Distinct carbon isotope fractionation signatures during biotic and abiotic reductive transformation of chlordecone

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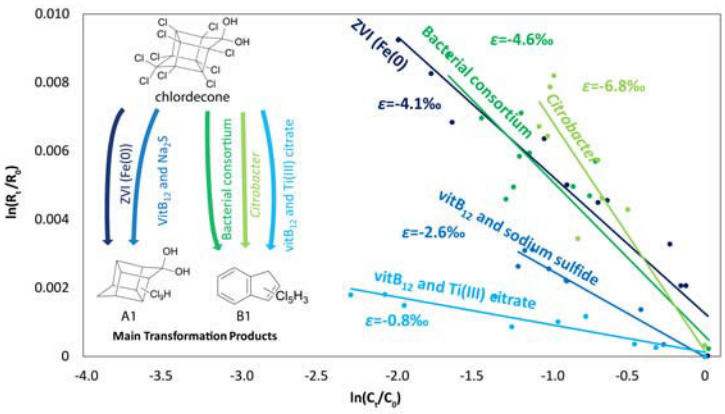
Abstract

Chlordecone is a synthetic organochlorine pesticide, extensively used in banana plantations of the French West Indies from 1972 to 1993. Due to its environmental persistence and bioaccumulation, it has dramatic public health and socio-economic impact. Here we describe a method for carbon-directed compound specific isotope analysis (CSIA) for chlordecone and apply it to monitor biotic and abiotic reductive transformation reactions, selected on the basis of their distinct product profiles (polychloroindenes versus lower chlorinated hydrochlordecones). Significant carbon isotopic enrichments were observed for all microbially mediated transformations (ε_{bulk}=-6.8‰ with a *Citrobacter* strain and ε_{bulk}=-4.6‰ with a bacterial consortium) and for two abiotic transformations (ε_{bulk}=-4.1‰ with zero-valent iron and ε_{bulk}=-2.6‰ with sodium sulfide and vitamin B_{12}). The reaction with titanium(III) citrate and vitamin B_{12}, which shows the product profile most similar to that observed in biotic transformation, did not lead to significant carbon isotope enrichment. The CSIA protocol was also applied on representative chlordecone formulations previously used in the French West Indies, giving similar chlordecone $\delta^{13}C$ values from -31.1 ± 0.2‰ to -34.2 ± 0.2‰ for all studied samples. This allows the in situ application of CSIA for the assessment of chlordecone persistence.

Graphical Abstract



Introduction

Chlordecone is a perchlorinated synthetic pesticide with a bis-homocubane structure (anhydrous form: $C_{10}Cl_{10}O$) that can be hydrated in the presence of water to a gem-diol form in place of the ketone moiety (Figure 1).[1-3] Between the 1960s and 1990s, chlordecone was the active ingredient of pesticide formulations in many countries, including the French West Indies, among them Kepone® which was introduced in 1958. Chlordecone toxicity was demonstrated in 1975 at the Hopewell chlordecone production plant (USA), where a lack of safety controls led to poisoning of workers and environmental pollution.[4-7] This disaster prompted the USA to prohibit the production and use of chlordecone in 1978. In spite of its ban in the USA, strong pest pressure led French authorities to allow the use of a new chlordecone formulation, Curlone®, to control the banana black weevil *Cosmopolites sordidus.* Curlone® was used in the French West Indies from 1981 until its final prohibition in 1993.[8-10] The massive use of 3 kg ha^{-1} $year^{-1}$ of chlordecone from 1972 to 1978 and from 1981 to 1993 resulted in extensive pollution of the French West Indies. More than 33% of the arable land is now contaminated with chlordecone (0.2 to 37.4 mg kg^{-1} dry weight equivalent),[11] and 80% of the rivers contain chlordecone (0.1 to 2 µg L^{-1}).[12,13] Due to its high concentration in soil, its low volatility and its strong adsorption to organic matter, chlordecone environmental pollution is estimated to persist for decades to centuries.[11] Long-term exposure to chlordecone through food and drinking water[14] can have severe impacts on human health such as an increase of the risk of prostate cancer and impacts on the neurological development of infants.[15] To decrease human exposure in the French West Indies, local regulations have been implemented. River and coastal fishing has been prohibited[10] and chlordecone levels in all fruits, vegetables or meat produced in the French West Indies are being monitored. These regulations massively impact cultural and economic life of the inhabitants.

Chlordecone is highly recalcitrant in the environment due to its bis-homocubane structure and the numerous chlorine atoms. However, according to thermodynamic calculations, the reductive transformation of chlordecone to less chlorinated products is exergonic.[16] Indeed, chlordecone has been described to undergo abiotic chemical transformation under reducing conditions: hydrochlordecones were observed as transformation products after incubation with zero-valent iron,[17] or when chlordecone was incubated under UV-light,[1] whereas apolar C_9 compounds were formed when vitamin B_{12} was used as catalyst in the presence of reducing agents.[18,19] Detection of C_9-compounds, assigned as polychloroindenes,[18,19] led the authors to propose a mechanism for chlordecone dechlorination in which the cage opening was mediated by vitamin B_{12}.

Few studies have addressed the microbial transformation of chlordecone under aerobic or anaerobic conditions. Under aerobic conditions, *Pseudomonas aeruginosa* strains as well as a mixed aerobic enrichment culture KO3, both originating from the Hopewell wastewater treatment plant, dehalogenated chlordecone to mono- and dihydrochlordecones.[20] Chlordecol, formed by the reduction

of the ketone moiety of chlordecone, was detected in trace amounts in KO3 cultures. Removal of one or two chlorine atoms was also demonstrated, when chlordecone was aerobically incubated with three other *Pseudomonas* species.[20] By using ^{14}C-labeled chlordecone Merlin et al. showed sorption of chlordecone to fungal biomass and traces of ^{14}C-metabolites were detected, indicating partial transformation.[21] In a study with the anaerobic archaeon *Methanosarcina thermophila* apolar and polar compounds were detected as transformation products,[22] while the removal of one or two chlorine atoms was demonstrated when chlordecone was incubated with bacterial cultures.[23] In a detailed study under anaerobic conditions, bacterial consortia and isolated *Citrobacter* strains transformed chlordecone to several different products: The major transformation product of this biotic reaction had the same mass spectrum as the abiotically produced C_9-compound from a previous study,[18,19] and was identified as $C_9Cl_5H_3$. Minor products such as mono-, di- and tri-hydrochlordecones and one $C_9Cl_4H_4$ compound were also detected.[24] The opening of the bis-homocubane cage, presumably generating compounds with an indene structure, and the loss of five chlorine atoms suggest that the mechanism is not as known for direct reductive dechlorination. Reductive dechlorination would result in the production of only hydrochlordecones.

In practical applications, activated carbon filters are used to remove chlordecone from drinking water.[25] However, high levels of chlordecone and the presence of other pesticides can saturate the filters fast. In Situ Chemical Reduction (ISCR) using zero-valent iron has been proposed as an alternative remediation technique to reduce chlordecone levels in soils. Field experiments with ISCR resulted in chlordecone removal from 22% to 74% depending on the soil type. Hydrochlordecones ($C_{10}Cl_{10-n}H_{n+2}O_2$, with n=1 to 5) were produced by ISCR.[26] However, in the French West Indies, 200 km^2 of surface is contaminated with chlordecone and such a wide-scale treatment by ISCR is expensive.[27] Therefore, a feasible remediation approach is urgently needed.

In highly polluted soils from the French West Indies, the only detected chlordecone derivative was 2-monohydrochlordecone (IUPAC nomenclature; 5b-monohydrochlordecone for CAS nomenclature). However, its concentration was much lower than that of chlordecone, on average 2% when observed.[28] Its detection by gas chromatography coupled to mass spectrometry (GC-MS) is relatively easy, due to the formation of characteristic fragment ions identical to those used for chlordecone quantification.[28] In contrast, other transformation products, especially those not bearing the bis-homocubane structure, are very difficult to detect and to quantify in complex environmental samples because standards are not available. To be able to monitor chlordecone transformation in environmental samples, an analytical method is therefore needed that is independent from the detection of transformation products.

Compound specific isotope analysis (CSIA) can detect chlordecone transformation in complex mixtures by measuring shifts in the isotope composition of the parent compound chlordecone. CSIA is based on the separation of compounds by gas chromatography (GC) and the compound-specific

detection by Combustion-Isotope Ratio Mass spectroscopy (GC-C-IRMS).[29-31] It has been successfully applied to differentiate transformation mechanisms of halogenated pollutants, such as chlorinated ethenes,[32] α-hexachlorocyclohexane[33] and chloroform[34]. It is also an efficient tool to assess transformation of pollutants in the environment.[31] In general, an enrichment of heavier isotopes (^{13}C) is observed in the pool of not-yet transformed (residual) pollutant molecules when transformation takes place. This results from the slightly faster reaction rate when the lighter isotope is involved in the chemical bond that is broken during the reaction. Using an experimentally determined isotope enrichment factor (ε), the change in the isotope ratio during transformation can be used for a quantitative evaluation of a transformation reaction.[31,35,36] The decisive advantage of CSIA in the context of chlordecone transformation is to enable assessment of transformation rates without detection of transformation products that might evade chemical analysis. [37-46]To our knowledge, isotope fractionation for molecules with a bis-homocubane structure such as chlordecone has not been described. CSIA could be a powerful tool to monitor chlordecone transformation in the laboratory and in the field.

The objective of this study was to assess if abiotic and biotic transformations of chlordecone are associated with carbon isotope fractionation. The occurrence of such isotope fractionation could be exploited for the detection of chlordecone transformation at contaminated sites. Therefore we developed a method to measure $^{13}C/^{12}C$ isotope ratios of chlordecone and applied it to samples incubated under different transformation conditions in the laboratory. We had two microbial inocula available to assess chlordecone transformation: a pure *Citrobacter* strain and a mixed consortium.[24] In addition we monitored three different conditions for carbon isotope fractionation under which abiotic reductive transformation of chlordecone occurred: zero-valent iron ("ZVI"); vitamin B_{12} together with sodium sulfide as reducing agent ("VSS") and vitamin B_{12} together with titanium(III) citrate as reducing agent ("VTC"). In the names the "V" indicates the presence of vitamin B_{12}, "SS" the presence of sodium sulfide and "TC" the presence of titanium(III) citrate. These five conditions were selected based on their known potential to generate distinct products and indeed, different carbon isotope fractionation values were found. In addition, commercial formulations of original pesticide batches of Kepone® and Curlone® were analyzed to evaluate the isotope ratio range for the various chlordecone sources spread in banana plantations of the French West Indies.

Material and Methods

Chemicals and Analytics

Used chemicals are described in detail in the supporting information (SI) (Table S1 and Supporting Methods). Also, the methods to extract chlordecone from samples and to analyze chlordecone and its transformation products are described in the SI Methods.

Chlordecone transformation protocols

In all five experimental approaches oxygen was excluded by working in an anoxic glove box (Coy lab products, Grass lake, MI, USA) (biotic experiments with *Citrobacter* strain 86_1 or with a mixed consortium) or by degassing with N_2 (abiotic experiments ZVI, VSS and VTC). In addition, reducing agents were amended (see below and Table S2). An overview of the experimental conditions is given in the SI (Table S2). For the exact determination of carbon isotopic compositions it was essential that either chlordecone was completely dissolved in the sample or that the sample was completely extracted (sacrificed). We sacrificed the whole experimental bottle in biotic experiments in which not all chlordecone was solubilizing due to the physiological pH and in experiments with ZVI in which chlordecone was not homogeneously distributed due to adsorption to the ZVI particles. Subsamples of a larger bottle were taken in the abiotic experiments VTC and VSS in which we adjusted the pH to 12-12.6 at which chlordecone was completely dissolved. Whereas biotic samples had 100 µM chlordecone, the abiotic set-ups were amended with 330 µM.

For biotic experiments, we used a pure culture, *Citrobacter* strain 86_1, and a mixed bacterial consortium 86_1 that contains *Citrobacter* strain 86_1.[24] The cultivation medium was as described[47] with modifications.[24] It contained 10 mM phosphate buffer (KH_2PO_4 and K_2HPO_4) at pH 7.5, 0.4 g L^{-1} (5.1 mM) sodium sulfide as reducing agent, 10 mM pyruvate, 2 g L^{-1} yeast extract, 2 g L^{-1} tryptone and resazurin as redox indicator. All handling procedures and incubations were done within the anoxic glove box containing a gas composition of 98% N_2 and 2% H_2. To obtain homogeneous pre-cultures, 1-L glass bottles with 500 ml of medium and a gas phase of N_2/H_2 98%/2% (v/v) were inoculated with the pure *Citrobacter* strain or the mixed consortium by adding 1% (v/v) active culture using sterile anoxic syringes. Bottles were incubated in the glove box at 25°C without shaking. After 6 h of incubation an absorption at 600 nm wavelength of 0.3 to 0.4 was reached. Then the culture liquid was distributed into 20-ml glass tubes, 10 ml per tube. Chlordecone was added to each tube to a final concentration of 100 µM from a 200 mM stock solution in dimethylformamide. Dimethylformamide was used because it dissolves chlordecone well, is water-miscible, not a carbon or nitrogen source for the bacteria, not oxidizing and was previously shown to not inhibit the used microbial cultures.[24] Every week two bottles were sacrificed and extracted for chlordecone and transformation product analysis.

ZVI: Abiotic reactions of chlordecone with zero-valent iron were performed at pH 6.8 in 12 glass bottles that contained 10 ml of a water/acetone mixture (3:1, v/v), chlordecone (330 µM) and Fe^0 (75 mg, 131 mM).[17] Two bottles were sacrificed every week over a time period of 5 weeks. Negative controls without zero-valent iron were monitored over the same time period.

VSS: For the reaction of chlordecone with B_{12} and sulfide, two bottles (total volume 100 ml) were filled with 30 ml of an N_2-purged oxygen-free aqueous solution of chlordecone (330 µM), sodium sulfide (94 mM) and vitamin B_{12} (96 µM). The pH in the bottles was 12.6. Chlordecone transformation

was monitored over 4 hours by taking 500 µl subsamples every hour with glass syringes. Control experiments contained chlordecone and sodium sulfide but no vitamin B_{12}.

VTC: To test the transformation of chlordecone in the presence of the strong reducing agent titanium(III) citrate and vitamin B_{12} as a catalyst two bottles (total volume 100 ml) were set up with 30 ml of anoxic water containing 330 µM chlordecone, 11.9 mM titanium(III) citrate, and 96 µM vitamin B_{12}. The pH was adjusted to pH 12.0 with 4 M NaOH to solubilize chlordecone. The bottles were then incubated for 100 minutes at 25°C without shaking. Two negative control experiments contained chlordecone and vitamin B_{12} but no titanium(III) citrate.

Chlordecone carbon isotope measurements from compound mixtures

Compound-specific isotopic analysis (CSIA) of chlordecone was done by GC-IRMS. A GC 7890A (Agilent Technologies, Germany) was equipped with a GC IsoLink interface containing a combustion reactor (combustion reactor tube no. 1255321, Thermo Fisher, Bremen, Germany) with a CuO/NiO catalyst which was held at 1,000°C. Two of these reactors were used successively over the measuring period because the first reactor reached the end of its lifetime during our measurements. In the text we refer to them as "reactor 1" and "reactor 2". The reactor was coupled via a ConFlo IV open split system to a MAT 253 IRMS (Thermo Fisher, Bremen, Germany). Samples were separated in the GC on a BPX5 column (50 m length, 0.32 mm inner diameter, 0.5 µm film thickness; SGE, Australia) at a constant helium carrier gas flow of 2.0 mL min^{-1} with the following temperature program: 80°C (hold for 7 min), increasing at 28°C min^{-1} to 220°C (0 min), increasing at 6°C min^{-1} to 300°C (hold for 3 min) and increasing at 20°C min^{-1} to 320°C (hold for 5 min). Samples were introduced into the GC via a splitless injection mode. For the analyses we used a split / splitless GC inlet liner with single taper and quartz wool and with the following specifications: outer diameter = 6.3 mm, inner diameter = 4.0 mm, length = 78.5 mm (Part No.: 092019, SGE Analytical Science, Germany). The splitless inlet program was as follows: purge time = 2 min, purge flow = 10 ml min^{-1}, septum purge = 2 ml min^{-1}. After each analysis, the combustion reactor was oxidized by flushing with O_2 for 6 min. After oxidation the reactor was re-equilibrated by flushing the system with helium for 6 min and purging the condition lines for 2 min with helium. Injection volumes were adapted between 1 and 5 µL to reach a stable response range in the GC-IRMS. The corresponding molar amount of carbon injected onto the column was 20 to 40 nmol (see SI Methods).

Isotope nomenclature is given according to Coplen,[48] using the letter *R* to describe ratios and the letter *N* to describe molar amounts of ^{13}C and ^{12}C isotopes. Carbon isotope ratios $R(N(^{13}C)/N(^{12}C))$, in the following abbreviated as $R(^{13}C/^{12}C)$, of samples R_{sample} and the Vienna Pee Dee Belemnite (VPDB) standard $R_{standard}$ were measured. From these *R* values relative differences of isotope ratios were calculated and are expressed in delta notation ($\delta^{13}C$) according to equation 1:

$$\delta^{13}C\ [‰] = \left(\frac{R_{sample} - R_{standard}}{R_{standard}}\right) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \quad (1)$$

Because of the typically small variations in the relative differences of carbon isotope ratios, δ-values are reported in parts per thousand (‰) and e.g. a δ^{13}C-value of 5‰ means a relative difference of isotope ratios of 0.005.[49,50] Each sample was measured in at least three technical replicates (three individual injections of the same sample into the GC-IRMS) to secure accuracy and reproducibility of the measurement. Additionally, a chlordecone standard was measured every 6 samples to ensure the reliability of the measurements. The maximal standard deviation tolerance derived from three replicates was set to 1‰ for δ^{13}C values of chlordecone.

For quantification of carbon isotope fractionation, a carbon isotope enrichment factor (ε_{bulk}) was calculated using the Rayleigh equation:[43,48,51]

$$\ln\left(\frac{R_t}{R_0}\right) = \ln\left(\frac{\delta^{13}C_t+1}{\delta^{13}C_0+1}\right) = \ln\left(\frac{C_t}{C_0}\right) \times \varepsilon_{bulk} \quad (2)$$

where ($\delta^{13}C_0$, C_0) and ($\delta^{13}C_t$, C_t) are the relative differences of isotope ratios and concentrations of the compound at time 0 and time t, respectively. The enrichment factor (ε_{bulk}) correlates the change in isotope ratios with the change in concentration.

Results and Discussion

Development of a CSIA method to measure the carbon isotope ratio of chlordecone from compound mixtures

Initial measurements of chlordecone-containing sample extracts by GC-IRMS showed that the highly oxidized bis-homocubane structure of chlordecone is more difficult to combust than chloroethenes[52,53] and that the combustion reactor was quickly deactivated. However, complete combustion is required to obtain stable values for the isotopic composition of a compound in GC-IRMS. An oxidation cycle was therefore introduced for the combustion reactor, previous to each single measurement increasing the oxidative capacity of the reactor, enabling full compound combustion and resulting in a stable and linear response for chlordecone concentrations ranging from 15 to 400 mg L^{-1} (Figures S1 and S2). Under the applied conditions, the response in signal intensity was linearly dependent on the injected sample volume between 1 and 5 µl. The established method enabled determination of the isotopic composition of the residual fraction of chlordecone from complex samples.

To determine the concentration range in which stable and reproducible chlordecone isotopic composition values could be obtained, detection limits were defined. The two criteria setting this detection limit were i) that the standard deviation of triplicate analyses at a particular concentration was less than ±1‰ δ^{13}C and ii) that the mean value of triplicate measurements for a particular concentration was within ±0.5‰ of the mean of all analyses over the range of tested concentrations applying a modified approach from Jochmann and colleagues.[54] Under these criteria and with 5-µl

injections the lower detection limit was at 200 mg L^{-1} whereas the upper quantification limit was at 400 mg L^{-1}. This represented 20-40 nmol of carbon and 20-40 nmol of chlorine on the column (see also SI Methods). For the same level of precision, these values are 4-400 times higher than for chloroethenes or nitroaromatic compounds for which a carbon sensitivity of 0.1-5 nmol with a precision of 0.1-0.3‰ was reported.[55-57]

The fluctuation of $\delta^{13}C$ values typically to lighter isotope values indicated incomplete combustion. Such difficulties to completely combust chlordecone have been described also by others in different contexts.[8,58,59] In general, highly halogenated compounds are complicated to combust quantitatively and aging of the reaction is frequently observed. Reasons for this might include the stability of the halogen-carbon bond, the need to break several bonds in the complete oxidation of chlordecone to CO_2 and deactivation of catalyst and copper oxides. However, our method allows reproducible determination of the ^{13}C isotope signature describing carbon isotope fractionation of chlordecone. The frequent reactor reoxidation mitigated the loss of oxidation capacity in our experiments.

Reported values of chlordecone contamination in the French West Indies are below the value of our CSIA detection limit. For example, chlordecone concentrations down to 9 mg kg^{-1} were described by Cabidoche et al.[11]. Levillain et al.[60] measured different concentrations in different soil types with a mean value of 2.1 mg kg^{-1}, whereas Crabit et al.[61] reported up to 3.4 mg kg^{-1}. Therefore, to apply CSIA in the field, soil extraction procedures to accumulate chlordecone from 20-200 g soil will have to be established.

To further investigate the conversion of chlordecone in the reactors, results from GC-IRMS were compared with those from an elemental analyzer. Two different chlordecone standards provided by Ehrenstorfer (96.7% purity) and Supelco (99.9% purity) were measured with both methods. The obtained $\delta^{13}C$ values for Ehrenstorfer chlordecone were -28.5 ± 0.3‰ (GC-IRMS, reactor 1), -30 ± 0.3‰ (GC-IRMS, reactor 2) and -26.2 ± 0.08‰ (elemental analyzer). The obtained $\delta^{13}C$ values for chlordecone from Supelco were -22.2 ± 0.3‰ (GC-IRMS, reactor 1), -21.4 ± 0.2‰ (GC-IRMS, reactor 2) and -21.3 ± 0.06‰ (elemental analyzer). There was a small offset in isotope composition in reactor 1, however, reactor 2 gave nearly identical values compared to the elementary analyzer results. The variability in the values for Ehrensdorfer chlordecone might be due to its low purity of 96.7%. Such high variability was not measured with the purer chlordecone from Supelco.

Biotic and abiotic transformation of chlordecone

We monitored biotic and abiotic transformation of chlordecone . Obtained transformation products fall into two families: hydrochlordecones with bis-homocubane structure and aromatic C_9-compounds (Figure 1).

Several different transformation products were detected when the isolated *Citrobacter* strain 86_1 or the mixed bacterial consortium 86_1 from which this strain was isolated, was incubated with

chlordecone. The main transformation product B1 ($C_9Cl_5H_3$) was identified as pentachloroindene based on its mass spectrum and retention time. Two other products referred to as B3 ($C_9Cl_4H_4$, tetrachloroindene) and A1 ($C_{10}Cl_9H_2O_2$, monohydrochlordecone) were detected in minor amounts (Figure S3). Control experiments without bacteria showed no chlordecone transformation and no formation of products. Cultures without chlordecone did not form these products indicating that they were formed from chlordecone.

For abiotic transformation (Table 1, Figure S4-S6), chlordecone was incubated with different reducing agents either with or without vitamin B_{12} as catalyst. Again, transformation products belonging to one of the two product families, hydrochlordecones and polychloroindenes were observed. Abiotic transformation with zero-valent iron (ZVI) approximates the in situ chemical reduction applied in the field[17] and was therefore investigated. After five weeks of incubation only monohydrochlordecone A1 (50% of the initial chlordecone) was detected (Figure S4), similar to what has been described for field applications. In control bottles without zero-valent iron no chlordecone transformation occurred. Since sodium sulfide was previously successfully employed as reducing agent in our microbial cultures, it was selected as a reducing agent in presence of vitamin B_{12}. In our experiments these VSS conditions allowed the conversion of chlordecone to monohydrochlordecone A1 as the major product (~50% of the initial chlordecone) and pentachloroindene B1 as a minor product after 4 hours (Figure S5). Negative controls with sodium sulfide but without vitamin B_{12} showed no chlordecone transformation even after one year of incubation at room temperature. In previous work, tetrachloromethane was dechlorinated with titanium(III) citrate and vitamin B_{12}.[62] When we incubated chlordecone with titanium(III) citrate and vitamin B_{12} (VTC) transformation occurred fast. After 95 minutes 88% of the initial chlordecone was transformed mainly to a compound with a mass equivalent to the molecular formula $C_9Cl_5H_3$, identified as pentachloroindene B1. Minor products were tetrachloroindenes B2 and B3 and monohydrochlordecone A1 (Figure S6). When chlordecone was incubated with vitamin B_{12} at pH 12.0 without titanium(III) citrate, no transformation occurred. A quantitative comparison of all negative control experiments is shown in Figures S7.

To verify the structure of compound B1 a platinum-catalyzed chemical reduction was tested. In this experiment compound B1 was incubated with palladium on charcoal with H_2 in a tetrahydrofuran/ethanol/water mixture (35:15:2 v/v) (see SI Methods for details). By this treatment compound B1 was reductively dechlorinated and one double bond was reduced resulting in several products including indane and cis-perhydroindane (Figure S8). This indicated that indeed compound B1 possesses an indene structure and that biotic incubations and VTC conditions resulted in the restructuring of the chlordecone cage into an indene ring system. In contrast, the two abiotic transformations ZVI and VSS produced only monohydrochlordecone A1, as described previously.[18] Belghit et al. reported that with zero-valent iron, monohydrochlordecone A1 can be further transformed to polyhydrochlordecones.[17] This transformation of monohydrochlordecone A1 to

polyhydrochlordecones did not occur in our experiments with sodium sulfide and vitamin B_{12} (VSS). The difference in reaction products between the systems VTC and VSS probably originates from the oxidation state of cobalt(I) and (II), respectively, responsible for vitamin B_{12} reactivity.[63]

Carbon isotope fractionation during biotic and abiotic transformation of chlordecone

Carbon isotope ratios (R_{Sample} and $R_{Standard}$) were measured for biotic and abiotic reactions (Figure 2 and S9). In pure *Citrobacter* cultures, 49% of the initial chlordecone was transformed, accompanied by a change of chlordecone isotope composition (δ^{13}C-values) from -31.0‰ to -26.0‰ representing an isotope enrichment of 5‰. The ^{13}C enrichment factor of this transformation was ε_{bulk}=–6.8 ± 2.2‰ (Figure 3). With the mixed bacterial consortium 86_1, ε_{bulk}=-4.6 ± 1.4‰ was calculated (Figure 3). Therefore, the two biotic transformations showed very high carbon isotope fractionation but could not be differentiated from each other on the basis of their carbon isotope fractionation as the confidence intervals overlap (Figure 3). This is consistent with the fact that *Citrobacter* 86_1 is a member of the bacterial consortium 86_1 and the results might indicate that a similar *Citrobacter* strain is responsible for chlordecone transformation in the consortium. Also, the fact that the same products were formed supports this conclusion.

After 86% conversion of chlordecone with zero-valent iron, the δ^{13}C-values of chlordecone showed an increase of 9.0‰ (Figure 2). Based on the Rayleigh equation the ^{13}C enrichment factor was ε_{bulk}=4.1 ± 0.4‰, similar to what was observed with the microbial consortium 86_1 (Figure 3).

In contrast to the relatively similar isotope fractionation with microbial cultures and under ZVI conditions, the abiotic reactions VSS and VTC showed significantly different ^{13}C enrichment factors. With sodium sulfide as reducing agent and vitamin B_{12} as catalyst (VSS), chlordecone δ^{13}C-values increased by an absolute value of 3.1‰ (Figure 2), which resulted in a ^{13}C enrichment factor of ε_{bulk}=-2.6 ± 0.4‰ (Figure 3).

With titanium(III) citrate and vitamin B_{12} (VTC) chlordecone transformation was fast and 88% of the initial chlordecone concentration was transformed within 95 minutes (Figure 2). This lead to small isotope fractionation with an enrichment factor of ε_{bulk}=-0.8 ± 0.4‰ (Figure 3).

The three abiotic transformations of chlordecone analyzed in our study showed distinct carbon isotope effects. Transformation involving zero-valent iron shows the most similar isotope effect to biotic transformations. However in biotic chlordecone transformations and the abiotic transformation with ZVI, the pH was close to neutral whereas for VSS and VTC conditions transformation occurred at around pH 12. Previous solubility tests showed that chlordecone solubility increases significantly above pH 9.4.[64] This solubility profile suggests that below pH 9.4 the chlordecone gem-diol group is in its acidic form whereas above pH 9.4, it loses a proton and becomes negatively charged (Figure 1). These different forms may play a crucial role in the mechanism and may contribute to ^{13}C enrichment. Hydrolysis of chlordecone at pH 12, as previously found e.g. with hexachlorocyclohexane,[33] was not

observed (Figures S5 and S6). Although single element isotope analysis can give mechanistic information it is prone to masking effects often resulting in ambiguous results..[50]

To differentiate reactions on the basis of their isotope fractionation factors, *e.g.* for the characterization of transformation in the field, significant differences between the factors are necessary. However, when the number of carbon atoms in a molecule increases, enrichment factors decrease due to a dilution effect of carbon atoms in the molecule not involved in the reaction. It has been stated in this regard, that no significant carbon isotope fractionation can be expected for polyaromatic hydrocarbons consisting of 11 or more carbons.[44] Under sulfate reducing conditions enrichment factors of -1.1‰ and -0.9‰ were observed for the microbial transformation of naphthalene (10 carbon atoms) and methylnaphthalene (11 carbon atoms).[43] However, CSIA was successfully applied onto the dechlorination of tri- and tetrachlorodibenzodioxins (12 carbon atoms) by *Dehalococcoides*-containing cultures.[45,46] These studies indicate that the isotopic characterization of polycyclic C_{10}-molecules like chlordecone is feasible. Indeed, the pronounced carbon isotope effect detected here for chlordecone transformation might allow field application. From our data we cannot calculate values for the apparent kinetic isotope effect because detailed information on the catalytic mechanism and the number of carbon atoms possibly involved in the biotic and abiotic reactions investigated here is missing.

Involvement of cofactor B_{12} in the formation of indene structures

Two product families were detected during abiotic and biotic transformation of chlordecone: hydrochlordecone derivatives and polychloroindenes (Figure 1). While formation of hydrochlordecones takes place in the biotic and abiotic reactions, formation of chlorinated indene products occurred only in biotic experiments and in the abiotic reactions containing vitamin B_{12} (VSS and VTC). Polychloroindenes were the major products in biotic experiments and in the incubations with vitamin B_{12} + titanium(III) citrate (VTC). Titanium(III) citrate has a stronger negative redox potential than sulfide and can reduce the central cobalt ion in vitamin B_{12} from the (+III) to the (+I) oxidation state.[62] This highly reduced B_{12} might be required for chlordecone ring-opening giving rise to indene derivatives. In contrast, sulfide can reduce vitamin B_{12} only to its (+II) state,[65] which can dechlorinate chlordecone to hydrochlordecones, but cannot open the bis-homocubane ring structure. As both *Citrobacter* strains and several species from bacterial consortium 86_1 encode the anaerobic corrinoid-biosynthesis pathway in their genomes,[24] it is possible that corrinoid-dependent enzymes are involved in chlordecone ring-opening in the biotic incubations. However, this hypothesis is not supported by the present isotope fractionation study since the largest difference in enrichment factors was observed between biotic transformation and abiotic vitamin B_{12} + titanium(III) citrate-mediated transformation (VTC). At the current stage, our data might indicate that other enzymes or free cofactors are involved.

$^{13}C/^{12}C$ isotope ratios of commercial formulations of chlordecone

Four different commercial formulations of chlordecone stemming from different production periods between 1978 and 1993 were analyzed. This analysis required the extraction of chlordecone from the commercial formulations as a first step. Comparing the extracted amounts with values reported in the literature for these commercial formulations[28] we calculated our extraction efficiency to be between 88 and 105% confirming good recovery. $\delta^{13}C$-values of the four batches were all close to each other: $\delta^{13}C_{Kepone}$=-33.0 ± 0.4‰, $\delta^{13}C_{Curlone\text{-}Cirad}$=-34.2 ± 0.2‰, $\delta^{13}C_{Curlone\text{-}IRD}$=-33.2 ± 0.1‰ and $\delta^{13}C_{Curlone\text{-}UAG}$=-31.1 ± 0.2‰.

At least 55 different commercial chlordecone-containing formulations have been applied in the French West Indies between 1972 and 1993[9]. These 55 different products have been manufactured at three different plants (Brazil, Martinique and Guadeloupe), during at least two distinguishable periods of time (before 1976 and in 1983). The four formulations available for our study (Table S1) were described as representatives for all chlordecone formulations used in the French West Indies.[28] The fact that these four representative formulations show very similar isotope signatures suggests that the isotope signatures of all other chlordecone formulations sprayed in the French West Indies might also be similar, which has to be investigated in more detail in the future. This situation is similar to the situation observed for hexachlorocyclohexane stereoisomers for which groups with homogeneous isotope composition over production time and area could be observed.[66]

If indeed the carbon isotope signatures of all chlordecone formulations originally used in the French West Indies are similar to each other, the quantitative assessment of chlordecone transformation by analyzing carbon isotopic signatures in different compartments in the field is possible. For such field monitoring, chlordecone $\delta^{13}C$-values at a contaminated site would be compared with $\delta^{13}C$-values of the original compounds and a shift in the $\delta^{13}C$-values would indicate transformation. According to our data, carbon isotope fractionation would occur when biotic transformation similar to that observed with the *Citrobacter* strain takes place. The pronounced enrichment factors as described above would allow quantitative assessment.

As a perspective to advance the understanding of chlordecone transformation, more accurate data could be obtained from dual-element isotope analysis combining $^{13}C/^{12}C$ and $^{37}Cl/^{35}Cl$ data. With such an approach, transformation could be detected with better sensitivity and without the influence of masking effects. This may also allow gaining insight into the mechanisms of biotic and abiotic chlordecone transformation.

Associated Content

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: …

Sources of chemicals, chlordecone extraction protocol, protocol to identify transformation products, method description for elemental analyzer and IRMS; figures on linear response of the IRMS signal, stable IRMS range, chromatograms of biotic and abiotic transformations, quantitative evaluation of negative controls, compound B conversion, IRMS of under all chosen conditions, table of analyzed chlordecone formulations, and table with detailed experimental information. (pdf)

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Notes

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Figures

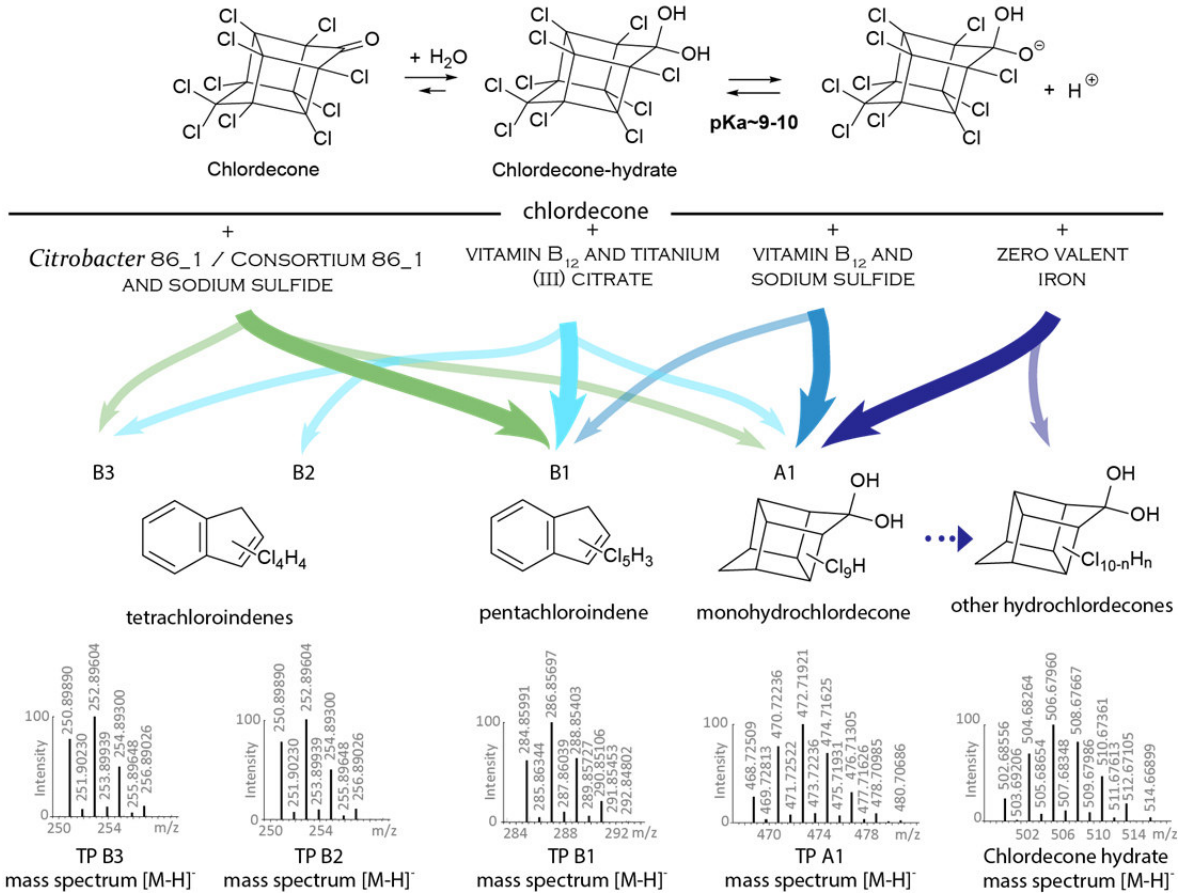
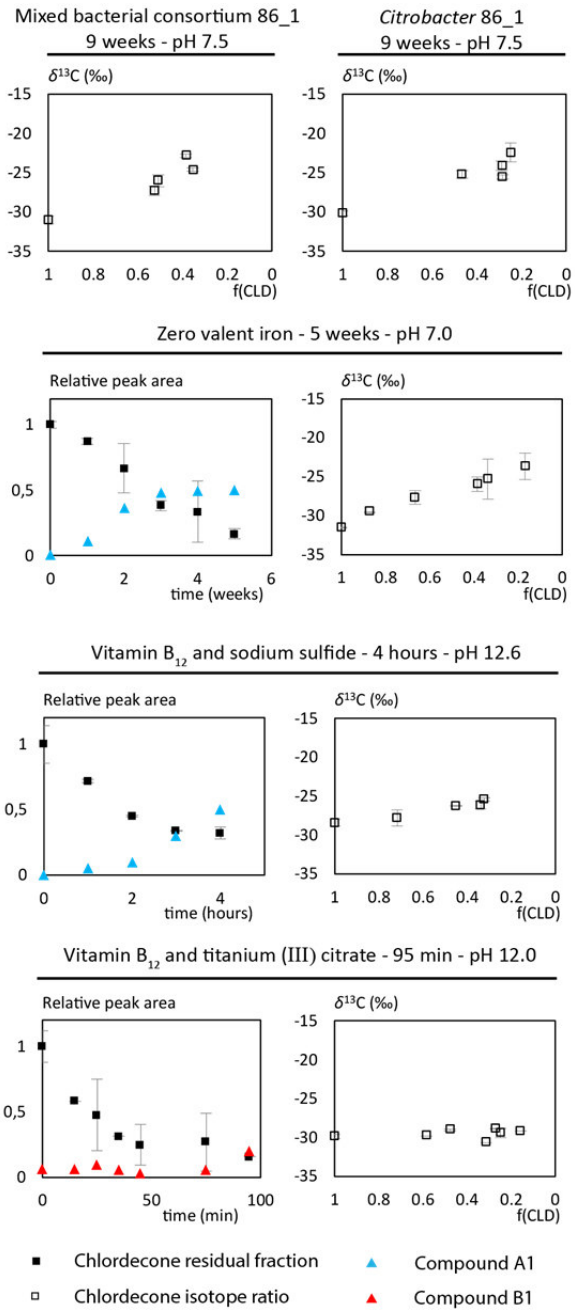
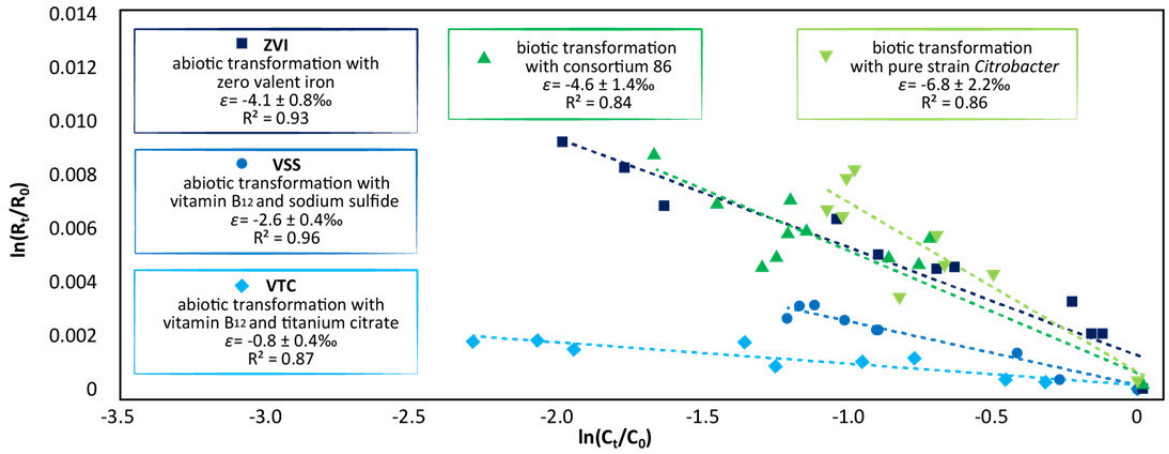


Figure 1. Overview on observed biotic and abiotic transformation paths and products (see Figures S3-S6 for details). In the top row the equilibrium of chlordecone between its ketone form (left), the gem-diol form (middle) and the better water-soluble deprotonated form (right) is shown. Below observed transformation products and their mass spectra $[M\text{-}H]^-$ are shown. TP: Transformation product. Symbols: thick arrows – major transformation path, thin arrow – minor transformation path, dotted arrow – transformation not observed here but described by others.



630

631 **Figure 2.** Determined $\delta^{13}C$ values in chlordecone over the chlordecone residual fractions
632 ($f(CLD)=[CLD]_t/[CLD]_0$) for the five tested biotic and abiotic reactions (upper row and right column),
633 and relative peak areas of chlordecone and its transformation products over time during abiotic
634 transformations (left panels in the lower three rows).



635

636 **Figure 3.** Rayleigh plot for carbon isotope fractionation during chlordecone transformation in biotic

637 (green) and abiotic (blue) experiments

Tables

Table 1. Incubation of chlordecone with different catalysts and reducing agents (see Figures S3-S6 and Table S2 for details).

Transformation conditions							Chlordecone transformation[a]			Carbon isotope fractionation					
		Catalyst	Reducing agent	pH	Incub. time[b]	CLD[c] conc.	Degree (%)	Main TP[d]	Minor TP[d]	Start (‰)	End (‰)	ε_{bulk} (‰)	CI (‰)	R^2	Number of replicates
biotic	Citro	Citrobacter 86_1	sulfide	7.5	9 weeks	100 µM	49	$C_9Cl_5H_3$	$C_{10}Cl_9O_2H_3$ $C_9Cl_4H_4$	-31.0[h]	-26.0[h]	-6.8	2.2	0.86	2
biotic	Cons	consortium 86_1	sulfide	7.5	9 weeks	100 µM	75	$C_9Cl_5H_3$	$C_{10}Cl_9O_2H_3$ $C_9Cl_4H_4$	-30.1[h]	-22.4[h]	-4.6	1.4	0.84	2
biotic	NC[f]	none	sulfide	7.5	9 weeks	100 µM	none	none	none	nm[e]	nm	nm	nm	nm	1
abiotic	ZVI	none	ZVI	6.8	5 weeks	330 µM	83	$C_{10}Cl_9O_2H_3$	$C_{10}Cl_8O_2H_4$ $C_{10}Cl_7O_2H_5$	-31.5[h]	-23.7[h]	-4.1	0.8	0.93	2
abiotic	NC	none	-	6.8	5 weeks	330 µM	none	none	none	nm	nm	nm	nm	nm	2
abiotic	VSS	vitamin B_{12}	sulfide	12.6	4 hours	330 µM	69	$C_{10}Cl_9O_2H_3$	$C_9Cl_5H_3$	-28.5[g]	-24.5[g]	-2.6	0.4	0.96	2
abiotic	NC	none	sulfide	12.6	30 hours	330 µM	none	none	none	nm	nm	nm	nm	nm	2
abiotic	VTC	vitamin B_{12}	Ti(III)	12.0	95 min	330 µM	88	$C_9Cl_5H_3$	$C_{10}Cl_9O_2H_3$ $C_9Cl_4H_4$	-30.4[h]	-28.9[h]	-0.8	0.4	0.87	2
abiotic	NC	vitamin B_{12}	-	12.0	2 hours	330 µM	none	none	none	nm	nm	nm	nm	nm	2

[a]Chlordecone transformation in % of the initial concentration; [b]incubation time of the reaction; [c]initial chlordecone concentration; [d]TP – transformation product; [e]nm – not measured; [f]NC – negative control; [g]chlordecone standard $\delta^{13}C$ = -28.5 ± 0.3‰ (measured with reactor 1), [h]chlordecone standard $\delta^{13}C$ = -30.2 ± 0.5‰ (measured with reactor 2).