AN ABSTRACT OF THE THESIS OF

Darin James Trobaugh for the degree of Master of Science in Civil Engineering presented on July 1, 1998. Title: Reductive Dechlorination of Sediment-Sorbed Polychlorinated Biphenyls by Vitamin B_{12s}.

Redacted for Privacy

Abstract approved:

Sandra L. Woods

The reductive dechlorination of chlorobiphenyls in sediment by titanium(III) citrate-reduced vitamin B_{12s} was studied in batch reactors. Long term ampoule studies demonstrated reductive dechlorination of sediment-sorbed 2,3,4,5,6-pentachlorobiphenyl (2,3,4,5,6-PeCB) to tetra-, tri-, di-, and monochlorobiphenyl products. Over 50% chlorine removal was observed over 160 days. The results of the ampoule experiment were compared to previous experiments with aqueous PCBs, and both systems appeared to follow the same pathway. Theoretical product distributions based on free energies of formation were compared to product distributions for the ampoule experiments, and both aqueous and sediment-sorbed PCB reductive dechlorination followed the thermodynamically favored pathway. Although chlorines were removed from all positions, reductive dechlorination was generally preferred at the *ortho* position.

Reductive Dechlorination of Sediment-Sorbed Polychlorinated Biphenyls by Vitamin B_{12s}

by

Darin James Trobaugh

A THESIS

submitted to

Oregon State University

In partial fulfillment of

the requirements for the

degree of

Master of Science

Presented July 1, 1998

Commencement June 1999

Master of Science thesis of Darin James Trobaugh presented on July 1, 1998

APPROVED:

Redacted for Privacy

Major Professor, representing Civil Engineering

Redacted for Privacy

Chair of Department of Civil, Construction, and Environmental Engineering

Redacted for Privacy

Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Redacted for Privacy

Darin James Trobaugh, Author

Acknowledgment

Funding for this study was provided by the Office of Research and Development, U.S. Environmental Protection Agency, under agreement R-815738-01 through the Western Region Hazardous Substance Research Center. The content of this paper does not necessarily represent the views of the Agency.

In addition to monetary funding, this project was also supported by many friends whom I greatly appreciate (including but not limited to the following...): Rob Murchison, Marcus Quigley, Jason and Jessica Cole, and Jeremy and Kathy Donaldson (and Pluto & Drooly!). Their companionship made my graduate studies in Oregon a truly rewarding experience. The opinions expressed herein do represent their views.

My family was also a great source of support for this degree. Jason, you were an inspiration to go the distance. Ange, you were a shoulder to lean on when things got weird. Dad, you were a voice of reason and unending fishing advice. Mom, you were a steady pressure to get a real job afterwards.

Finally, I would especially like to thank my advisor, Dr. Sandra Woods, whose thoughtful advice, outstanding patience, and keen sense of fashion were undeniably the greatest influence in the success of my graduate studies. And where would I be without the Gyramax? Thanks, Sandy!

Table of Contents

Table of Contents, Continued

List of Figures

List of Tables

List of Appendices

Preface

This thesis reports on a continuing study of the reductive dechlorination of polychlorobiphenyls (PCBs) by vitamin B_{12s}. The paper represents a compilation of my work and the work of Kim Carter and Dr. Sandra Woods. Kim performed experiments to demonstrate the vitamin B_{12s}-catalyzed reductive dechlorination of aqueous 2,3,4,5,6-pentachlorobiphenyl (2,3,4,5,6-PeCB) to monochlorobiphenyls and to determine the pathway for these reactions. My experiments demonstrated the reductive dechlorination of sediment-sorbed 2,3,4,5,6-PeCB by vitamin B_{12s}. Dr. Woods developed a model based on thermodynamic data, and this model was compared to Kim's and my results.

Chapter 1 contains an introduction to the background and theory of PCB reductive dechlorination, followed by a review of recent literature on topics related to this research. Chapters 2 and 3 contain a brief description of my experimental procedures and results. Chapter 4 contains a comparison of Kim's and my work, and Chapter 5 is a discussion of the thermodynamics and regiospecificity of Kim's and my observations based on Dr. Woods' model. Chapter 6 presents a summary of the project and its major conclusions. Chapter 7 is a discussion of the engineering significance of this research, and Chapter 8 includes some suggestions for future work on this project. The appendices contain details of the methods used in this study and the development of Dr. Woods' model.

Reductive Dechlorination of Sediment-Sorbed Polychlorinated Biphenyls by Vitamin B_{12s}

Chapter 1. Introduction

Rationale for Vitamin B_{12s}-Catalyzed Chlorobiphenyl Reductive Dechlorination

Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants due to widespread use and inadequate past disposal practices. The hydrophobic nature of heavily chlorinated PCBs causes them to partition into 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 4 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.

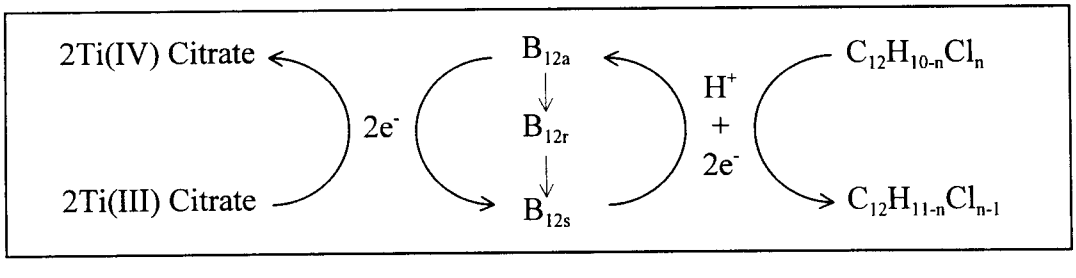
Until recently, laboratory and field studies generally indicated that microbial reductive dechlorination occurred almost exclusively at the *meta* and *para* positions (1). In recent years, laboratory studies have successfully demonstrated microbial PCB reductive dechlorination at the *ortho* position (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 the regiospecificity of reductive dechlorination. 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*, *ortho* reductive dechlorination) (4). However, Rhee et al. identified 2,3,5,6-TeCB, 2,4,6-TCB, and 2,6-DCB as reductive dechlorination products of 2,3,4,5,6-PeCB, indicating a preference for *meta* and *para* dechlorination (9). While such differences are common in microbial reaction pathways, chemically catalyzed reactions are typically much more predictable. The study of chemically catalyzed reductive dechlorination may assist us in understanding the regiospecificity of microbial reductive dechlorination processes. This research focuses on PCB reductive dechlorination by chemically reduced vitamin B_{12}.

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 exists in the +3 oxidation state; this common form is called cyanocob(III)alamin, or vitamin B_{12a}. Under sufficient reducing conditions, the cobalt can be reduced to +2 (B_{12r}) or +1 (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). As shown in Figure 1.1, the reduction of B_{12a} to B_{12s} can be accomplished by a strong reductant such as titanium(III) citrate; the B_{12s} then catalyzes the reductive dechlorination of a PCB molecule.

Figure 1.1. Diagram of B_{12} reduction by Ti(III) citrate and PCB reductive dechlorination by vitamin B_{12s}.



Vitamin B_{12s} is expected to be a much stronger electron donor than vitamin B_{12r} and consequently 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 sediment-sorbed chlorobiphenyls at all chlorine positions. The objectives of the study were as follows:

1) Demonstrate reductive dechlorination of sediment-sorbed PCBs by vitamin B_{12s}.
2) Compare the pathway observed for sediment-sorbed 2,3,4,5,6-PeCB reductive dechlorination by vitamin B_{12s} to the pathway observed in previous studies with aqueous PCBs.
3) Evaluate, based on thermodynamic theory, the regiospecificity of chlorobiphenyl reductive dechlorination by vitamin B_{12s}.

Thermodynamic Prediction of Reductive Dechlorination Pathways

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 (15,16). 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 (17-20).

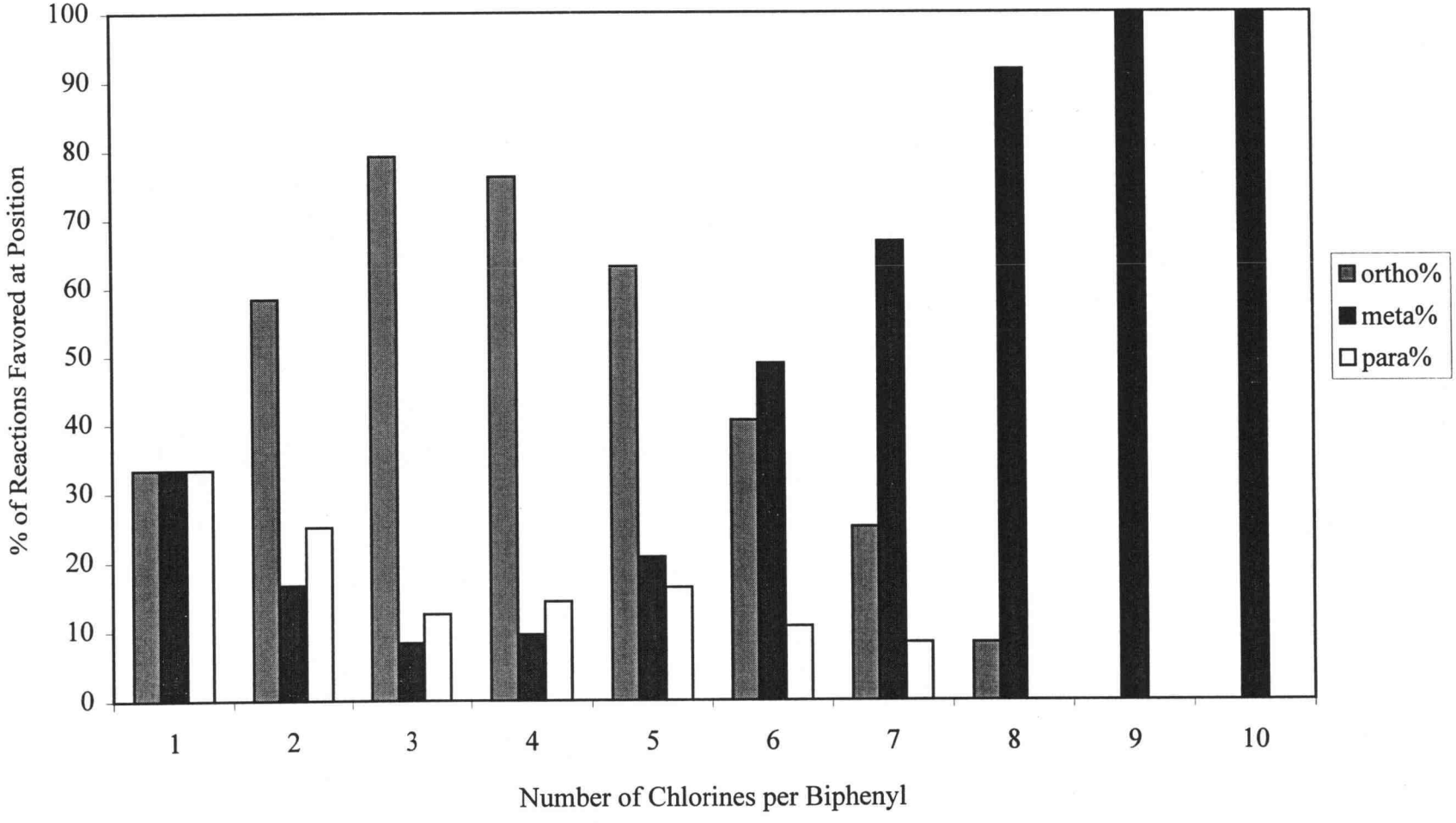
To test the validity of thermodynamic predictions for microbial PCB reductive dechlorination, experimentally observed results were evaluated based on Gibbs free energies of formation ($\Delta_f G°$'s) from Holmes et al. (21). A set of congener-specific microbial PCB reductive dechlorination reactions observed in the literature is compared to thermodynamic predictions in Table 1.1. For comparison with our research, the reactions included in the table are for PCB congeners with chlorines only on a single ring. For each reaction, the potential product with the lowest $\Delta_f G°$ was predicted to be the most thermodynamically favorable product. Reductive dechlorination at the *ortho* position was predicted to be the most thermodynamically favorable in 24 of 33 biological reactions, but it was only observed in 6 reactions. *Meta* dechlorination was observed in 24 reactions (6 predicted), and *para* dechlorination was observed in 15 reactions (3 predicted). Hence, *ortho* dechlorination was apparently less desirable to the microorganisms than either *meta* or *para* dechlorination, and the reactions were not governed solely by the $\Delta_f G°$ of the product compound.

To expand on the interpretation of Table 1.1, the favored chlorine position for reductive dechlorination (based on $\Delta_f G°$'s) was evaluated for all 209 PCB congeners. For each parent compound, the most favorable dechlorination reaction was recorded. Within each homolog group, the percent of reactions favoring reductive dechlorination at the *ortho*, *meta*, and *para* positions was then calculated (Figure 1.2). As illustrated in the figure, *ortho* dechlorination is heavily favored for PCBs with fewer than 6 chlorines, while *meta* dechlorination is favored for those congeners with more than 6 chlorines. Thus, highly chlorinated PCBs should be reductively dechlorinated at the *meta* position, while lower chlorinated congeners should be attacked more at the *ortho* position.

Table 1.1 Experimentally observed microbial PCB dechlorination products, and comparison to thermodynamic predictions.

Ref#	Parent Compound	Product(s)		Position Removed	
		Predicted	Observed	Predicted	Observed
9	2,3,4-	3,4-	2,4-	*Ortho*	*Meta*
	2,4,5-	3,4-	2,4-/2,5-	*Ortho*	*Meta*
	2,4,6-	2,4-	2,6-	*Ortho*	*Para*
	2,3,4,6-	**2,4,6-**	**2,4,6-**	***Meta***	***Meta***
	2,3,4,5,6-	**2,3,4,6-**	**2,3,4,6-**/2,3,5,6-	***Meta***	***Meta***/*Para*
2	2,4-	**4-**	2-/**4-**	***Ortho***	*Para*/***Ortho***
	2,3,6-	2,5-	2,6-	*Ortho*	*Meta*
	2,4,6-	**2,4-**	**2,4-**/2,6-	***Ortho***	***Ortho***/*Para*
	2,3,4,6-	**2,4,6-**	2,3,6-/**2,4,6-**	***Meta***	*Para*/***Meta***
23	2,3,4-	3,4-	2,3-/2,4-	*Ortho*	*Para/Meta*
	2,4,5-	3,4-	2,4-/2,5-	*Ortho*	*Meta/Para*
3	2,4-	**4-**	2-/**4-**	***Ortho***	*Para*/***Ortho***
	2,5-	3-	2-	*Ortho*	*Meta*
	3,4-	3-	4-	*Para*	*Meta*
	2,3,4-	3,4-	2,4-	*Ortho*	*Meta*
	2,3,5-	3,5-	2,5-	*Ortho*	*Meta*
	2,3,6-	2,5-	2,6-	*Ortho*	*Meta*
	2,4,5-	3,4-	2,5-	*Ortho*	*Para*
	2,4,6-	**2,4-**	**2,4-**/2,6-	***Ortho***	***Ortho***/*Para*
	3,4,5-	**3,5-**	3,4-/**3,5-**	***Para***	*Meta*/***Para***
24	2,3,6-	2,5-	2,6-	*Ortho*	*Meta*
	2,4,6-	2,4-	2,6-	*Ortho*	*Para*
4	2,4-	4-	2-	*Ortho*	*Para*
	2,4,6-	**2,4-**	**2,4-**	***Ortho***	***Ortho***
	2,3,4,6-	**2,4,6-**	**2,4,6-**	***Meta***	***Meta***
	2,3,4,5,6-	**2,3,4,6-**	**2,3,4,6-**	***Meta***	***Meta***
25	2,3,6-	2,5-	2,6-	*Ortho*	*Meta*
5	2,3,5-	3,5-	2,5-	*Meta*	*Meta*
	2,3,5,6-	**2,3,5-**	**2,3,5-**/2,3,6-	***Ortho***	***Ortho***/*Meta*
7	2,3-	3-	2-	*Ortho*	*Meta*
	3,4-	**3-**	**3-**	***Para***	***Para***
	2,4,5-	3,4-	2,4-/2,5-	*Ortho*	*Meta/Para*
	2,3,4,5-	3,4,5-	2,3,4-/2,4,5-	*Ortho*	*Meta/Meta*

Figure 1.2 Thermodynamically Favorable Reductive Dechlorination Positions for PCB Homolog Groups



Review of Microbial PCB Reductive Dechlorination Studies

Microbially mediated PCB reductive dechlorination has been studied extensively. A list of recent microbial PCB reductive dechlorination studies is shown in Table 1.2. In the majority of these studies, dechlorination at the *ortho* position was not observed, especially for congeners with fewer than 6 chlorines. This observation is contrary to the theoretical predictions in Figure 1.2. Also, *ortho* dechlorination was not observed in any of the field tests. These results are more evidence that many microbial systems may lack the ability to perform *ortho* dechlorination, even when it is apparently more energetically favorable than either *meta* or *para* dechlorination. The comparison of microbial PCB reductive dechlorination to the vitamin B_{12s} system may help explain such phenomena.

Table 1.2 Recent microbial PCB reductive dechlorination studies.

Ref. #	Year	Degree of Chlorination (Lo < 6 < Hi)	Was *ortho* observed? + yes, - no	Field Tests
5	'91	Lo	+	
9	'93	Lo	-	
26	'93	Lo	-	X
25	'93	Lo	-	
27	'93	Lo	-	
28	'93	Lo	-	
29	'93	Lo	-	
3	'94	Lo	+	
30	'95	Lo	-	
31	'95	Lo	-	
32	'96	Lo	-	
33	'96	Lo	-	
4	'96	Lo	+	
34	'96	Lo	+	
7	'96	Lo	+	
35	'97	Lo	-	
24	'97	Lo	-	
2	'97	Lo	+	
23	'94	Lo/Hi	-	X
36	'90	Hi	-	
37	'92	Hi	-	
38	'94	Hi	-	
8	'96	Hi	-	X
39	'96	Hi	-	
40	'96	Hi	-	
6	'96	Hi	+	
41	'97	Hi	-	
42	'97	Hi	-	

Chapter 2. Materials and Methods

The reductive dechlorination of sediment-sorbed chlorobiphenyls by vitamin B_{12s} was examined in batch studies. Experiments were conducted in sealed glass ampoules over a period of approximately 200 days. Additional details of the experimental procedures can be found in the appendices.

Chemicals

Vitamin B_{12} (Sigma, St. Louis, MO) was prepared in a stock solution at 0.5 mmol/L in 660 mmol/L Tris and adjusted to pH 8.2 with HCl (Appendix A). A stock solution of 241 mmol/L titanium(III) citrate in 660 mmol/L Tris at pH 8.2 was prepared from sodium citrate (Mallincrodt, Inc., St. Louis, MO) and titanium trichloride (Fluka Chemical Corp., Ronkonkoma, NY) as described in Appendix B. The 2,3,5-TCB standard was purchased from AccuStandard Inc. (North Haven, CT), and the remaining chlorobiphenyls were obtained from ULTRA Scientific (North Kingstown, RI).

Experimental Procedures

A 1 liter sediment sample was collected from the bank of the Willamette River in Corvallis, OR. The sediment was agitated to form a slurry, spiked with 10 mg 2,3,4,5,6-PeCB, and allowed to equilibrate for 8 months at 5°C. The sediment slurry was then sieved through a mesh screen (1.5 mm x 1.5 mm pore size) to achieve a saturated silty sand composition. The resulting 2,3,4,5,6-PeCB concentration in the sediment was 9.8±4.0 mg/kg dry weight.

Long term experiments were conducted in hermetically sealed glass ampoules to demonstrate reductive dechlorination of sediment-sorbed PCBs by vitamin B_{12s}. 108

replicate ampoules were prepared with 1 mL of 0.5 mmol/L vitamin B_{12} in 660 mmol/L Tris at pH 8.2. The PeCB-spiked sediment slurry was homogenized on a stir plate, and 25 μL of this slurry (approx. 0.036 μg 2,3,4,5,6-PeCB) was added to each ampoule with a repeating pipette. The necks of the ampoules were flame-stretched to a constriction of about 2 mm, and the solution was purged with oxygen-scavenged nitrogen for 10 minutes at 15 mL/min. After purging, 500 μL of 241 mmol/L Ti(III) citrate was added to the ampoule, and the reactor was quickly flame sealed at the constriction. The final reagent concentrations in the ampoules were 333 μmol/L vitamin B_{12}, 80 mmol/L titanium citrate, and 660 mmol/L Tris (pH 8.2). The ampoules were incubated in the dark on a swirling shaker at 30°C. Controls lacking vitamin B_{12} and/or titanium citrate were also prepared. Samples were taken in duplicate and averaged.

PCB Extraction and Analytical Procedures

All samples were extracted in hexane and analyzed using gas chromatography with electron capture detection (GC/ECD). The ampoule was broken at the neck, and 1 mL hexane was added directly to the ampoule. The ampoule was resealed with a Teflon/silicon cap, and the solution was shaken on a wrist shaker for a specified time (5 minutes in the aqueous experiments, 30 minutes in the sediment experiment). The hexane fraction was transferred to 2 mL amber GC autosampler vials with 300 μL inserts and black viton septa. The hexane contained 10 μg/L 2,3',4,4'-TeCB as an internal standard.

Analysis was performed using splitless injection of a 1.0 μL sample on a Hewlett Packard 7673 autosampler coupled to an HP 5890 or 6890 gas chromatograph with an electron capture detector. The column was a 0.32 mm I.D. fused silica capillary column with a DB-5 liquid phase (J & W Scientific, Inc., Folsom, CA) on a 0.25 μm film thickness. The GC parameters were set as outlined in Appendix G.

Chapter 3. Demonstration of Reductive Dechlorination of Sediment-Sorbed 2,3,4,5,6-PeCB by Vitamin B_{12s}

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

Overall Results

The overall results of the ampoule experiment are shown in Figures 3.1 and 3.2. Each homolog is shown as an average molar fraction of total chlorobiphenyls in 2 duplicate samples. As shown in Figure 3.1, most of the 2,3,4,5,6-PeCB fraction was removed in the first 40 days, and the controls showed little or no reductive dechlorination. As 2,3,4,5,6-PeCB was removed, reductive dechlorination products were observed (Figure 3.2). TeCBs and TCBs appeared almost immediately, and DCBs began to appear after 4 days. The TeCB fraction peaked at 60% around day 10, followed by the TCBs at 37% on day 58. The DCB 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 (data not shown).

After 200 days (data not shown), the remaining ampoules had turned to amber, indicating the oxidation of the vitamin B_{12s} to B_{12r}. Although no MCBs had been detected in single ampoules, four of these expired ampoules 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

Figure 3.1. PeCB fraction vs. time in the sediment ampoule experiment.

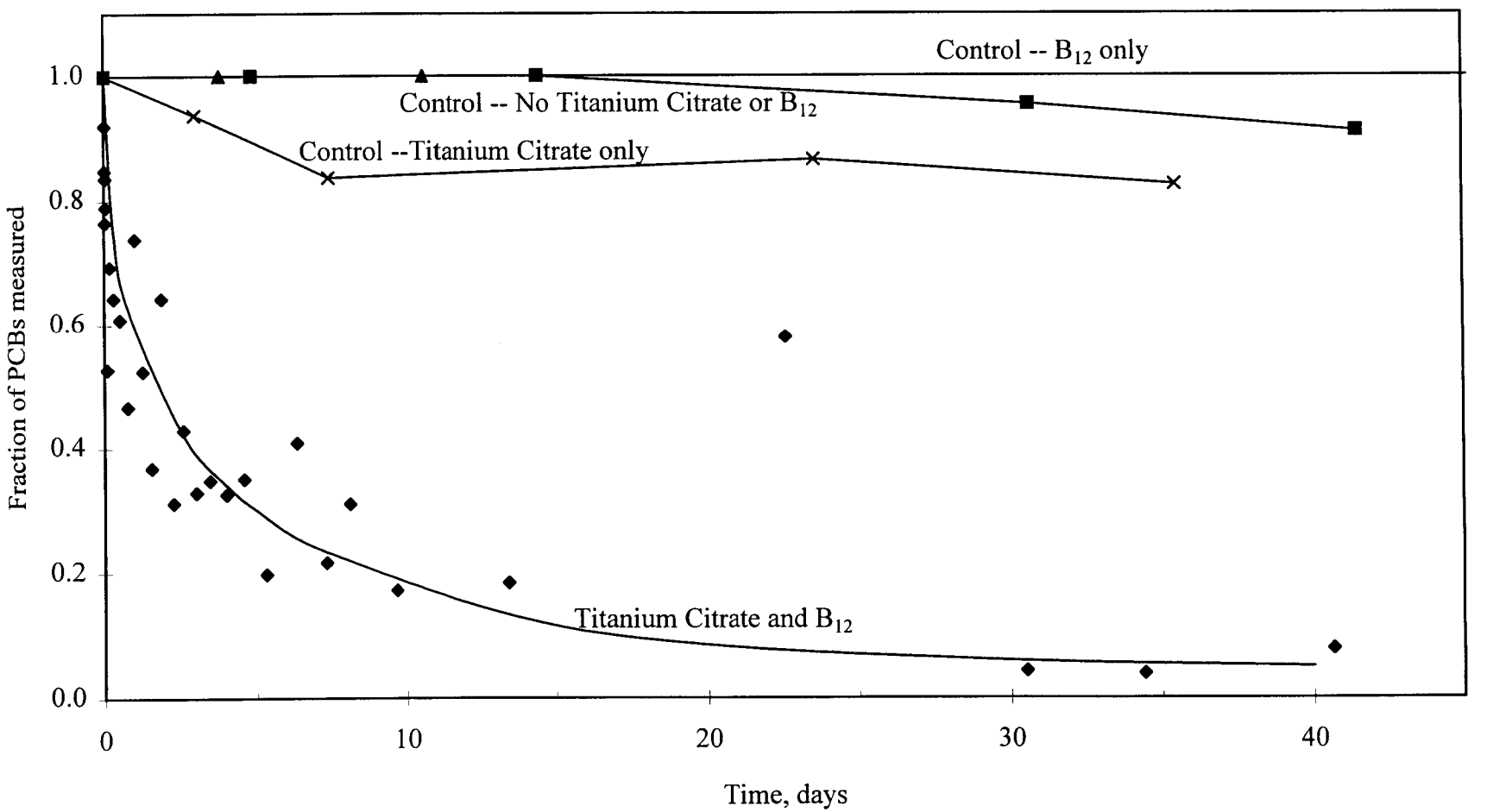
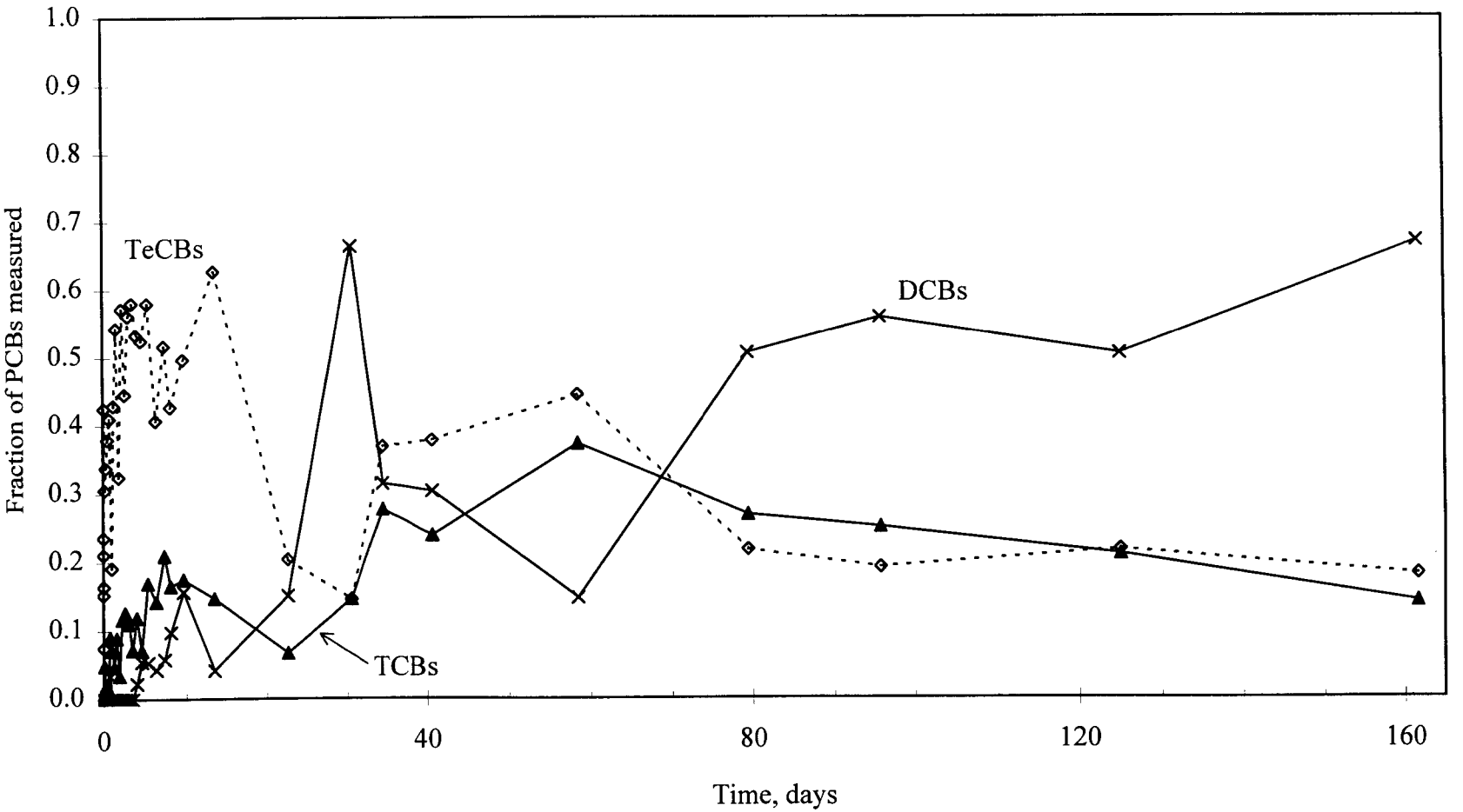


Figure 3.2. Summed TeCB, TCB, and DCB fractions vs. time in the sediment ampoule experiment.



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 appeared; this discrepancy may explain the apparent accumulation of DCBs after 100 days (Figure 3.2).

Distribution of Individual Congeners

The individual TeCB, TCB, and DCB congeners observed in the ampoule experiment are shown in Figures 3.3-3.5. Each congener is represented as an average molar fraction of the total chlorobiphenyls measured in two duplicate samples. The TeCB products observed in the experiment are shown in Figure 3.3. 2,3,4,6- and 2,3,5,6-TeCB are not separated by our analytical procedure, so they are presented as a combined fraction in the figure. GC analysis with very slow temperature ramping indicated the presence of both congeners. As Figure 3.3 shows, the 2,3,4,5-TeCB fraction was much lower than the fraction of the other TeCBs, indicating that either *ortho* dechlorination of 2,3,4,5,6-PeCB was not a favorable reaction, or 2,3,4,5-TeCB was more rapidly dechlorinated to TCB products.

The TCBs observed in the ampoule experiment are shown in Figure 3.4. Four TCB congeners (2,3,5-, 2,3,6-, 2,4,5-, and 2,4,6-TCB) were observed in similar fractions throughout the experiment, indicating they were produced and dechlorinated at approximately equal rates. The presence of these TCBs in significant amounts demonstrated that reductive dechlorination was occurring at *ortho*, *meta*, and *para* positions. The fraction of 2,3,4-TCB remained below 1%, and 3,4,5-TCB was not observed in the experiment; the low fractions of these congeners meant that they were either not produced, or they were dechlorinated more quickly than the other congeners.

As shown in Figure 3.5, all six potential DCB products were observed in the ampoule experiment. Because 2,4- and 2,5-DCB were not separated by our analysis, they are presented as a combined fraction. The fractions of 2,4-, 2,5-, and 3,5-DCB grew steadily throughout the experiment, while the other three DCB fractions remained below

Figure 3.3. Individual TeCB fractions vs. time in the sediment ampoule experiment.

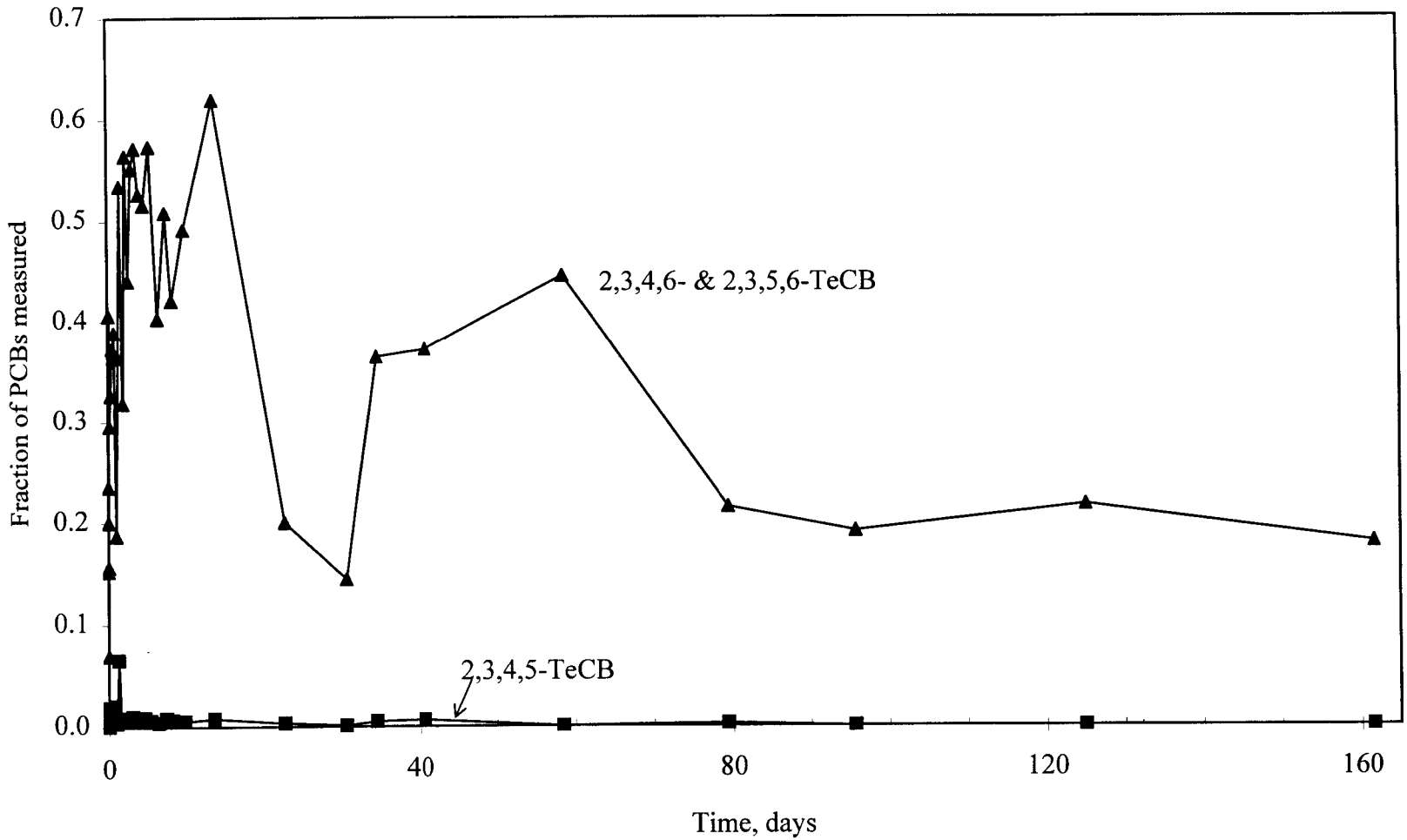


Figure 3.4. Individual TCB fractions vs. time in the sediment ampoule experiment.

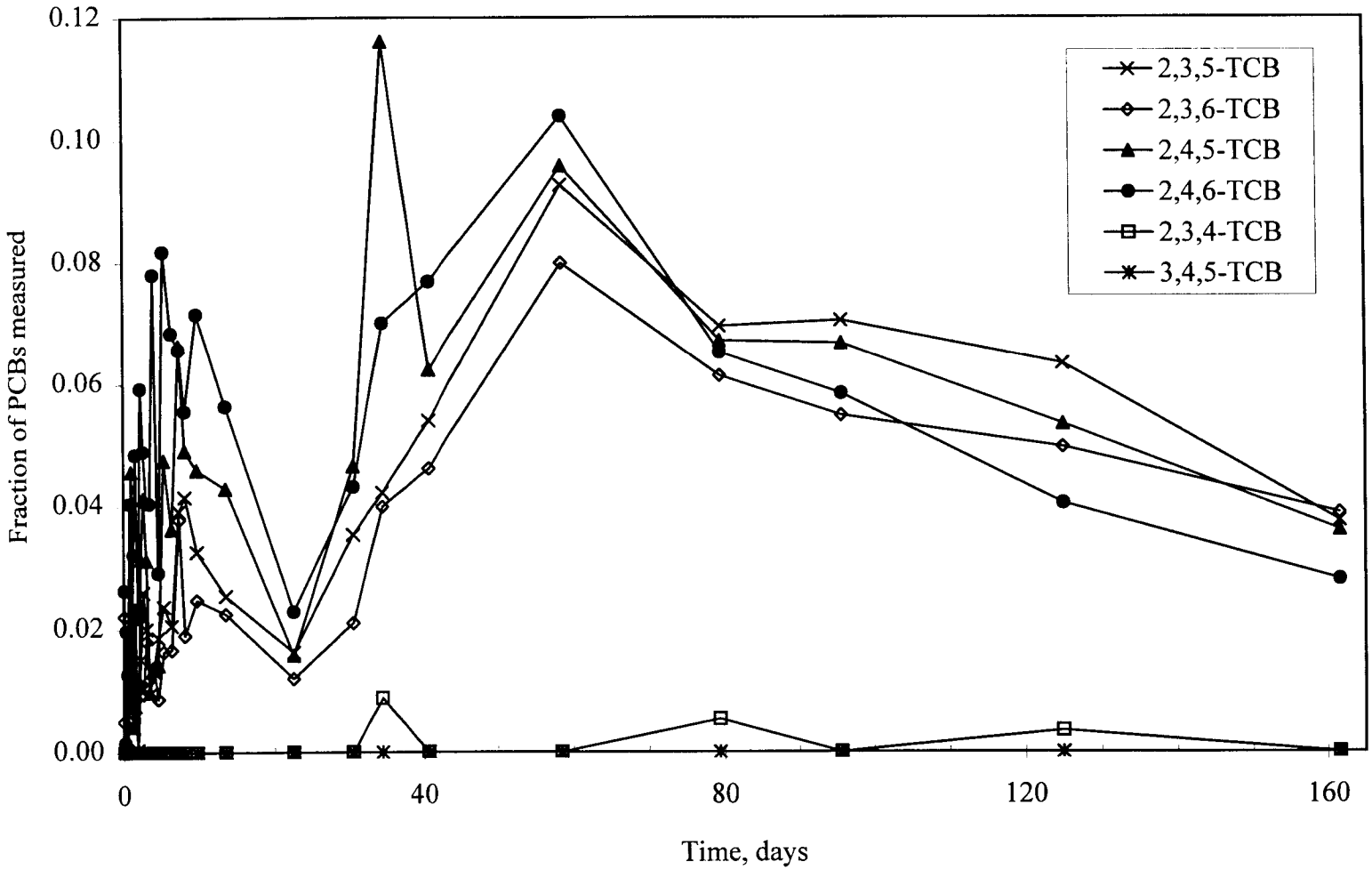
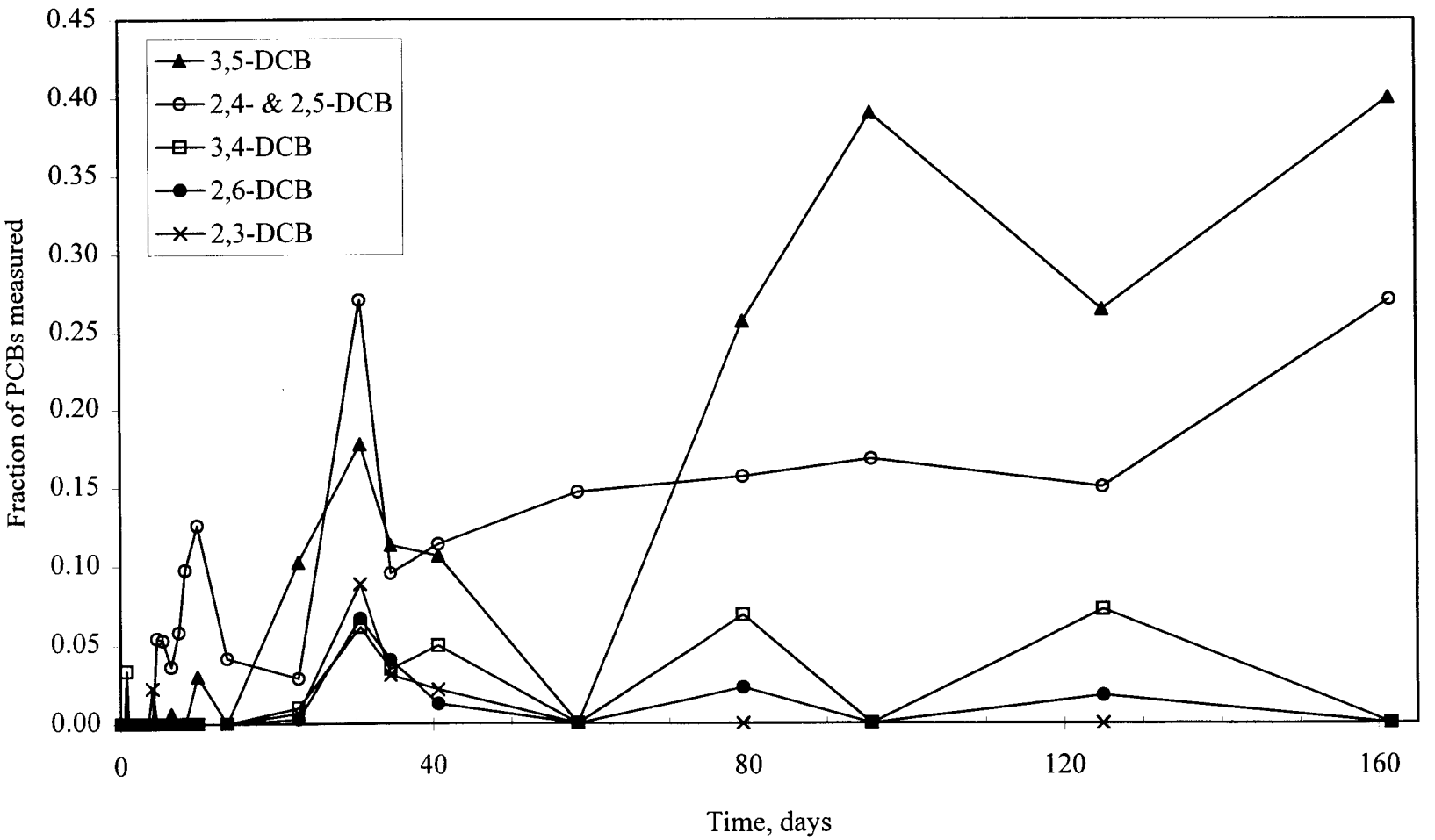


Figure 3.5. Individual DCB fractions vs. time in the sediment ampoule experiment.



10%. The fluctuations in DCB distributions could be due to their low response on the GC, since the measurement of low concentrations is inherently quite variable.

Distribution of Chlorines

The distribution of chlorines at the *ortho*, *meta*, and *para* positions with time in the ampoule experiment is shown in Figures 3.6 and 3.7. As shown in Figure 3.6, the average number of chlorines per PCB molecule decreased to less than 2.5 after 160 days (over 50% dechlorination), with chlorines removed from all positions. Because 2,3,4,5-TeCB was an insignificant product of 2,3,4,5,6-PeCB reductive dechlorination (Figure 3.3), *meta* and *para* chlorines were initially removed more rapidly than *ortho* chlorines. The percentage of chlorines at the *ortho* position increased to 47% in the first 8 days of the experiment, while the fractions of *meta* and *para* chlorines decreased to 37% and 16%, respectively (Figure 3.7). 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* (initially 40% *ortho*, 40% *meta*, 20% *para*).

Figure 3.6. Chlorines vs. time in the sediment ampoule experiment.

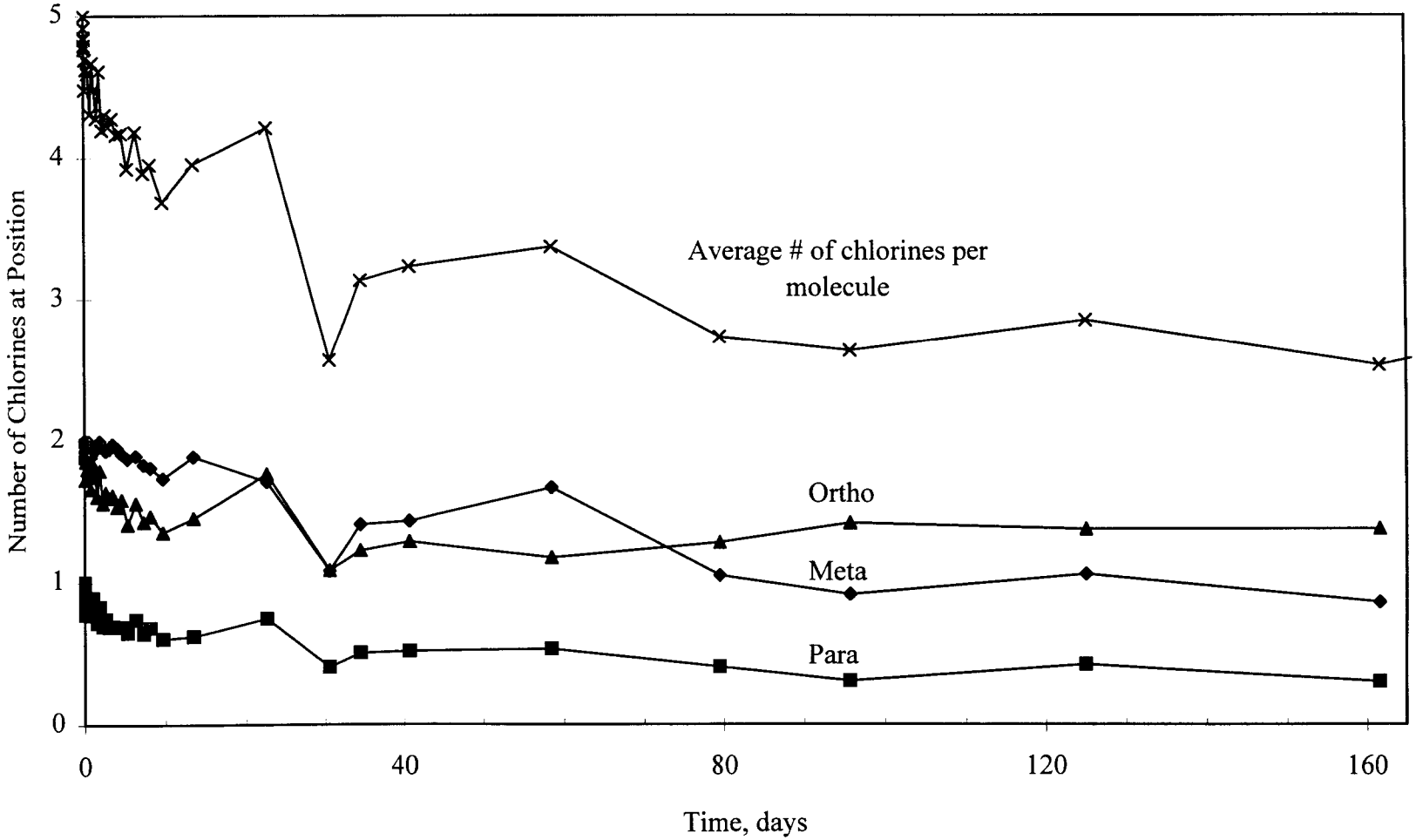
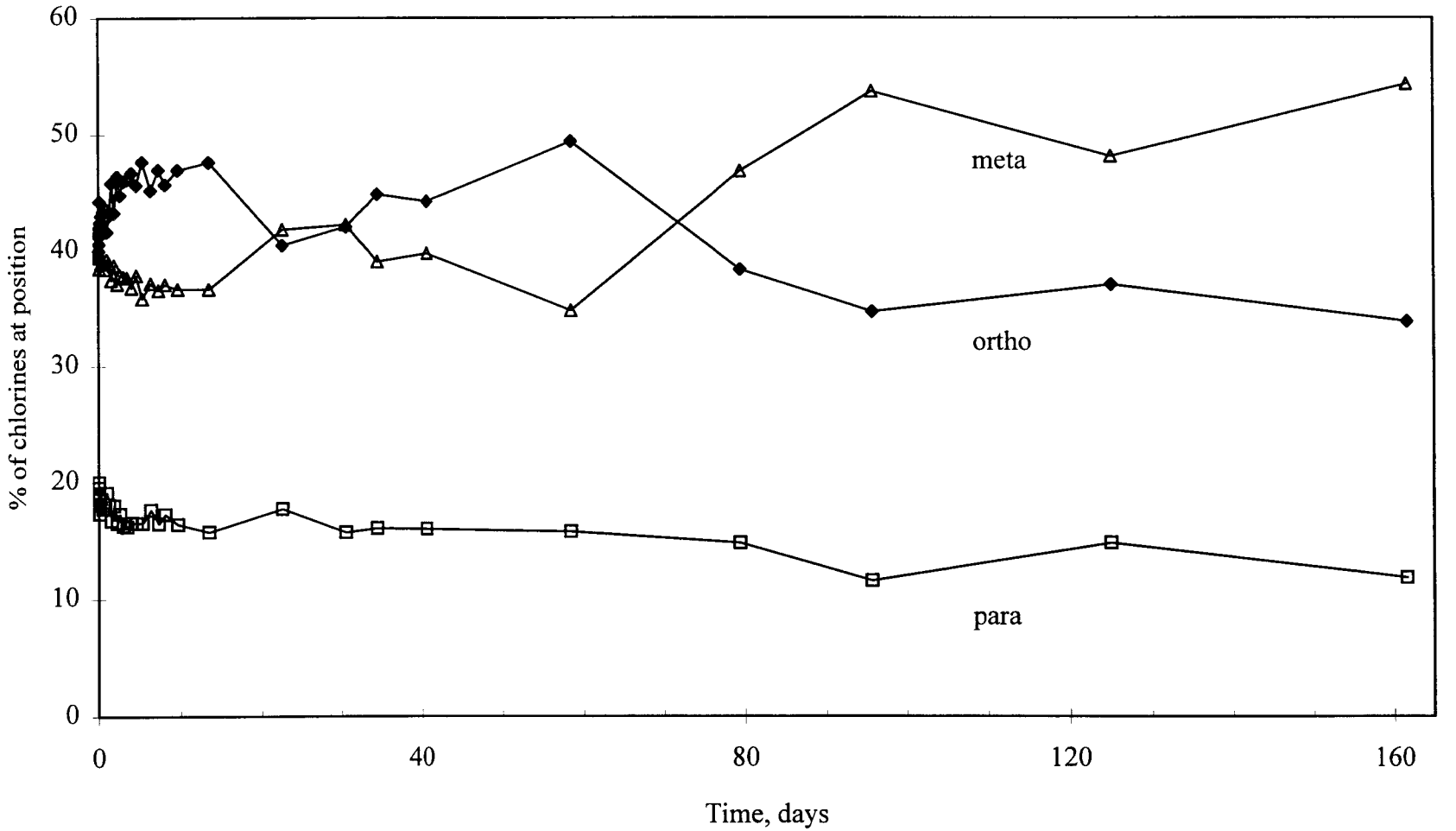


Figure 3.7. Relative chlorine distribution vs. time in the sediment ampoule experiment.



Chapter 4. Comparison of Aqueous and Sediment-Sorbed PCB Reductive Dechlorination by Vitamin B_{12s}

Since PCBs in the environment are commonly found in soils or sediments, it follows that environmentally relevant reactions should be studied in the presence of sediment. While aqueous systems are generally predictable and simple to analyze, the presence of sediment introduces a considerable amount of variability in the processes and reactions that occur in the system. With the convenience of aqueous studies and the more realistic aspects of sediment systems, the comparison of aqueous and sediment-sorbed PCB reductive dechlorination pathways can be a useful tool to study which processes or reactions are prevalent in each system.

The results of the sediment-sorbed 2,3,4,5,6-PCB experiment were compared to those of a similar ampoule study conducted previously with aqueous 2,3,4,5,6-PCB (43). For each experiment, the distribution of individual congeners within each homolog group was calculated for each sample and averaged over the course of the experiment. The average distributions are reported in Table 4.1. The most significant product observed in each reaction is printed in bold; for every homolog, this product was the same for the aqueous and sediment experiments. The pathways were very similar, with the exception of 2,6- and 2,3-DCB. The discrepancy in the distribution of these two DCBs was probably due to the propagation of small differences in the distribution of their parent compounds.

The results listed in Table 4.1 indicate that the pathway for the reductive dechlorination is independent of the presence of sediment in the system. This conclusion was expected, based on the assumption that the sediment-sorbed PCB reductive dechlorination reaction likely occurs in the aqueous phase. The availability of PCBs in the sediment system could be governed by sorption/desorption equilibria, which might explain some of the differences between the distributions observed in these systems.

Table 4.1 Distribution of congeners within homolog groups in sediment and aqueous ampoule experiments.

PCB Congener	Sediment	Aqueous
2,3,4,6- & 2,3,5,6-TeCB	**98**	**94**
2,3,4,5-TeCB	2	6
2,4,6-TCB	**41**	**38**
2,4,5-TCB	24	20
2,3,6-TCB	19	26
2,3,5-TCB	16	16
2,3,4-TCB	0	<0.1
3,4,5-TCB	0	0
2,4- & 2,5-DCB	**56**	**48**
3,5-DCB	25	21
3,4-DCB	10	13
2,6-DCB	2	18
2,3-DCB	7	2
4-CB	**100**	**100**
3-CB	0	0
2-CB	0	0

Chapter 5. Theoretical Evaluation and Modeling of Chlorobiphenyl Reductive Dechlorination by Vitamin B_{12s}

Model Development

The reductive dechlorination of 2,3,4,5,6-PeCB by vitamin B_{12s} was modeled for a batch process, as detailed in Appendix H. Equilibrium constants (Table 5.1) were calculated based on Gibbs free energies of formation ($\Delta_f G°$'s) reported in Holmes et al. (22). These constants were used to predict distributions of products for individual parent compounds, and the predictions were combined to predict the complete pathway for reductive dechlorination of 2,3,4,5,6-PeCB to MCBs. This analysis assumed equilibrium and, therefore, nearly complete conversion of the parent compound without subsequent conversion of the products.

Theoretical Evaluation of 2,3,4,5,6-PeCB Reductive Dechlorination

The product distributions listed in Table 5.1 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 vitamin B_{12s} and titanium citrate were assumed to be in excess. The concentration of each TeCB was determined for a system yielding theoretical values of 60% 2,3,4,6-TeCB, 36% 2,3,5,6-TeCB, and 4% 2,3,4,5-TeCB. Production and removal of the remaining chlorobiphenyls were calculated based upon the concentrations of their precursors and the theoretical product distributions. The predicted distribution of individual congeners within homolog groups was calculated and compared to observations in the sediment and aqueous ampoule experiments (Table 5.2). The observed average distributions of congeners within homolog groups were calculated as discussed in Chapter 4 (Table 4.1). All predicted distributions were calculated for a system at 25°C. Although the ampoule

Table 5.1. Thermodynamic model predictions for reductive dechlorination of individual parent compounds.

Parent Compound	Product Compound	Parent $\Delta_f G°$, kJ/mol	Product $\Delta_f G°$, kJ/mol	Equilibrium Constant	Predicted Distribution
2,3,4,5,6-PeCB	2,3,4,6-TeCB	197.1	203.4	7.45×10^{21}	60
	2,3,5,6-TeCB		204.7	4.41×10^{21}	36
	2,3,4,5-TeCB		210.2	4.80×10^{20}	4
2,3,4,6-TeCB	2,4,6-TCB	203.4	215.9	6.12×10^{20}	62
	2,4,5-TCB		218.0	2.62×10^{20}	27
	2,3,6-TCB		220.5	9.57×10^{19}	10
	2,3,4-TCB		226.3	9.23×10^{18}	1
2,3,5,6-TeCB	2,3,5-TCB	204.7	218.1	4.26×10^{20}	72
	2,3,6-TCB		220.5	1.62×10^{20}	28
2,3,4,5-TeCB	3,4,5-TCB	210.2	217.1	5.85×10^{21}	42
	2,4,5-TCB		218.0	4.07×10^{21}	29
	2,3,5-TCB		218.1	3.91×10^{21}	28
	2,3,4-TCB		226.3	1.43×10^{20}	1
2,4,6-TCB	2,4-DCB	215.9	235.4	3.64×10^{19}	83
	2,6-DCB		239.4	7.25×10^{18}	17
3,4,5-TCB	3,5-DCB	217.1	225.2	3.61×10^{21}	87
	3,4-DCB		229.9	5.42×10^{20}	13
2,4,5-TCB	3,4-DCB	218.0	229.9	7.79×10^{20}	82
	2,4-DCB		235.4	8.48×10^{19}	9
	2,5-DCB		235.4	8.48×10^{19}	9
2,3,5-TCB	3,5-DCB	218.1	225.2	5.40×10^{21}	98
	2,5-DCB		235.4	8.83×10^{19}	2
	2,3-DCB		241.7	6.96×10^{18}	0
2,3,6-TCB	2,5-DCB	220.5	235.4	2.32×10^{20}	78
	2,6-DCB		239.4	4.63×10^{19}	16
	2,3-DCB		241.7	1.83×10^{19}	6
2,3,4-TCB	3,4-DCB	226.3	229.9	2.21×10^{22}	89
	2,4-DCB		235.4	2.41×10^{21}	10
	2,3-DCB		241.7	1.90×10^{20}	1
3,5-DCB	3-MCB	225.2	249.0	6.42×10^{18}	100
3,4-DCB	3-MCB	229.9	249.0	4.27×10^{19}	60
	4-MCB		250.0	2.86×10^{19}	40
2,4-DCB	4-MCB	235.4	250.0	2.62×10^{20}	98
	2-MCB		259.6	5.47×10^{18}	2
2,5-DCB	3-MCB	235.4	249.0	3.93×10^{20}	99
	2-MCB		259.6	5.47×10^{18}	1
2,6-DCB	2-MCB	239.4	259.6	2.74×10^{19}	100
2,3-DCB	3-MCB	241.7	249.0	4.98×10^{21}	99
	2-MCB		259.6	6.93×10^{19}	1

experiments were conducted at 20°C (aqueous) and 30°C (sediment), the difference in predicted product distributions due to this temperature variation was less than 1%.

The thermodynamic characteristics of the TeCBs predicted their distributions in the ampoule experiments. The predicted distributions in Table 5.1 suggest that production of 2,3,4,6- and 2,3,5,6-TeCB would be much more energetically favorable than production of 2,3,4,5-TeCB. As shown in Table 5.2, 2,3,4,6- and 2,3,5,6-TeCB were indeed produced in significantly higher fractions than 2,3,4,5-TeCB, supporting the thermodynamic predictions.

Table 5.2. Distribution of congeners within homolog groups: comparison of model predictions to results of the ampoule experiments.

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

The lack of production of 2,3,4,5-TeCB affected the appearance of TCBs in the experiment. For example, 3,4,5- and 2,3,4-TCB were produced in very small or undetectable amounts. 3,4,5-TCB has the second lowest $\Delta_f G°$ of the possible TCBs (Table 5.1), but it was not detected in the system because it can only be formed from 2,3,4,5-TeCB. 2,3,4-TCB is a potential product of 2,3,4,5- and 2,3,4,6-TeCB, but its high $\Delta_f G°$ makes it a less favorable product. The four TCBs observed in significant amounts in the ampoule experiments (2,4,6-, 2,3,6-, 2,4,5-, and 2,3,5-TCB) all have relatively low $\Delta_f G°$'s (215.9 - 220.5 kJ/mol) and are possible products of 2,3,4,6- and/or 2,3,5,6-TeCB. Thus, the relative distribution of TCBs in the ampoule experiments agreed with predictions based upon thermodynamic data.

As the ampoule experiments progressed, the DCBs and MCBs were also produced in relative amounts similar to those predicted by the model. The combined fraction of 2,4- and 2,5-DCB was the highest of the DCBs observed and predicted, and 3,5-DCB was correctly predicted as the third highest DCB fraction. All other observed DCB distributions were within 10% of the predicted distributions. 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 5.2). These predictions supported the appearance of 4-MCB and the absence of 2-MCB in the ampoule experiments but the lack of 3-MCB in the ampoules was unexpected.

The TeCB, TCB, and DCB distributions observed in the aqueous and sediment ampoule experiments (Table 5.2) are compared to the model predictions graphically in Figure 5.1. 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.

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 compared to the results of the sediment ampoule experiment in Figure 5.2. The relative distribution of chlorines at the ortho, meta, and para positions is plotted versus average number of chlorines per biphenyl. The experimental data (symbols) follow

the predictions (lines) closely until the number of chlorines drops below 3. Both the predicted and observed data suggest that as reductive dechlorination progresses to DCBs and MCBs in the vitamin B_{12s} system, chlorine removal is favored at the ortho position.

Figure 5.1. Graphical comparison of theoretical predictions with aqueous and sediment ampoule experiment results (average distribution of individual congeners within homologs).

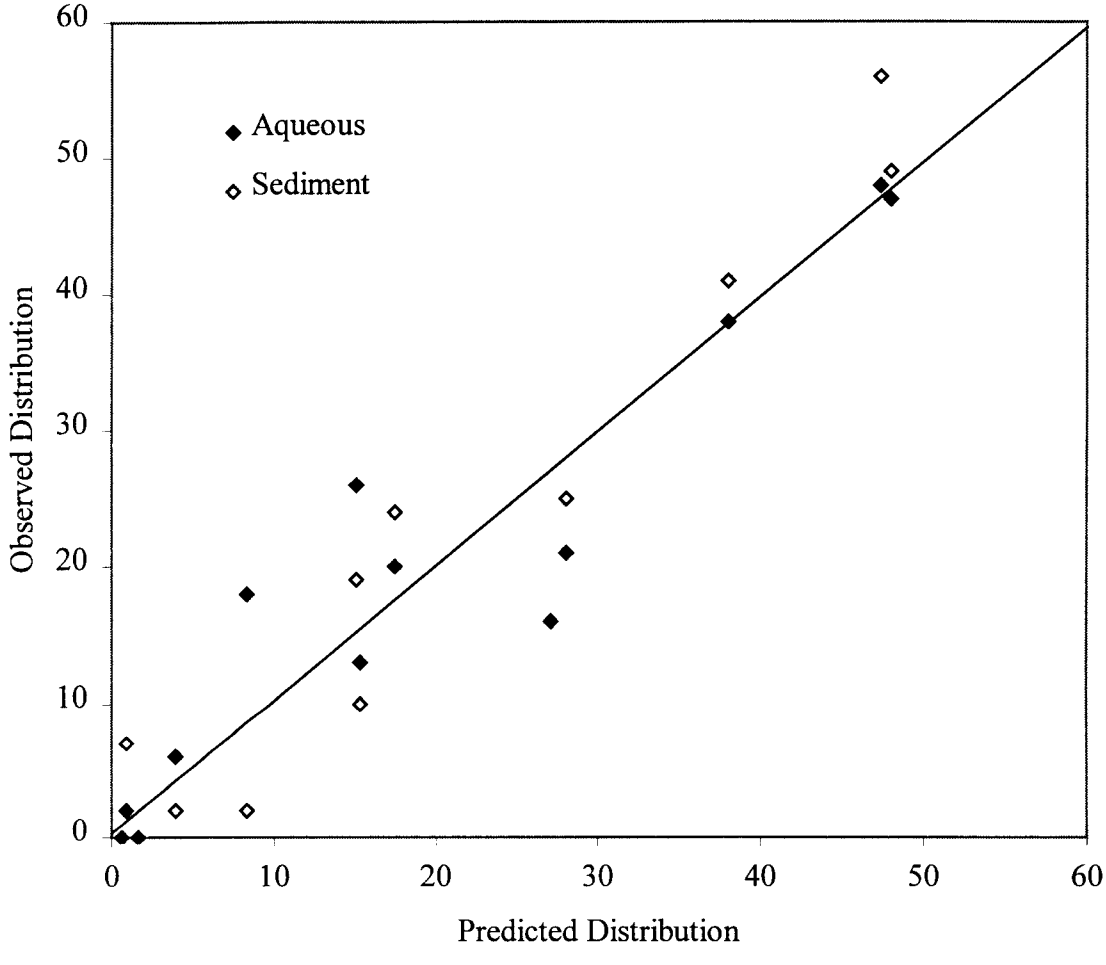
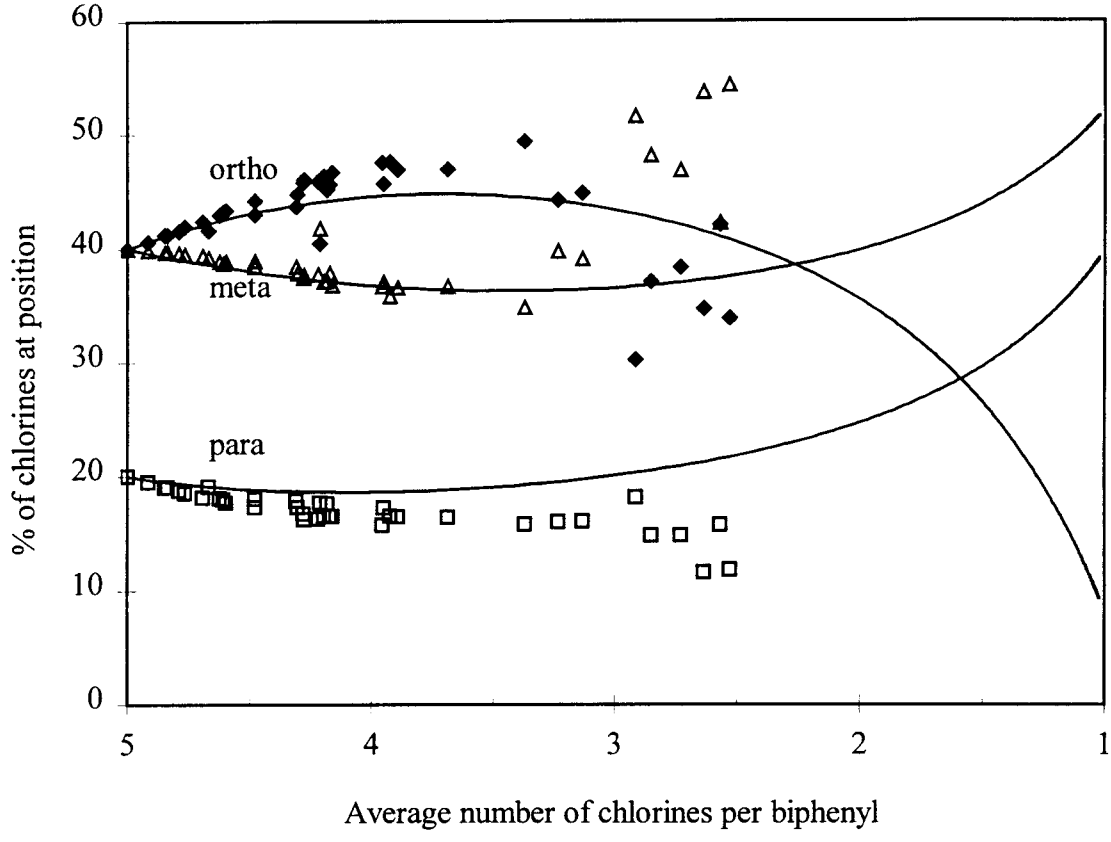


Figure 5.2. Comparison of chlorine distribution in the sediment ampoule experiment (symbols) to theoretical predictions (lines).



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 the vitamin B_{12s}-catalyzed pathway predicted in Table 5.1, except for the *para* dechlorination of 2,4-DCB. Conversely, Rhee et al. observed reductive dechlorination of 2,3,4,5,6-PeCB to produce 2,3,5,6-TeCB, 2,4,6-TCB, and 2,6-DCB, which does not agree well with our results (9). The similarities and discrepancies between these microbial pathways and the vitamin

B_{12s}-catalyzed pathway illustrate the need for further study into the mechanisms by which these reactions occur.

The vitamin B_{12s} system offers a useful tool for studying the differences between biological and abiotic reductive dechlorination process. The chemically catalyzed process followed theoretical predictions quite well (Table 5.2). However, biological reactions did not follow the theory (Table 1.1), especially where *ortho* dechlorination was predicted.

The poor ability of microorganisms to reductively dechlorinate PCBs at the *ortho* position could be due to many factors. The microbes may lack the appropriate enzymes, or they might be hindered by the physical positioning of the chlorine in this position. It is also possible that the reaction to remove an *ortho* chlorine, if it incorporates a different enzyme, is ultimately less favorable than the removal of a *meta* or *para* chlorine. The physical properties of *ortho*-substituted PCBs may decrease their availability to microorganisms directly (i.e., steric hindrance) or indirectly (i.e., partitioning into sediments and fat tissues). Further studies into the mechanisms of microbial reductive dechlorination are necessary to understand this phenomenon; exploration of the vitamin B_{12s} system should offer more information about both biological and abiotic chlorobiphenyl reductive dechlorination.

Chapter 6. Summary and Conclusions

The objectives of this study were to 1) demonstrate vitamin B_{12s}-catalyzed PCB reductive dechlorination; 2) compare the observed pathway in the sediment system to that of an aqueous system; and 3) evaluate the regiospecificity of these reactions based on thermodynamic relationships. The major conclusions of this research are as follows:

1. Titanium citrate-reduced vitamin B_{12s} is an effective catalyst for the reductive dechlorination of sediment-sorbed PCBs.
2. The vitamin B_{12s}-catalyzed reductive dechlorination pathway for sediment-sorbed PCBs is similar to that for aqueous PCBs.
3. The reductive dechlorination of PCBs by vitamin B_{12s} follows thermodynamically favorable reactions.
4. For 2,3,4,5,6-PeCB and its transformation products, reductive dechlorination at the *ortho* position was favored. This observation agreed with thermodynamic predictions; however, microbial reductive dechlorination studies in the literature do not follow this theory well.

Chapter 7. Engineering Significance

This research paper represents two significant contributions to the field of environmental engineering. First, the project improves our knowledge of chemically catalyzed PCB reductive dechlorination. With further investigation, the vitamin B_{12s} system could be used as part of a PCB remediation strategy to decrease the toxicity of the PCBs and reduce the risk of human and animal exposure. The project also refines our perception of the relationship between the vitamin B_{12s} system and microbial reductive dechlorination systems. Knowledge of both chemical and microbial processes in the environment allows us to improve our natural systems engineering practices and make them more effective, economical, and ecologically sound. Hence, the engineering significance of this project is twofold, enhancing our understanding of a specific engineering application and of similar processes in the natural world.

Chapter 8. Suggestions for Future Study

The vitamin B_{12s} system has two major aspects to be studied: the use of vitamin B_{12} as a practical application for remediation of PCBs and other halogenated compounds, and the comparison of vitamin B_{12s}-catalyzed reactions to microbially mediated reactions in the environment. Based on the findings of my research, I would suggest several studies to follow up this work and expand the overall scientific value of the project.

To study the value of vitamin B_{12} as a practical remediation strategy, several points should be considered. To minimize waste and costs, the system should use the lowest feasible concentration of B_{12}. Assuming the reaction does not require pure reagent grade catalyst, the B_{12} could be produced inexpensively on-site in a small bioreactor. The system could also be partially or fully reduced by microorganisms, to minimize the use of expensive reducing chemicals. Thus, research should be performed to examine the dependence of the system on the concentration and purity of vitamin B_{12s}, method of reduction, and optimal redox potential. With further investigation, the vitamin B_{12s} system may have potential for passive remediation, especially at sites where sediment disturbance must be kept to a minimum.

To support the growing popularity of bioremediation technologies, the vitamin B_{12s} system offers an excellent opportunity to improve our understanding of microbial reductive dechlorination processes. There are many comparisons to be examined between chemical and biological PCB reductive dechlorination systems, including concentration dependence, recalcitrance of certain congeners, reaction mechanisms, enzyme activity, and the importance of thermodynamic relationships. The course of such work should begin with congener-specific studies, and the information learned from these studies could be translated to field tests. Studying the similarities and differences between biological and chemical systems will increase our potential to implement effective natural remediation processes.

Bibliography

1. Abramowicz, D. A. "Aerobic and Anaerobic PCB Biodegradation in the Environment." 1995. *Environmental Health Perspectives* v. 103 supp. 5, 97-99.

2. Wu, Q.; Bedard, D. L.; Wiegel, J. "Effect of Incubation Temperature on the Route of Microbial Reductive Dechlorination of 2,3,4,6-Tetrachlorobiphenyl in Polychlorinated Biphenyl (PCB)-Contaminated and PCB-Free Freshwater Sediments." 1997. *Applied and Environmental Microbiology* 63, 2836-2843.

3. Williams, W. A. "Microbial Reductive Dechlorination of Trichlorobiphenyls in Anaerobic Sediment Slurries." 1994. *Environmental Science and Technology* 28, 630-635.

4. Natarajan, M. R.; Wu, W.-M.; Jain, M. K. "Dechlorination of Polychlorinated Biphenyl Congeners by an Anaerobic Microbial Consortium." 1996. *Applied Microbiology and Biotechnology* 46, 673-677.

5. Van Dort, H. M.; Bedard, D. L. "Reductive *Ortho* and *Meta* Dechlorination of a Polychlorinated Biphenyl Congener by Anaerobic Microorganisms." 1991. *Applied and Environmental Microbiology* 57, 1576-1578.

6. Hartkamp-Commandeur, L. C. M.; Gerritse, J.; Govers, H. A. J.; Parsons, J. R. "Reductive Dehalogenation of Polychlorinated Biphenyls by Anaerobic Microorganisms Enriched from Dutch Sediments." 1996. *Chemosphere* 32, 1275-1286.

7. Berkaw, M., Sowers, K. R.; May, H. D. "Anaerobic *ortho* Dechlorination of Polychlorinated Biphenyls by Estuarine Sediments from Baltimore Harbor." 1996. *Applied and Environmental Microbiology* 62, 2534-2539.

8. Bedard, D. L.; May, R. J. "Characterization of the Polychlorinated Biphenyls in the Sediments of Woods Pond: Evidence for Microbial Dechlorination of Aroclor 1260 *in Situ*." 1996. *Environmental Science and Technology* 30, 237-245.

9. Rhee, G-Y.; Sokol, R. C.; Bethoney, C. M.; Bush, B. "Dechlorination of Polychorinated Biphenyls by Hudson River Sediment Organisms: Specificity to the Chlorination Pattern of Congeners." 1993. *Environmental Science and Technology* 27, 1190-1192.

10. Glod, G.; Angst, W.; Holliger, C.; Schwarzenbach, R. P. "Corrinoid-Mediated Reduction of Tetrachloroethene, Trichloroethene, and Trichlorofluoroethene in

Homogenous Aqueous Solution: Reaction Kinetics and Reaction Mechanisms." 1997. *Environmental Science and Technology* 31, 253-260.

11. Burris, D.R., Delcomyn, C. A.; Roberts, A. L. "Reductive Dechlorination of Tetrachloroethylene and Trichloroethylene Catalyzed by Vitamin B_{12} in Homogeneous and Heterogeneous Systems." 1996. *Environmental Science and Technology* 30, 3047-3052.

12. Smith, M. H.; Woods, S. L. "Regiospecificity of Chlorophenol Reductive Dechlorination by Vitamin B_{12s}." 1994. *Applied and Environmental Microbiology* 60, 4111-4115.

13. Adriaens, P.; Chang, P. R.; Barkovskii, A. L. "Dechlorination of PCDD/F by Inorganic Electron Transfer Molecules in Reduced Environments." 1996. *Chemosphere* 32, 433-441.

14. Assaf-Anid, N.; Nies, L.; Vogel, T. M. "Reductive Dechlorination of a Polychlorinated Biphenyl Congener and Hexachlorobenzene By Vitamin B_{12}." 1992. *Applied and Environmental Microbiology* 58, 1057-1060.

15. Susarla, S.; Masunaga, S.; Yonezawa, Y. "Redox Potential as a Parameter to Predict the Reductive Dechlorination Pathway of Chloroanilines in Anaerobic Environments." 1997. *Microbial Ecology* 33, 252-256.

16. Buerskens, J. E. M., et al. "Dechlorination of Chlorinated Benzenes by an Anaerobic Microbial Consortium that Selectively Mediates the Thermodynamic Most Favorable Reactions." 1994. *Environmental Science and Technology* 28, 701-706.

17. Dolfing, J.; Harrison, B. K. "Redox and Reduction Potentials as Parameters to Predict the Degradation Pathway of Chlorinated Benzenes in Anaerobic Environments." 1993. *FEMS Microbiology Ecology* 13, 23-30.

18. Masunaga, S.; Susarla, S.; Gundersen, J. L.; Yonezawa, Y. "Pathway and Rate of Chlorophenol Transformation in Anaerobic Estuarine Sediment." 1996. *Environmental Science and Technology* 30, 1253-1260.

19. Dolfing, J. (Letter to the Editor) "Regiospecificity of Chlorophenol Reductive Dechlorination by Vitamin B_{12s}." 1995. *Applied and Environmental Microbiology* 61, 2450-2451.

20. Woods, S. L.; Smith, M. H. (Author's Reply) "Regiospecificity of Chlorophenol Reductive Dechlorination by Vitamin B_{12s}." 1995. *Applied and Environmental Microbiology* 61, 2450-2451.

21. Smith, M. H. "Reductive Dechlorination of Chlorophenols by Vitamin B_{12}." 1993. Ph.D. Dissertation, Oregon State University.

22. Holmes, D. A.; Harrison, B. K.; Dolfing, J. "Estimation of Gibbs Free Energies of Formation for Polychlorinated Biphenyls." 1993. *Environmental Science and Technology* 27, 725-731.

23. Rhee, G-Y.; Sokol, R. C.; Kwon, O-S. "Reductive dechlorination of polychlorinated biphenyls in St. Lawrence River sediments and variations in dechlorination characteristics." 1994. *Environmental Science and Technology* 28, 2054-2064.

24. Williams, W. A. "Stimulation and enrichment of two microbial polychlorinated biphenyl reductive dechlorination activities." 1997. *Chemosphere* 34, 655-669.

25. Boyle, A. W.; Blake, C. K.; Price, W. A. II. "Effects of Polychlorinated Biphenyl Congener and Sediment Supplementation on Rates of Methanogenesis and 2,3,6-Trichlorobiphenyl Dechlorination in an Anaerobic Enrichment." 1993. *Applied and Environmental Microbiology* 59, 3027-3031.

26. Abramowicz, D. A., Van Dort, H. M.; Gallagher, E. L. "Factors influencing the rate of polychlorinated biphenyl dechlorination in Hudson River sediments." 1993. *Environmental Science and Technology* 27, 1125-1131.

27. Rhee, G.-Y.; Sokol, R. C.; Bethoney, C. M.; Bush, B. "A Long-Term Study of Anaerobic Dechlorination of PCB Congeners by Sediment Microorganisms: Pathways and Mass Balance." 1993. *Environmental Toxicology and Chemistry* 12, 1829-1834.

28. Rhee, G.-Y., et al. "Reductive Dechlorination of Aroclor 1242 in Anaerobic Sediments: Pattern, Rate, and Concentration Dependence." 1993. *Environmental Toxicology and Chemistry* 12, 1025-1032.

29. Rhee, G.-Y., et al. "Anaerobic Dechlorination of Aroclor 1242 as Affected by Some Environmental Conditions." 1993. *Environmental Toxicology and Chemistry* 12, 1033-1039.

30. Pagano, J. J.; Scrudato, R. J.; Roberts, R. N.; Bemis, J. C. "Reductive Dechlorination of PCB-Contaminated Sediments in an Anaerobic Bioreactor System." 1995. *Environmental Science and Technology* 29, 2584-2589.

31. Sokol, R. C.; Bethoney, C. M.; Rhee, G-Y. "Effect of PCB concentration on reductive dechlorination and dechlorination potential in natural sediments." 1995. *Water Research* 29, 45-48.

32. Klasson, K. T.; Barton, J. W.; Evans, B. S.; Reeves, M. E. "Reductive Microbial Dechlorination of Indigenous Polychlorinated Biphenyls in Soil Using a Sediment-Free Inoculum." 1996. *Biotechnology Progress* 12, 310-315.

33. Liu, X., et al. "An Investigation of Factors Limiting the Reductive Dechlorination of Polychlorinated Biphenyls." 1996. *Environmental Toxicology and Chemistry* 15, 1738-1744.

34. McCue, J. J., et al. "Effects of Selected Reducing Agents on Microbiologically Mediated Reductive Dechlorination of Aroclor 1242." 1996. *Environmental Toxicology and Chemistry* 15, 1071-1082.

35. Kim, J.; Rhee, G-Y. "Population Dynamics of Polychlorinated Biphenyl-Dechlorinating Microorganisms in Contaminated Sediments." 1997. *Applied and Environmental Microbiology* 63, 1771-1776.

36. Quensen, J. F. III; Boyd, S. A.; Tiedje, J. M. "Dechlorination of Four Commercial Polychlorinated Biphenyl Mixtures (Aroclors) by Anaerobic Microorganisms from Sediments." 1990. *Applied and Environmental Microbiology* 56, 2360-2369.

37. Morris, P. J.; Mohn, W. M.; Quensen, J. F. III "Establishment of a Polychlorinated Biphenyl-Degrading Enrichment Culture with Predominantly *meta* Dechlorination." 1992. *Applied and Environmental Microbiology* 58, 3088-3094.

38. Ofjord, G. D.; Puhakka, J. A.; Ferguson, J. F. "Reductive dechlorination of Aroclor 1254 by marine sediment cultures." 1994. *Environmental Science and Technology* 28, 2286-2294.

39. Bedard, D. L.; Bunnell, S. C.; Smullen, L. A. "Stimulation of Microbial *para*-Dechlorination of Polychlorinated Biphenyls that have Persisted in Housatonic River Sediment for Decades." 1996. *Environmental Science and Technology* 30, 687-694.

40. Evans, B. S.; Dudley, C. A.; Klasson, K. T. "Sequential Anaerobic-Aerobic Biodegradation of PCBs in Soil Slurry Microcosms." 1996. *Applied Biochemistry and Biotechnology* 57/58, 885-894.

41. Van Dort, H. M.; Smullen, L. A.; May, R. J.; Bedard, D. L. "Priming Microbial *meta*-Dechlorination of Polychlorinated Biphenyls That Have Persisted in Housatonic River Sediments for Decades." 1997. *Environmental Science and Technology* 31, 3300-3307.

42. Natarajan, M.R., et al. "Reductive Dechlorination of PCB-Contaminated Raisin River Sediments by Anaerobic Microbial Granules." 1997. *Biotechnology and Bioengineering* 55, 182-190.

43. Carter, K. "Reductive Dechlorination of 2,3,4,5,6-Pentachlorobiphenyl by Vitamin B_{12s}: Experiments to Determine a Transformation Pathway." 1994. Master's Project, Oregon State University.

APPENDICES

Appendix A. Protocol for preparation of vitamin B_{12} stock solution

Objective

Preparation of 0.5 mmol/L vitamin B_{12} in 660 mmol/L Tris at pH 8.2.

Materials

- 200 mL volumetric flask
- glass funnel
- Disposable plastic weigh boats
- 2x125 mL amber jars with screw caps
- Solid Vitamin B_{12} (Sigma, St. Louis, MO)
- Tris pH buffer
- Concentrated hydrochloric acid (HCl)
- Deionized water
- Analytical balance, 1/10 mg precision
- pH probe and meter

Methods

1. Using the funnel, add 0.1355 g of vitamin B_{12}, 16.0 g Tris, and about 170 mL deionized water to the flask, and swirl until all solids are dissolved.
2. Slowly add HCl to adjust the pH of the solution to 8.2.
3. Dilute to 200 mL with deionized water and transfer to the amber jars.
4. Store in the dark at 4°C until ready for use.

Appendix B. Protocol for preparation of titanium(III) citrate stock solution

Objective

Preparation of 250 mmol/L titanium(III) citrate in 660 mmol/L Tris at pH 8.2.

Materials

- 250 mL beaker
- Disposable plastic weigh boats
- 4x25 mL serum bottles with teflon/butyl rubber septa and aluminum crimp seals
- Analytical balance, 1/10 g precision
- 13% Titanium(III) Chloride ($TiCl_3$) in 20% Hydrochloric Acid (HCl) (Fluka Chemical Corp., Ronkonkoma, NY)
- Sodium Citrate ($Na_3C_6H_5O_7$) (Mallincrodt, Inc., St. Louis, MO)
- Tris pH buffer
- Sodium Hydroxide (NaOH) flakes
- Deionized water
- Ice bath
- Purified Nitrogen gas for sparging
- pH probe and meter

Methods

1. Add 8.0 g Tris and 14.7 g sodium citrate to about 40 mL deionized water in the 250 mL beaker, and swirl to mix.
2. Place the beaker in the ice bath, and sparge the solution with nitrogen at 15 mL/min for 10 minutes.
3. With nitrogen still bubbling through the solution, add 30 mL of the $TiCl_3$ solution.
4. Swirling the beaker gently, adjust the pH to 8.2 with NaOH flakes. The ice bath should dissipate the heat generated by this reaction.
5. Dilute the solution to 100 mL with deionized water.
6. Dispense the solution into the serum bottles, crimp seal, and store in the freezer.

Appendix C. Protocol for creation of PCB standard curves

Objective

Preparation of standard solutions of chlorobiphenyls in hexane for calibration of the gas chromatograph.

Materials

- Volumetric flasks: 2 mL, 5 mL, 10 mL and 2x25 mL
- Glass-ground, stainless steel plunger syringes: 10 µL, 100 µL, and 500 µL
- Custom standard solutions CUS-1829 and CUS-1830 prepared by ULTRA Scientific (PCBs in hexane, sealed in 1 mL glass ampoules):

CUS-1829

Congener	Weight/mL
4-MCB	100.5 µg
2,5-DCB	10.0 µg
3,5-DCB	10.0 µg
2,3,6-TCB	4.0 µg
2,4,5-TCB	4.0 µg
2,4,6-TCB	4.0 µg
3,4,5-TCB	4.0 µg
2,3,4,6-TeCB	1.5 µg
2,3,4,5,6-PeCB	1.0 µg

CUS-1830

Congener	Weight/mL
2-MCB	100.5 µg
3-MCB	100.5 µg
2,3-DCB	10.0 µg
2,4-DCB	10.0 µg
2,6-DCB	10.0 µg
3,4-DCB	10.0 µg
2,3,4-TCB	4.0 µg
2,3,4,5-TeCB	1.5 µg
2,3,5,6-TeCB	1.5 µg

- GC grade hexane
- HP 5890 or 6890 gas chromatograph with electron capture detector (GC/ECD)

Methods

1. Make the following dilutions of each standard solution in the volumetric flasks:

Volume of Standard Solution	Flask Volume	Dilution
500 μL	2 mL	1:4
100 μL	5 mL	1:50
100 μL	25 mL	1:250
10 μL	10 mL	1:1000
5 μL	25 mL	1:5000

2. Analyze each solution on the GC according to the procedure in Appendix G.
3. Create standard curves of concentration vs. Peak Area using a 2^{nd} order polynomial fit.
4. Recreate standard curves periodically to adjust for changes in the gas chromatograph.

Appendix D. Protocol for Sediment Collection and Preparation

Objective

Collection and preparation of sediment for the ampoule experiment.

Materials

- Garden trowel or other suitable digging utensil
- 2x1-gallon wide-mouth glass jars with lids
- 1/4" x 8" glass stirring rod
- 2x6" squares of mesh window screen, 1.5 mm square pore size
- 2x500 mL beakers
- 10 mg 2,3,4,5,6-PeCB (neat)
- GC grade methanol
- pH probe and meter
- Permanent marker and labeling tape

Methods

1. Choose an accessible site along the bank of the Willamette River (near Crystal Lake Boat Ramp, Corvallis, OR).
2. Fill each 1 gallon jar half full with a slurry of water and sediment. Take the sample from sediment that is covered with at least 3 in. of standing water.
3. Swirl each jar of sediment to mix it completely, then record the pH of each slurry.
4. Add 10 mg of 2,3,4,5,6-PeCB in 4 mL methanol to one jar of sediment, swirl vigorously and stir with a large stirring rod. Reserve the other jar of sediment as a blank, and label the jars appropriately.
5. Store both jars of sediment in the dark at 4°C for 8 months to allow the 2,3,4,5,6-PeCB to sorb into the sediment.
6. Sieve each sediment slurry through a clean screen into a 500 mL beaker and label accordingly. Cover and store the prepared sediments in the dark at 4°C, and dispose of the remaining material appropriately.

Appendix E. Protocol for determining sediment density and PCB concentration

Objective

Sediment sample characterization, including density, water content, and PCB concentration.

Materials

- 10x10 mL borosilicate glass ampoules
- 20 mm teflon coated butyl septa with aluminum crimp seals
- 25 μL repeating pipette with disposable glass tips
- 3 mL repeating pipette with disposable plastic tips
- Disposable glass Pasteur pipettes and rubber pipette bulb
- 2 mL amber GC vials with black viton crimp caps
- 2,3,4,5,6-PeCB-spiked sediment (in 500 mL beaker) as prepared in Appendix D
- GC grade hexane
- Deionized water
- 1.5" magnetic stir bar and stir plate
- Analytical balance, 1/10 mg precision
- 105°C drying oven
- Wrist action shaker
- Hewlett Packard 5890 or 6890 Gas Chromatograph
- Inert chemical wipes
- Permanent marker
- 1/2" labeling tape

Methods

1. Label each ampoule with a unique number, and record the tare weight of each ampoule.
2. Add 3 mL deionized water to each ampoule, and reweigh.

3. With the stir bar in the sediment, turn the magnetic stirrer up to a speed that completely mixes the sediment.
4. Using the 25 μL pipette, add sediment to each ampoule as follows: First, draw and waste 2 samples to prepare the tip. Then, for each ampoule, draw a fresh sample into the pipette, wipe the tip clean, and dispense the sediment into the ampoule with the tip submersed in the liquid.
5. Add 3 mL hexane to each ampoule, tape a teflon/butyl cap onto each ampoule, and shake for 30 minutes.
6. Dispense the hexane fraction of each ampoule into a GC vial and cap. Analyze each sample for PCBs according to Appendix G.
7. Dry the ampoules at 105°C for at least 24 hours (until all the liquid is completely evaporated), and reweigh.
8. Calculate sediment dry weight and record the concentration of 2,3,4,5,6-PeCB in each ampoule in μg PeCB/kg sediment.

Appendix F. Protocol for sediment-sorbed PCB ampoule experiment

Objectives

- Demonstrate the reductive dechlorination of 2,3,4,5,6-PeCB by vitamin B_{12s}.
- Observe the distribution of dechlorinated products in the system and compare to previous studies and theoretical predictions.

Materials

- 500 µL syringe with 0.32 mm ID untreated fused silica capillary needle
- 150x2 mL prescored glass ampoules
- 0.5-5 mL repeating pipetter with disposable Pasteur pipette tips
- 25 µL repeating pipettor with disposable glass tips
- Teflon lined caps for 2 mL GC vials
- 1/2" labeling tape
- Pasteur pipettes with small rubber pipette bulb
- 2 mL amber GC vials with black viton caps and 300 µL cylindrical inserts
- Inert chemical wipes
- 1.5" magnetic stir bar and stir plate
- Sediment sample collected and prepared as in Appendix D
- 0.5 mmol/L vitamin B_{12} in 660 mmol/L Tris buffer at pH 8.2 (Appendix A)
- 250 mmol/L titanium(III) citrate in Tris buffer at pH 8.2 (Appendix B)
- Hexane with 10 µg/L 2,3',4,4'-tetrachlorobiphenyl as an internal standard
- Methanol for cleaning
- Bunsen burner with gas supply
- Purified Nitrogen purging apparatus with 4 0.32 mm ID untreated fused silica capillary needles and mass flow controller
- Water bath and constant temperature room at 30°C
- Wrist-action shaker with 30 minute timer
- HP 5890 or 6890 Gas Chromatograph equipped with Electron Capture Detector (GC/ECD) with autosampler

Methods

Ampoule preparation:

1. Wear lab coat, nitrile gloves, and eye protection.
2. Clearly label each ampoule with a 3-digit number, e.g. 001.
3. Using the 0.5-5 mL repeating pipettor, dispense 1 mL of the vitamin B_{12} solution into each ampoule (DI water for the no-B_{12} controls).
4. Place the stir bar in the sediment slurry and turn the stir plate up until the slurry becomes a homogeneous mixture.
5. Dispense the sediment slurry into each ampoule using the following procedure: Draw 25 μL of the sediment into the 25 μL repeating pipettor, and wipe the outer surface of the tip clean with a dry chemical wipe. Dip the tip of the pipettor into the B_{12} solution in the ampoule, and dispense the sediment completely. Wipe the pipettor clean between ampoules to avoid contamination of the sediment with vitamin B_{12}.
6. Heat the neck of each ampoule over the burner and stretch the neck to an inner diameter just wide enough to fit the purging capillary needle.
7. Store prepared ampoules at 4°C until ready to purge and seal.

Ampoule purging and sealing:

1. Remove 4 prepared ampoules from the refrigerator.
2. Purge each ampoule with purified N_2 gas for 10 minutes at 15 mL/min. For samples with less than 2 hours reaction time, place in water bath at 30°C during purging.
3. Add 500 μL titanium(III) citrate to an ampoule and quickly flame seal.
4. Record the sealing time for each ampoule.
5. Store ampoules on a swirling shaker at 30°C in the dark until ready to sample.

Ampoule sampling and analysis:

1. Select two ampoules that appear black/dark blue in color and do not have sediment above the neck.
2. Break open each ampoule at the neck.

3. Using the 0.5-5 mL repeating pipettor, add 1 mL hexane (with internal standard) to each ampoule. Use a clean tip for each ampoule to avoid contamination of the hexane.
4. Press a teflon lined GC vial cap onto each ampoule and tape it down tightly. Clearly print the ID number of each ampoule on the tape.
5. Shake ampoules on a wrist shaker for 30 minutes.
6. Record the time the hexane was added to each ampoule.
7. Using the Pasteur pipette bulb and a clean pipette for each ampoule, remove approximately 250 mL of the hexane fraction and dispense into a 2 mL amber GC vial. Seal and store at 4°C until ready to analyze on the GC.

Appendix G. Gas chromatograph program

Injector: 300°C

Detector: 300°C

Initial temperature: 45°C (hold for 2 minutes)

Ramp 1: 25°C/min to 120°C

Ramp 2: 3°C/min to 200°C

Ramp 3: 10°C/min to 245°C (hold for 10 minutes)

Column head pressure: 13 psig

Carrier gas: Helium

Makeup gas: 95% Argon, 5% Methane

Appendix H. Development of the theoretical model

Note: This appendix was written by Dr. Sandra Woods as a supplement to the project reported in this thesis.

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 evaluating the standard free energy for the following reduction half reaction using Gibbs free energies of formation ($\Delta_f G°$) values reported in Table 5.1, from Holmes et al. (22):

$$C_{12}H_{10-n}Cl_n + H^+ + 2e^- \rightarrow C_{12}H_{11-n}Cl_{n-1} + Cl^-$$

The following oxidation half reaction and overall reaction were used:

$$RED \rightarrow OX + 2e^-$$

$$C_{12}H_{10-n}Cl_n + H^+ + RED \rightarrow C_{12}H_{11-n}Cl_{n-1} + Cl^- + OX$$

In these equations, RED and OX are the reduced and oxidized forms of the electron donor, respectively. The standard free energy for the reaction ($\Delta G°$) is the sum of the standard free energies for the reduction and oxidation half reactions ($\Delta G°_{red}$ and $\Delta G°_{ox}$). At equilibrium,

$$-\Delta G° = RTlnK = -\Delta G°_{red} - \Delta G°_{ox}$$

$\Delta G°_{ox}$ is the same for each reaction (for comparison, we assumed that the electron donor is H_2, yielding a $\Delta G°_{ox}$ of zero), so it can be removed from the equation:

$$RTlnK = -\Delta G°_{red}$$

$$K = \exp\left(\frac{-\Delta G°_{red}}{RT}\right)$$

At 20°C, this equation can be expressed as follows:

$$K = \exp\frac{-\Delta G°_{red}\left(kJ/mol\right)}{8.29\times10^{-3}\,kJ/mol°K\,(293°K)} = \exp\left(\frac{-\Delta G°_{red}}{2.43}\right)$$

This equation was used to calculate equilibrium constants for each parent compound and its potential products, as shown in Table 5.1.

The calculated equilibrium constants were combined to estimate product distributions for individual reactions. For a product M_1 ($C_{12}H_{11-n}Cl_{n-1}$) and parent compound P ($C_{12}H_{10-n}Cl_n$),

$$K_1 = \frac{\{C_{12}H_{11-n}Cl_{n-1}\}\{Cl^-\}\{OX\}}{\{C_{12}H_{10-n}Cl_n\}\{H^+\}\{RED\}} = \frac{\{M_1\}\{Cl^-\}\{OX\}}{\{P\}\{H^+\}\{RED\}}$$

Activities of $\{Cl^-\}$, $\{OX\}$, $\{P\}$, $\{H^+\}$, and $\{RED\}$ are assumed to be equal for each comparison, so they can be represented by a single constant, C:

$$K_1 = \{M_1\}C$$

$$\{M_1\} = \frac{K_1}{C}$$

The distribution of products for a system with three potential products was estimated based upon the following mass balance:

$$\{M_T\} = \{M_1\} + \{M_2\} + \{M_3\} = \frac{K_1}{C} + \frac{K_2}{C} + \frac{K_3}{C}$$

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

$\{M_1\}/\{M_T\}$ 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 the aqueous and sediment ampoule experiments (Table 5.2).