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Polychlorinated Biphenyl Reductive Dechlorination by Vitamin B_{12s}: Thermodynamics and Regiospecificity

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Microbial reductive dechlorination reactions play an important role in determining the environmental fate of polychlorinated biphenyls (PCBs), especially for PCB congeners with more than four chlorines. Powerful chemical catalysts such as vitamin B_{12s} provide an effective tool for the study of reductive dechlorination reactions. The reductive dechlorination of PCBs by titanium(III) citrate-reduced vitamin B_{12s} was studied in batch reactors. Long-term experiments demonstrated reductive dechlorination of aqueous and sediment-sorbed 2,3,4,5,6-pentachlorobiphenyl (2,3,4,5,6-PeCB) to tetra-, tri-, di-, and monochlorobiphenyl products. Approximately 10% chlorine removal was observed in 36 days in aqueous experiments at 20 °C; the sediment experiment showed 40% chlorine removal in 42 days at 30 °C. Nearly all possible intermediates were produced and reductively dechlorinated, with no apparent accumulation of individual congeners. Short-term experiments were conducted to determine the pathway for vitamin B_{12s}-catalyzed reductive dechlorination of aqueous 2,3,4,5,6-PeCB and its dechlorinated products; relative product distributions were measured for all possible tetra- and trichlorobiphenyl reductive dechlorination reactions. Theoretical product distributions based on free energies of formation agreed with observed product distributions for short- and long-term experiments. Reductive dechlorination was favored at positions with adjacent chlorines; on average, chlorines were removed equally from ortho, meta, and para positions.

Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants due to widespread use and inadequate past disposal practices. The hydrophobic nature of heavily chlorinated biphenyls causes them to persist in soils, sediments, and animal fatty tissues (*1*). Under aerobic conditions, many chlorobiphenyls can be completely mineralized by microorganisms. Microbes preferentially degrade the lesser chlorinated congeners, and the molecule is usually attacked at adjacent ortho and meta positions. PCBs with more than four chlorines and those with chlorines in the 2,3-positions are typically degraded very slowly by aerobes, if at all (*1*). Microbial reductive dechlorination of heavily chlorinated PCBs, especially at the ortho and meta positions, has the potential to increase the bioavailability and biodegradability of these compounds.

Until recently, laboratory and field studies generally indicated that microbial PCB reductive dechlorination occurred almost exclusively at the meta and para positions (*1*). In recent years, laboratory studies have successfully demonstrated microbial PCB reductive dechlorination at the ortho position (*2*−*7*), but field tests continue to show accumulation of ortho-substituted congeners (*1*, *8*). This preferential removal of meta and para chlorines by microorganisms is not well understood; such selectivity can be studied through congener-specific analysis of reductive dechlorination pathways.

With chlorines distributed at all positions and readily distinguishable transformation products, 2,3,4,5,6-pentachlorobiphenyl (2,3,4,5,6-PeCB) is a convenient compound for the investigation of reductive dechlorination pathway regiospecificity. Natarajan et al. demonstrated microbial reductive dechlorination of 2,3,4,5,6-PeCB to 2,3,4,6-tetrachlorobiphenyl (2,3,4,6-TeCB), 2,4,6-trichlorobiphenyl (2,4,6-TCB), 2,4-dichlorobiphenyl (2,4-DCB), 2-monochlorobiphenyl (2-MCB), and biphenyl (meta, meta, ortho, para, and ortho reductive dechlorination) (*4*). The study of chemically catalyzed reductive dechlorination may assist in understanding the regiospecificity of microbial reductive dechlorination processes.

Vitamin B_{12}, or cyanocobalamin, consists of a cobalt atom coordinated at four positions by the nitrogens of a corrin ring. Under normal redox conditions, the cobalt atom exists in the +3 oxidation state, with two additional axial ligands (cyanides in cyanocobalamin). The cyanide ligands can be replaced with water to form aquocob(III)alamin, or vitamin B_{12a}. Under sufficient reducing conditions, the cobalt can be reduced to +2 (B_{12r}) or +1 (B_{12s}) (*9*). At B_{12} concentrations above 0.01 mmol/L, this reduction is easily visible as a color change from red (B_{12a}) to amber (B_{12r}) to dark blue (B_{12s}). Vitamin B_{12s} is a strong nucleophile that catalyzes rapid reductive dehalogenation of many organic compounds, including alkenes, chlorophenols, and chlorinated dioxins (*10*−*13*). Vitamin B_{12s} is expected to be a much stronger electron donor than vitamin B_{12r} and thus should be a more effective catalyst for reductive dechlorination.

Assaf-Anid et al. demonstrated reductive dechlorination of 2,3,4,5,6-PeCB at the meta and para positions by dithiothreitol-reduced vitamin B_{12r} (Co^{2+}), but product yields were low (3.5% of the initial parent concentration) (*14*). A stronger reductant, such as titanium(III) citrate, lowers the redox potential sufficiently to reduce the cobalt to +1 (vitamin B_{12s}), resulting in faster rates of reductive dechlorination. Powerful catalysts such as vitamin B_{12s} and other corrinoids represent a convenient research tool that may help us understand reductive dechlorination processes in the environment.

This paper represents research in which titanium citrate-reduced vitamin B_{12s} was used as a catalyst for the reductive dechlorination of chlorobiphenyls at all chlorine positions. The objectives of the study were to (i) demonstrate reductive dechlorination of aqueous and sediment-sorbed 2,3,4,5,6-PeCB by vitamin B_{12s}, (ii) determine the pathway for 2,3,4,5,6-PeCB reductive dechlorination by vitamin B_{12s}, and (iii) evaluate the regiospecificity of chlorobiphenyl reductive dechlorination by vitamin B_{12s}.

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Model Development

The use of thermodynamic constants to predict microbial transformation pathways has been evaluated for a variety of compounds. Redox potentials calculated from free energies of formation correctly predicted the microbial reductive dechlorination products of chloroanilines and chlorobenzenes (*15*−*17*). While redox potentials did not predict the preferential ortho dechlorination of pentachlorophenol in microbial systems, they correctly predicted the pathway for vitamin B_{12s}-catalyzed pentachlorophenol reductive dechlorination (*18*−*21*).

The regiospecificity of PCB reductive dechlorination was evaluated based upon thermodynamic data for 2,3,4,5,6-PeCB and its potential reductive dechlorination products. Theoretical product distributions were estimated for each congener by calculating equilibrium constants from Gibbs free energies of formation ($\Delta_f G°$) values reported by Holmes et al. (*22*).

The standard free energy for the reaction was estimated from the reductive dechlorination half reaction ($\Delta G°_{red}$). For comparison, we assumed the electron donor was H_2, yielding a $\Delta G°_{ox}$ of zero. At equilibrium,

$$RT \ln K_{eq} = -\Delta G°_{red}$$

At 25 °C, this equation can be expressed as follows:

$$K_{eq} = \exp\frac{-\Delta G°_{red}\ (\text{kJ/mol})}{8.314 \times 10^{-3}\ \text{kJ/mol·K (298 K)}} = \exp\left(\frac{-\Delta G°_{red}}{2.48}\right)$$

This equation was used to calculate equilibrium constants for each parent compound and its potential products, as shown in Table 1.

The calculated equilibrium constants were combined to estimate product distributions for individual reactions. Aside from the reductively dechlorinated products, activities of all compounds were assumed to be constant for each comparison. The distribution of products for a system with three potential products was estimated based upon the following expression:

$$\frac{M_1}{M_T} = \frac{K_1}{K_1 + K_2 + K_3}$$

M_1/M_T is the fraction of the products represented by product M_1. The product distributions calculated with this thermodynamic model were compared to distributions observed in experiments with 2,3,4,5,6-PeCB and its reductive dechlorination products (Tables 1 and 2).

Materials and Methods

The transformation of chlorobiphenyls by vitamin B_{12s} was examined in batch studies employing ampules and two-chambered reactors (TCRs) using the procedures described by Smith and Woods (*23*). Ampule experiments maintained reductive dechlorination reactions for periods up to 1 year, while TCRs typically remained reduced for up to 6 days.

Chemicals. All solutions were prepared in 660 mmol/L Tris buffer (Life Technologies, Inc., Gaithersburg, MD) and adjusted to pH 8.2 with HCl or NaOH. The concentration of the vitamin B_{12} (Sigma Chemical Company, St. Louis, MO) stock solution was 0.5 mmol/L. A solution of 241 mmol/L titanium(III) citrate was prepared from sodium citrate (Mallinckrodt Specialty Chemicals Co., Paris, KY) and titanium(III) trichloride (Fluka Chemical Corp., Ronkonkoma, NY) as described previously (*23*). 2,3,5-TCB was purchased from AccuStandard Inc. (New Haven, CT), and the remaining chlorobiphenyls were obtained from ULTRA Scientific (North Kingstown, RI). The purity of all chemicals was 99% or greater,

TABLE 1. Results of Thermodynamic Model Predictions and Their Comparison with Short-Term Experimental Results[a]

parent compd	product compd	equilibrium constant	distribution (%) calcd	distribution (%) obsd	position removed
2,3,4,5,6-PeCB	2,3,4,6-TeCB	2.24E+22	60	**94**[b]	meta
	2,3,5,6-TeCB	1.31E+22	36		para
	2,3,4,5-TeCB	1.36E+21	4	6	ortho
2,3,4,5-TeCB	3,4,5-TCB	1.75E+22	42	28	ortho
	2,4,5-TCB	1.21E+22	29	**48**	meta
	2,3,5-TCB	1.16E+22	28	24	para
	2,3,4-TCB	3.96E+20	1	0	meta
2,3,4,6-TeCB	2,4,6-TCB	1.74E+21	63	**63**	meta
	2,4,5-TCB	7.34E+20	27	24	ortho
	2,3,6-TCB	2.62E+20	9	10	para
	2,3,4-TCB	2.41E+19	1	3	ortho
2,3,5,6-TeCB	2,3,5-TCB	1.20E+21	73	**60**	ortho
	2,3,6-TCB	4.48E+20	27	40	meta
2,3,4-TCB	3,4-DCB	6.80E+22	90	**45**	ortho
	2,4-DCB	7.06E+21	9	35	meta
	2,3-DCB	5.28E+20	1	20	para
2,3,5-TCB	3,5-DCB	1.61E+22	98	**85**	ortho
	2,5-DCB	2.41E+20	1	15	meta
	2,3-DCB	1.80E+19	0	0	meta
2,3,6-TCB	2,5-DCB	6.48E+20	79	**53**	ortho
	2,6-DCB	1.25E+20	15	23	meta
	2,3-DCB	4.85E+19	6	23	ortho
2,4,5-TCB	3,4-DCB	2.23E+21	83	**68**	ortho
	2,4-DCB	2.32E+20	9	32[b]	meta
	2,5-DCB	2.32E+20	9		para
2,4,6-TCB	2,4-DCB	9.76E+19	84	**90**	ortho
	2,6-DCB	1.88E+19	16	10	para
3,4,5-TCB	3,5-DCB	1.07E+22	87	**60**	para
	3,4-DCB	1.54E+21	13	40	meta
2,3-DCB	3-MCB	1.48E+22	99		ortho
	2-MCB	1.89E+20	1		meta
2,4-DCB	4-MCB	7.34E+20	98		ortho
	2-MCB	1.41E+19	2		para
2,5-DCB	3-MCB	1.11E+21	99		ortho
	2-MCB	1.41E+19	1		meta
2,6-DCB	2-MCB	7.31E+19	100		ortho
3,4-DCB	3-MCB	1.15E+20	60		para
	4-MCB	7.62E+19	40		meta
3,5-DCB	3-MCB	1.66E+19	100		meta

[a] Maximum observed product percentages are printed in boldface. Product distributions were not measured for DCB parent compounds. [b] Combined.

TABLE 2. Comparison of Model Predictions to Long-Term Experimental Results[a]

PCB congener	predicted	aqueous	sediment
2,3,4,6- & 2,3,5,6-TeCB	**96**	**94**	**98**
2,3,4,5-TeCB	4	6	2
2,4,6-TCB	**38**	**38**	**41**
2,3,5-TCB	27	16	16
2,4,5-TCB	17	20	24
2,3,6-TCB	15	26	19
3,4,5-TCB	2	0	0
2,3,4-TCB	1	<0.1	0
2,4- & 2,5-DCB	**47**	**48**	**56**
3,5-DCB	28	21	25
3,4-DCB	15	13	10
2,6-DCB	8	18	2
2,3-DCB	1	2	7
3-CB	**52**	0	0
4-CB	40	**100**	**100**
2-CB	8	0	0

[a] Individual congeners are reported as fractions of each homolog. The highest fraction observed and predicted for each homolog is printed in boldface.

with no known chlorinated contaminants; no additional purification was performed.

In previous experiments, the presence of the three forms of vitamin B_{12} at various redox potentials was verified based on spectrophotometric comparison with published spectra. These studies confirmed that vitamin B_{12s} was the dominant form present in a solution containing excess titanium citrate (*21*). In the current work, the form of vitamin B_{12} was verified visually by observing the color change from red (B_{12a}) to amber (B_{12r}) to dark blue (B_{12s}).

Ampule Experiments. Long-term experiments were conducted in hermetically sealed glass ampules to demonstrate reductive dechlorination of PCBs by vitamin B_{12s}. Vitamin B_{12} and PCBs in water/methanol or sediment slurry were dispensed into 2 mL borosilicate glass ampules. The solution was purged with oxygen-scavenged nitrogen or argon (7−10 min at 15 mL/min). Titanium(III) citrate was added to the ampule using a syringe fitted with a 0.5 mm fused silica capillary needle, and the ampule was quickly flame sealed. In fully reduced ampules, the solution changed from bright red (vitamin B_{12a}) to amber (B_{12r}) to dark blue (B_{12s}) within a few minutes. Improperly sealed ampules changed back to amber or red and were discarded.

Ampules were periodically sacrificed for sampling. The ampule was broken at the neck, and 1 mL of hexane was added directly to the ampule. The ampule was resealed with a Teflon/silicon cap and shaken on a wrist shaker for a specified time (5 min in the aqueous experiments, 30 min in the sediment experiment). The hexane fraction was transferred to a 2 mL amber vial with a 300 μL glass insert and black viton septum for analysis. These experiments were conducted with PCB concentrations well above solubility limits; the addition of hexane directly to the ampule reduced error due to sorption/desorption phenomena. For the sediment ampule experiment, the hexane contained 10 μg/L 2,3′,4,4′-TeCB as an internal standard to monitor the repeatability of the extraction procedure. The reaction conditions varied for each ampule experiment and are detailed below.

Aqueous 2,3,4,5,6-PeCB Ampule Experiment. For the aqueous ampule experiment, a total of 40 replicate ampules was created at three separate times, and the results were combined to record the progress curve for reductive dechlorination of aqueous 2,3,4,5,6-PeCB by vitamin B_{12s}. Each ampule was prepared with 1 mL of a solution containing 0.5 mmol/L vitamin B_{12}, 7 mmol/L tianium(III) citrate, 234 mmol/L methanol, and 0.153 mmol/L 2,3,4,5,6-PeCB. Controls lacked either vitamin B_{12} or titanium(III) citrate. Ampules were incubated in the dark at 20 °C.

Sediment-Sorbed 2,3,4,5,6-PeCB Ampule Experiment. A 1 L sediment sample was collected from the bank of the Willamette River in Corvallis, OR. The sediment was spiked with 10 mg of 2,3,4,5,6-PeCB and allowed to equilibrate for 8 months at 5 °C. The sediment was then sieved through a mesh screen (1.5 mm pore size) to a final composition of approximately 42% sand, 41% silt, and 17% clay. The chemical properties of the sediment were as follows: pH 8.2 (pore water), 6.2% organic matter (loss on ignition), 300 mg/kg iron, 200 mg/kg manganese, and 9.8 ± 4.0 mg of 2,3,4,5,6-PeCB/kg of sediment. Deionized water was added to the sediment to produce a slurry that could be easily mixed with a magnetic stirrer.

Over 100 replicate ampules were prepared with 1 mL of the vitamin B_{12} stock solution. The PeCB-spiked sediment was homogenized with a magnetic stirrer, and 25 μL of the slurry was added to each ampule with a repeating pipet. After purging with nitrogen, 500 μL of titanium(III) citrate was added, and the ampule was sealed. Each ampule contained 0.33 mmol/L vitamin B_{12}, 80 mmol/L titanium(III) citrate, and 0.04 μg of 2,3,4,5,6-PeCB. Controls lacking vitamin B_{12} and/or titanium citrate were also prepared. The ampules were incubated in the dark on a swirling shaker at 30 °C, and samples were taken in duplicate and averaged.

Aqueous 2,3,5-TCB Ampule Experiment. An ampule experiment was conducted with aqueous 2,3,5-TCB to observe the product distribution for an individual step of the 2,3,4,5,6-PeCB reductive dechlorination pathway. Ampules were prepared with 0.5 mmol/L vitamin B_{12}, 7 mmol/L titanium citrate, and excess 2,3,5-TCB (200 μg/mL nominal). Ampules were incubated in the dark at 20 °C.

TCR Experiments. Two-chambered reactors (TCRs) allowed measurement of reductive dechlorination product distributions over short time periods. TCRs were created as described by Smith and Woods (*23*). The upper chamber of the reactor was continuously purged, and reductive dechlorination reactions continued for periods up to 8 days.

TCR experiments were conducted for 2,3,4,5-TeCB, 2,3,4,6-TeCB, 2,3,5,6-TeCB, 2,3,4-TCB, 2,3,6-TCB, 2,4,5-TCB, 2,4,6-TCB, and 3,4,5-TCB. For each PCB, 3 mL of a solution of 75 μg/mL PCB in 0.5 mmol/L B_{12} was introduced to a TCR. The solution was purged with purified argon or nitrogen at 15 mL/min for 10 min, and 30 μL of titanium(III) citrate was dispensed into the reactor. TCRs were held at 20 °C using a circulating water bath.

TCRs were sampled periodically to measure product distributions for each reaction. Approximately 120 μL of solution was drawn through the vent capillary into a small culture tube. From this sample, 100 μL was delivered into a 2.7 mL glass vial with a Teflon screw cap. One milliliter of hexane was added, and the vial was wrist shaken for 5 min. The hexane fraction was concentrated into a 150 μL conical autosampler vial to allow the detection of minor transformation products and a comparison of their relative concentrations.

Analytical Procedures. PCBs were analyzed by 1.0 μL splitless injection with a Hewlett-Packard 7673 autosampler coupled to an HP 5890 or 6890 gas chromatograph with electron capture detector. Individual congeners were identified by retention time comparison with pure standards. The column was a 30 m × 0.32 mm i.d. fused silica capillary column with a 0.25 μm thickness DB-5 liquid phase (J & W Scientific, Inc., Folsom, CA). Injector and detector temperatures were 300 °C. The oven temperature was programmed as follows: 45 °C for 2 min, 25 °C/min to 120 °C, 3.0 °C/min to 200 °C, 10 °C/min to 245°, hold for 10 min. The carrier gas was helium (13 psig head pressure), and the makeup gas was 95% argon/5% methane. The average error in our analysis was ±13% (SD/mean), based on recovery of the internal standard in the sediment ampule experiment.

Results and Discussion

Vitamin B_{12s}-Mediated Reductive Dechlorination of Aqueous and Sediment-Sorbed 2,3,4,5,6-PeCB. The reductive dechlorination of aqueous and sediment-sorbed 2,3,4,5,6-pentachlorobiphenyl (2,3,4,5,6-PeCB) by titanium citrate-reduced vitamin B_{12s} was evaluated over several months in experiments conducted in hermetically sealed glass ampules. Because ampules were created individually, PCB concentrations were converted to molar fractions to reduce variability between samples.

Aqueous 2,3,4,5,6-PeCB Ampule Experiment. For the aqueous ampule experiment, 40 replicate test ampules were created, each containing 50 μg of 2,3,4,5,6-PeCB, 0.5 mmol/L vitamin B_{12}, and 7 mmol/L titanium(III) citrate in 660 mmol/L Tris (pH 8.2). Two sets of controls were created, lacking either the reductant (titanium citrate) or vitamin B_{12}. Ampules were incubated in the dark at 20 °C.

Ampules were sampled to record the progress curve for 2,3,4,5,6-PeCB reductive dechlorination (Figure 1); each homologue or congener was reported as a molar fraction of the total chlorobiphenyls measured in each sample. The

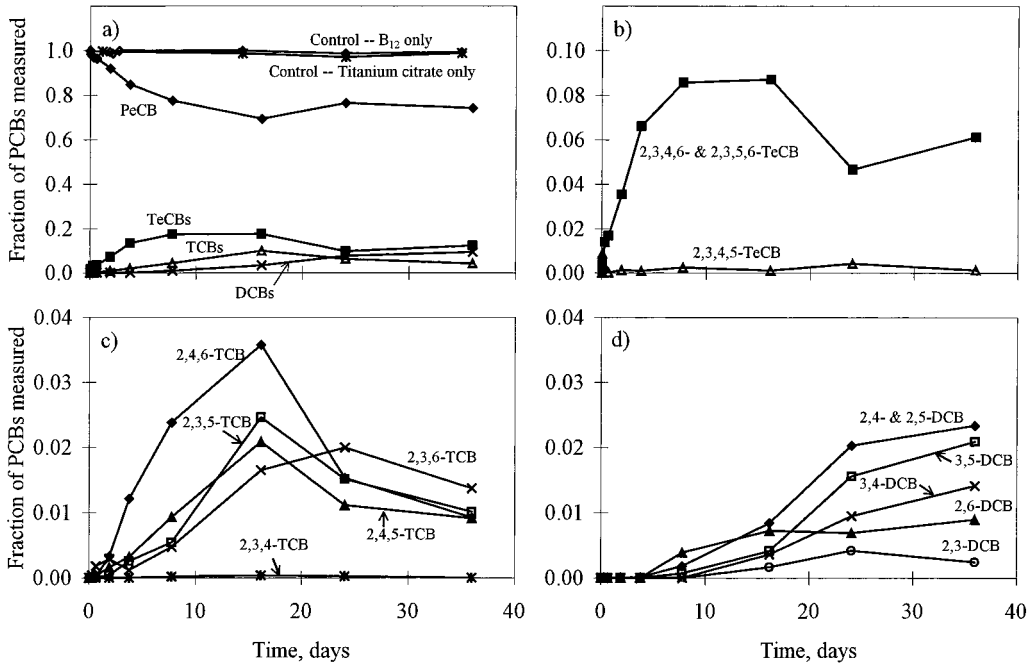

FIGURE 1. Results of aqueous ampule experiment: (a) 2,3,4,5,6-PeCB fractions (active and control samples) and homologue sums (active samples only) with time; (b–d) fractions of individual TeCBs, TCBs, and DCBs with time (active samples only).

progress of reductive dechlorination of aqueous 2,3,4,5,6-PeCB to tetra-, tri-, and dichlorobiphenyls is shown in Figure 1a. In samples containing vitamin B_{12} and titanium citrate, the 2,3,4,5,6-PeCB fraction decreased to 74% (of total chlorobiphenyls measured) over 36 days. The PeCB fraction remained above 97% in controls lacking either vitamin B_{12} or titanium citrate.

The fractions of individual TeCBs, TCBs, and DCBs observed in the aqueous ampule experiment are shown in Figure 1b–d. 2,3,4,6-TeCB and 2,3,5,6-TeCB are not easily separated chromatographically, so they are shown as a combined fraction in Figure 1b. The presence of both compounds was verified by gas chromatography with very slow-temperature ramping. The fraction of 2,3,4,5-TeCB remained low throughout the experiment. As indicated in Figure 1c, four of the six possible TCBs appeared in significant amounts in the experiment. The fraction of 2,3,4-TCB was less than 0.1%, and 3,4,5-TCB was not observed in the experiment. All six DCBs were observed in the experiment (Figure 1d), indicating that dechlorination was occurring at ortho, meta, and para positions. 2,4-DCB and 2,5-DCB were not separated by our analytical procedure, so they are presented as a combined fraction. After 12 months of incubation (data not shown), minor production of 4-monochlorobiphenyl (4-MCB) was observed; there was no evidence of production of 2- or 3-MCB.

The relative distribution of chlorines in the ortho, meta, and para positions in each sample were calculated based upon measured molar concentrations of each congener. The chlorines of 2,3,4,5,6-PeCB are 40% at the ortho, 40% at the meta, and 20% at the para positions. During the first 20 days of the experiment, there was a slight increase in the percentage of chlorines at the ortho position and a decrease in the percentage of chlorines at the meta position. This initial accumulation of ortho chlorines occurred because production of the 2,3,4,5,6-PeCB's ortho-dechlorinated product, 2,3,4,5-TeCB, was insignificant (Figure 1b). As the reaction proceeded past the TeCBs, however, ortho dechlorination occurred readily, indicated by a steady chlorine distribution after 25 days. After 36 days, the average number of chlorines per biphenyl had decreased to 4.5 (10% removal), with dechlorination occurring in similar amounts at all chlorine positions.

Sediment-Sorbed 2,3,4,5,6-PeCB Ampule Experiment. Experiments were conducted in sealed glass ampules to evaluate reductive dechlorination of sediment-sorbed 2,3,4,5,6-PeCB by vitamin B_{12s}. Over 100 replicate ampules were created, each containing 0.33 mmol/L vitamin B_{12}, 80 mmol/L titanium citrate, and 660 mmol/L Tris (pH 8.2). To this solution was added 25 μL of 2,3,4,5,6-PeCB spiked sediment (about 0.04 μg of 2,3,4,5,6-PeCB). The ampules were incubated in the dark on a swirling shaker at 30 °C.

The results of the sediment ampule experiment are shown in Figure 2. For each homolog, the line represents the average molar fraction of total chlorobiphenyls in two duplicate samples (symbols). As shown in Figure 2, most of the 2,3,4,5,6-PeCB fraction was removed in the first 40 days, with successive production of TeCBs, TCBs, and DCBs. There was little or no reductive dechlorination observed in the controls. The distribution of individual congeners in the sediment ampule experiment was consistent with that of the aqueous experiment, indicating that the two systems followed similar pathways.

Reduced conditions were maintained for over 160 days, and reductive dechlorination reactions continued throughout this time (data not shown). The fraction of tetrachlorobiphenyls peaked at 62% around day 14, followed by the trichlorobiphenyls at 37% on day 58. The DCBs fraction rose steadily for the duration of the experiment, representing nearly 70% of the total chlorobiphenyls at 160 days. The 2,3,4,5,6-PeCB fraction decreased to less than 1% at 160 days, and the average number of chlorines per molecule dropped below 2.5 (over 50% dechlorination).

After 200 days, the remaining ampules had turned to amber, indicating the oxidation of the vitamin B_{12s} to B_{12r}. Four of these expired ampules were sampled and concentrated together, revealing a substantial amount of 4-MCB (32% of total chlorobiphenyls measured). Thus, a significant fraction of chlorobiphenyls existed as 4-MCB at concentrations slightly below our detection limits. The presence of an undetectable MCB fraction suggests that the fractions of PeCB, TeCBs, TCBs, and DCBs were actually lower than they

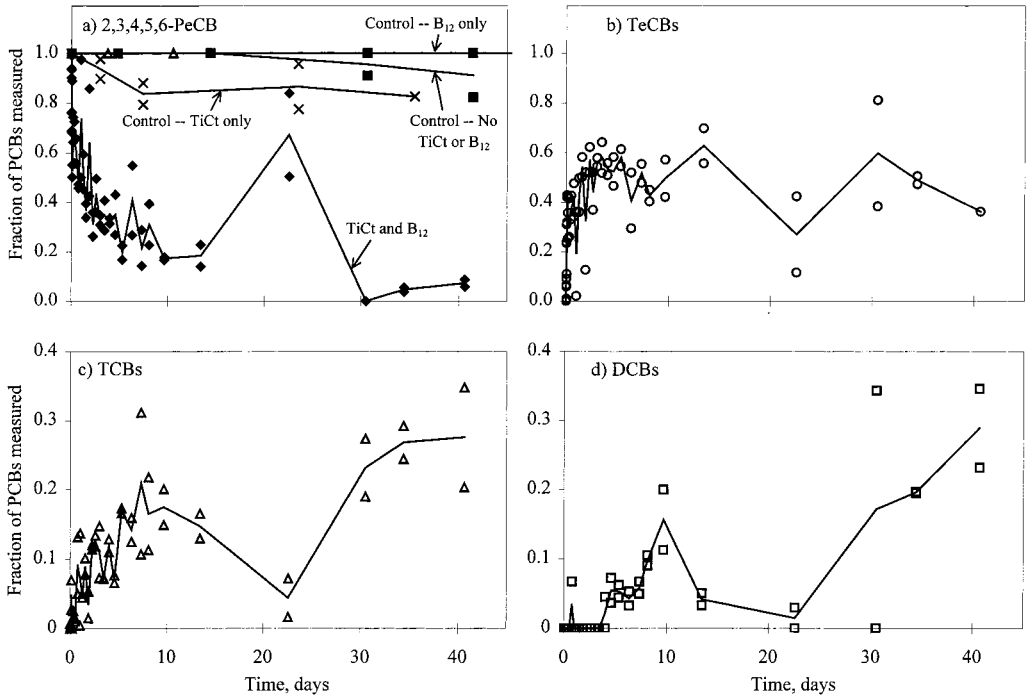

FIGURE 2. Results of sediment ampule experiment: (a) 2,3,4,5,6-PeCB fraction with time in active and control samples; (b−d) TeCB, TCB, and DCB sums with time in active samples. TiCt represents the reductant, titanium(III) citrate.

appeared; this discrepancy may explain the apparent accumulation of DCBs in the experiment.

The distribution of chlorines at the ortho, meta, and para positions with time in the sediment ampule experiment was similar to the pattern of dechlorination observed in the aqueous experiment. In the first 8 days, the percentage of chlorines at the ortho position increased to 47% with the production of 2,3,4,6- and 2,3,5,6-TeCB. The fractions of meta and para chlorines decreased to 37% and 16%, respectively. As the experiment progressed, reductive dechlorination was observed at all positions, and from 90 to 160 days the average distributions were 35 ± 2% ortho, 52 ± 3% meta, and 13 ± 2% para.

Determination of the Pathway for 2,3,4,5,6-PeCB Reductive Dechlorination by Vitamin B_{12s}. Aqueous experiments were conducted for all possible TeCB and TCB intermediates in the vitamin B_{12s}-catalyzed 2,3,4,5,6-PeCB reductive dechlorination pathway. Samples were taken at several times during each experiment, and the results were averaged to record the product distribution for each reductive dechlorination reaction. Experiments were conducted in TCRs (<6 days) and ampules (up to 80 days). The results of these individual studies were compiled to determine the pathway for the reductive dechlorination of 2,3,4,5,6-PeCB by vitamin B_{12s}, and the data were analyzed to examine the effect of chlorine position on the reactions.

Reductive Dechlorination Pathway. The compiled results of the individual congener experiments are reported in Figure 3 as the vitamin B_{12s}-catalyzed reductive dechlorination pathway for 2,3,4,5,6-PeCB. The pathway results elucidated significant dechlorination products and correspondingly the insignificant or absent products in the long-term ampule experiments with 2,3,4,5,6-PeCB. For example, 2,4,6-TCB represented a large fraction of the TCBs observed in the ampule experiments, while 3,4,5-TCB was not observed at all (Figure 1). Each of these congeners has one potential parent compound: 2,4,6-TCB can be formed only from 2,3,4,6-TeCB, and 3,4,5-TCB can be formed only from 2,3,4,5-TeCB. The preferential dechlorination of 2,3,4,5,6-PeCB at the meta and para positions and preferential dechlorination of 2,3,4,6-TeCB at the meta position (Figure 3) supported the high fractions of 2,4,6-TCB observed in the ampule experiments. Yet 3,4,5-TCB was not observed in the ampule experiments because its parent compound, 2,3,4,5-TeCB, was an insignificant product of 2,3,4,5,6-PeCB.

Observed Patterns of Dechlorination. The long-term aqueous and sediment ampule experiments demonstrated chlorine removal from all positions; however, studies with individual TeCB and TCB congeners exhibited regiospecificity. The results of these experiments were examined for correlations between product distribution and chlorine position, adjacent chlorines, or thermodynamic constants.

In Table 1, the dominant product experimentally observed in each reaction is printed in boldface. Reductive dechlorination was preferred at the ortho position in six reactions, while meta dechlorination was preferred in two reactions and para dechlorination was preferred once. Thus, reductive dechlorination at the ortho position was preferred. The presence of adjacent chlorines was also correlated with reaction preference. With the exception of 2,4,5-TCB, each congener exhibited a positive correlation between product distribution and the number of adjacent chlorines, i.e., chlorines were most often removed when adjacent chlorines were present. Thermodynamic constants were also strongly related to product distribution; for all the TeCB and TCB congeners, a lower product $\Delta_f G°$ was associated with a higher product percentage. This correlation was expected because a lower product $\Delta_f G°$ would indicate a more favorable reaction.

Modeling and Evaluation of PCB Reductive Dechlorination by Vitamin B_{12s}. The reductive dechlorination of 2,3,4,5,6-PeCB by vitamin B_{12s} was modeled for a batch process, as detailed in the model development section. Equilibrium constants based on free energies of formation were used to predict distributions of products for individual parent compounds, and these predictions were combined to predict the complete pathway for reductive dechlorination of 2,3,4,5,6-PeCB to MCBs. Vitamin B_{12s} and titanium citrate were assumed to be in excess. Predicted distributions were calculated for a system at 25 °C. Although the aqueous and

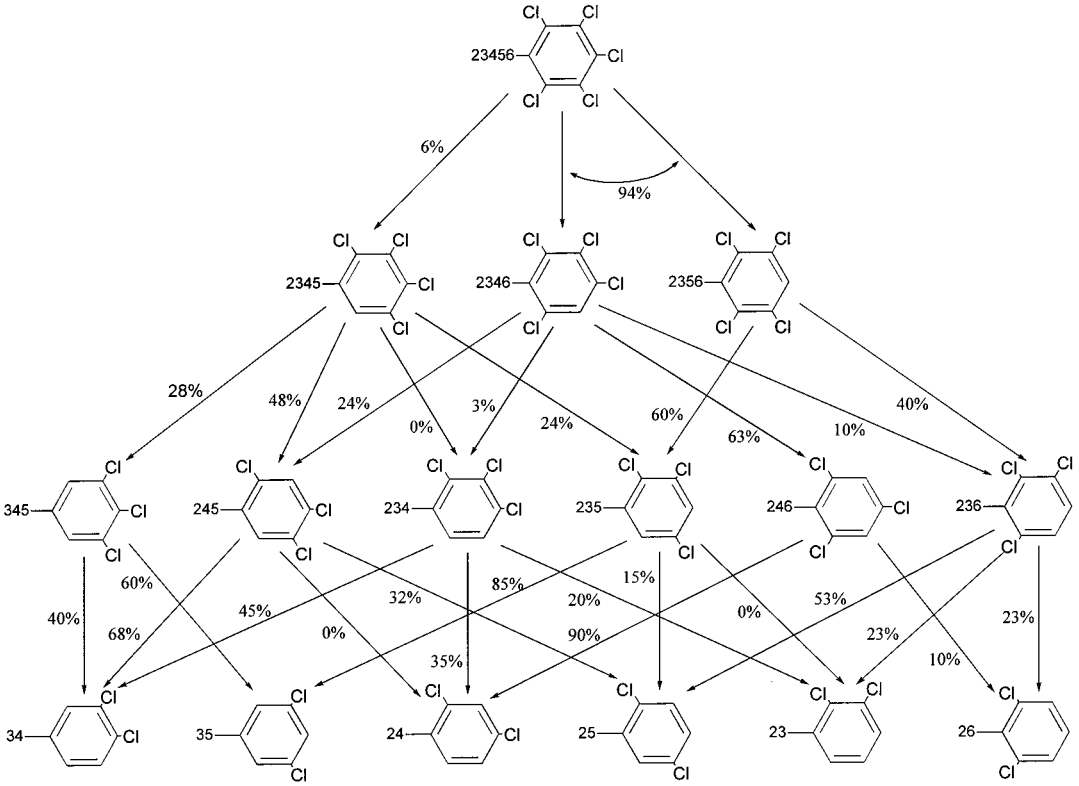

FIGURE 3. Pathway for reductive dechlorination of 2,3,4,5,6-PeCB by vitamin B_{12s}. The numbers next to the arrows represent the average product distributions for each parent compound.

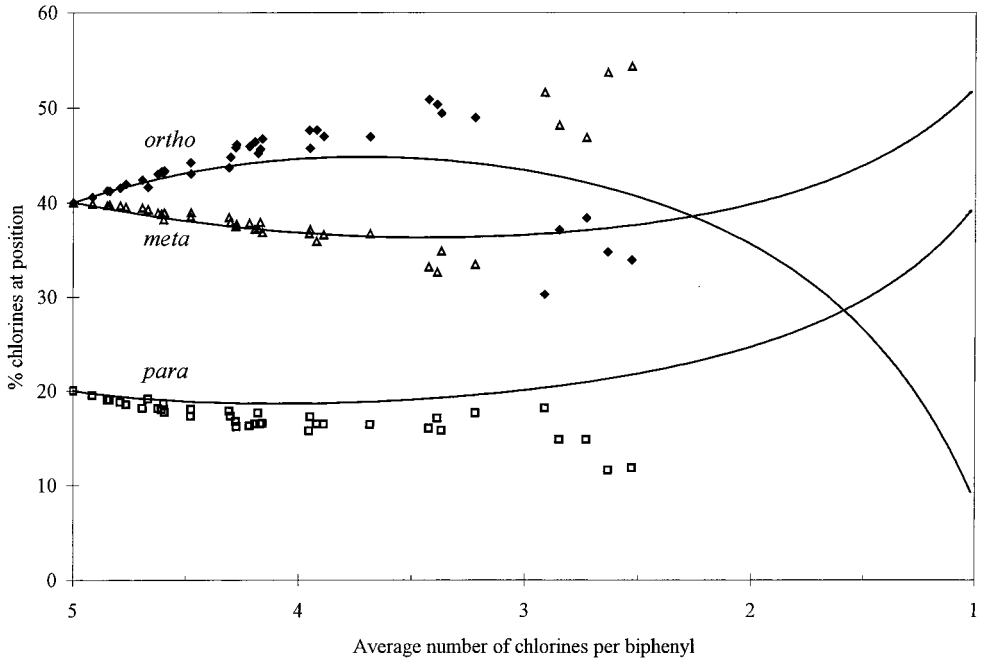

FIGURE 4. Chlorine distribution vs degree of chlorination from the pathway model (lines) and the sediment ampule experiment (symbols). The model lines extend past the observed data, indicating that ortho dechlorination is preferred as dechlorination continues.

sediment ampule experiments were conducted at 20 and 30 °C, the difference in product distributions due to this temperature variation was less than 1%. These analyses assumed equilibrium and, therefore, nearly complete conversion of the parent compound without subsequent conversion of the products. The rapid reductive dechlorination rates exhibited by vitamin B_{12s} improved the validity of the equilibrium assumption.

Evaluation of Individual Experiments. Model-predicted product distributions for individual parent compounds were compared to experimental results (Table 1). The best predictions were made for 2,3,4,6-TeCB, for which predicted fractions were within 3% of measured fractions for each of four products. Predictions of products in the highest and lowest fraction were correct in every case except that of 2,3,4,5-TeCB. In the case of 2,3,4,5-TeCB, model predictions and experimental observations agreed within 20%. Both the calculated and measured product distributions in Table 1 suggested that reductive dechlorination at the ortho position was favored in the vitamin B_{12s} system.

Evaluation of Complete Pathway. The product distributions shown in Table 1 were combined to predict the complete pathway for reductive dechlorination of 2,3,4,5,6-PeCB and its intermediates in a vitamin B_{12s} system. All compounds were assumed to decay at the same rate, and products were assumed to be formed in relative amounts according to the calculated distributions. The distribution of individual congeners within homologue groups was calculated and compared to observations in the aqueous and sediment ampule experiments (Table 2).

The TeCB, TCB, and DCB distributions observed in the aqueous and sediment ampule experiments (Table 2) were compared to the model predictions graphically by plotting observed versus predicted distributions (chart not shown). Because 2,3,4,6- and 2,3,5,6-TeCB were not separated by our analytical procedure, their fraction was plotted as an average. The best-fit linear correlation showed a slope of 0.99 (versus 1.0) with an r^2 value of 0.90, indicating a strong agreement between the model and the experimental results.

Of the MCBs, 3- and 4-MCB were expected to appear in the highest concentrations, and production of the ortho-substituted MCB was least favorable (Table 2). These predictions supported the appearance of 4-MCB and the absence of 2-MCB in the ampule experiments; the lack of 3-MCB in the ampules was unexpected.

The pathway model was also used to predict the change in chlorine distribution as the reductive dechlorination of 2,3,4,5,6-PeCB progresses. The results of these predictions are shown in Figure 4 with the results of the sediment ampule experiment. The relative distribution of chlorines at the ortho, meta, and para positions is plotted versus average number of chlorines per biphenyl. The model predictions were extended beyond the observed data to show that, as reductive dechlorination progresses in the vitamin B_{12s} system, removal is favored at the ortho position.

Comparison to Biological Experiments. The investigation of vitamin B_{12s}-catalyzed chlorobiphenyl reductive dechlorination may also have some relevance to similar biological reactions. Natarajan et al. observed a preferential pathway of 2,3,4,5,6-PeCB to 2,3,4,6-TeCB, 2,4,6-TCB, 2,4-DCB, 2-CB, and biphenyl (*4*). This pathway agrees with our predicted and observed results in Table 1, except for the para dechlorination of 2,4-DCB. The similarities and discrepancies between this microbial pathway and the vitamin B_{12s}-catalyzed pathway illustrate the importance for further study into the mechanisms by which these reactions occur. The vitamin B_{12s} system provides a powerful tool for studying both biological and abiotic chlorobiphenyl reductive dechlorination processes.

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