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Spills of Hydraulic Fracturing Chemicals on Agricultural Topsoil: Biodegradation, Sorption, and Co-contaminant Interactions

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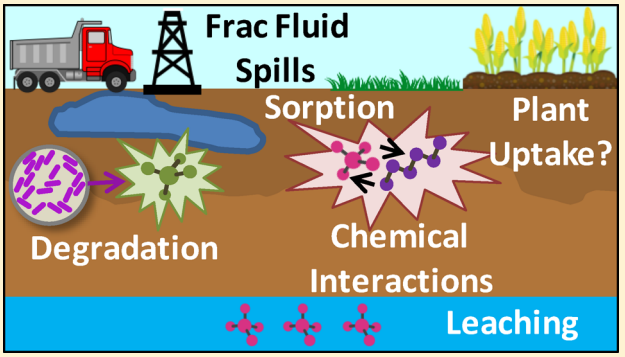
Supporting Information

ABSTRACT: Hydraulic fracturing frequently occurs on agricultural land. Yet the extent of sorption, transformation, and interactions among the numerous organic frac fluid and oil and gas wastewater constituents upon environmental release is hardly known. Thus, this study aims to advance our current understanding of processes that control the environmental fate and toxicity of commonly used hydraulic fracturing chemicals. Poly(ethylene glycol) surfactants were completely biodegraded in agricultural topsoil within 42−71 days, but their transformation was impeded in the presence of the biocide glutaraldehyde and was completely inhibited by salt at concentrations typical for oil and gas wastewater. At the same time, aqueous glutaraldehyde concentrations decreased due to sorption to soil and were completely biodegraded within 33−57 days. While no aqueous removal of polyacrylamide friction reducer was observed over a period of 6 months, it cross-linked with glutaraldehyde, further lowering the biocide's aqueous concentration. These findings highlight the necessity to consider co-contaminant effects when we evaluate the risk of frac fluid additives and oil and gas wastewater constituents in agricultural soils in order to fully understand their human health impacts, likelihood for crop uptake, and potential for groundwater contamination.



INTRODUCTION

Hydraulic fracturing (HF) is a widely used technology that enhances oil and gas extraction from unconventional formations such as low-permeability shale and source rock.[1] In the past decade, this extraction technique has become more economically viable due to the advancement of horizontal drilling followed by high-volume fluid injection.[2] These fluids are typically composed of about 90% water, ∼9% proppant, and 0.5−1% chemical additives.[2] Depending on formation geology and the number of stages in the well, between 3 and 50 million liters of water, and therefore tens of thousands of liters of chemicals, are injected into each well.[2−4] Hundreds of different chemicals have been used in HF fluid, however, only 4−28 of these chemicals are typically used per well,[3] including acids, friction reducers, biocides, surfactants, corrosion inhibitors, clay stabilizers, iron control agents, gelling agents, cross-linkers, breakers, oxygen scavengers, pH adjustors, and scale inhibitors.[2,5] Selection of chemical additives varies by well and can be based on pumping rates, formation composition, microbial activity, interactions with other additives, precedence, and other factors.[6,7]

Although natural gas has been touted as a bridge fuel to renewable energy, the substantial increase in HF activity has also raised concerns over the potential environmental and health impacts of the process.[3] Inadvertent fluid releases may occur during many different stages in the hydraulic fracturing and resource extraction process, including transportation (in pipelines or on trucks), chemical mixing, injection, production, and water disposal, which can include recycling, reuse, or treatment.[3,7] Surface spills on site or during transportation are the most commonly reported causes of contamination.[3,7−9] Currently, the most complete database (http://cogcc.state.co.us/) is managed by the Colorado Oil and Gas Conservation Commission (COGCC), listing all reported spills and releases of flowback and produced water in the state of Colorado that are 1 barrel (159 L) or larger outside and 5 barrels (795 L) or larger inside well pad berms.[10] In 2014, 838 spills were reported to the COGCC, which resulted in a total release of over 2 500 000 L. Ninety-three of these spills were reported as having contaminated groundwater, and eight contaminated surface water. Six hundred four of these spills (72%) were not

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contained within the well pad, suggesting that the surrounding environment (i.e., soil and/or water) was impacted.[10,11] It is worth noting that contaminations caused by spilled fluids in Colorado are solely registered on the basis of detection of select inorganic parameters as well as benzene, toluene, ethylbenzene, and xylenes (BTEX) and total petroleum hydrocarbons (TPH). Other organic chemicals injected during HF are not analyzed. Consequently, spills of fresh, uninjected HF fluids or pure chemical products may remain undetected and unreported. According to a report published by the COGCC, the majority of these spills were cause by equipment failure (67%) or human error (23%).[11] Additionally, nearly 78% of the spills occurred during the production phase and are therefore more likely to have involved produced or flowback water.[11] Another extensive spill database is the Alberta Energy Regulator (AER) Compliance Dashboard, which lists reportable releases of any substance potentially causing an adverse effect.[12] Here, 153 releases of produced water, frac fluid, or pure chemical product were reported for 2015. Since hydraulic fracturing frequently occurs in the vicinity of agricultural lands, these releases may lead to complex soil and water contamination.

Many of the chemicals used in HF have been applied previously in conventional oil and gas extraction or other industries. As a result, environmental impact studies are available for certain HF chemicals,[7,13−18] although vital data are still lacking for many other compounds and product mixtures.[8,9] In addition, critically needed environmental impact screening frameworks are slowly emerging.[19] The majority of these studies, however, evaluated the impact of these chemicals individually and in a general sense, without a specific focus on conditions relevant to the HF process. Recently, a few studies have addressed interactions within chemical mixtures; however, these studies mostly characterized complex HF fluid mixtures through bulk parameters rather than by a specific compound approach, as was done in this study.[20,21] A thorough understanding of chemical and biological interactions in (released) HF fluids is extremely important because the chemicals used in hydraulic fracturing have the ability to alter solubility, viscosity, microbial communities, pH, and other aspects of the soil environment that may affect the fate of other additives. Additionally, additives may impact each other directly through chemical reactions.[9]

Here, we study simulated spills of HF fluid additives on agricultural topsoil in order to advance our current understanding of processes that control their environmental fate and toxicity. The biocide glutaraldehyde (GA), poly(ethylene glycol) (PEG) surfactants, and a polyacrylamide (PAM)-based commercial friction reducer were chosen as target chemicals due to their widespread use in HF fluids and the fact that GA (transformation products) and PEGs have previously been identified in flowback and produced water.[22,23] Our specific objectives were to (1) determine their sorption, degradation kinetics, and mechanisms and (2) elucidate co-contaminant effects (i.e., combinations of GA, PEGs, PAM, and salt) on HF fluid additive transformation kinetics and products.

EXPERIMENTAL SECTION

Materials and Experimental Setup. To study the environmental fate and transport of glutaraldehyde (25% w/w in water, Sigma−Aldrich), poly(ethylene glycol) 400 (100%, Alfa Aesar), and friction reducer in agricultural topsoil, aerobic batch studies were conducted with Julesburg sandy loam collected as a 3-foot soil core from Weld County, CO, the U.S. county with the highest number of active oil and gas wells [global positioning system (GPS) coordinates −104.7458, 40.3098]. Prior to analysis, the top 30 cm of soil was thoroughly homogenized, including the A (0−15 cm), BA (15−25 cm), and a portion of the Bt1 (25−61 cm) horizon, and then sieved through a 2 mm mesh. Total organic carbon (TOC) content of the sieved soil was 7.9 g/kg, total organic nitrogen content was 0.78 g/kg, electrical conductivity was 80 mS/m, and pH was 6.8. Texture analysis showed that the sieved soil was 52% sand, 26% silt, and 22% clay. The commercial friction reducer product ASP900 (Nalco, 17.5% ± 2.5% anionic PAM) was used in these experiments. The other chemicals were used as received.

Aerobic batch studies were conducted in 250 mL Erlenmeyer flasks containing 20 g of topsoil and 200 mL of synthetic surface water (SSW) with 0−3 of the HF additives of interest and salt or no salt. Initial concentrations of these additives were 750 mg/L PEGs, 250 mg/L GA, 130 mg/L PAM (equivalent to 750 mg/L ASP900), and 30 g/L NaCl (99.9%, Fisher Scientific). SSW was filter-sterilized and was composed of 1.07 mM $MgSO_4$, 25.7 μM $CaCl_2{\cdot}2H_2O$, 6.00 μM $Ca(NO_3)_2{\cdot}4H_2O$, 1.12 mM Na_2SO_4, 0.506 mM $CaCO_3$, 0.783 mM $KHCO_3$, 1.76 mM $NaHCO_3$, and 19.5 mM NaH_2PO_4 as a buffer. All chemicals were obtained from Fisher Scientific (purity >98%) and used as received. Ten different chemical combinations were studied, including a control with SSW; a control with SSW and NaCl; three reactors with one HF additive each (GA, PEGs, and PAM); three reactors with two HF additives each (GA/PEGs, GA/PAM, and PEGs/PAM); one reactor with all three HF additives; and one reactor with all three HF additives and salt. Biotic and abiotic (microbial activity inhibited with 2.5 g/L NaN_3) reactors for each chemical treatment were prepared in triplicate. Abiotic flasks were redosed with NaN_3 twice over the course of the experiment in order to limit microbial activity. Reactors were plugged with a plastic foam stopper, to allow air exchange but prevent bacterial contamination, and placed on a rotary shaker at 100 rpm and 23 ± 1 °C. Flasks were monitored throughout the experiment to ensure that pH in the reactors containing GA did not rise above pH 7 to minimize polymerization, which rapidly occurs at pH > 8.[24]

Samples were obtained by use of sterile syringes and needles. A 2 mL sample was taken from each reactor at each time point. A 200 μL aliquot of each sample was collected in an autoclaved plastic centrifuge vial for adenosine triphosphate (ATP) analysis. The remainder of the sample was immediately passed through a filter (0.45 μm, cellulose acetate, VWR International, Radnor, PA) into an autoclaved 2 mL amber glass vial and capped [poly(tetrafluoroethylene) (PTFE)-lined silicone septum]. Aliquots for GA analysis were immediately derivatized as described in the next section to prevent polymer formation.

Glutaraldehyde Analysis. GA analysis was conducted with an Agilent 1200 series high-performance liquid chromatography (HPLC) system using a 4.6 × 150 mm Agilent Zorbax Eclipse XDB-C18 column (Agilent) with a diode-array detector (DAD) monitoring UV absorbance at 358 nm. GA was eluted by an isocratic method with A (acetonitrile) and B (deionized water with 0.1% formic acid) at a ratio of 70:30. Mobile-phase flow rate was 3.0 mL/min, injection volume was 10 μL, and column temperature was 40 °C. 2,4-Dinitrophenylhydrazine (DNPH) reagent was made by mixing 0.286 g of DNPH (30% water content) with 100 mL of ACS-grade acetonitrile. GA samples were derivatized by combining 50 μL of aqueous sample, 1.5

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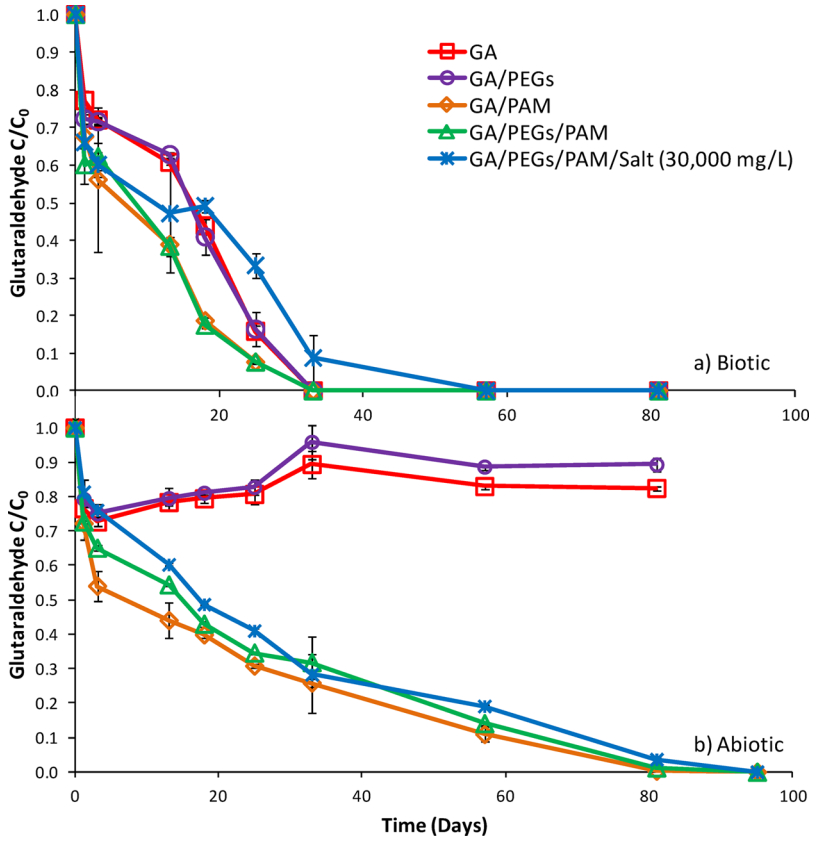

Figure 1. Glutaraldehyde removal from aqueous phase over time in (a) biotic and (b) abiotic reactors containing agricultural topsoil, synthetic surface water, varying HF fluid additives, and salt or no salt.

mL of DNPH reagent, and 450 μL of 0.12 M HCl. Samples were stored in the dark for at least 30 min before analysis.

Poly(ethylene glycol) Analysis. PEGs were analyzed on an Agilent 1100 series liquid chromatograph coupled with an Agilent G3250AA time-of-flight (ToF) mass spectrometer, via the method described by Thurman et al.[23] with the following exceptions. Mobile phases were A (0.1% formic acid) and B (acetonitrile with 0.1% formic acid). A gradient elution method was developed: 0−5 min, 10% B; 5−11 min, 10−34% B; 11−18 min, 34−90% B; 18−25 min, 10% B. Flow rate was 0.6 mL/min, injection volume was 5 μL, and temperature of the drying gas was 325 °C. Prior to analysis, samples were diluted 1:40 with sterile deionized water. Poly(propylene glycol) (300 ppm) was used as an internal standard (also diluted 1:40).

Polyacrylamide Analysis. PAM analysis was conducted via size-exclusion chromatography on an Agilent 1200 Series HPLC with a PL aquagel-OH 50 × 7.5 mm guard column followed by a PL aquagel-OH 60 300 × 7.5 mm column with the DAD monitoring absorbance at 215 nm. Mobile phase was 3 mM NaCl, flow rate was 1.0 mL/min, and injection volume was 50 μL.

ATP Analysis. ATP concentration was measured to ensure that the microbial activity in the abiotic samples was at least 2 orders of magnitude below the activity in (GA-free) biotic flasks, and to monitor for any correlation between ATP increases and biodegradation rates. This analysis was completed within 2 h of sampling by use of the BacTiter-Glo microbial cell viability assay (Promega Corp., Madison, WI). An opaque-walled 96-well plate was prepared with 100 μL of nonfiltered sample and 100 μL reagent in each well. The plate was allowed to equilibrate in the dark for 5 min and then immediately analyzed on a BioTek Synergy HT plate reader (BioTek Instruments, Winooski, VT). Abiotic and biotic samples were prepared on separate plates, and samples from different reactors were spaced out in order to avoid interference of fluorescence.

RESULTS

To elucidate both individual fate and mixture interactions among GA, PEGs, and PAM in agricultural topsoil, biotic and abiotic batch reactors with varying combinations of the three common HF fluid additives were set up at concentrations in a typical range for hydraulic fracturing fluids (GA ≈ 50−500 mg/L; PEGs ≈ 500−1000 mg/L; PAM ≈ 85−175 mg/L).[25]

Glutaraldehyde. Initial GA concentration was 250 mg/L in all abiotic and biotic reactors containing this biocide. As shown in Figure 1, a sharp decrease in aqueous GA concentration was observed in all biotic and abiotic reactors within the first day of the experiment, continuing through day 3 in most reactors. Because it occurred in both biotic and abiotic reactors, this decrease can be attributed to sorption of GA to the soil, while polymerization of GA was minimized via pH control, as evidenced by the lack of GA dimers and trimers present in the ToF mass spectra.

In the biotic reactors, between 23% (GA only) and 40% (GA/PEGs/PAM) of GA sorbed within 1 day, lowering the aqueous GA concentrations to between 150 and 193 mg/L. Aqueous GA concentrations kept declining past day 3 in all biotic reactors, and complete removal was observed within 33 days in all reactors except the one containing salt, where GA was removed from aqueous solution by day 57. The rate of GA removal was slightly faster in the biotic reactors that contained PAM and no salt (GA/PAM and GA/PEGs/PAM), as

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compared to the reactors that did not contain PAM (GA only and GA/PEGs). The observed pseudo-first-order rate constants, which were calculated between days 3 and 25 (Figure S2 in Supporting Information), were 0.0661 day^{-1} for GA only, 0.0654 day^{-1} for GA/PEGs, 0.0918 day^{-1} for GA/PAM, and 0.0971 $day^{-1}$for GA/PEGs/PAM (Table 1). In all biotic reactors, a slight lag phase until day 13 (day 18 in the reactor containing salt) was observed, after which removal rates increased. This lag phase was more pronounced in the reactors containing GA only and GA/PEGs, resulting in slower rate constants overall. Note that lag phases were included in observed rate constants due to their substantial impact on overall removal from the aqueous phase. Furthermore, in all five biotic reactors, there was a spike in ATP concentration when less than 10% of the original GA concentration (i.e., 25 mg/L) was left in solution (Figures S7−S11 in Supporting Information).

Table 1. Observed Pseudo-First-Order Rate Constants and Half-Lives[a]

Reactor Contents	k_{obs} (day^{-1})	$t_{1/2}$ (days)
GA, Biotic Reactor		
GA	0.0661	10
GA/PEGs	0.0654	11
GA/PAM	0.0918	7.6
GA/PEGs/PAM	0.0971	7.1
GA/PEGs/PAM/salt	0.0589	12
GA, Abiotic Reactor		
GA/PAM	0.0300	23
GA/PEGs/PAM	0.0285	24
GA/PEGs/PAM/salt	0.0266	26
PEGs, Biotic Reactor		
PEGs	0.0339	20
PEGs/GA	0.0230	30
PEGs/PAM	0.0435	15
PEGs/GA/PAM	0.0073	95

[a]For glutaraldehyde (GA) and poly(ethylene glycols) (PEGs) at 23 ± 1 °C and pH = 5.9−6.3 (biotic) or pH = 6.5−7.0 (abiotic).

In abiotic reactors containing both GA and PAM, GA was fully removed from the aqueous phase within 95 days. When the initial sorption phase was excluded, the observed pseudo-first-order rates of removal were 0.0300 day^{-1} for GA/PAM, 0.0285 day^{-1} for GA/PAM/PEGs, and 0.0266 day^{-1} for GA/PAM/PEGs/salt reactors (Table 1, Figure S3 in Supporting Information). In the two abiotic reactors that did not contain PAM, however, no further removal of GA from the aqueous phase was observed after initial sorption (also indicating that the nucleophilic azide ion, added to inhibit microbial activity, did not react or cross-link with GA).

Poly(ethylene glycol) Removal. Initial total PEG concentration was 750 mg/L in all abiotic and biotic reactors containing this additive. The only reactors in which PEGs were fully removed over the course of the experiments were the biotic PEGs-only and PEGs/PAM reactors, with complete removal occurring after 71 and 42 days, respectively (Figure 2). The observed pseudo-first-order rate constants were 0.0339 day^{-1} for PEGs-only and 0.0435 day^{-1} for PEGs/PAM (Table 1), as measured between the beginning of the experiment and day 33. Additionally, the concentration of ATP in the PEGs/PAM reactor was consistently higher (up to 1 order of magnitude) than in the PEGs-only reactor (Figure S16 in Supporting Information). The majority (92%) of PEGs were removed from the PEGs-only reactor by day 42; however, at this point the removal rate slowed. Biodegradation in the reactors containing both PEGs and GA but no salt (PEGs/GA and PEGs/GA/PAM) did not begin until day 33 and then proceeded slowly. ATP concentrations in both reactors were lowest when GA was present and in general increased slowly with time, except in the PEGs/GA/PAM reactor where there was a major ATP spike (0.0036 μM spike vs 0.0018 μM end point) once most of the GA had been removed by day 25 (Figures S12, S13, and S18 in Supporting Information). On day 95, degradation rates increased in both reactors containing GA. Between 95 and 173 days, the observed pseudo-first-order rate constants were 0.0230 day^{-1} in the PEGs/GA reactor and 0.0073 d^{-1}in the PEGs/GA/PAM reactor (Figure S1 in Supporting Information). The PEGs/GA/PAM/salt reactor neither showed indication of initial sorption of PEGs to the soil

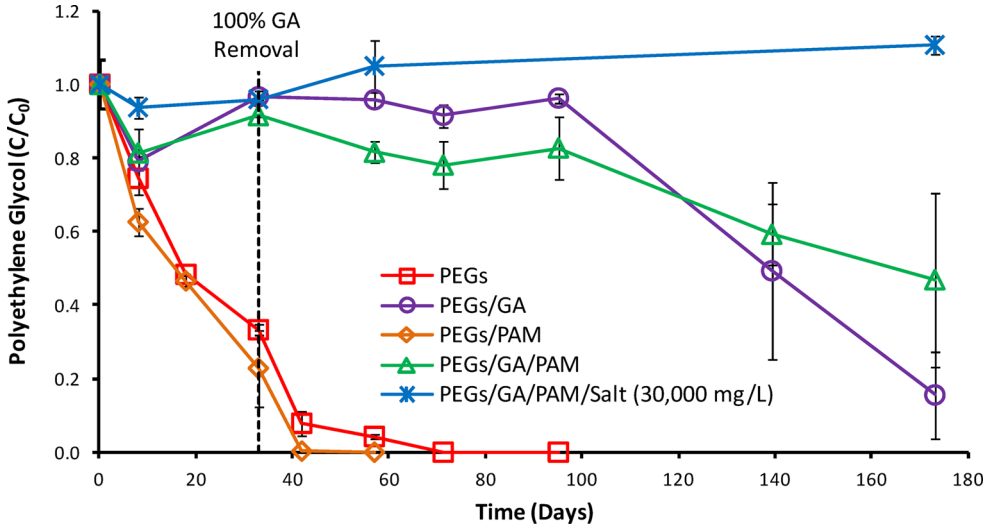

Figure 2. Total poly(ethylene glycol) (PEG) removal from aqueous phase over time in biotic reactors containing agricultural topsoil, synthetic surface water, varying additives, and salt or no salt. The dashed line shows the point in time when GA had been completely removed from biotic reactors without salt and reduced by more than 90% in the biotic reactor containing salt.

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nor signs of biodegradation by day 173. Aldehyde and carboxylate derivatives of PEGs were observed as oxidation intermediates in all biotic reactors where degradation occurred (Tables S1–S4 in Supporting Information).

In the abiotic reactors, there were no signs of PEG degradation over a period of 140 days, as peak areas remained fairly constant and no degradation intermediates were detected (Figure S29 in Supporting Information). ATP concentrations remained relatively constant with time in these reactors and were consistently lower than in the biotic reactors (by at least 1 order of magnitude as compared to biotic reactors without GA). Additionally, little (<7%) to no sorption of PEGs was observed in these reactors.

Poly(ethylene glycol) Speciation. Poly(ethylene glycol) is a polymeric surfactant with the structural formula HO-$(CH_2CH_2O)_nH$, where n represents the number of ethylene oxide (EO) units in the molecule. The PEG 400 product used in this study contains a mixture of homologous compounds with an average molecular mass of 400 g/mol, enabling assessment of the individual polymeric species after chromatographic separation and analysis.[23]

The initial PEG distribution was typical for a polydisperse mixture where the majority of PEGs was present in the midrange of molecular weights as EO10, and the minority was present at the highest and lowest detectable molecular weights. As shown in Figure 2, PEGs were removed from the biotic PEGs-only reactor over time, with full removal occurring by day 71. Figure 3a shows how the distribution of PEG species in this reactor shifted toward a higher average molecular weight over time as PEGs were removed from the aqueous phase. The initial PEG distribution was observed in the 4 h and 8 day samples. On day 33, when about 65% of the PEGs had been removed, this distribution had clearly begun to shift toward higher molecular weight species, as indicated by the nearly equal amounts of EO10 and EO11 species. Additionally, fractions of the smaller molecular weight species were decreasing. At this point, and for the remainder of the experiment, EO4 and EO5 species were no longer detectable in the reactor. Until day 42, when 92% of the PEGs had been degraded, the trend continued and EO11 became the major PEG species, while the fraction of smaller PEGs continued to decrease and the fraction of larger PEGs continued to increase. Finally, on day 57, when 95% of the PEGs had been removed from aqueous solution, EO12 and EO13 were the dominant PEG species. Additionally, the EO6 species was no longer detectable and the EO14 species was present at its highest fraction. A similar pattern was observed in all biotic reactors where degradation occurred except the PEGs/PAM/GA reactor (Figures S19–S21 in Supporting Information).

In the abiotic reactors and the biotic PEGs/PAM/GA/salt reactor, only small variations in PEG composition were detected. As shown in Figure 3b for the abiotic PEGs-only reactor, there was a slight yet statistically significant shift (at the 0.05 level) toward PEGs of lower molecular weight over time. This was observed in the other abiotic reactors as well as the biotic PEGs/PAM/GA/salt reactor (Figures S22–S27 in Supporting Information).

Polyacrylamide. Initial friction reducer concentration was 750 mg/L (~130 mg/L PAM) in all abiotic and biotic reactors containing this additive. In both abiotic and biotic reactors, there were no signs of PAM degradation based on shifts in chromatographic retention time or total peak area (data not shown). However, ATP concentrations were consistently higher (up to 1 order of magnitude) in reactors with PAM as compared to reactors without PAM (Figures S15–S18 in Supporting Information).

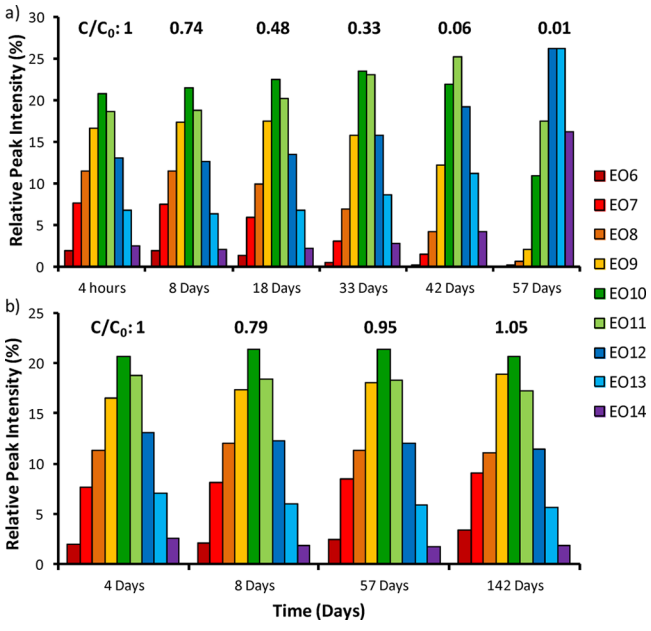

Figure 3. Changes in PEG relative peak intensity over time in (a) biotic and (b) abiotic reactors containing PEGs. A shift toward higher molecular weight PEG species (higher EO) was observed in the biotic reactors only. All PEGs were removed from the biotic reactor by day 71. Peak intensities are set in relation to the total peak area for the respective sample. Absolute abundance at each sampling time is shown as normalized concentration.

DISCUSSION

In this study, co-contaminant effects among three common HF additives and salt were investigated to better understand the complex interactions impacting their fate and transport after accidental release on agricultural soil. GA was present at an initial concentration of 250 mg/L, and thus above 200 mg/L, a typical concentration in HF fluids used to achieve a 5–6 log decrease in sulfate-reducing bacteria (SRB) and acid-producing bacteria (APB) growth.[23] However, microbial degradation of a biocide becomes possible when concentrations are reduced to sublethal levels (due to dilution, sorption, etc.) or when biocide efficacy is reduced due to produced water exposure and biocide-adapted bacteria proliferate.[26−28] Our results show that in the presence of soil, rapid sorption—either physisorption or chemisorption via cross-linking to amine functional groups in soil[29]—lowered the aqueous GA concentrations by as much as 40% within the first 3 days of the experiment. At this point, with concentrations ranging between 150 and 193 mg/L, biodegradation of GA proceeded in all biotic reactors. It should be noted that the soil/water ratio used here (1:10 on a mass basis) was substantially lower than under field conditions. Consequently, it is expected that exposure to higher soil/water ratios would lead to increased GA sorption and an even greater reduction in aqueous GA concentration.

For GA only, a half-life of 10 days was determined (Table 1), which is comparable to the range of aerobic biodegradation rates observed in wastewater treatment plants.[30] Biological GA removal can be attributed to two different mechanisms, namely, metabolization by organisms and covalent binding to microbial

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cells. GA is capable of covalently binding to microbial cell components such as proteins through the cross-linking action that constitutes its biocidal action. Previous measurements of O_2 consumption and CO_2 evolution during biodegradation of GA suggested that both of these mechanisms occur simultaneously.[30] In this study, ATP concentrations in the GA-containing reactors did not increase substantially during biotic GA removal (Figures S7−S11 in Supporting Information), suggesting that nonmetabolic cross-linking was at least partly responsible for GA removal. However, the initial microbial concentration was not sufficient to lead to significant GA removal through cross-linking with cell mass alone, as can be seen by comparison with abiotic controls without PAM, where GA concentrations remained constant after initial sorption to soil. Consequently, some microbial metabolism and growth must have occurred during the time of GA removal, further supported by our observation of a slight lag phase with a subsequent period of accelerated removal. Finally, substantially longer lag phases of PEG biodegradation in reactors where GA was present indicate that even while it was undergoing biodegradation, GA was still at inhibitory levels for some organisms within the microbial community (Figure 2).

The overall rate of GA removal was about 50% higher in the biotic reactors that contained PAM (Table 1), and complete abiotic GA removal was observed only in these reactors, suggesting that chemical interactions with PAM actively reduced the concentration of GA. Glutaraldehyde has been shown to be a potent cross-linker with amine groups.[31] Thus, it is very likely that a covalent double (i.e., imine) bond formed between the amine group on PAM and the aldehyde group on GA (Figure S5 in Supporting Information), which is extremely stable under both acidic and basic conditions.[32] The friction reducer that contained PAM (ASP900) also contained ammonium sulfate. Ammonia (NH_3, the deprotonated form of ammonium) also has the potential to cross-link with GA, following a similar mechanism as described above. Consequently, removal of GA from the aqueous phase in the abiotic PAM-containing reactors, after initial sorption to soil, can be fully attributed to the cross-linking reactions between GA/PAM and GA/NH_3. The observed rates of this abiotic process (Table 1) imply that about a third of the GA removal in PAM-containing biotic reactors can be attributed to GA cross-linking with PAM and NH_3, while the other two-thirds can be attributed to biological removal based on metabolism and/or covalent binding to cells. However, the extent of GA cross-linking in the biotic batch reactors may have been slightly lower than in the abiotic batch reactors due to the slightly lower pH (Table 1),[31,33] and its contribution therefore may be overestimated.

Abiotic processes such as sorption and covalent binding were far less pronounced in the case of PEGs. Their removal from the aqueous phase was almost exclusively due to biodegradation, which set in immediately upon the start of the experiment. Detection of aldehyde and carboxylate derivatives (Tables S1−S4 in Supporting Information) during biodegradation matches previous reports of aerobic microbial polyether transformation via oxidation at the terminal ethoxylate units with subsequent release of glyoxylate (Figure S4 in Supporting Information), producing homologues of lower molecular weight.[17] The presence of GA, however, substantially delayed biodegradation of PEGs (Figure 2). Degradation products were first detected in the PEGs/GA and PEGs/GA/PAM reactors after GA had been completely removed, followed by a prolonged lag phase of 62 days. After the lag phase, PEG biodegradation proceeded at a similar rate in the PEGs/GA reactor as it did in the PEGs-only reactor (0.0230 vs 0.0339 day^{-1}, respectively; Table 1). In the PEGs/GA/PAM reactor, however, the rate only increased slightly and was much slower than in the PEGs/PAM and PEGs/GA reactors (0.0073 vs 0.0435 vs 0.0230 day^{-1}, respectively). This suggests that the product formed by the cross-linking reaction between GA and PAM or NH_3 may have retained some biocidal activity through GA's second aldehyde functional group that remained unbound. Previous studies have shown that when one aldehyde group on the GA molecule binds with the amide group on PAM, the second aldehyde group is still available to cross-link with other free amino or amide groups present in proteins or nucleic acids.[33] Finally, the rate of PEG removal in the PEGs/PAM reactor (0.0435 day^{-1}) proceeded slightly faster than in the PEGs-only reactor (0.0339 day^{-1}). While this difference may be within experimental variation, it could have also been promoted by cross-linking of PAM or NH_3 with the observed aldehydic and carboxylated PEG transformation intermediates. This is supported by our observation that, in the PEGs-only reactor, aldehydic and carboxylated intermediates were observed during biodegradation and also after all parent species had been removed. In the PEGs/PAM, reactor, however, these intermediates were initially detected but no longer observed after less than 50% of the original PEG concentration remained.

PEG biodegradation was completely halted in the presence of salt, and no microbial transformation products were observed at the end of the 171-day experiment. A previous study compared degradation of PEGs in freshwater and artificial seawater and showed that while the rate of PEG degradation was decreased in seawater, PEGs up to 7400 Da (initial concentration 20 mg/L) were fully degraded within 130 days.[34] In contrast, GA was still removed in the presence of salt, even though slowed by a factor of 1.7 (Table 1), comparable to previous observations made for GA biodegradation in seawater.[30] These observations indicate that GA is metabolized by halotolerant microbial species.

In the absence of biodegradation, only slight (but statistically significant) shifts in PEG speciation toward homologues of lower molecular weight were observed in the abiotic reactors (Figure 3b). These findings indicate that sorption to the organic-rich topsoil increased with increasing hydrophobicity, which is consistent with previous reports of preferential sorption of PEGs containing a higher number of EO units (CH_2CH_2O).[35,36] In the biotic reactors, shifts in speciation over time toward homologues of higher molecular weight were much more pronounced due to preferential biodegradation of shorter-chain species (Figure 3a). This pattern was observed in all biotic reactors where degradation occurred except the PEGs/PAM/GA reactor. This is likely because the speciation trend in the other reactors became apparent only after 60% PEG degradation had occurred, and only 53% PEG degradation was achieved by day 173 in the PEGs/PAM/GA reactor. This pattern is in contrast to previous studies conducted on aerobic PEG biodegradation in freshwater, which have reported a shift toward lower molecular weight homologues with time.[34,37]

PAM analyses via size-exclusion chromatography did not provide any evidence for substantial PAM transformation other than the observed cross-linking to GA. PAM is generally regarded as stable in soil and water at temperatures up to 200 °C,[14] although one study found two bacterial strains capable of degrading PAM and using it as their sole source of carbon.

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These bacteria, however, had been subjected to PAM contamination in an oil field for an extended period of time prior to the study.[38] The presence of the friction reducer, however, did result in an increased ATP concentration (Figures S15−S18 in Supporting Information), possibly due to the use of ammonium or the amide groups in PAM as nitrogen source.[35] This may have led to increases in microbial activity and/or density as previously observed in PAM-treated soils,[35] and thus another potential reason for why the rate of PEG biodegradation was slightly higher in the PEGs/PAM reactors compared to PEGs only.

Implications for Environmental Fate. Our results clearly illustrate the necessity of considering mixture interactions among organic frac fluid and oil and gas wastewater constituents, not only for environmental impact assessment after spillage but also prior to application for other (beneficial) purposes such as crop irrigation. The chemical and biological transformation mechanisms and products revealed here for only a fraction of the many hundreds of HF chemical products used today show a complex picture of co-contaminant fate and toxicity that needs to be considered when environmental impact models are developed or expanded.[19] Biocidal inhibition of natural microbial attenuation processes has the potential to increase contaminant transport times and distances. This effect may be aggravated by the presence of salts, which are typically encountered at high concentrations in fluids returning from deep shale formations. In addition, surface-active agents such as surfactants, including PEGs, may increase the mobility of other organic HF additives through co-solvent effects and possibly solubilize otherwise immobile metals in the soil.[36] This also infers the need for considering mixture toxicity rather than relying on data for individual compounds.

On the other hand, sorption to soil and biodegradation, even of biocides at toxic levels,[7] are operative retardation and/or removal mechanisms. As a result, chemicals may be transported at different rates, thereby separating the mixtures and allowing for degradation. Conditions that do not favor degradation or transport may result in accumulation of HF additives in (agricultural) topsoil layers, with potential for uptake in crops or negative impacts on plant growth. Additionally, in areas where the water table is close to the surface or in soils with larger hydraulic conductivities, there is increased risk for water contamination as a result of HF fluid releases. This is especially concerning in areas where people rely on well water. As a consequence, monitoring and remediation strategies that go beyond current standard parameters[36] and target site-specific HF fluid additives are critically needed.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b00240.

Twenty-nine figures and four tables showing kinetic plots, aerobic PEG biodegradation pathway, cross-linking mechanism between GA and PAM, chromatographic and mass spectrometric data for PEGs and transformation products, ATP concentrations over time, PEG speciation plots, and abiotic PEG data (PDF)

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Notes

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