Spirodela polyrrhiza*[14], recently.

†Department of Biology, Faculty of Science and Arts, Kahramanmaras Sütcü Imam University, 46100 Kahramanmaras, Turkey.

‡Department of Industrial Engineering of Forest, Faculty of Forest, Kahramanmaras Sütcü Imam University, 46060 Kahramanmaras, Turkey.

Aspergillus species such as *Aspergillus niger, Aspergillus foeditus* have various functional groups such as carboxyl, amino, phosphate and sulfonate. These groups act as excellent binding sites in the biosorption of heavy metals and various dyes[15]. In this work, the first *Aspergillus wentii*, a fungus was produced in laboratory and then it was dead by drying at 80 ºC in an oven. Dead fungal biomass prepared was used as a biosorbent for the removal of crystal violet (CV) from aqueous solution. The effects of contact time, initial dye concentration, biomass dose, solution pH and temperature on biosorption of dye were investigated. The isotherm and kinetic studies of the biosorption process were performed.

EXPERIMENTAL

Preparation of biomass: *Aspergillus wentii* (ATCC #10584) was maintained on Potato Dextro Agar slants and grown on a Potato Dextro Agar solid medium for 7 days. Then, the fungus was cultivated in a liquid growth medium composed of 30 g of glucose, 2.0 g of $(NH_4)_2HPO_4$, 1.0 g of K_2HPO_4, 0.5 g of KCl, 3 g of $CaCO_3$, 0.5 g of $MgSO_4 \cdot 7H_2O$ and 0.01 g of $FeSO_4 \cdot 7H_2O$ in 1 L of distilled water[16]. The pH of the growth medium was adjusted to 5.30 using 1 M HCl before autoclaving. Then 100 mL of the liquid medium was transferred to Erlenmeyer flasks of 250 mL and it was autoclaved at 121 °C for 15 min. Subsequently, the test fungus was inoculated to each flask, which was then grown on a biologic incubator at 25 °C for 14 days. After incubation, the biomass was harvested from the growth medium and thoroughly washed with distilled water. Living fungal biomass (200 g wet weight) was dried at 80 ºC in an oven for 6 h for obtaining dead fungus. The dead fungal biomass pellicles obtained was powdered by using a mortar and a pestle. The powdered biomass was sieved through a molecular sieve of 80 mesh (Retsch AS-200). It was then utilized as a biosorbent for batch sorption studies.

Preparation of dye solutions: Crystal violet, a cationic dye, was purchased from Merck and was used as received without further purification. The structure of the dye containing secondary amine groups is shown in Fig. 1. Physico-chemical characteristics of dye are also given in Table-1. Stock solution of crystal violet of 500 ppm was prepared with pure distilled water and then they were diluted to the desired initial concentrations. The pH values of the solutions were adjusted with diluted NaOH and HCl solution using a pH meter (WTW pH Meter 320, Germany).

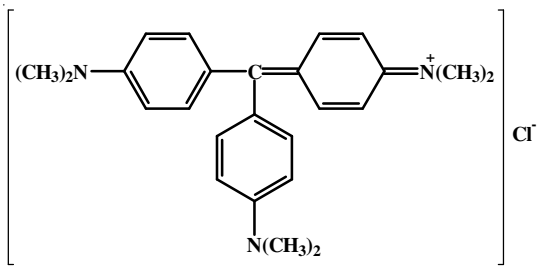

Fig. 1. Structure of crystal violet

TABLE-1
PYSICO-CHEMICAL CHARACTERISTICS OF CRYSTAL VIOLET

C.I. number	42555
C.I. name	Basic violet 3
Class	Triarylmethane
Chemical formula	$C_{25}H_{30}N_3Cl$
Molecular weight (g/mol)	408
Water solubility (g/L)	16 (at 25 ºC)

Batch biosorption studies: Batch biosorption experiments were performed using 0.075 g of the biomass (except for studies of biomass dose) with 25 mL of dye solution in 150 mL erlenmeyer flasks as functions of concentration, pH and temperature. The samples were shaken at 130 rpm in a temperature-controlled shaking incubator. After the desired contact time, the samples were taken from mixture and they were centrifuged at 3000 rpm for 15 min. Final absorbencies of dye were measured using a Perkin-Elmer UV/Vis spectrometer Lambda EZ 150 at a 590 nm which is a maximum absorbency. Then the concentrations of the samples were determined by using a standard curve.

The amounts of crystal violet removed by biomass were calculated using the equation:

$$q_e = (C_0-C_e)V/W \quad (1)$$

where C_0 and C_e are the initial and equilibrium concentrations of dye (in mg/L), W is the weight of biomass (g) and V is the volume of solution (L).

Desorption studies: The biomass used for the adsorption of initial dye concentration of 25 mg/L was separated from the dye solution. The dye-loaded biomass was washed gently to remove any unadsorbed dye with water. And then, dye-loaded biomass was stirred using a magnetic stirrer with 25 mL of pure water. The amount of dye desorbed was determined as mentioned before.

RESULTS AND DISCUSSION

Effect of contact time on biosorption: The effect of contact time on biosorption of crystal violet (CV) by *Aspergillus wentii* (biomass) was studied at varying times from 2 to 90 min for all initial dye concentrations at 25 °C. A very rapid biosorption is observed within a time of the first 5 min and thereafter the gradual increase in biosorption occurs with increasing contact time up to 45 min and at this time it is attained a maximum value of adsorption. After this time, sometimes very small decreases in the amounts of the dye adsorbed were observed. Therefore, the time of 45 min was fixed as the optimum contact time. For example; for initial dye concentration of 25 mg/L, while the amount of dye adsorbed per unit biomass is 4.15 mg/g (66.42 %) for 2 min, it is 5.65 mg/g (90.42 %) for 45 min. Similar results on equilibrium time have also been reported for the adsorption of congo red on various activated carbons in a work done by Kannan and Meenakshisundaram[17], for the

adsorption of an acid dye from aqueous solution by chitin in another work conducted by Annadurai and Krishnan[18] and for the adsorption of congo red from aqueous solution by fly ash in our previous work[4].

Effect of pH on biosorption: Effect of solution pH on biosorption of crystal violet by biomass was studied among pH 3 and 10. Effect of solution pH on biosorption for initial dye concentration of 25 mg/L at 25 °C is shown in Fig. 2. As shown in Fig. 2, the amount of crystal violet biosorbed per unit biomass increases with increasing of solution pH. For example, the amount of dye biosorbed by biomass increases from 3.80 (60.85 %) to 5.63 mg/g (90.08 %) while pH increases from 3 to 10. It is seen that basic pH is in favour of biosorption (Fig. 2). However, the most effective pH was 7. The maximum amount of dye adsorbed was 5.76 mg/g (92.15 %) at pH 7. After pH 7, the amount of biosorption was slightly decreased and it was 5.42 (86.66 %) and 5.63 mg/g (90.08 %) at pH 8 and 10, respectively. This situation may be attributed that the ionization of cationic crystal violet is different at various pH medium[19]. Therefore further experiments were performed at pH 7.

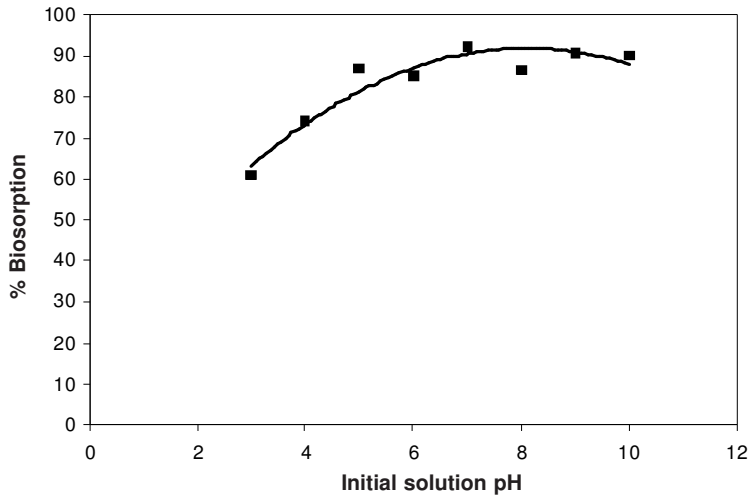

Fig. 2. Effect of initial solution pH on biosorption of crystal violet. (Conditions: Co = 25 mg/L, T = 25 ºC)

Effect of initial dye concentration on biosorption: Effect of initial dye concentration (initial concentrations of 10, 25, 35 and 50 mg/L) on biosorption was studied at 25 °C and pH 7. The results are demonstrated in Fig. 3. As shown in Fig. 3, the amount of the dye biosorbed increases with increasing initial dye concentrations. For example, the maximum amounts of the dye biosorbed are found to be 2.19, 5.65, 8.25 and 10.85 mg/g for initial dye concentrations of 10, 25, 35 and 50 mg/L, respectively. Furthermore, it is seen that the per cent of biosorption increases between 87.98 and 94.31 with increasing initial dye concentration from 10 to 50 mg/L.

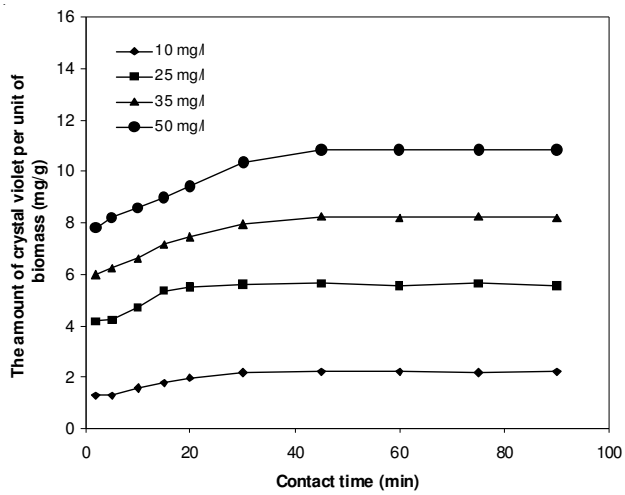

Fig. 3. Effect of initial dye concentration on biosorption of crystal violet as a contact time (Conditions: T = 25 ºC, pH = 7.0)

Effect of temperature on biosorption: The effect of temperature on biosorption was studied at the temperatures of 25, 30 and 40 °C for the initial concentration of 25 mg/L. The results were shown in Fig. 4. As shown in Fig. 4, the amount of dye biosorbed increases with increasing temperatures up to 15 min and then after the time this increase is slow and maintains slightly up to 45 min. End the of maximum contact time of 45 min, while the maximum amount of dye biosorbed by biomass is 5.65 mg/g (90.42 %) at 25 ºC, it is 5. 86 mg/g (93.89 %) at 40 °C. The temperature of 40 °C is optimum for maximum dye biosorption. This situation may be occurred as a function of the fast action of large dye molecules in higher temperature.

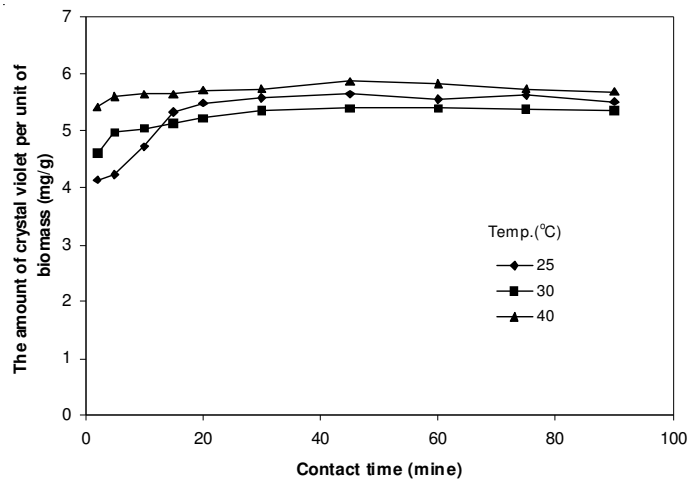

Fig. 4. Effect of temperature on biosorption of crystal violet as a function of contact time (Conditions: Co = 25 mg/L, pH = 7.0)

Effect of biomass dose on biosorption: The effect of biomass dose on biosorption was studied using amounts of 0.05, 0.075, 0.10, 0.20, 0.30 g for the initial concentration of 25 mg/L at 25 ºC, pH 7 and a contact time of 45 min. The percentage of the dye biosorbed increases with increasing biomass concentration. As shown in Fig. 5, while the amount of dye biosorbed is 75.67 % for biomass of 0.05 g, it is found to be 95.97 % in case of usage of 0.30 g biomass. This can be attributed to the existent of more sites on the surface of biomass with increasing biomass dose.

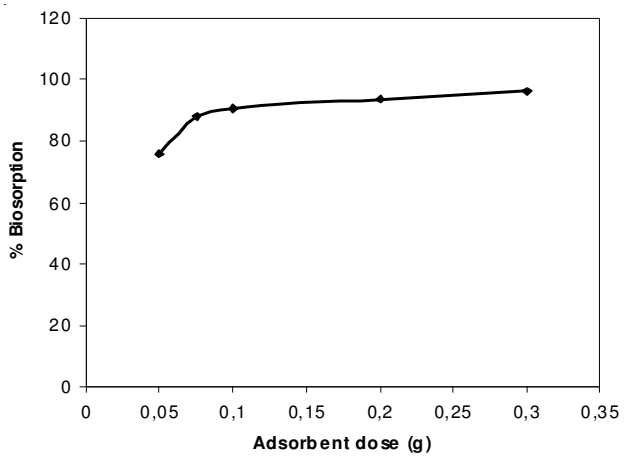

Fig. 5. Effect of adsorbent dose on biosorption of crystal violet (Conditions: Co = 25 mg/L, T = 25 ºC, pH = 7.0)

Adsorption isotherm: The adsorption equilibrium data were tested for Langmuir and Freundlich isotherms. The isotherm results indicate that the adsorption of crystal violet by biomass is in consistent with the Freundlich isotherm. Biosorption does not obey the Langmuir isotherm. Thus, Langmuir equation is not mentioned herein. Freundlich adsorption isotherm can be expressed as follows:

$$\ln q_e = \ln k_f + 1/n \ln C_e \quad (2)$$

where q_e is the amount of dye adsorbed at equilibrium time (mg/g), C_e is the equilibrium concentration of the dye in solution (mg/L). k_f and n are isotherm constants which indicates capacity and intensity of the adsorption, respectively. The plot of Freundlich isothrem is shown in Fig. 6. The plots of ln q_e against ln C_e at 25, 30 and 40 °C are in harmony with Freundlich adsorption model with correlation coefficients of 0.95, 0.95 and 0.98, respectively. The values of k_f and n are calculated from the slope and intercept of the plot ln q_e *vs.* ln C_e. These values are given in Table-2. It is seen that the n values are higher than 1. The fact that the values of n are in the range $1 < n < 10$ indicate the adsorption is favourable[8]. Similar results have been recorded for the adsorption of crystal violet on zeolite MCM-2219, for removal of various dyes from aqueous solutions by cellulosic waste orange peel[3].

TABLE-2
FREUNDLICH CONSTANTS OF BIOSORPTION OF CRYSTAL VIOLET ON BIOMASS AT DIFFERENT TEMPERATURES

Temperature (ºC)	n (L/g)	k (mg/g)	r^2
25	1.40	2.32	0.947
30	1.21	1.82	0.948
40	1.06	2.35	0.980

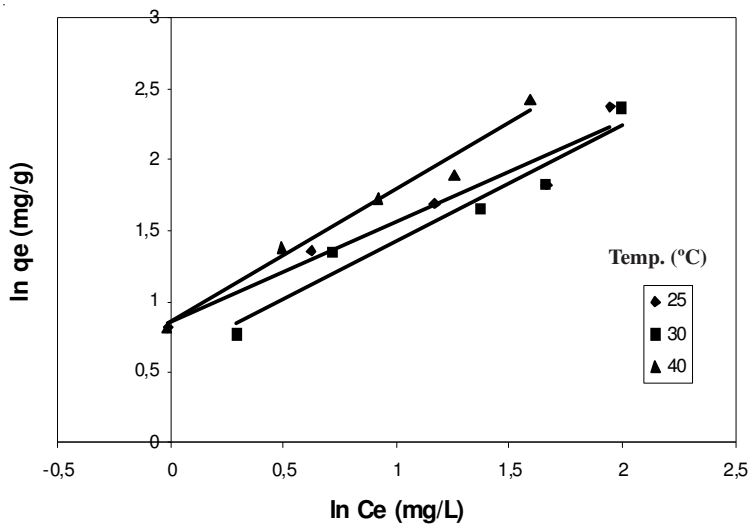

Fig. 6. Plots of Freundlich isotherms of biosorption of crystal violet at different temperatures

On the other hand, the fact that the biosorption obey Freundlich isotherm suggests that the surface of the biomass has some heterogeneity[20]. Thus isotherm has also no plateau and it may be suggested that no formation exists on a full monolayer of dye covering biomass surface.

Desorption of crystal violet: Herein, dye-loaded biomass (for each biomass treated with dye at contact times of 5.0-90 min) was stirred with 50 mL of pure water for 0.5 h, one by one. It was seen a very low desorption with pure water. It was determined that the dye desorbed was 6.50 % as average. The fact that desorption of dye from the biomass surface is very low indicates that the attachment of the dye on the biomass is not by weak bonds[4]. The low desorption of dye indicates that a chemical activation or chemisorption might be taken place between active sites of biomass and functional groups of crystal violet. And therefore, biosorption kinetic is also studied.

Kinetic study: Biosorption kinetic has been studied in terms of a pseudo-first order kinetic model of Lagergren[21], a pseudo-second order kinetic model of Ho and McKay[7,22] and intra-particle diffusion model of Weber and Morris[23]. It was seen

that biosorption obeyed well the pseudo-second order kinetic model. And therefore, there has been not presented the other kinetics equations herein. A pseudo-second order kinetic model of Ho and McKay[7,22] is as the following:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \tag{3}$$

where, k_2 is the rate constant for pseudo-second order reaction (g/mg min), q_e and q_t are the amounts solute sorbed at equilibrium and any time (in mg/g), respectively. The plot of pseudo-second order kinetic for the biosorbed of crystal violet onto *Aspergillus* is present in Fig. 7. The straight line plots of t/q_t *vs.* t are used to obtain the constants of pseudo-second reaction. Herein, the initial sorption rate is $h = k_2 q_e^2$ as mentioned by Ho and McKay[7]. q_e and k_2 were determined from slope and intercept of plot of t/q_t *vs.* t for an initial concentration of 25 mg/L at 25 ºC, respetively. q_e and k_2 were found to be 5.854 mg/g and 0.110 g/mg min, respectively. Initial sorption rate, h, was also 3.764 (mg/g min). The value q_e from pseudo-second order model was consistent with value q_e, which is the amount of dye sorbed at equilibrium obtained as experimental (5.651 mg/g). Moreover, biosorption process has a high correlation value of 0.99 from pseudo-second order equation. Both a high correlation value and agreement with experimental value of q_e obtained from the pseudo-second order equation indicate that biosorption process follows the pseudo-second order kinetic model. Thus, this situation indicates that a chemical activation can be occurred between crystal violet and biomass. Similar results have also been reported for the sorption of crystal violet onto zeolite MCM-2220, for adsorption of congo red by fly ash[4] and for sorption kinetics of methylene blue by perlite[5].

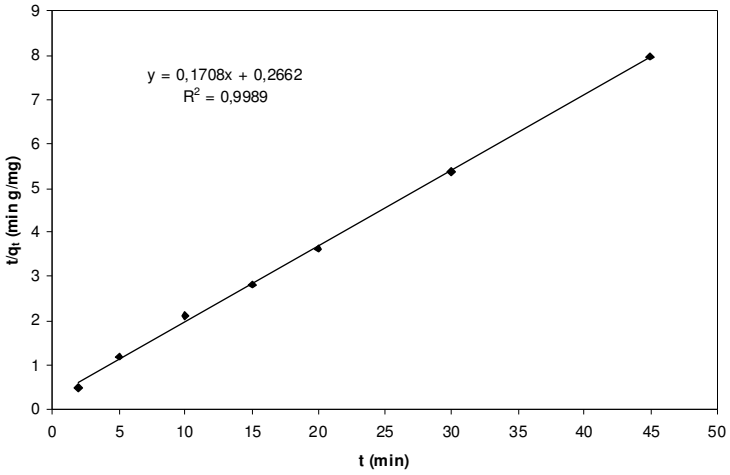

Fig. 7. Plot of pseudo-second order kinetics of biosorption of crystal violet by biomass (Conditions: Co = 25 mg/L, T = 25 ºC, pH = 7.0)

Conclusion

Aspergillus wentii was found to be used as a biosorbent for the removal of crystal violet from aqueous solution. Biosorption increased with an increase in initial dye concentration, temperature, biosorbent dose and solution pH. Required time for maximum dye biosorption was 45 min. Biosorption followed Freundlich isotherm. Biosorption kinetic obeyed well the pseudo-second order kinetic model. As a result of this study, it can be suggested that aspergillus wentii will be used as a biosorbent for the removal of another dyes from environment and aquatic mediums.

REFERENCES

1. M. Dogan and M. Alkan, *Chemosphere*, **50**, 517 (2003).
2. V.K. Garg, R. Gupta, A.B. Yadav and R. Kumar, *Bioresour. Technol.*, **89**, 121 (2004).
3. C. Namasivayam, N. Muniasamy, K. Gayatri, M. Rani and K. Ranganathan, *J. Biosour. Technol.*, **57**, 37 (1996).
4. B. Acemioglu, *J. Colloid. Interf. Sci.*, **274**, 371 (2004).
5. B. Acemioglu, *Chem. Eng. J.*, **106**, 73 (2005).
6. M. Dogan and M. Alkan, *J. Colloid. Interf. Sci.*, **267**, 32 (2003).
7. Y.S. Ho and G. McKay, *Chem. Eng. J.*, **70**, 115 (1998).
8. H.M. Asfour, O.A. Fadali, M.M. Nassar and M.S. El-Guendi, *J. Chem. Technol. Biotechnol.*, **35A**, 21 (1985).
9. V.K. Garg, R. Kumar and R. Gupta, *Dyes Pigm.*, **62**, 1 (2004).
10. Y. Fu and T. Viraraghavan, *Bioresour. Technol.*, **79**, 251 (2001).
11. S. Sumathi and B.S. Manju, *Enzyme Microb. Technol.*, **27**, 347 (2000).
12. M. Tatarko and A.J. Bumpus, *Wat. Res.*, **32**, 1713 (1998).
13. T. O'Mohany, E. Guibal and J.M. Tobin, *Enzyme Microb. Technol.*, **31**, 456 (2002).
14. P. Waranusantigul, P. Pokethitiyook, M. Krauatrachue and E.S. Upatham, *Environ. Pollut.*, **125**, 385 (2003).
15. A. Kapoor and T. Viraraghavan, *Bioresour. Technol.*, **63**, 109 (1998).
16. G.V. Sukhodolskaya, V.M. Nikolayeva, M.V. Donova, S.A. Gulevskaya, B.P. Baskunov, K.A. Koshcheyenko and K.F. Turchin, *Appl. Micobiol. Biotechnol.*, **53**, 695 (2000).
17. N. Kannan and M. Meenakshisundaram, *Water Air Soil Pollut.*, **138**, 289 (2002).
18. G. Annadurai and M.R.V. Krishnan, *Indian J. Chem. Technol.*, **4**, 217 (1997).
19. W. Shaobin, L. Huiting and X. Longya, *J. Colloid. Interf. Sci.*, **295**, 71 (2006).
20. G. Bayramoglu and M.Y. Arica, *J. Hazad. Mater.*, **143**, 135 (2007).
21. S. Lagergren, About the Theory of So-called Adsorption of Soluble Substances Kungliga Suenska Vetens kapsakademiens, Handlingar, Band 24, p. 1 (1898).
22. Y.S. Ho and G. McKay, *Process Biochem.*, **34**, 451 (1999).
23. W.J. Weber, *J. Saint. Eng. Div. ASCE*, **89**, 31 (1963).

(*Received*: 30 April 2009; *Accepted*: 28 October 2009) AJC-7996