Effective biosorption of phenol by the thermophilic cyanobacterium *Phormidium* sp.

Sevgi Ertuğrul Karatay, Gönül Dönmez and Zümriye Aksu

ABSTRACT

The use of microbial biomass as biosorbent for phenol removal has been extensively studied, but its removal by biosorption by thermophilic cyanobacterium *Phormidium* sp. has not been investigated to the best of our knowledge. In the present study, some important parameters for biosorption process were optimized, starting with testing the effects of different pH values ranging from 1 to 12, and then initial phenol concentrations of 45.1, 115.3, 181.4, 243.3, 339.9 mg/L on phenol uptake. The efficiency of removal from aqueous solution was higher within the pH 6–8 range, with the maximum of 100% at pH 7 after 24 hours of adsorption time. The highest specific rate was observed as 165.1 mg/g in the presence of 339.9 mg/l initial phenol concentration. The Freundlich adsorption models were fitted to the equilibrium data, which indicated that phenol ions were favourably adsorbed by *Phormidium* sp.

Key words | biosorption, cyanobacterium, phenol, *Phormidium* sp., thermophilic

Sevgi Ertuğrul Karatay (corresponding author)
Gönül Dönmez
Department of Biology,
Faculty of Science,
Ankara University,
Beşevler, Ankara 06100,
Turkey
E-mail: *sertugrul@ankara.edu.tr*

Zümriye Aksu
Department of Chemical Engineering,
Hacettepe University,
Beytepe,
Ankara 06800,
Turkey

INTRODUCTION

Phenol is a toxic, carcinogenic and mutagenic organic pollutant, which is typically present in industrial effluents from refineries, coking operations, coal processing plants and petrochemical industries (Singh & Singh 2002; Dotto *et al.* 2013; Mohseni *et al.* 2016). As a highly toxic and accumulative priority pollutant, the treatment of phenol and its derivatives in industrial effluents has long been of interest (Priyadharshini & Bakthavatsalam 2016). In order to increase the case-specific efficiency of the treatment procedures, different removal methods such as distillation, extraction, liquid–liquid extraction, adsorption, catalytic oxidation, ozonation and advanced oxidation processes are generally employed (Busca *et al.* 2008). Since these techniques are generally time consuming, costly and elaborate, biological processes including biodegradation and biosorption have been receiving interest as emerging technologies in the treatment of phenol-containing effluents. The reports on the successful phenol removal procedures utilizing the aerobic biodegradation capability of some bacteria and fungi are worth mentioning here (Cintia *et al.* 2016; Long *et al.* 2016).

Among the biological processes, biosorption is known as a cost-effective and eco-friendly treatment method for some pollutants such as phenol, offering advantages such as ease of operation, high efficiency and minimization of chemical sludge, in addition to the use of natural, low-cost and renewable biosorbents, namely microorganisms (Singh & Balomajumder 2016). The biomass of various microorganisms such fungi, yeasts, microalgae, cyanobacteria and bacteria are very well known for their ability to eliminate a wide variety of organic pollutants by biosorption (Singh *et al.* 2016). Among the microorganisms tested for some decades, cyanobacteria are known to have some additional advantages over the other groups with respect to their larger surface area, greater mucilage volume, and consequent higher binding affinity (Wang *et al.* 1998; Gupta & Rastogi 2008). Their ability to grow at higher pH values and in thermophilic conditions are important in preventing contamination by other organisms. Their simple nutrient requirements offer suitability for economical large-scale cultivation, make them very attractive for wastewater treatment management (Abed *et al.* 2009).

Despite the fact that cyanobacteria have been successfully used as biosorbents in wastewater treatment systems, there are no studies in the literature on the determination of phenol biosorption by cyanobacterial biomass. So, we thought it worthwhile to study the possibility of treating phenol efficiently and economically in effluents using the

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biosorption capacity of the thermophilic cyanobacterium *Phormidium* sp.

MATERIALS AND METHODS

The microorganism and growth conditions

Phormidium sp. was obtained from the current culture collections of Ankara University, Faculty of Science Laboratories. The cells were cultured in 250 ml Erlenmeyer flasks containing 100 ml BG-11 fermentation medium (Rippka 1988). Erlenmeyer flasks were incubated under continuous illumination for 14 days in a plant growth chamber (Lab-line Biotronette). A series of batch culture experiments were performed, in unshaken flasks, illuminated by cool white fluorescent lamps emitting 2,400 lux of light at 30 °C (Sadettin & Dönmez 2007).

Preparation of biosorbent and phenol solutions

The biomass was harvested from the medium after the growth period, washed twice with distilled water, and then dried at 60 °C for 24 hours. For the biosorption studies, a weighed amount of dried biomass was suspended in 100 ml of double-distilled water and homogenized in a homogenizer (Janke and Kunkel, IKALabortechnick, Ultra Turrax T25, Germany) at 8,000 rpm for 20 min and then stored in the refrigerator. At the beginning of biosorption, 10 ml of dried biomass suspension was mixed with 90 ml of solution at the desired pH, containing a known concentration of phenol in an Erlenmeyer flask. All the final solutions contained 1.0 g/L of biosorbent.

Chromatographic-grade phenol was purchased from Riedel-de Haën (Germany); its stock solution was prepared by dissolving the exact quantity of phenol in distilled water and diluting it to a concentration of 10 g/L. Appropriate volumes of the stock solution were added to the medium.

Biosorption studies

Sorption studies were conducted in a routine manner by the batch technique in 250 ml Erlenmeyer flasks containing 100 ml phenol solutions containing the desired concentrations at the beginning of the adsorption period. The flasks were continuously agitated on a shaker at 100 rpm constant shaking rate for 48 hours to ensure the required equilibrium was reached.

The effects of pH on the phenol uptake were investigated for pH 1–12 with aqueous solutions containing approximately 50 mg/L phenol, and measuring the phenol residue in the solution at the end of 24 hours bioremoval period. In order to examine the effect of initial phenol concentrations and contact time on phenol biosorption, 100 ml water was prepared at the optimum pH value with the addition of 45.1, 115, 181, 243, 340 mg/L phenol concentrations and aliquots were taken from the flasks at 0, 4, 24, 36 and 48 hours. The flasks were incubated in a rotary shaker (New Brunswick Scientific Innova 4230) at 25 ± 1 °C. Samples (5 ml) were taken before and after mixing the biosorbent and phenol bearing solutions at definite time intervals. The samples were taken and centrifuged at $3{,}421 \times g$ for 5 min, and the supernatant was analyzed for the remaining phenol ions. Studies were performed at a constant temperature of 25 °C, selected as typical ambient temperature. All the biosorption experiments were repeated three times to confirm the results. The data were the mean values of three replicates.

Analytical methods

The concentration of phenol in the supernatant was determined by high performance liquid chromatography (HPLC) technique by using a Shimadzu, Japan chromatograph, C-18 column (250 mm × 4.6 mm inner diameter: 5 μm particle size). The mobile phase was acetonitrile: water (60:40 v/v) pumped at 1 ml/min, and the detection was performed with a UV detector set at 275 nm (Karatay & Dönmez 2014).

RESULTS AND DISCUSSION

Biosorption of pollutants by microorganisms is affected by several different factors, such as the surface properties of microorganisms and the physico-chemical characteristics of the subject solution including pH, initial pollutant concentration etc. (Aksu & Akpınar 2001). In the current study the effect of parameters such as initial pH, initial phenol concentration and contact time on biosorption process were examined at typical ambient temperature.

Effect of initial pH

As is well known, the initial pH plays an important role in biosorption capacity, due to the interactions between the

sorbate and sorbent couples, through its effect on the surface charge distribution of the biosorbent and also on the ionization potentials along with some minor effects on biosorption process.

The effect of pH on both phenol uptake rate and capacity by *Phormidium* sp. was studied at about 50 mg/L initial phenol concentration, in order to find the optimum pH for the effective phenol biosorption by this thermophilic cyanobacterium at various initial pH values ranging from 1 to 12. The variation of phenol removal with initial pH obtained from batch system studies is given in Figure 1. The efficiency of removal of phenol from the aqueous solution was significantly efficient only within the pH 6–8 range, with maximum removal at pH 7 of 100% at the end of 24 hours adsorption period. This is a noticeable advantage in terms of corrosion prevention, which could become a limiting factor in the phenol removal process (Busca *et al.* 2008). Similar observations have been reported previously; for example, the biosorption of phenols by wood-rotting fungus *Funalia trogii* pellets was investigated as a function of initial solution pH. Higher adsorption capacities were also found around pH 8 (Bayramoglu *et al.* 2009). In another study, *Pseudomonas putida* (MTCC 1194) was used as biosorbent for simultaneous biosorption and bioaccumulation of phenol and cyanide from the liquid phase. At pH 7 and 8, phenol removal was nearly constant for both free and immobilized cells. In the study it was found that percentage removal of phenol decreased after pH 8 and maximum removal of phenol was found at pH 7–8 for both free and immobilized cells.

Effect of initial phenol concentration

The changes in biosorption capacity of the biomass with time at 45.1, 115.3, 181.4, 243.3 and 339.9 mg/l initial phenol concentrations at 25 °C when the initial pH 7.0 is presented in Figure 2. As seen in this figure, the sorption capacity increased with the increasing initial phenol concentrations up to 339.9 mg/l. In the first two concentrations studied, the biomass adsorbed all phenol molecules at the end of 48 hours, but at higher concentrations the establishment of equilibrium points were observed in the same incubation period.

Uptakes which reached an equilibrium and the related phenol removal yields obtained at different initial phenol concentrations are given in Table 1. As seen from Table 1, in the presence of 45.1 and 115.3 mg/l phenol concentrations, phenol uptake yields obtained were 100%. The equilibrium point of phenol sorption by biomass increased with increasing initial phenol concentrations up to 339.9 mg/l, higher concentrations adversely decreased the removal efficiencies of the cyanobacterial biomass. The highest uptake of phenol was observed as 165.1 mg/g in the presence of 339.9 mg/l initial phenol concentration. In a study the kinetics and equilibrium of phenol biosorption of fungal biomass were studied from aqueous solution using the batch technique at an initial pH of 5.5. For free *Phanerochaete chrysosporium* biomass, the maximum equilibrium uptake was found at 2.82 and 5.25 mg/g for phenol concentrations of 25 and 50 mg/l, respectively (Farkas *et al.* 2013).

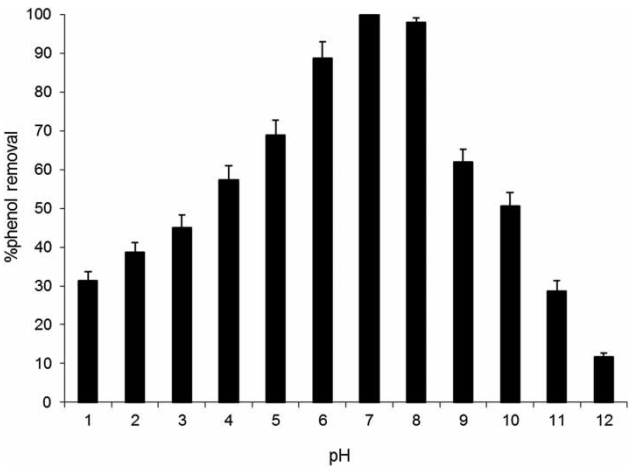

Figure 1 | The effect of initial pH value on the biosorption of phenol (C_o: 50 mg/L, temperature: 25 °C, agitation rate: 100 rpm, adsorption time 24 hours).

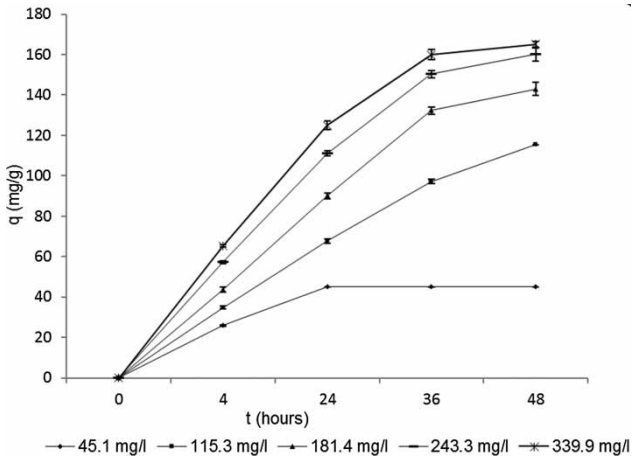

Figure 2 | The time course of changes in phenol uptake (q) by *Phormidium* sp. at different initial phenol concentrations (temperature: 25 °C, agitation rate: 100 rpm).

Table 1 | Uptake equilibria points and phenol removal yields obtained at different initial phenol concentrations (temperature: 25 °C, agitation rate: 100 rpm)

C_0 (mg/L)	q_{eq} (mg/g)	% phenol removal
45.0	45.0 ± 0.0	100 ± 0.0
115.3	115.3 ± 0.0	100 ± 0.0
181.4	143.0 ± 3.3	78.8 ± 1.8
243.3	160.1 ± 3.2	65.8 ± 1.3
339.9	165.1 ± 1.4	48.6 ± 1.4

q_{eq} = uptake of phenol by unit mass of biosorbent.

Equilibrium modeling of biosorption

Analysis of adsorption isotherms for phenol ions is important for developing an equation that may offer results suitable for design purposes, as conventionally used in such projects (Brouers & Al-Musawi 2015). Applying the experimental equilibrium data to Freundlich and Langmuir models, the linear isotherms obtained at 25 °C are presented in Figures 3 and 4, respectively. The model constants were determined as $K_F = 97.76$ and $n = 9.55$ for Freundlich model, and $Q^o = 175.0$ and $b = 0.119$ for Langmuir model. The parameters were summarized in Table 2.

As seen in Figure 3, the magnitude of K_F and n; the values of Freundlich constants showed the easy removal of phenol ions from wastewater with high adsorptive capacity of the dried biomass at pH 7. The data observed from Figure 2 also indicate that n is greater than 1, which means that phenol ions are favourably adsorbed by *Phormidium* sp. Values of Q^o and b for pH 7 have been calculated from the plots presented in Figure 4. The maximum

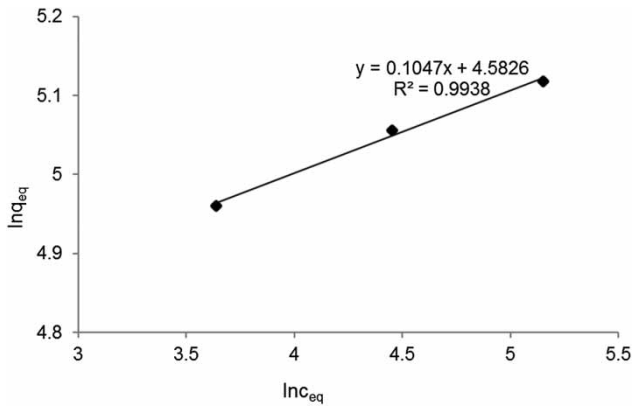

Figure 3 | The linearized Freundlich adsorption isotherms of phenol (temperature: 25 °C, agitation rate: 100 rpm). (ln q_{eq} = natural logarithm of the amount of adsorbed phenol per unit weight of biosorbent at equilibrium; ln c_{eq} = natural logarithm of unadsorbed phenol concentration in solution at equilibrium.)

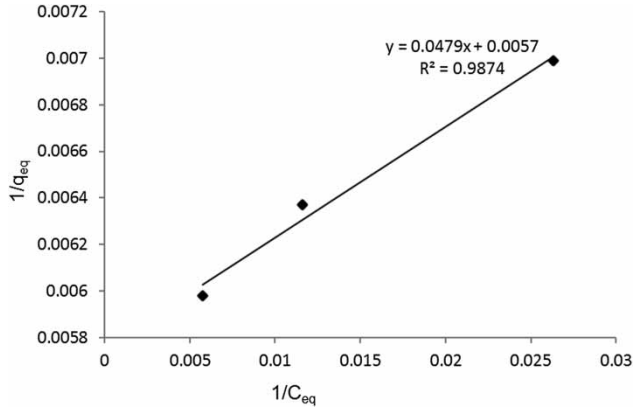

Figure 4 | The linearized Langmuir adsorption isotherms of phenol (temperature: 25 °C, agitation rate: 100 rpm).

Table 2 | A comparison of the Freundlich and Langmuir adsorption constants obtained from the Freundlich and Langmuir adsorption isotherms of phenol ions (temperature: 25 °C, agitation rate: 100 rpm)

Freundlich constants			Langmuir constants		
K_F	n	R^2	Q^o (mg/g)	b (L/mg)	R^2
97.76	9.55	0.99	175.0	0.119	0.98

capacity Q^o determined from the Langmuir isotherm, which defines the total adsorption capacity of the biosorbent for phenol ions. The magnitude of R^2 showed the equilibrium data fitted to Freundlich model much more accurately than that of Langmuir model. In a study, batch experiments were conducted to study the biosorption of phenolic compounds from aqueous solutions by non-living *Phanerochaete chrysosporium* mycelial pellets. The adsorption equilibrium of phenol from aqueous solutions by mycelial pellets could be well described with Freundlich equation (Wu & Yu 2006).

CONCLUSIONS

The primary goal of this study is to show the possibility of treating phenol efficiently and economically in effluents by using biosorption capacity of thermophilic cyanobacterium *Phormidium* sp. To our best knowledge this is the first report on the biosorption of phenol using thermophilic cyanobacterial biomass. The highest uptake of phenol was observed as 165.1 mg/g in the presence of 339.9 mg/l initial phenol concentration at pH 7. The magnitude of K_F and n and the values of Freundlich constants showed the easy removal of phenol ions from wastewater with high

adsorptive capacity of the dried biomass at pH 7. The data observed from the current work indicate that n is greater than 1, which means that phenol ions are favourably adsorbed by *Phormidium* sp.

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REFERENCES

Abed, R. M., Dobretsov, S. & Sudesh, K. 2009 Applications of cyanobacteria in biotechnology. *Journal of Applied Microbiology* **106**, 1–12.

Aksu, Z. & Akpinar, D. 2001 Competitive biosorption of phenol and chromium(VI) from binary mixtures onto dried anaerobic activated sludge. *Biochemical Engineering Journal* **7**, 183–193.

Bayramoglu, G., Gursel, İ., Tunali, Y. & Arica, M. Y. 2009 Biosorption of phenol and 2-chlorophenol by *Funalia trogii* pellets. *Bioresource Technology* **100**, 2685–2691.

Brouers, F. & Al-Musawi, F. T. 2015 On the optimal use of isotherm models for the characterization of biosorption of lead onto algae. *Journal of Molecular Liquids* **212**, 46–51.

Busca, G., Berardinelli, S., Resini, C. & Arrighi, L. 2008 Technologies for the removal of phenol from fluid streams: a short review of recent developments. *Journal of Hazardous Materials* **160**, 265–288.

Cintia, E. P., Melina, A. T., González, P. S., Magallanes-Noguera, C., Kurina-Sanz, M. & Agostini, E. 2016 Biotechnological tools to improve bioremediation of phenol by *Acinetobacter* sp. RTE1.4. *Environmental Technology* **37**, 2379–2390.

Dotto, G. L., Costa, J. A. V. & Pinto, L. A. A. 2013 Kinetic studies on the biosorption of phenol by nanoparticles from *Spirulina* sp. *LEB 18*. *Journal of Environmental Chemical Engineering* **1**, 1137–1143.

Farkas, V., Felinger, A., Hegedüsova, A., Dekany, I. & Pernyeszi, T. 2013 Comparative study of the kinetics and equilibrium of phenol biosorption on immobilized white-rot fungus *Phanerochaete chrysosporium* from aqueous solution. *Colloids and Surfaces B: Biointerfaces* **103**, 381–390.

Gupta, V. K. & Rastogi, A. 2008 Sorption and desorption studies of chromium(VI) from nonviable cyanobacterium *Nostoc muscorum* biomass. *Journal of Hazardous Materials* **154**, 347–354.

Karatay, S. E. & Dönmez, G. 2014 An economical phenol bioremoval method using *Aspergillus versicolor* and agricultural wastes as a carbon source. *Ecological Engineering* **73**, 224–228.

Long, Y., Yang, S., Xie, Z. & Cheng, L. 2016 Cloning, expression, and characterization of catechol 1,2-dioxygenase from a phenol-degrading *Candida tropicalis* JH8 strain. *Preparative Biochemistry & Biotechnology* **46**, 673–678.

Mohseni, M., Abdar, P. S. & Borghei, S. M. 2016 The highest inhibition coefficient of phenol biodegradation using an acclimated mixed culture. *Water Science and Technology* **73**, 1033–1040.

Priyadharshini, S. D. & Bakthavatsalam, A. K. 2016 Optimization of phenol degradation by the microalga *Chlorella pyrenoidosa* using Plackett–Burman Design and Response Surface Methodology. *Bioresource Technology* **207**, 150–156.

Rippka, R. 1988 Isolation and purification of cyanobacteria. *Methods Enzymology* **167**, 3–27.

Sadettin, S. & Dönmez, G. 2007 Simultaneous bioaccumulation of reactive dye and chromium(VI) by using thermophil, *Phormidium* sp. *Enzyme and Microbial Technology* **41**, 175–180.

Singh, N. & Balomajumder, C. 2016 Simultaneous biosorption and bioaccumulation of phenol and cyanide using coconut shell activated carbon immobilized *Pseudomonas putida* (MTCC 1194). *Journal of Environmental Chemical Engineering* **4**, 1604–1614.

Singh, N. & Singh, J. 2002 An enzymatic method for removal of phenol from industrial effluent. *Preparative Biochemistry& Biotechnology* **32**, 127–133.

Singh, M., Srivastava, P. K., Jaisvali, V. K. & Kharwar, R. N. 2016 Biotechnology: trends and applications. In: *Biotechnology: Trends and Applications* (R. Singh & M. Trivedi, eds). Studium Press LLC, Houston, Texas, USA, pp. 179–214.

Wang, T. C., Weissman, J. C., Ramesh, G., Varadarajan, J. & Benemann, R. 1998 Heavy metal binding and removal by *Phormidium*. *Bulletin of Environmental Contamination and Toxicology* **60**, 739–744.

Wu, J. & Yu, H.Q. 2006 Biosorption of phenol and chlorophenols from aqueous solutions by fungal mycelia. *Process Biochemistry* **41**, 44–49.

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