The Absence and Application of Stable Carbon Isotopic Fractionation during the Reductive Dechlorination of Polychlorinated Biphenyls

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A bacterial enrichment culture (specific to doubly flanked chlorine removal) reductively dechlorinated 2,3,4,5-tetrachlorobiphenyl (2,3,4,5-CB) to 2,3,5-trichlorobiphenyl (2,3,5-CB) in aqueous media. Approximately 90% conversion to 2,3,5-CB occurred after 90 days, with no other products formed. The δ13C values of 2,3,4,5-CB and 2,3,5-CB were relatively constant over the course of the reaction, indicating a very small or no isotope effect. In addition, compound-specific δ13C analysis performed for every congener in three different lots of Aroclor 1260 showed an intrinsic isotopic trend of decreasing 13C abundance with increasing chlorine content, similar to observations in other commercial mixtures of polychlorinated biphenyls (PCBs). The results of this laboratory study suggest that microbial reductive dechlorination of PCBs in contaminated sediments will create congeners with more depleted δ13C values than native PCBs of similar chlorination. Such information may provide additional evidence for the occurrence of this process and aid in further understanding the biogeochemistry of these compounds.

Introduction

Polychlorinated biphenyls (PCBs) are a class of 209 synthetic organic compounds that were sold to industry and widely used for a significant portion of the past century. In the United States, Monsanto sold these mixtures under the trademark Aroclor. Similar mixtures were sold under different trademarks in other countries, such as Clophen (Germany) and Phenoclor (France). At least 600–1100 million kg have been produced worldwide (1).

These mixtures had a variety of industrial applications including usage as dielectric fluids, plasticizers, flame-retardants, heat transfer fluids, printing inks, paints, and pesticides. Their utility was mostly due to their chemical inerntness and favorable physical properties, which include resistance to oxidation, low vapor pressures, and high flash points. Ironically, these attributes have resulted in the persistence and accumulation of PCBs in the environment, where they have the potential to endanger humans and perturb ecosystems. Production of PCBs in most industrial countries ceased in the 1970s (1).

There is substantial evidence that under reducing conditions some PCBs in contaminated sediments are dechlori- nated (2–5). Laboratory evidence has shown that natural populations of bacteria are responsible. This process has been observed in sediments from several sites including the Hudson River (NY), New Bedford Harbor (MA), and the St. Lawrence River (Canada) (6–9). Pattern comparisons, the monitoring of selective chlorine loss and novel congener formation, and parent/product mass balance considerations have all been used to tentatively validate the in situ occurrence of dechlorination and assign its associated pathways. However, since this apparent attenuation may be due to other chemical processes or variations in PCB inputs, additional evidence for confirming microbial reductive dechlorination would be beneficial.

To address this issue, we investigated whether stable carbon isotopic fractionation during reductive dechlorination of PCBs may be used as a tracer of this process. Similar studies of both carbon and chlorine fractionation during the alteration of other environmental contaminants have been reported (10–16). In this approach, carbon isotopic fractionation may complement the aforementioned pattern comparison/mass balance treatments. Here, we monitored the extent of carbon isotopic fractionation during the microbial dechlorination of 2,3,4,5-tetrachlorobiphenyl (2,3,4,5-CB) to 2,3,5-trichlorobiphenyl (2,3,5-CB) (Figure 1) and present an application based on these results.

Methods

**Bacterial Culture and Incubation.** A sediment-free culture, specific for removing doubly flanked chlorines (17), was pregrown for 1 month to a cell density of 3.3 × 10⁷ cells/mL. Anaerobically prepared media was inoculated with this culture to achieve an initial density of 1.0 × 10⁶ cells/mL. The medium was E-CI, containing vitamins and trace elements prepared and dispensed following Hungate procedures (18). It also contained cysteine+HCl (25 g/L), but 10 mM sodium formate was used as the carbon source (in lieu of short chain fatty acids). The medium (15 mL) was dispensed into N2:CO2 (4:1) glass test tubes (16 × 150 mm) and sealed with Teflon-lined butyl stoppers prior to autoclaving. Following autoclaving and cooling, the vitamins, 2,3,4,5-CB (15 μL of a ~67 μg/μL solution in acetone), and the cell inoculum were added to each tube under the N2:CO2 gas flow and ressealed. Control tubes received no cell inoculum.

All inoculated and control tubes were incubated statically in the dark at 30°C. Two to three inoculated tubes were...
harvested at various time intervals and stored frozen in the dark at −20 °C until analysis. At harvest points, epifluorescence microscopy was used to count cells stained with acridine orange (19). Cell density in the inoculated tubes reached $1 \times 10^7$ cells/mL after 4 days and grew to a maximum of $3.5 \times 10^7$ cells/mL after 25 days. Duplicate control tubes were also frozen at representative time points over the 3-month period.

**Extraction and Analysis.** Upon thawing, each whole sample was extracted in the tube with methyl tert-butyl ether (3 × 10 mL) by sonication for 15 min. The combined extracts (upper layer) were eluted through a small column of anhydrous sodium sulfate to remove residual water and reduced in volume by rotary evaporation. The percent conversion was monitored using a Hewlett-Packard 5890 Series II gas chromatograph (He carrier gas) equipped with a flame ionization detector (GC-FID) and a J&W Scientific DB-5 capillary column (60 m × 0.32 mm; 0.25 μm film thickness). The temperature of the gas chromatograph was initially held at 40 °C for 1 min, ramped at 20 °C/min until 320 °C, and then held for 2 min. Products other than 2,3,5-CB were not observed in select samples that were analyzed by high performance liquid chromatography (reversed phase and photodiode array detection), gas chromatography mass spectrometry, and direct mass spectrometry. Recoveries of 2,3,5-CB and 2,3,4,5-CB in the inoculated samples and controls were greater than 80%. Control samples showed no measurable conversion over the duration of the experiment.

**Isotope Ratio Monitoring Gas Chromatography Mass Spectrometry (irmGC-MS).** Compound-specific isotope analysis was performed on a Hewlett-Packard 6890 gas chromatograph coupled to a Finnigan DeltaS mass spectrometer via a modified Finnigan GC Combustion III interface. Analytes were separated on a Chrompack CP5 CB column (60 m × 0.25 mm; 0.25 μm film thickness) with He as the carrier gas. The oven temperature was the same one used for GC-FID analysis (see above). Carbon isotopic compositions are reported in the delta (δ) notation

$$\delta^{13}C (\%) = ((R_{sample}/R_{standard}) - 1)1000$$

where $R_{sample}$ and $R_{standard}$ are the $^{13}C/^{12}C$ ratios of the sample and reference standard, Vienna Pee Dee Belemnite (VPDB), respectively. The $\delta^{13}C$ values for 2,3,5-CB and 2,3,4,5-CB were determined relative to a coined standard, 2,2′,4,4′-tetrachlorobiphenyl (2,2′,4,4′-CB), rather than the reference CO$_2$. This technique improves accuracy by minimizing any bias associated with either the open split or the combustion efficiency, and because the peak shape of 2,2′,4,4′-CB more closely resembles that of the analytes. All three compounds were baseline resolved, with 2,2′,4,4′-CB eluting midway between 2,3,5-CB and 2,3,4,5-CB. Each culture extract was analyzed in triplicate, with corresponding standard deviations that ranged from 0.04 to 0.44% and 0.10 to 0.50% for 2,3,5-CB and 2,3,4,5-CB, respectively. Uncertainties for each data point are shown as the 95.5% confidence limits for each triplicate set of injections ($2\sigma/\sqrt{n}$). To test for any instrument drift, we compared the $\delta^{13}C$ values of the reference gas CO$_2$ relative to the coined standard and observed less than 0.1% drift. The $\delta^{13}C$ values of the control samples did not vary throughout the experiment.

**Results and Discussion**

**Dechlorination Results.** The disappearance of 2,3,4,5-CB and corresponding production of 2,3,5-CB for each incubation tube is shown in Figure 2. Approximately 90% conversion occurred after 90 days, similar to 86% conversion over the same period reported by Wu et al. (17). After a lag period of 15 days, we observed an average conversion of $\sim 11.5 \mu g$ of PCB/day, which is a substantially accelerated rate compared to estimates that are decadal or longer for contaminated sediments (8). A bacterial enrichment culture was employed in a sediment-free system in this study for multiple reasons. First, this bacterium has demonstrated short lag times (17) and is activity-specific for doubly flanked (chlorine on either side of target atom) dechlorination only, with para-dechlorination favored over its meta-counterpart. Only 2,3,5-CB was produced. This simplified the isotopic study, since no intermediate or secondary products were formed, which might have produced competing isotope effects. We also sought to compare our observed reaction dynamics (rate, specificity, etc.) to those of other workers and to provide a basis for comparison of isotope effects from different microbial species (16). In addition, this sediment-free system eliminated the need to account for possible isotopic fractionation by desorption from sediments.

**δ$^{13}C$ Results.** The $\delta^{13}C$ values of the unconsumed reactant (2,3,4,5-CB), and the pooled product (2,3,5-CB) versus percent conversion are shown in Figure 3. The isotopic
compositions of 2,3,4,5-CB ($x = -28.2\%_o, \sigma = 0.3\%_o, n = 38$) and 2,3,5-CB ($x = -27.8\%_o, \sigma = 0.2\%_o, n = 35$) remained virtually constant over the course of the experiment and suggest a very small or no isotope effect. To test whether there was any significant difference (and hence an isotope effect) between the isotopic compositions of the unconsumed reactant and pooled product, we performed a $t$-test between the individual differences in $\delta^{13}C$ values for five samples that had 48.5–54.2% conversion. These samples are approximately equivalent in terms of completion of the reaction and provide a good estimate of overall reproducibility. Also, since the concentrations of 2,3,5-CB and 2,3,4,5-CB are similar, any bias due to sample size is reduced. The average difference was 0.3\%, and the $\delta^{13}C$ values were significantly different at the 95% but not at the 98% confidence level. An identical average difference occurs when the whole data is compared, implying a small systematic error rather than an isotope effect, as the latter would have caused the isotopic composition of the unconsumed reactant and the pooled product to diverge as the reaction proceeded to completion (20). Several possible systematic errors include slightly different combustion efficiencies for a trichlorobiphenyl relative to a tetrachlorobiphenyl and/or water-induced protonation of carbon dioxide in the ion source (21).

Nevertheless, such differences are very small and are below the uncertainty often cited (0.5\%) for $\delta^{13}C$ values of chlorinated organic compounds analyzed by irmGC-MS in laboratory and field samples (15, 16).

The absence of a large fractionation is due, in part, to the dilution of signal by the eleven carbons on the biphenyl moiety that are not involved in the reaction. Similar results have been observed in studies that have investigated carbon isotopic fractionation of other semivolatile organic contaminants during microbial degradation, such as long chain n-alkanes (22) and polycyclic aromatic hydrocarbons (23, 24).

**Biogeochemical Implications and Potential Applications.** While the above results indicate that there is no observable carbon isotopic fractionation associated with the reductive dechlorination of PCBs, the absence of an isotope effect may prove equally valuable for examining this process. This is because of systematic internal variations in the $\delta^{13}C$ values of congeners in Aroclors and other PCB mixtures. Jarman et al. (25) showed that the $\delta^{13}C$ values of select congeners in Aroclor 1242 and 1254 (as well as some Clophens, Kanechloirs, and Phenoclors) generally decreased with increasing chlorine content. That is, the less chlorinated PCBs were more enriched in $^{13}C$ than their more highly chlorinated counterparts. We have also observed this trend in three different lots of Aroclor 1268 where the $\delta^{13}C$ values were measured on all of the major congeners (Figure 4). These mixtures represent three distinct lots because they exhibit slightly different congener distributions. As shown, the $\delta^{13}C$ values range from approximately $-28$ to $-21\%$ and display a clear pattern of decreasing $\delta^{13}C$ with increasing degree of chlorination. Thus, if these laboratory results accurately reflect what occurs in the field, then microbial reductive dechlorination will create congeners with more negative $\delta^{13}C$ values than native PCBs exhibiting the same degree of chlorination. This concept is depicted in Figure 5.

![Figure 4. Compound-specific $\delta^{13}C$ values for each congener in three different lots of Aroclor 1268. PCB congeners are denoted analogously to those in the text.](image-url)
for a hypothetical Aroclor mixture. In order for this approach to be useful, the $\delta^{13}C$ values for a specific PCB congener in the initial mixtures must not vary substantially. Isotopic measurements for each congener in the three different Aroclor 1268 mixtures yielded $\delta^{13}C$ values that were quite similar (within ~0.8%, Figure 4). These results suggest that the chlorination process for different lots of the same Aroclor was isotopically consistent and that the isotopic composition of the starting biphenyl was similar for each lot. However, Jarman et al. (25) also found that the $\delta^{13}C$ values of select congeners vary significantly between different Aroclor mixtures. For example, the $\delta^{13}C$ values of 2,2',5,5'-tetrachlorobiphenyl in Aroclor 1242 and 1254 were ~28.26 and ~23.25%, respectively. This is not surprising as this congener is one of the higher chlorinated congeners in Aroclor 1242 and one of the lower chlorinated congeners in Aroclor 1254. Therefore, the $\delta^{13}C$ values of PCBs will be most effective assessing microbial reductive dechlorination in areas contaminated with only one type of Aroclor.

In summary, we have observed an absence of stable carbon isotopic fractionation during the microbial reductive dechlorination of 2,3,4,5-CB to 2,3,5-CB. Furthermore, due to the intrinsic trend of $^{13}C$ depletion with increasing chlorine content observed in Aroclor mixtures, this approach may provide additional evidence for the in situ occurrence of this process and aid in further understanding the parent/daughter relationships and environmental chemistry of these toxic and bioaccumulating compounds. Future work will be directed at examining the isotopic variation within and between additional Aroclors, comparison to field samples, and examining isotope effects accompanying other biochemical and physiochemical processes that act upon them.

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Literature Cited