Assessment of enhancement in biodegradation of dichlorodiethyl ether (DCDE) by pre-oxidation

Milica Kaludjerski, Mirat D. Gurol*

San Diego State University, Department of Civil & Environmental Engineering, 5500 Campanile Drive, San Diego, CA 92182-1342, USA

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Abstract

The objective of this study is to assess the enhancement in biodegradation of dichlorodiethyl ether (DCDE) by ozonation and Fenton treatment. Acclimated and non-acclimated sludge cultures were used to test the biodegradability of the preoxidized DCDE solutions by three different tests: Short-term and long-term respirometry through continuous monitoring of oxygen consumption, and the mid-term test in which the amount of remaining organic matter was measured by TOC and COD tests. These tests were applied to solutions of DCDE preoxidized at the levels of 25%, 50%, 75%, and 100%.

The results indicated that the biodegradability of oxidized DCDE solutions improved substantially compared to nonoxidized solutions. Fenton-treated DCDE exhibited toxicity to microorganisms under long-term exposure. Higher levels of preoxidation of DCDE led to mineralization of larger amounts of organic matter during subsequent biodegradation. There was no significant difference in the rate of biodegradation of oxidized products by either acclimated or non-acclimated bacteria.

Keywords: Dichlorodiethyl ether; Biodegradability; Respirometry; Ozonation; Fenton treatment

1. Introduction

The discharge of wastewater containing hazardous compounds into municipal treatment systems is often prohibited because these compounds can persist in biological treatment systems and pass through the effluent or absorb to the biosolids without any transformation. Furthermore, some of these compounds can upset the biological process through toxicity. As a result, industries are forced to implement costly alternatives such as transfer to other facilities for off-site treatment and land disposal.

Application of chemical oxidation as a pre-treatment is known to be an effective way to transform many such compounds into bio-treatable reaction intermediates. The present work was undertaken with three main objectives: (1) to investigate the biodegradability of preoxidized dichlorodiethyl ether (DCDE), which has not been studied before, (2) to compare the effectiveness of two different oxidation processes, ozonation and Fenton treatment in terms of enhancement of biodegradability, and (3) to compare the responses of three different biodegradability tests: Short-term and long-term respirometry that use oxygen consumption, and the mid-term test, which measures the removal of organic matter by TOC and COD tests.

DCDE was used in this study as a model compound, because DCDE is classified under RCRA as a toxic compound (#U025), under Clean Air Act as a “hazardous air pollutant”, and under Clean Water Act as a “priority and toxic pollutant”, because it is reactive, flammable, toxic, mutagenic and a probably carcinogetic [1]. Furthermore, DCDE is known to be nonbiodegradable in conventional biological treatment systems [2].
For chemical oxidation of DCDE, ozone and Fenton reagent were used with the assumption that each method would produce different oxidation by-products, and as a result these methods would enhance the rate and extent of biodegradation differently. It is well known that ozone might react with organic matter as an electrophile, or through hydroxyl radical pathway. Whereas, hydroxyl radical is believed to be the main oxidant produced in the Fenton reagent, although an electron transfer mechanism for oxidation of organic compounds was proposed in the recent literature [3,4].

An extensive review of combined chemical and biological treatments was provided by Scott and Ollis [5]. These authors reviewed more than 80 papers pertaining to oxidative treatment processes for various types of contaminants and water types. Since that review a number of studies have been published using combined biological oxidation and chemical oxidation, including TiO2 photocatalytic treatment [6] ozonation [7–9], ozonation coupled with UV light or H2O2 [10,11], wet oxidation [12], Fenton treatment [13,14], ozone/hydrogen peroxide [15], TiO2 and ZnO photocatalysis [16], and UV/hydrogen peroxide [17]. A detailed review of these publications are available in Kaludjerski [18] and Christensen [19]. In general, it was concluded that the combined chemical oxidation and biological processes led to increased degradation of target compounds with a few exceptions, which noted no change or negative results.

Many of the earlier studies selected chemicals to chemical oxidation, the extent of which was either not noted, or decided arbitrarily at a single value. Furthermore the biodegradability of untreated and treated solutions of recalcitrant compounds were measured typically by the BOD test, which obviously is not suitable for assessment of the possible toxicity of the oxidation intermediates to the microorganisms because of extensive dilutions. Furthermore, very few of the earlier studies provided rate information, e.g., Monod constants of the oxidized solutions. Very few studies used both acclimated and non-acclimated organisms to compare their responses to the oxidized solutions, and none applied three different methods for evaluation of the biodegradability and the toxicity of the oxidized solutions.

This research is an attempt to provide a methodology to provide fundamental information and better insight into the process of integrated chemical oxidation/biodegradation. The approach consisted of oxidizing the model chemical, DCDE, at various levels (25%, 50%, 75%, and 100%), and then subjecting the preoxidized solutions to three different biodegradability tests. The rate and the extent of biodegradability of the oxidized DCDE solutions were assessed by short-term and long-term respirometric tests by measuring the oxygen consumption as a function of time. The results were then evaluated based on “non-parent TOC” a parameter used to represent the substrate concentration in oxidized solutions. In addition, the extent of organic matter mineralization during biodegradation was measured through a third test (mid-term) over a 6-h period by monitoring the COD and TOC of the test solutions. Finally, the responses of acclimated and non-acclimated cultures to oxidized DCDE were compared for all three biodegradation tests.

2. Materials and method

2.1. Oxidation tests

Both ozonation and Fenton tests were conducted at ambient temperature (22 ± 0.2°C) and the pH values noted. The ozonation was carried out using a typical experimental system. Fenton experiments were performed in a completely mixed batch reactor to which H2O2 and Fe2(SO4)3 were added in stepwise fashion with 10-min intervals, as described earlier [20]. During the reaction period the samples were taken by 10-min intervals, and the pH was restored to 7 by NaHCO3. Then the solution was filtered using 0.45 μm membrane filters for separation of the iron precipitate before any measurements. At the end, the oxidized solutions contained 5 mM NaHCO3. A catalase solution was used to quench any remaining H2O2 prior to the biodegradation studies. Short-term biodegradation experiments run as control with catalase and Fenton chemicals showed that neither catalase nor Fenton chemicals were toxic to the microorganisms, and that the catalase in the amounts added did not contribute to the biodegradability of Fenton-treated samples (data not shown).

All oxidation experiments were started with 200 mg/L of DCDE, and oxidized to provide 25%, 50%, 75% and 100% oxidation of DCDE as target values. These solutions were then made available for the biodegradation studies. After each oxidation experiment and before the biodegradation tests, the solutions were analyzed for DCDE to evaluate the exact percent oxidation levels, which changed slightly from the target values.

2.2. Activated sludge cultures

Heterogeneous microbial populations used in this study were grown in our laboratory in two 5 L reactors, both operated in semi-batch mode. The original biomass was obtained from the return line of the activated sludge reactor (RAS) in the North City Reclamation Plant, San Diego, CA. The MLSS concentration in the reactor was adjusted to and maintained at 3200 mg/L. The biomass was fed with a mixture of filtered primary effluent and chemical oxygen demand (COD) supplement solution to adjust the strength of the feed to 1500 mg/L as COD.
This mixture was added to the reactors daily at a volume of 4 L. The COD supplement solution was prepared using the modified recipe of Henze [21]. Twice daily, 2 L of the supernatant were withdrawn from the reactors to maintain the operating parameters of the reactors at the sludge residence time of 5 days, food to microorganisms ratio of 0.375 day\(^{-1}\), and a hydraulic residence time of 1.25 days. The reactors were aerated to maintain about 4 mg/L of dissolved oxygen. Once a week, 1 L from each reactor was replaced by fresh RAS from the same plant in order to maintain a diverse microbial community. In order to acclimate the microorganisms, one of the reactors was fed with 0.4 mg/L of DCDE in addition to the mixture of the primary effluent and the COD supplement solution for at least 4 weeks before the experiments were conducted.

Biomass used in biodegradation studies was harvested by centrifuging reactor contents and recovering and resuspending the biomass pellet in a mineral salt solution to achieve an MLSS concentration of 2000 mg/L.

2.3. Biodegradation studies

Biodegradation studies were conducted by using three different tests.

2.3.1. Short-term tests

The short-term biodegradation test involved the measurement of biomass respiration rate by dissolved oxygen (DO) probes for a period of 30 min. The biomass concentration was kept constant at 400 mg/L as MLSS, which was chosen to yield a significant oxygen decrease within 30 min of experimentation, thereby allowing accurate measurement of respiration rates. A modification of the Organization of Economic Cooperation (OECD) Method 209 [22] was followed as the experimental protocol. The experiments were conducted in BOD bottles using Orion, Model #97-08 DO probes. The tests were performed with both non-acclimated and acclimated microorganisms. The bottles contained oxidized DCDE solutions, biomass and a yeast extract (0.15 mg/L) which provided the necessary macro-nutrient for the microorganisms. A personal computer, with a Pentium II processor, equipped with a data acquisition board (IO Tech, Model DynaRes-8U) was set up to acquire the data during experiments and then to calculate the kinetic constants of the biodegradation rate. The data points were collected for each second during the experiment over a period of 30 min. The respiration rate of the microorganisms in each bottle was determined by calculating the slopes of DO versus time plots by Microsoft Excel program.

The endogenous respiration rate of microorganisms was obtained by running a control experiment with microorganisms alone. The net exogenous respiration rates for DCDE solutions were obtained by subtracting the endogenous rate from the observed exogenous respiration rates. The net exogenous respiration rates that are directly proportional to the substrate removal rate were substituted in the Monod equation to determine the specific substrate removal rate constants

\[
\frac{dS}{dt} = q = \frac{q_{max} S}{S + K_S}
\]

Non-parent TOC resulting from oxidation was used as the biodegradable substrate concentration (S) for parameter estimation. Non-parent TOC for this purpose was defined as the TOC contributed by the chemical oxidation products, and it was estimated by subtracting the TOC attributable to DCDE from the total TOC, i.e., non-parent TOC = npTOC = total TOC − 0.34C\(_{DCDE}\), where total TOC is the measured TOC value, C\(_{DCDE}\) is the measured concentration of DCDE, and 0.34 is the theoretical amount of TOC per mg of DCDE. The constants, q\(_{max}\) and K\(_S\) were determined through nonlinear curve fitting. The calculations of kinetic constants and statistical significance tests were performed by using Sigma Plot software for the 95% confidence level. Comparison of the kinetic constants obtained for the oxidized samples and the non-oxidized samples allowed the assessment of the extent of improvement of biodegradation.

2.3.2. Long-term tests

The long-term respirometric experiments were conducted over a period up to 14 days to obtain information on biodegradability and toxicity of the by-products of oxidation and of the parent compound. These data allowed us to see if prolonged exposure would prompt microorganisms to develop the necessary enzymes for degradation of DCDE and/or the oxidation by-products, or would result in toxicity and killing of the microorganisms.

A ten channel Micro Oxymax Respirometer (Columbus Instruments International Corporation, Columbus, OH), equipped with oxygen and carbon dioxide sensors and a computer interface was used for these tests. The respirometric technique involved placing the DCDE solutions with microbial inocula into sealed 250 mL bottles and continuously measuring oxygen and carbon dioxide concentrations under batch conditions over a period of up to 14 days. The data points were taken every 3 h and stored to a hard disc by the Micro Oxymax software.

The experiments were run in duplicates, and included three levels of controls, each with duplicates: (1) Test with no microorganisms (no mo), (2) Test with microorganisms and 100 mg/L of non-oxidized DCDE (no oxidation), and (3) Test with microorganisms alone (endogenous).
2.3.3. Mid-term tests

These experiments were designed to present data in terms of degradation of the biodegradable organic matter, as measured by TOC and COD contents of the solutions as a function of time. The total amount of oxidation by-products that were biodegradable within 6h was determined and compared among different treatments. Six hours was used as the time span because (1) the rate of biodegradation as measured by COD or TOC change leveled off beyond 6h, and (2) 6h is a typical hydraulic residence time in wastewater treatment plants.

The amount of TOC and COD due to the parent compound (DCDE) was subtracted from the total measured concentrations of TOC and COD, and the remaining amounts of TOC and COD were attributed to the oxidation products. The theoretical COD of 1 mg of DCDE is 160/143 = 1.12 mg. Hence, the COD contributed by the oxidation products, i.e., “the non-parent COD” was estimated as follows: npCOD = (Total COD−1.12CDCDE). The npTOC was defined earlier.

A different experimental system was set up to conduct the mid-term biodegradation tests. These experiments were conducted in duplicates in 1L glass bottles open to the atmosphere. Air was supplied with glass diffusers with a flow rate of 1.65 L/min. The concentration of microorganisms, measured as mixed liquor suspended solids (MLSS), was adjusted to 400 mg/L, with dilution of all samples by 50% once DCDE solution was added.

Over 24h, samples (20 mL) were withdrawn every 1.5h, filtered directly into 20 mL vials through 0.2 μm pore filter papers, and the filtrate immediately analyzed for DCDE concentration. The remaining filtrate was acidified with H2SO4 and stored at 4°C. The measurements of suspended solids, COD and TOC were performed within 24h.

2.4. Analytical methods

The DCDE concentration in the solutions was determined by a Shimadzu Gas Chromatograph equipped with a flame ionization detector. The aqueous samples were injected directly on a Nukol column (15 m x 0.53 mm with 0.5 mm film) that was obtained from Supelco (Bellefonte, PA). Helium was used as a carrier gas at 15 mL/min, while hydrogen and air were used to fuel the detector. The injector and detector were operated at temperatures of 150°C and 250°C, respectively. The initial column temperature was 80°C, and it was increased to 200°C at the rate of 5°C/min.

The TOC concentration was determined by using a Shimadzu Analyzer 5000A. The COD concentration was measured by the closed reflux, colorimetric method according to the Standard Methods [23]. Total suspended solids in samples were measured by the gravimetric method [23].

3. Research results and discussion

3.1. Short-term biodegradation test results

The tests conducted on a range of different concentrations of non-oxidized DCDE indicated that DCDE was not biodegradable by non-acclimated activated sludge culture. Microorganisms exposed for a period of more than 4 weeks to 0.4 mg/L of DCDE and thereby presumably acclimated to its presence were not able to degrade non-oxidized DCDE either. Only trivial exogenous respiration rates were measured (data not shown), confirming the results previously reported in Hazardous Chemicals Desk Reference book that DCDE is non-biodegradable in conventional biological treatment systems [2].

The results of short-term biodegradation tests conducted on oxidized solutions are presented in Figs. 1–4 in terms of net exogenous respiration rate (mg oxygen consumed per liter per minute) versus non-parent TOC, which represents the concentration of biodegradable substrates resulting from chemical oxidation of DCDE. The varied parameter in the figures is the percentage of DCDE oxidation prior to the biodegradation tests. The data point for non-oxidized DCDE, i.e., zero amount of non-parent TOC is represented by the point of origin in the figures. The results in all four figures show that an increase in the substrate concentration is reflected by a rapid increase in the net respiration rates, reaching a plateau at higher concentrations, a behavior predicted by the Monod equation. The net respiration rates for all levels of oxidation by ozone or Fenton reagent are significantly higher than the rate of non-oxidized DCDE, which is zero. This increase in the respiration rates is clearly due to existence of biodegradable chemicals produced as by-products of DCDE oxidation by ozone or Fenton reagent. Many of these products were identified in our laboratories as primarily carboxylic acids and aldehydes [20].

![Fig. 1. Respiration rates of non-acclimated cultures for ozone-treated DCDE solutions.](image-url)
rates. A slight drop in the maximum respiration rate ($q_{\text{max}}$) can be seen in the samples oxidized by 100%. However acclimated culture produced significantly different results for ozonated solutions (Fig. 2). The solution ozonated for 75% removal of DCDE produced significantly higher respiration rate than the other solutions. Furthermore, the respiration rate dropped significantly for 100% oxidized DCDE. The decreased respiration rate for 100% oxidation can be explained by formation of higher concentrations of by-products from oxidation of DCDE that might either be toxic or less biodegradable.

The results for DCDE solutions oxidized by Fenton reagent were not very different than those obtained for ozonated solutions (Figs. 3 and 4), i.e., the net respiration rate increased with increasing substrate concentration. For non-acclimated culture, higher oxidation rates, e.g., 75% and 100%, produced more enhancement in the respiration rate than the lower oxidation rates (Fig. 3). However, for the acclimated culture (Fig. 4) a lower maximum respiration rate was observed for 100% oxidized solution than the others. These samples are again highly oxidized and therefore, possibly contain higher concentrations of the by-products that are either toxic or less biodegradable. The acclimated cultures seem to be more sensitive to the oxidation by-products of DCDE. This higher sensitivity suggested for the acclimated cultures could be due to reduced resistance in a less diverse microbial culture.

The $K_S$ for acclimated and non-acclimated cultures exposed to ozone or Fenton-treated samples (Table 1) showed that the acclimated and non-acclimated cultures degraded the by-products equally well. The data imply that Fenton treated samples have more readily biodegradable by-products of oxidation (i.e., lower $K_S$), however, the presence of toxic by-products seemed to have resulted in a lower maximum respiration rate ($q_{\text{max}}$) when compared to ozonation (Table 1). Furthermore, ozone by-products induced higher respiration rate than Fenton reagent by-products at the 75% oxidation level.

3.2. Long-term biodegradation test results

The results of the long-term tests were presented for brevity in terms of oxygen consumption data only. Data on carbon dioxide formation are available elsewhere [18]. In Figs. 5–9, the decrease in oxygen concentration due to consumption was presented in mg/L as a function of time in days.

The results of the long-term biodegradation experiments for ozonated DCDE solutions are shown in Figs. 5 and 6, for non-acclimated and acclimated cultures, respectively. For non-acclimated culture, oxygen consumption for the endogenous control was consistently higher than the oxygen consumption observed for non-oxidized DCDE. This observation indicates that DCDE

For ozonated solutions subjected to non-acclimated culture (Fig. 1), different ozonation levels produced similar results in terms of enhancement in the respiration

![Graph](image-url)
was toxic at 100 mg/L. However, for the preoxidized DCDE samples, the oxygen consumption was significantly higher than the endogenous control resulting in an overall oxygen consumption of about 200 mg/L within 8 days for the two highly oxidized samples. The acclimated culture seemed to tolerate the presence of 100 mg/L of non-oxidized DCDE initially, but exhibited reduced oxygen consumption after about 126 h. The ozonated DCDE solutions consumed more oxygen compared to the endogenous rate, and the oxygen consumption increased as the oxidation level increased.

### Table 1

Monod constants for biodegradation of preoxidized DCDE by ozone and Fenton reagent using acclimated and non-acclimated cultures

<table>
<thead>
<tr>
<th>Percent oxidation of 200 mg/L of DCDE</th>
<th>0%</th>
<th>25%</th>
<th>50%</th>
<th>75%</th>
<th>100%</th>
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<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>$q_{\text{max}}$ (mg/L-min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O$_3$ acclimated</td>
<td>0</td>
<td>0.11 ± 0.01</td>
<td>0.15 ± 0.02</td>
<td>0.21 ± 0.03</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>O$_3$ non-acclimated</td>
<td>0</td>
<td>0.12 ± 0.02</td>
<td>0.14 ± 0.02</td>
<td>0.19 ± 0.03</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>Fenton acclimated</td>
<td>0</td>
<td>0.12 ± 0.02</td>
<td>0.14 ± 0.02</td>
<td>0.123 ± 0.007</td>
<td>0.090 ± 0.003</td>
</tr>
<tr>
<td>Fenton non-acclimated</td>
<td>0</td>
<td>0.07 ± 0.01</td>
<td>0.10 ± 0.01</td>
<td>0.123 ± 0.004</td>
<td>0.123 ± 0.004</td>
</tr>
<tr>
<td>$K_s$ (mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O$_3$ acclimated</td>
<td>—</td>
<td>6.9 ± 2.0</td>
<td>6.5 ± 5.1</td>
<td>8.1 ± 4.1</td>
<td>7.9 ± 3.6</td>
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<tr>
<td>O$_3$ non-acclimated</td>
<td>—</td>
<td>5.2 ± 2.1</td>
<td>6.5 ± 5.6</td>
<td>9.9 ± 3.9</td>
<td>9.4 ± 3.6</td>
</tr>
<tr>
<td>Fenton acclimated</td>
<td>—</td>
<td>4.2 ± 1.9</td>
<td>3.5 ± 2.3</td>
<td>1.5 ± 0.7</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>Fenton non-acclimated</td>
<td>—</td>
<td>3.0 ± 2.0</td>
<td>3.5 ± 1.7</td>
<td>1.2 ± 0.3</td>
<td>2.0 ± 0.1</td>
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</table>

Fig. 5. Oxygen decline during long-term respirometric tests for ozone-treated DCDE with non-acclimated culture.

Fig. 6. Oxygen decline during long-term respirometric tests for ozone-treated DCDE with acclimated culture.

Fig. 7. Oxygen decline during long-term respirometric tests for Fenton-treated DCDE with non-acclimated culture.

Fig. 8. Oxygen decline during long-term respirometric tests for Fenton-treated DCDE with acclimated culture.
The results of the long-term biodegradation experiments for Fenton-treated DCDE solutions are shown in Figs. 7 and 8, for non-acclimated and acclimated cultures, respectively. The non-acclimated culture showed high sensitivity to the oxidation by-products because the endogenous control started to consume more oxygen than the microorganisms with oxidized solutions within 2 days. These results show that after the initial consumption of easily biodegradable by-products of oxidation, microorganisms were not able to adapt to the possibly toxic by-products and could not continue to degrade them for long periods of time. The acclimated culture produced similar results.

For both cultures, the toxicity of the Fenton-treated solutions seemed to diminish with increasing levels of oxidation. It should be noted that the solutions of oxidized DCDE still contain DCDE, unless the oxidation is by 100%. However, independent toxicity tests conducted in our laboratories under comparable conditions indicated that the toxicity of DCDE to activated sludge was negligible if the DCDE concentration was below 50 mg/L [24]. Hence, it is obvious that the toxicity observed in the Fenton-treated samples (oxidation higher than 50%) was not due to DCDE but the oxidation products. Finally, the observation made in short-term tests that solutions preoxidized at 75% produced higher \( q_{\text{max}} \) values than those preoxidized by 100% was not obvious in the long-term tests.

Fig. 9 shows a comparison of the net oxygen consumption using the highest oxidized solutions by both oxidation processes and with both acclimated and non-acclimated cultures. The net oxygen consumption was calculated by subtracting the oxygen consumption of the endogenous control, i.e., microorganism alone, from the consumption by microorganisms in the presence of the oxidized solutions. The figure shows that for the Fenton-treated samples after the initial period of around 70 h for the acclimated culture and only 10 h for the non-acclimated culture, the oxygen consumption was lower than for the endogenous control, giving net positive oxygen levels in the cumulative scale. These results indicate that Fenton’s reagent produced toxic by-products that might have killed or inhibited the microorganisms. In ozone treated samples with acclimated cultures the rate of oxygen consumption was similar to the rate of endogenous control, after the initial period, resulting in a flat oxygen curve. That implies that the acclimated culture was not able to continue with biodegradation of the ozonation by-products. According to these results, the non-acclimated culture with ozone treated samples was the only culture that was able to continue to consume oxygen throughout the experiment.

Based on these results, ozone treatment seems to be producing less toxic by-products, and as such might be preferred over the Fenton treatment. Furthermore, the non-acclimated culture seems to degrade the ozonation by-products at a faster rate than the acclimated culture. The acclimation of the cultures was in contact with pure DCDE, while oxidation produced a wide range of by-products. The better performance of the non-acclimated cultures could be explained by reduced diversity of the microorganisms in the culture, which limits the utilization of the broad spectrum of by-products as substrates.

### 3.3. Mid-term biodegradation test results

The mid-term biodegradation experiments were conducted with DCDE solutions preoxidized at 75% and 100%. These two levels of oxidation were chosen because of the higher rate of respiration obtained for these levels with the short- and long-term tests. In fact, industry might need to oxidize recalcitrant chemicals by 100%, otherwise, the effluent stream could still contain concentrations higher than is permitted by the law, especially since these recalcitrant compounds are expected to pass through the treatment sequence without being removed.

The removal of npCOD and npTOC based on the average and range of data of the duplicate tests are compared in Table 2 for different treatments. Note that the measured values for percent oxidation of DCDE varied from the target values of 75% and 100% considerably, and the actual values are reported in table. About 25 ± 6 mg/L of non-parent COD was removed over the 6-h period for both ozonated and Fenton-treated DCDE at both high and low oxidation levels. This is about 22% of the theoretical initial COD value (the theoretical COD value of 100 mg/L of the original DCDE is 112 mg/L).

The treatments do not seem to be very different from one another in terms of npCOD removal efficiencies. Likewise, non-acclimated cultures and acclimated cultures performed about the same under these conditions.

In terms of mineralization of organic matter, about 9 mg/L (8.7 ± 2.5) of npTOC biodegraded during the 6-h
test period for the “high oxidation” samples, i.e., those oxidized by 90% or more. The apparent differences in npTOC removal among the first four samples are not significantly different from each other after taking into consideration the errors involved in the TOC measurements. However, there is a noticeable difference in the removal of the npTOC between the high and low oxidation levels for both ozone and Fenton treatments. On average, after low oxidation levels (72–73%), the microorganisms were able to remove only about 5 mg/L (4.6 ± 1.2) of npTOC.

These results indicate that higher oxidation levels produce higher concentrations of the by-products that are more readily biodegradable, and that leads to the mineralization of a larger fraction of the initial organic matter. This agrees with expectations that higher levels of chemical oxidation would lead to formation of more oxygenated and smaller weight products, e.g., carboxylic acids that can easily be converted to CO₂ by microorganisms.

However, even for high oxidation levels, the percentage of TOC removal is only 25% of the initial theoretical TOC (the theoretical TOC value of 100 mg/L of DCDE is 34 mg/L). In other words, only 25% of the original organic matter present in the solutions after chemical oxidation biodegraded under the described conditions. This relatively low organic matter removal may be attributed either to the formation of limited amounts of biodegradable oxidation products, and/or to formation of oxidation products that are inhibitory and/or toxic for the types of microorganisms present in the culture. The results of the long-term biodegradation tests showed the toxicity for the Fenton-treated samples. However, no toxicity was observed for the ozonated samples. On the other hand, it can be hypothesized that higher organic matter removal may be obtained in activated sludge systems that contain at least 5–6 times higher concentrations of microorganisms. Furthermore, longer exposures of the biomass might facilitate acclimation of the biomass to the oxidation products.

4. Conclusions

The following are the main conclusions of this study:

1. DCDE was basically non-biodegradable by the activated sludge cultures maintained in our laboratories. The acclimation of the cultures to DCDE did not improve its biodegradability.

2. All three tests used in this study showed that ozone and Fenton reagent were able to oxidize DCDE to biodegradable compounds. The oxidation products of DCDE were significantly more biodegradable than DCDE itself by both cultures that were non-acclimated and acclimated to DCDE.

3. Fenton-treated DCDE resulted in significantly reduced respiration rate of microorganisms in long-term respirometric studies, indicating formation of toxic or inhibitory oxidation by-products.

4. The amounts of organic matter removal by biodegradation following ozone and Fenton treatment were not significantly different from each other; however, even for the highly oxidized samples, only about 25% of total organic matter could be mineralized, which led to the conclusion that the rest of the by-products were non-biodegradable under the experimental conditions.

5. In general, the cultures acclimated to the parent compound showed more sensitivity to the toxicity of oxidation by-products than the non-acclimated cultures.

Three different laboratory tests used for the assessment of biodegradation of preoxidized DCDE provided complementary information and thus the needed insight into the integrated process that would otherwise be

<table>
<thead>
<tr>
<th>High oxidation levels</th>
<th>npCOD AVG (mg/L), REMOVED</th>
<th>npTOC AVG (mg/L), REMOVED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ozone, 94%, acclimated culture</td>
<td>25.1 (19.7–30.5)</td>
<td>9.5 (4.4–14.6)</td>
</tr>
<tr>
<td>Ozone, 94%, non-acclimated culture</td>
<td>19.0 (14.2–23.8)</td>
<td>8.2 (6.5–9.8)</td>
</tr>
<tr>
<td>Fenton reagent, 90% acclimated culture</td>
<td>35.0 (28.8–41.2)</td>
<td>10.9 (10.8–11)</td>
</tr>
<tr>
<td>Fenton reagent, 90% non-acclimated culture</td>
<td>21.6 (18.8–24.5)</td>
<td>6.2 (4.6–7.8)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Low oxidation levels</th>
<th>npCOD AVG (mg/L), REMOVED</th>
<th>npTOC AVG (mg/L), REMOVED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ozone, 73%, acclimated culture</td>
<td>22.7 (20.4–25.0)</td>
<td>4.9 (4.4–5.3)</td>
</tr>
<tr>
<td>Ozone, 73%, non-acclimated culture</td>
<td>26.5 (18.3–34.6)</td>
<td>3.9 (3.1–4.7)</td>
</tr>
<tr>
<td>Fenton reagent, 72%, acclimated culture</td>
<td>26.4 (23.0–29.8)</td>
<td>5.1 (4.7–5.4)</td>
</tr>
<tr>
<td>Fenton reagent, 72%, non-acclimated culture</td>
<td>22.6 (18.0–27.2)</td>
<td>4.7 (4.4–5)</td>
</tr>
</tbody>
</table>
difficult to obtain. However, the results of the mid-term tests would probably contribute the most to the practical application of the integrated process by allowing the prediction of the amount of expected organic matter removal. The long-term tests, on the other hand, might predict better the toxicity of the reaction by-products on the biomass over extended periods of exposure.

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