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In situ decay of polyfluorinated benzoic acids under anaerobic conditions

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ABSTRACT

Polyfluorinated benzoic acids (PBAs) can be used as non-reactive tracers to characterize reactive mass transport mechanisms in groundwater. The use of PBAs as non-reactive tracers assumes that their reactivities are negligible. If this assumption is not valid, PBAs may not be appropriate to use as non-reactive tracers. In this study, the reactivity of two PBAs, 2,6-difluorobenzoic acid (2,6-DFBA) and pentafluorobenzoic acid (PFBA), was tested in situ. A series of two single-well push-pull tests were conducted in two hydrogeologically similar, yet spatially distinct, groundwater monitoring wells. Bromide, 2,6-DFBA, and PFBA were added to the injection fluid and periodically measured in the extraction fluid along with chloride, nitrate, sulfate, and fluoride. Linear regression of the dilution-adjusted breakthrough curves of both PBAs indicated zero-order decay accompanied by nitrate and subsequent sulfate removal. The dilution-adjusted breakthrough curves of chloride, a non-reactive halide similar to bromide, showed no evidence of reactivity. These results strongly suggested that biodegradation of both PBAs occurred under anaerobic conditions. The results of this study implied that PBAs may not be appropriate to use as non-reactive tracers in certain hydrogeologic settings, presumably those where they can serve as carbon and/or electron donors to stimulate microbial activity. Future studies would benefit from using ring-¹⁴C-labeled PBAs to determine the fate of carbon combined with microbial analyses to characterize the PBA-degrading members of the microbial community.

1. Introduction

The use of tracers is important for the accurate characterization of physical, chemical, and biological processes in hydrologic settings. Davis et al. (1980) defined a tracer as matter or energy carried by water that will give information concerning the direction and/or velocity of the water as well as potential contaminants that could be transported by the water. Tracers can exist naturally or be released artificially and exist in many forms including dyes, halides, stable isotopes, radionuclides, colloids, bacteria, fluorocarbons, and heat (Abbott et al., 2016; Becker and Coplen, 2001; Davis et al., 1980; Flury and Wai, 2003; Herczeg and Leaney, 2011; Koeniger et al., 2016; Phillips, 1995; Rau et al., 2014; Thompson et al., 1974). Ideally, a hydrologic tracer should be non-toxic, inexpensive, easy to detect, high in specificity, and most importantly, non-reactive, meaning that it does not undergo transformations or attach to solids.

The in situ use of fluorocarbons as artificial and non-reactive

tracers, particularly polyfluorinated benzoic acids (PBAs), has been substantial in the past several decades due to their nearly-ideal characteristics (Bowman and Gibbens, 1992; Bowman and Rice, 1986; Dahan et al., 1999; Hu et al., 2001; Jaynes et al., 2001; Kung et al., 2000; Lu et al., 2011; Meigs and Beauheim, 2001; Queloz et al., 2015a; Reimus et al., 2003; Salve et al., 2004). PBAs can be especially useful for characterizing unique groundwater flow paths (Dahan et al., 1999; Jaynes et al., 2001; Kung et al., 2000). This is due to the many isomers of di-, tri-, and tetra-fluorobenzoic acids, and pentafluorobenzoic acid, all of which have nearly-identical transport characteristics yet are analytically discernable (Benson and Bowman, 1994; Hu and Moran, 2005). Perhaps the most common use of PBAs is to characterize diffusive mass transport from mobile to immobile pore and/or fracture matrices, i.e., matrix diffusion (Callahan et al., 2000; Hu and Brusseau, 1995; Hu et al., 2001; Lu et al., 2011; Meigs and Beauheim, 2001; Reimus et al., 2003; Salve et al., 2004). This is due to their relatively low aqueous diffusion coefficients with respect to commonly and

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concurrently used halide tracers such as bromide and chloride (Flury and Wai, 2003; Hu and Moran, 2005). However, it is well known that PBAs are not ideal under low-pH conditions where significant sorption has been observed onto soil organic matter (Benson and Bowman, 1994; Boggs and Adams, 1992; McCarthy et al., 2000; Seaman, 1998). Furthermore, there is relatively sparse, yet mounting, evidence that PBAs are susceptible to biodegradation under certain conditions (Cass et al., 1987; Nimmo et al., 1990; Queloz et al., 2015a; Queloz et al., 2015b).

A study by Cass et al. (1987) demonstrated biodegradation of 2,5-difluorobenzoic acid (2,5-DFBA) by *Pseudomonas putida* by showing that 85% of the total fluorine in 2,5-DFBA was biotransformed to fluoride ion in a laboratory experiment conducted with synthetic growth media under aerobic conditions. *P. putida*, once considered an obligate aerobe, has since been recognized as a ubiquitous and metabolically versatile bacterium with the genetic potential to degrade halogenated organic compounds under oxygen-limited conditions (dos Santos et al., 2004). More recently, *P. putida* strains have been isolated under anaerobic conditions (Freikowski et al., 2010; Pascual et al., 2015). Nimmo et al. (1990) demonstrated complete mineralization of ring-¹⁴C-labeled 2,6-difluorobenzoic acid (2,6-DFBA) to ¹⁴CO₂ in a laboratory experiment conducted with two different soils under aerobic conditions; it was assumed that degradation of 2,6-DFBA was microbial-mediated. More recently, Queloz et al. (2015a) provided evidence of microbial-mediated degradation of di- and tri-fluorobenzoic acids within highly-controlled and vegetated hydrologic mesocosms based on mass balance analyses; the experimental results were then corroborated with theoretical analyses and model simulations (Queloz et al., 2015b).

It is becoming clearer that PBAs are indeed susceptible to biodegradation in the laboratory and may also biodegrade under certain field conditions, presumably those where they can serve as carbon and/or electron donors to stimulate microbial activity. It is also clear that there is no such thing as a perfectly ideal tracer. Hu and Moran (2005) suggested that the transport behavior of any potential tracer under relevant geochemical conditions, preferably in the field, should be evaluated before beginning ambitious, large-scale field tracer experiments. The objective of this study was to test the in situ reactivity of four non-reactive tracers, bromide, chloride, 2,6-DFBA, and pentafluorobenzoic acid (PFBA), in a shallow, unconfined, and heterogeneous aquifer. The hypothesis of this study was that the PBAs would be susceptible to biodegradation. This hypothesis was informed by the characteristics of the aquifer that suggested biodegradation, as opposed to matrix diffusion or sorption, would likely be the dominant, non-ideal, mass transport/transformation mechanism.

2. Materials and methods

2.1. Study site

The study site is located within Area 2 of the United States Department of Energy (DOE) Oak Ridge Reservation (ORR) in Oak Ridge, Tennessee (Fig. 1). The subsurface within Area 2 is comprised of approximately 6 m of unconsolidated and heterogeneous fill materials, e.g., silty and clayey fill, related to historical construction activities, underlain by undisturbed and weathered bedrock (Moon et al., 2006; Watson et al., 2004) (Fig. 2). Slug and single-well pumping tests indicated that the hydraulic conductivity of the fill materials was approximately two orders of magnitude greater than the weathered bedrock, e.g., 10^{-6} versus 10^{-8} m/s (Paradis et al., 2018b) (Fig. 2). The study site contains 13 monitoring wells (FW218 through FW230), two of which were used as test wells (FW222 and FW224), and one as a source well (FW228) for the injection fluid for the push-pull tests (Fig. 1). The test wells were installed by direct push (Geoprobe® model 6610DT) using a 3.8-cm outside diameter drive casing. The test wells are constructed of 2.7-cm outside diameter and 1.9-cm inside diameter schedule-80 polyvinyl chloride (PVC) pipe (Fig. 2). The test wells are screened across the unconfined aquifer and in direct contact with the

fill materials from 3.7 to 6.1 m below ground surface (mbgs) (Fig. 2). The source well is constructed of 5.1-cm inside diameter PVC pipe and is screened within the fill materials. The shallow groundwater aquifer is unconfined and the depth to groundwater is approximately 3.5 mbgs. The average magnitude and direction of the hydraulic gradient is approximately -0.045 m/m and to the south/southwest, respectively (Fig. 1). Single-well pumping and push-pull tests indicated that the hydraulic conductivity and effective porosity of the porous media associated with test wells were relatively similar (Paradis et al., 2018a; Paradis et al., 2018b) (Table 1). Groundwater sampling indicated that the pH of the aquifer fluid associated with the test wells is near-neutral (Paradis et al., 2016) (Table 1). Microbial activity in Area 2 and in the monitoring wells of the study site is known to be limited by carbon and/or electron donors (Paradis et al., 2016; Watson et al., 2004).

2.2. Push-pull tests

The single-well push-pull tests were conducted according to the methodology of Istok (2013) and the data was analyzed according to the methodology of Paradis et al. (2018b). A push-pull test is conducted by injecting a volume of water containing one or more non-reactive and reactive tracers into a single well (push phase), followed by a non-pumping period (rest phase), and subsequent extraction of groundwater from the same well (pull phase). The extraction fluid is comprised of a mixture between the injection and aquifer fluids. The concentration of a reactive tracer in the extraction fluid can be adjusted for dilution based on the concentration of a non-reactive tracer to generate a dilution-adjusted concentration versus time elapsed profile (dilution-adjusted breakthrough curve). Any deviation of the dilution-adjusted breakthrough curve of the reactive tracer from its injected concentration can be attributed to reactivity if the mass transport characteristics both the non-reactive and reactive tracers, e.g., advection, mechanical dispersion, molecular diffusion, and sorption, are no different. Analysis of the dilution-adjusted breakthrough curve can be utilized to quantify the reaction rate of a tracer by fitting it an appropriate kinetic model, e.g., zero-order, first-order, Michaelis-Menton, etc.

Ten liters of groundwater were collected from the source well FW228 (Fig. 1) using a peristaltic pump and stored in a plastic carboy. Potassium bromide (KBr), 2,6-DFBA (C₇H₄O₂F₂), and PFBA (C₇HO₂F₅) (Sigma-Aldrich, Inc., > 98% purity) were added to the 10-liters of groundwater to create an injection fluid that contained 100 mg/L of bromide (Br⁻) and de-protonated 2,6-DFBA (C₇H₃O₂F₂⁻) and PFBA (C₇O₂F₅⁻). The injection fluid was thoroughly mixed by recirculation using a peristaltic pump. During mixing of the injection fluid, three samples were collected, filtered (0.2- μ m filter), stored in 20-mL scintillation vials, preserved at 4 °C, and promptly analyzed for bromide, chloride, 2,6-DFBA, PFBA, nitrate, sulfate, and fluoride by ion chromatography (Dionex™ ICS 5000⁺). Immediately prior to the injection, three samples were collected from the test well to determine the concentrations of bromide, chloride, 2,6-DFBA, PFBA, nitrate, sulfate, and fluoride in the aquifer fluid associated with the test well; these samples were processed identically to the injection fluid samples. The 10-liter injection fluid was pumped into the test well at approximately 250 to 500 mL/min. Immediately after the injection, a 35-minute resting, i.e., non-pumping, period occurred. Groundwater, comprised of a mixture of the injection and aquifer fluids, was then continuously extracted from the test well at approximately 125 mL/min until 22 to 24 l was collected. Samples were collected every liter for the first 10 l of extracted fluid and every two liters thereafter, e.g., at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, etc. liters extracted; these samples were processed identically to the injection and aquifer fluid samples.

The first push-pull test was conducted in test well FW222 because it is located approximately 6 m down –/side-gradient of test well FW224 (Fig. 1) and therefore unlikely to have influenced the aquifer fluid associated with test well FW224. The radius of influence of the injection fluid can be estimated from Istok (2013) as given by:

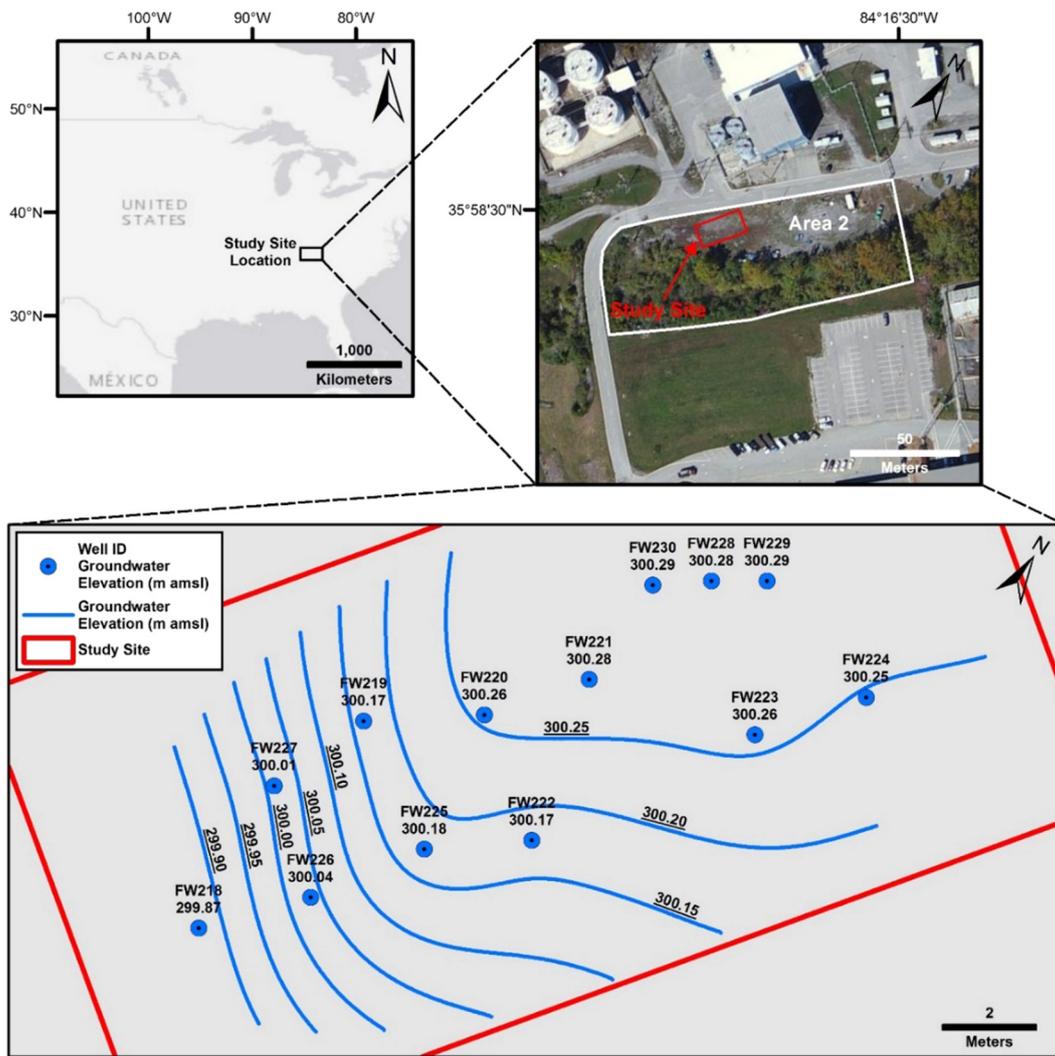


Fig. 1. Plan-view maps of the study site from Paradis et al. (2018a; 2018b), clockwise from upper left, country map showing study site location in the southeastern United States, area map showing study site location in Area 2 of ORR, and study site map showing location of test wells FW222 and FW224, source well FW228, groundwater elevations, and groundwater elevation isocontours, m amsl = meters above mean sea level.

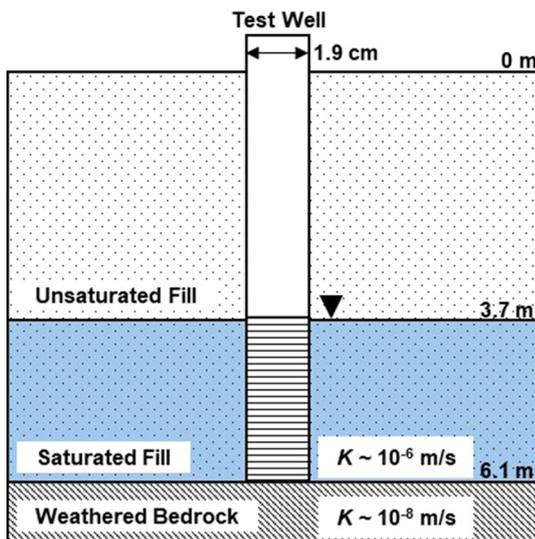


Fig. 2. Vertical-view conceptual model of the shallow, unconfined, aquifer and construction details of a test well from Paradis et al. (2018a), horizontal exaggeration is 50-fold.

Table 1

Hydraulic conductivity, effective porosity, and pH of test wells FW222 and FW224 from Paradis et al. (2016) and Paradis et al. (2018a; 2018b).

Parameter	FW222	FW224
Hydraulic conductivity	6.9×10^{-6} m/s	1.6×10^{-5} m/s
Effective porosity	3.3%	2.3%
pH	7.8	7.7

$$r_i = \left[\frac{V_i}{\pi b n_e} + r_w^2 \right]^{1/2} \quad (1)$$

where

- r_i = radius of influence [L]
- V_i = volume of injection fluid [L³]
- b = saturated aquifer thickness [L]
- n_e = effective porosity [dimensionless]
- r_w = radius of test well [L]

Eq. (1) assumes that the aquifer is confined, homogeneous, and isotropic and that the transport of the injection fluid is radial, governed by advection, and not affected by ambient groundwater flow.

Therefore, the radius of influence of the 10-liter injection fluid was approximately 0.25 m.

2.3. Data analysis

The dilution-adjusted breakthrough curves of bromide, chloride, DFBA, PFBA, nitrate, sulfate, and fluoride were generated according to the method by Paradis et al. (2018b) as given by:

$$C_e^{2*} = C_e^2 \left[\frac{C_i^2}{C_e^{2e}} \right] \quad (2)$$

where

C_e^{2*} = dilution-adjusted concentration of reactive tracer in the extraction fluid [M/L³]
 C_e^2 = concentration of reactive tracer in the extraction fluid [M/L³]
 C_i^2 = concentration of reactive tracer in the injection fluid [M/L³]
 C_e^{2e} = expected concentration of reactive tracer in the extraction fluid [M/L³]

The expected concentration of the reactive tracer in the extraction fluid (C_e^{2e}) is given by:

$$C_e^{2e} = \left(\frac{[C_e^1 - C_a^1]}{[C_i^1 - C_a^1]} \right) [C_i^2 - C_a^2] + C_a^2 \quad (3)$$

where

C_e^1 = concentration of non-reactive tracer in the extraction fluid [M/L³]
 C_a^1 = concentration of non-reactive tracer in the aquifer fluid [M/L³]
 C_i^1 = concentration of non-reactive tracer in the injection fluid [M/L³]
 C_a^2 = concentration of reactive tracer in the aquifer fluid [M/L³]

Eq. (3) assumes the following: (1) the concentrations of both tracers are equal to their injection concentrations at time equal to zero, (2) the concentrations of both tracers are equal to their aquifer concentrations as time approaches infinity, and (3) the mass transport characteristics both the non-reactive and reactive tracers, e.g., advection, mechanical dispersion, molecular diffusion, and sorption, are no different. During a push-pull test each independent variable in Eq. (3) is measured. Therefore, Eq. (3) can be used to compare the expected concentration of a reactive tracer (C_e^{2e}) to the measured concentration of a reactive tracer (C_e^2). Any difference between the two concentrations can be attributed to reactivity. Eq. (2) accounts for such differences and allows for generating a dilution-adjusted breakthrough curve of a reactive tracer. The dilution-adjusted breakthrough curve from Eq. (2) can be utilized to quantify the reaction rate of a tracer. Linear regression of the dilution-adjusted breakthrough curves was performed using the Microsoft Excel Data Analysis Regression Tool.

3. Results and discussion

3.1. Pre-test conditions

The concentrations of bromide, 2,6-DFBA, and PFBA in the injection fluid were approximately 400-times greater than within the aquifer fluid (Table 2). The relatively high concentrations of bromide, 2,6-DFBA, and PFBA were expected because these tracers were added to the injection fluid to establish a sufficiently high signal to noise ratio with respect to the aquifer fluid. The concentrations of nitrate and sulfate in the injection fluid were approximately 17- and 3-times greater than within the aquifer fluid, respectively (Table 2) and their relatively high concentrations were not expected because these tracers were not added

Table 2

Concentrations of tracers in the injection and aquifer fluids immediately prior to conducting the push-pull tests in wells FW222 and FW224.

Tracer	Fluid	FW222	FW224
		(mg/L)	(mg/L)
Bromide	Injection	100	100
	Aquifer	0.23	0.25
Chloride	Injection	18.0	19.6
	Aquifer	24.8	23.2
2,6-DFBA	Injection	100	100
	Aquifer	0.20	0.17
PFBA	Injection	100	100
	Aquifer	0.29	0.37
Nitrate	Injection	7.72	13.7
	Aquifer	1.09	0.12
Sulfate	Injection	19.5	14.2
	Aquifer	2.46	8.92
Fluoride	Injection	0.19	0.18
	Aquifer	0.44	0.20

to the injection fluid and were therefore naturally present within the aquifer fluid obtained from the nearby and up-gradient source well FW228 (Fig. 1). These results suggested that the spatial variability of some tracers, e.g., nitrate and sulfate, within the shallow and unconfined aquifer were considerable. The concentrations of chloride and fluoride in the injection and aquifer fluids were not identical but nevertheless very similar (Table 2). Despite the spatial variability of nitrate and sulfate, the concentrations of other tracers within the injection and aquifer fluids of the test wells were relatively similar (Table 2). Moreover, the physical characteristics of the porous media and the pH (near-neutral) of the aquifer fluid associated with the test wells were relatively similar (Table 1). Therefore, the test wells were hydrogeologically similar (Tables 1 and 2) yet spatially distinct, as indicated by test well FW222 being located down-gradient with respect to test well FW224 (Fig. 1), making the pair of wells ideal for in situ experimentation.

3.2. Push-pull tests

3.2.1. Bromide and chloride

The dilution-adjusted breakthrough curves of bromide were identical to their injected concentrations of 100 mg/L (Table 2) for the duration of the tests in both wells (Fig. 3). According to Eqs. (2) and (3), the dilution-adjusted breakthrough curve of the pre-determined non-reactive tracer, e.g., bromide, will always be equal to its injected concentration if the assumptions of the analytical method by Paradis et al. (2018b) are valid. Therefore, any deviation of the dilution-adjusted breakthrough curves of all other tracers, e.g., chloride, 2,6-DFBA, PFBA, nitrate, sulfate, and fluoride, from their injected concentrations can be attributed to reactivity.

The dilution-adjusted breakthrough curves of chloride were nearly identical to their injected concentrations of 18.0 and 19.6 mg/L (Table 2) for the duration of the tests in wells FW222 and FW224, respectively (Fig. 3). These results strongly suggested that the mass transport characteristics of chloride, e.g., advection, mechanical dispersion, molecular diffusion, and sorption, were no different than bromide. These results were expected based on the nearly identical physical properties of bromide and chloride, e.g., aqueous solubility and aqueous diffusion coefficients and their observed non-reactive transport in a wide range of hydrogeological settings (Davis et al., 1980; Eggenkamp and Coleman, 2009; Flury and Wai, 2003; Hu and Moran, 2005).

3.2.2. 2,6-DFBA and PFBA

The dilution-adjusted breakthrough curves of 2,6-DFBA and PFBA showed strong and linear decreases starting from slightly above their

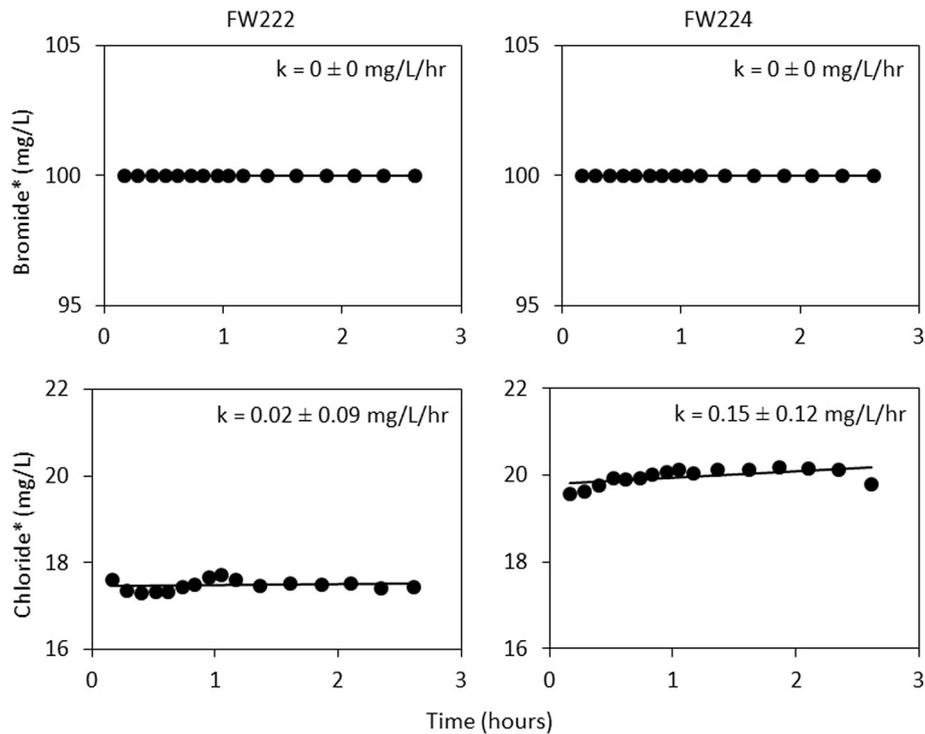


Fig. 3. Dilution-adjusted breakthrough curves of bromide and chloride for wells FW222 and FW224, * indicates dilution-adjusted concentrations per Eq. (2) and (3) from Paradis et al. (2018b).

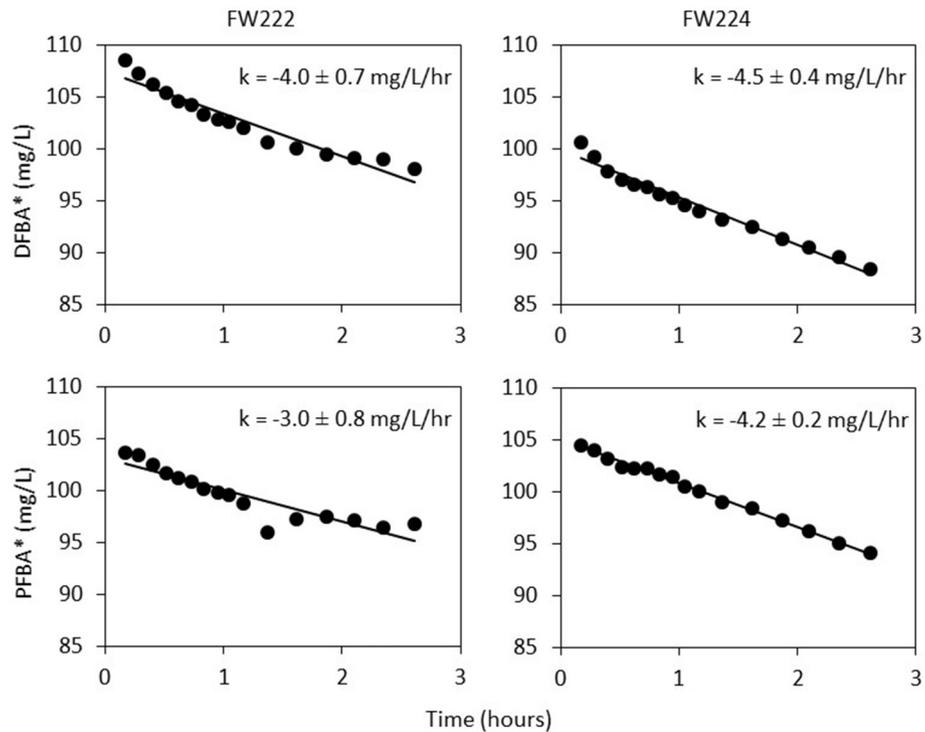


Fig. 4. Dilution-adjusted breakthrough curves of 2,6-DFBA and PFBA for wells FW222 and FW224, * indicates dilution-adjusted concentrations per Eq. (2) and (3) from Paradis et al. (2018b).

injected concentrations of 100 mg/L (Table 2) for the duration of the tests in both wells (Fig. 4).

The zero-order decay coefficients for 2,6-DFBA and PFBA in both wells were similar, statistically significant, and ranged from -3.0 ± 0.8 to -4.5 ± 0.4 mg/L/h (Fig. 4). These results strongly suggested that the mass transfer and/or transformation processes that

affected 2,6-DFBA and PFBA were significantly different in magnitude and/or type than those that affected bromide. The dilution-adjusted concentrations of 2,6-DFBA and PFBA were slightly above their injected concentrations of 100 mg/L. These elevated values were likely due to their difference in diffusion coefficient with respect to bromide. The diffusion coefficient of bromide is approximately 3 times greater than

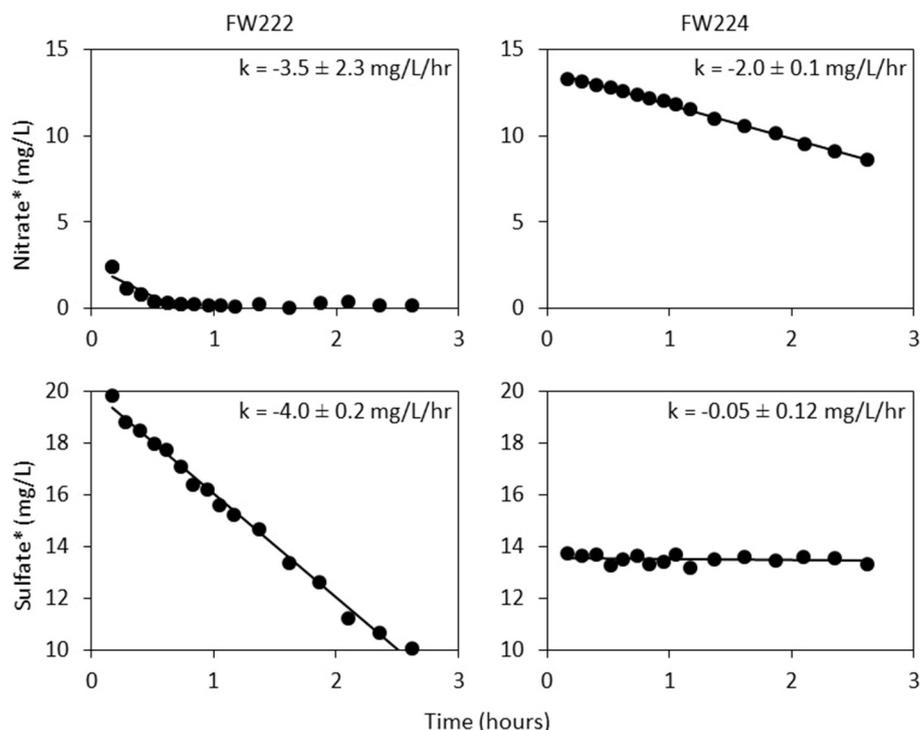


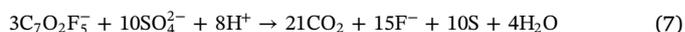
Fig. 5. Dilution-adjusted breakthrough curves of nitrate and sulfate for wells FW222 and FW224, * indicates dilution-adjusted concentrations per Eq. (2) and (3) from Paradis et al. (2018b).

those of 2,6-DFBA and PFBA (Flury and Wai, 2003; Hu and Moran, 2005). Therefore, preferential diffusive mass transport of bromide would likely result in expected concentrations of 2,6-DFBA and PFBA lower than their measured concentrations. An inspection of Eq. (2) shows that when the expected concentration of a reactive tracer (C_e^{2e}) is less than its measured concentration (C_e^2) the dilution-adjusted concentration (C_e^{2*}) is greater than its injected concentration (C_i^2). Sorption of 2,6-DFBA and PFBA was likely negligible based on a previous study by McCarthy et al. (2000) that demonstrated that sorption of 2,6-DFBA, PFBA, and bromide were nearly identical in laboratory experiments conducted with sediments from within the Oak Ridge Reserve under circumneutral pH conditions; the pH of the aquifer fluid associated with the test wells is near-neutral (Paradis et al., 2016).

Degradation of 2,6-DFBA was possible based on a previous study by Nimmo et al. (1990) that demonstrated that ring- ^{14}C -labeled 2,6-DFBA was degraded to $^{14}\text{CO}_2$ in a laboratory experiment conducted with two different soils under aerobic conditions; it was assumed that degradation of 2,6-DFBA was microbial-mediated. Moreover, microbial-mediated biotransformation of 2,5-DFBA, a nearly identical isomer of 2,6-DFBA, by *P. putida* was demonstrated in a study by Cass et al. (1987) that showed that 85% of the total fluorine in 2,5-DFBA was biotransformed to fluoride ion in a laboratory experiment conducted with synthetic growth media; *Pseudomonas* species have been commonly detected in sediments and groundwater at the Oak Ridge Reserve (Cardenas et al., 2008; Fields et al., 2005). Although degradation of PFBA was also possible, the only studies to date that have demonstrated PFBA degradation were associated with ultraviolet-assisted photo-defluorination (Ravichandran et al., 2007; Ravichandran et al., 2010). In contrast, numerous studies, both in the laboratory and in situ, have demonstrated that the mass transport of 2,6-DFBA and PFBA in unsaturated and saturated porous media were nearly identical to bromide (Becker and Coplen, 2001; Bowman and Gibbens, 1992; Hu and Moran, 2005; McCarthy et al., 2000) which makes the results presented here relatively interesting. However, previous studies typically compared percent mass recovery and mean retention or arrival times (Becker and Coplen, 2001; Bowman and Gibbens, 1992; Hu and Moran, 2005;

McCarthy et al., 2000) which theoretically can be similar if the effects of diffusive mass transport of bromide and biodegradation of 2,6-DFBA and PFBA result in similar breakthrough curves. Moreover, relatively few previous studies were conducted in situ and those that were, were not conducted in anaerobic aquifers (Becker and Coplen, 2001; Bowman and Gibbens, 1992); microbial-mediated anaerobic degradation of mono-fluorobenzoic acids, e.g., 2-, 3-, and 4-fluorobenzoic acids, under nitrate- and sulfate-reducing conditions has been well documented in the literature (Kiel and Engesser, 2015). Therefore, the zero-order decay of 2,6-DFBA and PFBA was likely due to biodegradation under anaerobic conditions as opposed to matrix diffusion or sorption.

Nitrate and sulfate are the predominant dissolved-phase electron acceptors at the study site (Paradis et al., 2016) and were measured in the injection and aquifer fluids immediately prior to conducting the push-pull tests (Table 2). Therefore, if the zero-order decay of 2,6-DFBA and PFBA was primarily due to biodegradation under anaerobic conditions, it would be expected that sequential nitrate- and sulfate-reduction would occur. Nitrate is a higher energy yielding electron acceptor than sulfate and would theoretically be preferentially depleted before the onset of sulfate reduction (Essaid et al., 2015). It would also be expected that defluorination of 2,6-DFBA and PFBA would occur and therefore result in the production of fluoride ion (Kiel and Engesser, 2015). The complete oxidation of 2,6-DFBA and PFBA to carbon dioxide coupled to reduction of nitrate and sulfate can be described by the following equations:



It is important to note that solid-phase electron acceptors, e.g., manganese and ferric oxides, are known to be present within Area 2 (Moon et al., 2006) and therefore had the potential to serve as electron acceptors in addition to nitrate and sulfate; manganese and ferric oxides

are typically less energetically favorable than nitrate, but more energetically favorable than sulfate, as electron acceptors (Essaid et al., 2015).

3.2.3. Sulfate, nitrate, and fluoride

The dilution-adjusted breakthrough curves of nitrate for wells FW222 and FW224 showed strong and linear decreases starting from slightly below or near their injected concentrations of 7.72 and 13.7 mg/L (Table 2), respectively (Fig. 5). The zero-order decay coefficients of nitrate for wells FW222 and FW224 were comparable, statistically significant, and ranged from -3.5 ± 2.3 to -2.0 ± 0.1 mg/L/h, respectively (Fig. 5). These results strongly suggested that nitrate reduction occurred in both wells. The dilution-adjusted breakthrough curve of sulfate for well FW222 showed a strong and linear decrease starting from near its injected concentration of 19.5 mg/L (Table 2) for the duration of the test whereas the breakthrough of sulfate for well FW224 was nearly identical to its injected concentration of 14.2 mg/L (Table 2) (Fig. 5).

These results strongly suggested that sulfate reduction occurred in well FW222 and did not occur in well FW224. These results further suggested that sequential nitrate- and sulfate-reduction occurred as predicted by Eqs. (4)–(7). For example, for well FW222, nitrate concentrations decreased to below 1 mg/L within the first half-hour followed by a significant and sustained decrease in sulfate concentrations from approximately 20 to 10 mg/L (Fig. 5). These results suggested that nitrate was rapidly and effectively depleted to sufficiently low enough concentrations to trigger the onset of sulfate-reducing conditions. In contrast, for well FW224, nitrate concentrations decreased from approximately 14 mg/L at the beginning of the test to approximately 8 mg/L by the end of the test during the time sulfate concentrations were nearly identical to their injected concentrations (Fig. 5). These results suggested that nitrate was rapidly but not effectively depleted to sufficiently low enough concentrations to trigger the onset of sulfate-reducing conditions. It is important to note that a previous study by Paradis et al. (2016) stimulated microbial-mediated nitrate- and sulfate-reduction in wells FW222 and FW224 that was coupled to ethanol oxidation; ethanol, like 2,6-DFBA and PFBA in this study, was added to the injection fluid during the push-pull tests, bromide was also added to the injection fluid to serve as a non-reactive tracer.

The dilution-adjusted breakthrough curves of fluoride were nearly identical to their injected concentrations of 0.19 and 0.18 mg/L (Table 2) for the duration of the tests in wells FW222 and FW224, respectively (Fig. 6).

The zero-order decay coefficients of fluoride for wells FW222 and FW224 were -0.012 ± 0.003 mg/L/h and -0.003 ± 0.002 mg/L/h, respectively, and relatively negligible (Fig. 6). These results were not expected based on Eqs. (4)–(7) that predicted that defluorination of 2,6-DFBA and PFBA would result in the production of fluoride ion and

based on previous studies that demonstrated substantial production of fluoride ion resulting from microbial-mediated defluorination of mono- and/or polyfluorobenzoic acids (Cass et al., 1987; Kiel and Engesser, 2015). However, the previous studies that demonstrated the production of fluoride ion were conducted using synthetic growth media in the laboratory (Cass et al., 1987; Kiel and Engesser, 2015) as opposed to natural porous media in the field. The porous media within Area 2 (Fig. 1) and the Oak Ridge Reserve is known to contain ferric oxides (Hu and Moran, 2005; Moon et al., 2006) and significant sorption of fluoride ion to ferric oxides has been demonstrated in previous studies (Farrah and Pickering, 1986) including those conducted with porous media from the Oak Ridge Reserve and numerous other DOE sites (Hu and Moran, 2005). According to Hu and Moran (2005), fluoride is the least appropriate halide for use as a hydrologic tracer because of its strong specific chemical binding to mineral surfaces. Therefore, if microbial-mediated defluorination of 2,6-DFBA and PFBA under nitrate- and/or sulfate-reducing occurred, as the data suggested (Figs. 4 and 5), it would be reasonable to assume that significant sorption of fluoride ion occurred which may explain the negligible production of dissolved-phase fluoride (Fig. 6).

The Eqs. (4)–(7) can be used to predict the rates of sulfate and nitrate decreases and fluoride increases based on the calculated rates of 2,6-DFBA and PFBA decreases (Table 3). For example, for well FW222, it would be predicted that 15.1 ± 2.3 mg/L/h of sulfate would be reduced (Table 3) if 4.0 ± 0.7 and 3.0 ± 0.8 mg/L/h of 2,6-DFBA and PFBA, respectively (Fig. 4), were completely oxidized to carbon dioxide. However, the results for well FW222 suggested that only 4.0 ± 0.2 mg/L/h of sulfate was reduced (Table 3). The calculated rates of nitrate and sulfate reduction for both wells were substantially less than the prediction rates (Table 3). As previously noted, solid-phase electron acceptors, e.g., manganese and ferric oxides, are known to be present within Area 2 (Moon et al., 2006) and therefore had the potential to serve as electron acceptors in addition to nitrate and sulfate. Therefore, it would be reasonable to assume that substantial reduction of manganese and ferric oxides occurred which may explain the lower-than-predicted rates of nitrate and sulfate reduction (Table 3). The calculated rates of fluoride production for both wells were negligible and therefore substantially less than the prediction rates (Table 3). As previously noted, significant sorption of fluoride ion was assumed to have occurred which may explain the negligible production of dissolved-phase fluoride. Therefore, the predicted rates of fluoride production are presented here simply for completeness and for reference of future studies (Table 3).

4. Conclusions

The in situ decay of two PBAs, 2,6-DFBA and PFBA was accompanied by nitrate and subsequent sulfate removal during single-well

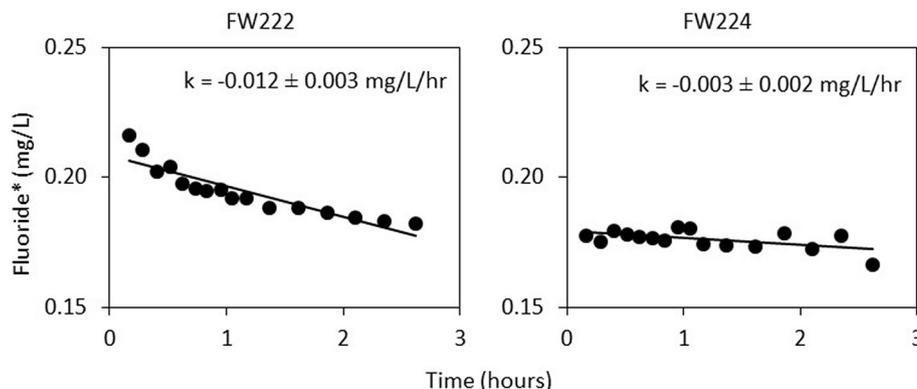


Fig. 6. Dilution-adjusted breakthrough curves of fluoride for wells FW222 and FW224, * indicates dilution-adjusted concentrations per Eq. (2) and (3) from Paradis et al. (2018b).

Table 3

Comparison of calculated versus predicted reaction rates for nitrate and sulfate reduction and fluoride production, calculated rates derived from linear regression of breakthrough curves (Figs. 4 and 5), predicted rates derived from stoichiometry in Eqs. (4)–(7), reaction rates equal to zero are either not statistically significant ($p > .05$) or negligible in magnitude, NA = not applicable.

Tracer	Reaction rate	FW222 (mg/L/h)	FW224 (mg/L/h)
–	–		
Nitrate	Calculated	-3.5 ± 2.3	-2.0 ± 0.1
	Predicted	-11.7 ± 1.8	-14.2 ± 0.7
Sulfate	Calculated	-4.0 ± 0.2	0
	Predicted	-15.1 ± 2.3	NA
Fluoride	Calculated	0	0
	Predicted	$+ 2.3 \pm 0.3$	$+ 3.0 \pm 0.2$

push-pull tests conducted in two hydrogeologically similar, yet spatially distinct, groundwater monitoring wells. These results strongly suggested that biodegradation of both PBAs occurred under anaerobic conditions and implied that PBAs may not be appropriate to use as non-reactive tracers in certain hydrogeologic settings. The hydrogeologic setting of the study site is low in carbon and electron donors. Therefore, it was likely that the PBAs served as carbon and/or electron donors to stimulate microbial activity. However, future studies are needed to provide stronger evidence of in situ biodegradation of PBAs. Such studies would benefit from using ring- ^{14}C -labeled PBAs to determine the fate of carbon combined with microbial analyses to assess any changes in biomass and community structure/function in both groundwater and sediments.

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Conflict-of-interest

None.

Financial disclosure

None.

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