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Sequential Anaerobic Dechlorination of Pentachlorophenol: Competitive Inhibition Effects and a Kinetic Model

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The fate and dechlorination kinetics of PCP and its intermediates were studied in two fluidized-bed reactors (FBRs) with methanogenic enrichments. The two FBRs were operated at a 1-day hydraulic retention time and fed lactate at 400 mg/L and pentachlorophenol (PCP) at 5 mg/L (18.8 μmol/L). PCP was meta-dechlorinated to 2,3,4,6-tetrachlorophenol (2346-TeCP). Approximately 75% of the 2346-TeCP was meta-dechlorinated to 2,4,6-trichlorophenol (246-TCP), followed by sequential *o*-chlorine removals to 4-monochlorophenol (4-MCP); the remaining 2346-TeCP was ortho-dechlorinated via 245-TCP to 3,4-dichlorophenol (34-DCP). Neither 34-DCP nor 4-MCP were dechlorinated throughout the 18-month testing period. In mixed-CP batch tests, CPs competed with each other, resulting in reduced dechlorination rates; competition was position-specific with respect to ortho- and meta-dechlorination reactions. Michaelis−Menten dechlorination kinetic coefficients and linear solid−liquid partition coefficients were determined for PCP, 2346-TeCP, 246-TCP, 245-TCP, 24-DCP, 34-DCP, and 4-MCP. These coefficients were used in a Michaelis−Menten, sequential dechlorination, competitive inhibition kinetic model that used the experimentally determined CP half-saturation coefficients (K_s) for respective inhibition coefficients. The model reasonably predicted CP concentrations over time in batch tests fed 246-TCP or PCP. Without competitive inhibition, the model increasingly overestimated CP dechlorination rates with each successive dechlorination step and underestimated the time for dechlorination.

Introduction

Pentachlorophenol (PCP) and other chlorophenols (CPs) have been widely used as biocides and herbicides in the United States since the 1950s. PCP has since been detected in surface waters, sediments, rainwater, drinking water, aquatic organisms, soils, and food and has been identified in at least 235 of the 1300 hazardous waste sites on the National Priorities List (*1*). Within the last two decades, extensive research has been conducted to determine the ability of anaerobic microorganisms to degrade PCP and other chloroorganic compounds in the environment and in controlled processes (for reviews, see refs *2* and *3*).

Anaerobic biotransformation of CPs occurs via reductive dechlorination, in which chlorine removal and substitution with hydrogen results in a reduced organic compound with fewer chlorines (*3*). Reports of anaerobic CP dechlorination using pure cultures (*4*−*9*) and undefined enrichment cultures (*10*−*16*) demonstrate that the dechlorination of PCP and other CPs is carried out by a variety of bacteria that catalyze ortho-, meta-, or para-position-specific dechlorination reactions.

Despite our growing knowledge of CP anaerobic dechlorination pathways and bacteria, few studies have rigorously examined the dechlorination kinetics of CPs in mixed anaerobic cultures. PCP dechlorination proceeds sequentially with ortho-, meta-, and para-dechlorination reactions to produce various chlorinated intermediates. Such position-specific dechlorination raises the possibility that more than one compound could compete for the same dechlorination enzyme. The likelihood and the effect of competitive inhibition on dechlorination kinetics in CP mixtures were of primary interest in this study.

The fate and degradation kinetics of PCP and its dechlorination intermediates were studied in two anaerobic fluidized-bed reactors (FBRs) with methanogenic enrichments. The FBRs were used to develop PCP-dechlorinating enrichments and to conduct dechlorination kinetic tests using single- and multiple-CPs. Kinetic coefficients from the single-CP tests were incorporated into a Michaelis−Menten competitive inhibition model that described sequential dechlorination of PCP and its dechlorination intermediates using competitive inhibition kinetics. A specific goal of this research was to determine for which CPs competitive inhibition occurs and whether the competing CP compounds could be characterized by the number of chlorines or by position specificity of dechlorination.

Materials and Methods

FBR Design and Operation. PCP-dechlorinating cultures were enriched and maintained in two FBRs for 18 months. The FBRs (FBR-1 and FBR-2) were 450-mL glass reactors (4 cm diameter by 35 cm tall) maintained in a 35 °C incubation chamber (*10, 17*). Each reactor contained 50 g of support media, Celite (Type R-633; World Minerals, Inc.; Lompoc, CA). Type R-633 Celite had a 30/50 mesh size (i.e., 80% of the particles are between 300 and 600 μm in diameter) and a specific surface area (including the pore volume) of 1.3 m^2/g (World Minerals, Inc.; Lompoc, CA). Sampling ports allowed liquid or support media/biomass sampling. Peristaltic pumps were used for liquid recycling and feeding, and Viton tubing (Cole-Parmer; Vernon Hills, IL) was used for feed and recycle lines to minimize CP partitioning. Neoprene tubing (Cole-Parmer; Vernon Hills, IL) was used for the peristaltic pumps. The Celite was fluidized under a recycle flow rate of approximately 570 mL/min, providing 100% bed expansion. The empty bed hydraulic residence time was 24 h, based on the 450-mL reactor volume.

The FBRs were operated with continuous feeding of a reduced nutrient medium (RNM) buffered with sodium bicarbonate (2 g/L) and supplemented with 18 μmol/L (5 mg/L) PCP plus 400 mg/L lactate as an electron donor. The RNM media was modified from Shelton and Tiedje (*18*) and included 0.27 g/L KH_2PO_4, 0.35 g/L K_2HPO_4, 0.53 g/L NH_4Cl, 75 mg/L $CaCl_2 \cdot 2H_2O$, 100 mg/L $MgCl_2 \cdot 6H_2O$, 20 mg/L $FeCl_2 \cdot 4H_2O$, 0.5 mg/L $MnCl_2 \cdot 4H_2O$, 0.05 mg/L H_3BO_3, 0.05 mg/L

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$ZnCl_2$, 0.03 mg/L $CuCl_2$, 0.01 mg/L $NaMoO_4 \cdot 2H_2O$, 0.5 mg/L $CoCl_2 \cdot 6H_2O$, 0.05 mg/L $NiCl_2 \cdot 6H_2O$, 0.05 mg/L Na_2SeO_3, and 1.0 g/L $NaHCO_3$. RNM feed solutions were boiled under a stream of N_2 to remove oxygen, and sulfide ($Na_2S \cdot 9H_2O$; 5 mg/L as Na_2S) was added as a reducing agent. Feed solutions were prepared once every 4 days and were stored in closed, 4-L Erlenmeyer glass flasks to maintain reduced conditions during feeding. Reduced conditions in the feed solutions and in the FBRs were confirmed by the presence of Resazurin (Supelco, Inc.) (*19*), which remained clear throughout the experimental period. Feed lines entered the closed flasks through 3-cm-thick rubber stoppers, and N_2 was supplied continuously at low pressures (less than 1 psi) to replace the liquid medium pumped from the feed flasks. The feed solutions were maintained at ambient temperatures.

The FBRs were inoculated with a PCP-degrading enrichment culture obtained from laboratory-scale sludge digesters that had been fed PCP for approximately 2 years (*17*). The PCP feed concentration was increased gradually from 1.5 (0.4 mg/L) to 18.8 μmol/L (5 mg/L) over a 150-day acclimation period. The lactate concentration was increased from 100 to 400 mg/L by day 84. PCP was dechlorinated to 34-DCP and 4-MCP in the FBRs under normal operating conditions.

FBR Batch Dechlorination Rate Tests Conducted for Single-CP Compounds. Single-CP batch dechlorination rate tests were conducted with PCP and each of its dechlorination intermediates using FBR-1. Over a 1-month period, each CP was tested at three to four initial CP concentrations, except for 2345-TeCP, which was tested only at a single concentration. Each CP was spiked directly into the FBR, and reactor feeding was discontinued while maintaining recycle flows. Lactate (100 mg/L) was spiked at the beginning of each test and once every 30 min thereafter to maintain excess electron donor levels.

The FBR aqueous phase was monitored over time until all parent CP compounds and their dechlorination intermediates were dechlorinated to 34-DCP or 4-MCP, the final PCP dechlorination products. The first sample point, taken at 1 min after CP spiking to allow for complete mixing, was established as time zero.

Effect of CP Mixtures on Batch Dechlorination Rates. Using both FBRs, batch dechlorination tests were conducted with CP mixtures to determine whether CP dechlorination rates were affected by the type and concentration of other CPs. The hypothesis for this work was that CP compounds with the same position-specific dechlorination reaction would exhibit competitive inhibition when combined in the FBRs. The goal was to observe the effect of CP mixtures on initial dechlorination rates; dechlorination rates of single-CP compounds were compared with rates in CP mixtures. Three sets of tests were conducted: the first set of tests combined ortho-dechlorinated CPs or meta-dechlorinated CPs; the second set of tests combined CPs that underwent both ortho- and meta-dechlorination reactions; the third set of tests combined CPs that underwent solely ortho-dechlorination reactions with those that underwent solely meta-dechlorination reactions. These tests were conducted over a 3-month period, approximately 1 year after inoculating the FBRs and 2 months after conducting the single-CP batch dechlorination kinetic tests.

Because tests were conducted chronologically, there was a need to verify that changes in dechlorination rates were due to inhibition rather than other environmental changes (i.e., population changes, long-term toxicity, or changes in the feed media). Therefore, selected single-CP batch tests were repeated to confirm the stability of the cultures. Furthermore, the sequence of tests was randomized.

Analytical Methods. The PCP-fed FBRs were monitored for aqueous-phase CP concentrations. One-milliliter aqueous-phase samples were obtained from the FBRs by syringe. Phenol and the CPs were acetylated by adding 40 μL of concentrated K_2CO_3 (720 g/L) buffer plus 40 μL of reagent-grade acetic anhydride/1 mL aqueous-phase sample in 4-mL glass vials. An internal standard (2,4,6-tribromophenol [246-TBP]) was added to each sample to account for extraction losses. The CPs and 246-TBP were extracted into 1 mL of hexane, followed by the addition of 200 μL of tetrabutylammonium hydrogen sulfide to remove sulfides. Hexane extractions were stored at 4 °C until GC analysis. Eight-point standards were prepared gravimetrically for quantification of each of the 19 possible CPs plus phenol. The CP standards were extracted and analyzed using the same methods described above, and aqueous CP concentrations were determined by comparison with the standard curves.

Hexane extractions were analyzed using a Perkin-Elmer Autosystem GC equipped with an electron capture detector (ECD) connected to a 30-m RtX-1 (0.32 mm i.d.; 1.00 μm df; Restek Corporation; Bellefonte, PA) and a flame ionization detector (FID) connected to a DB-5 megabore column (0.548 mm i.d.; 1.50 μm df; J&W Scientific; Folsom, CA). DCPs, TCPs, TeCPs, and PCP were analyzed using the ECD, and phenol and MCPs were analyzed using the FID. The carrier gas was helium (He), and the ECD makeup gas consisted of 95% argon plus 5% methane; 1-μL samples were injected onto the GC and split 90:1. FID operating temperatures were 100 °C for 2 min to 250 °C at 5.5 °C/min, held at 225 °C for 5 min; ECD operating temperatures were 175 to 225 °C at 5 °C/min, held at 225 °C for 5 min. Detection limits were as follows: 0.04 μM PCP; 0.11 μM TeCPs; 0.13 μM TCPs, except for 0.25 μM 246-TCP; 0.61 μM DCPs, except for 0.31 μM 23-DCP and 1.5 μM 24-DCP; 3.9 μM 2-MCP and 4-MCP and 2.0 μM 3-MCP.

FBR biomass concentrations were determined by measuring the mass of volatile solids (VS) per unit Celite mass. Biomass/Celite samples were withdrawn from the FBRs and dried overnight at 110 °C in preweighed, pretreated (550 °C for 1 h) aluminum trays to determine the mass (g) of total solids (TS). The samples subsequently were heated at 550 °C for 30 min to burn off the VS and to determine the mass of nonvolatile solids (NVS), which was assumed to equal the Celite mass (g) in the samples. The VS mass (g) was the difference between the TS and the NVS and was normalized to NVS mass. Total reactor VS concentrations were estimated by multiplying the milligrams of VS/gram of Celite by 50 grams of Celite/FBR (110 g of Celite/L-reactor).

Phenol and chlorinated phenols were obtained from Aldrich Chemical Co. (Milwaukee, WI). All compounds were greater than 99% purity except for 3-MCP, 25-DCP, 26-DCP, 246-TCP, and 2345-TeCP (which were 98% pure) and 2346-TeCP (which was 90% pure). The impurity in 2346-TeCP, which primarily consisted of PCP, was corrected for when calculating 2346-TeCP dechlorination rates. Concentrated stock solutions of phenolic compounds were prepared gravimetrically in organic-free deionized (DI) water and were pH-adjusted with sodium hydroxide (NaOH). Lactate was obtained from Aldrich Chemical Co. (Milwaukee, WI).

Modeling CP Dechlorination in the FBRs. CP removal in the FBRs is described by a sequential Michaelis−Menten dechlorination model that relates CP dechlorination rates to aqueous-phase CP concentrations. The reactor biomass concentration and CP solid−liquid partitioning coefficients for desorption were included to account for the total CP mass in the reactor. Because the dechlorination reaction rates were relatively slow as compared to CP desorption kinetics (*17*), solid−liquid partitioning equilibrium was assumed, and partitioning rates were not incorporated into the model. No transfer to the gas phase was assumed due to the low Henry's constants for chlorinated phenols, which were added to the reactors as sodium salts.

At pH 7.3−7.4, for all samples, the desorption partition coefficient ($K_{d,n}$) values obtained and used with the kinetic

test data are as follows (*17*): $K_{p,PCP} = 0.273$ L/g; $K_{p,2346-TeCP} = 0.139$ L/g; $K_{p,2345-TeCP} = 0.286$ L/g; $K_{p,246-TCP} = 0.149$ L/g; $K_{p,245-TCP} = 0.187$ L/g; $K_{p,345-TCP} = 0.401$ L/g; $K_{p,24-DCP} = 0.121$ L/g; $K_{p,34-DCP} = 0.252$ L/g; $K_{p,4-MCP} = 0.092$ L/g. FBR VS concentrations were 9.8 ± 0.7 mg of VS/g of Celite in FBR-1 and 11.4 ± 1.1 mg of VS/g of Celite in FBR-2. Thus, the fraction of CPs adsorbed onto the Celite biomass ranged from 9% for 4-MCP to 31% for 345-TCP.

The general mass balance equation describing the fate of a single-CP in the liquid phase is shown in eq 1:

$$\Phi(1 + K_{d,n}\cdot VS)\frac{dCP_n}{dt} = Q\cdot CP_{ni} - Q\cdot CP_n + \Phi r \quad (1)$$

where Φ is the FBR liquid volume (L); Q is the liquid flow rate (L/d); $K_{d,n}$ is the CP solid−liquid partition coefficient (L/g of VS) $= X_n/M\cdot CP_n$; X_n is the CP_n mass sorbed (μg); M is the mass of volatile solids (g of VS); VS is the volatile solids concentration (g/L); CP_n is the reactor aqueous-phase CP concentration (μmol/L); CP_{ni} is the influent CP concentration (μmol/L); r is the net CP transformation rate (μmol/L-day); and n is the number of chlorines, identifying the CP congener.

The CP transformation kinetics are described in eq 2. For each CP intermediate, the model included a production term due to the dechlorination of a previous, more chlorinated CP (CP_{n+1}) and included a depletion term due to the dechlorination of the intermediate (CP_n):

$$r = -\frac{V_{m,n}\cdot CP_n}{K_{s,n}^{app} + CP_n} + \frac{V_{m,n+1}\cdot CP_{n+1}}{K_{s,n+1}^{app} + CP_{n+1}} \quad (2)$$

where $V_{m,n}$ is the maximum dechlorination rate of CP_n (μmol/L-day) and $K_{s,n}^{app}$ is the apparent half-saturation constant for CP_n (μmol/L).

The FBR biomass included fermentation, methanogenic, and dechlorinating bacteria. Because the dechlorinating biomass concentration could not be separated from the overall biomass concentration in the FBRs, the term V_m was used to represent the maximum dechlorination rate (kX'), where k equals the maximum specific dechlorination rate (μmol/g-day) and X' equals the CP-dechlorinating biomass concentration (g/L). Where appropriate, the apparent half-saturation constant accounts for competitive inhibition, as shown in eq 3:

$$K_{s,n}^{app} = K_{s,n}\left(1 + \frac{CP_{n\pm1}}{K_{i,n\pm1}} + \frac{CP_{n\pm2}}{K_{i,n\pm2}} + ...\right) \quad (3)$$

where $CP_{n\pm1}$, $CP_{n\pm2}$, etc. are the concentrations of inhibiting CPs ($n \pm 1$) and ($n \pm 2$) (μmol/L) and $K_{i,n\pm1}$, $K_{i,n\pm2}$, etc. are the inhibitor constants (K_s) for CPs ($n \pm 1$) and ($n \pm 2$) (μmol/L). When no competitive inhibition was found, $K_{s,n}^{app}$ equals $K_{s,n}$, which was determined in the single-compound tests. Although kinetic models have been developed by empirically selecting K_i values (*20*), in this competitive inhibition model the experimentally determined K_s value for a CP was used as its K_i value in determining $K_{s,n}^{app}$ values (*21*).

The maximum CP dechlorination rates (V_m) and the half-saturation coefficients (K_s) were determined individually for PCP and each CP intermediate from the single-compound batch dechlorination tests. The dechlorination rates accounted for changes in the total CP mass over time, including aqueous- and solid-phase concentrations, based on aqueous-phase V_m values ($V_{m(aq)}$) and CP partition coefficients (eq 4). $V_{m(aq)}$ values were determined from linear regressions of initial changes in aqueous-phase CP concentrations versus time. The single-compound K_s values were determined using a statistical data-fitting program (*22*). Competitive inhibition between parent compounds and their competing CP intermediates was factored into the analysis of K_s values for competing CPs, as shown in eq 4:

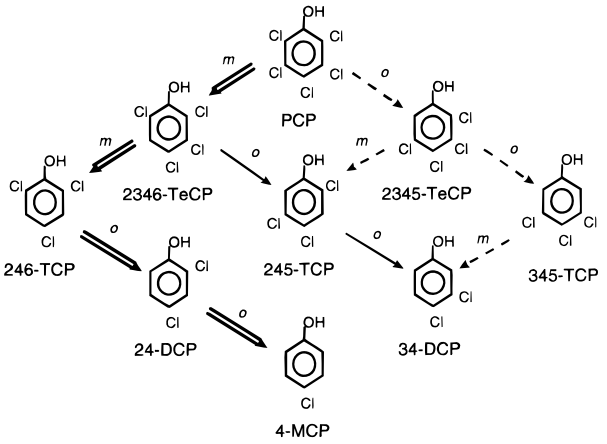

FIGURE 1. PCP dechlorination pathways in both FBRs. Arrows directed toward the right represent ortho-dechlorination reactions. Those directed toward the left represent meta-dechlorination reactions. Solid lines represent the PCP dechlorination pathways appearing during the first 14 months of operation. Dashed lines represent a minor pathway that first appeared after 14 months of operation. Double lines indicate the dominant dechlorination pathway observed throughout the experimental period.

$$V_m = V_{m(aq)}(1 + K_{d,n}\cdot VS) \quad (4)$$

A basic assumption for the model was that biofilm diffusion limitations were not significant. This assumption was supported by transmission electron microscopy (TEM) studies (*17*) that showed a thin biofilm on the outer surface of the Celite particles of about 15−50 μm (see Supporting Information). An estimated biofilm thickness of 10 μm was calculated (*17*), based on the FBR VS concentrations and the total external (spherical) Celite surface area (see Supporting Information), supporting the thin biofilm assumption.

The model was solved using Stella II, Version 3.0.5 software (*23*). Stella calculates the dynamic response for multiple differential equations using Runge−Kutta analysis by incorporating a series of simultaneous differential equations for each compound and its metabolites in the solid and liquid phases.

Results and Discussion

CP-Dechlorination Pathways. During startup of the methanogenic FBRs, PCP dechlorinated via initial *o*- and *m*-chlorine removals, producing 2345- and 2346-TeCP; 345-, 245-, and 246-TCP; and 24-DCP as intermediates. Low concentrations ($\leq 5\,\mu$mol/L) of dechlorination intermediates were measured in the FBRs, but after 6.5 months, the concentrations of intermediates were below their detection limits; 34-DCP and 4-MCP were the sole end products of PCP dechlorination.

Figure 1 shows the PCP-dechlorination pathways in the FBRs. Solid (single- or double-lined) arrows represent the PCP-dechlorination pathways from the 3rd to 14th months of operation after startup. During this time, which was when the single-CP batch kinetic tests were conducted (month 8), PCP was solely meta-dechlorinated to 2346-TeCP, and more than 80% of the 2346-TeCP produced via PCP dechlorination was meta-dechlorinated to 246-TCP. The 246-TCP was dechlorinated sequentially to 4-MCP. Double-lined arrows represent this pathway. The remaining 2346-TeCP was dechlorinated sequentially via 245-TCP to 34-DCP, represented by single-lined arrows. Despite the prolonged exposure to 34-DCP and 4-MCP (over 2 years) and the high degree of meta-dechlorination in the FBRs, para-dechlorination was

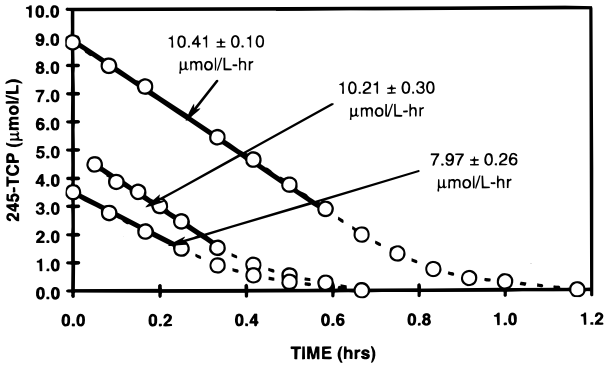

FIGURE 2. Aqueous-phase 245-TCP concentrations versus time for batch 245-TCP dechlorination tests in FBR-1. Solid lines highlight aqueous-phase, zero-order dechlorination rates with calculated values.

not observed throughout the study period, and 245-TCP and 34-DCP were never meta-dechlorinated.

After 14 months of continuous operation, ortho-PCP dechlorination was seen with the appearance of 2345-TeCP; this pathway is represented by dashed arrows in Figure 1. The 2345-TeCP subsequently was dechlorinated to 34-DCP via 345-TCP or 245-TCP. The first appearance of 2345-TeCP occurred after a series of 246-TCP and 245-TCP batch dechlorination tests, suggesting that the repeated application of 246-TCP and/or 245-TCP in the FBRs enriched for ortho-PCP dechlorination to 345-TCP. Enrichment of position-specific dechlorination using mono-, di-, or trichlorophenols has been reported by others (*11, 13, 15, 16*), and several ortho-dechlorinating strains that dechlorinate higher CPs have been reported (*4*−*8*). Thus, the reappearance of ortho-PCP dechlorination after the TCP kinetic tests showed that the PCP dechlorination pathways in the FBRs were subject to change over time and that the ortho-dechlorination pathway could be stimulated by the growth of ortho-TCP dechlorinators.

Determination of Michaelis−Menten Kinetic Coefficients for Single-CP Compound Batch Kinetic Tests in the FBRs. Batch tests were conducted using single-CP compounds, to establish Michaelis−Menten kinetic rate coefficients, for each CP in the PCP-dechlorination pathway. Examples of the single-compound CP batch kinetic test results are shown in Figures 2 and 3, where measured parent compound aqueous-phase concentrations are plotted for multiple batch kinetic tests. The batch 245-TCP dechlorination test results (Figure 2) are shown as an example of a CP that underwent a single dechlorination step, in this case to 34-DCP. The 2346-TeCP dechlorination results (Figure 3) are shown as an example CP that underwent multiple dechlorination steps, ending with 34-DCP and 4-MCP.

Initial linear dechlorination rates ($V_{(aq)}$) were determined from each batch kinetic test, as shown in Figures 2 and 3. The maximum measured $V_{(aq)}$ value for each CP was taken as $V_{m(aq)}$. For 2346-TeCP, the dechlorination rates were corrected for initial PCP dechlorination to 2346-TeCP. The V_m and K_s values determined from the single-CP-compound tests are shown in Table 1, along with confidence limits for K_s. V_m values are highest for PCP and 2345-TeCP, followed by 2346-TeCP, 245-TCP, and 345-TCP, and are lowest for 246-TCP and 24-DCP. All K_s values were below 1.0 μmol/L, which is in agreement with the fact that CP-intermediate concentrations were below their respective detection limits during steady-state FBR operation.

Competition between CPs. Examination of the position-specific dechlorination reactions shown in Figure 1 shows that some CPs were dechlorinated only via ortho or meta reactions, while others were dechlorinated at both positions with one being the dominant reaction.

TABLE 1. Michaelis−Menten CP Dechlorination Kinetic Values Determined from Batch FBR Tests

chlorophenol	V_m (μmol L^{-1} h^{-1})	K_s (μmol/L)	K_s 95% confidence limits	
			lower 95%	upper 95%
PCP	16.6	0.41	0.29	0.53
2345-TeCP	17.0	0.38	0.17	0.59
2346-TeCP	11.4	0.06	0.03	0.10
345-TCP	9.7	0.23	0.07	0.39
245-TCP	12.4	0.82	0.58	1.05
246-TCP	6.4	0.11	0.06	0.17
24-DCP	6.0	0.30	0.02	0.58

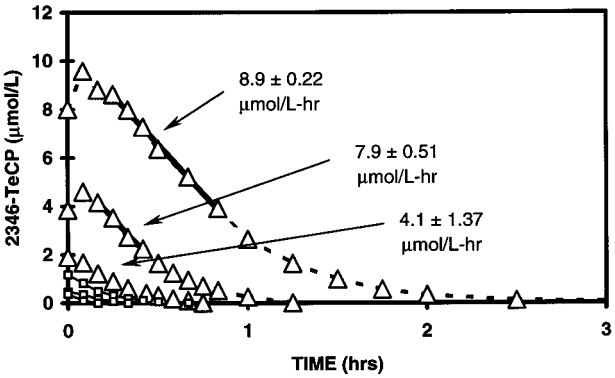

FIGURE 3. Aqueous-phase 2346-TeCP (△) concentrations and PCP concentrations (□) versus time for batch 2346-TeCP dechlorination tests in FBR-1. Solid lines highlight aqueous-phase, zero-order dechlorination rates with calculated values.

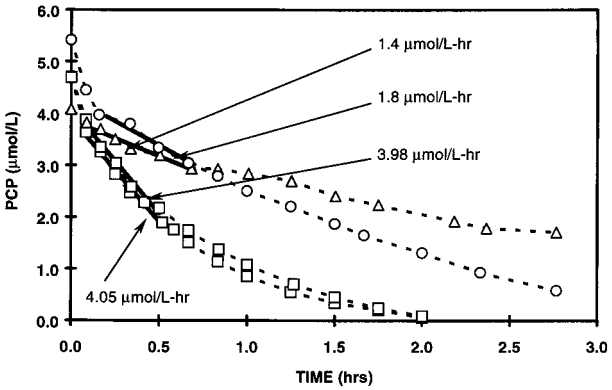

FIGURE 4. Aqueous-phase PCP concentration versus time with 5.6 μmol/L 345-TCP (△) or 8.1 μmol/L 2345-TeCP (○) in mixed-CP batch-fed dechlorination tests. Duplicate PCP-only tests (□) are shown to demonstrate the reproducibility of the tests.

A series of batch tests was carried out to test the hypothesis that the same enzymes are used to attack CPs with the same position-specific dechlorination reactions, resulting in competition between these compounds. If competition exists, CP dechlorination rates should decrease in the presence of competing CPs. For some CPs where both ortho and meta reactions could occur (i.e., PCP and 2346-TeCP), the metabolite distribution also was evaluated, because competition at one reaction site could shift more of the dechlorination to the other site.

Competition between meta-Dechlorinated CPs. Figure 4 shows that the presence of 345-TCP or 2345-TeCP decreased the rate of PCP dechlorination. Because 345-TCP was solely meta-dechlorinated and both PCP and 2345-TeCP were primarily meta-dechlorinated, these results demonstrate meta-specific competition. Initial PCP dechlorination rates were determined for each batch test using linear regressions

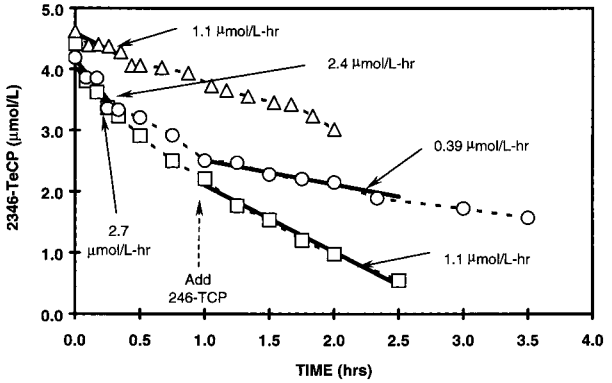

FIGURE 5. Aqueous-phase 2346-TeCP concentrations versus time with 11 μmol/L 246-TCP added with 2346-TeCP (△) or added 60 min after spiking with 2346-TeCP (○) in mixed-CP batch-fed dechlorination tests. One test includes 2346-TeCP only (□) without 246-TCP addition.

of the first three to four time points (i.e., the first 10−15 min) of each curve in Figure 4.

The PCP-only dechlorination test was replicated to determine the variation in dechlorination rates during the 2-week testing period for these tests. Initial dechlorination rates are shown in the figure. The PCP dechlorination rates of the duplicate batch tests averaged 4.02 ± 0.05 μmol L^{-1} h^{-1}, demonstrating reproducibility of the PCP-only batch tests. In the presence of 5.6 μmol/L 345-TCP, the initial PCP dechlorination rate was reduced by approximately 65%, and in the presence of 8.1 μmol/L 2345-TeCP, the initial PCP dechlorination rate was reduced by 55%.

Increasing initial 345-TCP and 2345-TeCP concentrations also led to increasing ortho-dechlorination metabolite concentrations and decreasing meta-dechlorination metabolite concentrations (data not shown) supporting the hypothesis that the inhibition of PCP dechlorination by 345-TCP and 2345-TeCP primarily was meta-specific.

Competition between ortho-Dechlorinated CPs. Figure 5 shows that the presence of 246-TCP decreased the 2346-TeCP dechlorination rate, whether 246-TCP was introduced at the onset of the test or 1 h after spiking the reactor with 2346-TeCP. Initial 2346-TeCP dechlorination rates with and without 246-TCP are shown in the figure. The addition of 246-TCP resulted in a 56% reduction in the 2346-TeCP-dechlorination rate when added at the onset of the experiment and resulted in a 65% reduction when added 60 min after 2346-TeCP. 2346-TeCP also was tested for ortho-specific inhibition using 245-TCP. The addition of 5.2 μmol/L 245-TCP with 4.2 μmol/L 2346-TeCP resulted in a 57% reduction in the initial 2346-TeCP-dechlorination rate (results not shown). Because 246-TCP and 245-TCP were solely ortho-dechlorinated, these results are used to demonstrate ortho-specific inhibition of 2346-TeCP.

246-TCP (15 μM) was tested with PCP (5 μM) to examine ortho-specific PCP inhibition. Because PCP primarily was meta-dechlorinated, reduced ortho-PCP dechlorination had a negligible effect on the PCP dechlorination rate; i.e., PCP dechlorination rates with and without 246-TCP averaged 4.0 ± 0.24 μmol L^{-1} h^{-1} (results not shown). However, the addition of 246-TCP led to decreased 2345-TeCP and 34-DCP intermediate concentrations (peak concentrations decreased from 0.4 μmol/L to below detection limit and from 3.0 to 1.5 μmol/L, respectively) and increased 2346-TeCP intermediate concentrations (peak concentrations increased from 1.4 to 2.5 μmol/L). Thus for PCP, competition at the ortho-dechlorination site shifted more of the dechlorination to the meta site, suggesting that ortho-specific inhibition of PCP dechlorination occurred although PCP dechlorination rates were unaffected.

TABLE 2. Effect of 345-TCP on 246-TCP Dechlorination and That of 246-TCP on 345-TCP Dechlorination in Batch FBR Tests[a]

initial CP concn (μmol/L)		dechlorination rate (μmol L^{-1} h^{-1})	
246-TCP	345-TCP	246-TCP	345-TCP
12.8		3.1 (12.8−11.4)	
14.4	1.5	3.3 (13.5−12.2)	2.2 (1.4−0.2)
	1.4		2.0 (1.5−0.5)

[a] Parentheses ( ) identify concentration ranges over which dechlorination rates were determined.

TABLE 3. Effect of 345-TCP on 245-TCP Dechlorination and That of 245-TCP on 345-TCP Dechlorination in Batch FBR Tests[a]

initial CP concn (μmol/L)		dechlorination rate (μmol L^{-1} h^{-1})	
245-TCP	345-TCP	245-TCP	345-TCP
6.7		4.4 (6.7−3.0)	
6.7	1.6	4.9 (6.7−3.2)	2.3 (1.4−0.5)
	1.4		2.0 (0.7−0.3)

[a] Parentheses ( ) identify concentration ranges over which dechlorination rates were determined.

Competition between ortho-Dechlorinated CPs and meta-Dechlorinated CPs. In separate tests, 345-TCP was combined with 246-TCP or 245-TCP to investigate inhibition between ortho- and meta-dechlorinated CPs. Tables 2 and 3show results of the single- and mixed-CP batch tests for 246-TCP and 345-TCP and for 246-TCP and 345-TCP, respectively. The presence of 345-TCP did not affect the 245-TCP and 246-TCP dechlorination rates, and the presence of 245-TCP and 246-TCP did not affect 345-TCP dechlorination rates. These results support the hypothesis that competition is position-specific, where CP dechlorination rates were unaffected by CPs dechlorinated at strictly different positions.

Competition between CPs with both ortho- and meta-Dechlorination Reactions. Batch PCP dechlorination tests were conducted with initial PCP concentrations of 3.5 ± 0.3 μmol/L and initial 2346-TeCP concentrations of 0, 5.2, and 8.6 μmol/L to examine inhibition between PCP and 2346-TeCP. Surprisingly, 2346-TeCP did not affect PCP dechlorination rates (PCP dechlorination rates were 11.4 ± 0.7 μmol L^{-1} h^{-1}), and the distribution of ortho- and meta-dechlorination reactions did not reveal position-specific inhibition (not shown). The effect of PCP on 2346-TeCP was difficult to ascertain because 2346-TeCP was a metabolite of PCP.

Table 4 summarizes the observed position-specific competitive inhibition for CP dechlorination. 245-TCP did not appear to compete with 246-TCP or 24-DCP, although all three CPs were ortho-dechlorinated and both TCPs competed with common CPs (i.e., both competed with PCP, 2345-TeCP, and 2346-TeCP). This observation could be explained if multiple ortho-dechlorinating microorganisms were present in the FBRs, masking the effect of competition between these CPs. Although this conclusion could not be verified by the results, it is supported by the variety of ortho-dechlorinating bacteria identified in the literature.

The competition studies provide evidence for at least three different position-specific groups of CP-dechlorinating bacteria in the FBRs, one meta- and two ortho-dechlorinating groups. Indirectly, they also suggest a fourth group of

TABLE 4. Summary of Position-Specific CP Competition in the FBRs[a,b]

	2346-TeCP	2345-TeCP	246-TCP	245-TCP	345-TCP	24-DCP
PCP	none	ortho and meta	ortho	ortho	meta	none
2346-TeCP		ortho and meta	ortho	ortho	meta	ortho[c]
2345-TeCP			ortho	ortho	meta	ortho[c]
246-TCP				none	none	ortho
245-TCP					none	none
345-TCP						none[c]

[a] Ortho and meta indicate the type of position-specific competition included in K_s^{app}. None indicates that competitive inhibition was not included between those CPs. [b] Not all test results are presented in this paper (*19*). [c] CPs were not tested together, and competitive inhibition behavior was assumed in the model.

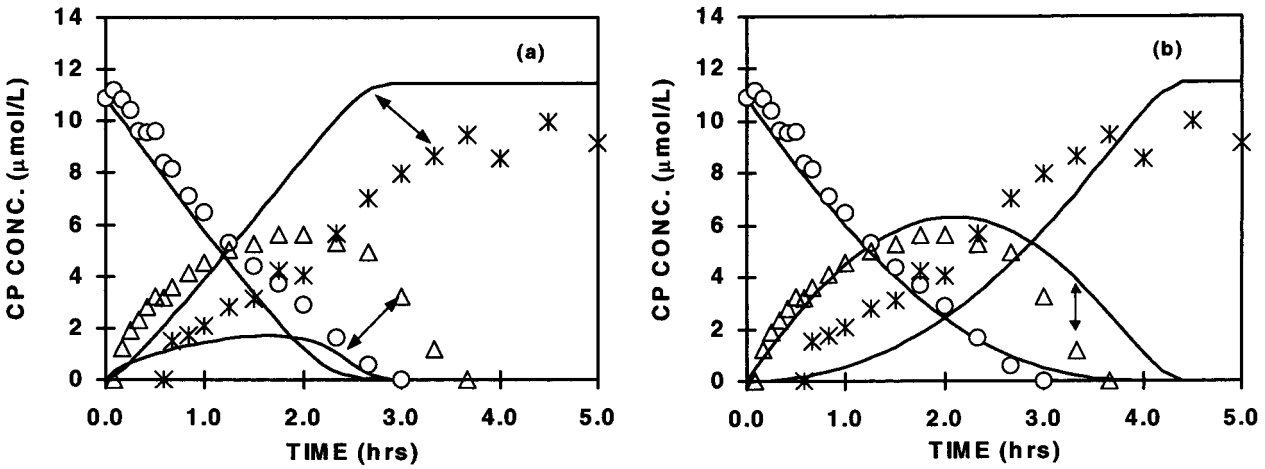

FIGURE 6. Model predictions of aqueous-phase CP concentrations compared to measured concentrations during a batch 246-TCP dechlorination test for 246-TCP (○) dechlorination to 4-MCP (*) via 24-DCP (△). Solid lines represent model predictions (a) without competitive inhibition between 246-TCP and 24-DCP and (b) with competitive inhibition between 246-TCP and 24-DCP.

dechlorinating bacteria not present in the FBRs, which would be required for para-dechlorination.

Modeling CP Dechlorination in the FBRs for Single-Compound Batch Tests. The V_m and K_s values obtained from the single-CP batch tests were incorporated into a sequential dechlorination Michaelis−Menten model to predict aqueous-phase CP concentrations in batch dechlorination tests. The model assumed no competition, competitive inhibition, noncompetitive inhibition, or mixed inhibition between CPs. In each case, competition was modeled using Michaelis−Menten kinetics, where K_s values of competing CPs equaled their corresponding K_i values (*21*). The noncompetitive and mixed inhibition models significantly overestimated the extent of competition and underestimated the dechlorination rates of parent CPs and their dechlorination intermediates (*17*), resulting in overestimated CP concentrations over time (results not shown). For this reason, the noncompetitive and mixed inhibition models were ruled out.

Figure 6 shows model-predicted CP concentrations for a 246-TCP batch dechlorination experiment, with and without competitive inhibition. The model without competitive inhibition (Figure 6a) overestimates the 246-TCP and 24-DCP dechlorination rates. When competitive inhibition is included (Figure 6b), the predicted aqueous-phase CP concentrations were close to their corresponding measured concentrations. These results support the observed ortho competition between 246-TCP and 24-DCP and suggest that Michaelis−Menten competitive inhibition kinetics can be used to describe the two-step dechlorination process.

Figure 7 shows the results of a batch PCP dechlorination test with model-predicted CP concentrations. Once again, the model is plotted with and without competitive inhibition. Whereas, both models closely approximate the PCP concentrations (Figure 7, panels a and d), the model without competitive inhibition (Figure 7a−c) increasingly overestimates intermediate CP dechlorination rates with each dechlorination step and substantially overestimates 4-MCP concentrations. Consequently, without competitive inhibition, the model predicts PCP dechlorination to 34-DCP and 4-MCP in less than 2 h, whereas approximately 3 h was required. The competitive inhibition model (Figure 7d−f) provides a much better approximation of the 2346-TeCP, 246-TCP, 24-DCP, and 4-MCP concentrations during PCP dechlorination and predicts approximately 3 h for PCP dechlorination to 34-DCP and 4-MCP. These results also support the observed competition between CPs and show that PCP dechlorination can be predicted reasonably well using the Michaelis−Menten competitive inhibition model, where K_i values equal the K_s values of competing compounds.

The results shown in Figures 6 and 7 were from tests conducted during the 8th month of operation, when PCP was solely meta-dechlorinated. Consequently, the model assumed 100% meta-PCP dechlorination. On the other hand, 2346-TeCP was ortho- and meta-dechlorinated; the meta-dechlorination pathway led to 4-MCP and the ortho-dechlorination pathway led to 34-DCP. The distribution between ortho- and meta-2346-TeCP dechlorination reactions employed by the model was determined by matching model predictions of 4-MCP and 34-DCP concentrations with measured final aqueous-phase concentrations, where final distribution of CP products in Figure 7 was 69% 4-MCP and 31% 34-DCP. As expected, this approach led to different distributions of ortho- and meta-dechlorination reactions depending on whether competition was included in the model. When competitive inhibition was included, the model predicted that 2346-TeCP was 75% meta-dechlorinated; without competitive inhibition, the model predicted 82.5% meta-2346-TeCP-dechlorination.

An alternative explanation for the observed reduced dechlorination rates in CP mixtures could be toxicity. CPs

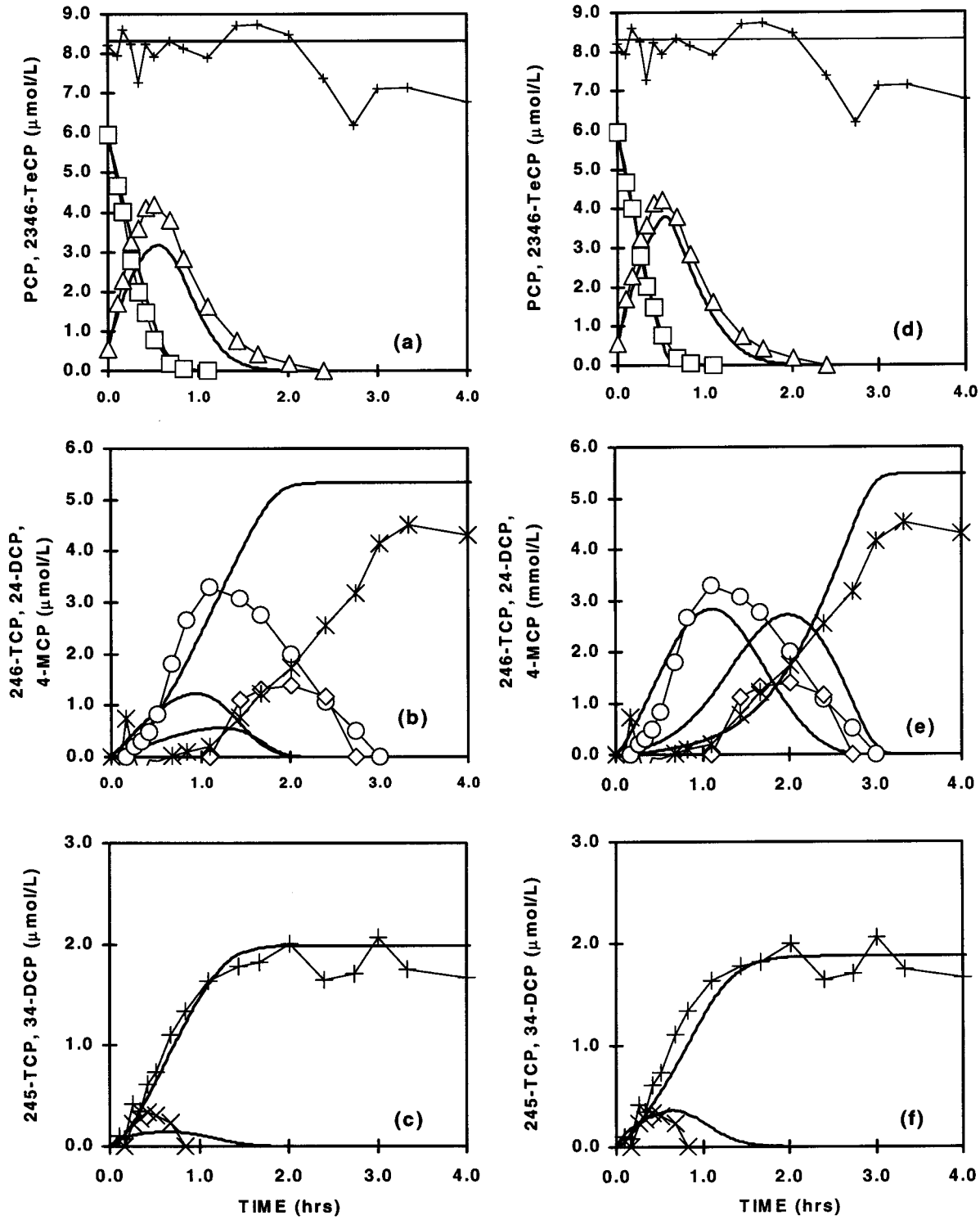

FIGURE 7. Model predictions of aqueous-phase CP concentrations compared to measured concentrations during a batch PCP dechlorination test where PCP (□) dechlorinated to 34-DCP (+) and 4-MCP (*) via 2346-TeCP (△), 245-TCP (×), 246-TCP (○), and 24-DCP (◇). Solid lines without symbols represent model predictions (a–c) without competitive inhibition and (d–f) with competitive inhibition among CPs.

are known uncouplers of oxidative phosphorylation and are toxic to anaerobic organisms. Three factors indicate the inhibition observed in the mixed-CP batch tests was not due to toxicity. First, toxicity can be reduced by acclimation to CPs. Thus, the FBR cultures were expected to be more resistant to CP toxicity than unacclimated cultures. Second, the appearance of position-specific inhibition makes toxicity an unlikely explanation for the observed inhibition effects between CPs. Although toxicity may be considered "isomer-specific" (i.e., certain CPs could be more toxic than others could), it is not likely that toxicity would result in the selective inhibition of position-specific dechlorination pathways. Third, the sequential dechlorination model, which did not account for toxicity, demonstrated that CP dechlorination could be modeled using Michaelis–Menten competitive inhibition kinetics.

A greater understanding of kinetic behavior and the effects of competition among CPs should lead to more efficient ex situ anaerobic treatment reactor designs and better predictions of the behavior of PCP and other CPs during anaerobic

in situ bioremediation. Historically, groundwater fate and transport models, such as Bioplume I and II (*24*), relied on simple first-order degradation rates. However, newer models, such as RT3D (*25*) or BioF&T-3D (*26*), increasingly use Michaelis−Menten kinetics. Models that do not incorporate Michaelis−Menten kinetics cannot address competitive inhibition. Furthermore, design models that do not include competition between CPs risk over predicting CP dechlorination rates in CP mixtures and under predicting the time for remediation.

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Supporting Information Available

TEM examination of biofilm depth on the Celite support matrix, including four figures (8 pages). This material is available free of charge via the Internet at http://pubs.acs.org.

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