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# EFFECT OF HIGH CONCENTRATIONS OF 2,4-DCP ON ACTIVATED SLUDGE

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## ABSTRACT

Biodegradation of 2,4-dichlorophenol (2,4-DCP) was investigated with a mixed culture in a continuous activated sludge bioreactor. Glucose was used as co-substrate. Experiments were carried out at the feed 2,4-DCP concentrations between 5-450 mg/l, and the removal efficiencies of 2,4-DCP and COD were determined. The removal efficiencies and specific removal rates of 2,4-DCP for feed 2,4-DCP concentration up to 350 mg/l varied between 96.3-98.6 % and 0.007-1.15 mg/mg X.day respectively. Removal efficiencies and specific removal rates ranged between 75.66-92.0 % and 0.43-1.29 mg/mg X.day for COD. When 2,4-DCP concentration was increased from 350 to 450 mg/l, the removal efficiency and specific removal rate of 2,4-DCP decreased to 42.6 % and 0.77 mg/mg X.day due to the substrate inhibition on the microorganisms. Also, these values decreased to 7.85 % and 0.18 mg/mg X.day for COD. These results indicate that the adaptation of activated sludge could tolerate as high as 350 mg/l 2,4-DCP concentration as to the removal of 2,4-DCP and COD.

**Key Words :** Activated sludge, Biodegradation, 2,4-Dichlorophenol

## AKTİF ÇAMUR ÜZERİNE YÜKSEK KONSANTRASYONLARDAKİ 2,4-DKF'ÜN ETKİSİ

### ÖZET

2,4-diklorofenol'ün (2,4-DKF) biyolojik olarak ayrışması sürekli işletimli aktif çamur biyoreaktöründe karışık kültür kullanılarak araştırılmıştır. Glukoz büyüme maddesi olarak kullanılmıştır. Deneyler 5 ile 450 mg/l aralığındaki giriş 2,4-DKF konsantrasyonlarında yapılarak, 2,4-DKF ve KOİ arıtım verimleri belirlenmiştir. 350 mg/l'ye kadar olan giriş 2,4-DKF konsantrasyonları için 2,4-DKF giderim verimleri % 96.3-98.6 aralığında ve spesifik giderim hızları 0.007-1.15 mg/mg biyokütle.gün aralığında olmuştur. KOİ için giderim verimleri % 75.66-92.0 aralığında ve spesifik giderim hızları 0.43-1.29 mg/mg biyokütle.gün aralığında olmuştur. 2,4-DKF konsantrasyonu 350 mg/l'den 450 mg/l'ye yükseltildiğinde, 2,4-DKF'ün mikroorganizmalar üzerine olan inhibe edici etkisinden dolayı 2,4-DKF giderim verimi % 42.6'ya ve spesifik giderim verimi 0.77 mg/mg.biyokütle.gün'e azalmıştır. KOİ için giderim verimi % 7.85'e ve spesifik giderim verimi 0.18 mg/mg.biyokütle.gün'e azalmıştır. Bu sonuçlar, aktif çamurun adaptasyonu ile 350 mg/l gibi yüksek 2,4-DKF konsantrasyonuna kadar 2,4-DKF ve KOİ giderim verimlerinin olumsuz olarak etkilenmediğini göstermiştir.

**Anahtar Kelimeler :** Aktif çamur, Biyolojik ayrışma, 2,4-Diklorofenol

## 1. INTRODUCTION

Chlorophenolic compounds are found in waste discharges of many industries including petrochemical, oil refinery, plastic, pesticides, biocides, wood preservers, pulp and insulation materials (Wang et al., 2000; Quan et al., 2003).

Due to their high toxicity, strong odor emission and persistence in the environment and suspected carcinogenic and mutagenic properties, chlorophenols pose serious ecological problems as environmental pollutant (Quan et al., 2003). Hence, the removal of phenol and chlorinated organic compounds from wastewater is a necessary task to conserve the quality of natural water resources (Ha et al., 2000).

Several physical, chemical and biological methods including activated carbon adsorption, ion exchange, incineration and biological degradation have been proposed for treating or recovering chlorophenolic compounds (Raung, 1984). The biological treatment is superior to physicochemical methods such as activated carbon adsorption and incineration because the latter have high treatment costs and possibilities of causing a secondary pollution (Wang et al., 2000).

Biodegradability of chlorophenols depends on the number and position of chlorine groups on the aromatic ring. Usually, biodegradability decreases with increasing number of chlorine groups (Annachhatre and Gheewala, 1996).

2,4-DCP, one of the most recalcitrant chlorophenols, has been detected dominantly in chlorophenol-containing industrial effluents. Biological degradation of 2,4-DCP has been studied in different reactors like sequencing batch reactor (SBR), air-lift honeycomb-like ceramic reactor by many investigators (Ha et al., 2000; Ouan et al., 2003; Quan et al., 2003a). However, activated sludge process is being applied worldwide in municipal and industrial wastewater treatment. In general, it is recommended to use the activated sludge to treat toxic compounds due to its microbial diversity (Spain and Van Veld, 1983; Watson, 1993). It is, therefore, important and necessary to investigate the effect of 2,4-DCP on the performance of activated sludge.

High concentrations of chlorophenols are usually inhibitory to microorganisms. However, adaptation of microorganisms to chlorophenols was found to improve the biodegradative ability of the organisms and alleviate inhibitory effects to some extent (Annachhatre and Gheewala, 1996). The degradation capacities of an activated sludge can be enhanced by the acclimation of the inoculum (Buitron and

Capdeville, 1995). An adequate acclimation has been the key issue to achieve the degradation of some recalcitrant compounds (Spain and Van Veld, 1983). Little attention has been paid to the adaptation of inoculum in 2,4-DCP biodegradation in an activated sludge bioreactor. Therefore, the major goal of this study was to investigate the biodegradation of 2,4-DCP with adaptation of activated sludge.

Mixed cultures are particularly important when the emphasis is placed on complete mineralization of toxic organics. Many pure-culture studies have shown that toxic intermediates accumulate during biodegradation, because a single organism culture may not have the ability to completely mineralize the xenobiotics (Buitron and Gonzalez, 1996). Therefore, the treatment of chlorophenols using an activated sludge process in which a mixed culture is in action would be more meaningful, informative, and practical. The main advantage achieved by the microbial consortium formed by activated sludge is the interaction between all the species present in the flocs (Sahinkaya and Dilek, 2005).

Biodegradation of 2,4-DCP by activated sludge in continuous systems has been studied by several investigators (Beltrame et al., 1982; Chudoba et al., 1989; Kargi et al., 2005). However, the 2,4-DCP concentration range was less than 250 mg/l and effect of high 2,4-DCP loading rate on microbial growth was not determined. The aim of the present study was to investigate biodegradation of high concentrations of 2,4-DCP with adaptation of mixed culture in an activated sludge bioreactor and to determine the effect of 2,4-DCP on the microbial growth, specific removal rate and COD removal efficiency.

## 2. MATERIALS AND METHODS

### 2. 1. Experimental System

This study was performed, using activated sludge bioreactor consisting of aeration and sedimentation tank. Volume of aerobic reactor was 8.75 l and volume of settling unit was 1.15 l. Aerobic reactor was aerated by an air pump. Aeration and sedimentation tanks were separated by an inclined plate. Effluent wastewater passage from the aeration tank to sedimentation tank was through the holes on the inclined plate. The effluent of sedimentation tank was collected in an effluent tank and it was regularly discharged.

## 2. 2. Organisms and Wastewater Composition

Mixed culture was used in aerobic reactors. Activated sludge culture was obtained from the wastewater treatment plant of Pak Maya Bakers Yeast Company in Izmir, Turkey. The aerobic reactor was inoculated with this culture.

The synthetic wastewater used throughout the study was composed of glucose as carbon source, urea as nitrogen source (50 mg/l),  $\text{KH}_2\text{PO}_4$  as phosphorus source (10 mg/l),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (75 mg/l),  $\text{CaCl}_2$  (50 mg/l),  $\text{FeCl}_3$  (2 mg/l) and various concentrations of 2,4-DCP (0-450 mg/l). Nitrogen and phosphorus concentrations were adjusted to maintain C/N/P=100/10/2 ratio during the experiments. While COD concentration was kept constant as 500 mg/l up to 350 mg/l 2,4-DCP concentration, it was adjusted to 575 mg/l for the 450 mg/l 2,4-DCP concentration. The feed 2,4-DCP concentration was increased stepwise from 5 to 450 mg/l during the operation period. In this case, in order to keep COD concentration at a constant value, glucose concentration was decreased when the feed 2,4-DCP concentration was increased. Therefore, 2,4-DCP/glucose ratio gradually increased in the system.

## 2. 3. Experimental Procedure

Experiments were started batchwise. Activated sludge from an industrial wastewater treatment plant was added to the reactor as seed source. The synthetic wastewater containing glucose and nutrients was inoculated with a mixture of activated sludge. The media was aerated vigorously for several days until a dense culture was obtained. Continuous operation was realized by pumping the feed wastewater to the aeration tank by a feed pump with a known flow rate. The reactor was initially fed with glucose as the carbon source in order to determine its performance in the absence of 2,4-DCP and then gradually acclimatized to 2,4-DCP.

pH values were controlled at  $\text{pH} = 7 \pm 0.6$  during the experiments. Dissolved oxygen (DO) concentration was kept around 3 mg/l. Hydraulic retention time ( $\text{HRT}=\theta_H$ ) was kept constant at 17 h through the operation period. 2,4-DCP loading rate was changed by adjusting the 2,4-DCP concentration. Sludge retention time (SRT) was controlled at about 15 days by removing a certain volume of mixed liquor from the aeration zone of the activated sludge bioreactor. Every experiment was conducted until the system reached the steady-state yielding the same 2,4-DCP and COD contents in the effluent for the last 3 days.

## 2. 4. Analytical Methods

Samples were centrifuged at 6000 rpm for 25 minutes to remove biomass and other solids from the liquid medium. Clear supernatant was analyzed for COD and 2,4-DCP. Standard methods based on digestion and reflux was used for COD analysis (APHA, 1992). The 2,4-DCP analysis were carried out on clear supernatant using 4-aminoantipyrine colorimetric method based on the procedure detailed in Standard Methods for the Examination of Water and Wastewater (Anon., 1992). Biomass concentrations in the liquid phase were determined by filtering samples from 0.45  $\mu\text{m}$  pore size membrane filters and drying the filter paper in an oven at 103 °C until a constant weight is reached. DO and pH measurements were carried out by using the DO and pH meter probes and WTW MultiLine P3 pH/OXI-SET Analyser.

## 3. RESULTS AND DISCUSSION

### 3. 1. 2,4-DCP Biodegradation

The reactor containing 1500 mg/l MLSS (mixed liquor suspended solids) was operated with only glucose at the beginning of operation period for 12 days. After stable COD removal condition (92 %) was obtained, 5 mg/l 2,4-DCP was added as starting concentration. At the end of the 2,4-DCP pre-adaptation period within 10 days, about 98 % 2,4-DCP removal efficiency and 89 % COD removal efficiency was obtained in the reactor at the steady-state conditions. In the 2,4-DCP pre-adaptation stage, low 2,4-DCP concentration was applied. In this case, 10 days was enough to obtain steady-state conditions. If the higher 2,4-DCP concentration was applied in the pre-adaptation phase, the longer time would be required to achieve steady-state. Lora et al. (2000) indicate that adaptation period for 5 mg/l 2,4,6-TCP was 13 days. After acclimation period, the reactor was operated at a range of 30-450 mg/l 2,4-DCP concentrations with progressive adaptation of the mixed culture.

Effluent 2,4-DCP concentrations and corresponding removal efficiencies for feed 2,4-DCP concentrations of 5-350 mg/l varied between 0.1-4.9 mg/l and 96.3-98.6 % respectively (as seen Figure 1). At this range of 2,4-DCP concentrations, 2,4-DCP removal efficiencies remained stable over 96 %. These results demonstrate the possibility elimination of 2,4-DCP shocks after the adaptation phase. Biodegradation with adapted sludge can be expected to cope with quite high load of inhibitory substance.

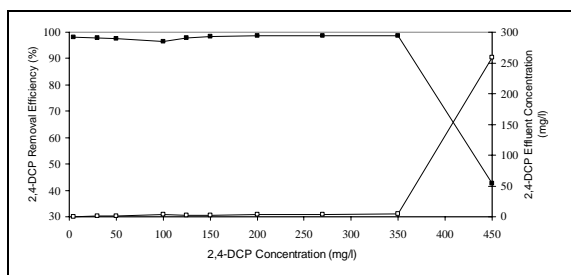


Figure 1. Variation of 2,4-DCP removal efficiency and the effluent 2,4-DCP concentration with the feed 2,4-DCP concentration (■) 2,4-DCP removal efficiency, (□) 2,4-DCP effluent concentrations.

2,4-DCP removal efficiency was decreased from 98.6 to 42.6 % when 2,4-DCP concentration was increased from 350 to 450 mg/l which could be related to the inhibitory effect on microorganisms. Increase in 2,4-DCP in the inlet also resulted in the reduction of degradation capacities of the biomass.

2,4-DCP volumetric removal rate ( $R_{DCP}=(S_o-S)/\theta_H$ ) was plotted against the feed and the steady-state aeration tank (reactor) 2,4-DCP concentrations as given in Figure 2. It could be observed from Figure 2 that different 2,4-DCP concentrations have different biodegradation rates. The rate of 2,4-DCP degradation increased up to 20.3 mg/l.h when feed 2,4-DCP concentration was raised to 350 mg/l (up to 4.9 mg/l reactor 2,4-DCP concentrations), and decreased sharply when the 2,4-DCP concentration was increased further to 450 mg/l (reactor 2,4-DCP concentration 258.3 mg/l) because of inhibition effect on microorganisms of high 2,4-DCP levels in the reactor. It could be conceived that decrease of 2,4-DCP volumetric removal rate at 450 mg/l 2,4-DCP concentration was due to the lowest concentration of microorganisms in the system (as seen in Figure 4). For low reactor 2,4-DCP concentrations (2,4-DCP < 5 mg/l), the volumetric removal rate of 2,4-DCP increased with increasing 2,4-DCP concentrations due to non-inhibitory 2,4-DCP levels in the reactor.

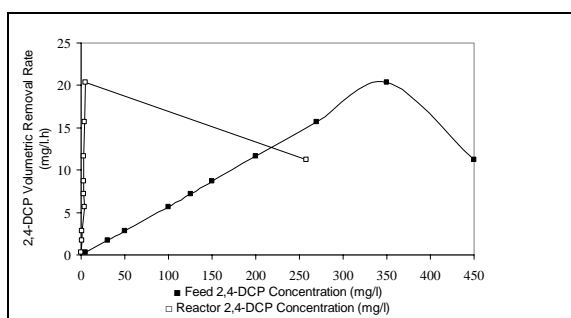


Figure 2. Variation of the volumetric removal rate with the feed and reactor (aeration tank) 2,4-DCP concentrations.

Increases in specific 2,4-DCP removal rates [ $R_{DCP}=(S_o-S)/(\theta_H \times X)$ ] from 0.007 to 1.15 mg/mg X.day with increasing feed 2,4-DCP concentrations up to 350 mg/l (Figure 3) were due to reductions in biomass concentrations starting from 50 mg/l 2,4-DCP in the feed as shown in Figure 4. In the 450 mg/l 2,4-DCP concentration, specific 2,4-DCP removal rate decreased to 0.77 mg/mg X.day due to substrate inhibition.

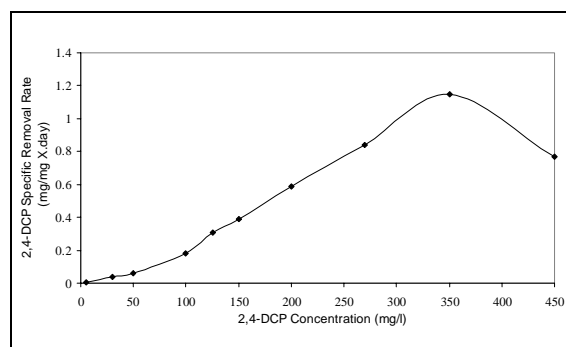


Figure 3. Variation of the specific removal rate of 2,4-DCP with the feed 2,4-DCP concentration.

During 2,4-DCP pre-adaptation stage at 5 mg/l feed concentration, some biomass loss was observed (Figure 4). After this stage, 2,4-DCP increase up to 50 mg/l resulted in biomass production due to pre-adaptation. No inhibition effect occurred during this phase because of the low 2,4-DCP loading rate applied. Further increases in 2,4-DCP concentrations up to 450 mg/l resulted in decrease in the biomass concentration, and the lowest biomass concentration was observed at 450 mg/l 2,4-DCP concentration due to high maintenance requirements of the organism at inhibitory 2,4-DCP concentrations. Kargi et al. (2005) also reported that biomass concentrations decreased with increasing feed 2,4-DCP content in the range between 25 and 250 mg/l.

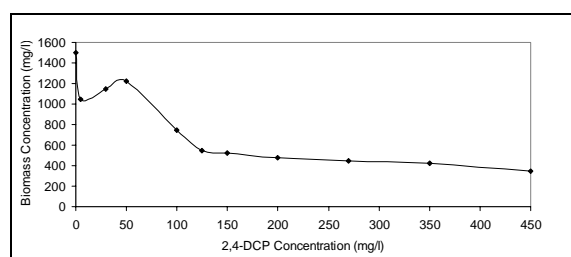


Figure 4. Variation of biomass concentration with respect to the feed 2,4-DCP concentration.

### 3. 2. Effect of 2,4-DCP on COD Removal Efficiency

Figure 5 illustrates variation of effluent COD concentrations at different 2,4-DCP concentrations

in the feed. When 2,4-DCP concentration was increased from 5 to 50 mg/l, COD removal efficiency decreased from 89.00 to 78.98 %. Figure 5 indicates that the COD removal efficiency remains almost constant at further increases in 2,4-DCP concentration, even when it was increased to 350 mg/l. The system could tolerate up to 350 mg/l 2,4-DCP concentration and the COD removal efficiency was not adversely affected by the increase due to the adaptation of microorganisms. The removal efficiency of COD remained between 75.66-78.58 % when 2,4-DCP concentration ranged between 100-350 mg/l.

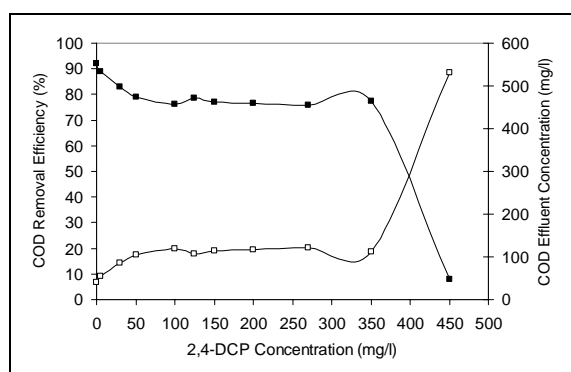


Figure 5. Variation of COD removal efficiency and effluent COD concentration with respect to the feed 2,4-DCP concentration (■) COD removal efficiency, (□) Effluent COD concentrations.

COD removal efficiency decreased sharply to 7.85 % and effluent COD concentration increased from 112.7 to 529.86 mg/l when 2,4-DCP concentration was increased from 350 to 450 mg/l. 450 mg/l 2,4-DCP concentration has caused a toxic shock on microorganisms

Figure 6 illustrates COD volumetric removal rates for the feed 2,4-DCP concentrations and the reactor 2,4-DCP concentrations at steady-state. The rate of COD degradation slowly decreased with increasing feed 2,4-DCP concentration up to 350 mg/l. When 2,4-DCP concentration was increased from 350 to 450 mg/l, COD volumetric removal rate was decreased to the lowest value of 2.66 mg/l.h due to inhibitory effect of 258.3 mg/l reactor 2,4-DCP concentration.

The relationship between the feed 2,4-DCP concentration and specific removal rate of COD is given in Figure 7. While specific removal rates of COD decreased at 2,4-DCP concentrations up to 50 mg/l with increasing biomass, they increased up to 350 mg/l 2,4-DCP concentration due to decreasing biomass concentrations. The specific removal rate of COD decreased from 1.29 mg/mg X.day at 2,4-

DCP=350 mg/l to 0.18 mg/mg X.day at 2,4-DCP=450 mg/l.

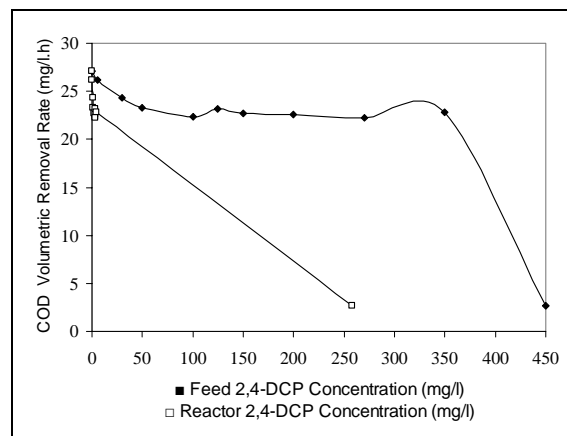


Figure 6. Variation of volumetric removal rate of COD with the feed and reactor (aeration tank) 2,4-DCP concentrations.

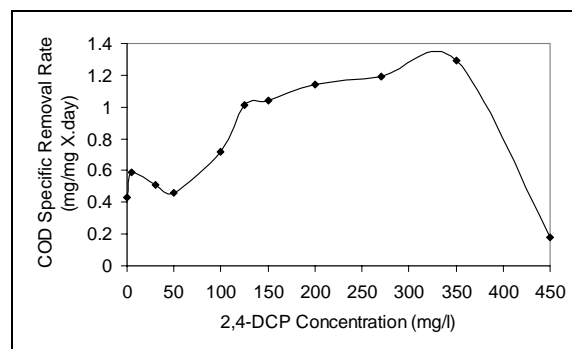


Figure 7. Variation of the specific removal rate of COD with the feed 2,4-DCP concentration.

## 4. CONCLUSIONS

The progressive adaptation of mixed culture to 2,4-DCP was achieved in the experimental study. Adaptation of microorganisms was very important in biodegradation and they could tolerate high concentrations of 2,4-DCP.

Activated sludge could tolerate about as high as 350 mg/l 2,4-DCP concentration in this study, although Xiangcuhun et al. (2003a) reported that 2,4-DCP up to concentration 100 mg/l could be treated in the air-lift honeycomb-like ceramic reactor. Quan et al. (2003) reported that SBR system failed to treat influent 2,4-DCP at concentration of 166 mg/l. Kargi et al. (2005) reported that inhibition effects of 2,4-DCP were pronounced for the feed 2,4-DCP contents above 150 mg/l. Experiments with 2,4-DCP concentrations between 10-200 mg/l showed that higher concentrations of 2,4-DCP (50-200 mg/l) are

inhibitive to the growth of either suspended or immobilized *Bacillus insolitus* by Wang et al. (2000).

The progressive increase in feed 2,4-DCP could enhance the degradation capacities of the biomass. During the adaptation stage, some biomass loss observed. When the microorganisms was adapted to 2,4-DCP, it has generated very little effect on COD removal up to a certain 2,4-DCP concentration. The removal efficiencies of 2,4-DCP for feed 2,4-DCP concentration up to 350 mg/l, varied between 96.3-98.6 %. For COD, it varied between 75.66-92 %. When the 2,4-DCP load was raised up to 450 mg/l, the system was subjected to substrate inhibition which resulted in lowering of biodegradation. When 2,4-DCP concentration was increased from 350 to 450 mg/l, the removal efficiencies of 2,4-DCP and COD decreased to 42.6 % and 7.85 % respectively. Therefore, as long as the 2,4-DCP concentration was kept below inhibitory concentration, it did not effect the removal rate of COD and 2,4-DCP due to adaptation of microorganisms to 2,4-DCP.

## 5. ACKNOWLEDGEMENTS

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