

Original Research

Treatability of Wastewater Containing 2,4 Dichlorophenol Using Acclimated Activated Sludge Microorganisms in Packed Upflow Column Bioreactor

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Abstract

2,4-Dichlorophenol (2,4-DCP) acclimated activated sludge culture was immobilized in polyurethane foam and supplemented to a packed upflow column bioreactor (PUCB) to treat 2,4-DCP containing wastewater. The impact of sludge age and 2,4-DCP concentration on biodegradation performance were studied. Results show that sludge age affected biodegradation in the PUCB. The residence time was adjusted by feeding with synthetic wastewater without DCP. The optimum phenol concentration was determined in 5-, 10-, and 15-day-old sludge. According to the results obtained, the removal of 99% phenol and 95% COD were determined in 10-day-old sludge and 500 mg/L 2,4-DCP concentration. Packing medium was examined both visually and by SEM, and a significant amount of organisms was observed accumulating on the surface, which indicated that diffusion limitations were controlling biofilm thickness.

Keywords: 2,4-DCP biodegradation, acclimation, immobilization, packed upflow column bioreactor, polyurethane foam

Introduction

Waters containing phenol-derivative chemicals such as chlorinated hydrocarbons must be purified and discharged because of their carcinogenic and teratogenic effects, even at low concentrations. Phenol and phenolic compounds are toxic to soil ecosystems and water microecosystems, aquatic life, and plants, and have negative effects on human health even at low concentrations. They have been reported to cause liver

and kidney damage, cardiac toxicity, reproductive and developmental toxicity, neurotoxicity, cardiac depression, and reduced blood pressure in humans. For this reason these compounds must be removed from the environment with advanced or conventional treatment [1-2]. Phenol and chlorophenolic compounds are widely used in industry in coal businesses, coal distillation units, petroleum refining in the petrochemical industry, pulp and paper bleaching plants, resins, disinfectants, herbicide and insecticide production, and plastics production [3-4]. 2,4-DCP is 2,4-dichlorophenol or 1-hydroxy-2,4-dichlorobenzene; the molecular formula is $C_6H_4Cl_2O$ and molecular weight is 162.997 g/mol

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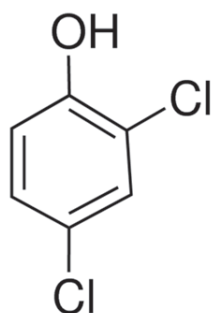


Fig. 1. 2,4-Dichlorophenol.

(Fig. 1). 2,4-DCP that has been used in the study is a type of chlorophenol, typically used in synthesizing insecticides, herbicides, preservatives, antifungal agents, and dyes [3-4]. As a result of chlorination of the phenols, as the byproducts of the hypochlorite-phenolic acids reactions, as biocides or as degradation products of phenoxy herbicides, chlorophenols are seen in drinking water. 2-chlorophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol are the byproducts that have the greatest possibility of being seen in drinking water. However, in drinking water taste thresholds for chlorophenols are low [5].

Phenolic and chlorinated phenolic compounds inhibit microorganisms found in municipal biological wastewater treatment plants due to their high toxicity even at low concentrations [6]. They can easily degrade most of the pollutants that can be biodegradable due to the presence of various populations of microorganisms in activated sludge. Species that have important roles for biological degradation in activated sludge are: *pseudomonas*, *flavobacterium*, *achromobacter*, *rhombacterium*, *azobacter*, *micrococcus*, *bacillus alkaligenes*, *arthrobacter*, *ycobacterium*, *aeromonas*, *nocardia*, and *lophomonas*. Mixed culture containing multiple microorganisms such as pure culture or activated sludge can be used for degrading chlorinated aromatic compounds [7-8]. Microorganisms such as *Arthrobacter*, *Pseudomonas*, *Alcaligenes*, and *Phanerochaete* have been reported to break down these compounds [4, 9-11]. This toxic compounds such as 2,4,6-trichlorophenol (TCP) inhibited phenol biodegradation and biomass growth. Song et al. [12] in their work on UV photolysis converted TCP into dichlorocatechol, monochlorophenol, and dichlorophenol. After UV photolysis were executed, phenol-removal and biomass-growth rates importantly gained speed. That is why after UV photolysis was executed the monod maximum specific growth rate (μ max) increased by 9% and the half-maximum-rate concentration (K_s) decreased by 36%. Various bioreactors are used that are batch and continuously working in determining the biodegradation performance [7, 13-16]. In addition to conventional activated sludge systems [17], immobilized within calcium alginate gel beads for the fluidized bed bioreactor [18], jet-loop

bioreactor [19], sequencing batch reactor (SBR) [20], moving bed sequencing batch reactor (MSBR) [21], and membrane biofilm bioreactor [22] are used for refining. In biofilm reactors, whether they are fluid bed or filled column reactors, plastic materials such as PVA gel [23] and polyurethane [24] or ceramic balls [9, 25] can also be preferred as filling material, as well as natural materials such as sand, clay, anthracite, and slag.

In this study, microporous polyurethane (sponge) was used as filling material for the immobilization of upflow filled column reactor and activated flour acclimated in the reactor. It was developed by feeding synthetic wastewater that does not contain 2,4-DCP to the acclimated sludge support material. The addition of 2,4-DCP was carried out after immobilization, and the bioreactor was operated in a batch system for 18 h hydraulic retention time. System performance was determined by chemical oxygen demand (COD), phenol concentration, and time-dependent monitoring of physical parameters. The advantages of this system are small footprint, economical packing medium, and high removal efficiency. The micro flora were protected by immobilization of the sludge, and this was able to degrade phenol concentrations over 1,000 mg/L.

Material and Methods

Culture

Activated sludge cultures to be used as initial inoculant were obtained from the Greater Municipality of Kayseri Domestic Wastewater Treatment Plant. The activated sludge culture was taken from the reactor fed with synthetic wastewater free of chlorophenol and used in the first inoculum as equilibrated cultures for all experiments. Activated sludge culture growth media content is: K_2HPO_4 , 0.5 g/L; $(NH_4)_2SO_4$, 0.94 g/L; KH_2PO_4 , 0.25 g/L; and whey powder, 1.0 g/L. Growth medium was prepared using tap water.

The stock synthetic wastewater was prepared to contain 1 g/L glucose, 0.1 g/L peptone, 0.2 g/L urea, 0.1 g/L $(NH_4)_2SO_4$, 0.1 g/L KH_2PO_4 , and 0.01 g/L K_2HPO_4 . Micronutrients included $CaCl_2$ (15 mg/L), $MgSO_4 \cdot 7H_2O$ (30 mg/L), $CuSO_4 \cdot 5H_2O$ (0.75 mg/L), $FeSO_4 \cdot 7H_2O$ (15 mg/L), $ZnSO_4 \cdot 7H_2O$ (0.5 mg/L), $MnSO_4 \cdot 7H_2O$ (30 mg/L), Citric Acid (60 mg/L), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.5 mg/L), H_3BO_3 (0.01 mg/L), and KI (0.01 mg/L). $NaHCO_3$ was used as buffer to adjust the influent pH to 6.8 ± 0.2 . All the chemicals were of analytical grade. In the acclimation incubation, the sludge had $2,000 \pm 24$ mg/L mixed liquor volatile suspended solids (MLVSS). The acclimation study was carried out at a working volume of 100 mL in a 250 mL Erlenmeyer in a shaking incubator. The incubation was completed by treating the 5-, 10-, and 15-day-old sludge with 250 mg/L 2-DCP following 2,4-DCP (linear formula $Cl_2C_6H_3OH$, molecular weight 163.00, CAS number 120-83-2) (2-DCP) small stepwise

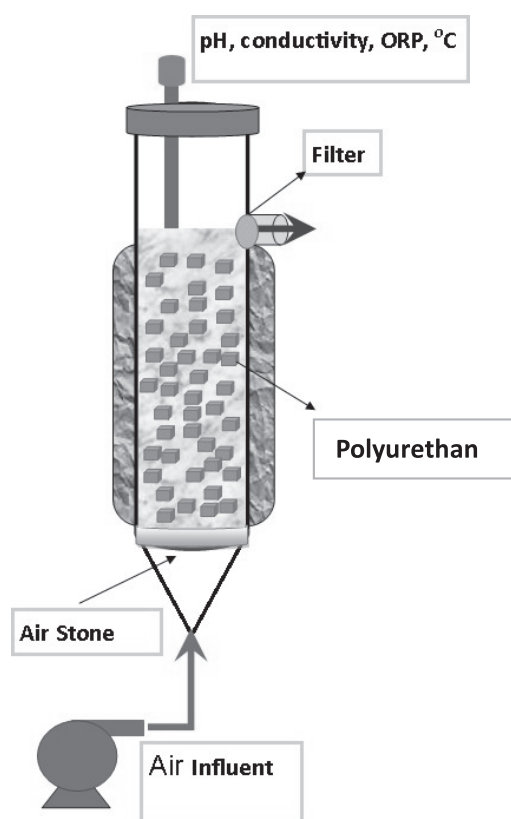


Fig. 2. PUCB used in the study.

increments, during 4 weeks. Synthetic chlorophenolic wastewater was prepared by dissolving 0.4 g/L NaOH in 1 L distilled water and adding 5 g/L 2,4-DCP (Merck). All acclimation incubation was operated at 25°C and aeration was provided at 200 rpm shaking.

Active sludge is used for the immobilized and suspended column bioreactor. In the immobilized bioreactor, the biomass's adsorption and proliferation on the porous packing medium could be noticed visually due to its being faster. At the end of day 10, all of the

packing medium was covered with biofilm. Following immobilization, synthetic wastewater was filled into the system and the solution was re-circulated in batch mode for 10 days. And the bioreactor was run in continuous mode when COD removal reached a value of 97% (based on the initial COD concentration). The pH levels were kept at 6.5 ± 0.5 on a daily basis by monitoring. With a scanning electron microscope (SEM) (ZEISS EVO 40 EP Electron microscope), the samples of polyurethane foam were examined during the immobilization of the culture. 3.0% glutaraldehyde in 0.1 M phosphate buffer was used to fix the foam samples at pH 7.2, and the samples were dehydrated with ethanol (95%) and dried at 65°C. Before examination with SEM, the samples were coated with gold [26].

Optimization Study

An optimization study planned for the disintegration of phenolic compounds of acclimated sludge cultures was carried out in both a laboratory-scale aerated up-flow packed column bioreactor and a suspended column bioreactor, with the reactor volume of 1,000 mL, working volume of 750 mL, pH of 6.5 ± 0.5 and temperature of $24 \pm 2^\circ\text{C}$ (Fig. 2). Aeration in the reactor is provided by adjustable aquarium pumps with the air flow of 2 L/min. A coarse bubble diffuser was located at the base of the column and through it, and the bioreactor was aerated. The operating conditions of the bioreactors are presented in Table 1. The study was carried out with 5-, 10-, and 15-day-old sludge cultures at different concentrations of 2,4-DCP in the range 100-1,000 mg/L.

Within the scope of this study, the optimum sludge and phenol concentrations were determined by examining the removal efficiency of the age of the sludge and various phenol concentrations in the filled column.

Analytical Methods

For determining COD and phenol, samples from the bioreactors are filtered through membrane filters having a pore size of 0.45 μm after centrifugation (8 min at 15,000 rpm). pH, conductivity, dissolved oxygen, and ORP were directly monitored by a multi-meter in bioreactors. This was done according to pH, conductivity, dissolved oxygen, ORP, and standard methods [27-28]. Total phenol determined that the absorbance of the colored complex of phenol and p-nitroaniline was read at 470 nm [29]. The COD was analyzed using a thermoreactor (Giant QBH2) by closed reflux method using potassium dichromate solution as oxidant according to standard methods [30]. The COD and phenol analyses were done triple times and their average values were used afterward.

Table 1. Operational PUCB parameters.

Operational parameter	Value
Detention time (with packing)	18 hours
Air flow rate	2 L/min
Operating temperature	$25 \pm 1^\circ\text{C}$
Packing media (foam) pore size (average)	295 μm
Size of foam pieces	$0.5 \times 0.5 \text{ cm}$
Porosity	0.8
Water sorption capacity of the media	8.17 g/g
Total column height	52 cm
Total packing media height	38 cm
Port – height from base (exit port)	38 cm

Results and Discussion

Effect of Initial Phenol Concentration on 2,4-DCP Removal

In this work, two bioreactors (suspended and PUCB) were used and optimum conditions for the increase of synthetic wastewater containing synthetic 2,4-DCP in the acclimated activated sludge microorganisms in both reactors were determined. Activated sludge microorganisms used in the study were immobilized to microporous polyurethane material. The hydraulic retention time of the bioreactor was identified to be 18 hours in order to determine the initial phenol concentration. The result in the suspended bioreactor was worse than the PUCB. Therefore, it only gave up flow-packed column bioreactor results in this study.

As seen in Fig. 3, the initial phenol concentration is quite effective in the biodegradation of 2,4- DCP. According to the results of the hour 8 of hydraulic retention time, 100 mg/L COD removal was found to be 98%, phenol removal was found to be 99%, 250 mg/L COD removal was found to be 96%, phenol removal was found to be 99%, 500 mg/L COD removal was found to be 97%, total phenol removal was found to be

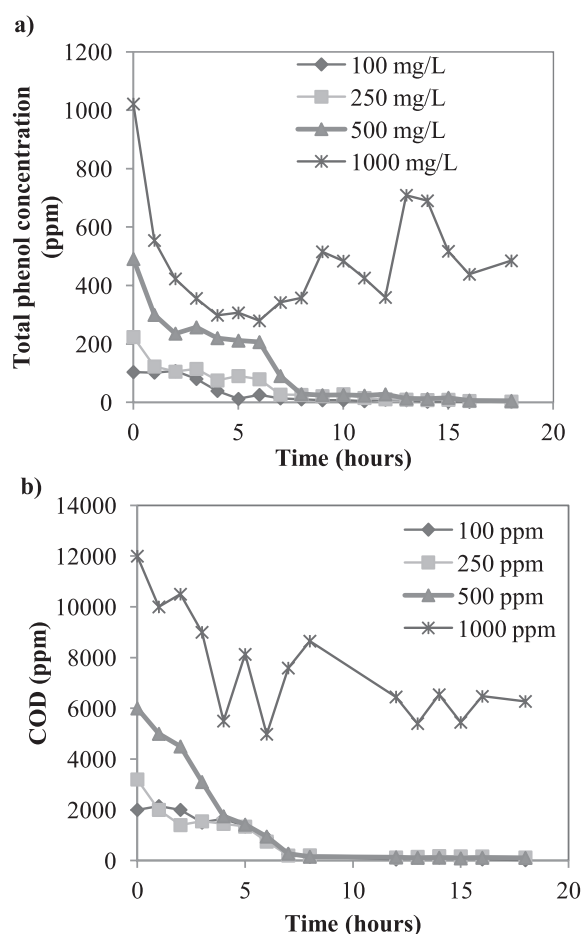


Fig. 3. Effect of initial phenol concentration on 2,4-DCP removal in upflow: a) total phenol change and b) COD change.

99%, 1,000 mg/L COD removal was found to be 48%, and phenol removal was found to be 52%. At 18-hour retention time, 99% of 2 close COD and phenol removal were achieved except for 1,000 mg/L 2,4 DCP. The results of suspended bioreactor of hour 8 of hydraulic retention time, 100 mg/L COD removal was found to be 58%, phenol removal was found to be 79%, 250 mg/L COD removal was found to be 56%, phenol removal was found to be 71%, 500 mg/L COD removal was found to

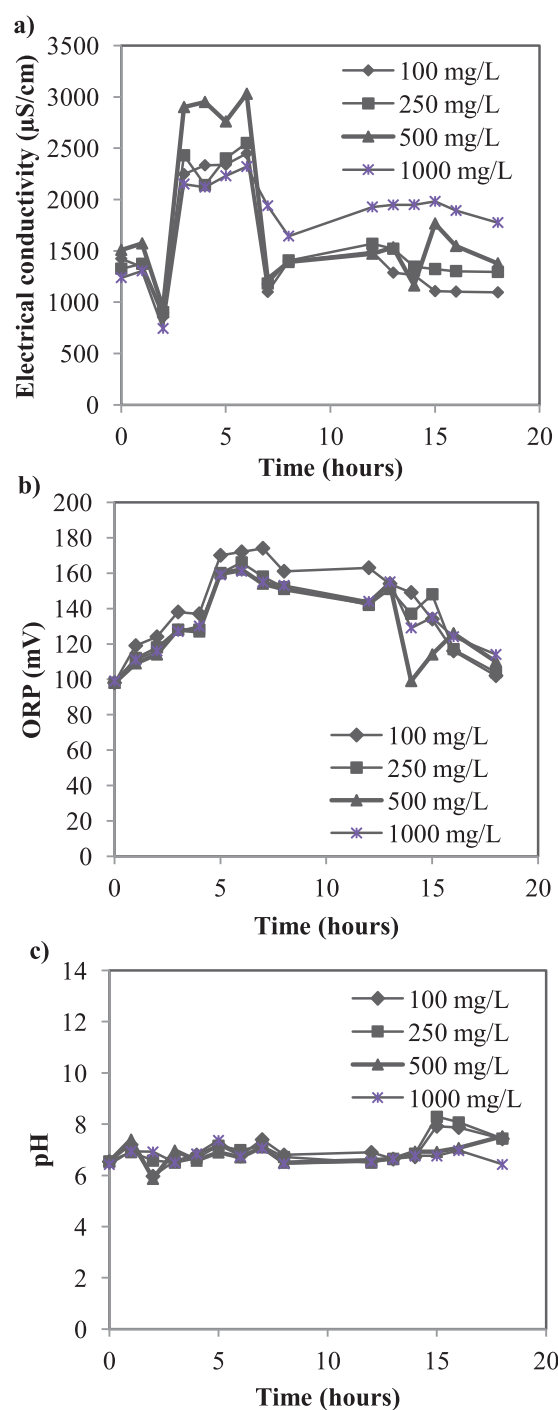


Fig. 4. Effect of initial phenol concentration on 2,4-chlorophenol removal (sludge age 10 days): a) electrical conductivity, b) ORP, and c) pH.

be 66%, total phenol removal was found to be 64%, and 1,000 mg/L COD removal was found to be 43%, phenol removal was found to be 45%. At 18 hour retention time, 99% of 2 close COD and phenol removal in up-flow packed bioreactor and approximately 65% were achieved except for 1,000 mg/L 2,4 DCP. According to the results obtained, the phenol toxicity level of confined microorganisms was determined as 500 mg/L. As the phenol has a toxic effect over 500 mg/L, the removal rate is considerably reduced.

The effect of physical parameters in determining the effect of initial 2,4-DCP concentration on removal was monitored. According to the obtained results, it was determined that pH value did not change significantly, but the electrical conductivity value showed a significant change in the time interval when the biodegradation was intense and the ORP value reached the highest values during degradation (Fig. 4). The time of biodegradation that the incubation partially completed is 7-8 hours, as can be seen from Fig. 3. [30] concluded that measurements of conductivity and turbidity can be used to estimate the COD of wastewater to some extent. Acclimated activated sludge microorganisms can also complete biodegradation in a shorter time, also with the effect of immobilization. Abuhamed et al. [31] studied the biodegradation of *Pseudomonas putida* FIATCC 700007 with benzene toluene and phenol in an aerobic reactor. In the study they showed that biodegradation is shorter in acclimated cultures than in non-acclimated cultures. The acclimated microorganisms degraded in 6 hours while the non-acclimated 90 mg/L benzene and toluene-containing wastewater degraded in 24 hours. Jiang et al. [32] conducted a phenol removal study with *Alcaligenes faecalis*. During the latter phase of the exponential growth stages, where 1,600 mg L⁻¹ phenol was completely degraded in 76 hours, maximum phenol degradation was observed. A large quantity of phenol hydroxylase was secreted and accumulated by *A. faecalis* in this physiological phase. Then the cells were able to quickly utilize the phenol solely as a carbon and energy source. Unlike other studies, changes in physical parameters have also been associated with this study. This important change can be caused by biodegradation and by-products because similar fluctuations were observed in the same periods, even at different rates in each parameter. Furthermore, when time-dependent changes in COD and phenol removal are evaluated together, physical parameters also show a change in the same time interval.

The results presented in this paper showed that the maximum concentration of 2,4-DCP was 500 mg/l in immobilized bioreactors and suspended column bioreactors. 2,4-DCP removal efficiency is 95% in an up-flow packed column bioreactor an 66% in a suspended column bioreactor. Compared with other studies, the minimal inhibitory concentration of 2,4-DCP to 125 mg/l for *P. putida* and for *P. alcaligenes* was 220 mg/l [33]. Şahinkaya and Dilek [34] showed that un-acclimated and acclimated activated sludges

were examined for their ability to degrade 4-CP (4-chlorophenol) using aerobic batch reactors. According to results obtained, a considerable decrease was observed in the un-acclimated culture, while the increase in 4-CP concentration up to 300 mg/L had no adverse effect on the acclimated culture's COD removal efficiency. In this study, acclimated sludge using a PUCB were able to remove 500 mg/L 2,4-DCP. Lin has shown that the removal efficiency for 2,4-DCP was ~89% in a steady-state condition [9]. Patel and Kumar (2015) indicated that *Bacillus endophyticus*

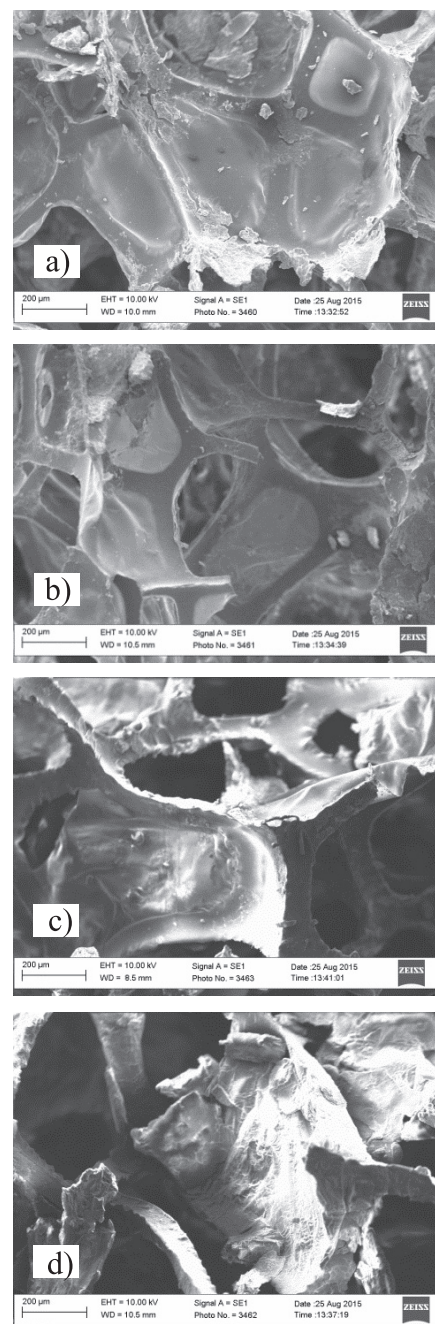


Fig. 5. SEM micrographs of immobilized active sludge biofilm from the bioreactor: a) 100 mg/L 2,4-DCP, b) 250 mg/L 2,4-DCP, c) 500 mg/L 2,4-DCP, d) 1,000 mg/L 2,4-DCP (sludge age 10 days, pH 7.02±0.5, 25°C).

strain degraded 400 mg/L 2,4-DCP for optimizing experimental parameters. Optimization of experimental parameters was determined using response surface methodology [35].

This work is a biological system with a significant advantage with its economical packing medium content. For the immobilization of activated sludge culture, polyurethane foam can be considered as an alternative sustainable media for bioreactors due to its suitable characteristic. However, the bioreactors, which utilize biofilms (i.e., immobilized cultures), have some operational difficulties. Biofilm loses its integrity as it matures. Thus, the biosolids should be removed periodically. This might be done either by restarting the system or with a backwash step to allow detachment and removal of the biomass from the packing medium. Once the biofilm is detached, it will be able to be separated easily from the packing media while losing its integrity, with its dewatering ability and due to its containing concentrated forms of biosolids. As the media has a flexible and light nature, it feeds the flows and allows the packing to be adjusted for possible short circuits of the air [26, 36]. SEM images of polyurethane in determining the effect of 2,4-DCP concentration on the treatability at hour 8 of incubation were given in Fig. 5, which shows how the increase in 2,4-DCP concentration affected biofilm formation. A concentration of 1,000 mg/L 2,4-DCP reduced biofilm thickness and width. For this reason, 500 mg/L 2,4-DCP is optimum concentration for microbial activity and toxicity.

Effect of Sludge Age on Chlorophenol Removal

Five-, 10-, and 15-day-old sludges were used to determine the effect of sludge age on phenol removal. The concentration used was 500 mg/L for 2,4-chlorophenol in the incubation medium. Total phenol, COD, electrical conductivity, pH, and ORP analyses were performed on the samples taken at every hour during incubation. The time-dependent changes of phenol and COD results from the obtained data are given in Fig. 5.

As can be seen in Fig. 6, sludge is quite effective in the biodegradation of 2,4-DCP. Although it is observed that older cells are more resistant than younger cells, 10-day-old cells are observed to be more capable of biodegradation. According to the obtained results, the chlorophenol removal by the sludge age is observed as follows: in the up-flow packed column bioreactor for 5-day-old sludge COD removal is 83% and total phenol removal is 85%, for 10-day-old sludge COD removal is 95% and total phenol removal is 99%, for 15-day-old sludge COD removal is 53% and total phenol removal is 79%; and in the suspended column bioreactor for 5-day-old sludge COD removal is 61% and total phenol removal is 64%, for 10-day-old sludge COD removal is 70% and total phenol removal is 75%, and for 15-day-old sludge COD removal is 34% and total phenol removal

is 42%. In the study of Quan et al. [37], 2,4-DCP degrading mixed culture was immobilized in polyvinyl alcohol gel beads, and for treating the wastewater that contained 2,4-DCP they were supplemented by sequencing batch reactors (SBR). They have studied the effects of the bioaugmentation level on the performance of bioaugmented systems. According to the results, the inoculum sizes have affected the start-up times of the SBR systems. Nine days were needed for the non-augmented SBR system to start-up; while it only took six, four, three, and two days for the SBRs with 1.9%, 3.7%, 5.6%, and 9.3% immobilized cultures, respectively. In this study, sludge age was determined as 10 days. Kargı and Eker [38] used sludge with sludge ages for 5-30 days and according to the results obtained they found that as the sludge age increased, COD and toxicity decreased. The study emphasizes that sludge age is important in the removal of 2,4-DCP. Under the experimental conditions, when a sludge of 25 days age was tested the results were: more than 90% COD and nearly 100% DCP and toxicity removal with an SVI value of 108 ml g⁻¹. In another study of Kargı and Eker [39], with a hybrid-loop bioreactor system consisting of a packed column biofilm and an aerated tank bioreactor with 400 mg/L 3-chlorophenol, 20 days of sludge age and 90% removal were identified. In this study, on day

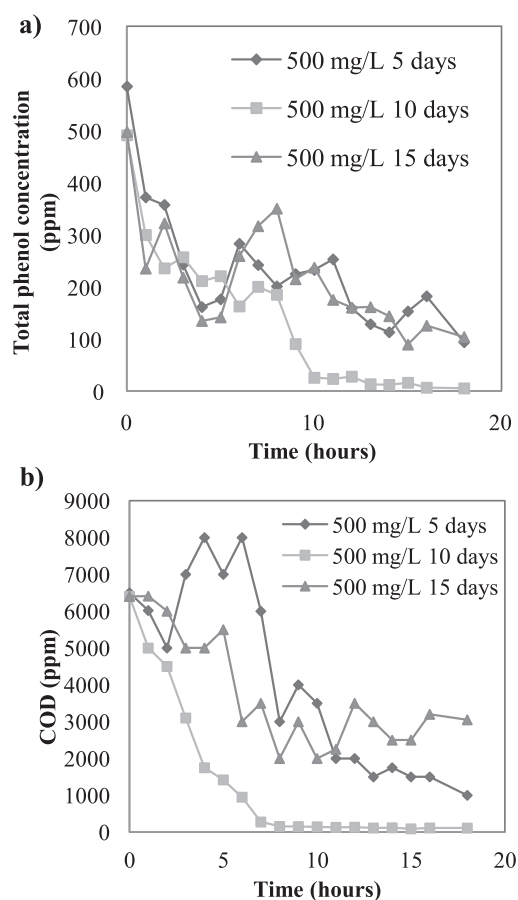


Fig. 6. Effect of sludge age on 2,4-DCP removal: a) total phenol change, b) COD change.

10, which was determined as optimum sludge age, 99% 2,4-DCP and 95% COD removal were determined.

pH, electrical conductivity, and ORP analyses were carried out at regular intervals during the determination of the effect of sludge age on 2,4-DCP removal in an up-flow packed column bioreactor (Fig. 7). According to the obtained results, it was observed that pH value did not change significantly but that electrical conductivity value showed a significant change in the time interval when biodegradation was intense, and the ORP value decreased after degradation. At hour 8, which was determined as optimum hydraulic retention

time, conductivity and ORP reached a certain balance. COD removal and phenol removal were parallel to each other at hour 8, which was determined as the optimal 2,4-DCP degradation time. Uysal and Turkman [40] studied the effect of surfactants on the biodegradation of 2,4-DCP by activated sludge microorganisms. It has been determined in the study that microorganisms that are acclimated degrade more efficiently than non-microorganisms, and that the surfactant has positive effects on removal efficiency. The DCP removal efficiency was determined to be in the range of 97.4-97.7% for 30 mg/L phenol and 99.7-99.8% for 100 mg/L phenol. It has been determined in the study that sludge age is effective in phenol removal and that older cells are more efficient due to their toxicity levels. Sludge age was determined to be 10 days in the study we conducted.

Microporous polyurethane (sponge) is preferred as an immobilization material due to its easy supply

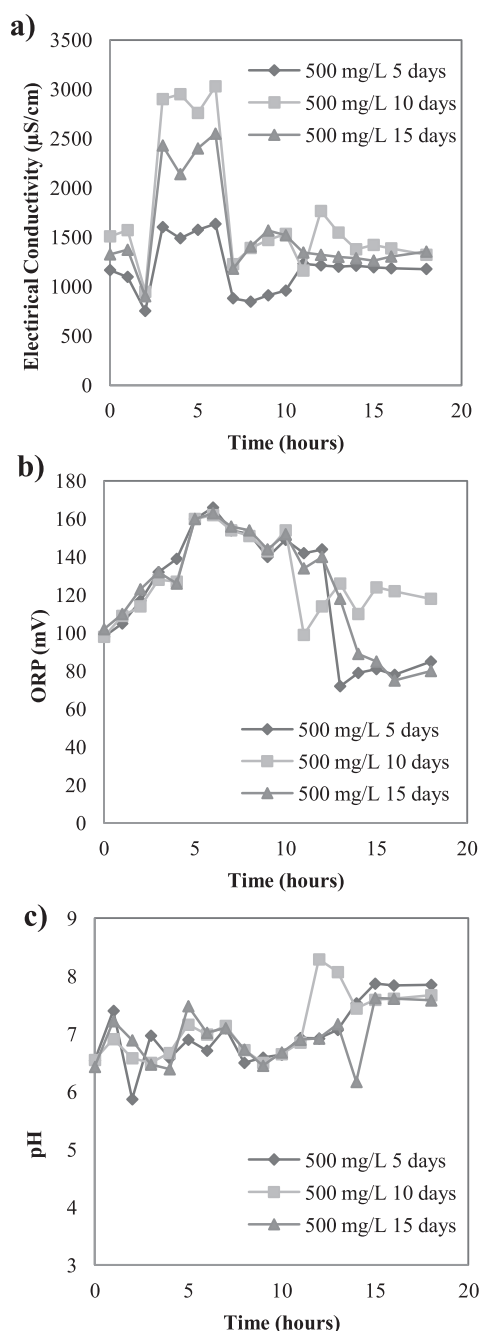


Fig. 7. Effect of sludge age on 2,4-DCP removal (2,4-DCP concentration 500 mg/L): a) electrical conductivity, b) ORP, and c) pH.

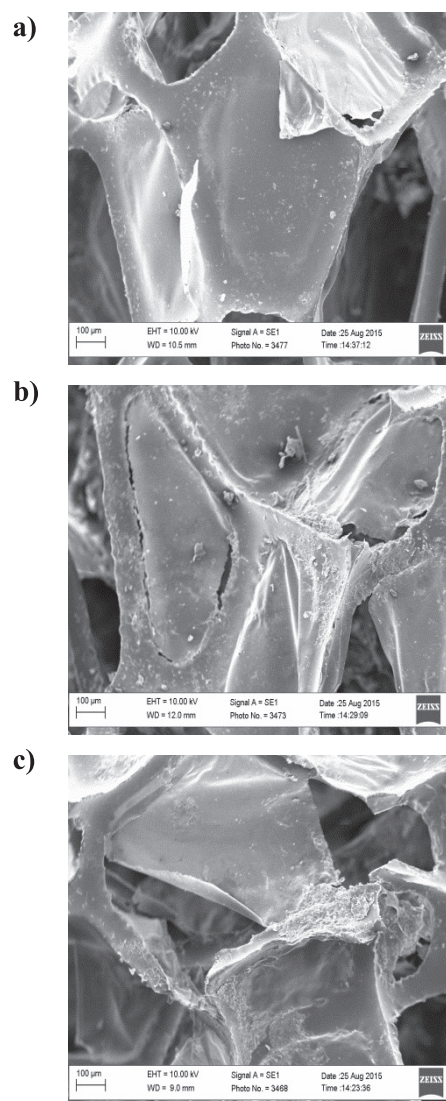


Fig. 8. SEM image of the immobilized material polyurethane used in the study: a) sludge age 5 days, b) sludge age 10 days, and c) sludge age 15 days) (500 ppm 2,4-DCP, pH 7.02±0.5).

availability and low cost. The polyurethane material taken from the 500 mg/L 2,4-DCP optimization medium, which is determined as the optimum condition to determine the biofilm thickness on the microporous polyurethane at a certain level, is imaged in SEM (scanning electron microscope) and the images are given in Fig. 8. When SEM images are compared, it is seen that older cells in Fig. 8c) have a thinner film thickness and more breaks from the immobilization material, suggesting that 10 days is the optimum sludge age at 2,4-DCP removal. According to the images obtained, we determined that biofilm thickness was formed up to a certain level and immobilization was achieved in the experimental work.

Conclusions

In our study, 2,4-DCP removal was carried out with microporous polyurethane (sponge) immobilization material and acclimated active sludge. Even with very toxic 2,4-DCP we accomplished 95% COD removal.

Experiments were conducted to determine optimal parameters for 10-day sludge age at 18 hours of hydraulic retention time and at 500 mg/L 2,4-DCP concentration to provide 95% COD and 99% 2,4-DCP removal. As the initial concentrations of 100 mg/L, 250 mg/L, 500 mg/L, and 1,000 mg/L 2,4-DCP increased, 2,4-DCP and COD removal efficiencies were obtained effectively until 500 mg/L 2,4-DCP. At concentrations higher than 500 mg/L 2,4-DCP, the removal efficiency is considerably reduced. This is an important result for the removal of 2,4-DCP and it can be supported by several advanced oxidation processes, indicating that the removal can be taken to µg/L levels.

On the other hand, in this study it was emphasized that physical parameters such as electrical conductivity, ORP, and pH are important besides COD and phenol analysis in monitoring biodegradation. The significant change in electrical conductivity at the time of incubation, where biodegradation is intense, adds specificity to the study.

In addition to being an economical and easily available immobilization material and an alternative filling material option, the system can be considered to be feasible with the positive effect of immobilization on the treatment. Polyurethane is a cheap and easily supplied material. It has been determined that the material is suitable for immobilization and that it can easily be attached to the biofilm polyurethane and has an important effect on the efficiency of the treatment.

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