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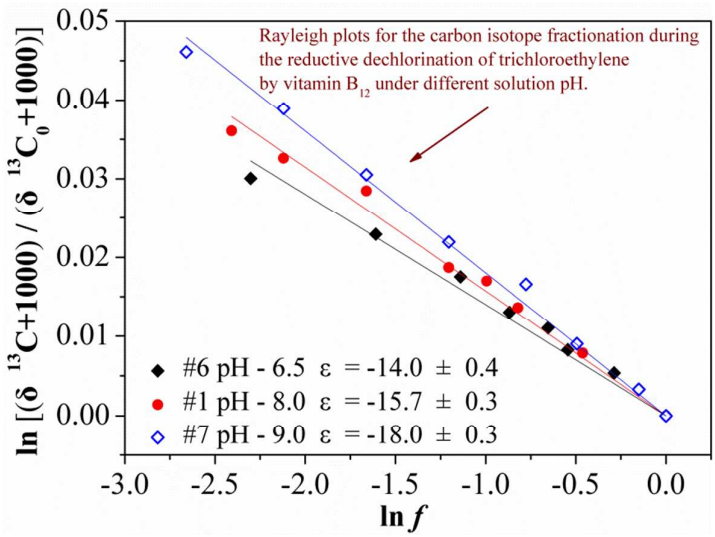

The initial solution pH from 6.5 to 9.0 caused a notable change in the ε values, from −14.0‰ to −18.0‰.

Environmental impact

Trichloroethene is one of the most common chlorinated organic contaminants of groundwater which has an influence on both the environment and on human health. Remediation technologies based on reductive dechlorination by vitamin B_{12} have been developed and have potential for treating chlorinated organic contaminants in groundwater. Effective remediation also requires detailed knowledge of the extent to which the contaminants at a site are degraded. Compound specific isotope analysis is an analytical technique that can be used to monitor the transformation of organic contaminants during remediation. In this study significant carbon isotope fractionation observed implies that stable carbon isotope analysis can be used to distinguish dechlorination by vitamin B_{12} from non-fractionating processes such as sorption, dissolution and volatilization. And if the carbon fractionation factor is reproducible, it can be used to assess the efficacy of in situ remediation of TCE implemented by vitamin B_{12} dechlorination. This study demonstrates that even if similar reactants are involved, stable isotope fractionation may differ significantly because of different reaction conditions such as solution pH. Therefore, the selection of an appropriate fractionation factor is crucial for a quantitative assessment of in situ remediation by means of compound specific isotope analysis.

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ARTICLE TYPE

Variability in the carbon isotope fractionation of trichloroethene on its reductive dechlorination by vitamin B_{12}

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Stable carbon isotope fractionation through the reductive dechlorination of trichloroethylene by vitamin B_{12} was determined to assess the possibility of using stable carbon isotope analysis to determine the efficacy of remediation of trichloroethylene using vitamin B_{12}. We elucidated the effects of environmental conditions, including the pH, reaction temperature, and vitamin B_{12} concentration, on the carbon isotope enrichment factor (ε). The ε values were relatively insensitive to the reaction temperature and vitamin B_{12} concentration, ranging from −15.7‰ to −16.2‰, with a mean of −15.9±0.2‰, at different temperatures and vitamin B_{12} concentrations. Such a reproducible ε value could be particularly useful for estimating the extent of degradation in reactions in which a mass balance is difficult to achieve. However, changing the initial solution pH from 6.5 to 9.0 caused a notable change in the ε values, from −14.0‰ to −18.0‰. Reactions were investigated by calculating the apparent kinetic isotope effects for carbon, which, at 1.029–1.037, were smaller than the kinetic isotope effect values previously found for C–Cl bond cleavage. This indicates that a reaction other than the elimination of chloride may be a competitive degradation pathway. The dominant degradation pathway may be different for different initial solution pH values, and this will clearly influence carbon isotope fractionation. Therefore, if the ε value varies with reaction conditions, such as the solution pH, the calculations should take into account the actual environmental conditions that affect the rate limiting pathways.

Introduction

Remediation technologies based on reductive dechlorination by vitamin B_{12} have been developed and have potential for treating chlorinated organic contaminants in groundwater.[1–4] It is important to investigate the reaction pathways involved in remedial technologies. Effective remediation also requires detailed knowledge of the extent to which the contaminants at a site are degraded. Compound specific isotope analysis is an analytical technique that can be used to monitor the transformation of organic contaminants during remediation.[5] In particular, stable carbon isotope analysis is an effective and powerful tool for investigating and monitoring contaminant remediation.[6] Carbon isotope ratios often change when organic contaminants are degraded, and these changes are highly reproducible and can be modeled using the Rayleigh model.[7] The change can be expressed as a fractionation factor, α, which relates the isotopic composition of the reactant to the extent of degradation, as shown in Eq. 1.

$$ln\left[\left(\delta^{13}C+1000\right)/\left(\delta^{13}C_0+1000\right)\right]=\left(\varepsilon/1000\right)\times ln\, f \qquad (1)$$

δ^{13}C and $\delta^{13}C_0$ are the isotopic compositions of the reactant at a given time and the initial time, respectively. And it has become common practice in recent years to replace the fractionation factor α by the enrichment factor ε, which is defined as ε=1000(α−1), ‰. The ε value is generally determined in laboratory-scale studies, and) f is the fraction of the parent compound that has not been transformed at a site.[8] Slater et al.[9] found large enrichment factors, of −17.2‰ and −16.6‰, for the reductive dechlorination of trichloroethene (TCE) by vitamin B_{12} in laboratory microcosms.[26]

Carbon isotopic fractionation during the dechlorination of chlorinated organic contaminants has been well documented and has been found to vary depending on the degradation pathway.[10–13] Variations in the ε value may be caused by different rate-determining steps dominating different degradation processes, and these differences can be used to assess remediation technologies.[14–16] The selection of an appropriate ε value to represent the degradation pathway in the field is crucial for a quantitative assessment of degradation using compound specific isotope analysis. It is critical to understand the magnitudes of and variability in the ε values associated with different reaction conditions that affect the transformation efficiency of the target materials.[17–20]

TCE is toxic, but is a useful chlorinated solvent that has been widely used, and it is frequently detected in soil and groundwater. TCE was chosen as a model compound for the research presented here. The reaction processes involved in the reduction of TCE by vitamin B_{12} with titanium (III) as an electron donor have been the focus of a number of studies.[21–23] Many factors control the transformation efficiency of TCE by vitamin B_{12}, which may affect the carbon isotope fractionation and result in variability of

carbon isotope enrichment factor. We assessed the isotopic effects associated with the reductive dechlorination of TCE at different vitamin concentrations, solution pH values, and temperatures. The long-term goal of this research is to explore the possibility of using carbon isotopic analysis to investigate the mechanisms involved in dechlorination, and its effectiveness in remediation, both in the laboratory and in the field.

Experimental

Chemicals

TCE (C_2HCl_3; 99.0%) and methanol (99.5%) were purchased from Tianjin Hengxing Chemical Reagent Co., Ltd. (Tanjin, China). Vitamin B_{12} (cobalamin; >98%), titanium (III) chloride ($TiCl_3$; 20% in HCl), trisodium citrate ($C_6H_5Na_3O_7{\cdot}2H_2O$; analytical reagent (AR) grade), oxalic acid ($H_2C_2O_4$; AR grade), and sodium carbonate (Na_2CO_3; AR grade) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All aqueous solutions were prepared in 18.0 MΩ·cm deionized water (NW10UV; Heal Force, , Hong Kong, China).

Experimental design

Each test was conducted in a 300 mL serum flask sealed with an open screw cap and a Teflon-faced silicone septum. Each serum flask contained 250 mL deionized water containing 3.0 g oxalic acid, 12 g sodium citrate, and 5.133 g $TiCl_3$. The experimental conditions are listed in Table 1. The *Control* vials were not treated with vitamin B_{12}. Experiments *#1*, *#2*, and *#3* were used to determine the effect of the vitamin B_{12} concentration on carbon isotope fractionation, and 5 mL, 15 mL, and 25 mL of 1.844 mmol L^{-1} vitamin B_{12} stock solution was added to the *#1*, *#2*, and *#3* serum flasks, respectively, to give different vitamin B_{12} concentrations (Table 1). Experiments *#1*, *#4*, and *#5* were used to determine the effect of the reaction temperature on carbon isotope fractionation. Experiments *#1*, *#6*, and *#7* were used to determine the effect of the initial solution pH on carbon isotope fractionation. The pH was adjusted using 4.7 g, 6.5 g, or 7.5 g of sodium carbonate in experiments *#6*, *#1*, and *#7*, respectively (Table 1). The final volume in each vial was adjusted to 290 mL with deionized water. The reaction mixtures were prepared in an isolation glove box. TCE was added to each flask by injecting 60 µL of a 547,500 mg L^{-1} TCE in MeOH stock solution through the septum, to produce a theoretical TCE concentration of 113 mg L^{-1}. The well-sealed vials were then placed on a temperature-controlled circular action shaker for at least 10 h, shaking at 150 rpm.

Table 1 Experimental conditions used for the tests of the degradation of trichloroethylene catalyzed by vitamin B_{12}

ID	$[B_{12}]$ (µmol L^{-1})	$NaCO_3$ (g) / pH	T (°C)
Control	0	6.5 / 8.0	30
#1	95	6.5 / 8.0	30
#2	32	6.5 / 8.0	30
#3	159	6.5 / 8.0	30
#4	95	6.5 / 8.0	20
#5	95	6.5 / 8.0	40
#6	95	4.7 / 6.5	30
#7	95	7.5 / 9.0	30

TCE analysis

The TCE residue fraction was determined using an Agilent 6890N (Agilent Technologies Inc., Santa Clara, CA, USA) gas chromatography (GC) instrument fitted with an electron capture detector (ECD) and a 30 m long, Φ 0.25 mm, 1.4 µm film thickness DB-624 column (J&W Scientific, Folsom, CA, USA). A solid phase microextraction (SPME) device was used to extract the TCE from the samples and introduce the TCE into the GC. At each sampling time, an aqueous sample (10–20 µL) was withdrawn from the flask using a gas-tight micro-syringe and added to an 8 mL vial containing 4 mL deionized water. The vial was sealed with an open screw cap and a Teflon-faced silicone septum. The SPME fiber (with a 100 µm polydimethylsiloxane coating; Supelco, Bellefonte, PA, USA) was exposed to the headspace (4 mL) for 5 min, then the analytes were thermally desorbed from the SPME fiber for 2 min in the GC injection chamber (at 230 °C with a 1:10 split ratio). The GC oven temperature program was 50 °C for 1.0 min, 15 °C min^{-1} to 130 °C, 130 °C for 1 min. The flow rate of the nitrogen carrier gas was 1.0 mL min^{-1}. The ECD temperature was 230 °C and the make up gas (nitrogen) flow rate was 60 mL min^{-1}. The analytical precision for the TCE determination was 10% (n=3). The method detection limit (MDL) for TCE was 0.7 µg L^{-1}.

Isotope analysis

Stable carbon isotope analysis was performed using SPMEs and gas chromatography/combustion–isotope ratio mass spectrometry (GC/C-IRMS; Thermo Fisher Scientific, Bremen, Germany). The GC instrument was equipped with a 30 m long, Φ 0.25 mm, 0.25 µm film thickness DB-5 capillary column (J&W Scientific). The GC oven temperature program was 35 °C for 1.5 min, 10 °C/min to 50 °C, 30 °C min^{-1} to 140 °C, 140 °C for 1 min. The flow rate of the helium carrier gas was 1.0 mL min^{-1}. The inlet temperature was 230 °C, and the combustion interface temperature was 940 °C. The GC/C-IRMS instrument was calibrated against CO_2 and an external TCE standard with a known carbon isotope ratio, which was obtained using a dual inlet-IRMS instrument. The carbon isotope values are reported in conventional delta notation relative to the Vienna Peedee belemnite (VPDB) standard. The $\delta^{13}C$ value is defined as

$$\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 1000, \quad (2)$$

where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ ratios of the sample and VPDB reference standard, respectively. The total uncertainty for the $\delta^{13}C$ measurements, incorporating both reproducibility and accuracy, was ±0.5‰. SPME extraction was found to be an efficient preconcentration technique, giving MDLs of 100–150 µg L^{-1}.

Results

Degradation kinetics

The degradation products (and intermediates) were consistent with those reported in the literature,[21] and included *cis*-1,2-dichloroethylene (*c*DCE), acetylene, ethene, small amounts of *trans*-1,2-dichloroethylene (*t*DCE) and vinyl chloride, and a trace amount of chloroacetylene, but these products were not quantified in this study. The *Control* experiment contained $[Ti(III)]_0$ at 6.65 mmol L^{-1} and no vitamin B_{12}. According to the

electronic supplementary information, the TCE $\delta^{13}C$ in the *Control* batch changed only within the analytical error range (±0.5‰) as did the TCE concentration (±10%). No changes in the TCE $\delta^{13}C$ value or concentration were, therefore, observed in the *Control* experiment.

Significant reductive dechlorination of TCE was observed when vitamin B_{12} was present. The rate of disappearance of the TCE during its dechlorination in aqueous vitamin B_{12} could be described using a pseudo-first-order equation (Eq. 3).

$$\ln\frac{C_t}{C_0} = -k_{obs}t \quad (3)$$

where C_t is the TCE concentration at reaction time t and C_0 is the initial TCE concentration. The pseudo-first-order rate coefficients (k_{obs}) for the experiments shown in Table 1 were calculated using Eq. 3. The 95% confidence interval (CI) was determined for the slope of each linear regression (each of which was forced through the origin). The statistical significance of each linear relationship was tested using the F-statistic (analysis of variance [ANOVA]). The linear models were considered to significantly fit the data at $P < 0.01$. All of the data were found to be significantly fitted using the linear models. The pseudo-first-order rate coefficients (k_{obs}) that were calculated are listed in Table 2.

Experiments *#1*, *#2*, and *#3* contained different vitamin B_{12} concentrations (Table 1). As the vitamin B_{12} concentration was increased from 32 to 159 μmol L^{-1}, the TCE dechlorination reaction constant k_{obs} increased from 0.068 to 0.355 h^{-1} (Table 2). Experiments *#1*, *#4*, and *#5* were conducted to determine the influence of temperature on TCE degradation by vitamin B_{12}. As the reaction temperature was increased from 20 to 40 °C, the TCE dechlorination reaction constant k_{obs} increased from 0.102 to 0.361 h^{-1} (Table 2). The effect of the initial solution pH on TCE dechlorination was investigated over the pH range 6.5–9.0 in experiments *#1*, *#6*, and *#7*, and we found that the pH had a significant effect on the degradation kinetics. The TCE dechlorination reaction constant k_{obs} increased from 0.091 to 0.322 h^{-1} as the pH was increased from 6.5 to 9.0 (Table 2).

Table 2 Pseudo-first-order rate constants (k_{obs}) and enrichment factors (ε) for TCE dechlorination by vitamin B_{12} under the experimental conditions shown in Table 1

ID	k_{obs} (h^{-1})	r^2	n	ε (‰)	r^2	n
#1	0.233 ± 0.008	0.99	6	−15.7 ± 0.3	1.00	9
#2	0.068 ± 0.003	0.99	6	−16.1 ± 0.5	0.99	8
#3	0.355 ± 0.011	1.00	6	−16.2 ± 0.4	1.00	8
#4	0.102 ± 0.005	0.99	6	−15.8 ± 0.5	0.99	8
#5	0.361 ± 0.013	0.99	6	−15.8 ± 0.5	0.99	8
#6	0.091 ± 0.004	0.99	6	−14.0 ± 0.4	0.99	8
#7	0.322 ± 0.010	1.00	6	−18.0 ± 0.3	1.00	8

The errors for k_{obs} and ε are the 95% confidence limits.

Isotope fractionation associated with degradation

The isotopic fractionation trends observed could be described using the Rayleigh fractionation model. The Rayleigh model[24] (Eq. 1) can be applied to irreversible reactions. In the work presented here, $\delta^{13}C$ and $\delta^{13}C_0$ are the isotopic compositions of the TCE at a given time and the initial time, respectively, and f is the remaining TCE fraction. According to the Eq. 1, the ε values were calculated by plotting ln$f$ against $\ln[(\delta^{13}C+1000)/(\delta^{13}C_0+1000)]$, and were used to compare the isotope fractionation magnitudes during the experiments. The 95% CI was determined for the slope of each linear regression, in ‰ units. The statistical significance of each linear relationship was tested using the F-statistic (ANOVA). The linear models were considered to significantly fit the data at $P < 0.01$. All of the data were found to be fitted significantly by the linear models. The slope m was determined using a least-squares regression, where $\varepsilon = m \times 1000$ (‰).

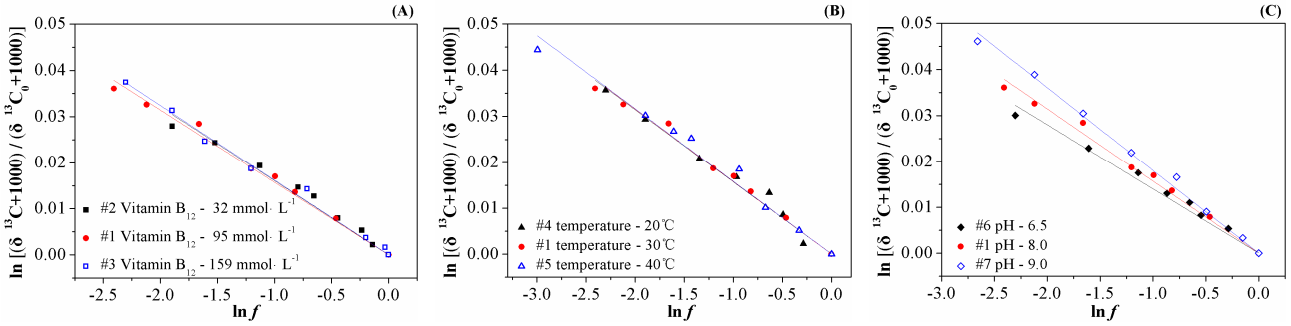

Fig. 1 Rayleigh plots for the carbon isotope fractionation during the reductive dechlorination of trichloroethylene by vitamin B_{12} under different experimental conditions (see Tables 1 and 2)

Fig. 1 shows the Rayleigh plots for the carbon isotope fractionation found during the reductive dechlorination of TCE by vitamin B_{12} under the different experimental conditions. Fig. 1A, B, and C demonstrate that there were good linear least-squares fits between ln$f$ and $\ln[(\delta^{13}C+1000)/(\delta^{13}C_0+1000)]$ using different vitamin B_{12} concentrations, different temperatures, and different initial pH values. The slopes of the regressions shown in Fig. 1 were used to calculate the ε values according to Eq. 1, and these are listed in Table 2. The ε values ranged from −14.0 ± 0.4‰ to −18.0 ± 0.3‰ (Table 2) under the different conditions used for the TCE dechlorination experiments. A significant degree of carbon fractionation was observed during the degradation of TCE by vitamin B_{12}.

Discussion

TCE molecules containing the lighter carbon isotope (^{12}C) tend to react more rapidly than TCE molecules containing the heavy stable isotope (^{13}C) during the reaction of TCE with vitamin B_{12}. The carbon isotope composition of TCE that has not reacted will,

therefore, change as the reductive dechlorination reaction proceeds. No significant changes in the TCE carbon isotope composition ($\delta^{13}C$) were observed when no vitamin B_{12} was present, and TCE was not consumed (see the the electronic supplementary information). These results prove that the dechlorination of TCE could not occur without vitamin B_{12}.

TCE can be reduced microbially, and enzymes containing a tetrapyrrole cofactor, such as cobalamin (vitamin B_{12}), are thought to be involved in dehalogenation reactions.[25] Vitamin B_{12} is believed to be the key species in the catalytic cycle in the presence of a stoichiometric, sacrificial reducing agent, such as titanium (III).[26] TCE was clearly consumed in experiments *#1–#7* when vitamin B_{12} was present, and the pseudo-first-order rate coefficients k_{obs} were 0.068–0.361 h^{-1} (Table 2). As is shown in Table 2, the ε values ranged from −14.0‰ to −18.0‰. This indicates that the notable reductive dechlorination of TCE by vitamin B_{12} was accompanied by strong carbon isotope fractionation. Significant carbon isotope fractionation during the dechlorination of TCE has also been observed in previous studies,[12,17,27] in which ε values of from −10.9‰ to −22.9‰ were found.

Liang et al. suggested that differences in isotope fractionation can be used to distinguish between two different dechlorination processes.[11] However, if isotope fractionation is to be used to predict or monitor one of the degradation processes, the isotopic enrichment factor of a contaminant during this process or pathway must be reproducible. It is therefore necessary to understand the influence of important factors, such as the vitamin B_{12} concentration, reaction temperature, and pH, which control the activity of the tetrapyrrole cofactor, on isotope fractionation. As is shown in Table 2, the reaction efficiency was dependent on the vitamin B_{12} concentration, temperature, and pH when there was an excess of the substrate and the electron donor. Nevertheless, there was no notable relationship between the ε values and the reaction rate constants k_{obs} (Fig. 2). Specifically, the k_{obs} varied between 0.068 and 0.361 h^{-1} in experiments *#1–#5*, but the ε values only varied between −15.7‰ and −16.2‰, with a mean of −15.9 ± 0.2‰. Previous studies have indicated that the degradation rate does not seem to have a significant impact on the ε value.[8,19,28,29] Elsner et al. found that kinetic isotope effects (KIEs) do not generally directly correlate with reaction rates. Isotope effects arise only from those changes in molecular energy that are mass-sensitive, in particular from changes in vibrational energies.[14] Consequently, although the experimental conditions such as the vitamin B_{12} concentration and the reaction temperature controlled the reaction rate, the ε values were reproducible.

As is shown in Fig. 3, the ε values changed significantly as the pH was increased from 6.5 to 9.0. Slater et al. found carbon ε values for the TCE dechlorination by vitamin B_{12} at pH 8.8 of −17.2‰ and −16.6‰.[9] These ε values are lower than those we found in experiment *#7*, in which the solution pH was 9.0. Variability in carbon isotope fractionation caused by different initial solution pH values has also been observed in studies of the reductive dechlorination of tetrachloroethylene by vitamin B_{12} (−13.2‰ at pH 8.0 and −16.2‰ at pH 8.8).[9,30]

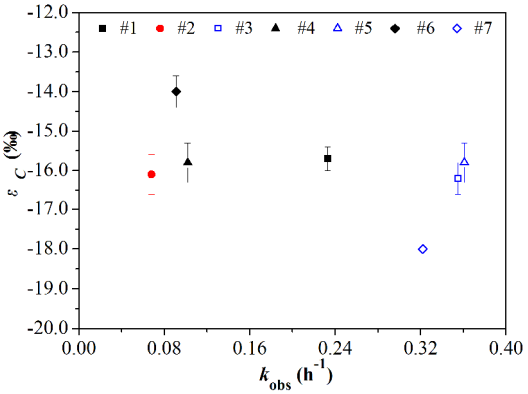

Fig. 2 Trichloroethylene isotope enrichment factor (ε) plotted against the pseudo-first-order rate constant (k_{obs})

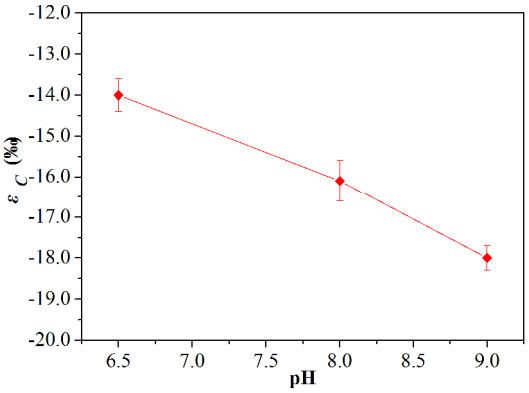

Fig. 3 Trichloroethylene isotope enrichment factors (ε) plotted against the initial solution pH

Systematic differences in ε values can be attributed to bonds involving the light carbon isotope, ^{12}C (with reactions described by the rate constant ^{12}k) being more readily broken than are bonds involving ^{13}C (with reactions described by the rate constant ^{13}k). The intrinsic KIE_C (subscript C indicating that it is for carbon), can be defined as shown in Eq. 4,

$$KIE_C = \frac{^{12}k}{^{13}k} \quad (4)$$

and it is often strongly dependent on the reaction mechanism as well as the strength of the bonds being broken or formed.[10] Except for the differences in the ε values, the results may be in good agreement in terms of the reaction pathways. Fig. 4 shows the proposed pathways for the reduction of TCE by vitamin B_{12} using titanium citrate as the bulk reductant, which have been modified from previous work.[21,23,31,32] The formation of products *c*DCE and *t*DCE and the chloroacetylene intermediate in these pathways has been supported by their direct detection.[21] Gold et al. found that the solution pH influences the proportions of the products formed.[23] This means that different degradation pathways may be dominant at different initial solution pH values, and this means that different solution pH values will cause different ε values.

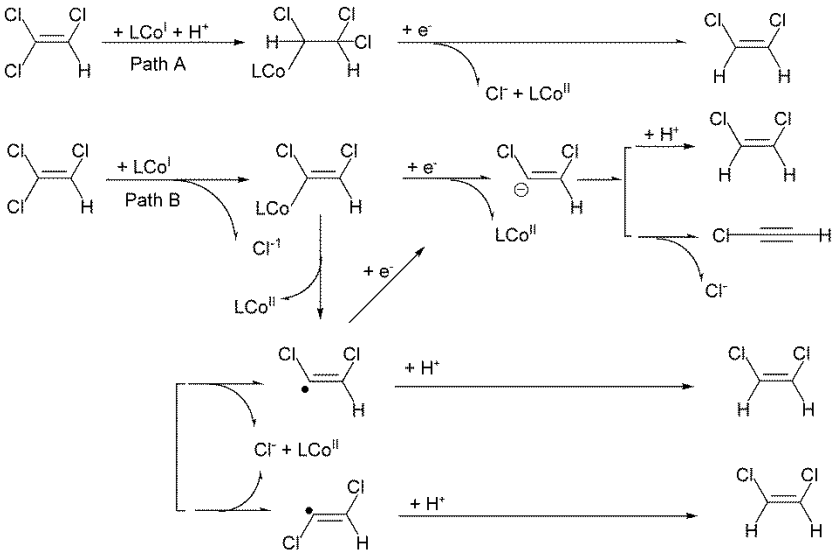

Fig. 4 Simplified schematic of the reductive dechlorination of trichloroethylene by vitamin B_{12}.

Previous experiments have indicated that the primary pathways involved in TCE reduction by vitamin B_{12} are reductive hydrogenolysis (Path A) and β-elimination (Path B) (Fig. 4). As can be seen in Fig. 4, the cob(I)alamin nucleophile can attack TCE and form either a 1,2,2-trichloroethyl complex (Path A) or eliminate chloride to form a dichlorovinyl complex (Path B). There are two ways for dichlorovinyl anions to be formed from the reduced dichlorovinyl complex, direct heterolytic cleavage of the Co–C bond, or homolytic cleavage of the Co–C bond followed by fast in-cage electron transfer from Co^{I} to the dichlorovinyl radical.[33] Dichlorovinyl anions and the dichlorovinyl radical have several possible fates, forming the products *c*DCE, *t*DCE, or chloroacetylene. Carbon isotope fractionation is affected when the isotope of interest is present in two positions in the TCE molecule, of which only one site is reactive (Fig. 4). This leads to one of the carbon isotopes not directly taking part in the reaction, and the bulk ε values becoming smaller because of "dilution" effects by the isotopes at nonreactive positions. To address the meaning of and the variability in the experimental ε values, the ε values need to be related to the apparent kinetic isotope effect (AKIE), using Eq. 7.[24]

$$\mathrm{AKIE}=\frac{1}{1+z\ n\ \varepsilon/x\ 1000}, \qquad (5)$$

where z is the number of atoms that are in intramolecular competition, n is the total number of atoms that are in the molecule, x is the number of atoms that could experience isotope effects in the given mechanistic pathway, and ε is experimentally observed. The reactions were investigated by calculating the $AKIE_C$ (i.e., for carbon) values. For our study, z, n, and x were 1, 2, and 1, respectively, according to Eq. 5. Using the experimentally observed ε values (Table 2), the $AKIE_C$ values were 1.029–1.037. These can be compared with the KIE_C values for the well-defined reactions described above. The theoretical value for intrinsic carbon kinetic isotope effects during C-Cl bond cleavage (KIE_C) is ~1.03, based on the rough estimation of 50% bond cleavage in the transition state. The value of KIE_C 1.057 in Elsner et al. is the maximum value based on 100% bond cleavage, and, assuming that bond cleavage is the rate limiting step, determined that the AKIE and KIE values should be the same.[14] However, the theoretical KIE_C value for 100% C–Cl bond cleavage was higher than the $AKIE_C$ derived from our experimental carbon ε values. This indicates that hydrogenolysis (Path A) is a competitive degradation pathway and has a clear influence on carbon isotope fractionation.

As shown in Fig. 4, both of the two primary pathways are involved in the redox potential. Glod et al. found that the reduction potential of Ti(IV)/Ti(III) was strongly pH-dependent in the pH range used in our experiments.[34] The reduction potential of vitamin B_{12} itself is not pH-dependent in this pH range, but reduced vitamin B_{12} exists in its base-on form at pH>3.[34] Furthermore, Zehnder and Wuhrmann showed that increasing the pH increases the reducing potential of titanium (III) citrate, causing the active cobalt (I) catalyst to regenerate more quickly.[35] This is the reason why the pseudo-first-order rate constants (k_{obs}) increased as the pH increased in our study (Table 2). In relation to the potential for hydrolysis, the intermediate chlorinated acetylenes have been shown to hydrolyze in hydroxylic solvents under alkaline conditions via nucleophilic attack at the Cl.[21] The hydroxyl groups are likely to act as bridging ligands between titanium and cobalt, providing a pathway for electron transfer.[36] This will lead to the proportion of β-elimination increasing as the solution pH increases. The important phenomenon seen in our study was that carbon isotope effects were stronger at higher solution pH values than at relatively low pH values (Fig. 4). Elsner et al reevaluated ε values for typical reductive cleavage reactions of C–Cl bonds from the literature and found $AKIE_C$ values of 1.02–1.03 (at pH 8.0–8.8).[9,14,30] These results are a little lower than the $AKIE_C$ value of

1.037 we found in this study (at pH 9.0). This indicates that different solution pH values cause carbon isotope effects that arise from changes in the balance between the competitive reductive hydrogenolysis (Path A) and β-elimination (Path B) degradation pathways.

Conclusion

We used cobalamin (vitamin B_{12}) as a model catalyst to assess carbon isotope fractionation caused by the reactive center of trichloroethylene. Our results imply that complexation of a chlorinated ethene to vitamin B_{12} is the rate-determining step, and, therefore, that significant carbon isotopic fractionation is associated with the reductive dechlorination of TCE by vitamin B_{12}. This fractionation can be described using a Rayleigh model, and ε values of −14.0‰ to −18.0‰ were found in all of the experiments we performed. The observation of such a large ε value for this reaction indicates that isotopic analysis can provide clear evidence of the occurrence of reductive dechlorination by vitamin B_{12} in groundwater remediation applications.

Batch experiments were performed to determine whether rate limitation plays a role in isotope fractionation during the dechlorination of TCE by vitamin B_{12}. The degradation rate does not seem to significantly affect the observed ε value. The k_{obs} changed from 0.068 to 0.361 h^{-1} at different vitamin B_{12} concentrations and reaction temperatures, but the ε values ranged only from −15.7‰ to −16.2‰, with a mean of −15.9 ± 0.2‰. Such a reproducible ε value may be particularly useful in estimating the extent of degradation through reactions for which mass balances are difficult to achieve. Nevertheless, if the ε values vary with the reaction conditions (such as the solution pH) the estimation needs to be performed taking the reaction conditions that influence the rate limiting pathways into account.

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Notes and references

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