Biosorption Characteristics of Ectomycorrhizal Fungal Mycelium for Anthracene[1]

YI HUANG, SHU-YING ZHANG, MING-JI LV, AND SHU-GUANG XIE*

College of Environmental Sciences and Engineering, Peking University, Beijing 100871, China

Objective To investigate the potential of *Gomphidius viscidus*, a kind of ectomycorrhizal fungi, for phytoremediation of anthracene in soil. **Methods** Absorptioe changes of micro-habitat were studied in detail. **Conclusion** Ectomycorrhizal plants have a strong potential for remediation of polycyclic aromatic hydrocarn characteristics of both active and inactivated mycelia. **Results** A high calculated adsorption capacity of 1 886.79 mg/g and 1 515.15 mg/g at 25 ℃, pH 6.0 for active and inactivated mycelia respectively, was obtained based on Langmuir model. The ANT biosorption was more ideally characterized by the Langmuir model than by the Freundlich model. The biosorption of anthracene to biomass was extremely fast and could be modeled with pseudo-second order adsorption kinetics. Moreover, ectomycorrhizal mycelia demonstrated a strong ability to adjust the physiological process to get adapted to the change of micro-habitat.

Key words: Biosorption; Phytoremediation; Anthrocene; Ectomycorrhizal fungi

INTRODUCTION

Mycorrhizas, as a widespread symbiosis between plant roots and fungi, are well-known for their facilitating plants' tolerance for nutrient exhaustion, excessive heavy metal, salinity of soil and so on[1]. Therefore, mycorrhizas have been paid increasing attention due to their possible involvement in phytoremediation, such as helping establish vegetation on heavy metal polluted sites[2-3]. Recently, increase of anthropic organic pollutants has led to the speculation that mycorrhizal plants may potentially be applied to degrade organic pollutants through phytoremediation[2,4], which has been supported by some laboratories studies. *In vitro*, various ectomycorrhizal fungi show an ability to degrade certain pesticides, PCBs, and PAHs[5-7].

Even though it is still unclear whether the capacity of ectomycorrhizal fungi is maintained and can be exploited in soil when the fungi take part in an active symbiosis, the response of mycelium *in vitro* to pollutants is referred to in the behavior of extra-mycelium of mycorrhiza in soil. In the symbiosis, extra-mycelium of ectomycorrizas is a "bridge" connecting the plant and soil. Pollutants firstly contact the extra-mycelia surface before being degraded or adsorbed by roots. The interaction process on the mycelia surface has potential effects on degradation, bioavailability, and absorption of organic pollutants in soil and then conducts detoxification for the host plant[8]. Especially, biosorption covers a number of metabolism-independent processes, including physical and chemical adsorption, complexation, chelation, and microprecipitation in the cell wall[9]. The characteristics of biosorption of ectomycorrhizal mycelia affect the fate of organic pollutants and their degradation rates, and may underlie the degradation of organic pollutants by ectomycorrhizas.

Therefore, this study was aimed to investigate mycelia adsorption characteristics for anthracene (ANT), a common POP in China[10-12] and the possible mechanism of degradation of organic pollutants by ectomycorrhizal fungi, and thus to exploit potential application of ectomycorrhizas to phytoremediation of PAH-contaminated soil.

MATERIALS AND METHODS

Mycelia Preparation and Medium

Ectomycorrhizal fungal *Gomphidius viscidus* (*G. viscidus*) was collected from an unpolluted mixed broadleaf-conifer forest in Beijing Western Hills and identified by Professor Zeng-Pu LEI (Laboratory of

[1]This research was supported by the National Natural Science Foundation of China (No. 50979002 and No. 20677003).

[2]Correspondence should be addressed to: Shu-Guang XIE, College of Environmental Sciences and Engineering, Peking University, Beijing 100871, China. Tel: 86-10-62751923. Fax: 86-10-62751923. E-mail: xiesg@pku.edu.cn

[3]Biographical note of the first author: Yi HUANG, Female, born in 1964, Ph. D., majoring in enverоment al biology and ecology.

0895-3988/2010
CN 11-2816/Q

Forest Pathology, Beijing Forestry University). Mycelia isolate was cultured on agar media[13] to be used as inoculums. To obtain enough mycelia for absorption experiments, *G.viscidus* mycelia on agar was inoculated into modified Kottke nutrient solution with pH 5.5, and incubated in a thermostatic chamber at 25 ℃ with continuous aeration. After two-week cultivation in liquid media, the mycelia were harvested by centrifugation and washed twice with deionized water. Half of the mycelia were autoclaved for inactivation at 121 ℃ for 20 min. Then the active and inactivated mycelia were mixed by stirring tempestuously for 5 min with a blender and ready for use in the following experiments. Biomass was measured by dry weight with mycelia being dried in oven at 60 ℃ for 24 h.

Adsorption Experiments

ANT of 99% purity (J&K Chemical, China) was dissolved in methanol to prepare stock solution at a concentration of 200 mg/L and stored at dark to avoid photodegradation. Solvents were of HPLC grade while other chemicals were of analytical reagent grade. Adsorption experiments were carried out in a batch mode.

Adsorption Time

200 mg of prepared active and inactivated mycelia were added to 250 mL glass Erlenmeyer flasks containing 200 mL ANT solution at the concentration of 150 mg/L. Then flasks were shaken at 130 rpm. Samples (1 mL) were taken at given time intervals (1, 2, 3, 4, 5, 8, 10, 15, 20, 25, 30, 40, 50, 60, 90, and 120 min) and the residual concentrations of ANT in liquids were analyzed immediately with HPLC.

Initial Concentration of ANT and Temperature Impact

Two sets of flasks containing 100 mL ANT solution at concentrations of 100, 120, 130, 140, 150, 160, and 170 mg/L respectively were prepared. Each flask in the first set received 100 mg active mycelia while each in the other set received 100 mg inactivated mycelia. Then the two sets of flasks were shaken at 130 rpm in an orbital shaker at 25 ℃. Also, another two sets were prepared in the same way and shaken at 35 ℃. The concentrations of the residual ANT in liquids were measured immediately after 16 h.

pH Impact

pH of ANT solution at the concentration of 150mg/L was adjusted to different values (4.0-12.0) by adding freshly prepared NaOH or HCl solutions. Then mycelia were added to the solution and the residual ANT in liquids was measured immediately after 16 h to investigate pH influence on the mycelia absorption rate.

Analytical Methods

0.45 μm pore size filter was used to remove mycelia from liquids. The analysis of ANT concentrations in the liquid phase was conducted with a HPLC apparatus (Shimadzu LC-10Avp, Agilent Technologies) equipped with a LC-10AT pump, a UV-detector, a Venusil PAH column (Agela Technologies) by using methanol-water (95:5) as the mobile phase at a flow rate of 1 mL/min. ANT in liquid was detected by absorbance at 254 nm. The injection volume was 20 μL and the retention time was 8.5 min.

The amount of adsorption at equilibrium, q (mg/g), was calculated as follows:

$$q=(C_0-C_e)\times V/m \quad (1)$$

Where C_0 and C_e are the initial and equilibrium liquid phase concentrations (mg/L) respectively; V is the volume of the solution; and m is the weight of the dry biomass used (g).

RESULTS AND DISCUSSIONS

Adsorption Time

Figure 1 shows the adsorption equilibrium of ANT with active or inactivated mycelia of *G. viscidus* at 25 ℃ with the initial dosage of 150 mg/L. As shown in this figure, although the adsorption capacity was different, the ANT adsorption of both active and inactivated mycelia was rapid in the initial period and the equilibriums were gradually reached within 40 or 60 min respectively for active and inactivated mycelia. As described by the pseudo-second-order kinetic model[14], the kinetics of ANT uptake by active and inactivated mycelia had a good correlation (Fig. 1; Table 1). These results indicated that active mycelia had a higher ANT adsorption capacity than inactivated mycelia.

Different reactions to ANT on the surfaces of active and inactivated mycelia might account for the difference in adsorption capacity. Without involvement in any biological process, the dead mycelia adsorption observed in the research should be the sole role of physical-chemical interaction[15-16]. The adsorption of ANT by living mycelia cells, however, was more diverse and possibly involved both biological adsorption and surface active reactions.

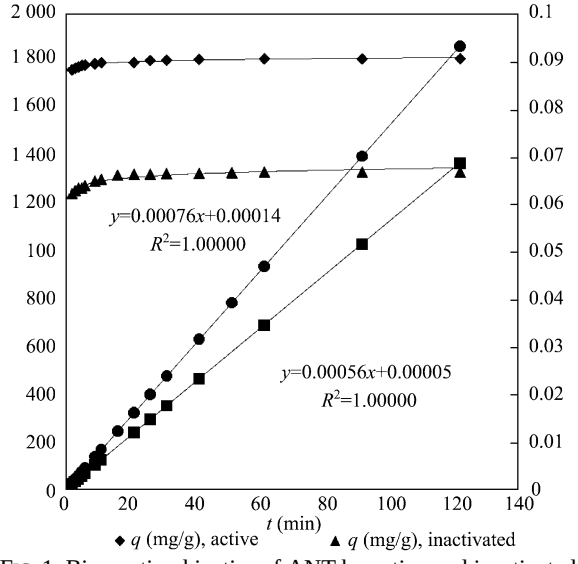

FIG. 1. Biosorption kinetics of ANT by active and inactivated mycelia.

TABLE 1

Pseudo-second Order Kinetics Model Parameters of ANT Adsorption on Mycelia (25 ℃, pH6.0)

Equation	$t/q=t/q_e+1/Kq_e^2$	
	Active	Inactivated
q_e(mg/g)	1 785.71	1 315.78
K(min^{-1})	6.27	4.13

Chung *et al.*[17] used brown seaweed Sargassum hemiphyllum as sorbent to remove phenanthrene from solution, and the loading capacity of biomass was 450.6 ± 4.4 mg/g at 25 ℃. William and Lisa[18] reported a K_p (the ratio of mass of phenanthrene adsorbed per gram of bacterial dry weight to mass of dissolved phenanthrene per liter solution) of 11-36 for five bacterial strains isolated from an activated sludge system, while in this study the K_p was 47-100 for active mycelia and 22-49 for inactivated mycelia at 25 ℃ respectively. Therefore, *G.viscidus* shows a strong biosorption capacity for PAHs, which makes it an effective tool to remediate PAHs polluted soil.

Impact of Initial Concentration of ANT on Sorption

The ANT adsorption capacity of either active or inactivated mycelia increased with the increase of initial concentration of ANT both at 25 ℃ and 35 ℃. It is in accordance with logarithmic form and its r-squares are up to 0.919 at least (Fig. 2).

Many researches reported that relatively high initial concentration of organic pollutants could accelerate biosorption[9]. The initial concentration might provide an important driving force to overcome all mass transfer resistances of adsorbate between the aqueous and solid phases, thus increasing the rate at which adsorbate molecules pass from the bulk solution to the adsorbent surface[19].

Impact of Temperature on Sorption

To determine the impact of temperature on the equilibrium loading, batch studies were carried out at different temperatures (Fig. 2). Figure 2 shows that the amount of ANT adsorbed at equilibrium was found to decrease with the rise of temperature. Many previous researches had also reported that the biosorption capacity of microorganism to lindane and 2, 4-dichlorophenol increased with the decrease of temperature[19-20]. A similar phenomenon was also observed in removal of lindane from aqueous solution with a fungal biosorbent[21]. It was due to the biosorption process involving an exothermically physical, rather than chemical mechanism[15, 22-24]. As shown in Fig. 2, the inactivated mycelia was more sensitive to temperature changes than the active one. Ectomycorrhizal mycelia may have a strong ability to adjust the physiological process and get adapted to the change of micro-habitat in a certain range[25-26].

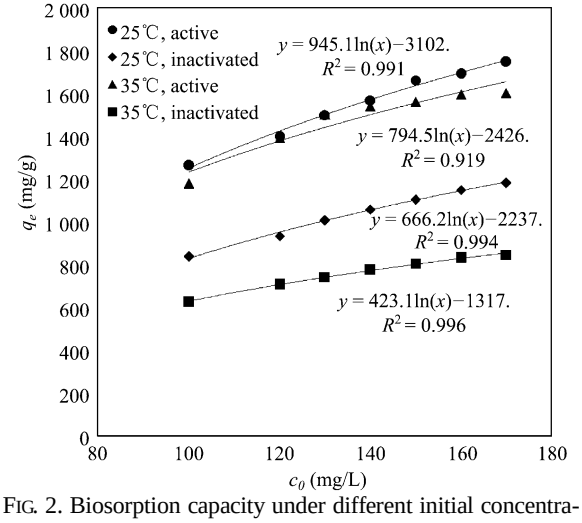

FIG. 2. Biosorption capacity under different initial concentrations of ANT at 25 ℃ and 35 ℃.

The Langmuir isotherm and Freundlich isotherm were applied to describe the adsorption characteristics of *G. viscidus*. The linearized Langmuir adsorption isotherms of ANT obtained at 25 ℃ and 35 ℃ were shown in Fig. 3. The values of Q^0 and b calculated from the slope and intercept of the plots were also shown in Table 2. High correlation coefficients (>0.99) were observed at all the temperatures studied, suggesting that the adsorption characteristics of *G. viscidus* were more ideally described by the Langmuir model. The increase of temperature lowered Langmuir constant Q^0, while the variation of constant b was not significant, indicating that lower temperatures favored the adsorption capacity.

TABLE 2

The Langmuir and Freundlich Isotherm Constants of ANT Adsorption on Mycelia at Different Temperatures (pH 6.0)

t (℃)		Langmuir Constants $C_e/q_e=C_e/Q^0+1/(Q^0b)$				Freundlich Constants $\lg(q_e)=\lg(K_F)+n\lg(C_e)$		
		Q^0 (mg/g)	b (l/mg)	R^2		K_F (mg/g)	n	R^2
25	Active	1886.79	0.28	0.993	Active	990.83	0.15	0.928
	Inactivated	1515.15	0.07	0.993	Inactivated	336.51	0.32	0.979
35	Active	1724.14	0.28	0.997	Active	870.96	0.17	0.741
	Inactivated	1190.48	0.03	0.999	Inactivated	163.30	0.37	0.987

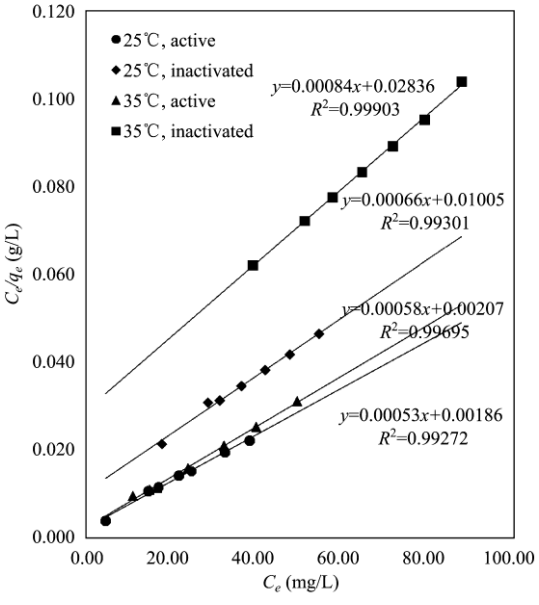

FIG. 3. Langmiur isotherm of active and inactivated mycelia at 25 ℃ and 35 ℃.

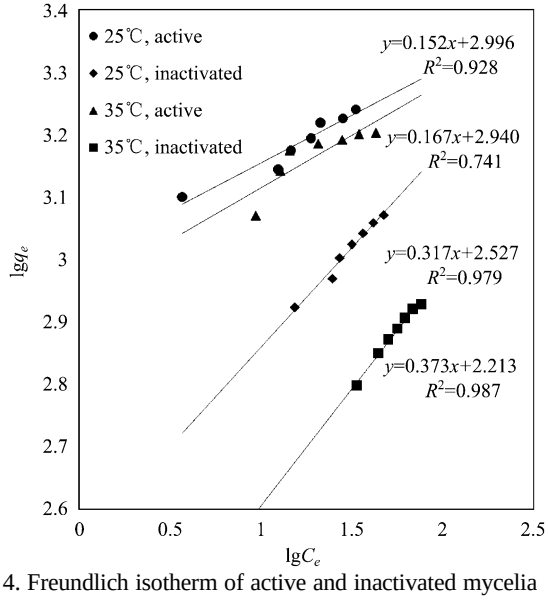

FIG. 4. Freundlich isotherm of active and inactivated mycelia at 25 ℃ and 35 ℃.

The linearized Freundlich adsorption isotherms of ANT obtained at different temperatures were shown in Fig.4. The values of K_F and n calculated from the plot were also shown in Table 2 with the correlation coefficients. The parameter K_F related to the sorption capacity increased with the decrease of temperature. The correlation coefficients of the Freundlich model suggested that it could not describe the biosorption equilibrium well except for active mycelia at 35 ℃ (R^2=0.741). Therefore, ANT biosorption was more ideally characterized by the Langmuir model than by the Freundlich model.

Inactivated mycelia values of Q^0 and K_F were constantly lower than those of active mycelia, indicating that the former had lower uptakes. Freundlich constant n was a measure of the deviation from linearity of the adsorption, and from these parameters, it was evident that the biosorption process of active mycelia had a greater deviation from linearity. And n<1 indicated a synergetic adsorption involving strong interactions between the molecules of adsorbates[27].

Impact of pH on Adsorption

To determine the impact of pH on ANT adsorption, equilibrium adsorption studies were carried out at different pH values (Fig. 5). A maximum amount of ANT was adsorbed at pH 5.5 for active mycelia and at pH 9.0 for inactivated mycelia. As active mycelia adsorption involved complex biological activities and the appropriate pH for *G.viscidus* was between 4 and 7, a slightly better adsorption was observed when the aquatic environment was weakly acidic.

Rao and Viraraghavan[28] used inactivated cells of *Aspergillus niger* to remove phenol from an aqueous solution by adsorption and revealed that the maximum removal of phenol occurred at an initial pH 5.1 for the biomass powder treated by sulfuric acid.

Other works on removing PCP by *Aspergillus niger* mycelia absorption demonstrated that PCP removal decreased with the increase of pH[29-30]. Some researches suggested that at a lower pH, more adsorbate functional groups were uncharged, and adsorbate had a lower solubility and was thus more adsorbable. Moreover, the lower pH resulted in high concentrations of protons, which neutralized the negative charge on both adsorbate and adsorbent, leading to enhanced sorption[31].

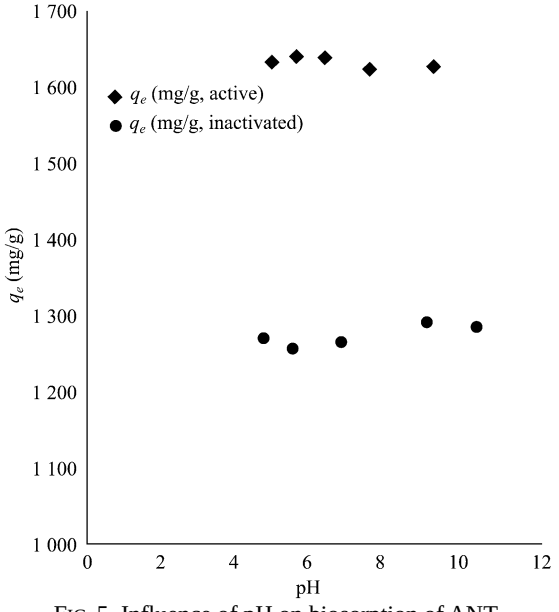

FIG. 5. Influence of pH on biosorption of ANT.

CONCLUSION

Adsorption of 1 886.79 mg/g and 1 515.15 mg/g of ANT by active and inactivated mycelia of *Gomphidius viscidus* implied ectomycorrhizal plants' powerful remediation potential for POPs polluted soil. Under the experimental conditions in this study, the process of equilibrium could be reached within 40-60 min and described well by the Langmuir isotherm model and pseudo-second order sorption kinetics. The adsorption capacity increased with an increase of the initial ANT concentration, but experiments at 25 ℃ and 35 ℃ indicated that the rise of temperature lowered the sorption capacity. pH only had a slight impact on sorption capacity. The marked differences of the parameters calculated from Langmuir and Freundlich models characterizing the sorption of active and inactivated mycelia, suggested that they differed in their sorption mechanisms.

REFERENCES

1. Huang Y, Zwiazek J (2008). Responses of ectomycorrhizal Populus tremuloides and Betula papyrifera seedlings to salinity. *Environmental and Experimental Botany.* **62,** 357-363.
2. Meharg A A, Cairney, J W G (2000). Ectomycorrhizasd extending the capacities of rhizosphere remediation? *Soil Biology and Biochemistry* **32,** 1475-1484.
3. Leyval C, Joner E J, del Val C, Haselwandter K (2002). Potential of arbuscular mycorrhizal fungi for bioremediation. In: Gianinazzi, S, Schüepp, H., Barea, J. M, Haselwandter, K. (Eds.), Mycorrhizal Technology in Agriculture: From Genes to Bioproducts. Birkhaüser, Basel, pp. 175-186.
4. Donnelly P K, Fletcher J S (1994). Potential use of mycorrhizal fungi as bioremediation agents. In: Anderson, T A Coats, J R. (Eds.), Bioremediation Through Rhizosphere Technology. American Chemical Society, Washington, pp. 93-99.
5. Donnelly P K, Fletcher J S (1995). PCB metabolism by ectomycorrhizal fungi. *Bulletin of Environmental Contamination Toxicology* **54,** 507-513.
6. Braun-Lüllemann A, Hüttermann A, Majcherczyk A (1999). Screening of ectomycorrhizal fungi for degradation of polycyclic aromatic hydrocarbons. *Applied Microbiology and Biotechnology* **53**, 127-132.
7. Huang Y, Zhao X, Luan S J (2007). Uptake and Biodegradation of DDT by 4 Ectomycorrhizal Fungi; *Science of the Total Environment* **385**, 235-241.
8. Jones M D, Hutchinson TC (1986). The effect of mycorrhizal infeetion on the response of betula-papyrifera to nickel and copper. *New Phytologist* **102**(3), 429-442.
9. Aksu Z (2005). Application of biosorption for the removal of organic pollutants: a review. *Process Biochemistry* **40**, 997-1026.
10. Tang L L, Tang H Y, Zhu Y G (2004). The distribution of polycyclic aromatic hydrocarbons in soil of Beijing. Journal of Nanjing PLA University of Science and Technology. **2**, 95-99.
11. Xiao R Wang, Q. H, Du, X.M, *et al*. (2006). The vertical distribution of polycyclic aromatic hydrocarbons in typical sewage irrigated soil. *Environmental Science* **19**(6)**,** 49-53.
12. Zhang T B,Wan H F, Yang G Y, *et al*. (2008). The distribution of polycyclic aromatic hydrocarbons in soil and vegetables of typical cities in Chu Chiang Delta. *Journal of Environmental Science* **11,** 2375-2384.
13. Kottke I, Guttenberger M, Hammpp R, *et al*. (1987). Actionspectrum for an enhancement of endogenous respiration by light in Chlorella.*Plant Physiol* **42**, 672-676.
14. Ho Ys, Mckay G (1999). Pseudo-second order model for sorption processes. *Process Biochemistry* **34** (5), 451-465.
15. Tsezos M, Seto W (1986). The adsorption of chloroethanes by microbial biomass. *Water Research* **20,** 851-858.
16. Vremont D L, Seigle-murandi F, and Benoit-guyod J (1998). Removal of pcnb from aqueous solution by a fungal adsorption process. *Water Research* **32**(12)**,** 3601-3606.
17. Chung M K, Martin T K T, Cheung K C (2007). Removal of aqueous phenanthrene by brown seaweed *Sargassum hemiphyllum*: Sorption-kinetic and equilibrium studies. *Separation and Purification Technology* **4,** 355-362.
18. William T, Lisa A C (1999). Evaluating the relationship between the sorption of PAHs to bacterial biomass and biodegradation. *Water Research* **33**(11)**,** 2535-2544.
19. Wu J Yu, H Q (2006). Biosorption of 2,4-dichlorophenol from aqueous solution by Phanerochaete chrysosporium biomass:Isotherms, kinetics and thermodynamics. *Journal of Hazardous Materials* **137**, 498-508.
20. Ju Y H, Chen T C, Liu J C (1997). A study on the biosorption of lindane. *Colloids and Surfaces B:Biointerfaces* **9(3-4)**, 187-196.
21. Young E Banks, C J (1998). The removal of lindane from aqueous solution using a fungal biosorbent: The influence of pH, temperature, biomass concentration, and culture age. *Environmental Technology* **19**(6)**,** 619-625.
22. Bell J P, Tsezos M (1987). Removal of hazardous organic pollutants by biomass adsorption. J. Water Pollut. *Control Fed*

59, 191-198.
23. Bell J P (1996). Biosorption of hazardous organic pollutants. Ph. D. thesis, McMaster University, Hamilton, Ont.
24. Huang Y, Tao S (2004). Influences of excessive Cu on photosynthesis and growth in ectomycorrhizal Pinus sylvestris seedlings. *Journal of Environmental Science* **16(3)**, 414-419.
25. Bell J P, Tsezos M (1988). The selectivity of biosorption of hazardous organics by microbial biomass. *Water Research* **22**, 1245-1251.
26. Huang Y Li T, Huang Z J, Fei Y H (2008). Ectomycorrhizal fungus induced change of Cu and Cd speciation in the rhizosphere of Pinus tabulaeformis seedlings. *Pedosphere* **18(6)**, 758-765; ISSN 1002-0160/CN 32-1315/Pref:806427.
27. Khezami L, Capart R (2005). Removal of chromium(VI) from aqueous solution by activated carbons: Kinetic and equilibrium studies. *Journal of Hazardous Materials* **123 (1-3)**, 223-231.
28. Rao J R, Viraraghavan T (2002). Biosorption of phenol from anaqueous solution by Aspergillus niger biomass. *Bioresource Technology* **85**, 165-71.
29. Wang J L, Qian Y Horan N, Ed Stentiford (2000). Bioadsorption of pentachlorophenol from aqueous solution by activated sludge biomass. *Bioresource Technology* **75**, 157-161.
30. Mathialagana T, Viraraghavan T (2009). Biosorption of pentachlorophenol from aqueous solutions by a fungal biomass. *Bioresource Technology* **100**, 549-558.
31. Zhou J L, and Banks C J (1993). Mechanism of humic-acid color removal from natural-waters by fungal biomass biosorption. *Chemosphere* **27**(4), 607-620.

(*Received January* 7, 2010 *Accepted August* 12, 2010)