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Enhanced TCDD degradation by Fenton's reagent preoxidation

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Abstract

The dioxin isomer 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has been called the most toxic compound known to man. Because of its poor bioavailability and low biodegradibility, bioremediation technology cannot effectively degrade TCDD when used alone. In this study, chemical pretreatment (partial oxidation) in combination with biodegradation technique was developed to efficiently remediate TCDD-contaminated soils. An oxidizing reagent [Fenton's Reagent (FR)] was applied in a slurry reactor to transform TCDD with a concentration of 96 μg per kg of soil to compounds more amenable to biodegradation. Up to 99% TCDD was transformed after the chemical pretreatment process. The slurry reactor was then converted to a bioreactor for the following biodegradation experiment. The detected TCDD oxidation byproducts including chlorophenols (CPs) and chlorobenzenes (CBs) were transformed in this bioreactor under aerobic conditions. Two other biodegradation experiments were performed in parallel to investigate the biodegradabiliy of TCDD under aerobic and anaerobic conditions without chemical pretreatment. Approximately 53% TCDD was transformed under anaerobic conditions possibly due to the reductive dechlorination process using organic materials contained in the activated sludge as the primary substrates. No TCDD degradation was observed under aerobic conditions. Results show that FR can oxidize TCDD to less-chlorinated and less-toxic byproducts, promoting their bioavailability to microbial communities. The bench-scale results indicate that the two-stage (partial oxidation followed by biodegradation) system has the potential to be developed to remediate TCDD-contaminated soils on-site.

Keywords: Dioxins; TCDD; Partial oxidation; Slurry reactor; Bioreactor

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1. Introduction

Polychlorinated dibenzo-*p*-dioxins, also called dioxins, comprise a group of compounds that consists of 75 individual isomers. Dioxins can enter the environment from numerous sources including municipal solid waste incinerator emissions, pulp and paper mill waste discharges, and through the manufacture and use of organochlorine pesticides [1–3]. Dioxins have also been identified at many hazardous waste sites. The most toxic dioxin isomer, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), as well as many of the other isomers, have been detected in sediments, soils, and in the adipose tissue of livestock and fish in both rural and industrialized areas [3,4].

The isomer TCDD has been called the most toxic compound known to man. Many adverse health effects have been associated with dioxins, including skin lesions (chloracne), stomach cancer, soft tissue sarcomas, lymphomas, immune system effects, and neurological effects [5–7]. TCDD has the LD_{50} (dose at which 50% of a population dies) of 0.04 mg/kg for rats. However, other dioxin isomers have LD_{50} values up to 100 mg/kg for rats [5,8,9]. Thus, the public health risk from environmental exposure to dioxins from contaminated sites can be significant. As a result, the clean-up of environmental dioxin contamination is an area requiring more attention.

Beyond the toxicity of TCDD and its presence in the environment, many researchers have shown the compound to be highly resistant to biodegradation. Part of this resistance may be due to its poor bioavailability [1,10]. The physical properties controlling environmental transport of TCDD are water solubility (19.3 ng/l), octanol–water partition coefficient (1.4×10^6), vapor pressure (7.4×10^{-10} Torr at 25°C), and molecular weight (321.974). With its low vapor pressure and aqueous solubility, strong sorption to soils, and hydrophobicity, the mobility of TCDD in a soil environment is low [10,11]. TCDD in sediment and soil will tend to biologically, and chemically, degrade slowly. It also has a strong potential to bioaccumulate within ecosystems.

1.1. Treatment options

Oxidation converts hazardous contaminants to nonhazardous or less-toxic compounds. The oxidizing agents most commonly used for the treatment of hazardous contaminants are ultraviolet radiation, ozone, chlorine dioxide, hydrogen peroxide (H_2O_2), and Fenton's reagent (FR) [H_2O_2 oxidation in the presence of ferrous iron (Fe^{2+})] [12–22]. Chemical oxidation has been used to treat liquids, slurry soils, and sludges that contain oxidizable contaminants [15,16]. However, oxidation processes have been demonstrated to be ineffective or partially effective for some highly toxic and stable compounds including pesticides, organic cyanides, polychlorinated biphenyls (PCBs), and dioxins/furans [15,16]. Therefore, using chemical oxidation alone may not be an effective and appropriate method for the remediation of TCDD-contaminated soils.

Bioremediation represents a cost-effective technology that can achieve complete mineralization of pollutants to CO_2 and H_2O, which results in detoxification at contaminated sites. However, some highly chlorinated compounds (such as TCDD) are usually recalcitrant to biodegradation. Despite this difficulty, chlorinated organic molecules still can be biologically dechlorinated through reductive metabolic pathways.

In fact, attempts to develop biotreatment schemes using reductive, anaerobic conditions have been moderately successful [10,23–29]. However, maintaining reductive, anaerobic conditions in either a full-scale ex situ treatment system or in situ in the vadose zone is likely to be difficult.

In this technology development study, a concept of preoxidation followed by biodegradation was developed for TCDD-contaminated soil remediation. This concept was tested in the laboratory using a bench-scale, two-stage, slurry-phase treatment system. In this study, a chemical reagent was used to transform TCDD to compounds more amenable to biodegradation, and then biodegradation was employed to convert the byproduct compounds of the chemical pretreatment. The first stage (partial oxidation or chemical pretreatment) in the treatment is a chemical ''softening'' step not intended to achieve complete chemical destruction. Therefore, the cost for chemical oxidation can be significantly reduced. The chemical reagent used in this project for the pretreatment process was FR.

1.2. TCDD preoxidation by FR

FR has been proposed as a very effective oxidizing agent for organochlorine compounds [2,15–21,30]. The chain reactions induced by FR first produce the highly reactive hydroxyl radical, $^{\cdot}OH$. The reaction is shown below:

$$H_2O_2 + Fe^{2+} \rightarrow {}^{\cdot}Fe^{3+} + OH + OH^- \quad (1)$$

Hydroxyl radical generation is enhanced at low pH (2.5 to 4.5), and oxygen evolution is the predominant route of H_2O_2 decomposition at neutral pH. In the presence of TCDD, the hydroxyl radical could be postulated to react in one of the following two mechanisms. (1) At low concentrations of H_2O_2 and Fe^{2+} (less than the amount for the completion of stoichiometric reaction) with uniform distribution due to adequate mixing, complete stoichiometric reactions cannot occur. The produced hydroxyl radical would break C—Cl or O—C bond. Fig. 1a, b, and c presents three of the possible cleavage patterns during the dioxin degradation [2,30,31]. Fig. 1a shows the symmetrical cleavage which involves breaking the O—C bond to produce two molecules of 3,4-dichlorophenol (3,4-DCP). Fig. 1b presents the asymmetrical cleavage which produces 4,5-dichlorocatechol (dihydroxybenzene) (4,5-DCC) and 1,2-dichlorobenzene (1,2-DCB). Fig. 1c shows the cleavage of C—Cl bond which forms less-chlorinated dioxins [e.g., mono-CDD (MCDD), di-CDD (DCDD), tri-CDD]. (2) At high concentrations of H_2O_2 and Fe^{2+} (more than the amount for the completion of stoichiometric reaction), total TCDD could occur as shown in the following equation:

$$C_{12}O_2H_4Cl_4 + 22H_2O_2 \rightarrow 12CO_2 + 22H_2O + 4H^+ + 4Cl^- \quad (2)$$

Based on the dioxin cleavage patterns presented in Fig. 1a–c, the ring cleavage processes and subsequent oxidation of these ring cleavage products would result in less-chlorinated and less-toxic products. These oxidation byproducts are more water soluble then TCDD by orders of magnitude, and therefore, can be more easily removed from soil systems through biodegradation or other means.

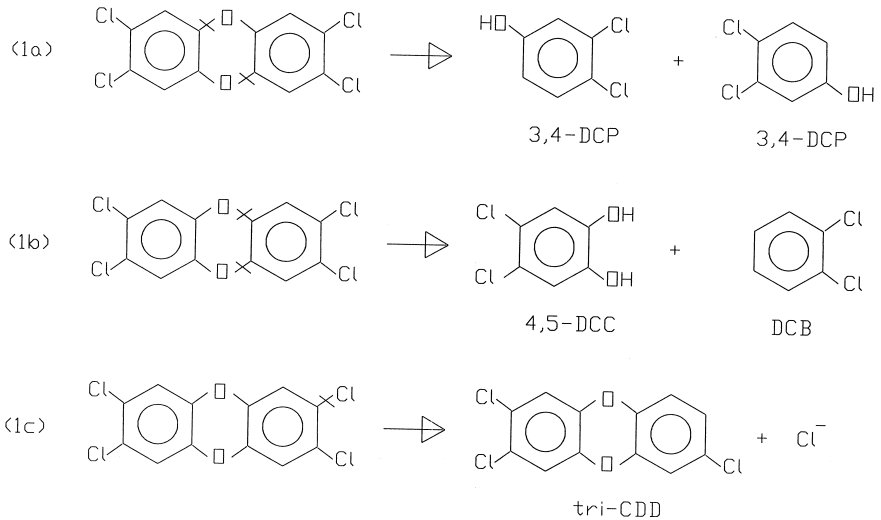

Fig. 1. Schematic diagram showing TCDD cleavage. (a) Symmetrical cleavage produces two molecules of 3,4-dichlorophenol. (b) Asymmetrical cleavage produces 4,5-dichlorocatechol and 1,2-dichlorobenzene. (c) Cleavage of C−Cl bond forms less-chlorinated dioxins.

Many studies have investigated the mechanisms and ideal FR oxidizing conditions for organochlorine contaminant destruction [14,15,17–24]. FR technology has been demonstrated in both laboratory and field-scale studies [2,15–24]. Results show that FR can successfully remove variety of organic contaminants from wastewater, groundwater, soils, and sediments. However, TCDD has not been widely used for the target compound for the FR process. Except for the Fe^{2+} used in the FR reaction, iron minerals (geothite, hematite, and magnetite) can also provide efficient contaminant transformation [32]. Recently, a Fenton-like reaction using zero-valent iron instead of Fe^{2+} has been found to be effective in degradation of organic pollutants in wastewater and soil as well. This zero-valent iron process could minimize the amount of sludge produced due to the production of ferric iron [e.g., $Fe(Cl)_3$, $Fe(OH)_3$] [21,33–35].

2. Materials and methods

2.1. Soil spiking procedures

To produce the dioxin-contaminated soils used in this study, uncontaminated soils from Eglin Air Force Base (FL, USA) were spiked with TCDD. Based on the results from grain-size distribution analysis, the collected soils consisted of mainly sands. Ten kilograms of soil was mechanically homogenized in a stainless steel container. The soil was spiked with 1250 μg of TCDD dispersed in 1.5 l of a 45/55% (v/v) acetone/hexane solution. The soil was then further homogenized. The solvents were allowed to evaporate from the soil by placing the container of spiked soil in a fume hood, thus

leaving behind the TCDD in the soil at a theoretical concentration of 125 ng/g or 125 μg/kg of soil. The effectiveness of the spiking procedure was verified through extraction and analysis of 10-g subsamples immediately after preparation of the spiked soil. Five subsamples were analyzed and the average concentration was 96.25 μg/kg. The average recovery was 77% with a coefficient of variation of 4.8.

2.2. Soil and water extraction procedures

Soil extraction procedures were applied to extract TCDD in soils after the oxidation experiment. The extraction procedures included internal standardization, overnight soxhlet extraction with toluene (16 to 24 h), snyder column concentration, and nitrogen gas vaporization to a final volume of 50 μl for analysis. The isotopically labeled internal standards (^{13}C-1,2,3,4-TCDD and ^{13}C-1,2,3,7,8,9-hexaCDD) were added before the extraction to measure the recovery efficiency. For each soil extraction process, duplicate slurry soil samples (10 g each) were collected and centrifuged, then the settlements were weighed and used for the soil extraction process.

The liquid–liquid extraction techniques were applied to extract TCDD in liquid phase after the oxidation experiment. For each liquid extraction process, duplicate liquid (supernatant) samples (10 ml each) from the oxidation reactor was collected and centrifuged, then the supernatant was used for the following extraction process. The supernatant volume was measured and mixed with 10-ml toluene, shaken for approximately 1 min, then the upper toluene phase was drawn. This procedure was repeated five times to achieve complete TCDD extraction from the liquid phase. After the extraction, collected toluene was passed through a sodium sulfate column to remove any water from the toluene solution, then concentrated down to 50 μl using snyder column concentration and nitrogen gas evaporation for gas chromatography (GC) analysis. For this liquid extraction process, isotopically labeled internal standards (^{13}C-1,2,3,4-TCDD and ^{13}C-1,2,3,7,8,9-hexaCDD) were also added before the extraction to measure the recovery efficiency.

Oxidation and biodegradation byproducts were analyzed and verified qualitatively by GC/mass spectrometer (MS). Liquid–liquid extraction was used to extract byproducts for both the soil and liquid samples collected from the reactor after the oxidation process. For the soil-phase extraction, 10 g of sodium sulfate was added to the soil sample (after the centrifuge process described above), followed by acetone/methylene chloride (50/50) extraction. After five passes of extraction, the collected solvent was concentrated down to 1 ml for GC/MS analysis. For the liquid-phase extraction, sulfuric acid was used to adjust the liquid sample (after the centrifuge process described above) to a pH of 1 to 2, then methylene chloride was used to perform the liquid–liquid extraction using a separatory funnel. After five passes of extraction, the volume was concentrated down to 1 ml using a K-D concentrator and nitrogen gas evaporation for GC/MS analysis.

2.3. Analytical methodology

Analysis of TCDD was performed using a Hewlett-Packard 5890A GC equipped with an electron capture detector. A 20-m × 0.25-mm DB-5 capillary column with a 0.25-μm

film was used to separate compounds. The injector temperature was 290°C and the detector temperature was 300°C. The oven temperature was programmed to increase from 150°C (5 min) to 290°C (35 min) at 3°C/min. He was used as the carried gas and N_2 was used as the make-up gas. The detection limit for TCDD was 0.5 ppb. Oxidation byproducts were analyzed by GC/MS using a Finnigan 4023 MS with Incos data system. The compounds were resolved on a 30-m × 0.32-mm DB-5 capillary column with a 0.25-μm film. The injector temperature was 290°C and the detector temperature was 300°C. The oven temperature was programmed to increase from 60°C (5 min) to 180°C (30 min) at 5°C/min. The detection limits for the oxidation products were 1 ppb. H_2O_2 concentrations were measured using a Hach titration kit. Fe^{2+} concentrations were measured using a Hach test kit and a spectrophotometer (Hach). pH (Orion Ross) values were monitored continuously during the oxidation process. The detection limits for H_2O_2 and Fe^{2+} were 0.2 and 0.01 mg/l, respectively.

2.4. Introduction to experimental procedures

In the first part of this study (oxidation experiment), FR was applied to transform TCDD to compounds more amenable to biodegradation. In the second part of this study (biodegradation experiment), the biodegradability of detected TCDD oxidation byproducts were determined under aerobic conditions. Moreover, biodegradation of TCDD was also evaluated under aerobic and anaerobic conditions. A total of five reactors (labelled as A to F) were prepared. Table 1 presents the characteristics of each oxidation and biodegradation reactors. Reactors A to C were used in the oxidation experiment, then Reactor B was transformed to a bioreactor to study the biodegradability of the oxidation byproducts. Reactors (or bioreactors) D to F were used in the biodegradation experiment for TCDD biodegradation evaluation. Details of the experimental procedures were described in the following two sections.

2.5. Oxidation experiment procedures

Results from the previous oxidation studies indicate that TCDD oxidation by FR occurred immediately without any lag period [1,2]. The early stage TCDD daughter compounds (e.g., MCDD, DCDD, tri-CDD) could be subsequently transformed under the sequential oxidation process with a lower FR addition during each oxidation step.

Table 1
Characteristics of oxidation and biodegradation reactors

Reactor	Treatment	Addition	pH
A	Oxidation	FR	3.5
B	Oxidation followed by aerobic biodegradation	FR followed by activated sludge	3.5 then 7
C	Oxidation control	No	3.5
D	Aerobic biodegradation	Activated sludge	7
E	Anaerobic biodegradation	Anaerobic sludge	7
F	Biodegradation control (aerobic conditions)	Activated sludge + $HgCl_2$ + NaN_3	7

Therefore, using the sequential oxidation process should be able to effectively transform the early stage TCDD byproducts without addition a significant amount of FR. Thus, in this study a three-step sequential oxidation process was conducted to transform TCDD to less-chlorinated products by adding ferrous sulfate ($FeSO_4 \cdot 7H_2O$) and H_2O_2 (30%) into the reactor. For each oxidation step, ferrous sulfate and H_2O_2 were added to achieve a final Fe^{2+} concentration of 28 mg/l and a final H_2O_2 concentration of 20 mg/l in the reactor.

Three 2-l serum bottles (batch reactors) (labelled as A, B, and C) were used to perform the partial oxidation experiment. Each reactor capped with a Teflon-lined rubber stopper containing 500 g of TCDD-spiked soils and 500 ml of deionized water had the following features: (1) continuous monitoring of pH, and (2) a three-way outlet attached on the top of the reactor for water/soil sample collection and reagent injection. The pH in each reactor was adjusted to 3.5 by adding H_2SO_4, and the slurry was magnetically stirred continuously. A stepwise addition of FR was carried out at half-hour intervals for Reactors A and B. Reactor C was used as the control reactor and no FR was added. Three subsamples (each contained 10-ml supernatant and 10 g soil) were collected from Reactors A and C after each oxidation process, and analyzed for the components of byproducts, TCDD, Fe^{2+}, and H_2O_2 concentrations. Reactor B was transformed to a batch bioreactor for the following biodegradation experiment.

2.6. Biodegradation experiment procedures

2.6.1. Bioreactor preparation

Bioreactor B (transformed from oxidation Reactor B) as well as three other 2-l serum bottles (labeled as D, E, and F) containing 500 g of TCDD-spiked soils, 400 ml of deionized water, 50 ml of activated sludge, and 50 ml of stock mineral buffer solution were used to perform the biodegradation study. The solution in Bioreactor B was adjusted to pH of 7 with 1 N sodium hydroxide. All bioreactors had the same features described in the previous section. The aerobic and anaerobic microbial cultures used in this study were the activated sludges obtained from the aerobic activated sludge basin and the anaerobic sludge basin of a pulp and paper wastewater treatment plant located in New Burn, NC. The mixed-liquor suspended solids (MLSS) for the aerobic and anaerobic sludges were approximately 4500 and 6500 mg/l, respectively. More than 10^8 cells/ml of total heterotrophic bacteria were detected in both sludges using plate count techniques. Aerobic and anaerobic sludges were purged with air and nitrogen to remove any residual volatile and semi-volatile organics. Sludges were also analyzed by GC/MS to insure the ''cleanup (purge)'' performance. Because the industrial wastewater contained CBs, CPs, and other aromatic hydrocarbons, the microbial cultures (activated sludges) were acclimated to part of the TCDD oxidation byproducts before use. A stock mineral buffer solution provided essential inorganic nutrients for microbial growth. Fifty milliliters of the stock mineral buffer solution was added into each bioreactor. The final mineral medium in each reactor consisted of (milligram per liter of H_2O) $(NH_4)_2SO_4$, 100; K_2HPO_4, 174; KH_2PO_4, 136; $CaCl_2$, 0.4; $FeCl_3.6H_2O$, 0.3; H_3BO_4, 0.03; concentrated HCl, 0.25, $MgSO_4 \cdot 7H_2O$, 3; $MnSO_4 \cdot H_2O$, 0.09; and $NaMoO_4 \cdot 2H_2O$, 0.01.

2.6.2. Bioreactor incubation and analysis

Bioreactor B was used to evaluate the effects on TCDD transformation by chemical pretreatment followed by aerobic biodegradation. Bioreactor D was prepared to evaluate the biodegradability of TCDD under aerobic conditions. Therefore, stock mineral buffer solution and deionized water were aerated before the addition and the headspace in the bioreactor contained air to provide the oxygen for aerobic degradation. Bioreactor E was used to evaluate the biodegradability of TCDD under anaerobic conditions. Therefore, Bioreactor E was prepared in an anaerobic glovebox to preclude intrusion of oxygen. Hungate techniques were used to prepare anaerobic solutions [36]. Bioreactor E also contained 20% H_2 and 80% CO_2 in the headspace. A redox indicator (0.0002% resazurin) and reducing agent (1 mM sodium sulfide) were added to insure the anaerobic conditions throughout the experiment. Sodium sulfide was chosen because it would not serve as a carbon source, and it has a redox potential (-571 mv) low enough to reduce resazurin [36]. During the sample collection process, Bioreactor E was transferred into the anaerobic glovebox for sample collection.

Bioreactor F was used as the control bottle, which contained 250 mg/l mercuric chloride ($HgCl_2$) and sodium azide 500 mg/l (NaN_3). Because the control bottle was used to correct for the physical/chemical effects of dilution, volatilization, decay, and sorption mechanisms, this reactor was operated under aerobic conditions and no anaerobic control reactor was prepared. Bioreactors were incubated in the dark at 20°C. Duplicate supernatant and slurry soil samples were collected periodically (0, 2, 4, 6, 8, 10, 12, 14, 16, 25, 30, 35, 40, 50, 60, 70, 80, and 90 days after incubation) from each bioreactor through the three-way outlet during the 3-month incubation period for the analysis of TCDD concentrations and the components of byproducts.

3. Results and discussion

3.1. Partial oxidation

Three reactors (A, B, and C) were used for the partial oxidation experiment. Reactors A and B were treated by the sequential addition of FR, and Reactor C was used as the control reactor. Because reactor B was converted to the bioreactor for the following biodegradation experiment, analysis was only conducted for Reactors A and C. Table 2 presents the TCDD concentrations in both soil and water phases after each oxidation process. Approximately 19% of the TCDD was desorbed from the soil phase to the aqueous phase, 35% of the TCDD remained bound to the soil particles, and 46% of the TCDD was oxidized by FR after the first oxidation step in Reactor A. Therefore, the transformation of TCDD from the soil particles was demonstrated to be through either oxidation or desorption.

After the second oxidation step, approximately 8% of the TCDD was desorbed from the soil phase to the aqueous phase, 12% of the TCDD remained bound to the soil particles, and 34% of the TCDD was oxidized by FR. Therefore, a total of 80% of the TCDD was removed after the first and second oxidation steps. After the third oxidation, approximately 0.6% of the original TCDD was desorbed to the aqueous phase, 0.52%

Table 2
TCDD concentrations before and after each oxidation process in Reactor A

Oxidation step	TCDD in soil and water (before)[a] (μg/kg)	TCDD in soil (after)[b] (μg/kg)	TCDD in water (after) (μg/l)	% TCDD remained in soil and water (after)	% Total TCDD oxidized
1	96.25 ± 4.8[c]	33.78 ± 2.9 (35%)	18.2 ± 2.1 (19%)	54	46
2	96.25 × 0.54 = 51.98	11.22 ± 2 (12%)	8.14 ± 1.3 (8%)	20	80
3	96.25 × 0.2 = 19.25	0.52 (0.5%)	0.61 (0.6%)	1.1	98.9

[a] Before = before oxidation.
[b] After = after oxidation.
[c] Mean ± standard deviation.

remained in the soil phase, and 19% was oxidized. Results show that after the three-step sequential oxidation, up to 99% of the original TCDD was transformed, and only 1% of the original TCDD remained in the slurry (soil and water phases). The final TCDD concentration remained in the slurry was 1.06 μg/kg (Table 2). No TCDD transformation was observed in Reactor C without FR addition during the oxidation experiment operation period.

Table 3 presents the oxidation byproducts detected in Reactor A after each oxidation process. Those identified byproducts include the following: phenol, benzene, CB, 1,2-diCB, 3 + 4-CP, 2,4-dimethylphenol, 4-chloro-3-methylphenol, *o*-cresol, *m* + *p*-cresol, 4,5-dichlorocatechol, MCDD, DCDD, and tri-CDD. It can be hypothesized that the formation of the dioxin isomers (e.g., MCDD, DCDD, tri-CDD) were caused by an insufficient quantity of FR. Complete transformation of dioxin isomers was observed after the second oxidation process. Moreover, 4-chloro-3-methylphenol was also removed after the third oxidation step. After the sequential oxidation, dioxin isomers were removed and the remaining byproducts were mainly aromatic hydrocarbons (e.g., CBs, CPs). No degradation byproducts were observed in the control reactor (Reactor C), and this indicates that the observed byproducts in Reactor A were due to the TCDD oxidation.

After the sequential oxidation processes, it was found that FR can be used as both an oxidant and a solubilizing agent, which matched with the results from other study [2]. It

Table 3
Detected oxidation byproducts after each oxidation process

Oxidation step	Observed byproducts
1	MCDD, DCDD, Tri-CDD, 4-chloro-3-methylphenol, 2,4-dimethylphenol, 4,5-dichlorocatechol, 1,2-diCB
2	4-chloro-3-methylphenol, 2,4-dimethylphenol, CB, *o*-cresol, 1,2-diCB, 4,5-dichlorocatechol, 3 + 4-CP, *m* + *p*-cresol
3	2,4-dimethylphenol, 4,5-dichlorocatechol, CB, 1,2-diCB, 3 + 4-CP, *o*-cresol, *m* + *p*-cresol, phenol, benzene

can oxidize TCDD to less-chlorinated byproducts, and it appears to lower the soil/water distribution coefficient, shifting the sorption equilibrium, and releasing adsorbed TCDD for dissolution in the aqueous phase [2]. This shift would increase the aqueous concentration of TCDD, thereby increasing the treatment efficiency. From an engineering point of view, soluble TCDD is much easier to remove by either physical (activated carbon adsorption), chemical (oxidation), or biological (biodegradation) processes. In this study, soluble TCDD was further oxidized by FR to make the following biodegradation more effective.

Changes in Fe^{2+} and H_2O_2 concentrations resulting from each oxidation step are presented in Table 4. Analytical results show that H_2O_2 and Fe^{2+} concentrations dropped significantly after each oxidation. This indicates that half-hour reaction time was not a limiting factor for the oxidation process. Based on the previous study [2], treatment efficiency is sensitive to the iron amendment. Therefore, the decrease in Fe^{2+} concentrations indicates the decrease in reaction rate.

3.2. Biodegradation study

Four bioreactors (B, D, E, and F) were used in this study. Bioreactors B and D were operated under aerobic conditions, and Bioreactor E was operated under anaerobic conditions. Bioreactor F was used as the control group under aerobic conditions. Supernatant and slurry soil samples were analyzed for TCDD and its byproducts during the 3-month incubation period. Fig. 2 presents the TCDD concentrations in the slurry soil samples collected from Bioreactors D, E, and F. TCDD in water phase (supernatant) was below detection limit (0.5 ppb) after the incubation in Bioreactors D to F. Results also reveal that no TCDD transformation and no oxidation byproducts were observed in Bioreactors D and F after a 3-month incubation period. This indicates that TCDD is recalcitrant to the aerobic biodegradation using activated sludge as the inocula. In Bioreactor E (anaerobic), significant TCDD removal was observed (declined from 96 to 45 μg/kg) after 10 days of incubation. However, TCDD degradation ceased after 16 days, and no further concentration decrease was observed.

One of the possible causes of TCDD removal in Bioreactor E was due to the occurrence of anaerobic transformation. Another possible explanation for the TCDD reduction was the occurrence of reductive dechlorination. The provided sludge contained some biodegradable organics, which served as the primary substrates during the

Table 4
Fe^{2+} and H_2O_2 concentrations before and after each oxidation process in Reactor A

Oxidation step	Fe^{2+} (before)[a] (mg/l)	Fe^{2+} (after)[b] (mg/l)	H_2O_2 (before) (mg/l)	H_2O_2 (after) (mg/l)
1	27 ± 2.1[c]	3 ± 0.5	20 ± 2.4	4 ± 1.4
2	$27 + 3 = 30$	8 ± 1.3	$20 + 4 = 24$	6 ± 0.6
3	$27 + 8 = 35$	7 ± 1.1	$20 + 7 = 27$	5 ± 0.5

[a] Before = concentrations of Fe^{2+} and H_2O_2 before oxidation.
[b] After = concentrations of Fe^{2+} and H_2O_2 after oxidation.
[c] Mean ± standard deviation.

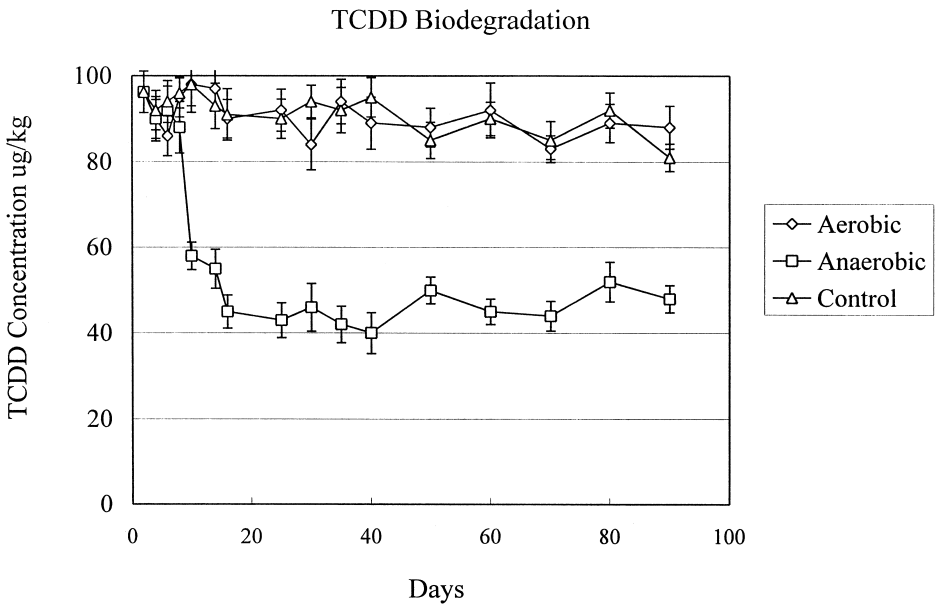

Fig. 2. TCDD biodegradation under aerobic and anaerobic conditions (error bars show standard deviation in duplicate samples).

reductive dechlorination process. However, after the depletion of degradable substrates, the reductive dechlorination cannot proceed. Only three TCDD biodegradation byproducts (phenol, diCB, and CB) were detected in Bioreactor E on day 10. This might be due to the occurrence of subsequent anaerobic biodegradation processes (in addition to reductive dechlorination) caused the immediate transformation of produced byproducts. The detected phenol, diCB, and CB were removed by day 16. Some TCDD degradation might be caused by the abiotic processes [37,38]. However, due to the lack of anaerobic control reactor, this cannot be confirmed in this study.

Table 5 presents the TCDD oxidation byproducts degradation results in Bioreactor B. Complete biodegradation of nonchlorinated aromatic compounds (e.g., benzene, phenol) was observed at the early stage of incubation (within 4 days of incubation). However, the transformation of chlorinated compounds (e.g., CBs, CPs) was observed after 8 incubation days. Among those observed oxidation byproducts, o-cresol and 4,5-dichlorocatechol were the least biodegradable compounds under aerobic conditions, and their transformation was observed after 25 and 35 days, respectively. Therefore, detected oxidation byproducts were transformed after 35 days of incubation under aerobic conditions using activated sludge as the seeded microorganisms. The acclimation of the microbial culture by the industrial wastewater containing mixed chlorinated aromatic compounds caused some of those byproducts biotransformed efficiently. Because *o*-cresol and 4,5-dichlorocatechol were the most recalcitrant compounds among those byproducts under aerobic biodegradation conditions, performing the microbial enrichment using these two compounds could possibly reduce the incubation time. No

Table 5
Removal of the oxidation byproducts by biodegradation process in aerobic Bioreactor B

Byproduct	Biodegradation time (days)											
	0	2	4	6	8	10	12	14	16	25	30	35
2,4-dimethylphenol	○	○	○	○	○	○	○	–	–	–	–	–
4,5-dichlorocatechol	○	○	○	○	○	○	○	○	○	○	○	–
1,2-diCB	○	○	○	○	○	○	–	–	–	–	–	–
3+4-CP	○	○	○	○	○	–	–	–	–	–	–	–
CB	○	○	○	○	–	–	–	–	–	–	–	–
CP	○	○	○	○	–	–	–	–	–	–	–	–
o-cresol	○	○	○	○	○	○	○	○	○	–	–	–
phenol	○	○	–	–	–	–	–	–	–	–	–	–
benzene	○	○	–	–	–	–	–	–	–	–	–	–

''○'': detected; ''–'': not detected.

significant pH variations (within ± 0.2) were observed in all bioreactors. Again, some TCDD removal and byproduct production might be due to the abiotic processes. Because no control treatment (oxidation followed by aerobic biodegradation) was performed in parallel with this reactor, the occurrence of abiotic TCDD removal and byproduct production cannot be confirmed in this study.

4. Conclusions

In this technology development project, a two-stage, slurry-phase treatment system for TCDD-contaminated soils was designed and tested. In the first part of this study, TCDD was partially oxidized due to the increased level and activity of hydroxyl radical formed with the FR. Without sufficient addition of FR, dechlorination byproducts (e.g., MCDD, DCDD, tri-CDD), rather than cleavage byproducts (e.g., CPs, CBs) were observed. Therefore, FR was added sequentially to achieve complete cleavage of all dioxin isomers.

More than 10 oxidation byproducts were detected after the sequential oxidation process. Some of these byproducts (e.g., phenol, benzene) are among priority pollutants listed by the U.S. EPA [39]. Because those byproducts have higher water solubilities and lower soil/water partition coefficients, incomplete TCDD oxidation may cause even severe surface water or groundwater contamination problems. Therefore, complete TCDD removal and subsequent environmental monitoring is necessary for TCDD site remediation project. Among those detected oxidation byproducts, *o*-cresol and 4,5-dichlorocatechol were the least biodegradable compounds under aerobic biodegradation conditions. In the future study, those two byproducts can be used as the target compounds for monitoring purpose.

The biodegradability of TCDD and its oxidation byproducts was evaluated in the second part of this study. TCDD was recalcitrant under aerobic conditions (Bioreactor D), and approximately 53% TCDD was removed in anaerobic Bioreactor E. Reductive dechlorination process using activated sludge as the primary substrates was one of the

possible cause of the observed TCDD removal. Another possible cause of TCDD removal in Bioreactor E was due to the occurrence of abiotic transformation in the reactor.

Bioreactor B treated with biodegradation following partial oxidation by FR was the only treatment option showing promising remediation results in this study. In situ bioremediation using TCDD as the sole carbon source may not be a feasible technology to clean up TCDD contaminated site. Because TCDD is highly chlorinated, application of other carbon sources (e.g., acetate) to enact the reductive dechlorination process is probably an appropriate way to enhance its biodegradation. To further evaluate the performance of the proposed system and the effects of abiotic processes on TCDD removal, the following tasks need to be performed in the future study: (1) mass balance between TCDD and oxidation/biodegradation byproducts; (2) FR treatment followed by anaerobic biodegradation; (3) control experiments for Reactor B (oxidation followed by aerobic biodegradation) and Reactor E (anaerobic biodegradation); (4) cost analysis based on a pilot-scale study.

Overall, the resulting partial dechlorination of TCDD by FR provided appropriate conditions for the subsequent enhancement of microbial biodegradation compared to untreated TCDD. Therefore, pretreatment with FR before biological treatment would be more effective and feasible than direct biological treatment to remediate TCDD-contaminated soils. The bench-scale results indicate that the two-stage (partial oxidation followed by biodegradation) system has the potential to be developed into a promising on-site remediation technology.

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