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Carbon and Hydrogen Isotope Fractionation of Benzene, Toluene, and *o*-Xylene During Chemical Oxidation by Persulfate

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Abstract

Experiments were performed to investigate the carbon and hydrogen isotope fractionation of benzene, toluene, and *o*-xylene (BTX) during chemical oxidation by unactivated persulfate at two concentrations (8 and 20 g/L). Carbon enrichment (ε_C) values of -1.7 ± 0.1‰ for benzene, -0.64 ± 0.1‰ for toluene and -0.36 ± 0.04‰ for *o*-xylene were obtained. No significant hydrogen enrichment (ε_H) was observed for benzene, while the hydrogen enrichment for toluene and *o*-xylene were -20 ± 3‰ and -23 ± 2‰, respectively. The dual isotope plot ($\Delta\delta^{13}C$ vs. $\Delta\delta^{2}H$) for benzene and *o*-xylene revealed a distinct fractionation trend compared to the majority of the biodegradation data compiled from the literature; however, no unique trend was observed for toluene. The significant carbon and/or hydrogen enrichment, and the distinct trend observed on the dual isotope plot suggest that compound specific isotope analysis (CSIA) can potentially be used to monitor the chemical oxidation of BTX by persulfate, and to distinguish treatment areas where persulfate or biodegradation reactions are occurring for benzene and *o*-xylene.

Introduction

Benzene, toluene, ethylbenzene, and xylenes (BTEX) are the most common petroleum hydrocarbon compounds (PHC) found in groundwater at contaminated sites (USEPA 2004). Due to their widespread presence, toxicity, and tendency to persist (particularly benzene) in soil and groundwater, remediation of these compounds is critical (Zogorski et al. 2006). Among the suite of remediation technologies available for consideration, in situ chemical oxidation (ISCO) has become a remedy of choice for the treatment of source zones and dissolved plumes (Siegrist et al. 2011; USEPA 2013).

ISCO involves the injection or release of a chemical reagent into the subsurface with the potential for mineralization of target organic compounds. Historically, strong oxidants such as Fenton's reagent (hydrogen peroxide with ferrous iron), permanganate, and ozone have been applied (Nelson and Brown 1994; Ferguson et al. 2004; Tiburtius et al. 2005). Fenton's reagent or catalyzed hydrogen propagation (CHP) reactions with target organic compounds are usually fast but are often affected by competing reactions, and the in situ persistence of these oxidants is limited (Huling et al. 1998; Petigara et al. 2002; Xu and Thomson 2010). While permanganate has a greater in situ persistence than peroxide, it is incapable of treating some PHCs (Huling and Pivetz 2006), and is typically consumed by competing reactions with other naturally present reductants (e.g., natural organic matter, and Fe or Mn species) which affects treatment efficiency (Siegrist et al. 2001; Xu and Thomson 2008). The effectiveness of ozone is inhibited by poor in situ gas phase distribution, and hence reaction rates in the aqueous and gas phases are mass transfer-limited (Ahlfeld et al. 1994; Brooks et al. 1999; Clayton 2000; Thomson and Johnson 2000; Tomlinson et al. 2003; Chao et al. 2008).

Persulfate, which also has a greater in situ persistence than peroxide, has recently been shown to degrade BTEX and several other volatile organic compounds (VOCs), including chlorinated compounds. Persulfate can be used alone (Watts and Teel 2006; Sra et al. 2013a, 2013b) or in combination with activation schemes involving chelated metals, heat, peroxide, or alkaline conditions that result in the generation of a suite of energetic species (Huang et al. 2002; Forsey 2004; Liang et al. 2004, 2008). A potential benefit of using persulfate is that dissolved sulfate is generated from the decomposition of persulfate, which can enhance subsequent biodegradation processes that rely on sulfate reducing bacteria (Kulik et al. 2006; Sutton et al. 2010; Cassidy et al. 2015; Shayan et al. 2017).

The performance evaluation of a persulfate-based ISCO treatment system is typically confounded by other processes (e.g., sorption, biodegradation or dilution) that may decrease the concentration of the target contaminants (Thomson et al.

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2007, 2008). In addition, contaminant rebound as a result of dissolution from remaining source mass can also limit performance assessment efforts (Thomson et al. 2007, 2008) and hence the interpretation of concentration data alone may be ambiguous (Hunkeler et al. 2003).

Compound specific isotope analysis (CSIA) has been extensively explored as a potential tool to investigate the efficacy of biotic and abiotic treatment systems, and to elucidate different degradation pathways of PHCs and chlorinated organic compounds (Hunkeler et al. 2001, 2003; Poulson and Naraoka 2002; Marchesi et al. 2012, 2013; Wijker et al. 2013). During biotic or abiotic degradation of an organic compound the lighter isotopes (e.g., ^{12}C or ^{1}H) may react faster leading to a fractionation and a subsequent enrichment of the heavier isotopes (e.g., ^{13}C or ^{2}H) in the residual contaminant (Hunkeler et al. 1999; Aelion et al. 2009). Thus there is a need to evaluate the potential magnitude of isotopic fractionation generated by the reaction between BTEX and persulfate to determine whether it is differentiable from the fractionation generated by other mass reduction processes (e.g., biodegradation). If persulfate and biodegradation reactions produced similar carbon and hydrogen enrichment factors this would confound the interpretation of the role of persulfate in situations where biodegradation processes are already occurring.

CSIA can also be used to identify if non-aqueous phase liquids (NAPLs) remain in a treatment zone following an injection episode. If residual NAPL remains after treatment, the isotopic signature of the dissolved PHCs will shift back from enriched to initial (background) values (Hunkeler et al. 2003; Marchesi et al. 2012). In situations where dissolved PHC plumes are of interest, CSIA data can be used to estimate the extent of in situ biodegradation that has occurred by applying the Rayleigh model (Hunkeler et al. 1999; Aelion et al. 2009) and to assess remedial system effectiveness. Moreover the use of CSIA on two elements of a molecule, the so-called dual isotope approach (2D–CSIA), can be used to distinguish between processes which can potentially co-occur during treatment (Fischer et al. 2008, 2009; Palau et al. 2014; Tobler et al. 2008; Vogt et al. 2008). For example, Palau et al. (2014) showed significant differences for 2D–CSIA carbon and chlorine fractionation during 1,1,1-trichloroethane (1,1,1-TCA) transformation by heat activated persulfate, hydrolysis/dehydrohalogenation, and zero valent iron. Fischer et al. (2008) and Vogt et al. (2008) showed the potential of carbon and hydrogen 2D–CSIA to distinguish between aerobic and anaerobic degradation pathways of benzene and toluene, respectively.

In contrast to biodegradation processes, the fractionation of BTX during chemical oxidation has not been extensively reported in the literature to date (e.g., Ahad and Slater 2008; Wijker et al. 2013; Zhang et al. 2016). The objective of this study was to investigate the potential of carbon and hydrogen fractionation, and 2D–CSIA to assess the performance of chemical oxidation of BTX using unactivated persulfate. A series of batch experiments were conducted to estimate the ^{13}C and ^{2}H enrichment factors under two persulfate concentrations. 2D–CSIA plots of the generated ^{13}C and ^{2}H data were then compared to the aerobic and anaerobic degradation data available from the literature to evaluate the potential of the 2D–CSIA approach to differentiate between biodegradation and chemical oxidation using unactivated persulfate.

Materials and Methods

Experimental Design

Two series of aqueous batch experiments using different initial persulfate concentrations (8 and 20 g/L) were conducted at room temperature (20 °C) to quantify the carbon and hydrogen enrichment factors for BTX during chemical oxidation by persulfate. These initial persulfate concentrations are representative of the lower range of concentrations used in field applications (Siegrist et al. 2011) and were selected to yield slower reaction rates so that samples could be collected over a reasonable time period. Each series was prepared in duplicate using sacrificial 42 mL glass vials (reactors) filled with BTX and persulfate solutions to 40 mL. Two (2) mL of headspace was intentionally left to accommodate gas formation (e.g., CO_2). The BTX stock solution was prepared by injecting pure BTX compounds (benzene ≥99%, toluene ≥95%, and *o*-xylene ≥98%, Thermo Fisher Scientific, Fair Lawn, New Jersey) into a reservoir containing Milli-Q water and stirring for approximately 48 h. The sodium persulfate stock solution ($Na_2S_2O_8$, Sigma-Aldrich, St. Louis, Missouri) was also prepared in advance and stirred for 12 h in the dark. The BTX solution was added into the reactors followed by the sodium persulfate solution to obtain an approximate initial concentration of 25, 15 and 10 mg/L of BTX, respectively, and an initial persulfate concentration of 8 or 20 g/L. The reactors were immediately capped (Teflon septa) and stored in the dark. Between 6 and 10 sampling episodes were performed over a period of 31 days. For each sampling episode, 2 reactors were analyzed for BTX concentration, 2 reactors for carbon isotopes, 2 reactors for hydrogen isotopes, and 1 treatment reactor for persulfate and pH. Data from the two reactors were averaged and used for data interpretation, while duplicate analysis of persulfate and pH were performed on the same reactor and the average reported. At each sampling episode 1 mL of saturated ascorbic acid solution (420 mM) was injected into the reactors to quench the remaining persulfate (Huling et al. 2011). The preferential reaction between ascorbic acid and persulfate prevented further BTX oxidation. Control reactors were prepared with BTX and Milli-Q water (instead of the persulfate solution) and handled in the same manner as the treatment reactors to ensure that other processes (e.g., volatilization) were not affecting BTX concentrations or isotopic composition during the 31-day experimental period.

Analytical Methods

BTX concentrations were quantified by gas chromatography (GC). The sample was first extracted with 2 mL of methylene chloride, and then injected (3 µL of solvent) into a HP 5890 GC-FID equipped with an auto-sampler and a DB-5 capillary column. Concentrations were adjusted according to the dilution resulting from the ascorbic acid or Milli-Q water (for controls) added. Residual persulfate analysis was conducted following Huang et al. (2002) by adding a 0.1 mL solution of 0.4 N ACS grade ferrous ammonium sulfate (Sigma-Aldrich), 0.6 N ammonium thiocyanate (J.T. Baker, Phillipsbourgh,

New Jersey) and sulfuric acid into 0.9 mL of Milli-Q water, and reading the absorbance with a spectrophotometer at a wavelength of 450 nm. For carbon and hydrogen isotope analysis, BTX were analyzed using a purge and trap system (Teledyne Tekmar Dohrmann, Mason, Ohio) coupled to an isotope ratio mass spectrometer through a GC (GC-IRMS, Thermo Scientific) following the operational procedures described by Hunkeler and Aravena (2000). Each sample was analyzed in duplicate. Reference samples were sequentially analyzed to ensure measurement stability. Average analytical errors for carbon and hydrogen isotope of 0.3‰ and 5‰, respectively, were obtained for the reference samples.

Data Analyses

Isotopic ratios are expressed by the $\delta^{13}C$ and $\delta^{2}H$ notations in units of permil (‰) deviation from the international standard Vienna Pee Dee Belemnite (V-PDB) and Vienna—Standard Mean Ocean Water (V-SMOW). For carbon, this is expressed as

$$\delta^{13}C = [(^{13}C/^{12}C_s - {}^{13}C/^{12}C_{std})/(^{13}C/^{12}C_{std})] \quad (1)$$

where $^{13}C/^{12}C_s$ and $^{13}C/^{12}C_{std}$ is the isotope ratio of the sample and the standard, respectively. The value obtained was then multiplied by 1000 to conveniently express it in units of permil (‰). The Rayleigh equation (Clark and Fritz 1997; Hunkeler et al. 1999) was used to estimate fractionation factors as expressed by the following for carbon

$$\ln(R_t/R_o) = (\alpha - 1)\ln(C_t/C_o) \quad (2)$$

where R_t is the ratio of $(^{13}C/^{12}C)_t$ and R_o is the ratio of $(^{13}C/^{12}C)_o$ at time t and $t=0$, respectively; C_o and C_t are the initial concentration and the remaining concentration of the compound at time t, respectively; and α is the fractionation factor. The fractionation factor was determined from the slope of the best linear least-squares fit to the Rayleigh equation and not forcing the fit through the origin as suggested by Scott et al. (2004). Since the fractionation factor is small, the value can be transformed to an enrichment factor (ε) expressed in permil (‰) by multiplying (α-1) by 1000.

To provide critical information that can be used to distinguish between the different mass degradation processes potentially occurring, a dual carbon and hydrogen isotope plot was constructed. The data pair plotted are the $\Delta\delta^{13}C$ and $\Delta\delta^{2}H$ values which represent changes in isotope fractionation from $t=0$ to time t (e.g., $\Delta\delta^{13}C = \delta^{13}C_t - \delta^{13}C_o$), and the slope of the best linear fit is used to estimate the ratio of $\Delta\delta^{13}C/\Delta\delta^{2}H$ (denoted by Λ).

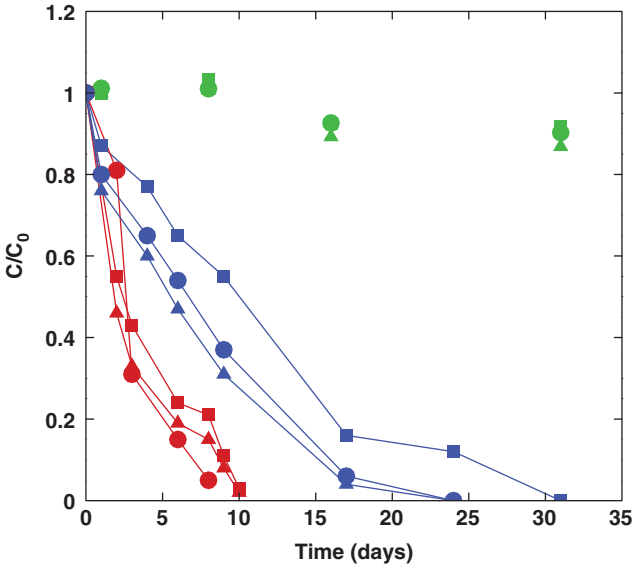

Figure 1. Temporal benzene (■), toluene (▲) and *o*-xylene (●) concentration profiles from the control and treatment reactors. Green symbols are data from the control series, while blue and red symbols are data from the 8 and 20 g/L persulfate concentration series, respectively. Each data point represents the average from duplicate reactors.

Results and Discussion

Extent of Carbon and Hydrogen Isotope Fractionation

BTX concentrations did not change significantly in the control reactors, suggesting that potential mass loss processes (e.g., volatilization, biodegradation) were insignificant over the experimental period (Figure 1). Almost complete destruction of BTX was observed in both treatment series with an overall decrease in concentration between 95 and 99% (Figure 1 and Table 1). Toluene and *o*-xylene were

Table 1

Change in $\delta^{13}C$ and $\delta^{2}H$ Observed in the Presence of Persulfate and Associated Enrichment Factors

			$\delta^{13}C$ (‰)[1]			$\delta^{2}H$ (‰)		
Compound	Persulfate (g/L)	Degradation	Initial	Final	ε_C (‰)[2] (r^2)[3]	Initial	Final	ε_H (‰) (r^2)
Benzene	8	97%	−28.1 ± 0.3	−21.5 ± 0.5	−1.7 ± 0.2 (0.96)	−72 ± 5	−63 ± 5	–[4]
	20	98%	−28.6 ± 0.2	−22.8 ± 0.6	−1.7 ± 0.2 (0.95)	−68 ± 2	−67 ± 3	–[4]
Toluene	8	98%	−27.3 ± 0.3	−24.2 ± 0.5	−0.69 ± 0.12 (0.94)	−73 ± 5	+10 ± 5	−22 ± 2 (0.99)
	20	>99%	−27.5 ± 0.3	−25.6 ± 0.4	−0.59 ± 0.14 (0.86)	−61 ± 3	+52 ± 2	−19 ± 4 (0.92)
o-Xylene	8	95%	−30.7 ± 0.1	−29.6 ± 0.3	−0.36 ± 0.09 (0.90)	−111 ± 5	−44 ± 5	−24 ± 3 (0.99)
	20	>99%	−30.6 ± 0.1	−28.7 ± 0.3	−0.36 ± 0.07 (0.94)	−116 ± 8	−12 ± 3	−22 ± 3 (0.96)

[1]Uncertainty corresponds to the standard deviation (n = 10) for different dilutions from duplicate reactors.
[2]Uncertainty corresponds to the 95% confidence interval obtained from the regression analysis.
[3]Coefficient of determination in brackets.
[4]No significant isotope fractionation observed.

preferentially oxidized relative to benzene, which is consistent with observations by Sra et al. (2013b). Similar to previous studies (Huang et al. 2005; Liang et al. 2008; Sra et al. 2013b), persulfate concentration decreased on average by 20% in both treatment series over the experimental period. The pH decreased from 4.1 to 2.3 after 5 days due to the H^+ produced from persulfate consumption, and remained stable until the end of the experimental period. A low pH (< 3) can further enhance the oxidation rate through acid catalyzation of persulfate and formation of peroxymonosulfate (House 1962).

The control series showed no significant change in $\delta^{13}C$ and δ^2H values over the experimental period. The average values for BTX were -28.3 ± 0.3‰, -27.4 ± 0.4‰ and -30.6 ± 0.1‰ for $\delta^{13}C$, and -70 ± 5‰, -67 ± 6‰ and -113 ± 9‰ for δ^2H, respectively. These values were adopted as the initial isotopic composition used to estimate the fractionation factors.

Increases in $\delta^{13}C$ and δ^2H values were observed in both treatment series throughout the experiment period (Table 1). For example, the $\delta^{13}C$ value increased by 6.6 ± 0.8‰ for benzene, 3.1 ± 0.8‰ for toluene, and 1.1 ± 0.4‰ for *o*-xylene for the 8 g/L series. The maximum increase in δ^2H was similar for toluene and *o*-xylene (113 ± 5‰ and 104 ± 11‰, respectively) while no significant enrichment was observed for benzene. These observed increases in the $\delta^{13}C$ and δ^2H values in the residual BTX indicate that carbon and hydrogen isotope analyses can be used to estimate the degradation of BTX compounds resulting from chemical oxidation using persulfate.

The good fit ($r^2 > 0.86$) between ln (C_t/C_0) and the isotopic shift for both carbon and hydrogen confirmed that the observed enrichments followed the Rayleigh model (Table 1 and Figure 2). Since there was no statistically significant difference between the enrichment factors determined for the 8 and 20 g/L series, the data were pooled and a single enrichment factor was determined. This resulted in a carbon enrichment (ε_C) value of -1.7 ± 0.1‰ ($r^2 = 0.96$) for benzene, -0.64 ± 0.1‰ ($r^2 = 0.88$) for toluene and -0.36 ± 0.04‰ ($r^2 = 0.93$) for *o*-xylene, and a hydrogen enrichment (ε_H) value of -20 ± 3‰ ($r^2 = 0.93$) for toluene and -23 ± 2‰ ($r^2 = 0.97$) for *o*-xylene. Overall, the estimated ε_C (for benzene) and ε_H (for toluene and *o*-xylene) values are deemed to be sufficiently significant to suggest the potential of CSIA to monitor the progress of chemical oxidation of BTX by unactivated persulfate in the field. Moreover, since there was no significant difference in enrichment factors for the two persulfate to BTX concentration ratios used in this study, the persulfate concentration may not influence the magnitude of the enrichment, a necessary prerequisite for using $\delta^{13}C$ and δ^2H data in the field.

Carbon and hydrogen enrichment factors for benzene biodegradation have been extensively reported in the literature with significant variation, for example, ε_C from -1.4‰ to -3.5‰ and ε_H from no significant fractionation to -13‰, for aerobic conditions (Hunkeler et al. 2001), and from -0.6‰ to -4.3‰ and -11‰ to -75‰ for anaerobic conditions (Mancini et al. 2003, 2008; Fischer et al. 2008, 2009; Bergmann et al. 2011). Reported enrichment factors for toluene are less constrained with respect to aerobic and anaerobic

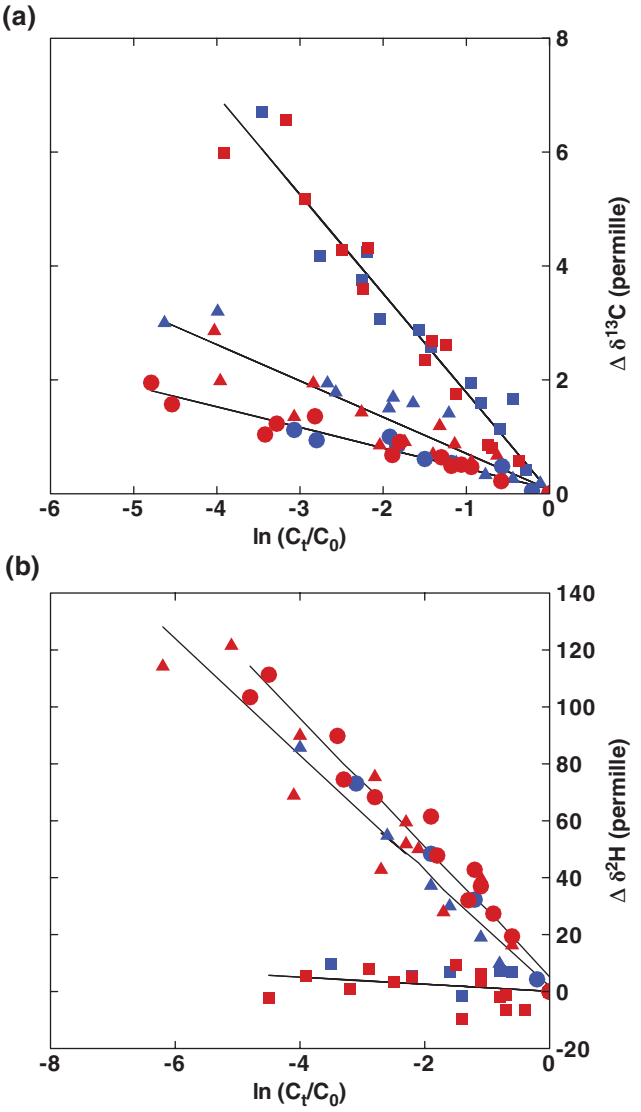

Figure 2. Linearized plot of (a) $\Delta\delta^{13}C$ and (b) $\Delta\delta^2H$ enrichment vs. normalized concentration for benzene (■), toluene (▲) and *o*-xylene (●) for each sample analyzed. Blue and red symbols represent data from the 8 and 20 g/L persulfate concentration series, respectively. The solid lines are the best linear fit to the pooled data from each series.

conditions, with variations from -0.4‰ to -5.6‰ for carbon and from -2‰ to -159‰ for hydrogen (Mancini et al. 2006; Ahad and Slater 2008; Tobler et al. 2008; Vogt et al. 2008; Herrmann et al. 2009). The reported carbon and hydrogen enrichment under anaerobic (sulfate reducing) conditions for *o*-xylene vary from -0.7‰ to -8.1‰ and from -25‰ to -41‰, respectively (Richnow et al. 2003; Steinbach et al. 2004; Herrmann et al. 2009). The data compiled in Table 2 indicate that, in general, similar ε_C but different ε_H values have been reported for benzene biodegradation as compared to those estimated in this study for the chemical oxidation of benzene using unactivated persulfate. Carbon and hydrogen enrichment factors for toluene exposed to persulfate were in the range of those reported for toluene degradation under aerobic and anaerobic conditions. Despite the limited data available for ε_C and ε_H for *o*-xylene, a slightly lower ε_C and comparable ε_H were observed during the degradation of

Table 2

Carbon and Hydrogen Enrichment Factors and Λ (=$\varepsilon_H/\varepsilon_C$) Values Representative of Chemical Oxidation and Biodegradation of BTX Compiled from the Literature

Compound	System[1]		ε_C (‰)	ε_H (‰)	$\Lambda^2 \approx \varepsilon_H/\varepsilon_C$	Reference
Benzene	Persulfate		−1.7 ± 0.1	No enrichment (± 5‰)	NA[2]	This study
Benzene	Pure culture (Acinetobacter sp)	Aerobic	−1.4 ± 0.06	−12.8 ± 0.7	9 to 9.2	Hunkeler et al. (2001)
	Pure culture (Burkholderia sp)	Aerobic	−3.5 ± 0.25	−11.2 ± 1.8	2.5 to 4	
Benzene	Enrichment culture	Nitrate reducing	−2.4 ± 0.1 and −2.2 ± 0.4	−29 ± 4 and −35 ± 6	10 to 14.3 and 11 to 23	Mancini et al. (2003)
		Sulfate reducing	−3.6 ± 0.3	−79 ± 4	19 to 25	
		Methanogenic	−1.9 ± 0.1 and −2.1 ± 0.1	−60 ± 3 and −59 ± 4	28.5 to 35 and 25 to 31.5	
Benzene	Enrichment culture	Nitrate reducing	−2.8 ± 0.6	−31 ± 7 to −47 ± 11	9.2 to 31.6 and 10.6 to 26.4	Mancini et al. (2008)
		Methanogenic	−0.8 ± 0.2 to −1.1 ± 0.1	−34 ± 8 to −38 ± 6	26 to 70 and 31.2 to 38.6	
Benzene	Pure culture (*Rh. opacus B-4)*	Aerobic	−1.3 ± 0.2	No enrichment (±5‰)	NA[3]	Fischer et al. (2008)
	Pure culture (*P. putida ML2)*	Aerobic	−0.7 ± 0.1	No enrichment (±5‰)	NA[3]	
	Pure culture (*R. pickettii PKO1)*	Aerobic	−1.7 ± 0.2	−11 ± 4	3 ± 1	
	Pure culture (*C. necator ATCC 17697)*	Aerobic	−4.3 ± 0.4	−17 ± 11	11 ± 6	
	Isolated from enrichment culture (*A. denitrificans strain BC)*	Aerobic	−2.6 ± 0.8	−16 ± 4	5 ± 2	
		Chlorate reducing	−1.5 ± 0.5	−18 ± 6	10 ± 4	
	Mixed culture	Sulfate reducing	−1.9 ± 0.3	−59 ± 10	28 ± 3	
Benzene	Enrichment culture	Sulfate reducing	−3 ± 0.4	−75 ± 8	23 ± 5	Fischer et al. (2009)
	Enrichment culture	Sulfate reducing	−1.6 ± 0.2	−49 ± 8	28 ± 3	
	Field sample	Sulfate reducing	−0.6 ± 0.2	−16 ± 5	24 ± 2	
Benzene	Enrichment culture (strain BF)	Iron reducing	−3.0 ± 0.5	−56 ± 8	17 ± 1	Bergmann et al. (2011)
	Enrichment culture (strain BPL)	Sulfate reducing	−2.5 ± 0.2	−55 ± 4	20 ± 2	
Benzene	UV/H_2O_2		−0.7 ± 0.1	−20 ± 2	24 ± 4	Zhang et al. (2016)
Toluene	Persulfate		−0.64 ± 0.1	−20 ± 3	35 ± 10	This study
Toluene	Pure culture (Pseudomonas putida mt-2)	Aerobic	−1.8 ± 0.2 to −2.5 ± 0.3	−97 ± 5 to −159 ± 11	46.5 to 63 and 53 to 77	Mancini et al. (2006)

Table 2 (*Continued*)

Compound	System[1]		ε_C (‰)	ε_H (‰)	$\Lambda^2 \approx \varepsilon_H/\varepsilon_C$	Reference
Toluene	Mixed culture	Methanogenic and sulfate-reducing	−0.5 and −0.8, respectively	NA[3]	NA[3]	Ahad et al. (2000)
Toluene	Pure culture (Geobacter metallireducens)	Anaerobic	−1.3 ± 0.1 to −3.6 ± 0.7	−34.6 ± 0.9 to −98.4 ± 3	24 to 29.6 and 22 to 35	Tobler et al. (2008)
Toluene	Pure strain (Rh. opacus B-4)	Aerobic	−1.8 ± 0.3	−2 ± 5	1 ± 2	Vogt et al. (2008)
	Pure strain (C. sphaerospermum)	Aerobic	−0.4 ± 0.2	−8.6 ± 3.7	16 ± 6	
	Pure strain (*P. putida mt-2)*	Aerobic	−2.8 ± 0.2	−140 ± 8	53 ± 5	
	Pure strain (T. aromatic)	Nitrate reducing	−2.7 ± 0.1	−35 ± 14	11 ± 5	
	Pure strain (Azoarcus T)	Nitrate reducing	−6.2 ± 1.1	−79 ± 21	11 ± 2	
	Pure strain (Azoarcus EbN1)	Nitrate reducing	−3 ± 0.1	−45 ± 15	14 ± 4	
	Pure strain (B. sulfoviridis)	Anaerobic	−4 ± 0.5	−23 ± 6	4 ± 3	
	Mixed culture (strain TRM1 and Zz 53–56)	Sulfate reducing	−2 ± 0.2 and −2.7 ± 0.2	−66 ± 19 and−88 ± 5	27 ± 4 and 31 ± 11	
Toluene	Pure culture (Desulfosarcina ovata)	Sulfate reducing	−2.5 ± 0.5	−107 ± 23	41 ± 8	Herrmann et al. (2009)
Toluene	Permanganate (50 mM)		−6 ± 0.4	−222 ± 9	34 ± 2.2	Wijker et al. (2013)
Toluene	UV/H_2O_2		−0.36 ± 0.05	−14 ± 2	34 ± 6	Zhang et al. (2016)
o-xylene	Persulfate		−0.36 ± 0.04	−23 ± 2	55 ± 9	This study
o-xylene	Consortium and pure culture (Desulfosarcina ovata)	Sulfate reducing	−0.7 ± 0.1 to −2.3 ± 0.4	−25 ± 3 to −41 ± 9	29 ± 5 to 15 ± 4	Herrmann et al. (2009)
o-xylene	Field sample	-	−2.6 ± 0.7	−29.6	9 to 15.5	Steinbach et al. (2004)
o-xylene	Field sample	Anaerobic	−8.1 ± 2.2	NA[3]	NA[3]	Richnow et al. (2003)
o-xylene	UV/H_2O_2		−0.27 ± 0.02	−3.2 ± 0.8	11 ± 4	Zhang et al. (2016)

[1]For biological systems the culture and redox conditions are provided if available in the cited reference.
[2]Λ value reported by authors, or calculated considering the lower and upper range of ε_C and ε_H reported.
[3]Not available.

o-xylene by persulfate compared to the majority of enrichment factors reported for *o*-xylene biodegradation.

In comparison with other chemical oxidants, Wijker et al. (2013) reported a large carbon and hydrogen isotope fractionation during the oxidation of toluene by permanganate, with enrichment factors of −6 ± 0.4‰ and −222 ± 9‰, respectively. In contrast, Ahad and Slater (2008) reported no significant carbon isotope fractionation of toluene during Fenton-like hydroxyl radical oxidation. Zhang et al. (2016) observed small carbon isotope enrichment factors during the oxidation of benzene, toluene and *o*-xylene by peroxide (H_2O_2) activated by UV light that are in the same range, particularly for toluene and *o*-xylene, with those observed in this study with persulfate (Table 2). In contrast, hydrogen enrichment factors for BTX reported by Zhang et al. (2016) are significantly different than those observed for persulfate (Table 2). Since enrichment factors are, in general, reaction mechanism specific it is not surprising that different chemical oxidants give rise to different values. There are numerous persulfate systems that can be employed (e.g., heat, chelated-Fe, alkaline) which potentially result in different degradation mechanisms (Liang et al. 2008; Furman et al. 2010), therefore the enrichment factors provided in this study should be considered relevant to unactivated persulfate applications.

Dual Carbon and Hydrogen Isotope Application (2D–CSIA)

The $\Delta\delta^{13}C$, $\Delta\delta^{2}H$, and Λ data for BTX obtained from this study were plotted along with Λ data for biodegradation processes (Figure 3). The biodegradation zones illustrated in Figure 3 were established using the smallest and the largest Λ value from the literature for aerobic and anaerobic conditions (Table 2). Note that the insignificant hydrogen enrichment factors reported for benzene by Fisher et al. (2008) were not used as these data are not reflective of field conditions.

For benzene, the $\Delta\delta^{13}C/\Delta\delta^{2}H$ plot shows that the relative large ε_C and negligible ε_H resulted in a distinct benzene isotope fractionation trend for chemical oxidation using unactivated persulfate compared to anaerobic and aerobic biodegradation processes (Figure 3(a)). The $\Delta\delta^{13}C/\Delta\delta^{2}H$ plot for toluene in the presence of unactivated persulfate generated a similar trend to the isotopic data expected for either anaerobic or aerobic biodegradation of toluene (generally under sulfate reducing conditions) (Figure 3(b)). This similarity in dual isotope trends limits the ability of the dual isotope approach to distinguish between the two mass removal processes for toluene. However, if the reaction kinetics between persulfate and toluene are assumed to be faster than biodegradation, then any change in the isotopic signature within a few weeks following persulfate injection could be used as an indication that chemical oxidation rather than biodegradation is degrading toluene mass. Carbon and hydrogen isotope data available in the literature for *o*-xylene for biodegradation processes are limited, especially for hydrogen under aerobic conditions. The $\Delta\delta^{13}C/\Delta\delta^{2}H$ plot shows that the trend observed for *o*-xylene oxidation by persulfate is distinct from trends expected for anaerobic biodegradation (Figure 3(c)). Except for toluene, such a distinction between the $\Delta\delta^{13}C/\Delta\delta^{2}H$ trends and Λ

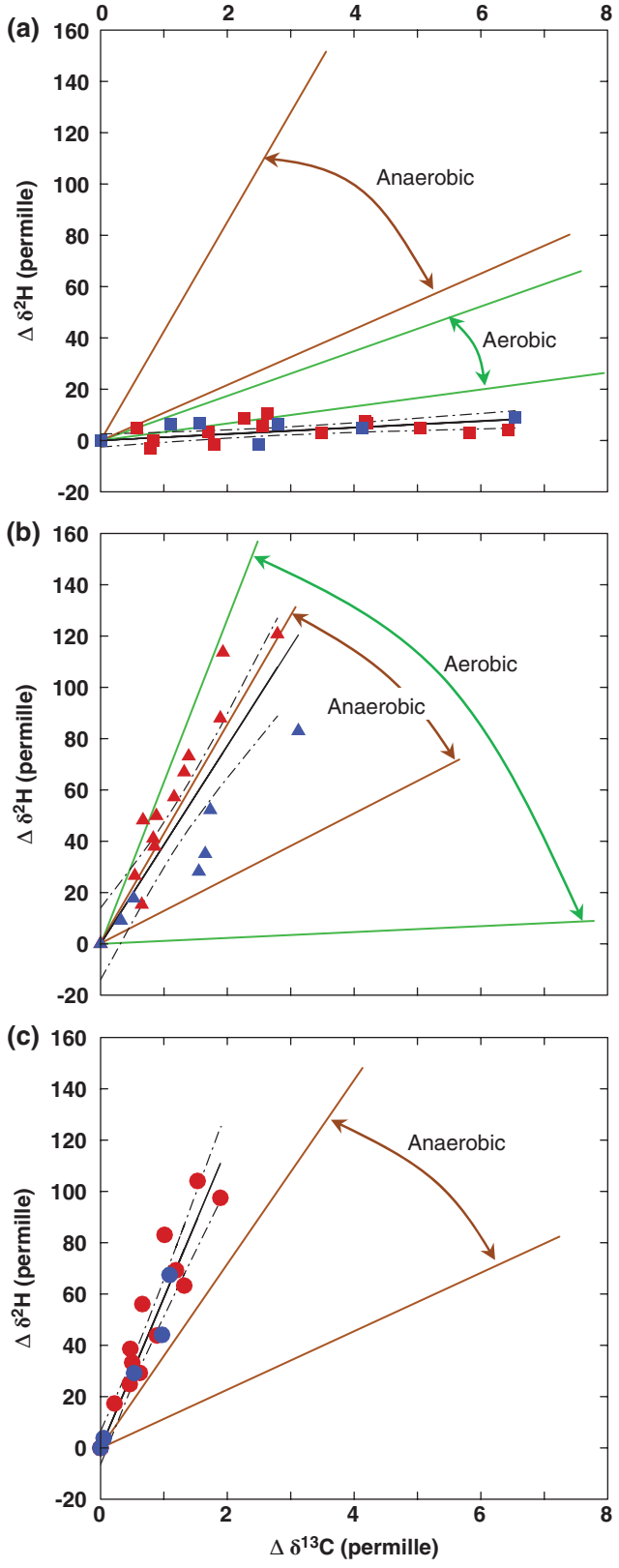

Figure 3. Dual $\Delta\delta^{13}C/\Delta\delta^{2}H$ isotope plot (2D–CSIA) for (a) benzene, (b) toluene, and (c) *o*-xylene. Blue and red symbols represent data from the 8 and 20 g/L persulfate concentration series, respectively. The solid black line represents Λ, and dashed lines represent the associated 95% confidence interval envelope for Λ. Also shown are aerobic (green lines) and anaerobic (brown lines) biodegradation zones established using the smallest and the largest Λ reported in Table 2.

(considering the 95% of confidence interval), suggests that the dual isotope approach can be used to distinguish the contribution of persulfate from biodegradation processes. The distinction between the dual isotope trend for persulfate and biodegradation under sulfate reducing conditions which could eventually be stimulated following persulfate injection also supports the potential for dual isotope trends to distinguish between these two processes.

Although it may not be possible to perform a detailed investigation of the degradation mechanism in complex systems using CSIA data only, we speculate that the lack of hydrogen isotope fractionation for benzene could be related to the absence of a C-H bond cleavage in the initial reaction step. Such absence or minor enrichment has also been observed for biological degradation of benzene (Gibson et al. 1968; Wilkins et al. 1994; Fischer et al. 2008). It has been suggested that in the initial step of benzene oxidation by persulfate an electron is abstracted from benzene to produce a short-lived cation radical (Norman et al. 1970; Huie and Neta 1984; Aravindakumar et al. 2003; Anipsitakis et al. 2006; Liu et al. 2015). In contrast to benzene, the hydrogen presents a greater tendency to get enriched relative to carbon during chemical oxidation of toluene and *o*-xylene by persulfate. This hydrogen enrichment suggests an initial reaction step involving a C-H cleavage in the alkyl group. This is in agreement with Long et al. (2014) who proposed hydrogen atom abstraction from the methyl group of toluene leading to a benzyl radical during oxidation in an iron activated persulfate system. A similar mechanism was proposed also for permanganate oxidation although with a greater hydrogen enrichment of −222‰ (Wijker et al. 2013). Additional studies are required to elucidate the mechanism of chemical oxidation of BTX compounds using persulfate.

Summary

This study quantified carbon and hydrogen isotope fractionation during the chemical oxidation of BTX by unactivated persulfate. Carbon enrichment (ε_C) values of -1.7 ± 0.1‰ for benzene, -0.64 ± 0.1‰ for toluene and -0.36 ± 0.04‰ for *o*-xylene were obtained. No significant hydrogen enrichment (ε_H) was observed for benzene, while toluene and *o*-xylene presented values of -20 ± 3‰ and -23 ± 2‰, respectively. This investigation showed that for the persulfate to BTX concentration ratios investigated, there were no significant differences in the magnitude of the observed isotope enrichment. The significant ε_C and/or ε_H indicates the utility of CSIA to monitor the chemical oxidation of BTX by unactivated persulfate.

The potential to use a dual isotope plot to distinguish different mass destruction processes that might occur at a PHC contaminated site was investigated. The benzene and *o*-xylene data generated during the chemical oxidation by persulfate produced a distinct isotope fractionation trend compared to most of the biodegradation processes reported in the literature. This distinction is more evident when compared to biodegradation under sulfate reducing conditions which might occur following a persulfate application as a result of the sulfate generated. The dual isotope plot for toluene was coincident with the trends for aerobic and anaerobic biodegradation, the latter generally under sulfate reducing conditions. The distinct pattern associated with the oxidation of benzene and *o*-xylene by persulfate compared to biodegradation processes on the dual isotope plot supports the utility of the dual isotope approach to distinguish between oxidation by unactivated persulfate and biodegradation as the main contaminant removal process occurring in the field.

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