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-^{14}C-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

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-^{14}C-labeled 2,6-difluorobenzoic acid (2,6-DFBA) to $^{14}CO_2$ in a laboratory experiment conducted with two different soils under aerobic conditions; it was assumed that degradation of 2,6-DFBA was microbial-mediated. 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 dimeter 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 ($C_7H_4O_2F_2$), and PFBA ($C_7HO_2F_5$) (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 ($C_7H_3O_2F_2^-$) and PFBA ($C_7O_2F_5^-$). 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-μm 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: