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# Continuous combined Fenton's oxidation and biodegradation for the treatment of pentachlorophenol-contaminated water

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ABSTRACT

Pentachlorophenol (PCP) was studied as a model recalcitrant compound for a sequential chemical oxidation and biodegradation treatment, in a continuous laboratory-scale system that combined a Fenton's chemical reactor and a packed-bed bioreactor.

PCP degradation and dechlorination were observed in the Fenton's reactor at a residence time of 1.5 h, although no reduction of total organic carbon (TOC) was observed. Both PCP degradation and dechlorination were strongly dependent on the $H_2O_2$ dose to the chemical reactor. The PCP degradation intermediates tetrachlorohydroquinone and dichloromaleic acid were identified in this reactor. Further treatment of the Fenton's reactor effluent with a packed-bed bioreactor (operating at a residence time of 5.5 h) resulted in partial biodegradation of PCP degradation intermediates and reduction in TOC, although no further reduction of PCP or dechlorination was achieved in the bioreactor. Increased residence time in the bioreactor had no significant impact on degradation of TOC. Recycle of the effluent from the bioreactor to the chemical reactor increased the TOC degradation, but not the extent of the PCP degradation or dechlorination.

A mathematical model of the combined Fenton's oxidation and biodegradation system supported the experimental results. While the model over-predicted the PCP and TOC degradation in the combined system, it adequately predicted the sensitivity of these parameters to different $H_2O_2$ doses and recycle rates. The model indicated that high recycle rates would improve TOC degradation.



## 1. Introduction

Pentachlorophenol is a common water contaminant, released into the environment from wood treating and biocide formulation operations. It often occurs in mixtures with other contaminants, constituting the most recalcitrant fraction of such mixtures (Graves and Joyce, 1994).

PCP biodegradation has been reported both aerobically and anaerobically, although rates are too slow for practical treatment, owing to the need to induce specific enzymes (Hale et al., 1994). Specialized cultures can achieve faster degradation rates (Puhakka and Melin, 1996), but might not be resilient when exposed to environmental disturbances (Scott and Ollis, 1995).

Due to this reported recalcitrance of PCP and other compounds to biodegradation, there has been increased interest in alternative treatment technologies. Advanced oxidation processes (AOPs) rely on the generation of hydroxyl free radicals (·OH), a highly reactive chemical species (Venkatadri and Peters, 1993). Complete mineralization of

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**List of symbols and abbreviations**

| | |
|---|---|
| $\alpha$ | recycle rate, equal to the ratio of the flow rate of the effluent from the bioreactor that is recycled back to the chemical reactor with respect to the inlet flow rate to the combined system |
| $\tau_{BR}$ | residence time (time units) in the bioreactor |
| $\tau_{ChRx}$ | residence time (time units) in the chemical (Fenton's) reactor |
| $\mu_i$ | microbial growth rate (1/h) |
| $\mu_{i,max}$ | Monod's maximum microbial growth rate (1/h) |
| ·OH | hydroxyl free radical |
| DCMA | dichloromaleic acid |
| Fe(II) | ferrous iron |
| Fe(III) | ferric iron |
| $H_2O_2$ | hydrogen peroxide |
| $k_i$ | Monod's half saturation microbial degradation constant for compound i (mol/L) |
| $k_j$ | second-order kinetic constant for the Fenton's system reaction j (L/mol·s) |
| $m_{i,j}$ | stoichiometric coefficient for reactant i in Fenton's reaction j PCP pentachlorophenol |
| $R_{i,j}$ | rate law for Fenton's reaction j (in which i is a reaction member) |
| $rate_{i,Bio}$ | rate of reaction of chemical species i under biodegradation treatment |
| $rate_{i,Ch}$ | rate of reaction of chemical species i under the Fenton's reaction system |
| $S_{i,BioRx}$ | concentration (mol/L) of the chemical species i in the bioreactor |
| $S_{iChRx}$ | concentration (mol/L) of the chemical species i in the chemical reactor |
| $S_{i,FbioRx}$ | concentration (mol/L) of the chemical species i in the feed to the bioreactor (which in the combined model was equal to the concentration of this species i n the chemical reactor) |
| $S_{i,FChRx}$ | concentration (mol/L) of the chemical species i in the feed to the chemical reactor |
| TCHQ | tetrachlorohydroquinone |
| TOC | total organic carbon |
| $X_T$ | packed bed biomass content (mg) |
| $Y_{X/Si}$ | yield of biomass to carbon substrate consumption (as TOC) in mg of biomass/mol of total organic carbon |

recalcitrant compounds can be achieved using these technologies (Scott and Ollis, 1995), although long treatment times and strong doses of the oxidizing reagents are required (Esplugas et al., 2004; Marco et al., 1997; Pera-Titus et al., 2004). Often, the chemically-oxidized intermediates are less recalcitrant than the parent compound (Comninellis et al., 2008). Since biological processes are typically less expensive than chemical processes, addition of a biodegradation stage can improve the economy of the overall process, particularly for low concentration wastewater (Pera-Titus et al., 2004).

Examples of this combined treatment have been presented (Pera-Titus et al., 2004; Marco et al., 1997; Scott and Ollis, 1995), but the mechanistic information required for the combined reactor system analysis and design remains scarce (Comninellis et al., 2008; Esplugas et al., 2004; Mantzavinos and Psillakis, 2004). As a result of the complexity of the processes and the lack of mechanistic data, process integration has typically been experimentally evaluated on a case-by-case basis. For chemical oxidation alone, a widely accepted idea is that kinetics modeling requires a complete reaction pathway for the organic substrate (Duesterberg and Waite, 2006; Kang et al., 2002; Rivas et al., 2001). Although simplified kinetics models have provided significant insight into reactor design (Esplugas et al., 2004), they typically are based on first-order reactions. Such simplified analysis has the limitation of neglecting competitive effects of the chemically oxidized by-products on the target parent compound, an essential feature of AOPs that in practice precludes complete treatment of target contaminants.

The purpose of this work was to study the combination of Fenton's oxidation (the combination of $H_2O_2$ and ferrous iron) and biodegradation of PCP-contaminated water. Experiments were conducted in a continuous stirred-tank reactor (CSTR) in which Fenton's degradation of PCP occurred. The effluent from this reactor was continuously treated in a second reactor system that included a packed-bed bioreactor to degrade the PCP intermediates. The effects of $H_2O_2$ dose, residence time in the bioreactor, and recycle of the bioreactor effluent back to the chemical reactor on the combined system performance were studied. A mathematical model was developed for the combined system, based on a kinetics model simplified using a lumping approach, for both chemical oxidation and biodegradation. In this approach, a non-PCP fraction was defined to account for ·OH scavenging effects of the by-products on the chemical oxidation of PCP, and as the biodegradable fraction on the biodegradation process. The model was used to test the consistency of the experimental data and provide insights into reactor design and operating strategies (i.e., the recycle of effluent from the second stage bioreactor back to the chemical reactor).

## 2. Kinetics modeling

### 2.1. *Fenton's oxidation kinetics model*

A mathematical model was developed previously for the chemical oxidation kinetics and validated using data from batch experiments (Zimbron and Reardon, 2009). This Fenton's kinetics model included 11 reactions between inorganic species involved in the Fenton's system (i.e., $H_2O_2$, Fe(II)/Fe(III) and initiation and propagation reactions involving radicals ·OH, $\cdot O_2H/\cdot O_2^-$). The mass balances were rewritten for the CSTR used in the combined Fenton's-biodegradation system.

The termination reaction for PCP with ·OH (calculated using the competitive kinetics method) was included with a value of $4.4 \times 10^{09}$ L/mol·s. The scavenging effects of the PCP by-products on the degradation of PCP (and the rest of the

reaction system) were included as a lumped reaction with a kinetic constant (Zimbron and Reardon, 2009). This lumping approach has been successfully applied to estimate the degradation of contaminants by different AOPs, including photo-Fenton's reactions (Zepp and Scholtzhauer, 1979), ultraviolet radiation (Haag and Hoigne, 1985), and soil slurries (Huling et al., 1998).

Using the above mentioned set of reactions, the reaction rate for each reactant or product $i$ in the Fenton's reaction system is assumed to follow second-order kinetics, generalized as:

$$rate_{i,Ch} = \sum_{j=1}^{n} m_{i,j} R_{i,j} = \sum_{j=1}^{n} m_{i,j} k_j S_{i,jChRx} S_{h,jChRx} \quad (1)$$

in which $S_{i,jChRx}$ indicates the molar concentration of chemical species, $i$. For each specific Fenton's reaction $j$, $R_{i,j}$ and $m_{i,j}$ are the rate law and the stoichiometric coefficient of reactant $i$ (negative for reactants, positive for products), respectively, $k_j$ is the reaction rate constant, and the subscript $h$ indicates other chemical species involved in that particular reaction rate (e.g., ·OH).

The resulting mass balance for the chemical reactor (including a recirculation stream from the bioreactor to the chemical reactor) is:

$$S_{i,ChRx} = \frac{S_{i,FChRx} + rate_{i,Ch}\tau_{ChRx} + \alpha S_{i,BioRx}}{1+\alpha} \quad (2)$$

in which $S_i$ is the molar concentration of the reactive species i, the subscripts *ChRx* and *FChRx* indicate the chemical reactor and feed stream to this reactor, $rate_i$ is the rate of reaction of the chemical species $i$ (mol/L·s), $\tau_{ChRx}$ is the residence time ($s$) in the chemical reactor, and $\alpha$ is the ratio of recycle flow rate from the bioreactor to the chemical reactor to the total flow rate to the system.

### 2.2. Biodegradation kinetics model

Kinetics data from the observed biodegradation of PCP by-products was incorporated into a biodegradation kinetics model. The mass balances for PCP and TOC were calculated, based on the non-PCP TOC fraction (the difference of PCP concentrations in the feed and the chemical reactor, multiplied by 6, the PCP stoichiometric carbon molecular content).

Monod-type biodegradation kinetic parameters were obtained based on the non-PCP TOC as substrate (at different feed concentrations), as supported by experimental data. The Monod model for the rate of biodegradation of substrate i, $rate_{i,Bio}$, depends on the total concentration of biomass contained in the bioreactor ($X_T$, assumed constant for an immobilized biomass reactor over the period of the experiment), the yield of biomass to substrate $i$ ($Y_{X/Si}$), and the molar concentration of substrate ($S_{i,BioRx}$):

$$rate_{i,Bio} = -\mu_i \frac{X_T}{Y_{X/Si}} = -\frac{\mu_{i,max} S_{i,BioRx}}{k_i + S_{i,BioRx}} \frac{X_T}{Y_{X/Si}} \quad (3)$$

The combined model assumed that chemical oxidation and biodegradation were mutually exclusive. Furthermore, upon recycle, biomass and excess nutrients incorporated into the bioreactor stream were assumed not to affect the degradation of PCP by Fenton's reaction. These assumptions were verified in batch experiments (data not shown).

The mass balance for the bioreactor is given by:

$$S_{i,BioRx} = S_{i,ChRx} + \frac{rate_{i,Bio}\tau_{BioRx}}{1+\alpha} \quad (4)$$

in which $\tau_{BioRx}$ indicates the residence time (in time units) in the bioreactor. The combined reactor model results in a system of coupled algebraic equations that was solved using Engineering Equation Solver (F-chart Software).

This model represents a large simplification of the combined system, for both the chemical oxidation process and the biodegradation process. However, the mass balance approach to model development has the potential to provide insights into the study of the process and chemical reactor design. The need for such simplified, yet mechanistic models for process integration has been highlighted by others (Esplugas et al., 2004; Mantzavinos and Psillakis, 2004).

## 3. Material and methods

### 3.1. Chemicals

Pentachlorophenol (99%), the extraction surrogate (dibromophenol, 95%) and the internal standard (dibromobenzene, 98%) were purchased from Sigma. $FeSO_4 \cdot 7H_2O$ (99%+) was purchased from Baxter. $H_2O_2$ (non-stabilized, 31.4%), sodium chloride (99.9%) and sulfuric acid (conc.) were all purchased from Fisher. Solvents (chloroform and ethyl acetate) for the analysis of PCP and organic PCP by-products were pesticide-grade (Fisher). Deionized and dechlorinated water was used for PCP solution and plate media preparation, while water used for preparation of all other reactants was Reagent Type I Grade water (Nanopure, 18 mΩ conductivity).

### 3.2. Continuous system apparatus

The continuous combined Fenton's and biodegradation system consisted of two CSTRs in series (2 L Bioflo C-30, New Brunswick Scientific) (Fig. 1). In the first reactor (with a liquid volume of 250 mL and residence time of 1.5 h), Fenton's treatment of the PCP-contaminated water was performed at pH 3.5, within the reported optimum range for Fenton's treatment (for example Venkatadri and Peters, 1993; Zepp and Scholtzhauer, 1979). A Markson controller with a combination pH electrode and $H_2SO_4$ 0.1 N were used to control pH at 3.5. Ferrous iron and $H_2O_2$ were dosed to achieve specific concentrations as described in the next section. The chemical reactor and reactant feeds were covered with aluminum foil.

The effluent from the Fenton's reactor was fed to the bioreactor at pH 7, adding supplementary nutrient solution (phosphates, trace minerals and nitrogen; Section 3.3). pH control was achieved with NaOH 0.2 N solution and an Omega pH/ORP controller with a combination pH electrode. The bioreactor residence time was either 5.5 or 10 h, achieved by adjusting the bioreactor liquid volume (either 750 or 1500 mL,

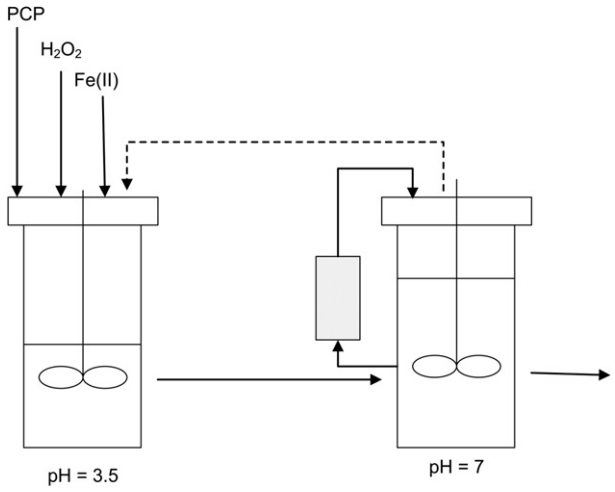


**Fig. 1 – Continuous combined reactor system for Fenton's oxidation and biodegradation of PCP-contaminated water.**

respectively). In some reactor configurations, the effluent from the bioreactor was recycled to the chemical reactor.

The low organic carbon concentration in the feed stream (due to the low PCP solubility) resulted in oligotrophic (low nutrient) conditions, under which suspended cell cultures might be washed out of the reactor. Thus, a packed-bed column (glass, 3.8 cm OD, 14.5 cm long) filled with 38.8 g of Celite biocatalyst carrier R-635 (Manville) was provided, to which the contents of the main bioreactor vessel were recirculated (in an upflow configuration) at a rate 20 times higher than the feed to the stirred tank to ensure that the liquid contents were well mixed. The bioreactor was initially inoculated with 3 mL of Fort Collins wastewater treatment plant aerobic activated sludge.

The reactor tubing consisted of chemically resistant PTFE when possible, or Norprene (Masterflex). All other parts in the system were stainless steel or glass. No PCP sorption or degradation was detected on these materials or the Celite biomass support through batch 24 h sorption experiments.

### 3.3. *Reactants to the continuous system*

The PCP solution was prepared with deionized and dechlorinated water adjusted to pH 10 with 0.8 mL of 0.1 M NaOH to facilitate PCP dissolution. PCP was added to the reported solubility at neutral pH (14 ppm). The flow rate of this solution to the reactor system was 3.5 L/day.

The flow rate of the $H_2O_2$ feed solution reactant was 85 mL/day, equivalent to 2.5% of the total flow rate to the combined system.At this flow rate, the $H_2O_2$ feed of 880 μM and 1800 μM solutions yielded the low and high doses of $[H_2O_2] = 220$ and $[H_2O_2] = 370$ μM to the chemical reactor, respectively.

The iron feed solution was prepared by dissolving 2.3 g of $FeSO_4 \cdot 7H_2O$ in water, with 6.4 mL of concentrated $H_2SO_4$ to avoid Fe(II) oxidation, and diluted to 1.0 L to a final concentration of [Fe(II)] = 8300 μM. The flow rate of this reactant was 85 mL/day, resulting in an actual dose of [Fe(II)] = 200 μM to the chemical reactor.

The supplementary nutrient medium was the PAS mineral salts medium (see Section 3.5), modified by replacing calcium chloride with calcium sulfate to eliminate background chloride concentrations. Nitrogen in the supplementary nutrient medium was stoichiometric to carbon in the PCP solution at a 1:5 N:C molar ratio (Shuler and Kargi, 1992). This nutrient solution was fed to the bioreactor at a rate of 85 mL/day (approximately 2.5% of the total flow rate to the system).

### 3.4. *Analytical methods*

Concentrations of PCP and the identified PCP degradation by-products tetrachlorohydroquinone and dichloromaleic acid were determined with a HP 5890 Series II GC-MS, after solvent extraction. Ferrous iron, chloride ion, and $H_2O_2$ analyses were done spectrophotometrically, using the 1,10-phenanthroline, thiocyanate (Hach Method 20635-00), and titanium sulfate methods, respectively (APHA et al., 1980). Additional details about these analyses are available from a previous report (Zimbron and Reardon, 2009).

Dissolved oxygen was measured with a temperature-compensated oxygen electrode (Phoenix Electrode), and a Cole Parmer 01971-00 analyzer. Purgeable total organic carbon (TOC) was analyzed with a Dohrmann DC-80 TOC Analyzer (detection limit lower than 1 mg/L).

### 3.5. *Biomass analysis*

A protein quantitative assay was used to measure biomass indirectly, since iron precipitates precluded direct estimation by optical density (600 nm). Samples were collected and frozen until analysis. Samples were centrifuged in 250-mL Teflon bottles for 25 min at 13200×g. The biomass was resuspended with phosphate 0.1 M pH 7 buffer and centrifuged again twice, then reconstituted to 3.0–10.0 mL. The biomass concentrate was lysed with a sonicator (UPXL, Heath Systems) for 20 min on ice and analyzed for protein using the micro-BCA assay (Pierce), comparing absorbance (562 nm) to that of bovine serum albumin standards.

The ability of the bioreactor effluent biomass to degrade PCP was tested by plating on an agar medium with PCP as the only carbon source (PCP medium) at a concentration of 0.5 mg/L (to prevent inhibitory effects).This medium included 7.5 g of Bacto-Agar with 38.5 mL PAS concentrate (13.8 g $NH_4Cl$, 10.97 g $KH_2PO_4$ and 28.39 g $K_2HPO_4$ diluted to 500 mL) and 5 mL of PAS 100× salts solution (0.15 g $CaCl_2 \cdot 2H_2O$, 0. 5 g $FeSO_4 \cdot 7H_2O$, 2.5 g $MnSO_4 \cdot H_2O$, 9.75 g $MgSO_4$, 2 drops $H_2SO_4$ diluted to 500 mL), diluted to 500 mL (Bedard et al., 1986) and autoclaved.

The ability of the biomass to grow on a readily available carbon source was tested by plating on a non-selective medium, Trypticase Soy Broth (TSB), diluted to 1:4 to simulate an oligotrophic (low nutrient) environment.

### 3.6. *Experiments*

In addition to the basic sequential chemical oxidation and biodegradation reactor configuration, the effect of recycling part of the bioreactor effluent back to the chemical reactor was evaluated, at the low residence time of 5.5 h in the bioreactor, for both oxidation strengths. Two recycle rates

(0.2 and 0.4) were used for this purpose. This recycle rate ($\alpha$) is defined as the ratio of the bioreactor effluent flow rate sent back to the chemical reactor (Fig. 1) to the total inlet flow to the system.

After a new operating condition was established, the system ran for 48 h to achieve steady state before sampling. This represents 32 and 9 residence times for the chemical reactor and the bioreactor, respectively. A set of samples at a $H_2O_2$ dose of 220 μM taken at 36 h yielded similar PCP and TOC results to those at 48 h, suggesting that 36 h was sufficient for the system to achieve steady state.

## 4. Results and discussion

### 4.1. Experimental results

#### 4.1.1. Hydrogen peroxide consumption in side reactions

Control experiments without Fe(II) in the continuous Fenton's reactor showed that $H_2O_2$ consumption occurred in that reactor in the absence of Fe(II), and that neither significant PCP degradation nor dechlorination resulted. The measured $H_2O_2$ concentrations were 29% and 30% lower than the mass balance around the chemical reactor at $H_2O_2$ doses of 178 and 406 μM, respectively, despite the lack of measured losses in the feed flask. These losses likely occurred at iron precipitate-coated reactor surfaces (due to the long term operation). The reactivities of such surfaces toward $H_2O_2$ have been documented (Watts et al., 1997). For these reasons, all reported $H_2O_2$ doses tested in this work were corrected to 30% lower than the actual dose, as estimates of the $H_2O_2$ available for Fenton's mechanisms (i.e., resulting in PCP degradation). These are referred hereafter as the effective hydrogen peroxide doses.

#### 4.1.2. Biomass characterization

The system operated for several months without recycle at constant conditions (Fe(II) dose of 200 μM and an effective $H_2O_2$ of 260 μM) to establish a stable microbial community. At these conditions, the PCP degradation (with respect to the feed concentration) achieved in the chemical reactor was 65% and the TOC degradation achieved in the bioreactor was 13%. A bioreactor effluent aliquot was analyzed for protein, yielding a concentration of 0.41 mg/L (c.v. = 11%). This effluent contained $1.3 \times 10^{05}$ CFU, obtained by plating on ¼-strength TSB medium (after 48 h of incubation).

Upon plating the bioreactor effluent on PCP medium and incubating for one week, no colonies developed, although growth in the non-selective TSB medium occurred at 2 days, showing the lack of PCP degraders within this microbial population.

Two samples of five pellets each were taken from the packed bed and analyzed for dry and organic matter (by drying at 35 °C and incinerating). The estimated organic content of the pellets was 0.41% (±0.13%), or 160 mg of biomass (dry weight) for the entire packed column.

Three pellets from the column were crushed, sonicated, and analyzed for protein. The organic-free dry weight was estimated by incineration. The resulting approximate protein content of biomass (organic matter) in the bioreactor was 69%, within the reported range of 40%–70% (Shuler and Kargi, 1992). Biomass and protein measurements in the column and the bioreactor effluent confirmed that more than 99% of the biomass present in the bioreactor was located in the packed bed, where most of the microbial activity occurred.

#### 4.1.3. Effect of hydrogen peroxide dose

PCP degradation achieved in the chemical reactor (with a residence time of 1.5 h without recycle) and the resulting chloride production under a constant dose of Fe(II) of 200 μM and variable doses of hydrogen peroxide is shown in Fig. 2. Also shown are the model-predicted Fenton's-driven degradation of PCP and dechlorination in the chemical reactor at these conditions. The model over-predicted PCP degradation in the continuous system, although it achieved considerably more accurate predictions for batch experiments data (Zimbron and Reardon, 2009). The lowest experimental PCP dimensionless concentrations (approximately 45%) were higher than the lowest model-predicted PCP dimensionless concentration (25%, corresponding to $H_2O_2$ doses of 200 μM or higher).

The lack of agreement between experimental and model-predicted PCP degradation is attributed to side reactions of free radicals at reactive surfaces in the continuous system. These side reactions consumed $H_2O_2$, but as shown by the no-Fe(II) control experiment with a high dose of $H_2O_2$, $H_2O_2$-scavenging reactions did not yield significant PCP degradation. Detailed evaluation of the nature of the interactions of active surfaces with Fenton's reactive species (i.e., free radicals) was beyond the scope of this work.

Both PCP degradation and $Cl^-$ release depended strongly on the $H_2O_2$ dose, up to a concentration of 200 μM (Fig. 2). The mean observed-to-theoretical chloride concentration ratio

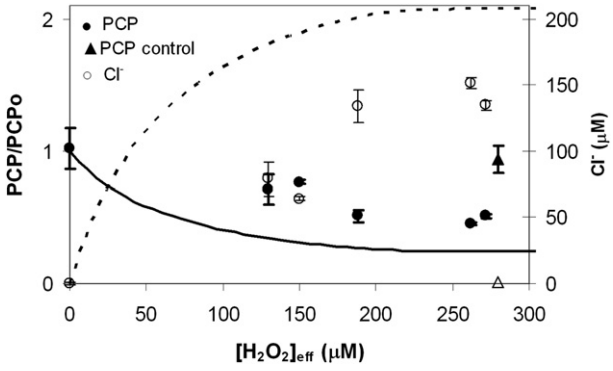


**Fig. 2 – Pentachlorophenol and chloride concentrations achieved at different effective $H_2O_2$ doses in the continuous chemical reactor. [Fe(II)] = 200 μM. Error bars represent the standard deviation of duplicate samples. Filled (●) and open (○) circles represent dimensionless concentrations of PCP in the chemical reactor (with respect to the feed concentration) and measured Cl- concentrations, respectively. The open triangle symbol is the result of a control experiment with a high dose of $H_2O_2$ and no Fe(II). The solid and dotted line represent the model-predicted PCP degradation and chloride production based on complete PCP dechlorination in the chemical reactor, respectively.**

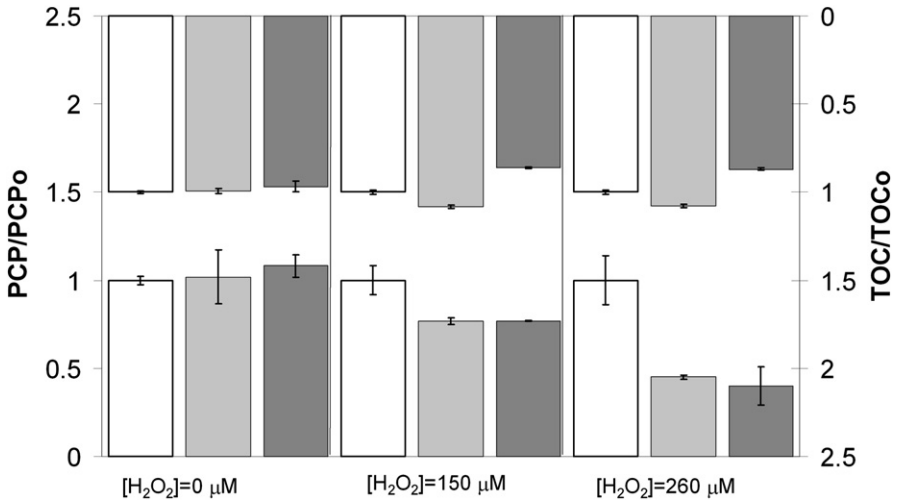


**Fig. 3 – Dimensionless concentrations of PCP (bottom) and TOC (top) at different hydrogen peroxide doses. Bars in white, light grey, and dark grey represent concentrations in the feed, chemical reactor, and bioreactor, respectively. Residence times in the chemical and bioreactor were 1.5 and 5.5 h, respectively. Error bars represent the standard deviation of duplicate samples.**

(assuming complete dechlorination of the degraded PCP) was 84% (±9%). Previous work on electrochemical oxidation of PCP in soil suspension reported about 80% dechlorination (Hanna et al., 2005), while studies on ozonation and UV/$H_2O_2$ have reported lower partial dechlorination (in the order of 30–60%) (Hirvonen et al., 2000).

The PCP and TOC concentrations in the feed, chemical reactor, and bioreactor are presented in Fig. 3. The average TOC concentration of the saturated PCP feed was 4.7 mg/L (±0.43), slightly higher than the theoretical TOC concentration of saturated PCP solution (4.0 ppm) (probably due to calibration error). Triplicate feed water blanks yielded values of 0.208 (±0.07) mg/L, showing that the feed water contained very low levels of extraneous carbon. The chloride (at the same three points along the experimental apparatus) and protein concentrations in the bioreactor effluent are shown in Fig. 4.

In agreement with the plating test results, Figs. 3 and 4 indicate that microbial growth (based on protein measurements) was supported by the partial degradation of the non-PCP TOC as a carbon source. In addition to the lack of PCP biodegradation (beyond the level achieved by chemical oxidation), no further dechlorination occurred at the bioreactor. As indicated by the TOC reduction observed in the bioreactor, the bioreactor microbial population had a preference for non-chlorinated intermediates over chlorinated ones, consistent with previous findings for PCP treated by electro-Fenton's (Hanna et al., 2005).

No PCP losses in the chemical reactor (or the bioreactor) occurred in control experiments without $H_2O_2$. Cell wash-out due to the lack of nutrients at these conditions might have caused the non-zero protein concentration in the bioreactor effluent.

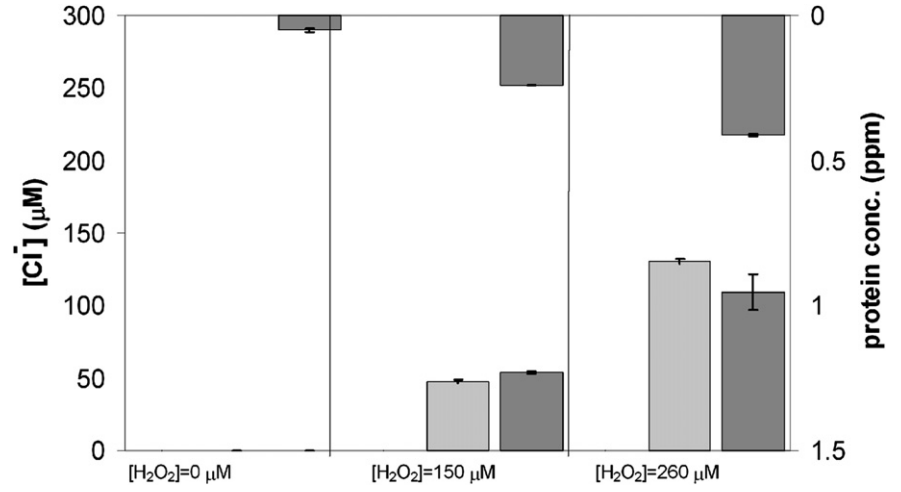


**Fig. 4 – Concentrations of chloride (bottom) and protein (top) at different hydrogen peroxide doses. Bars in white, light grey, and dark grey represent concentrations in the feed, chemical reactor, and bioreactor, respectively. Residence times in the chemical and bioreactor were 1.5 and 5.5 h, respectively. Error bars represent the standard deviation of duplicate samples.**

Tetrachlorohydroquinone (TCHQ) and dichloromaleic acid (DCMA) were identified as PCP intermediates of Fenton's oxidation in the continuous system, consistent with a previous batch reactor study (Zimbron and Reardon, 2009) and in agreement with previous reports of AOP treatment of PCP (Benitez et al., 2003; Hong and Zeng, 2002; Mills and Hoffman, 1993; Wong and Crosby, 1981; Zhao et al., 2006). DCMA was observed as a PCP by-product (and that of TCHQ and tetrachlorocatechol) under treatment with different AOPs (Hanna et al., 2005; Hong and Zeng, 2002; Sen Gupta et al., 2002; Shen et al., 2009; Wong and Crosby, 1981). The concentrations of TCHQ and DCMA in the chemical reactor and bioreactor are shown in Fig. 5. Attempts to identify other PCP intermediates were not successful.

Owing to partial degradation of PCP in the Fenton's system, the non-PCP TOC fraction was less than 55% of the PCP-saturated solution equivalent of 330 μM TOC. At a residence time of 5.5 h in the bioreactor, the maximum TOC reductions were about 14%. Further increases of the retention time in the bioreactor (with reactor conditions in the chemical reactor held constant) at the same effective $H_2O_2$ doses of 0, 150 μM, and 260 μM did not significantly increase TOC degradation (nor protein production) (95% confidence level, data not shown).

Low biodegradation rates are typically observed at high substrate or at very low biomass concentrations (conditions that lead to zero-order biodegradation rates). These potential causes seem unlikely in this system, due to the low soluble TOC (limited by PCP solubility) and the large amount of biomass present in the bioreactor column. A better explanation is that a simple Monod-type biodegradation model might not adequately describe the complex mixture kinetics of the Fenton's-treated PCP. The different biodegradability of the observed intermediates (Fig. 5) supports this. The observed lack of dechlorination, and reported toxicity of highly chlorinated compounds (such as PCP) (Pera-Titus et al., 2004), might explain the observed lack of sensitivity to extended residence time and the low extent of biodegradation of non-PCP TOC.

Analysis of the biomass in the effluent indicated a yield of 7.5 g of biomass (dry weight)/mol TOC consumed. This measured yield was only 30% of the theoretical maximum of 25.5 g biomass dry weight/mol TOC, obtained assuming a theoretical biomass formula of $CH_2N_{0.25}O_{0.5}$ (Shuler and Kargi, 1992). The remainder (70%) of the reduction of TOC in the bioreactor is an order of magnitude estimate of the mineralization extent to $CO_2$ (rather than incorporation into biomass).

The TCHQ yields upon PCP degradation in the continuous chemical reactor were 3.0 mol%, at both $H_2O_2$ doses (150 μM and 260 μM). The DCMA yields measured during continuous operation of the chemical reactor were 3.8 and 3.3 mol% at low and high $H_2O_2$ dose, respectively. These yields for TCHQ and DCMA were consistent with batch experiments results (Zimbron and Reardon, 2009).

#### 4.1.4. *Effect of recycle*

The effect of recycling the effluent from the bioreactor back to the chemical reactor for further treatment was tested at a residence time of 1.5 h in the chemical reactor and 5.5 h in the bioreactor. For this, 20% and 40% of the total flow rate to the system was recycled from the bioreactor back to the chemical reactor ($\alpha = 0.2$ and $\alpha = 0.4$, respectively). These experiments were conducted at both low (150 μM) and high (260 μM) effective $H_2O_2$ doses. Two-way ANOVA indicated that PCP and chloride concentrations in the effluent were not affected by the recycle level (20% or 40%), compared with no recycle (confidence level = 95%). At the low hydrogen peroxide dose there were no significant effects of recycle (at both recycle levels tested) on TOC biodegradation. In contrast, the recycle rate effects (for both recycle ratios of 0.2 and 0.4) on the TOC biodegradation were significant at the high $H_2O_2$ dose (Fig. 6). At this high $H_2O_2$ dose, recycle had the effect of increasing the TOC reduction that occurred at the biodegradation stage with respect to no recycle. This improved TOC degradation upon recycling was confirmed by protein analysis that indicated increased biomass production (data not shown).

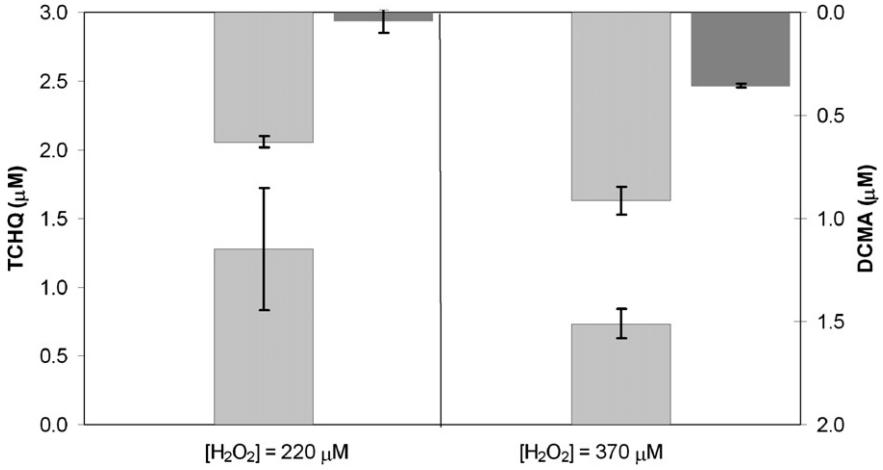


**Fig. 5 – Concentration of two observed PCP intermediates in the combined system. Tetrachlorohydroquinone (TCHQ) concentrations (bottom) and dichloromaleic acid (DCMA) concentrations (top) at different hydrogen peroxide doses. Bars in white, light grey, and dark grey represent concentrations in the feed (not present), chemical reactor, and bioreactor, respectively. Residence times in the chemical and bioreactor were 1.5 and 5.5 h, respectively. Error bars represent the standard deviation of duplicate samples.**

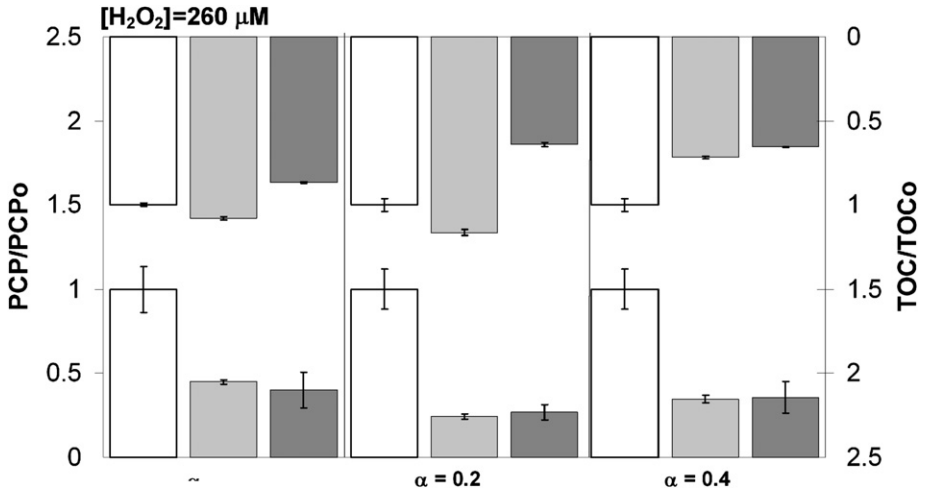


**Fig. 6 – Dimensionless concentrations of PCP (bottom) and TOC (top) at three different recycle ratios (0, 0.2, and 0.4) and a high hydrogen peroxide effective dose of 260 μM. Bars in white, light grey, and dark grey represent concentrations in the feed, chemical reactor, and bioreactor, respectively. Residence times in the chemical and bioreactor were 1.5 and 5.5 h, respectively. Error bars represent the standard deviation of duplicate samples (except for feed concentrations, for which error bars represent the standard deviation of 10 measurements).**

### 4.2. *Kinetics modeling results*

The biodegradation data presented in Figs. 3 and 6, in addition to data obtained at the longer bioreactor residence time (11 h, for a total of 6 data points) were used to estimate the Monod biodegradation kinetics of the non-PCP TOC. The yield coefficient was estimated as 5.2 (±2.2) g/L of protein produced per mol/L of TOC consumed, based on the measured concentrations of protein in the effluent from the bioreactor. The linearized Monod equation (Shuler and Kargi, 1992) was used, resulting in the Monod values of $k_s = 250$ μM TOC and $\mu_{max} = 3.1 \times 1^{-03}\ h^{-1}$ (with a correlation coefficient of 0.74).

The model-predicted TOC and PCP concentrations at different recycle ratios (0, 0.4, and 2.0) were estimated for two different scenarios:

a) Variable biodegradation residence time, with constant residence time in the chemical reactor (1.5 h) and fixed $H_2O_2$ and ferrous iron doses (300 μM and 200 μM, respectively).
b) Variable $H_2O_2$ dose, with constant residence times in both chemical and biodegradation reactors (1.5 h and 5.5, respectively), and Fe(II) = 200 μM).

At constant oxidizer dose and variable biodegradation residence time, the model-predicted PCP dimensionless concentrations (Fig. 7) were limited by the degradation of PCP achieved in the chemical reactor, because Fenton's reaction was the only mechanism to degrade PCP in the combined system. Increases in recycle rate (from 0 to 0.4 and 2) achieved only small increases in the degradation of PCP in the chemical reactor. This is in agreement with the observed lack of sensitivity of PCP degradation in the experimental combined system at recycle rates of 0.2 and 0.4. In contrast, the model-predicted degradation of TOC under the same conditions was more sensitive to the recycle of the bioreactor effluent back to the chemical reactor (in agreement with the experimental results).

Model-predicted PCP and TOC dimensionless concentrations at a variable $H_2O_2$ dose, [Fe(II)] = 200 μM, recycle ratios of 0.4 and 2.0, and constant residence time in each reactor (1.5 h in the chemical reactor and 5.5 h in the bioreactor) are shown in Fig. 8. At the three tested recycle rates (0, 0.4, and 2), the PCP degradation was not sensitive to additional $H_2O_2$ doses above 200 μM. Recycle rates of 0.4 and 2.0 achieve small increases in the degradation of PCP in the combined system. The observed lack of sensitivity of PCP degradation achieved by the experimental combined system at recycle rates of 0.2 and 0.4 can be explained in terms of the lack of sensitivity of the PCP and chloride analysis to detect the expected small changes.

Model-predicted TOC and PCP degradation in the combined system increased with higher $H_2O_2$ doses (at constant

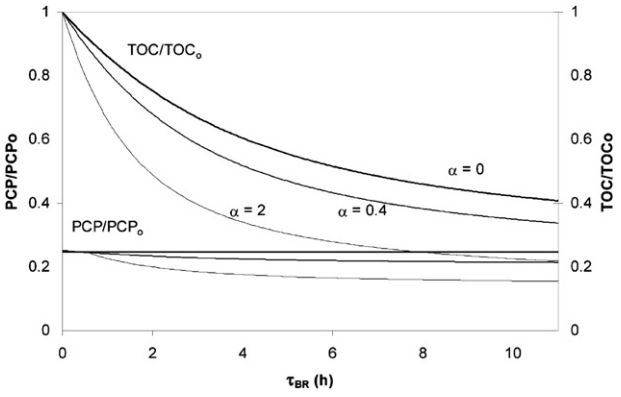


**Fig. 7 – Model-predicted dimensionless degradation of PCP and TOC as a function of residence time in the bioreactor. The residence time in the chemical reactor was 1.5 h [$H_2O_2$] = 300 μM and [Fe(II)] = 200 μM. Thicker lines represent model solutions at lower recycle rates.**

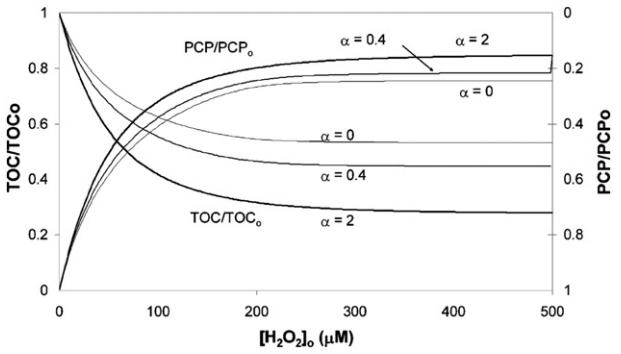


**Fig. 8 – Model-predicted dimensionless degradation of TOC and PCP in the combined system at variable hydrogen peroxide dose. The PCP degradation scale (right) is reversed to better separate the data. Residence time in the chemical reactor was 1.5 h, and 5.5 h in the bioreactor. [Fe(II)] = 200 μM. Thicker lines represent model solutions at lower recycle rates.**

residence time), as shown in Fig. 8. However, the magnitude of the increases was larger for TOC than for PCP degradation. This higher sensitivity of the model-predicted TOC degradation over that of PCP degradation can be explained in terms of the biodegradation kinetics constants for TOC and the scavenging effects of the PCP by-products on the chemical oxidation of PCP. The two limiting cases for the Monod-type kinetics are zero- and first-order kinetics, which occur under high and low substrate concentrations (compared to $k_i$), respectively. The TOC degradation would be sensitive to increases in PCP degradation achieved in the chemical reactor only under a regime of first-order degradation kinetics (i.e., at low substrate concentrations) as determined by the magnitude of the ratio of the Monod's constants $\mu_{i,max}$ and $k_i$ (which determines the value of the first-order constant).

## 5. Conclusions

- The combined system achieved both PCP and TOC degradation. All of the PCP degradation (which was correlated with dechlorination) occurred in the chemical reactor, while all of the TOC degradation occurred at the bioreactor. TOC biodegradation only occurred upon Fenton's oxidation of PCP in the chemical reactor.
- Plating tests and bioreactor performance indicated that the microbial population could not grow on PCP as a single carbon source. During bioreactor operation, this population partially mineralized the non-PCP fraction of the TOC without further significant dechlorination of the Fenton's reactor effluent. The observed Fenton's oxidation intermediates differed in their biodegradability: TCHQ was completely biodegraded, while DCMA was only partially biodegraded.
- Increased bioreactor residence time did not yield higher TOC biodegradation extents, possibly because of (a) a limited amount of biodegradable intermediates (with respect to the non-PCP TOC) and/or (b) the partial biodegradability of these intermediates.
- Recycling of the waste from the bioreactor back to the chemical reactor proved useful in achieving additional TOC degradation, but achieved only marginal additional PCP degradation.
- The model developed in this work was useful to explain some of the key experimental findings: the sensitivity of PCP degradation to $H_2O_2$ doses up to 200 μM, the lack of the system sensitivity to increases in bioreactor residence time, and the increased sensitivity of TOC degradation to recycling effects, compared to PCP degradation. This supports the idea that within the model limitations, the lumped chemical approach presented in this work is a useful tool for design and study of these combined treatment systems.

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