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Kinetics of the Sequential Microbial Reductive Dechlorination of Hexachlorobenzene

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The reductive dechlorination kinetics of hexachlorobenzene and all other chlorinated benzene congeners (CBs) were evaluated using a mixed enrichment culture derived from a contaminated estuarine sediment. The predominant, sequential hexachlorobenzene dechlorination pathway was hexachlorobenzene → pentachlorobenzene → 1,2,3,5-tetrachlorobenzene → 1,3,5-trichlorobenzene → 1,3-dichlorobenzene. Very low levels of 1,2,4,5-tetrachlorobenzene, 1,2,4-trichlorobenzene, and 1,4-dichlorobenzene were also detected. Batch dechlorination assays were performed separately for all CBs with an initial biomass concentration of 330 mg/L as volatile suspended solids, initial concentrations of CBs of 180 nM, and electron donor in excess. The maximum dechlorination rate (K') and the half-saturation coefficient (K_S) as well as the pseudo-first-order rate constant (k_{obs}) for eight dechlorination reactions were estimated by nonlinear regression of CBs progress curves data based on a Michaelis−Menten-type and the pseudo-first-order model, respectively. The K' and K_S values (± standard error) ranged from 4.6 ± 2 to 208 ± 2 nM/d and from 5 ± 2 to 122 ± 10 nM, respectively. The k_{obs} values (± standard error) ranged from 0.035 ± 0.002 to 1.455 ± 0.124 d^{-1}. On the basis of the Michaelis−Menten model and the parameter values estimated in independent batch assays, an overall model was developed that successfully described the batch sequential reductive dechlorination of hexachlorobenzene and other polychlorinated benzene congeners to predominantly 1,3-dichlorobenzene within 25 d of incubation. Instead, when the pseudo-first-order model and the estimated k_{obs} values were used, a predicted incubation period of about 100 d was required for the conversion of hexachlorobenzene to dichlorobenzene. On the basis of the results of this study, Michaelis−Menten kinetics are recommended over first-order kinetics for the modeling of the reductive dechlorination of CBs.

Introduction

Hexachlorobenzene (HCB) was widely used in the United States as a fungicide until 1965 as well as in the production of fireworks, ammunition, and synthetic rubber (*1*). Although HCB has not been produced commercially in the United States since 1982, it is formed as a byproduct during the manufacture of solvents, other chlorine-containing compounds, and pesticides. Small amounts of HCB are produced during the incineration of municipal waste and are also found in the waste streams of chloroalkali and wood-preserving plants (*1*). HCB residues have been detected in soil, wildlife, fish, and food samples collected worldwide. As a result of its hydrophobic nature and low volatility (aqueous solubility = 0.005 mg/L at 25 °C; $\log K_{ow} = 5.73$; vapor pressure = 0.00034 kPa at 25 °C) (*2*), HCB partitions into soils and sediments and bioaccumulates in animal and plant tissues. The U.S. Environmental Protection Agency has classified HCB as a probable human carcinogen and established national primary drinking water standards as follows: maximum contaminant level (MCL) of 0.001 mg/L; maximum contaminant level goal (MCLG) of 0 (*3*).

HCB and other polychlorinated benzene congeners are persistent under aerobic conditions, whereas the less chlorinated benzene congeners (e.g., dichlorobenzenes) are amenable to aerobic degradation (*4*−*7*). However, reductive dechlorination of polychlorinated benzene congeners and accumulation of less chlorinated benzene congeners (CBs) has been documented for reduced environments such as soils rich in organic matter and sediments (*8*−*11*). The most often cited predominant pathway of the microbial reductive dechlorination of HCB is as follows: HCB → pentachlorobenzene (PeCB) → 1,2,3,5-tetrachlorobenzene (1,2,3,5-TeCB) → 1,3,5-trichlorobenzene (1,3,5-TrCB). 1,3,5-TrCB usually accumulates, although it can be further reduced via 1,3-dichlorobenzene (1,3-DCB) to monochlorobenzene or even benzene (*12*−*20*). However, these latter reactions tend to occur much more slowly and result in trace levels of dechlorination products. Other possible tetrachlorobenzene (1,2,3,4-TeCB and 1,2,4,5-TeCB), trichlorobenzene (1,2,3-TrCB and 1,2,3-TrCB), and dichlorobenzene (1,4-DCB and 1,2-DCB) isomers have been observed either less frequently or at low levels as compared with the above-discussed predominant HCB sequential dechlorination pathway (*12, 15, 17*−*21*). However, despite the relatively large number of studies conducted with respect to the reductive dechlorination of HCB and other polychlorinated benzene congeners, there is a lack of information on the kinetics of the reductive dechlorination of all CBs. The few studies that have examined the reductive dechlorination kinetics of CBs used first-order kinetics and reported pseudo-first-order rate constants, mainly for the disappearance of the parent compound(s) (*11, 12, 18, 22*).

The objective of the work reported here was the systematic assessment of the reductive dechlorination kinetics of all CBs. A mixed culture derived from a contaminated sediment and enriched using HCB as the primary contaminant was used in this study. On the basis of the estimated values of the kinetic parameters for individual CBs, an overall kinetic model was developed that successfully described the sequential reductive dechlorination of HCB to DCB isomers. The work reported here was part of a broader investigation into the contaminant fate processes, in particular microbial reductive dechlorination, taking place in the sediments of Bayou d'Inde, a tributary of the Calcasieu River, LA (*9, 10, 23*).

Materials and Methods

Enrichment Culture. The enrichment culture was derived from a contaminated sediment sample collected from Bayou d'Inde, LA. Wet sediment (120 g dry weight) was added to a 9-L Pyrex bottle equipped with glass/Teflon valves for the sampling of both gas and liquid phases. The bottle was flushed with helium gas and then filled up to a total liquid volume

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of 6 L with anaerobic media. The composition of the media was as follows (in mg/L): K_2HPO_4, 900; KH_2PO_4, 500; NH_4Cl, 500; $CaCl_2 \cdot 2H_2O$, 100; $MgCl_2 \cdot 6H_2O$, 200; $FeCl_2 \cdot 4H_2O$, 100; $Na_2S \cdot 9H_2O$, 500; $NaHCO_3$, 1200; and resazurin, 2. One milliliter of each trace metal (*24*) and vitamin solution (*25*) was also added per liter of media. Glucose and yeast extract were added from a stock solution, resulting in initial concentrations of 300 and 30 mg/L, respectively. HCB dissolved in methanol was also added, resulting in initial concentrations of methanol and HCB equal to 50 mg/L and 100 nM, respectively. Incubation was carried out at 22 °C with only periodic mixing (5 min/d) using a magnetic stir plate (200 rpm). The culture pH ranged between 7.1 and 7.3. Reductive dechlorination of HCB in this culture was observed without any lag and was confirmed by the sequential production and depletion of less chlorinated benzene congeners. After three feeding cycles, 100 mL of the culture was used as inoculum, and a second generation culture was developed in the same manner as described above. This procedure was repeated four more times. The culture was then maintained with weekly glucose/yeast extract additions and replacement of 1 L of culture with fresh media every 14 d, which resulted in a solids retention time of 84 d. The enrichment culture has sustained its HCB dechlorination capacity for over 5 yr.

Dechlorination Assays. Prior to the start of each assay, a portion of the enrichment culture was anaerobically transferred to a 4-L helium-preflushed bottle and fed only glucose/yeast extract (initial concentration 300 and 30 mg/L, respectively) to reduce all of the intermediate dechlorination products to the more volatile tri- and dichlorobenzene congeners. A He/CO_2 gas mixture (10% CO_2 in He) was then bubbled through the culture liquid to strip out all transformation products. Liquid/liquid extractions of culture aliquots were performed to confirm that all CBs had been removed, and particulate organic carbon (POC) was measured to determine the biomass concentration. The culture was amended with glucose/yeast extract and contaminant (HCB or other chlorinated benzene) as required for each particular assay, pressurized with helium, and kept well mixed. Then, 20-mL culture aliquots were dispensed anaerobically by syringe into helium-flushed 28-mL serum tubes capped with Teflon-lined butyl rubber stoppers. The serum tubes were inverted and incubated at 22 °C in the dark. During incubation, the tubes were mixed once or twice a day by gently inverting the tube racks several times. The serum tubes were sacrificially sampled in duplicate to monitor total gas and methane production, biomass content, and CBs concentrations. Unless otherwise stated, the initial biomass concentration in all assays was 330 mg/L as volatile suspended solids (VSS); the initial glucose, yeast extract, and methanol concentrations were 300, 30 and 50 mg/L, respectively. Each of the CBs—from HCB to the three dichlorobenzene isomers—were tested individually as the primary contaminant in separate culture series developed with the sediment-derived, HCB-dechlorinating enrichment culture at an initial concentration of about 180 nM. The rationale for using relatively low initial concentrations of CBs in the batch dechlorination assays was to match contaminant levels found in the Bayou d'Inde-contaminated sediment (*9, 10, 23*). For the conditions of the batch dechlorination assays, 85–90% of the initially added CBs sorbed to biomass within 2 h. As a result, aqueous-phase CBs were not measured, but rather the contents of serum tubes were extracted to quantify the CBs (see below). CBs concentrations in the serum tubes reported herein are therefore nominal concentrations, i.e., the total mass of CBs in each tube normalized to the liquid volume. For consistency among all batch dechlorination experiments, transfer of aliquots from the enrichment, stock culture into the 4-L bottle and then into the serum tubes followed the exact same protocol. Care was taken to maintain the same initial biomass concentration in all batch assays.

Three types of controls were also tested: media controls containing the reduced media and CBs; azide-amended enrichment culture and CBs containing 1 g/L sodium azide; and 2-bromoethanesulfonate (BES)-amended enrichment culture and CBs containing 25 mM BES. In the case of azide- and BES-amended controls, the inhibitors were added, and these culture series were preincubated for 3 d before the addition of the CBs. All controls were prepared in a manner similar to that of the active culture samples.

Analytical Methods. CBs were quantified by liquid/liquid extracting culture samples with isooctane, and the extracts were then analyzed by gas chromatography (electron capture detection) as previously described (*10*). Calibration curves were prepared using neat CBs dissolved in isooctane. 1,3,5-Tribromobenzene (TBB) was used as the internal standard. For the liquid/liquid extraction method, the detection limit of monochlorobenzene (MCB) was 10 mg/L, and that of all other CBs was 0.2 μg/L. In the case of dechlorination assays, the CBs were monitored by extracting the entire contents of replicate, sacrificial serum tubes with 2 mL of TBB-containing isooctane injected through the stopper. The tubes were centrifuged for 25 min at 3000 rpm to separate the solvent layer from the culture media. The solvent layer was transferred into a glass autosampler vial and then capped using a Teflon-lined rubber septum and an aluminum crimp in preparation for GC analysis. Recoveries of TBB and CBs were consistently between 95 and 105%. Total biomass was estimated by measuring POC and converting it to VSS units based on a linear correlation developed with the enrichment culture. POC measurements were performed by first filtering culture samples through Whatman GF/C glass fiber filters. The filters and retained biomass were then rinsed several times with deionized water and dried at 95 °C. The total organic carbon of filtered solids was measured using a Shimadzu TOC-5050A total carbon analyzer equipped with an SSM-5000A solid sample module and a nondispersive infrared detector. Methane was determined by gas chromatography (thermal conductivity detection) as previously described (*10*).

Chemicals. Neat CBs, TBB, sodium azide, and BES were obtained from Aldrich Chemical Co. (Milwaukee, WI). Isooctane was obtained from Fisher Scientific (Pittsburgh, PA).

Modeling Batch Dechlorination of CBs and Data Analysis. The rate of dehalogenation of a haloorganic compound may be affected by such factors as the concentrations of the electron donor and electron acceptor (i.e., the haloorganic compound), the toxicity of the haloorganic compound, and the competitive inhibition (*26, 27*). However, in all the dechlorination assays conducted in the present study, relatively low initial concentrations of the CBs were used, thus avoiding toxicity and competitive inhibition (see Results section). The dechlorination rate of CBs can be described by a Michaelis–Menten-type equation:

$$-\frac{dC}{dt} = \frac{kXC}{K_S + C}\,\frac{D}{K_D + D} \qquad (1)$$

where C is the CB concentration (nM), k is the maximum CB dechlorination rate per unit biomass (nmol (mg of VSS)$^{-1}$ d^{-1}), X is the CB-dechlorinating biomass concentration (mg of VSS/L), K_S is the half-velocity coefficient for CB dechlorination (nM), D is the electron donor(s) concentration (mM), and K_D is the half-velocity coefficient for electron donor use (mM). The concentration of electron donor(s), and thus the level of reducing equivalents, throughout the batch experiments was kept in excess as compared to the CB concentration. All batch dechlorination assays were con-

ducted with the same initial biomass concentration following the same protocol for all assays. Biomass increase during the batch assays was consistently less than 10%. Under the imposed experimental conditions and because the specific CB-dechlorinating biomass concentration could not be measured, the dechlorination rate of CBs was described by the following equation:

$$-\frac{dC}{dt} = \frac{k'C}{K_S + C} \quad (2)$$

where $k' = kX \approx$ constant is the maximum CB dechlorination rate (nM/d) for a constant biomass concentration and other imposed experimental conditions. Integration of eq 2 yields

$$C = C_o - K_S \ln (C/C_o) - k't \quad (3)$$

Although several studies have successfully described dehalogenation kinetics using Michaelis–Menten-type expressions (*26–29*) and contaminant fate and transport models have begun to use such kinetic expressions, several previously conducted biotransformation studies as well as contaminant transport and fate models have used first-order kinetics for contaminant degradation (see citations in ref *26*). In the present study, for the purpose of comparison, in addition to the Michaelis–Menten kinetics, pseudo-first-order kinetics were also considered for the dechlorination of CBs as follows:

$$-\frac{dC}{dt} = k_{obs}C \quad (4)$$

which upon integration yields

$$C = C_o e^{-k_{obs}t} \quad (5)$$

where C_o is the initial CB concentration (nM), and k_{obs} is the pseudo-first-order rate constant (d^{-1}). A nonlinear regression procedure based on the Marquardt–Levenberg algorithm (*30*) was used to fit eqs 3 and 5 to a CB time course data by minimizing the residual standard error; estimates of K_S and k' as well as k_{obs} were obtained. In the case of pseudo-first-order kinetics, transformation rates can be compared in terms of the half-lifetime, i.e., the time required for the contaminant concentration to be reduced by one-half:

$$t_h = \frac{\ln 2}{k_{obs}} \quad (6)$$

For use in general comparisons between dechlorination rates in the present work, a Michaelis–Menten half-lifetime equation was derived by evaluating eq 3 for the condition of $S = 0.5\ S_o$:

$$t_{h,MM} = \frac{0.5C_o - K_S \ln (0.5)}{k'} \quad (7)$$

Unlike the half-lifetime expression for pseudo-first-order kinetics (eq 6), the Michaelis–Menten half-lifetime expression (eq 7) is dependent on the initial contaminant concentration, C_o. Therefore, comparisons of the dechlorination rates of CBs using eq 7 are only meaningful when applied at the same initial CB concentration, as done in this study.

Results and Discussion

Dechlorination Activity and Pathways. The sediment-derived enrichment culture rapidly reduced HCB to isomers of TrCB and DCB. The predominant, sequential HCB dechlorination pathway was as follows: HCB → PeCB → 1,2,3,5-TeCB → 1,3,5-TrCB → 1,3-DCB. Very low levels of 1,2,4,5-TeCB, 1,2,4-TrCB, and 1,4-DCB were also detected. This pathway is the most frequently observed HCB dechlorination pathway (*12, 15, 17, 19*). However, three other possible isomers—1,2,3,4-TeCB, 1,2,3-TrCB, and 1,2-DCB—were not detected in the HCB-amended culture. The lack of 1,2,3,4-TeCB production from the dechlorination of either HCB or PeCB is in agreement with the results of previous studies (*12, 15, 17*). The enrichment culture used in the present study did not dechlorinate any of the three DCB isomers in short dechlorination assays (see below). However, deliberate, long-term enrichment with DCBs added to the mixed culture was not performed, and the only DCBs present in this culture were those resulting from the sequential dechlorination of HCB added. Several studies have reported accumulation of DCBs (*15, 17, 19*); yet others have reported further dechlorination (*14, 18, 19, 21*). Reported half-lifetimes for the dechlorination of DCBs have been in the range from 37 to 433 d. Therefore, the apparently slow dechlorination and the relatively low concentration of DCBs (mainly 1,3-DCB) typically maintained in the enrichment culture may not have provided the necessary conditions for the enrichment of DCB-dechlorinating species in the mixed culture.

Abiotic controls of all CB congeners confirmed their chemical stability in the reduced culture media. In addition, the presence of actively respiring organisms was necessary for reductive dechlorination to take place, as no biotransformation occurred in azide-amended cultures. In contrast, although methane was not produced in BES-amended culture series, sequential reductive dechlorination of HCB took place in these culture series at a comparable rate and a pattern similar to those observed in the control culture series (*20*). These results indicate that methanogens may not have been directly responsible for the observed reductive dechlorination of HCB and the other CBs.

Applicability of Michaelis–Menten Kinetics to HCB Dechlorination. An assessment of the suitability of the Michaelis–Menten-type model was performed by using the data obtained from culture series prepared to determine the effect of the biomass concentration on the rate of HCB dechlorination. As discussed above, the maximum dechlorination rate at given experimental conditions should be proportional to the biomass concentration (i.e., $k' = kX$). To test this condition, nonlinear regression of eq 3 was performed with HCB time course data obtained from four culture series over an incubation period of 5 d. Each series was prepared with different initial biomass concentration (i.e., undiluted culture and three diluted culture series containing 75, 50, and 25% of the undiluted culture biomass concentration). The net increase in total biomass concentration over the 5-d incubation period was less than 10% of the initially added biomass in each culture series. It is noteworthy that dechlorination assays performed at initial concentrations of glucose, yeast extract, and methanol higher than the normal feeding level (300, 30, and 50 mg/L, respectively) resulted in comparable initial HCB dechlorination rates and confirmed that electron donor saturation conditions were achieved in the batch dechlorination assays (*20*). The ratio of the k' values for the dechlorination of HCB by each of the three diluted culture series as compared to that of the undiluted culture was 0.70, 0.56, and 0.30, corresponding to the three initial inoculum levels of 75, 50, and 25%, respectively. Therefore, the HCB dechlorination rate in each culture series was almost directly proportional to the initial biomass concentration. These results indicate that the Michaelis–Menten-type model (eq 3) can be used to describe batch dechlorination kinetics of HCB as long as the initial biomass and electron-donor concentrations are high enough to ensure that the conditions of constant biomass concentration and electron-donor saturation, for which eq 2 was derived, are satisfied.

Dechlorination of Individual CBs. The dechlorination pathways and kinetics of all CBs from HCB to the three

TABLE 1. Dechlorination Reactions Carried Out by the Sediment-Derived, HCB-Dechlorinating, Enrichment Culture and Product Distribution

parent compound	possible products[a]	obsd product distribution (%)[b]
HCB	PeCB	100
PeCB	1,2,3,4-TeCB	0
	1,2,4,5-TeCB	9
	1,2,3,5-TeCB	91
1,2,3,4-TeCB	1,2,3-TrCB	0
	1,2,4-TrCB	100
1,2,3,5-TeCB	1,2,3-TrCB	0
	1,2,4-TrCB	7
	1,3,5-TrCB	93
1,2,4,5-TeCB	1,2,4-TrCB	100
1,2,3-TrCB	1,2-DCB	1
	1,3-DCB	99
1,2,4-TrCB	1,2-DCB	0
	1,3-DCB	4
	1,4-DCB	96
1,3,5-TrCB	1,3-DCB	100
1,2-DCB	MCB	*c*
1,3-DCB	MCB	*c*
1,4-DCB	MCB	*c*

[a] After one dechlorination step (predominant reaction and product shown in boldface type). [b] Relative fractions among observed dechlorination products (molar basis). [c] Reaction not carried-out by the enrichment culture during the short dechlorination assays.

dichlorobenzene isomers were evaluated using the HCB-acclimated, enrichment culture. The dechlorination of monochlorobenzene was not tested because it was never detected in any of the dechlorination assays, and none of the three dichlorobenzene isomers were dechlorinated by the enrichment culture as discussed above. For each CB congener tested, the dechlorination reactions mediated by the enrichment culture as well as the observed product distribution are summarized in Table 1. The dechlorination product distribution represents the relative mole fractions among the dechlorination products for each CB congener tested and should not be interpreted to mean that all CB congeners were completely transformed within the 2-week incubation period. It is noteworthy that the two compounds that were never observed as products of HCB dechlorination—i.e., 1,2,3,4-TeCB and 1,2,3-DCB—were readily transformed by the enrichment culture.

Pathway branching and multiple dechlorination products were observed for four CB congeners: PeCB, 1,2,3,5-TeCB, 1,2,3-TrCB, and 1,2,4-TrCB. In all four cases, one of the possible dechlorination products was overwhelmingly predominant (equal to or more than 91%) (see Table 1). On the basis of the observed product distribution during the dechlorination of each of these four CBs when tested separately, the relative molar fractions of 1,3-DCB and 1,4-DCB resulting from the sequential dechlorination of either HCB or PeCB were calculated as 0.852/0.148, which agree well with observed data. Branching in the dechlorination pathways of polychlorinated benzene congeners has been observed in all reported studies. Although differences exist among several studies with respect to the relative product distribution, in most cases one product predominated over other possible isomers (*12, 15, 17*–*19*).

The preference for a predominant dechlorination reaction appears to be related to dechlorination at a site with adjacent chlorines, although exceptions to this pattern have been observed. The preference for the main HCB and PeCB dechlorination pathway—i.e., formation of 1,3-DCB via the intermediate production of 1,2,3,5-TeCB—has been rationalized based on free energy considerations and the hypothesis that reactions with the highest energy release have preference over other possible dechlorination reactions (*12, 31*). However, even dechlorination reactions not mediated by a given consortium or proceeding as minor reactions are also highly exergonic (*32*), which shows that, beyond the thermodynamic potential for these reactions, other factors (e.g., kinetic, steric, etc.) play an important role. Peijnenburg et al. (*22*) developed an empirical quantitative structure–activity relationship (QSAR) by relating the pseudo-first-order disappearance rate constants of halogenated aromatic hydrocarbons in anoxic sediments to four molecular descriptors: carbon–halogen bond strength, the sum of the Hammett σ constants, inductive constants, and steric factors of additional substituents. On the basis of the highest dechlorination rate as predicted by this QSAR relative to alternative dechlorination reactions for the same CB congener, the frequently observed HCB and PeCB dechlorination pathway to 1,2,3,5-TeCB and then to 1,3,5-TrCB was rationalized. However, certain dechlorination reactions predicted as the least preferred reactions on the grounds of lower dechlorination rates as calculated by the QSAR were observed in the present study and proceeded at even higher rates than other possible reactions among isomers (see below).

The profiles over the incubation period of the four predominantly produced CBs during the sequential dechlorination of HCB, including their dechlorination products during batch dechlorination assays using separate enrichment culture series for each CB congener, as well as those of other CBs tested are shown in Figures 1 and 2, respectively. During the dechlorination of HCB, 1,3,5-TrCB was the predominant intermediate while 1,3-DCB was the predominant HCB dechlorination product. On the basis of these results, it was again confirmed that the predominant HCB dechlorination pathway was as follows: HCB → PeCB → 1,2,3,5-TeCB → 1,3,5-TrCB → 1,3-DCB. It is noteworthy that dechlorination of intermediates such as 1,2,3,5-TeCB and 1,3,5-TrCB proceeded regardless if they were produced as intermediates or were added to the culture as the parent compounds. In contrast, 1,2,4,5-TeCB was dechlorinated by another enrichment culture only if it was produced from PeCB (*12*).

The dechlorination pattern observed in the dechlorination assays conducted with the sediment-derived, enrichment culture matched the distribution of CBs detected in the study sediment (*9, 10, 23*). The lack of 1,2,3,4-TeCB and 1,2,3-TrCB, and the dominant accumulation of 1,3,5-TrCB and DCBs (primarily 1,3-DCB) in the sediment indicate that microbial dechlorination is probably the dominant process controlling the distribution of these contaminants in the Bayou d'Inde sediments (*9, 20*). The match of the dechlorination product distribution between the enrichment culture and the sediment also indicates that the dechlorination capacity of the sediment-derived culture continued to represent that occurring in situ.

Methane production was monitored in each culture series during the batch dechlorination assays. As an example, the cumulative methane produced in the HCB-amended culture series is shown in Figure 3 along with the cumulative dechlorination, both expressed in terms of electron equivalents (eequiv). These calculations were based on 8 eequiv/mol of methane produced and 2 eequiv/mol of chlorine substituent removed. The relatively constant (i.e., linear) rate of methanogenesis in contrast to the declining dechlorination rate after approximately 6-d incubation confirms that the initial electron donor concentration was sufficient to guarantee that CB dechlorination was not electron donor limited in these assays throughout the incubation period. As mentioned above, batch dechlorination assays conducted with initial electron donor concentrations higher than the normal culture feeding level resulted in comparable initial dechlorination rates of CBs, which further confirms that the

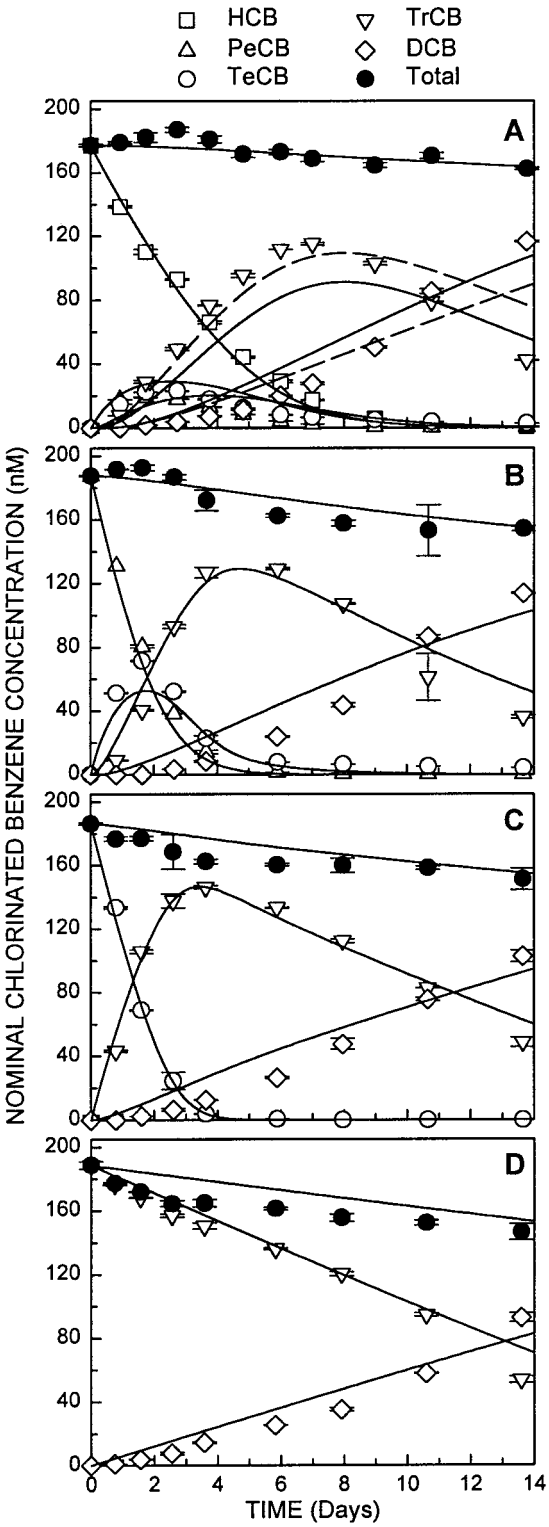

FIGURE 1. Time course of HCB (A), PeCB (B), 1,2,3,5-TeCB (C), and 1,3,5-TrCB (D) and their dechlorination products during four batch dechlorination assays conducted with each CB tested independently using the HCB-enriched culture. Error bars represent mean values ± 1 SD. Lines are model predictions based on the Michaelis–Menten model (for details related to the broken lines in panel A, see text).

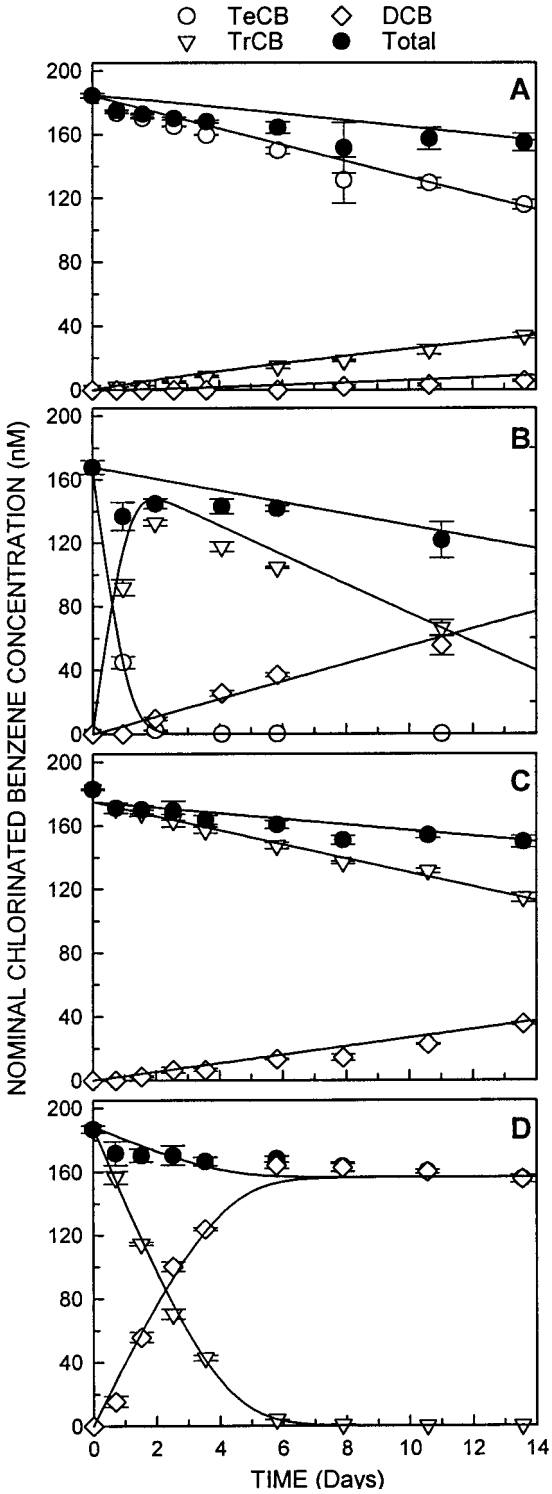

FIGURE 2. Time course of 1,2,4,5-TeCB (A), 1,2,3,4-TeCB (B), 1,2,4-TrCB (C), and 1,2,3-TrCB (D) and their dechlorination products during four batch dechlorination assays conducted with each CB tested independently using the HCB-enriched culture. Error bars represent mean values ± 1 SD. Lines are model predictions based on the Michaelis–Menten model.

batch dechlorination assays were conducted under electron donor saturation conditions (*20*).

It is noteworthy that even at an incubation period of about 4 d, while relatively constant rates of both methanogenesis and dechlorination were observed (Figure 3) relative to methanogenesis, only 0.0025% eequiv was diverted to the dechlorination reactions. Similar results were obtained with all culture series amended with the other CBs. These results agree with previous observations where the fraction of electrons used by mixed cultures for the co-metabolic reductive dechlorination of haloorganic compounds is

TABLE 2. Maximum Dechlorination Rates (k'), Half-Velocity Coefficients (K_S), and Pseudo-First-Order Rate Constants (k_{obs}) for Each CB Tested in Separate Batch Dechlorination Assays[a]

	Michaelis–Menten model				pseudo-first-order model		
parent compd	k' (nM/d)	K_S (nM)	r^2 [b]	$t_{h,MM}$[c] (d)	k_{obs} (d^{-1})	r^2 [b]	t_h (d)
HCB	52 ± 4	84 ± 9	0.981	2.8	0.282 ± 0.011	0.988	2.5
PeCB	131 ± 8	122 ± 10	0.992	1.3	0.557 ± 0.030	0.990	1.2
1,2,3,4-TeCB	208 ± 2	58 ± 4	0.999	0.6	1.455 ± 0.124	0.996	0.5
1,2,3,5-TeCB	94 ± 6	41 ± 7	0.995	1.2	0.639 ± 0.070	0.967	1.1
1,2,4,5-TeCB	5.5 ± 3	11 ± 3	0.951	17.5	0.037 ± 0.002	0.973	18.7
1,2,3-TrCB	60 ± 3	43 ± 5	0.996	2.0	0.387 ± 0.032	0.975	1.8
1,3,5-TrCB	9.5 ± 1.5	15 ± 2	0.984	10.4	0.069 ± 0.005	0.952	10.1
1,2,4-TrCB	4.6 ± 2	5 ± 2	0.951	20.0	0.035 ± 0.002	0.969	19.8

[a] Initial biomass concentration in all culture series equal to 330 mg/L VSS. [b] Nonlinear regression best estimate ± standard error. [c] Half-lifetimes for C_o = 180 nM (see eq 7).

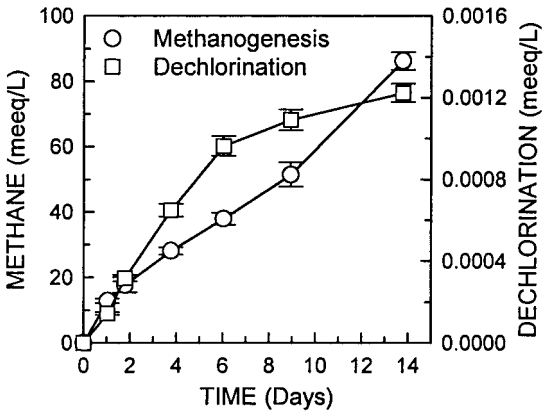

FIGURE 3. Cumulative methane produced and dechlorination equivalents during the batch HCB dechlorination assay. Error bars represent mean values ± 1 SD (meequiv = milli-electron equivalents; see text).

relatively very small as compared to that in halorespiring pure cultures (*10, 33*). It has been observed that as high as 70% of the total eequiv was used for the dechlorination process in halorespiring pure cultures (*33*). Therefore, the observed CB biotransformations by the enrichment mixed culture in the present study are considered to be co-metabolic reductive dechlorinations, although the possibility of the existence of chlororespiring species at relatively low population densities in this culture should not be discounted.

Modeling of Individual CB Congeners Dechlorination. On the basis of eqs 3 and 5 and time course data of all eight CBs tested in separate batch dechlorination assays, nonlinear regressions were performed. The resulting estimates of k', K_S, and k_{obs} are summarized in Table 2. The eight CBs concentration profiles over the incubation period of each dechlorination assay and the nonlinear fit of the data to eq 3 are shown in Figures 1 and 2. The Michaelis–Menten model described the transformation of CBs in the enrichment culture very well as judged by the relatively high values of correlation coefficients (r^2) and the relatively low values of the standard errors (see Table 2). The total biomass-normalized maximum dechlorination rates (k) measured in the present study ranged from 1.6×10^{-5} to 6×10^{-4} mol (mg of VSS)$^{-1}$ d^{-1}. It should be emphasized that the dechlorination rates depend on the concentration of the specific dechlorinating microorganisms, which in the case of mixed cultures represents a certain fraction of the total biomass. In turn, the fraction of dechlorinating microorganisms in mixed cultures is expected to vary and depend on experimental conditions such as inoculum source, enrichment method and duration, type and level of electron donor, reactor type, and many other experimental conditions. Therefore, caution is advised in interpreting and comparing dechlorination rates reported by different studies conducted with mixed cultures under widely varying experimental conditions. In spite of the exhaustive literature search performed, volumetric dechlorination rates for all CBs (with or without normalization to biomass) were not found, but rather dechlorination rates based on the pseudo-first order model have been reported more frequently as discussed below.

On the basis of the observed dechlorination kinetics (Table 2, Michaelis-Menten model), the eight CB congeners that were dechlorinated by the enrichment culture form the following series (listed in a decreasing dechlorination rate order; the dechlorination rate relative to HCB is given in parentheses): 1,2,3,4-TeCB (4.7); 1,2,3,5-TeCB (2.3); PeCB (2.2); 1,2,3-TrCB (1.4); HCB (1.0); 1,3,5-TrCB (0.3); 1,2,4,5-TeCB (0.2); 1,2,4-TrCB (0.1). Note that the first four CBs in this series with a lower chlorine content than HCB were dechlorinated at relatively higher rates than HCB. Previous studies (*12, 18*) also observed dechlorination of PeCB at substantially higher rates than that of HCB, confirming the results of the present study. The fastest dechlorination rate was observed for 1,2,3,4-TeCB, although this compound was not detected during the sequential dechlorination of either HCB or PeCB. Similarly, although 1,2,3-TrCB was not detected during the sequential dechlorination of either HCB, PeCB, 1,2,3,4-TeCB, or 1,2,3,5-TeCB, it was dechlorinated faster than HCB but more slowly than 1,2,3,4-TeCB. The two minor CBs detected during the sequential dechlorination of either HCB or PeCB—1,2,4,5-TeCB and 1,2,4-TrCB—had the lowest dechlorination rates.

Efforts made to correlate the dechlorination rates of CBs observed in the present study with three parameters—the free energy change of the overall dechlorination reaction, the charge differential between the C and Cl at the observed dechlorination site, and the HOMO–LUMO gap—obtained using a semiempirical molecular modeling method were met with mixed success (*20*). The observed relative dechlorination rates of CBs can be rationalized based on Cl-substituent location/steric hindrance considerations. As expected, an increase in the dechlorination rates is observed with an increase in the number of ortho Cl substituents. Thus, the dechlorination rates should form the following series (listed with a decreasing order of dechlorination rate): 1,2,3,4-TeCB > 1,2,3,5-TeCB > 1,2,4,5-TeCB; 1,2,3,4-TeCB > 1,2,3-TrCB; 1,2,3-TrCB > 1,2,4-TrCB > 1,3,5-TrCB. With the exception of 1,2,4-TrCB, which was dechlorinated at a relatively slower rate than that of 1,3,5-TrCB, the observed dechlorination rates among the tetra- and tri-chlorinated compounds followed the above presented series. As to the polychlorinated compounds, the observed decreasing dechlorination series 1,2,3,4-TeCB > PeCB > HCB may be rationalized on grounds of increasing steric hindrance with increasing degree of chlorination.

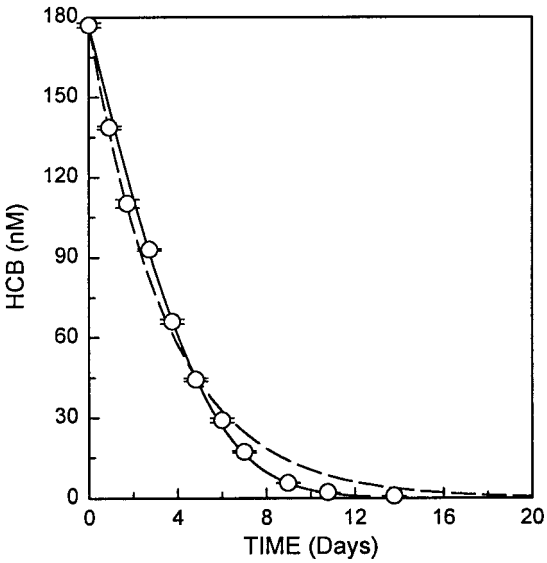

FIGURE 4. Time course of HCB during the batch dechlorination assay and nonlinear regression fit of the HCB data to the Michaelis–Menten (solid line) and pseudo-first-order (broken line) models. Error bars represent mean values ± 1 SD.

Although, an overall good fit of the time course data for single CB dechlorination was obtained when the pseudo-first-order model (eq 5) was used—as judged by the statistics reported in Table 2—this model systematically overpredicted the observed initial dechlorination rates and severely underpredicted the dechlorination rates at relatively low CB concentrations (i.e., CB concentrations at or below their K_S values) for all eight CBs tested. This behavior is exemplified in Figure 4 for HCB. The small deviations of the pseudo-first-order model from the CB time course data for single-step dechlorination reactions as a result of the compounded effect of such deviations through multiple dechlorination steps led to significant differences among the two models in the prediction of the sequential dechlorination of CBs, as discussed below. However, the half-life time estimates based on the two models (eqs 6 and 7) were very comparable (see Table 2).

Beurskens et al. (*12*) reported pseudo-first-order rate constant values ranging from 0.72 to 1.70 d^{-1} for the dechlorination of HCB by a sediment-free, enrichment culture derived from a freshwater sediment contaminated with CBs. Pseudo-first-order rate constants for the dechlorination of HCB in freshly spiked sediment slurry microcosms have been reported as equal to 0.021 d^{-1} (*11*) and 0.0256 d^{-1} (*18*). These dechlorination rates correspond to half-lifetimes of 33 and 27 d, which are at least an order of magnitude higher than HCB half-lifetimes measured in sediment-free, enrichment cultures by Beurskens et al. (*12*) and in the present study. The relatively lower dechlorination rates reported for freshly spiked sediment slurry microcosms may reflect sorption of added CBs to the sediment, thus presenting contaminant mass transfer limitations, and/or the presence of a relatively lower population density of dechlorinating microorganisms as compared to the enrichment cultures. In contrast to these studies, the pseudo-first-order HCB dechlorination rate in microcosms developed with the Bayou d'Inde-contaminated sediment (i.e., with sediment-bound HCB) was 0.0022 d^{-1}, which corresponds to a half-lifetime of 315 d (*10*). The large difference in the observed HCB dechlorination rates in sediment slurry microcosms developed with freshly added HCB, and sediment-bound HCB is attributed to the high degree of sequestration and thus limited bioavailability of the contaminant in the chronically contaminated sediments.

Modeling the Sequential Dechlorination of CBs. On the basis of eq 2 and the independently measured values of k' and K_S for each CB congener, a sequential CB dechlorination model was developed by accounting for both the consumption of the more chlorinated CB congeners and the simultaneous production of less chlorinated congeners as well as the degree of branching and product distribution in each dechlorination step. The sequential dechlorination pattern was modeled via the following pathway: HCB → PeCB; PeCB → 0.91(1,2,3,5-TeCB) + 0.09(1,2,4,5-TeCB); 1,2,3,5-TeCB → 0.93(1,3,5-TrCB) + 0.07(1,2,4-TrCB); 1,2,4,5-TeCB → 1,2,4-TrCB; 1,3,5-TrCB → 1,3-DCB; and 1,2,4-TrCB → 0.96(1,4-DCB) + 0.04(1,3-DCB). The resulting system of the eight simultaneous ordinary differential equations was solved numerically with Simnon, Version 3.0 software using the Runge–Kutta-Fehlberg 4/5 integration algorithm (*34*). The model-predicted data (i.e., CBs concentration vs time) for the eight CBs tested as parent compounds in the dechlorination assays are plotted against the experimental data in Figures 1 and 2. Because it was experimentally found that as much as 15% of the resulting lower CBs, especially the DCBs, was not accounted for at the end of the 14-d incubation period (believed to have diffused through the Teflon layer and partitioned in the culture tube rubber stoppers), the numerical dechlorination model also took into account these losses. Note that although the model predicted the two observed isomers for the TeCBs, TrCBs and DCBs, because of the overwhelming predominance of only one isomer, the sum of all isomers is shown in Figures 1 and 2. A comparison of the model predictions with the actual CBs data shows a very good match. It should be emphasized that the estimates of k' and K_S used in the numerical model to simulate the sequential dechlorination of all CBs are those obtained in independently performed, batch dechlorination assays where each of the eight CBs was added as the parent compound. The only significant deviation of the model prediction from the discrete experimental data is the underpredicted maximum levels of 1,3,5-TrCB and the earlier accumulation of DCBs as shown in Figure 1A. However, a closer look at the PeCB and TeCB data and comparison with the model predictions shows that, in the case of the HCB-amended culture series, PeCB and to a lesser degree TeCB (mainly 1,2,3,5-TeCB) dechlorinated at faster rates (200 and 110 nM/d, respectively) than in the respective cultures series amended with each of these CBs separately. When these relatively faster dechlorination rates were used in the model of the sequential dechlorination of HCB, the model-predicted TrCB concentrations better matched the experimental data (broken lines in Figure 1A).

Because the estimation of the kinetic parameters for all CBs was based on experimental data obtained from independently performed batch dechlorination assays in which each CB congener was added to separate culture series as the parent compound, the question arises as to whether competitive inhibitory effects were in effect when several CBs were present in the same culture series, especially in the case of accumulated intermediates. Such competitive inhibitory effects have been observed during the sequential reductive dechlorination of chlorobenzoates in anoxic sediment slurries and methanogenic cultures (*28*) as well as the sequential dechlorination of chlorophenols in fluidized-bed reactors (*29*). However, both studies were performed with contaminants added in the low [M to mM ranges, whereas in the present study all CBs tested were added at an initial concentration of around 180 nM, i.e., concentrations 2–4 orders of magnitude lower than in the two previously performed studies. Therefore, competitive inhibitory effects among the various CBs were not expected. In addition, numerical simulations of the sequential dechlorination of HCB were performed using the above presented sequential

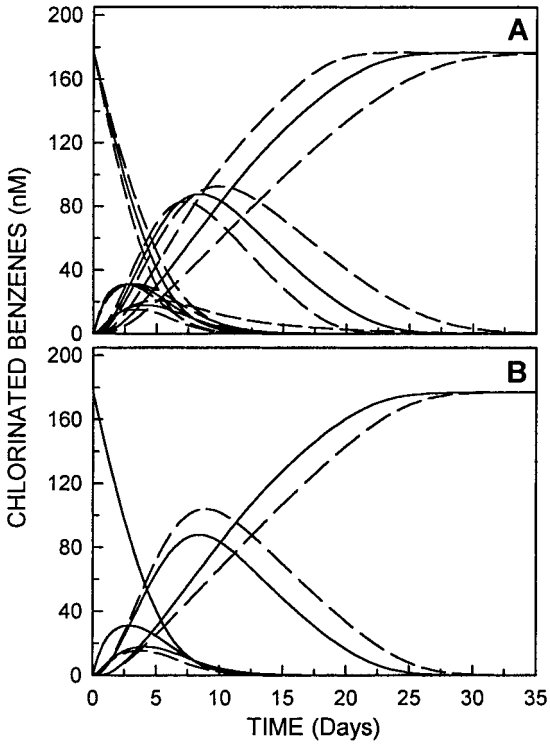

FIGURE 5. Simulated time course of CBs during the batch sequential dechlorination of HCB based on the Michaelis–Menten model. (A) Branched dechlorination pathway based on the observed product distribution (see Table 1) with k' and K_S values at their estimated mean ± standard error. (B) Comparison of the branched (solid lines) to the "linear" (broken lines) HCB dechlorination pathway (see text).

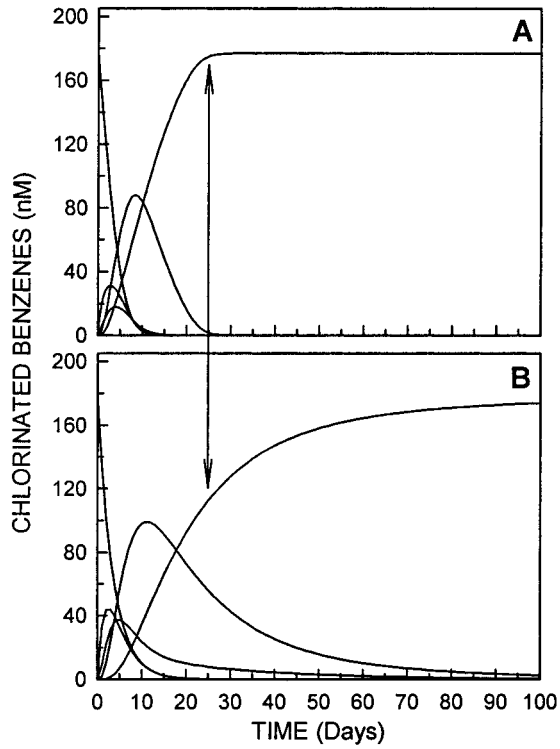

FIGURE 6. Comparison of the time course of CBs during the sequential HCB dechlorination simulated based on the Michaelis–Menten (A) and the pseudo-first-order (B) models. Arrow indicates the time (25 d) predicted by the Michaelis–Menten model for the complete conversion of HCB to DCB.

dechlorination model and incorporating competitive inhibitory effects. These effects were modeled by use of apparent half-velocity coefficients for CB dechlorination (i.e., K_S values modulated by the concentration of CBs and their inhibition constants, K_I) by setting K_I equal to the K_S value for each CB tested following a previously reported procedure (*29*). These simulations included several scenarios with combinations of CBs either being inhibited and/or acting as inhibitors. Compared to the baseline model simulation of HCB dechlorination without inhibitory effects, the individual CB profiles changed with the incorporation of such effects (mainly the individual CB curves became broader and the highest concentration of the intermediate CBs became lower, especially in the case where the dechlorination kinetics of all CBs were affected) (data not shown). However, these changes did not represent the experimental data. On the basis of the competitive inhibition simulation results as well as the above presented excellent fit of the model predictions to the experimental data, we conclude that such effects during the sequential dechlorination of polychlorinated CBs at the relatively low contaminant concentrations used in the present study were not in effect.

To demonstrate the cummulative effect of the uncertainty in the k' and K_S estimates on the overall prediction of the sequential dechlorination of HCB, the numerical model was run with the parameter estimates at their mean ± standard error values (see Table 2). The lower predictions were obtained by setting the k' values of all CBs at their lower estimate and the K_S values at their higher estimate, and vice versa for the upper predictions. The results of the three model simulations are shown in Figure 5A. As expected, relatively small differences in the dechlorination rates of HCB, PeCB, and TeCBs resulted in very small differences in the highest level of TrCB and had a more pronounced effect on the predicted concentrations of both TrCB and DCB, especially after the peak TrCB concentration was observed.

The effect of pathway branching and product distribution observed during the batch dechlorination assays (see Table 1) on the overall sequential dechlorination of HCB is shown in Figure 5B where the model simulation based on the mean estimates of k' and K_S for all CBs and taking into consideration the observed dechlorination pathway branching is compared with a "linear" pathway which ignores the production of the observed relatively low levels of 1,2,4,5-TeCB, 1,2,4-TrCB, and 1,4-DCB. As expected, there is no difference in the predicted concentrations of HCB and PeCB in either of the two pathway cases. However, as a result of the exclusive production of 1,2,3,5-TeCB, which has a relatively higher dechlorination rate than the 1,2,4,5-TeCB, relatively lower levels of TeCB accumulated in the case of the "linear" dechlorination pathway. Neglecting pathway branching resulted in earlier and higher accumulation of TrCB, which in turn delayed the production of DCB. It is noteworthy that although the observed degree of pathway branching was considered to be less significant; as a result of sequential dechlorination, the cummulative effect especially on the TrCB and DCB profiles was significant.

Comparison of the Michaelis–Menten and Pseudo-First-Order Dechlorination Models. A comparison of the sequential dechlorination of HCB simulated by the Michaelis–Menten and pseudo-first order models was conducted. The mean parameter estimates reported in Table 2 were used for these simulations with results shown in Figure 6. The primary difference between these two model simulations is the relatively slow depletion of the intermediates after attaining their maximum value, predicted by the pseudo-first-order model. As a result, an incubation period of about 100 d was required for the conversion of HCB to DCB whereas in the case of the Michaelis–Menten model such conversion was complete in 25 d. For comparison, at an incubation time of

25 d, the TrCB conversion to DCB was complete by only 63.5% based on the pseudo-first-order model. It is noteworthy that although both models resulted in very comparable overall dechlorination rates of individual CBs (see Table 2), when applied to the case of a sequential dechlorination, the underprediction of the CB depletion rates at relatively low concentrations (see Figure 4) becomes further compounded leading to a much longer predicted time for the completion of the sequential dechlorination.

The use of Michaelis–Menten kinetics to model the dechlorination process is recommended over the pseudo-first-order model. Depending on the haloorganic contaminant concentration relative to the value of the half-velocity coefficient (K_S), the Michaelis–Menten model can take the form of a first-order to a zero-order model for relatively low to relatively high contaminant concentrations, respectively. Thus, the dechlorination rate adjusts in response to the contaminant concentration, a feature that is significant for field applications. Also, the Michaelis–Menten model incorporates the effect of biomass concentration on the dechlorination rate and can serve as the basis to mathematically describe specific cases such as the thermodynamic influence of product formation on the rate of fermentation (*26*) and inhibitory effects (*28*, *29*) to mention but a few. In addition to mere modeling considerations, as more dechlorinating species are isolated and progress in the enzymology of reductive dechlorination is made, it will become even more appropriate to use the Michaelis–Menten model to describe microbial reductive dechlorination kinetics.

Acknowledgments

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