

Stimulation of Phenol Removal Efficiency of *Aspergillus versicolor* by Surfactants, a Promising Way to Treat Phenol-Containing Waste Waters

Sevgi Ertuğrul Karatay · Ülküye Dudu Gül ·
Gönül Dönmez

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Abstract The effect of the cationic surfactant, dodecyl trimethyl ammonium bromide (DTAB), on phenol bioremoval efficiency of an *Aspergillus versicolor* strain was examined. The strain was grown in mineral salt (MS) medium and the effect of DTAB was investigated as a function of different pH values, phenol and surfactant concentrations. The effect of pH was tested within the range of 4–7 and the maximum bioremoval was found at pH 4. Initial phenol concentrations investigated ranged from 100 to 600 mg/L, and the effects of surfactant concentrations on the removal were tested with 0, 0.25, 0.5 and 1 mM DTAB, which showed that 0.5 mM surfactant was the most effective concentration. The maximum bioremoval rates found after 72 h incubation were 99.48 and 99.15 % in 100 and 200 mg/L initial phenol-containing samples, respectively, where the phenol removal capacity of the fungus was only 142.373 mg/g in the DTAB blank samples. The maximum phenol uptake capacity of 267.162 mg/g was measured in the presence of 0.5 mM DTAB at 200 mg/L initial phenol concentration. These results showed that DTAB considerably increased the bioremoval efficiency of the strain tested at relatively lower phenol concentrations. The feasibility of this bioremoval method for industrial wastewater treatment is discussed.

Keywords *A. versicolor* · Bioremoval · Phenol · Cationic surfactant · Wastewater treatment

S. Ertuğrul Karatay (✉) · G. Dönmez
Department of Biology, Faculty of Science, Ankara University,
Beşevler, 06100 Ankara, Turkey
e-mail: sertugrul@ankara.edu.tr

Ü. D. Gül
Vocational School of Health Services, Bilecik Şeyh Edebali
University, Gölümbe, 11210 Bilecik, Turkey

Introduction

Phenols, which are produced by pulp and paper, petroleum and petrochemical, coal conversion, and phenol-producing industries, are considered as priority pollutants due to their harmful effects on organisms at low concentrations [1]. According to the US Environmental Protection Agency (EPA), the acceptable level of phenol in effluents is less than 1 mg/L [2]. Hence, different water treatment technologies, such as oxidation [3], adsorption [2] and bioremoval by microorganisms [4], are used to remove phenolic pollutants.

Biological wastewater treatment is often considered as the most economical and ecofriendly process compared to the physical and chemical ones [5]. It is reported that some microorganisms such as *Thauera aromatica* K172 can use phenol as a carbon and energy source [6]. After reviewing the literature on the removal of phenol, it was found that most of the work was done using bacterial strains and some yeasts [7–9]. In addition to this, the use of fungi such as *Phanerochaete chrysosporium* in the removal of environmental pollutants is a field of interest in environmental science and engineering [10, 11]. In fact, there are some studies that report experimental results showing effective phenol removal by some fungal strains [12, 13].

The surface-active agents called surfactants are amphiphilic organic molecules that reduce interfacial tension at low concentrations [14]. This phenomenon can only be observed in their diluted solutions containing monomeric molecules. As the surfactants are widely used by various sectors such as petroleum, food, cosmetic and textile industries [15], they typically represent one of the largest fractions in industrial wastewater effluents. Some recent studies focused on using surfactants to enhance efficiency of wastewater treatment techniques such as adsorption [16].

Another example is bioremoval of dyes by *A. versicolor* strains, in the presence of the cationic surfactant dodecyl trimethyl ammonium bromide (DTAB), which was also used to enhance decolorization of reactive dyes [17]. Several researchers investigated the chemical removal of phenols by using the surfactants to enhance the process [18–20]. There was also a study reporting the stimulatory effect of surfactants on phenol removal by a yeast named *Candida tropicalis* [21]. When it comes to enhancement of fungal phenol removal by surfactants, the number of published reports is limited and the reports are on certain selected aspects of the process. The present work is aimed to investigate the effect of the cationic surfactant DTAB on removal of phenol by the filamentous fungus species *Aspergillus versicolor*. The purpose of the present study is to demonstrate the technical feasibility of a simple and low-cost procedure increasing the phenol removal capacity of the fungi to a practically applicable level. To the best of our knowledge, this is the first report attempting to investigate the effects of the cationic surfactant DTAB on phenol removal by *Aspergillus versicolor*.

Experimental

Chemicals and Microorganism

The filamentous fungus *Aspergillus versicolor* strain used in the experiments was previously isolated in our laboratory [22]. The isolate was cultivated in a rotary shaker by rotating at 100 rpm and 30 °C for 144 h in a 250-ml flask containing 100 ml of mineral salt (MS) medium with 1 g/L glucose. The composition of MS medium was KH_2PO_4 , 1.7 g/L; $(\text{NH}_4)_2\text{SO}_4$, 2.69 g/L; MgSO_4 , 0.2 g/L and CaCl_2 , 0.03 g/L [23].

Chromatographic grade phenol was purchased from Riedel-de Haen; glucose and inorganic salts used in preparing the microbial growth media were obtained from Merck. The cationic surfactant dodecyl trimethyl ammonium bromide (DTAB) $[\text{CH}_3(\text{CH}_2)_{11}\text{N}(\text{Br})(\text{CH}_3)_3]$, MW: 308.34 g/mol) was supplied by Fluka. A 1.0 g/L stock surfactant solution was prepared by dissolving the weighed amount in the necessary volume of double-distilled water. Appropriate volumes of the stock solutions were added to the media.

Phenol Removal Experiments

The effect of initial pH on phenol removal was investigated at pH 4, 5, 6 and 7 in the medium supplied with 100 mg/L phenol and 0.5 mM (154.2 mg/L) DTAB. To determine the effect of initial phenol concentrations on phenol removal, the cultures were grown in MS media containing 100, 200, 400 and 600 mg/L phenol and 0.5 mM DTAB at pH 4. The

effect of contact time on phenol removal was examined at 200 mg/L phenol in the presence (0.5 mM DTAB) and absence of surfactant. The removal rates were followed in DTAB-containing samples and the blanks for 168 h. To examine the effect of surfactant concentration on bioremoval, *A. versicolor* was inoculated into MS media containing 0.25, 0.5 and 1 DTAB and 200 mg/L phenol. The studies were performed at a constant temperature of 30 °C [13], which was an optimal temperature for fungal growth.

Uninoculated Erlenmeyer flasks containing phenol and surfactant were used as control samples to observe any reactions of the media with the phenol and surfactant.

Analytical Methods

From each of the flasks, 4 ml aliquots were taken and were centrifuged at $3,421 \times g$ for 10 min to remove suspended biomass, before determination of phenol concentration in the supernatant by high performance liquid chromatography, using a Shimadzu (Japan) chromatograph, C-18 column (250 mm \times 4.6 mm inner diameter: 5 mm 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.

For the measurement of microbial growth, dry weight of the fungal biomass was measured at the end of the incubation period, filtering the contents of each of the flasks through pre-weighed filter paper and washing twice with distilled water. The liquid medium was removed and the filtered biomass was dried to a constant weight at 80 °C. Dry weight was expressed in terms of g of biomass per liter of culture. Centrifugation was performed using a Hettich EBA12 model centrifuge (Germany). The experiments were set up in a completely randomized design with three replicates.

Phenol removal by *A. versicolor* in the presence of surfactants was investigated as a function of initial values of pH, phenol and surfactant concentrations. The percentage of phenol removal was calculated by using its initial (C_o) and final (C_f) concentrations from the equation (1) below:

$$\text{Phenol Removal (PR \%)} = [C_o - C_f / C_o] \times 100 \quad (1)$$

Phenol removal capacity (q_m) is the concentration of phenol found in the biomass and its value can be calculated on the mass balance principle (Equation 2):

$$q_m = (C_o - C_f) / X_m \quad (2)$$

In these two equations, q_m (the maximum specific phenol degradation) represents the maximum amount of phenol per unit dry weight of microbial cells (mg/g), X_m is the measure of maximum dried cell mass (g/L), C_o and C_f are the initial and final concentrations (mg/L), respectively.

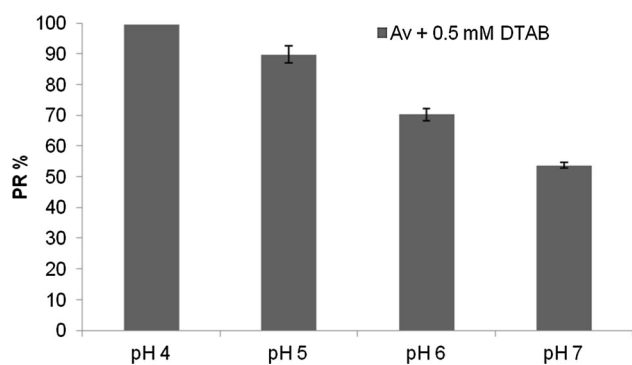


Fig. 1 Effect of initial pH on total phenol removal (PR %) by *A. versicolor* (Av) in MS with 100 mg/L phenol and 0.5 mM DTAB (T: 30 °C; incubation period: 72 h)

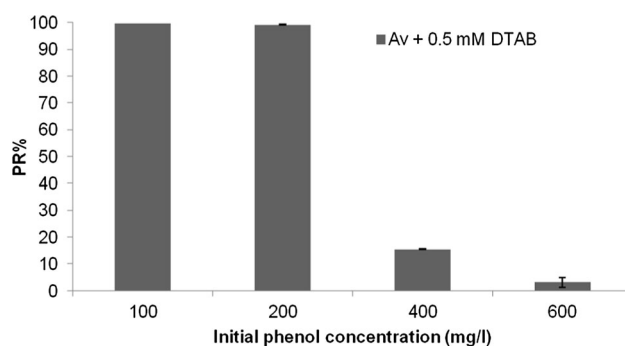


Fig. 2 Effect of initial phenol concentration on total phenol removal (PR %) by *A. versicolor* (Av) in MS with 0.5 mM DTAB (T: 30 °C; incubation period: 72 h)

Results and Discussion

Effect of pH

The effect of medium pH value on phenol removal was examined at the end of a 72 h incubation of the samples containing ~100 mg/L initial phenol concentration. Four different pH values (4, 5, 6 and 7) were tested to find the most appropriate pH for the highest phenol removal by *Aspergillus versicolor* in the presence of 0.5 mM DTAB (Fig. 1). Garcia et al. [24] showed that *A. niger* and *A. terreus* removed 76 and 64 % of the total phenol at the end of 115 and 112 h, respectively, at pH 4. The effect of pH on phenol removal by *A. versicolor* was investigated in the absence of surfactant previously, and it was found that the optimal pH was 6 (unpublished). The addition of surfactant changed the optimal pH value. The maximum phenol removal found in the present study was 99.48 %. This level of bioremoval was also obtained at pH 4, but in the presence of DTAB.

Effects of Bioremoval Period, Phenol and Surfactant Concentrations

The phenol removal rates in terms of percentage of 100, 200, 400, and 600 mg/L initial concentrations by *A. versicolor* in MS media are shown in Fig. 2. The removal rate decreased with increases in phenol concentration as expected. The maximum removal rates were 99.48 and 99.15 % at concentrations of 100 and 200 mg/L phenol, respectively, after 72 h incubation, in the presence of 0.5 mM DTAB (Fig. 2). *A. versicolor* could tolerate phenol concentrations up to 600 mg/L. The specific phenol removals by *A. versicolor* at 200, 400 and 600 initial concentrations are shown in Table 1. The fungus was negatively affected by the toxic effect of phenol above the 600 mg/L

Table 1 Effect of DTAB and phenol concentration on maximum specific phenol removal capacity (q_m) of *A. versicolor* (Av)

Initial phenol concentration (mg/L)	Initial DTAB concentration (mM)	q_m (mg/g)
200	0	142.373
	0.25	144.96
	0.5	267.162
	1	153.027
400	0.5	66.632
600	0.5	19.85

concentration. Strains of *Pseudomonas* sp. have been the most widely used bacteria for phenol biodegradation [25]. El-Naas et al. [25] examined the effect of phenol concentration ranging from 5 to 150 mg/L on phenol removal by *Pseudomonas* sp., and showed that phenol biodegradation by bacteria was decreased due to the toxic effect of increasing phenol concentration from 75 to 150 mg/L. Santos and Linardi (2004) tested 30 newly isolated filamentous fungal strains for phenol tolerance [13]. They reported that fifteen strains of the genera *Fusarium*, *Aspergillus*, *Penicillium* and *Graphium* tolerated the addition of 10 mM phenol in the medium, but not all of the spp. of the genera were tested. It was assumed that fungal strains have more tolerance than bacteria at high concentrations of phenol. The effect of phenol concentrations on microbial growth was studied previously [25–27]. The increase in phenol concentration resulted in an augmentation of phenol removal by microbial strains, but further increase in phenol concentrations reduced the removal rate due to the inhibitory effect of phenol on microbial growth [26, 27]. The phenol uptake in the presence of 0.5 mM surfactant was measured as 267.162, 66.632 and 19.85 mg/g at 200, 400 and 600 mg/L phenol concentrations, respectively (Table 1). Phenol removal capacity was

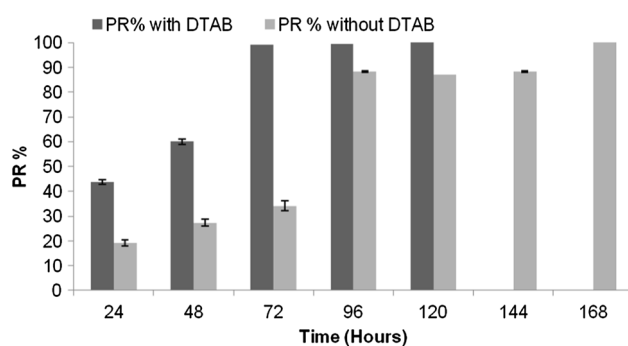


Fig. 3 Effect of contact time on total phenol removal (PR %) by *A. versicolor* (Av) in MS with 200 mg/L phenol in the absence and presence of 0.5 DTAB (T: 30 °C)

decreased due to the toxic effect of the highest phenol concentration on fungal growth; in fact, it was reported that surfactants had toxic effects on microbial growth, which were related to their structure [28]. The toxicity of the surfactants with the shortest alkyl chains such as DTAB, was lower than for the others.

The effect of contact time on 200 mg/L phenol uptake of fungus both in the presence (0.5 mM) and absence of DTAB within a 0–168-h time range was determined. The removal of phenol by *A. versicolor* increased considerably in a 168-h period. The phenol removed reached 100 % in a 120-h period in the presence of 0.5 mM DTAB; it took 168 h in the absence of the surfactant (Fig. 3). The addition of surfactant considerably shortened the completion of removal procedure. There were no significant difference between 72, 96 and 120 h (Fig. 3). Furthermore, the phenol removal rates were very close together between 96 and 120 h. Based on these results, the contact time was fixed at 96 h for the rest of the batch experiments to make sure that equilibrium was reached in all.

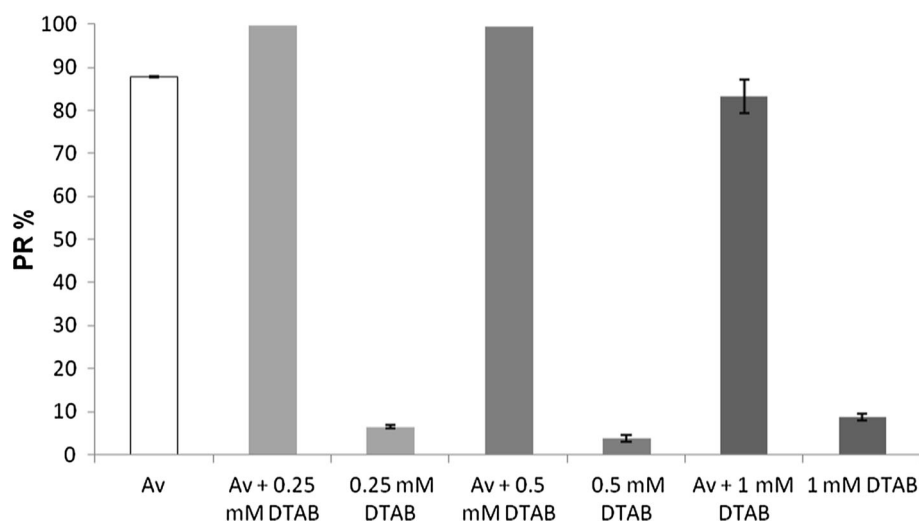
In order to examine the effect of DTAB concentration on the removal of 200 mg/L phenol by *A. niger* at pH 4, its concentration was varied within the 0, 0.25, 0.5 and 1 mM range and it was observed that increasing the concentration of DTAB up to 0.5 mM enhanced the removal efficiency from 88.7 to 99.15 % (Fig. 4). In some recent studies, surfactants were used to increase phenol removal capacity of chemical adsorbents [1, 18], and the positive effect of DTAB on dye removal by a fungal strain was also reported [17]. In a study on the solubilization and interaction of azo-dye with DTAB, it was shown that the solubility of the dye was increased by increasing the surfactant concentration as the result of surfactant-dye complex aggregation at surfactant concentrations below Critical Micelle Concentration (CMC) [29]. In the literature, we encountered no report regarding the effect of concentration of any surfactant on phenol removal by fungi. In this study it was observed that low DTAB concentrations such as 0.25 and

0.5 mM enhanced the phenol removal efficiency of the *A. versicolor* strain (Fig. 4) at a 200 mg/L phenol concentration. The decreases in phenol removal efficiency at higher phenol concentrations is in agreement with the conclusions reached by Zhong et al. [30], who reported that there were polar interactions between surfactant molecules and the chemical groups at the microbial cell surface. They added that hydrophobic moiety of surfactant molecules increased the cell surface hydrophobicity at lower concentrations of aqueous surfactant solutions. The hydrophobicity, stability and permeability of cell surface were decreased by higher surfactant concentrations due to this multiple surfactant layer formation or hemicelle accumulation [30]. The effect of presence of 0.25, 0.5 and 1 mM DTAB on 200 mg/L phenol removal is presented in Table 1, and in the presence of 0.5 mM DTAB, the removal rates of 200, 400 and 600 mg/L phenol concentrations were 99.39, 15.46 and 2.19 %, respectively. The highest specific phenol uptake capacity was 262.167 mg/L in the presence of 0.5 mM DTAB at a 200 mg/L initial phenol concentration.

The major mechanism of fungal degradation of phenol was related to enzymatic reactions [11]. Srinivasan and Viraraghavan [10] emphasized that the major mechanism involved in removal was biodegradation by the living cells of microorganisms, which could produce some degrading enzymes such as manganese peroxidase, laccase, and lignin peroxidase [11]. The *A. versicolor* strain used in this study is known to be a producer of an extracellular laccase enzyme [31], which is an oxidative enzyme involved in the degradation of phenol [32]. Additionally, there are reports showing the stimulation of some microbial enzymes by surfactants, or their manipulation to modify enzyme conformation in a manner altering enzyme activity, stability and/or specificity [33]. Liu et al. [34], for example, reported that the ramnolipid surfactant exerted a positive effect on the activity of laccase, the phenol-removing enzyme. They discussed that the interactions between the head group of the surfactant and the charged amino acid residues of the enzyme, or the hydrophobic interactions between the alkyl chains of the surfactant and the hydrophobic amino acid residues of the enzyme, might enhance phenol removal [34].

It is assumed that the positive effect of DTAB surfactant on phenol removal was related to the laccase activity of the fungus, as both phenol and the surfactant formed hydrogen bonds and hydrophobic interactions with laccase, which might enhance phenol removal by increasing the enzyme activity [35]. The addition of an octylphenol ethoxylate surfactant stabilized the laccase enzyme by maintaining a less polar environment to act on hydrophobic amino acid residues of laccase, or by changing the enzyme conformation due to enhanced removal of bisphenol A [36]. The results of the present study indicated that DTAB might

Fig. 4 Effect of initial DTAB concentration on total phenol removal (PR %) by *A. versicolor* (Av) in MS with 200 mg/L phenol (T: 30 °C; incubation period: 96 h)



enhance phenol bioremoval by altering laccase enzyme activity, its stability, or specificity. It was reported that the laccase enzyme had a role in removal of phenol [32], which means that there was a correlation between laccase activity and phenol removal. The phenol removal rate showed the activity of laccase, so if the phenol removal process was enhanced by the surfactant, the effect of the surfactant was related to the enhancement of laccase activity.

Conclusions

The effect of cationic DTAB surfactant on phenol removal by a living *A. versicolor* strain in MS medium was examined in this study. DTAB enhanced phenol removal capacity of the fungus and increased the maximum phenol bioremoval capacity to 267.162 mg/g in 200 mg/L phenol concentration at its 0.5 mM concentration at pH 4.

Considering the presence of both of phenols and surfactants in real industrial wastewater effluents and the use of fungal strains as inexpensive agents in biological wastewater treatment, the results presented in this study show that the addition of cationic surfactant DTAB increased the fungal performance of the treatment of phenol-based wastewater to a satisfactory level, and it can be concluded that *A. versicolor* can be used in the effective removal of phenols. Surfactant-induced fungal bioremoval technology may be taken as a feasible approach to remove phenols from industrial wastewaters.

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Sevgi Ertuğrul Karatay completed her undergraduate and Ph.D. degrees at Ankara University. She participated in postdoctoral studies at Ankara University and Ghent University (Belgium). She is currently an Associate Professor in the biology department of the Faculty of Science at Ankara University, Turkey. Her research interests are wastewater treatment technologies, environmental biotechnology, microbiology and biofuels.

Ülküye Dudu Gül graduated with a BS from the biology department of Ankara University, where she was also awarded her Ph.D. During her master's degree studies, she completed an internship program at Iowa State University, USA as a Fulbright Scholar. She is currently an Assistant Professor at Bilecik Şeyh Edebali University, Turkey. Her research interests are environmental education, environmental biotechnology, wastewater treatment technology, surfactants, microbiology, and algae technology.

Gönül Dönmez completed her undergraduate, Ph.D., and postdoctoral studies at Ankara University, Turkey. She is currently a Professor in the biology department of the Faculty of Science at Ankara University, where she has been a faculty member since 1987. Her research interests are biosorption, biodegradation, bioaccumulation, environmental biotechnology, wastewater treatment, microbiology and algae technology.