

Original Article**Biosorption of Pentachlorophenol from Aqueous Solutions by *Aspergillus Niger* Biomass**Reza Shokoohi¹, Salah Azizi², Seyed Amir Ghiasian^{*3}, Ali Poormohammadi⁴

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ABSTRACT

Background: This study aimed to investigate the biosorption of pentachlorophenol on *Aspergillus niger* biomass as a method for removal of pentachlorophenol from aqueous solutions.

Methods: Modified *A. niger* biomass with NaOH was used to absorb the pentachlorophenol. The impacts of various experimental parameters like primary pentachlorophenol concentration, pH of the solution, contact time, and biomass dosage on the biosorption of pentachlorophenol were investigated.

Results: The correlation of contact time, pH and initial concentration with the biosorption of pentachlorophenol by *A. niger* biomass was statistically significant ($P < 0.001$). Pentachlorophenol removal increased with decreasing pH of the solution and the maximum efficiency was obtained at pH=3. The equilibrium adsorption capacity was increased from 4.23 to 11.65 mg/g by increasing initial pentachlorophenol concentration from 10 to 40 mg/L, while pentachlorophenol removal efficiency decreased from 87 to 55%. Both Langmuir and Freundlich isotherms efficiently described adsorption equilibrium of pentachlorophenol on *A. niger* biomass. Correlation coefficients for the second order kinetic model were almost equal to one.

Conclusion: *A. niger* biomass can be used to reduce the toxicity of aqueous solutions containing pentachlorophenol in acidic pH conditions.

Keywords: Absorption, *Aspergillus niger*, Carcinogenic, Pentachlorophenol.

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INTRODUCTION

Pentachlorophenol (PCP) is a phenolic compound and a priority pollutant extensively used in wood preservation industries, and as a herbicide, fungicide and molluscicide [1]. In recent years, PCP's concentration in water and soil has been increased significantly due to its wide range of application worldwide. There are millions of microsites throughout the world that are contaminated with PCP. PCP has been detected in a variety of environmental media, including rivers and streams, surface water systems and seawater [2]. It is broken down by sunlight and some bacteria after reaching the soil and then evaporates from the upper layers of soil or leaks into groundwaters [3].

This compound is one of the most important environmental pollutants due to its high water solubility and toxicity to humans, animals and aquatic life [4]. PCP is readily adsorbed through the gastrointestinal tracts, lungs, and skin both in humans and animals. Prolonged exposure to PCP in high risk environments has been extensively studied. Although a direct causal effect on many pathological findings have not been established but PCP is categorized as one of the potential carcinogenic compounds by some epidemiological studies. PCP has been classified in Group 2B by International Agency for Research on Cancer (IARC) [5]. In humans, malignant lymphoma and leukemia have been attributed to occupational exposure to this compound. In addition, PCP is toxic to the liver of rats, mice, and dogs. The

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toxicity associated with PCP even occurs at low doses and the major target organs of this toxicity are liver, kidney and central nervous system [6]. Due to its adverse effects and poor biodegradability, PCP must be removed from wastewaters before release into water bodies. Improving an efficient method for removing contaminants from water has always been a priority for investigators [7, 8].

The main objective of this study was to investigate the biosorption of PCP by *Aspergillus niger* biomass as an efficient technology for removing PCP from aqueous solutions. Furthermore, the effect of several parameters such as sorption kinetics, sorption isotherms and pH were also evaluated on the adsorption efficiency.

MATERIALS AND METHODS

Biomass Preparation

A. niger strain was bought from Persian Type Culture Collection of Iranian Research Organization for Science and Technology (PTCC 5210). This strain of was held on a potato dextrose agar. The initial *A. niger* biomass was obtained in a Sabouraud's broth medium using a shake flask method. The pH of the growth medium was adjusted to 5.0 with diluted HNO₃ solution. The fungus was grown in 250 ml Erlenmeyer flasks (containing 100 ml of the growth medium) at room temperature and agitated on a rotary shaker at 120 rpm for 3–4 d. *A. Niger* grew as pellicles and after five days, its biomass was harvested using filtering and successive washing with tap water.

In order to make the fungus nonviable the washed biomass was autoclaved for about 30 min at 121°C and 124 kPa (18 psi). The autoclaved biomass were washed again and dried out in an oven at 60 °C and then dry milled into a powder using a commonly available commercial coffee grinder. The powdery biomass was boiled in 0.5 N NaOH solutions for 15 min and after chemical conditioning. The final mixture was filtered using a cotton filter cloth and the filtered product was washed by distilled water [8]. The treated product was dried at 40–60 °C (in an oven) and was kept for further studies.

Batch Biosorption Studies

Deionized water was used to prepare the working solutions. Stock solution of PCP (1000 mg/L) was prepared with PCP crystals (99% purity). Different concentrations of PCP were

prepared from stock solutions by appropriate dilutions and 0.1 M HNO₃ and 0.1 M NaOH, were applied for pH adjustments of solutions. Studies of pH were conducted by shaking 100 mL of 25 mg/L PCP solution, separately, with 0.5 g of NaOH conditioned *A. niger* biomass. We prepared and used PCP concentrations of 10, 15, 20, 25, 30, 35 and 40 mg per in separate experiments. The samples were shaken for 2 h at 120 rpm in 125 mL conical flasks then sealed with parafilm and filtered after 2 h using a 0.45 micron cellulose acetate filter. An optimum pH was selected for further studies. Control samples were used to check the volatilization and adsorption of PCP to the glass walls of the conical flasks during the course of the experiments.

Kinetic Studies

Kinetic studies were conducted at an optimum pH of five with 250 mL of 15, 20, 25, 30 and 35 mg/L of PCP solutions and 0.5 g of biomass. The samples were collected at various contact times of 5, 10, 15, 30, 45, 60, 90 and 120 min. Upon collection, samples were filtered and extracted for PCP.

Isotherm Studies

Isotherm studies were conducted at pH 5 with 100 mL of PCP solution of varying concentrations of 10, 15, 20, 25, 30, 35 and 40 mg/L, in increments of 0.5 g of conditioned biomass. The samples were shaken for 2 h at room temperature (21 ± 1 °C) to ensure that equilibrium was reached, and then filtered and extracted for PCP.

PCP Estimation

For quantitative analysis of PCP, samples were separated by reverse phase High Performance Liquid Chromatography (HPLC) [9] (KNAUER Co, Germany, model Smartline Autosampler 3950 HPLC, A5005-1) with C18 column. The mobile phase was acetonitrile and distilled water (0.01 M, pH 6) in a ratio of 60:40 v/v and PCP was detected using a UV detector at 254 nm. PCP concentrations were measured with a calibration curve with a correlation coefficient of 0.998. Experiments were conducted in accordance with standard methods.

RESULTS

Every batch test was conducted twice and their means were represented by a point in the figures. No significant loss of PCP due to volatilization or adsorption to the glass walls of

the conical flasks was observed in the control studies.

Factors Influencing Biosorption of PCP

Effect of Contact Time and Initial PCP Concentration

As shown in Figure 1, the removal efficiency of PCP by *A. niger* biomass increased with increasing contact time and reached to the maximum value after 2 h. After this equilibrium time, the removal efficiency of PCP did not change. With increasing initial PCP concentrations, the equilibrium sorption capacity increased from 10 to 40 mg/L (16.92 mg/g for 10 mg/L vs. 45.46 mg/g for 40 mg/L), while the PCP adsorption efficiency showed an opposite trend (Figure. 2). The equilibrium adsorption capacity was increased from 4.23 to 11.65 mg/g by increasing initial pentachlorophenol concentration from 10 to 40 mg/L, while pentachlorophenol removal efficiency decreased from 87 to 55%.

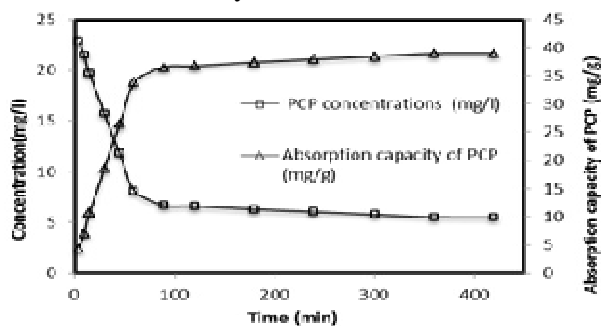


Figure 1. Pentachlorophenol concentration variations and absorption capacity over time (pH=5, amount of biomass 0.5 g in 250 ml, concentration 25 mg/l of pentachlorophenol).

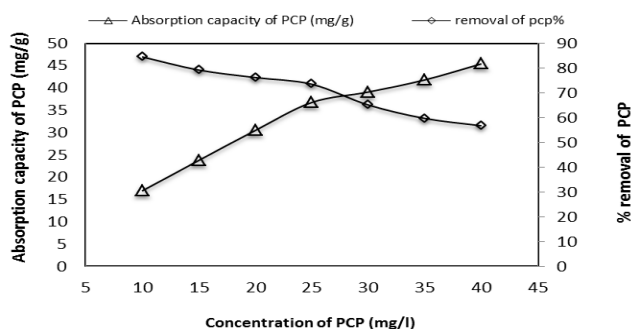


Figure 2. Percentage removal of pentachlorophenol at various initial pentachlorophenol concentration different by *A.niger* biomass. (pH= 5; contact time = 2 h. amount of biomass 0.5 g in 250 ml).

Effect of Initial PH

Figure 3 shows the effect of initial pH of the solution on the removal efficiency of PCP by *A. niger* at pH 3.0–8.0 and $25 \pm 1^\circ\text{C}$. Based on the results, the variation of solution's pH value in the range 3-8, had a significant effect on the PCP biosorption by fungi ($P < 0.001$). In general, the percentage removal of PCP decreased with an increase in pH for *A. niger* biomass (81.35% for pH=3 vs. 36.82% for pH=8).

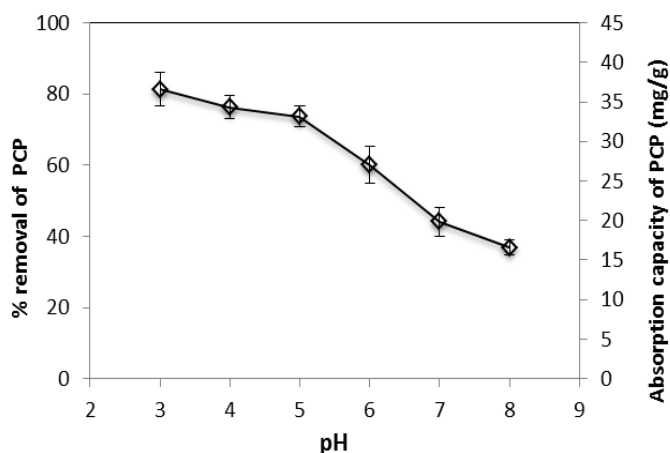


Figure 3. Percentage removal of pentachlorophenol at various pH different by *A.niger* biomass. (Initial pentachlorophenol concentration=25 mg/L; Contact Time= 2 h.).

Isotherm Studies

The linearized Langmuir and Freundlich adsorption isotherm models, the values of their model coefficients, and the regression values are listed in Table 1. The Langmuir model ($R^2 = 0.989$) could fit the data better than that of the Freundlich model ($R^2 = 0.96$) for removing PCP in the concentration range.

Kinetic Studies

To evaluate the biosorption kinetics of PCP, two kinetic models (the pseudo-first-order and pseudo-second-order models) were studied at different initial concentrations. Figure 4 and Table 1 show that PCP removal efficiency increased with increasing contact time. As a result, the second-order rate constant $k_{2,ad}$ decreased with the increase in PCP concentration.

Table 1. Isotherm models equations.

Isotherm models	Model parameters	R ²	References
Langmuir isotherm $\frac{1}{q_e} = \frac{1}{q_{ml}b} \left(\frac{1}{C_e} \right) + \frac{1}{q_{ml}}$	b=0.305 q _{ml} = 13.02	R ² = 0.9897	Langmuir ¹⁰
Freundlich isotherm $\log q_e = \log k + \frac{1}{n} \log c_e$	k _F =3.81 n=2.5	R ² = 0.96	Freundlich ¹¹

q_{eq}: mg of pentachlorophenol adsorbed/g of biomass; C_{eq}: equilibrium concentration of pentachlorophenol in the solution, mg/L; q_{ml}, b – the Langmuir model constants; K_F, n: the Freundlich model constants.

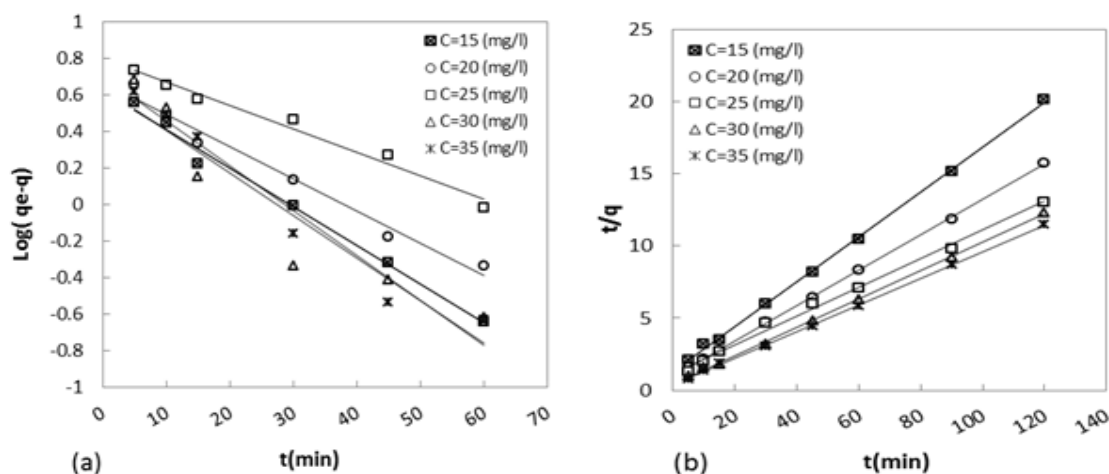


Figure 4. Linearized by pseudo-first order (a) and pseudo second-order (b) kinetic model for pentachlorophenol sorption by *A. niger* biomass at different initial concentration. (pH 5.0, biomass concentration: 0.5 g in 250 ml and agitation: 150 rpm).

DISCUSSION

Factors Influencing Biosorption of PCP, Sorption Time and Initial Concentration

The uptake of PCP by *A. niger* biomass increased with increasing sorption time. This can be related to larger surface area of the *A. niger* biomass being available at beginning for PCP adsorption [9]. Two hours was selected as the optimum retention time in this experiment and after that the PCP removal became steady. This finding was in line with previous studies [12-14], in which the times required for equilibrium were 2, 3 and 2 h, respectively. Different initial concentrations of PCP of 10 - 40 mg/L were chosen to compare the effect of initial PCP concentration on uptake capacity. The initial pollutant concentration is an important factor in the adsorption process, because a higher initial concentration of PCP increases the rate of its molecules binding to the biomass surface [15,16].

Therefore, a high initial concentration of PCP can enhance the adsorption process. This increase in adsorption capacity might result from a higher probability of collision between the PCP molecules and biomass.

Initial PH

The results of the pH studies showed that, the biosorption of PCP by *A. niger* biomass was dramatically influenced by solution pH variation (in the range 3–8) ($P < 0.001$). Solutions pH affects the surface property of *A. niger* biomass, and also affects PCP speciation in solution. PCP, the strongest acid of the phenol family has a pKa value of 4.75. PCP, in acidic pH, exists in the undissociated form, whereas at alkaline pH, PCP exists in the anionic form. Between these pH values, a combination of both forms are present [17]. The ionic and molecular species of PCP are hydrophobic, but the negative form is less so; consequently, sorption is ordinarily observed to

decrease where $\text{pH} > \text{pKa}$. We observed that an increase in pH value above 5 (pKa of PCP = 4.75), substantially decreased PCP removal capacity.

The surface charge on biomass is predominantly negative at pH 3.0–10.0 [8, 18]. At pH range of 7–10 there is generally electrostatic repulsion between anionic PCP molecules and biomass surface, which reduces absorption. Therefore, a decrease in pH may remove electrostatic barriers between the biomass and PCP, and facilitate biosorption [19]. Removal of PCP depends on pH values and an increase in solution pH resulted in a decrease in PCP removal [20]. PCP is represented by a combination of both molecular and anionic species in pH=5, therefore, we selected this figure as operating pH.

Isotherm Studies

Isotherm studies give important information on the equilibrium distribution of a solute between the liquid and solid phases within a system at a constant temperature. The equilibrium distribution of PCP between the biomass and aqueous phases can be represented mathematically by equations called sorption isotherms. Two models, i.e., Langmuir and Freundlich isotherms, are most frequently used for equilibrium modelling of biosorption processes [21, 22] investigated in our study.

The Langmuir isotherm is valid for monolayer adsorption onto the adsorbent surface. Freundlich equation is based on sorption on a heterogeneous surface. The values of q_m and b (the Langmuir model constants) were determined (Table 1), and were equal to 13.02 mg/g and 0.3051/g, respectively. The Freundlich constants K_f and n were 3.81 and 2.5, respectively. The magnitudes of K_f and n show easy separation of PCP from the aqueous solution and indicate optimal adsorption. Unlike Langmuir isotherm,

the Freundlich model is more extensively used but does not provide information about monolayer adsorption [8, 21]. Langmuir and Freundlich isotherms can both help in describing adsorption equilibrium of PCP on *A. niger* biomass [23, 24].

Kinetic Studies

Adsorption kinetic models are used to determine the adsorption rate and adsorption system design. The adsorption rate coefficients are important physicochemical parameters in the adsorption process [25]. In this study, the kinetic data were described by pseudo-first-order and pseudo-second-order models (Figure. 4 and Table 2).

The pseudo first-order rate expression of Lagergren model [26] is expressed as follows:

$$\log(q_{eq} - q) = \log q_{eq} - k_{1,ad} \frac{t}{2.303} \quad (1)$$

The plots of $\log(q_{eq} - q)$ are shown in Figure. 4. Table 2 shows the rate constants $k_{1,ad}$ and theoretical values of q_{eq} .

Pseudo second-order rate expression is expressed as follows:

$$\frac{t}{q} = \frac{1}{k_{2,ad} q_{eq}^2} + \frac{t}{q_{eq}} \quad (2)$$

For all cases, the correlation coefficients (R^2) for the second order kinetic model were closer to one and the theoretical values of q_{eq} also fitted well with the experimental data. The data did not fit the first-order model [27]. The second-order model [28] was used to depict chemisorption [29]. Coefficients with high correlations, indicated that possible chemisorption occurred between the modified biomass and PCP (Figure 4). Moreover, the pseudo second-order model described the experimental data well.

Table 2. Kinetic parameters of pseudo-first-order and pseudo-second-order equations for pentachlorophenol sorption on the aspergillus niger biomass.

C_0 (mg/L)	Lagergren-first order kinetic model			Pseudo-second order kinetic model			
	$K_{1,ad}$ (min^{-1})	$q_{eq,cal}$ (mg/g)	R^2	$K_{2,ad}$ (g/(mg min))	$q_{eq,cal}$ (mg/g)	R^2	$q_{eq,exp}$ (mg/g)
15	0.0488	4.23	0.9902	0.018	6.44	0.9992	5.97
20	0.04	4.69	0.9786	0.015	8.17	0.9998	7.65
25	0.029	6.3	0.9779	0.0083	10.09	0.9948	9.22
30	0.053	4.38	0.892	0.023	10.11	0.9994	10.06
35	0.056	5.06	0.9604	0.023	10.86	0.9997	10.48

q_e : sorption amount at equilibrium, mg/g; $K_{1,ad}$: rate constant of pseudo-first-order adsorption, min^{-1} ; $K_{2,ad}$: rate constant of pseudo-second-order adsorption, g/(mg min).

CONCLUSION

The main objective of this study was to investigate the PCP biosorption on *A. niger* biomass as a method for PCP removal from aqueous solutions. PCP removal increased with decreasing pH of the solution and maximum efficiency was obtained at pH 3. The equilibrium adsorption capacity was amplified by increasing initial PCP concentration from 10 to 40 mg/L of 4.23 to 11.65 mg/g, while the PCP removal efficiency decreased from 87 to 55%. Therefore, *A. niger* biomass can be used to absorb PCP from aqueous solutions in acidic pH conditions.

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