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# An OUR-based approach to determine the toxic effects of 2,4-dichlorophenoxyacetic acid in activated sludge

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**Abstract**

This study uses the oxygen uptake rate (OUR) measurement to measure toxicity effects of 2,4-dichlorophenoxyacetic acid (2,4-D) on activated sludges fed with the wastewater from a small domestic wastewater treatment plant and peptone-based synthetic wastewater. Two 2 l lab-scale batch reactors were run in parallel with the same F/M ratios (0.4 mg COD per mg VSS per day) to assess the inhibition effects of 2,4-D concentrations between 25 and 75 mg $l^{-1}$ considering at least a 100% dilution rate, as compared with a pesticide industry effluent containing 20,000–40,000 mg $l^{-1}$ COD, reaches a central treatment plant. It was noted that the OUR was decreased to 15 and 30%, respectively, when adding 75 mg $l^{-1}$ of 2,4-D to the domestic and synthetic reactors,. Meanwhile, the addition of 25 plus 50 mg $l^{-1}$ of 2,4-D in sequence to the domestic wastewater reactor did not significantly affect the OUR profile.

The OUR-based inhibition definition has been used in this research since the OUR methods have been frequently used and cited in the literature to study toxicity effects. However, the origin of the sludge used in the testing is also important. Synthetic wastewater may simulate the toxicity studies but with a higher response than actual systems, since the microorganisms are considerably becoming substrate-selective.



*Keywords:* Activated sludge; 2,4-D; OUR; Synthetic wastewater; Toxicity

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## 1. Introduction

Most phenolics are known as priority pollutants because of their different effects on humans (e.g. teratogenic, mutagenic, carcinogenic, or toxic) and on the environment. These pollutants reach the municipal treatment systems via surface run-off or industrial wastewaters [1,2].

2,4-D is one of the most common chlorinated herbicides. It mainly originates from the pesticide industry as well as from, e.g. pharmaceutical or chemical industries. It causes toxicity in receiving waters and inhibition of biological treatment systems even at low concentrations [3]. The removal of 2,4-D in the activated sludge system has been reported to be limited [2]. A 25% median removal of 2,4-D was observed in an activated sludge plant treating 3800 $m^3$ per day [4]. The importance of an acclimation phase was pointed out for 2,4-D and other chlorinated hydrocarbons [5]. The rate of 2,4-D removal in activated sludge was suggested to exhibit substrate inhibition characteristics at high substrate concentrations [6]. Meriç et al. [7] proposed a new kinetic model to explain the substrate inhibition behavior in the activated sludge for treating phenol and phenolics such as 2,4-D. Talınlı and Görgün [8] studied inhibition effects of eight micropollutants including 2,4-D on bacterial growth using BOD bottles. They determined the $IC_{50}$ value of 2,4-D as 400 mg $l^{-1}$ and they proposed a 2:4:1 THP classification indicating moderate inhibition effects to biological treatment.

Hartman et al. [9] studied the toxic effects of 2,4-D on *Daphnia magna*, on Microtox® measurements, and on the oxygen uptake rate (OUR) of potato starch-containing wastewaters. Addition of 20 and 40 mg $l^{-1}$ of 2,4-D to the potato starch reactor caused a decrease in the OUR relative to a control reactor (potato starch with 1000 mg $l^{-1}$ COD equivalent only). OUR was defined as 10.2, 8.7, and 7.6 (mg $O_2$ per g VSS per h) for the control, for 20 mg $l^{-1}$ and for 40 mg $l^{-1}$ additions of 2,4-D, respectively.

Chemical oxidation treatment processes have been used to treat chlorophenolics by breaking the aromatic ring thus producing more biodegradable by-products. Tünay et al. [10] reported that 2,4-D production wastewaters contain 20,000–40,000 mg $l^{-1}$ COD. They used chemical oxidation to remove COD at pH 3 using 3000 mg $l^{-1}$ of $Fe^{3+}$. A 3:1 molar ratio of $H_2O_2$/COD resulted in a complete COD removal. Yu and Hu [11] studied peroxidation of chlorophenolic wastewaters for their subsequent biological treatment in acidic and alkaline conditions. They observed that acidic conditions resulted in a better secondary treatment. Basu et al. [12] reported that Fenton's reagent was effective for toxicity reduction of 2,4,6-TCP. Phodegradation of dichloroacetic acid and 2,4-dicholorophenol by ferrioxalate/$H_2O_2$ was also reported with a high efficiency [13].

Respirometric techniques have been used to test the toxicity effects of different pollutants on activated sludge. Volskay and Grady [14] tested the joint toxic effects of 33 phenolics in a lab-scale continuous activated sludge reactor fed with a complex, synthetic feed designed to mimic domestic wastewater using respiration inhibition kinetic analysis (RIKA) assay [14]. Volskay and Grady [14] compared their toxicity results with the OECD 209 method [15] and they concluded that their method was more precise to determine the toxic effects of a number pollutants of different origins than the OECD method [15]. De Bel et al. [16] determined the toxic effects of different wastewaters on domestic wastewater treatment plants including the nitrification process using OUR and they calculated inhibiton levels. Rozzi et al. [17] conducted a study for the definition of toxic effects of industrial wastewaters from textile

and leather tanning processes wastewaters on an activated sludge system on the basis of OUR measurement. Madoni et al. [18] studied the inhibition effects of heavy metals on full scale process measuring OUR and ammonia uptake rate (NUR). They reported that OUR measurements were more meaningful than NUR to determine the toxic effects of heavy metals. An evaluation of wastewater toxicity measured by Microtox® and activated sludge oxygen uptake inhibition has been done by Guitérrez et al. [20]. According to their results, Microtox® proved to have a higher sensitivity to toxicants but was less representative of effects on activated sludge compared to respirometry.

The OUR measurements are infact quite meaningful in the determination of the growth response to any change in the conditions, since it instantaneously expresses the growth conditions that is why the OUR measurements have been adopted as the main tool in determining the growth characteristics of biological treatment systems [19].

This study was aimed at explaining the toxic behavior of 2,4-D using the OUR measurement which was considered more representative for toxic effects of pollutants on activated sludge. A modified ISO 8192 method [21] was applied to emphasize the importance of acclimation of activated sludge regarding the inhibitor concentration.

## 2. Materials and methods

### 2.1. Sampling and analyses

Raw domestic sewage and activated sludge samples were taken from a small wastewater plant 65 km southwest from Istanbul (Turkey) with two aeration basins working in parallel. The samples were cooled for transport to the laboratory and stored at 4 °C without adding any chemical.

The composition of synthetic wastewater according to the ISO 8192 [21] procedure is given in Table 1. Tap water was used to dilute the synthetic sewage to the required COD concentrations. A 1 g aliquot of synthetic wastewater mixture yielded 0.85 g of COD. The COD equivalent of 1 mg 2,4-D was measured as 2.1 mg.

All chemical analyses were performed according to Standard Methods [22]. The samples were filtered using AP40 Sartorious filter paper for TSS and filtrated COD analyses. Sludge characteristics were also defined measuring MLSS, MLVSS and sludge volume index (SVI)

Table 1
The composition of synthetic sewage (diluted, 1:100; pH, 7.5 ± 0.5)

| Components | Amount (g) |
|---|---|
| Peptone | 16 |
| Meat extract | 11 |
| Urea | 3 |
| Sodium chloride (NaCl) | 0.7 |
| Calcium chloride ($CaCl_2 \cdot 2H_2O$) | 0.4 |
| Magnesium sulfate ($MgSO_4 \cdot 7H_2O$) | 0.2 |
| Potassium diphosphate ($K_2HPO_4$) | 2.8 |
| Distilled water | 1000 ml |

Table 2
The characteristics of activated sludges taken from basins 1 and 2 working in parallel

| | MLSS ($mg\,l^{-1}$) | MLVSS ($mg\,l^{-1}$) | SVI ($ml\,g^{-1}$) | MLVSS/MLSS (%) |
|---|---|---|---|---|
| Basin 1 | 2860 | 1840 | 75 | 65 |
| Basin 2 | 4810 | 3540 | 275 | 74 |

after 30 min settling time as illustrated in Table 2. Although the MLVSS/MLSS ratio was higher (74%) in the basin 2, the sludge of basin 1 was used in OUR measurements, since it reflected a better SVI value and microbial diversity in terms of filamentous bacteria. The reason why the SVI value of the basin 2 is so high is due to recirculation pump technical error which was recently changed.

All analyses were done in duplicates and the results were presented as average.

All chemicals used were of analytical grade. Solutions were prepared using distilled water.

### 2.2. Experimental

Activated sludge taken from the treatment plant and synthetic wastewater fed in the laboratory were aerated continuously in the 2 l fill and draw batch reactors. The reactors were kept in room temperature (20 °C) and pH was controlled (Orion model 700). During experiment, 1 l volume closed reactor was used and it was continuously mixed by a magnetic stirrer as shown in Fig. 1. The reactors were aerated periodically. The oxygen consumption was measured by oximeter (model 580 WTW) connected to printer.

The OUR profiles of the reactors were drawn for determining optimum F/M ratios considering proper OUR profiles representing 25–30 min first plateu. The OUR measurements were realized on both domestic and synthetic wastewater reactors without (DWB and SWB) and adding 2,4-D (DW1, DW2 and SW1). Nitrification inhibitor (HACH, Formula 2533) was also added to the reactors.

The addition of 2,4-D was planned in two phases as either $75\,mg\,l^{-1}$ at once (DW1, SW1), or 25 plus $50\,mg\,l^{-1}$ in sequence (DW2). These concentrations were chosen to

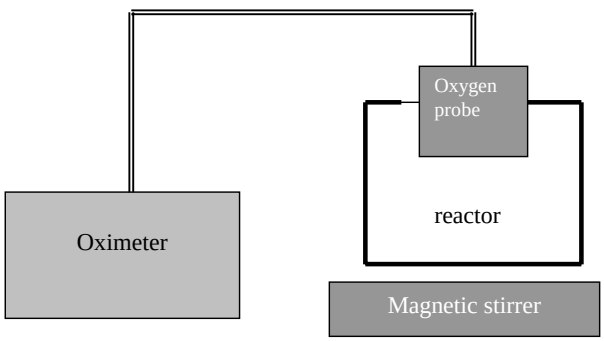


Fig. 1. Experimental set-up.

account for the COD addition percentage of 2,4 D which ranged from 5% to 20% to the total reactor COD (720 mg $l^{-1}$) considering the COD influent of the treatment plant. Synthetic wastewater acclimated reactor (SW) was run in parallel with the same COD content of DW reactor.

It was also considered that the reactors were not acclimated to 2,4-D. 2,4-D was added to the reactors in the phase of soluble substrate consumption where inhibitor substrate which is in soluble form could compete with or inhibit the exogenous substrate utilization in the reactors.

## 3. Results and discussion

### 3.1. Characteristics of treatment plant and wastewater

The existing treatment plant was designed as conventional activated sludge with 10 days of sludge age and 8 h of hydraulic retention time. It was built rectangular shape with two aeration basins working parallel. Influent characteristics were assumed 240 mg $l^{-1}$ of $BOD_5$ and 40 mg $l^{-1}$ of TKN while effluent characteristics would be 100 mg $l^{-1}$ of COD, 50 mg $l^{-1}$ of $BOD_5$, 15 mg $l^{-1}$ of TSS, and 10 mg $l^{-1}$ of TKN. On the other hand, chemical analyses of nine samples taken from the different points of the treatment plant between April 2001 and January 2002 presented strong domestic wastewater characteristics as COD = 500–700 mg $l^{-1}$, $BOD_5$ = 250–400 mg $l^{-1}$, SS = 190–220 mg $l^{-1}$, TKN = 70–85 mg $l^{-1}$, and TP = 9–15 mg $l^{-1}$, respectively.

### 3.2. OUR profiles and inhibition evaluation

The OUR profile of the activated sludge from basin 1 fed with domestic wastewater and considered as domestic wastewater blank reactor (DWB) is illustrated in Fig. 2a. As seen from Fig. 2a, a proper F/M ratio (0.4 mg COD per mg VSS per day) was used according to previous study performed on domestic wastewater [19].

Seventy-five milligram per liter of 2,4-D (DW1) was added at the 25th minute during the first plateau of the OUR profile where soluble substrate is consumed. The OUR profile of DW1 reactor is shown in Fig. 2b. As seen in Fig. 2b, the OUR decreased to approximately 15% with 4% standard deviation compared to DW reactor. After a sudden decrease, the OUR was observed to recover itself 20 min later. When 2,4-D was added to domestic wastewater fed reactor (DW2) in sequence as 25 mg $l^{-1}$ at the 25th minute (first plateau), the OUR decreased by $6 \pm 3\%$. Addition of 50 mg $l^{-1}$ of 2,4-D at the period where the hydrolysis phase occurs simultaneously in the reactor, did not affect the system OUR as seen in Fig. 2c.

The OUR profile of the synthetic wastewater fed blank reactor (SWB) is illustrated in Fig. 3a. The OUR of the SWB reactor was similar with the DWB reactor at the beginning but substrate was consumed at a longer period with respect to DWB reactor. This may be explained from the synthetic wastewater composition given in Table 1. Although it is completely soluble, especially peptone has different consumption pattern by the bacteria than domestic wastewater.

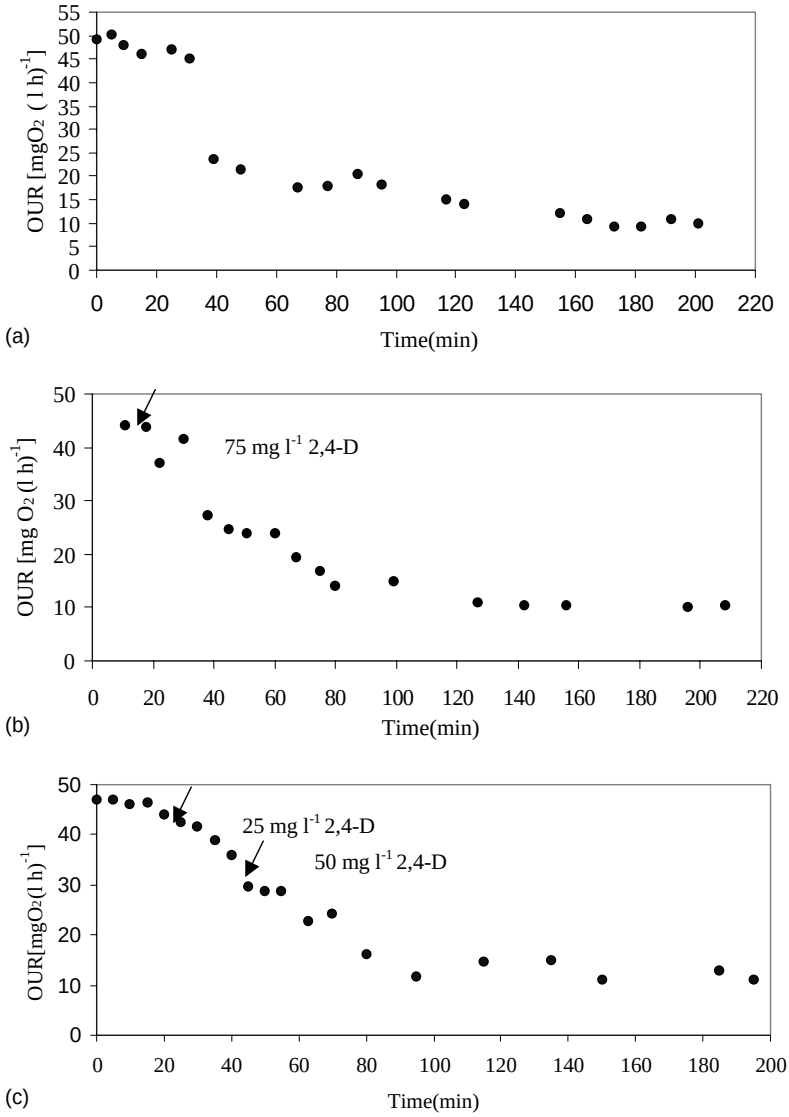


Fig. 2. The OUR profiles of domestic wastewater fed reactors: DWB (a), DW1 (b), and DW2 (c) at 0.4 mg COD per mg VSS per day of F/M ratio.

Since 2,4-D did not result in any significant inhibitory effect on DW2 reactor, 75 mg $l^{-1}$ of 2,4-D was only added at once to the synthetic wastewater fed reactor (SW1). The resulting OUR profile of SW1 reactor is shown in Fig. 3b. The OUR was affected for 30 ± 4% and the system could not recover as fast as DW1 reactor.

The slope of the OUR values after recovering time for each reactor can be indicative more for explaining the inhibiting action of 2,4-D. For example, if 40 and 20 mg $l^{-1}$ $h^{-1}$

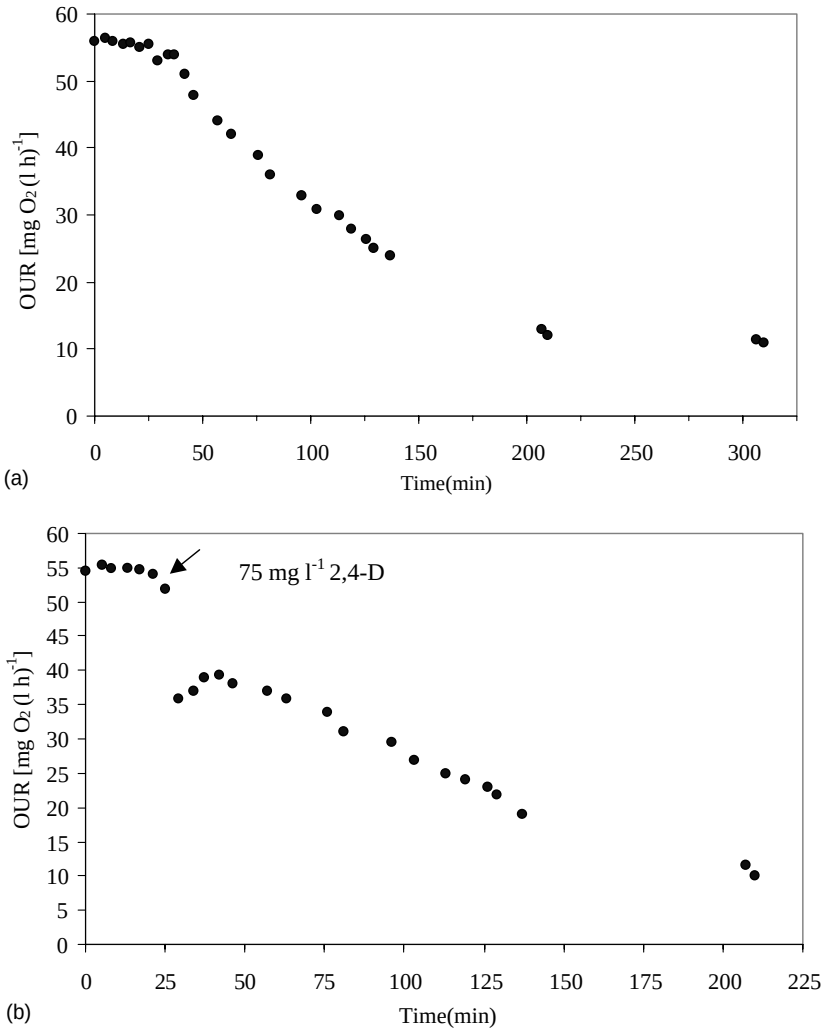


Fig. 3. The OUR profiles of synthetic wastewater fed reactors: SWB (a) and SW1 (b) at 0.4 mg COD per mg VSS per day of F/M ratio.

OUR interval is considered, the slope of DW1 reactor is approximately two-fold that of the SW1 reactor. The same can be observed from comparing each reactor with their controls (DW and SW).

The evidence for significant inhibition of 2,4-D in DW1 and SW1 reactors confirmed the literature data [7,9]. A further continuation of this study may be used to define the kinetic constants in the presence of 2,4-D in sludges of different origin. Running the RIKA [14] test and OECD [15] method in parallel could provide detailed data such as defining $IC_{50}$ of 2,4-D to explain the behavior of 2,4-D in the activated sludge.

## 4. Conclusions

OUR-based inhibition definition has been employed in this paper as a more reliable method, since it is based on the active biomass in the system compared to total biomass-based measurements. The inhibition effect of $75\,mg\,l^{-1}$ of 2,4-D was measured as $15 \pm 4\%$ and $30 \pm 4\%$ on domestic wastewater and synthetic wastewater fed reactors, respectively. However, 2,4-D did not display any significant inhibition effect when it was added as $25\,mg\,l^{-1}$ plus $50\,mg\,l^{-1}$ into domestic wastewater fed reactor. Inhibition effect of 2,4-D on activated sludge was evaluated considering the recovery time of each reactor.

Since these concentrations of 2,4-D are relatively high for municipal treatment plants, the results of this study may be evaluated in the context of the pretreatment or in-plant control applications.

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