THESIS

# ENVIRONMENTAL FATE OF HYDRAULIC FRACTURING FLUID ADDITIVES AFTER SPILLAGE ON AGRICULTURAL TOPSOIL

Submitted by

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# ABSTRACT

## ENVIRONMENTAL FATE OF HYDRAULIC FRACTURING FLUID ADDITIVES AFTER SPILLAGE ON AGRICULTURAL TOPSOIL

Inadvertent releases of hydraulic fracturing fluid may occur at many different stages, with surface spills being the most commonly reported cause of contamination. Hydraulic fracturing (HF) frequently occurs on agricultural land, where surface spills have the potential to impact soil, groundwater and surface water quality. However, the extent of sorption, transformation, and interactions among the numerous organic HF fluid and oil & 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 with a specific focus on co-contaminant effects.

Hydraulic fracturing fluid releases were simulated using aerobic batch studies conducted with a topsoil collected from Weld County, Colorado, an area where reservoirs are frequently stimulated. Each batch reactor contained varying combinations of the biocide glutaraldehyde (GA), polyethylene glycol (PEG) surfactants, and a polyacrylamide (PAM)-based friction reducer, three widely used hydraulic fracturing fluid components. Furthermore, the presence of salt was investigated in the experiments, often present at high concentration in produced water from hydraulic fracturing operations.

Results showed that aqueous GA concentrations decreased by as much as 40% in the first three days of the experiment as a result of sorption to soil. Complete biodegradation of this biocide occurred in all reactors in 33 to 57 days, with the slowest removal occurring in the reactor containing salt. The fastest removal of GA was observed in the reactors containing PAM friction

reducer, where degradation rates increased by 50% as compared to reactors without PAM. This increase in removal is attributed to the cross-linking reaction between GA and primary amine functional groups in the friction reducer.

In the absence of GA and salt, PEG surfactants were completely biodegraded in agricultural topsoil within 42 to 71 days. Their transformation was impeded, however, in the presence of the biocide GA, and completely inhibited in the presence of 30 g/L sodium chloride, a concentration in the typical range for oil and gas wastewater. No aqueous removal of PAM was observed over a period of six months. However, adenosine triphosphate (ATP) concentrations were consistently higher in reactors containing PAM friction reducer, suggesting this additive supplied an easily accessible source of nitrogen to the microbial soil community.

The findings of this study 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.

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# 1. INTRODUCTION

## 1.1 Reservoir Stimulation via Hydraulic Fracturing

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] The oil and gas that is contained within these unconventional reservoirs was not considered an economically-viable resource until the early 2,000s. However, as a result of advancements in horizontal drilling followed by high-volume fluid injection, the use of this extraction technique has increased substantially since.[2] In 2007, shale gas accounted for 1% of U.S. natural gas production.[3] By 2014, this number had increased to 44%.[4-5] Additionally, between 2007 and 2014, annual natural gas production in the United States increased by 27% (from 24,663,656 MMcf to 31,345,546 MMcf) and shale gas production increased nearly 600% (from 1,990,145 MMcf to 13,754,150 MMcf) (Figure 1). As result of this, the United States is now the world's largest natural gas producer.[5]

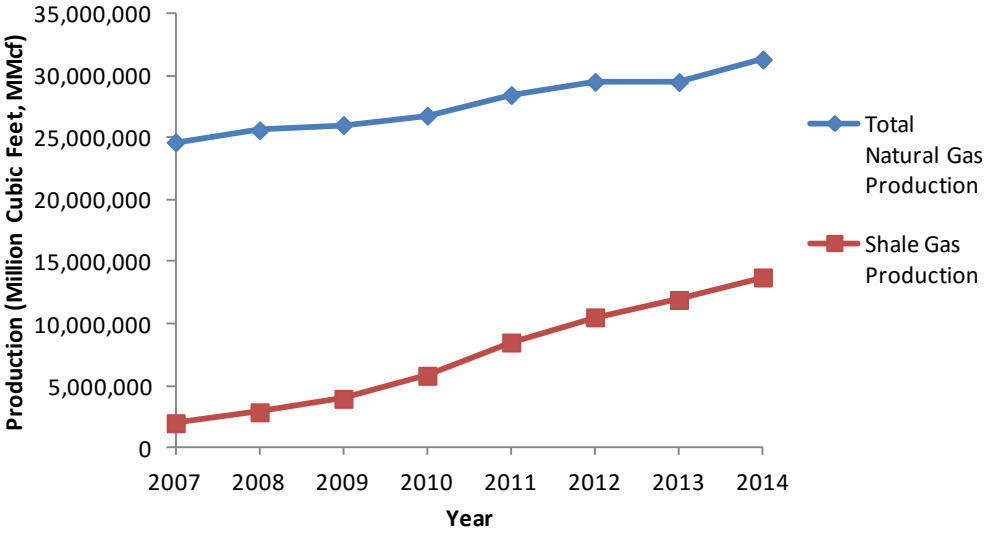



**Figure 1.** Total natural gas production and total shale gas production between 2007 and 2014. Data obtained from www.eia.gov.

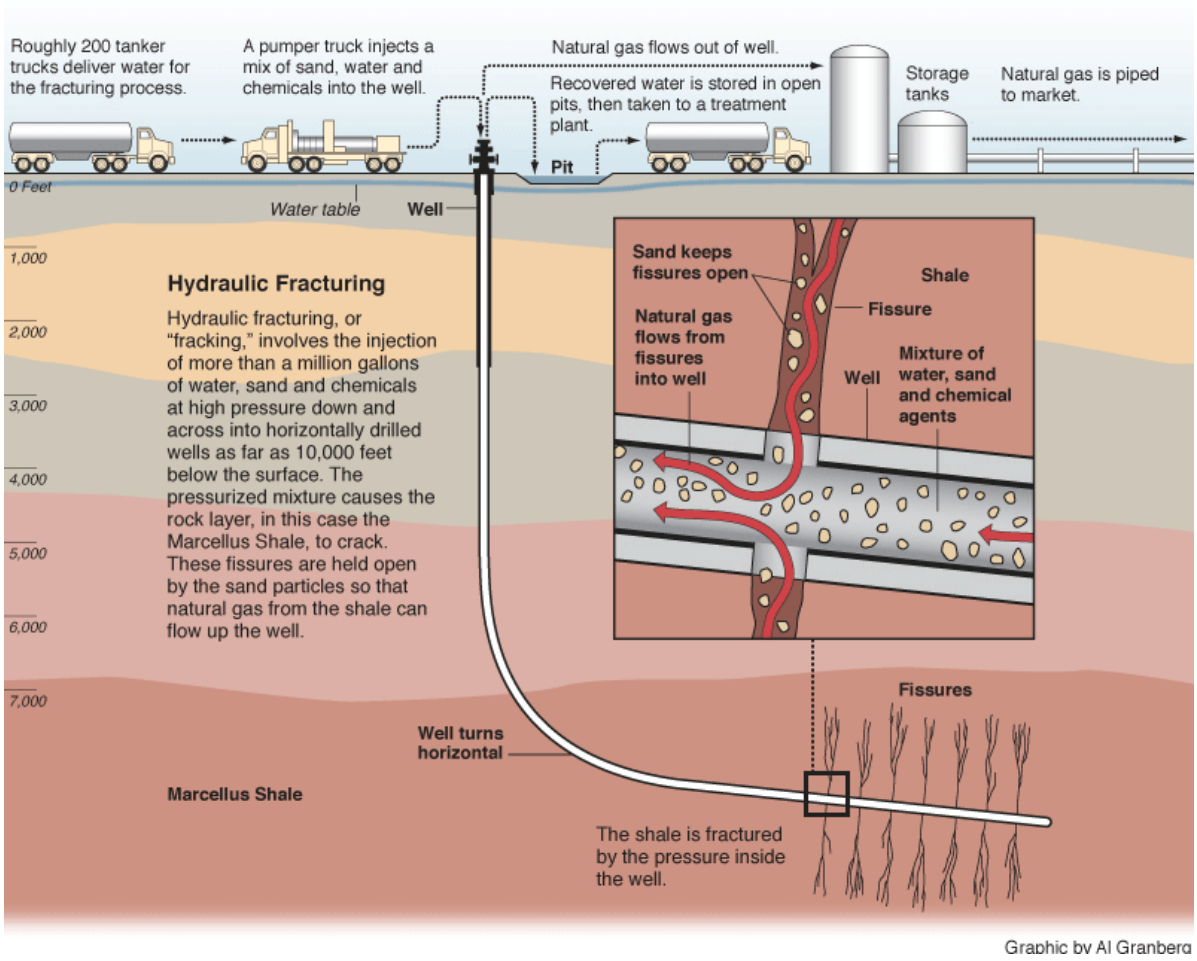



**Figure 2.** Schematic of the hydraulic fracturing process. Source: www.propublica.org.

Hydraulic fracturing in the United States has occurred at depths ranging between 250 - 4,500 meters (800-15,000 ft.) below ground, however, the average well is drilled to a depth of 2,500 m (8,000 ft.) and wells less than 900 m (3,000 ft.) deep are rare.[6] Once the desired formation is reached, the well turns horizontal, as shown in Figure 2, and continues for an average of 500 - 2,000 m in this direction.[7] To fracture the well, millions of liters of hydraulic fracturing fluid are injected downhole at a high pressure, inducing fractures in the formation and thereby increasing the reservoir's permeability. The proppant that is contained in the hydraulic fracturing fluid

infiltrates the fractures and props them open, allowing oil and gas to flow out and back to the surface, where these fuels are captured and eventually sold.

Many have touted natural gas as a bridge fuel to renewable energy, however, the substantial increase in HF activity has raised concerns over the potential environmental and health impacts of the process, especially in regards drinking water.[8] Concerns about this process stem from the fact that hydraulic fracturing is excluded from a number of federal environmental statutes. This includes but is not limited to: the Safe Drinking Water Act, the Clean Water Act, the Clean Air Act, the Superfund Act, the Toxic Release Inventory under the Emergency Planning and Community Right-to-Know Act. [9-11]. Additionally, the HF industry was not initially required to disclose the contents of HF fluid. Today, however, many of the states in which HF occurs have mandatory disclosure laws, including Colorado, Wyoming, Pennsylvania, Texas, Oklahoma, Ohio, Arkansas and more.[10] These laws apply to all chemicals used in the process, but exceptions for proprietary additives still exist. The reporting format in these databases is not well standardized and companies are not required to disclose the contents of HF fluid until after the job is complete. Consequently, there are still steps that can be taken towards transparency.[10, 12]

### 1.2 Hydraulic Fracturing Fluids

HF fluids are typically composed of about 90% water, 10% proppant and 0.5-1% chemical additives. Sand is the most commonly used proppant, while ceramic proppants are also employed.[13] Depending on formation geology and number of stages in the well, between 3-50 million liters of water, and therefore tens of thousands of liters of chemicals, are injected into each well.[2, 8, 14] Hundreds of different chemicals have been used in HF fluid, however, only 4-28 of these chemicals are typically used per well.[8] There are variety of additives used in HF fluids (Table

1) and also a wide variety of chemicals that can be used for each of these functions. Additive selection varies by well and can be based on many factors including: pumping rates, formation composition, microbial activity, interactions with other additives, and precedence.[13, 15]

**Table 1.** Common additives used in hydraulic fracturing fluids and their purpose, adapted from Gregory et. al, 2011.[16]

| Constituent | Purpose | Example |
|---|---|---|
| Acid | Dissolves minerals and initiates cracks in rock | Hydrochloric acid |
| Friction reducer | Minimizes friction between the fluid and the pipe | Polyacrylamide or mineral oil |
| Surfactant | Enhances water recovery | Polyethylene glycols |
| Salt | Creates a brine carrier fluid | Potassium chloride |
| Scale inhibitor | Prevents scale deposits in pipes | Phosphonates |
| pH-adjusting agent | Maintains effectiveness of chemical additives | Sodium or potassium carbonate |
| Iron control | Prevent precipitation of metal oxides | Citric acid |
| Biocide | Inhibits bacteria that produce corrosive and toxic by-products | Glutaraldehyde |
| Clay Stabilizer | Stabilizes clay to prevent swelling and shifting | Potassium chloride[1] |
| Cross-linker | Binds gel polymer molecules to increase viscosity | Borate salts[12] |
| Breaker | Breaks down viscosity of fluid at later stages in HF process | Peroxydisulfates[1] |
| Oxygen Scavenger | Prevents corrosion of metal pipes | Sulfites |
| Gelling Agents | Increase viscosity, suspend proppant during transport[11] | Guar gum |

Not all additive types are used in each well. For example, friction reducers are used in almost all slickwater HF jobs[16], whereas breakers are only used when the formation is not hot enough to naturally degrade the friction reducer.[1] Acids and biocides are also used in the majority of fracturing jobs. Clay stabilizers, however, are only used when the clay content of the formation, which can be as high as 50% in a shale, is so large that the induced permeability in the formation is unstable.[1, 13]

### 1.3 Hydraulic Fracturing Fluid Releases

Inadvertent releases of HF fluid 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.[8, 15] Surface spills on site or during transportation are the most commonly reported causes of contamination.[8, 15] Currently, the most complete spill 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.[17] In 2014, 838 spills were reported to the COGCC which resulted in a total release of over 2,500,000 L. Ninety-three (93) of these spills were reported to have contaminated groundwater and eight contaminated surface water. Six hundred and four (604) of these spills (72%) were not contained within the well pad, suggesting that the surrounding environment (i.e., soil and/or water) was impacted.[17-18] Additionally, chemicals are often stored on site to be mixed at the wellhead, presenting a greater issue if they are released in their concentrated form. This phenomenon likely occurred during the Colorado floods in September 2013 where 1,614 wells were in the impacted zone and 50 spills were reported in less than a week.[17]

It is worth noting that contaminations caused by spilled fluids in Colorado are solely analyzed based on detections of select inorganic parameters as well as BTEX (benzene, toluene, ethylbenzene and xylenes) and total petroleum hydrocarbons (TPH). Other organic chemicals injected during HF are not analyzed, except in extremely rare cases. Consequently, spills of fresh, uninjected HF fluids or pure chemical products may not be fully characterized and potential contaminants may be unreported. Furthermore, according to a report published by the COGCC, the majority of HF fluid releases were cause by equipment failure (67%) or human error (23%).[18] Nearly 78% of the spills occurred during the production phase and are therefore more likely to have involved produced or flowback water.[18] Since hydraulic fracturing frequently occurs in the vicinity of agricultural lands, these releases may lead to complex soil and water contamination.

During the hydraulic fracturing process, about 30-50% of the fluid that was injected downhole returns to the surface, although values ranging between 5-85% have been reported.[12] The aqueous fluid that flows out of the well is referred to as flowback or produced water. Flowback water is a mixture of the injected HF fluid and the fluid that is naturally contained in the formation, whereas produced water is composed entirely of the hypersaline solution naturally found in the formation.[14] The vast majority of the flowback water resurfaces in the first week of the operation while the produced water flows for the entire lifespan of the well. The transition between these two fluids is often hard to discern, however, the rate of flow for flowback water is generally about an order of magnitude greater than that for produced water. Produced water contains high total dissolved solids and high organic matter content as well as oil, bitumen and other hydrocarbon condensates. This fluid also contains toxic elements including barium, strontium, selenium, arsenic and naturally occurring radioactive materials (NORMs, e.g., radium).[14] Due to downhole mixing, flowback water contains many of these elements as well, although at lower

concentrations. Additionally, both flowback and produced waters contain any HF chemical additives that were originally injected downhole, as well as any transformation products that may have been formed as a result of the high temperature, high pressure, high saline downhole environment.[15] In an effort to reduce water usage, the majority of wells are fractured with recycled flowback and/or produced water.[13] As a result, it is important to understand the chemical characteristics of this fluid as well, although that is not a major consideration of this study.

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,[15, 19-24] although critical data are still lacking for many other compounds and product mixtures.[12, 25] These studies have shown that the majority of HF chemicals are water soluble, many associate strongly with soil and organic matter and few are volatile[8]. HF chemicals therefore have a potential to persist in soil and water. Additionally, a literature review of health data revealed that 37% of chemicals used in HF can affect the endocrine system; 25% have the potential to cause cancer or mutations; 10-50% could affect the brain/nervous system, immune system and cardiovascular system; and 75% can affect skin, eyes and other sensory organs as well as the respiratory and gastrointestinal systems.[11] It is important to note that the authors of this study searched for 941 HF chemicals and were only able to find information on 353, for which the above numbers were calculated.

Additionally, the majority of the previous studies investigated the impact of HF 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 using a specific compound approach, as was done in this study.[26-27] 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.[12]

### 1.4 Biocides in Hydraulic Fracturing Fluids

Biocides are used in HF fluid to inhibit microbes that 1) clog the well screen, 2) produce highly toxic $H_2S$ gas, 3) biodegrade other HF additives, and 4) cause corrosion of well equipment.[1, 15] If allowed to persist, these issues would reduce well and chemical efficiency as well as endanger worker health. Bacterial contamination can be found at all stages of the HF process and in most, if not all, of the materials used including water, proppant and drilling mud as well as storage tanks and pits. Because bacterial contamination is so ubiquitous, biocides are one of the most frequently used hydraulic fracturing additives.[13, 15] This additive accounts for about 0.005 - 0.05% of the total HF fluid volume,[28] and upwards of 1,000 gallons of biocide can be used per well.[15] In the hotter formations, such as the Haynesville in Texas, where downhole temperatures can reach 200°C, biocides are not always injected downhole because it is believed that the high temperatures will prevent bacterial growth despite cooling effects induced by the injected HF fluid. In these cases, however, biocides are still used in the above ground storage facilities to prevent bacterial issues there.[15]

Biocides can be categorized based on their mode of action and include lytic, oxidizing and electrophilic biocides. Lytic biocides, which include quaternary ammonium compounds (QACs), such as dimethylammonium chloride (DDAC), bind to anionic functional groups on the cell, eventually leading to lysis and destruction of the cell. Oxidizing biocides, which include chlorine or bromine-based species such as sodium hypochlorite (NaClO), release free radicals that attack

cells. These biocides can also cause corrosion and other equipment issues and are therefore rarely used in HF fluid. Finally, electrophilic biocides, which includes glutaraldehyde, cross-link with amino and other electron rich chemical groups on the cell wall, thereby preventing transport of essential nutrients into the cell and leading to cell death.[29]

Glutaraldehyde is the most widely used biocide in HF.[15, 28, 30] As a result of high prices and other factors, however, use of this additive has recently decreased, from 80% in 2012 to 27% in 2014.[15, 28] Some companies are switching to chlorine-based biocides while others are using biocide combinations that increase the biocidal activity of one or both chemicals. For example, when GA and QACs are combined, the surfactant properties of QACs make it easier for glutaraldehyde to penetrate the bacterial cell wall, thereby increasing its effectiveness.[29] Glutaraldehyde has previously been used by the oil and gas industry in off-shore drilling and also during secondary oil recovery in water flooding operations. This biocide is also widely used in medical sanitation, food preparation and water treatment.[15, 31]

Because glutaraldehyde is designed to kill microorganisms, it is not surprising that this chemical is highly toxic via the oral route and also extremely irritating to eyes and skin.[19] Because of its toxicity, this chemical is rarely, if ever, sold in its concentrated form and is instead diluted (e.g., 25% v/v) prior to sale. Glutaraldehyde is not believed to be carcinogenic, mutagenic or genotoxic.[19] It is, however, acutely toxic to aquatic organisms (3-12 mg/L, depending on species[32]) with greater adverse impact on freshwater organisms than those in salt water.[32] The median lethal concentration ($LD_{50}$) of this chemical in avian and mammalian species is 5,000 ppm. As a result of its cross-linking mode of action, glutaraldehyde is most effective against small organisms with little to no protective covering, including bacteria and fish.[33]

Glutaraldehyde is fully miscible in water. It is also moderately mobile in soil, as suggested by its moderate $K_{oc}$ value, but some sorption to organic matter is expected.[12] GA is unlikely to enter the atmosphere as evidenced by its low Henry's constant. Previous research has shown that GA is likely to remain in the aqueous phase and it is unlikely to bioaccumulate, as evidenced by its low $K_{ow}$ value (Table 2).[32]

Previous research has shown that glutaraldehyde is readily biodegradable (removed within 30 days) in both aqueous aerobic and anaerobic conditions at concentrations up to 50 mg/L.[32] Aqueous biodegradability studies have not been conducted at higher concentrations than this under the assumption that biodegradability would not occur if the glutaraldehyde concentration was inhibitory to bacteria.[32] Industry sources, however, have reported that glutaraldehyde is removed from produced water samples at concentrations as high as 1,000 mg/L, although the removal mechanism was not specified.[30] Under aerobic conditions, glutaraldehyde is fully metabolized to $CO_2$ after breakdown to glutaric acid. In anaerobic conditions, glutaraldehyde is metabolized to 1,5-pentanediol.[30] Studies have also shown that the rate of GA degradation is decreased in saltwater. Additionally, degradation increases with increasing temperature.[32]

Hydrolysis is also a potential removal mechanism for GA at higher pH. At pH = 5, the hydrolysis half life is 628 days but at pH = 9, the half life has decreases to 63.8 days. GA is stable to sunlight in aqueous solutions at pH = 5.[32] Finally, at pH > 8, GA reacts with itself to form a polymer. It is clear from these studies that solution pH should remain below pH = 7 and temperatures should remain low in order to minimize transformation of GA.

### 1.5 Surfactants in Hydraulic Fracturing Fluids

Surfactants are used in HF to 1) reduce the surface tension between the HF fluid and geologic formation, 2) increase fluid recovery after hydraulic fracturing and 3) maintain optimum viscosity

of the HF fluid.[12] Along with friction reducers, surfactants are generally one of the greatest additives by volume and account for 0.05-0.1% of the total fluid.[28] Surfactants are defined by their amphiphilic character, meaning that each molecule consists of a hydrophilic and a hydrophobic moiety. As a result of this amphiphilic character, one side of the surfactant will associate with hydrophobic or non-polar molecules while the other side will associate with hydrophilic or polar molecules, thereby reducing the surface tension between two immiscible phases. A wide variety of surfactants can be used for HF including amphoteric, anionic and non-ionic compounds.[12] Whether a surfactant is anionic, cationic, nonionic or amphoteric is determined by the hydrophilic moiety.[34] Examples of surfactants commonly used in oil and gas operations include lauryl sulfate and polypropylene glycol. A selected surfactant may have multiple purposes and can also act as a biocide, cross-linker or gelling agent in some cases.[12]

Polyethylene glycols are a commonly used in HF and have been found in flowback water.[35] They have also been found in the effluent of wastewater treatment plants that are treating HF fluid waste.[36] PEGs are non-ionic, linear alcohol ethoxylates and are produced by reacting fatty alcohols with ethylene oxide.[34] Polypropylene glycol (PPG) is produced in a similar manner. Because they are nonionic, these molecules have a low sensitivity to changes in pH and are compatible with charged molecules.[34] The carbon chain of a PEG is the hydrophobic part of the molecule, whereas the ethylene oxide is the hydrophilic moeity. PEGs are produced in a greater quantity (millions of tons per year[37]) than any other polyether and are frequently released to the environment as a result.[23] For example, PEG 550 has been found in river water and seawater at concentrations of 0.5 and 68 µg/L, respectively.[37] PEGs are widely used in household items and industrial processes because they are good emulsifiers and solubilizers and are relatively low in toxicity.[24] PEGs can be

found in detergents, cosmetics, lubricants, inks and shoe polish. They are used by the pharmaceutical industry and also in plastics production.[34, 37]

As a result of their amphiphilic character, surfactants can increase the solubility of other compounds in soil/aqueous systems.[38] Because of this property, surfactants have been used in remediation efforts to increase solubility, and therefore biodegradation, of contaminants in soil.[39] At a critical level, however, when surfactant concentration increases, the surfactants form micelles. In many instances, the formation of micelles results in delayed degradation because the contaminant has partitioned into the micelles and is less bioavailable.[39] Additionally, it has been shown that PEGs solubility decreases with increasing temperature.[34]

The molecular weight of PEGs can vary widely and as a result the properties of these chemicals vary from viscous liquids to waxy solids.[24] PEG 400, which was used in this study, is a clear, low viscosity liquid. PEG 400 is fully miscible in water as are PEGs up to a few million daltons (Table 2).[23] This is not the case for PPG, however, which are water soluble only up to 1,000 Da.[23] Previous studies have shown that PEGs are biodegradable in aqueous solutions up to 57,800 Daltons and that degradation rates increase with increasing size.[37] Level of PEG degradation (% mineralized) was not influenced by MW.[37] Concentrations up to 75 g/L have shown signs of biodegradation.[40] It has also been shown that degradation rates increase with increasing temperature.[40] PEGs can be fully mineralized to $CO_2$ when degraded.[40] Anaerobic degradation of PEGs is possible, however, it proceeds much slower than aerobic degradation.[37] Abiotic degradation of PEGs has not been observed.[24] Most surfactants are not toxic to organisms at concentrations present in the environment but some show toxic effects above 0.1 mg/L.[41] Specific toxicity data on PEG 400 was not readily available, however, this chemical has a median lethal concentration ($LD_{50}$) in rats of 30 mL/kg (Table 2).[1]

### 1.6 Friction Reducers in Hydraulic Fracturing Fluids

Friction reducers are used in HF to reduce the friction pressure during pumping so that a higher pressure can be achieved in the formation while using fewer pumps and less energy.[13, 28] There are two main types of HF operations: slick water and gel fracturing operations. The fluids used in slick water fracturing jobs contain a friction reducer and are lower viscosity (2-3 cP). The fluids used in gel fracturing jobs contain a gelling agent, such as guar gum, and can have viscosities as high as 1000 cP. In the vast majority of slick water jobs (near 100%) a polyacrylamide based friction reducer is included in the HF fluid.[28] Along with surfactants, friction reducers are the highest chemical additive by volume, accounting for 0.05 - 0.1% of the total volume.[28]

There are three main types of polyacrylamide based friction reducers including anionic, cationic and nonionic.[13] Friction reducer choice is strongly dictated by the compatibility with other additives, most importantly the biocide and breaker, as well as high salt concentrations, if present.[13] An anionic-polyacrylamide-based friction reducer, which is the type most commonly used in industry, was selected for this experiment. These friction reducers are generally compatible with higher salt concentrations.[13] As a result of their negative charge, however, anionic polyacrylamide is not compatible with QACs or another positively charged molecule and a different type of friction reducer would be necessary. Cationic polyacrylamide is generally compatible with other cationic molecules, however, is not compatible with higher salt concentrations.[13] Additionally, it can have deleterious impacts on aquatic life and therefore is used less than anionic polyacrylamide. Nonionic polyacrylamide is compatible with charged molecules but less efficient than both cationic and anionic polyacrylamide. As a result, it is clear that many factors must be considered when selecting a friction reducer product.

Similarly to polyethylene glycols, polyacrylamide size can vary widely, ranging up to tens of millions of daltons. These molecules can be linear or branched and both size and structure are optimized for different applications. For example, linear, anionic polyacrylamide (1-2 x $10^7$ Da) is commonly used in agriculture to reduce soil erosion.[42] The anionic character of these large molecules produces a stabilizing effect via electrostatic interactions with the soil and can reduce erosion by as much as 94%.[42] Polyacrylamide is also used in other products including agricultural soil stabilizer, baby diapers, cosmetics, biomedical applications and as a flocculant in water purification. It is also important to note that polyacrylamide has been used in the oil and gas industry for decades, prior to its role in HF fluid.[43]

Previous studies have shown that polyacrylamide is water soluble and non-volatile.[12] It has a low toxicity, with median lethal concentrations ($LD_{50}$) in mice and rabbits equal to 12,950 mg/kg and 11,250 mg/kg, respectively (Table 2).[12, 20] Polyacrylamide is stable up to 200 °C but begins to decompose at temperatures above 300 °C.[20, 28] Few microorganisms have been found that are able to degrade it, however many studies have shown that microorganisms are able to use polyacrylamide as a nitrogen source, despite being unable to break down the carbon chain.[20, 44] Since polyacrylamide is so widely used, a significant effort was made to determine if this molecule could break down into acrylamide, a known neurotoxin and possible carcinogen. It has been determined that in the presence of sunlight and at temperatures up to 200 °C, breakdown into acrylamide is not a significant issue.[20, 43] Additionally, in the few instances where polyacrylamide was biodegraded, no acrylamide was detected.[44-45]

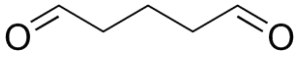
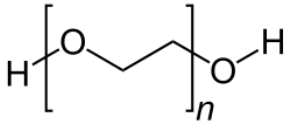
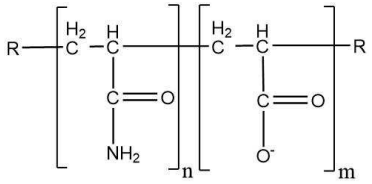
### 1.7 Research Objectives

In this study, spills of HF fluid additives on agricultural topsoil were simulated in order to advance our current understanding of processes that control their environmental fate and toxicity.

**Objective 1** was to determine the degradation kinetics and mechanisms of three widely used HF fluid components, namely, the biocide glutaraldehyde (GA), polyethylene glycol (PEG) surfactants, and a polyacrylamide (PAM)-based friction reducer. **Objective 2** was to elucidate co-contaminant effects (i.e., combinations of GA, PEGs, PAM, and salt) on HF fluid additive transformation kinetics and products.

This work was submitted on January 15th, 2016 to the American Chemical Society journal *Environmental Science and Technology* for review.

**Table 2.** Chemical, physical and oral toxicity values for Glutaraldehyde, Polyethylene Glycol 400 and Polyacrylamide.

| **Compound** | **Formula** | **Structure** | **Properties**[12, 15, 19, 46] |
|---|---|---|---|
| Glutaraldehyde | $C_5H_8O_2$ |  | Molecular Weight = 100.11 g/mol<br>$\log(K_{ow}) = -0.18$<br>$K_H = 1.10 \times 10^{-7}$ atm $m^3$ $mol^{-1}$<br>$K_{OC} = 120 - 500$<br>Density = 1.06 g $cm^{-3}$ (at 25°C)<br>Melting Point = -14°C<br>Boiling Point = 188°C<br>Vapor Pressure = 0.6 mmHg (at 30°C)<br>Solubility in Water = Miscible<br>Viscosity = 20.15 cP (at 20°C)<br>Oral Toxicity ($LD_{50}$), rat = 134 - 1470 mg $kg^{-1}$<br>Oral Toxicity ($LD_{50}$), mouse = 100 mg $kg^{-1}$<br>Oral Toxicity ($LD_{50}$), rabbit = 1.59 mg $kg^{-1}$ (50% aqueous solution) |
| Polyethylene Glycol 400 | $HO(CH_2CH_2O)_nH$ |  | Molecular weight = 400 g/mol (average value)<br>$\log(K_{ow}) = -2.1$<br>Density = 1.128 g $cm^{-3}$ (at 20°C)<br>Melting Point = 4-8°C<br>Solubility in Water = Miscible<br>Viscosity = 0.012 cP (at 20°C)<br>Oral Toxicity ($LD_{50}$), rat = 30 mL $kg^{-1}$ |
| Polyacrylamide | $(C_3H_5NO)_n(C_3H_3O_2^-)_m$ |  | Molecular Weight = 10-20 x $10^6$ g/mol (average range)<br>Oral Toxicity ($LD_{50}$), rat = > 1000 mg $kg^{-1}$<br>Oral Toxicity ($LD_{50}$), mouse = 12,950 mg $kg^{-1}$<br>Oral Toxicity ($LD_{50}$), rabbit = 11,250 mg $kg^{-1}$<br>Solubility in Water = Miscible<br>Vapor Pressure = Non-volatile |

# 2. EXPERIMENTAL METHODS

## 2.1 Experimental Set-up

To study the environmental fate and transport of glutaraldehyde (25% w/w in water, Sigma Aldrich), polyethylene glycol 400 (100%, Alfa Aesar), and friction reducer in agricultural topsoil, aerobic batch studies were conducted using Julesburg sandy loam collected from Weld County, Colorado. 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.

### *2.1.1 Field sample collection*

The topsoil used in these batch experiments was collected from Weld County, Colorado (GPS coordinates: -104.7458°, 40.3098°) using a 3-foot long, stainless steel soil core lined with a polyethylene tube. Prior to analysis, the top 30 cm of soil was thoroughly homogenized including the A (0-15cm), BA (15-25 cm) and a portion of the Bt1 (25-61 cm) horizon. Soil was sieved through a 2 mm mesh and stored at 23±1 °C.

### *2.1.2 Soil Characterization*

Total organic carbon (TOC) content of the soil was determined using the combustion method. This analysis was conducted in triplicate. Soil was weighed out (15 g) into three porcelain crucibles and dried in the oven at 105°C for 24 hours. The soil was then heated in the muffle furnace at 375°C for 16 hours, thereby combusting any carbon contained in the soil. After cooling, the soil was weighed again to determine the amount that had combusted and therefore the TOC content of the soil. TOC content of the soil was 7.9 ± 0.5 g/kg

Soil texture was determined using the 24 hour ASTM D422 hydrometer method. Texture analysis showed that the sieved soil was 52% sand, 26% silt and 22% clay. Soil grains larger than 2 mm were not included in this texture analysis. As a result, the soil used in this experiment was determined to be a sandy clay loam. This is slightly different than the sandy loam classification of the original soil core, which did contain grains larger than 2 mm.

**Figure 3.** A Julesburg sandy loam soil collected from Weld County, Colorado (GPS coordinates: -104.7458, 40.3098) was sieved through a 2 mm mesh prior to the start of the experiment.

### *2.1.3 Stock solutions*

The composition of synthetic surface water (SSW) was determined from Troyer, 2014[47] and prepared using a protocol described by Smith, et al.[48] The final concentration of SSW is shown in Table 3. This solution was filter-sterilized into autoclaved bottles. All chemicals used for the SSW were obtained from Fisher Scientific (purity > 98%) and used as received.

**Table 3.** Final composition of the synthetic surface water used in the batch experiments.

| Chemical | mM |
|---|---|
| $MgSO_4$ | 1.07 |
| $CaCl_2 \cdot 2H_2O$ | 0.0257 |
| $Ca(NO_3)_2 \cdot 4H_2O$ | 0.00600 |
| $Na_2SO_4$ | 1.12 |
| $CaCO_3$ | 0.506 |
| $KHCO_3$ | 0.783 |
| $NaHCO_3$ | 1.76 |
| $NaH_2PO_4$ (buffer) | 19.5 |

To ensure homogenous application of additives between each batch reactor, stock solutions were prepared for GA (26,000 mg/L), PEG (75,000 mg/L) and PAM (438 mg/L PAM; 2500 mg/L ASP900). All stock solutions were prepared in autoclaved bottles using filter sterilized Milli-Q water and sterilized materials. The GA solution was made immediately prior to the start of the experiment to prevent polymerization of GA. Aliquots of these stock solutions (1.9 mL GA stock, 2 mL PEG stock and 60 mL PAM stock) were then added to each reactor to achieve the desired starting concentration.

Additionally, prior to the start of the experiment, soil and SSW were incubated in a 1 L flask at a concentration of 100 g/L. The flask was placed on a shaker table for 1 week in order to encourage the growth of native bacteria. A 5-mL aliquot of the aqueous portion of this incubation was added to each biotic flask at the start of the experiment to ensure a more homogenous microbial community among the batch reactors. A similar incubation was performed for the abiotic reactors, however, this incubation was sterilized using 2.5 g/L $NaN_3$. An ATP test (see

below for method details) was conducted on the aqueous solution of this incubation prior to the start of the experiment to ensure microbial activity was at a minimum.

### *2.1.4 Batch reactor set-up*

Aerobic batch studies were conducted in 250-mL Erlenmeyer flasks containing 20 g of topsoil and 200 mL of synthetic surface water 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). All materials and glassware used in this experiment were sterilized prior to the experiment or purchased as sterile.

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 only, PEGs only, PAM only); three reactors with two HF additives each (GA/PEGs, GA/PAM, 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 2.5g/L $NaN_3$) reactors for each chemical treatment were prepared in triplicate. Soil and sterilized SSW in the abiotic reactors were set up 3 days prior to the start of the experiment to ensure sterilization of the soil. Abiotic flasks were re-dosed 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.[49]

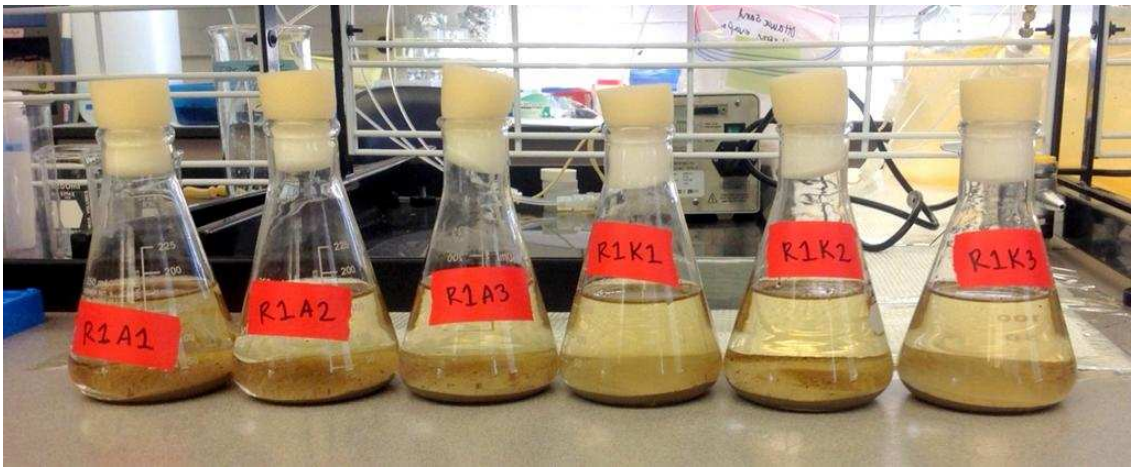



**Figure 4.** Aerobic batch studies were conducted in 250-mL Erlenmeyer flasks containing 20 g of topsoil and 200 mL of aqueous solution. As shown above for the control reactor with SSW (R1), biotic (three reactors on the left) and abiotic reactors (three reactors on the right) for each chemical treatment were prepared in triplicate.


**Figure 5.** Reactors were plugged with a plastic foam stopper and placed on a rotary shaker at 100 rpm and 23±1 °C.

Samples were obtained using sterile 5-mL syringes (Becton Dickinson, Franklin Lakes, NJ) and sterile 1.2mm x 40mm needles (Becton Dickinson, Franklin Lakes, NJ). 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 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 (PTFE-lined silicone septum). Aliquots for GA analysis were immediately derivatized as described below to prevent polymer formation.

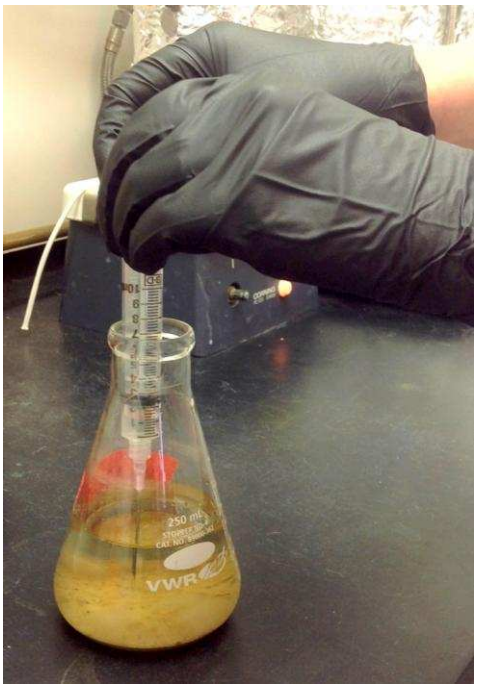



**Figure 6.** 2-mL samples were obtained using sterile syringes and needles.

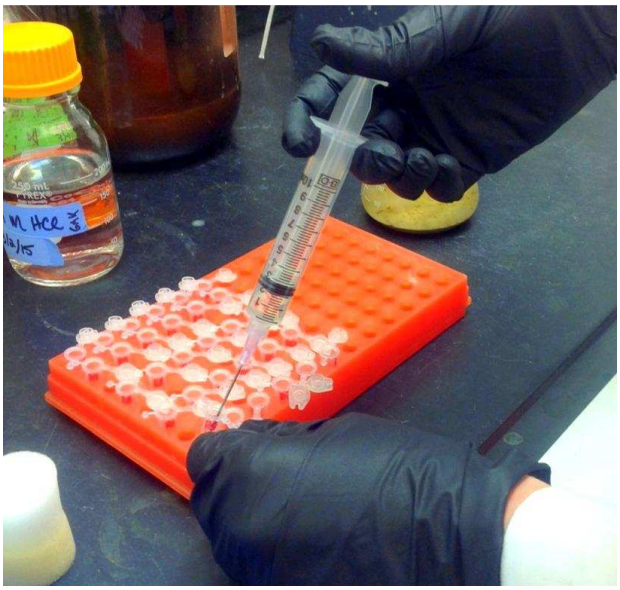


**Figure 7.** Immediately after sampling (before filtering), a 200-µL aliquot of each sample was collected in an autoclaved plastic centrifuge vial for ATP analysis.

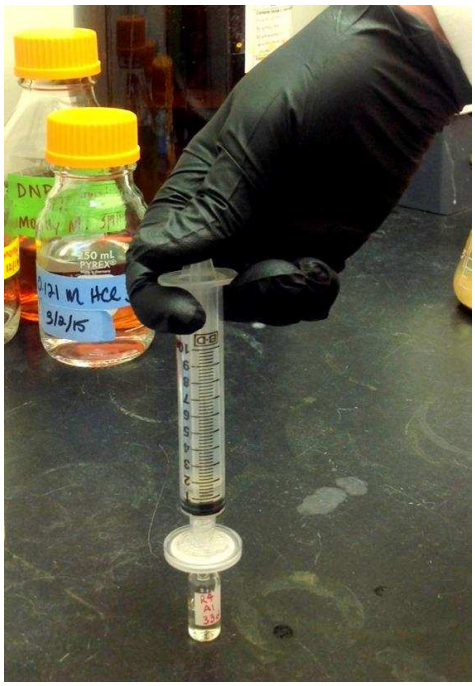


**Figure 8.** After an aliquot of the sample was collected for ATP analysis, the remainder of the sample was immediately passed through a 0.45-µm cellulose acetate filter into an autoclaved 2-mL amber glass vial and capped. The first 0.5 mL of this sample was discarded.

## 2.2 Ammonium Sulfate and Glutaraldehyde Studies

The friction reducer product, ASP900, contains ammonium sulfate (AS), in addition to the PAM that was a focus of this study. In order to interpret the differences in GA removal between reactors with and without ASP900, additional batch studies were conducted to determine if chemical GA removal was dominated by interactions with PAM, ammonium sulfate or both. These studies were conducted after the conclusion of the batch studies that were the main focus of this experiment and were set up as previously described. Three different chemical combinations were studied including: GA/AS, GA/AS/Soil and GA/ASP900. Reactors were set up in triplicate. Initial concentrations of these additives were 250 mg/L GA; 750 mg/L ASP900 and 245 mg/L AS, which was the initial concentration in the original reactors containing ASP900, as determined using ion chromatography. Samples were taken at the beginning of the experiment as well as at 30 days (for AS) and 45 days (for GA).

## 2.3 Analysis

The contaminants of interest (GA, PEG and PAM) were analyzed at decreasing intervals over the course of the experiment. Samples were analyzed within 48 hours of sampling.

### *2.3.1 Glutaraldehyde Analysis*

GA analysis was conducted with an Agilent 1200 Series high-performance liquid chromatography (HPLC) system using a 4.6 x 150 mm Agilent ZORBAX Eclipse XDB-C18 Column (Agilent) with a diode array detector (DAD) monitoring UV absorbance at 358 nm. GA was eluted using an isocratic method with A (acetonitrile) and B (de-ionized water with 0.1% formic acid) at a ratio of 70:30. The mobile phase flow rate was 3.0 mL/min, the injection volume was 10 µL and the column temperature was 40°C. A 2,4-dinitrophenylhydrazine (DNPH) reagent was made by

mixing 0.286 g DNPH (30% water content) with 100 mL ACS-grade acetonitrile. GA samples were derivatized by combining 50 µL aqueous sample, 1.5 mL DNPH reagent and 450 µL 0.12 M HCl. Samples were stored in the dark for at least 30 minutes before analysis.

### *2.3.2 Polyethylene Glycol Analysis*

PEGs were analyzed with an Agilent 1100 Series liquid chromatograph coupled with an Agilent G3250AA time-of-flight (ToF) mass spectrometer equipped with an electrospray ionization (ESI) source. Analysis was conducted following the method described in Thurman et al. (2014)[35] with some exceptions, full details of which are described here. Mobile phases were A (0.1% formic acid) and B (acetonitrile with 0.1% formic acid). A gradient elution method was developed with 0-5 minutes, 10% B; 5-11 min, 10-34% B; 11-18 min, 34-90% B; 18-25 min, 10% B. The flow rate was 0.6 mL/min, the injection volume was 5 µL, and the temperature of the drying gas was 325°C. A 4.6 x 150 mm Agilent ZORBAX Eclipse XDB-C8 column was used. The capillary, fragmentation and skimmer voltages were 4000 V, 190 V and 45 V, respectively. The temperature of the drying gas ($N_2$) was 325 °C, the flow rate of the drying gas was 10 L/min, and the pressure of the nebulizer gas ($N_2$) was 45 psi. LC/MS accurate mass spectra were recorded across the range 100 - 3,000 m/z.

Prior to analysis, samples were diluted 1:40 with sterile deionized water. Polypropylene glycol (300 ppm) was used as an internal standard (also diluted 1:40). Calibration curves were created by relating the concentration of PEGs in the initial sample (prior to the 1:40 dilution) to the ratio of the PEGs/PPGs response in total peak area.

An automated calibrant delivery system using a low flow calibrating solution (calibrant solution A, Agilent) was used to obtain accurate mass measurements of each peak from the total ion chromatograms. This information was analyzed in the Mass Hunter Qualitative Analysis

software using the "Find by Formula" method, which displays potential chemical formulas and corresponding mass accuracy for each identified mass of PEG surfactant or by-product.

### *2.3.3 Polyacrylamide Analysis*

PAM analysis was conducted via size-exclusion chromatography using the Agilent 1200 Series HPLC described above with a PL aquagel-OH Guard 50 x 7.5 mm column followed by a PL aquagel-OH 60 300 x 7.5 mm column with the DAD monitoring absorbance at 215 nm. The mobile phase was 3 mM NaCl, flow rate was 1.0 mL/min and the injection volume was 50 µL.

### *2.3.4 ATP Analysis*

ATP concentration was measured to ensure that the microbial activity in the abiotic samples was at least two 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 two hours of sampling using the BacTiter-Glo$^{TM}$ Microbial Cell Viability Assay (Promega Corporation, Madison, WI). An opaque-walled 96-well plate was prepared with 100 µL of non-filtered sample and 100 µL reagent in each well. The plate was allowed to equilibrate in the dark for five minutes 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.

# 3. 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: ~500-1,000 mg/L; PAM: ~85-175 mg/L).[28]

## 3.1 Glutaraldehyde Removal

Initial GA concentration was 250 mg/L in all abiotic and biotic reactors containing this biocide. As shown in Figure 9, 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. Polymerization of GA was minimized via pH control, as evidenced by the lack of GA dimers and trimers present in the ToF mass spectra.[50]

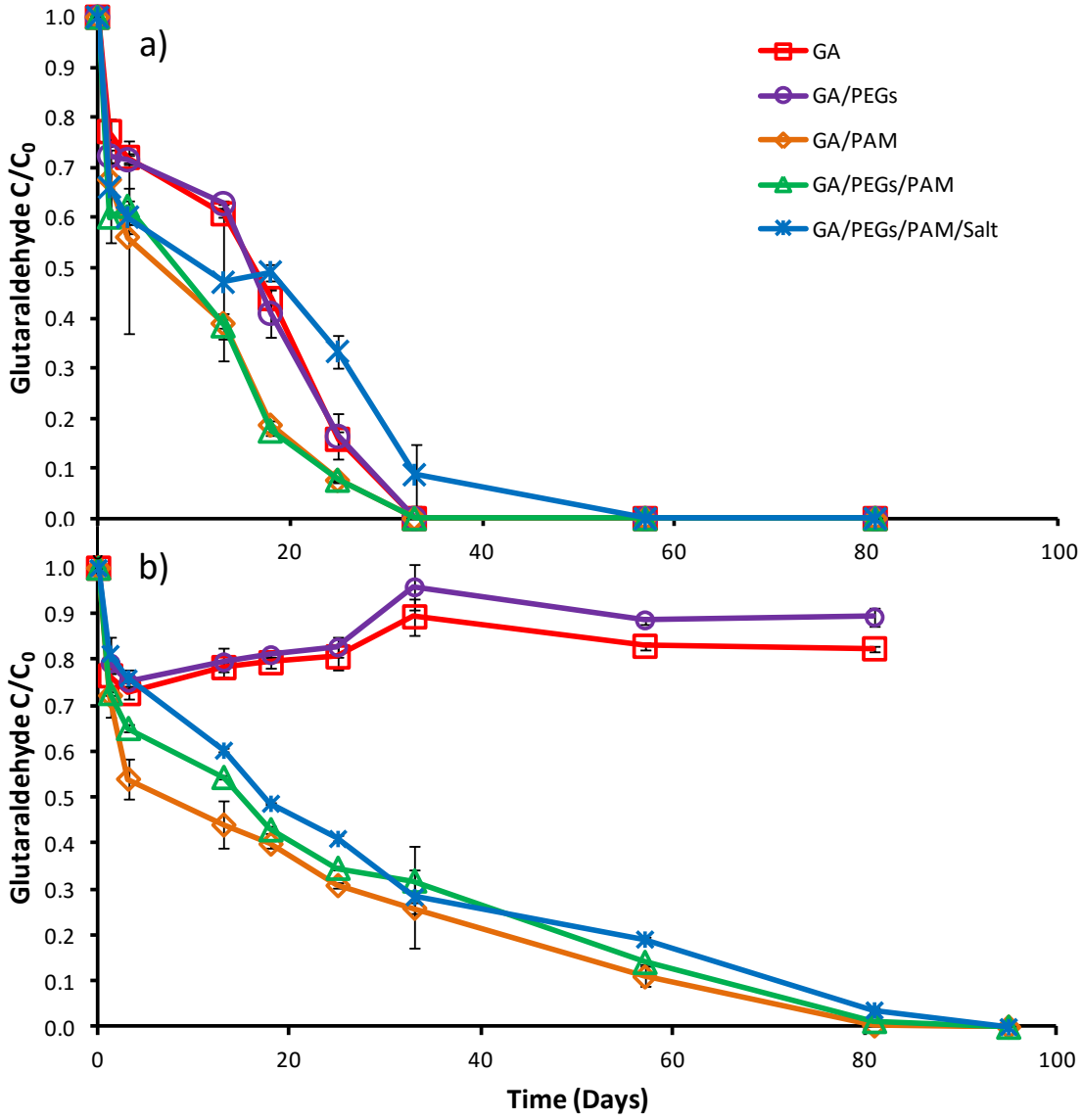



**Figure 9.** Glutaraldehyde removal from the aqueous phase over time in a) biotic and b) abiotic reactors containing agricultural topsoil, synthetic surface water, varying HF fluid additives and salt or no salt.

In the biotic reactors, between 23% (GA only) and 40% (GA/PEGs/PAM) of GA sorbed within one day, lowering the aqueous GA concentrations to between 150 and 193 mg/L (Figure 9a). Aqueous GA concentrations continued to decline 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 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, for GA only, GA/PEGs, GA/PAM and GA/PEGs/PAM were 0.0661 $d^{-1}$, 0.0654 $d^{-1}$, 0.0918 $d^{-1}$ and 0.0971 $d^{-1}$, respectively (Figure 10, Table 4). 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 (Figure 11).

**Table 4.** Observed pseudo-first order rate constants ($k_{obs}$) and half-lives ($t_{1/2}$) for glutaraldehyde (GA) and polyethylene glycols (PEGs) at 23±1 °C and pH = 5.9-6.3 (biotic) or pH = 6.5-7.0 (abiotic).

| Reactor Contents | $k_{obs}$ ($d^{-1}$) | $t_{½}$ (d) |
|---|---|---|
| **GA - Biotic** | | |
| 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** | | |
| GA/PAM | 0.0300 | 23 |
| GA/PEGs/PAM | 0.0285 | 24 |
| GA/PEGs/PAM/Salt | 0.0266 | 26 |
| **PEGs - Biotic** | | |
| PEGs | 0.0339 | 20 |
| PEGs, GA | 0.0230 | 30 |
| PEGs, PAM | 0.0435 | 15 |
| PEGs, GA, PAM | 0.0073 | 95 |

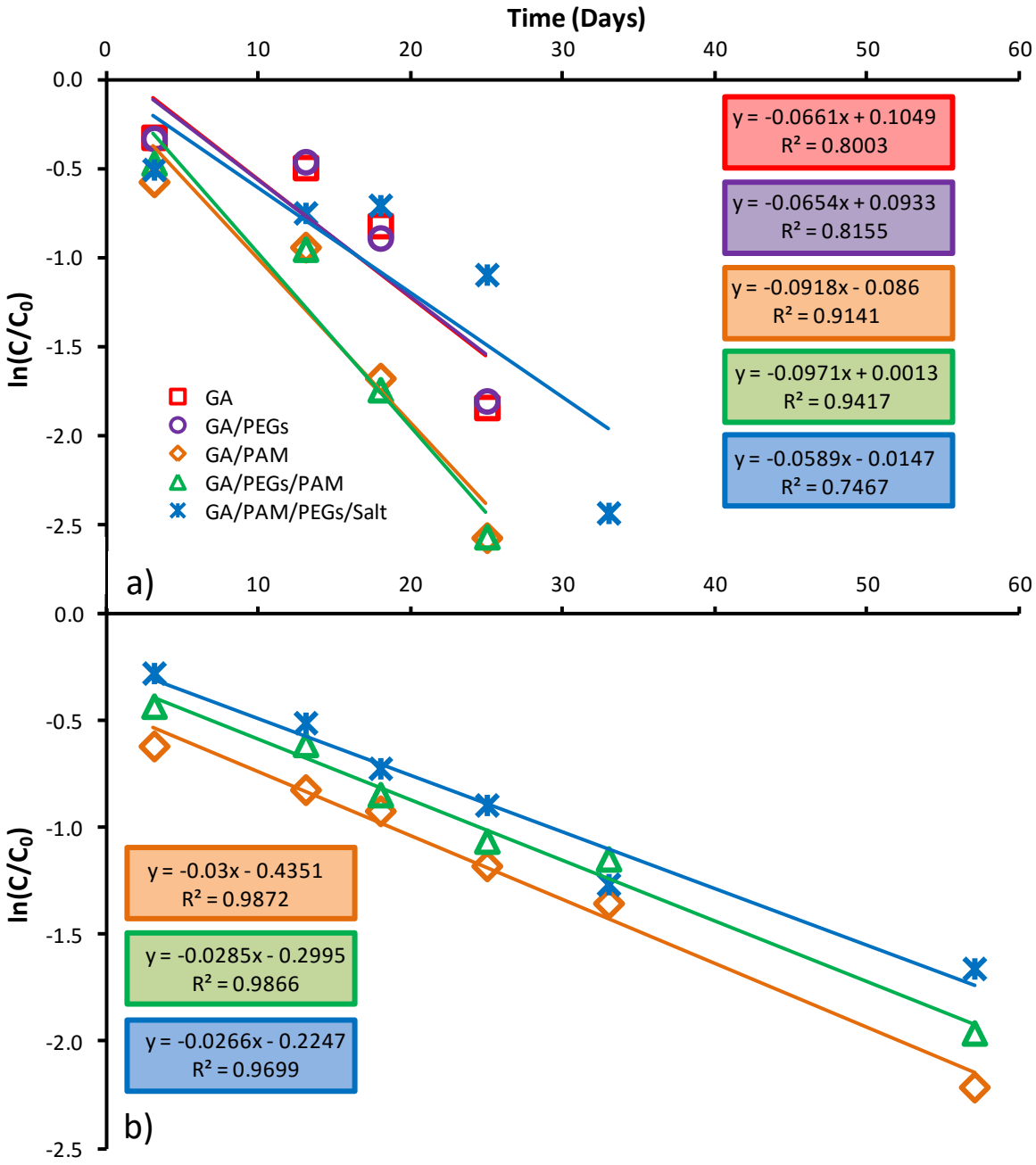



**Figure 10.** Pseudo-first order reaction rates for a) biotic and b) abiotic GA reactors. No removal after the initial sorption phase was observed in the abiotic GA and GA/PEGs reactors so reaction rates were not calculated.

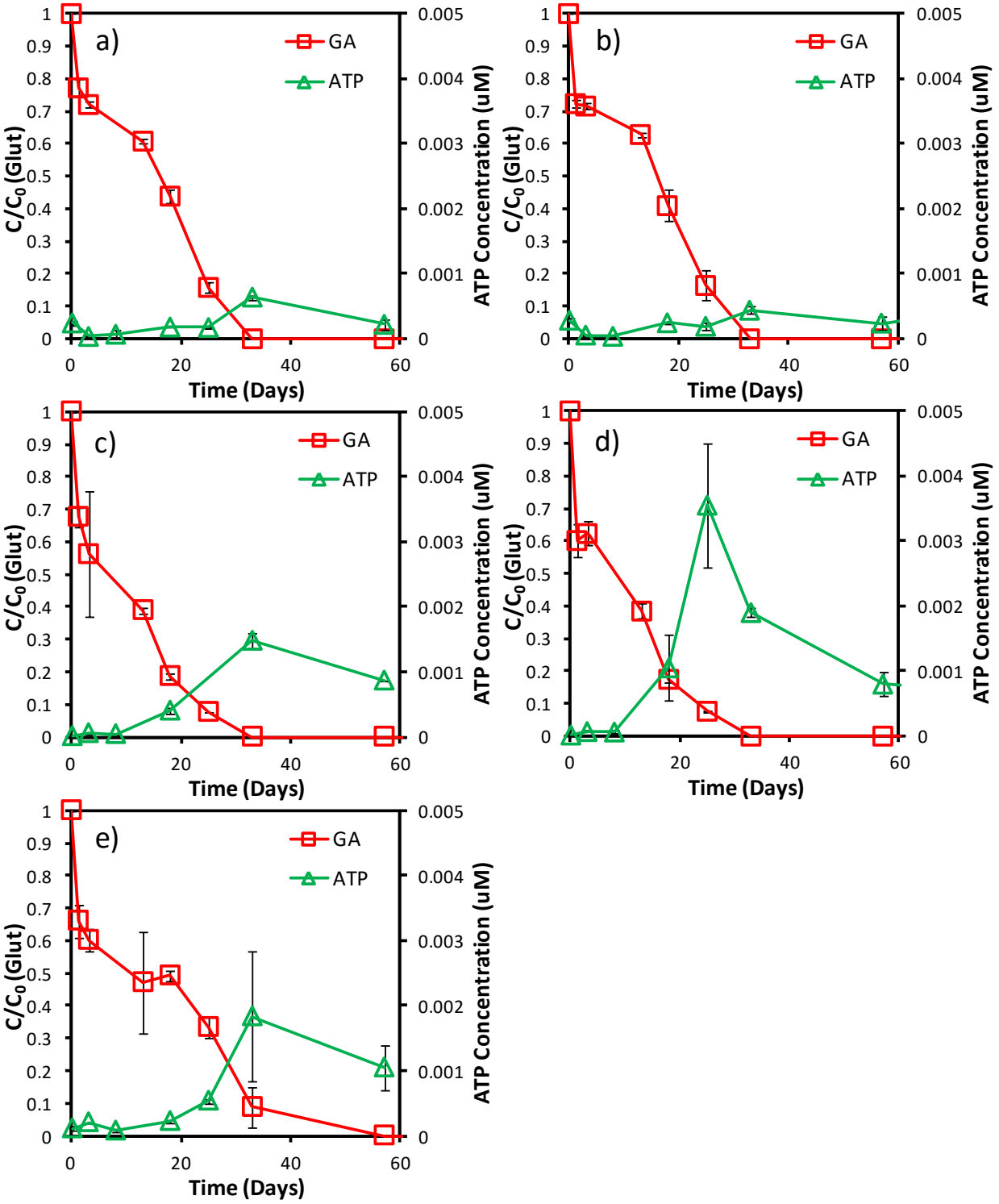



**Figure 11.** Normalized GA concentration (red squares) and ATP concentration (green triangles) in the biotic a) GA only, b) GA/PEG, c) GA/PAM, d) GA/PEG/PAM and e) GA/PEG/PAM/Salt reactors for first 60 days of experiment to highlight spike in ATP concentration at/ near the time GA is removed.

In abiotic reactors containing both GA and PAM, GA was fully removed from the aqueous phase within 95 days (Figure 9b). Excluding the initial sorption phase, the observed pseudo-first order rates of removal in the GA/PAM, GA/PAM/PEGs and GA/PAM/PEGs/Salt reactors were 0.0300 $d^{-1}$, 0.0285 $d^{-1}$ and 0.0266 $d^{-1}$, respectively (Figure 10, Table 4). 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.

### *3.1.1 Potential Biofilm in GA reactors*

On day 33 of the experiment, an off-white, gelatinous substance was observed in all biotic reactors containing GA, as shown in Figure 12. At this point, GA was fully removed from all biotic reactors except the GA/PAM/PEGs/Salt reactor (Figure 9a). This substance was found lightly attached to the soil or suspended in solution surrounding a soil aggregate. The substance did not dissolve in water and a spectrophotometric analysis was inconclusive. This substance was only observed in biotic GA reactors.

Previous studies have shown that sulfate-reducing bacteria form biofilms as a way to protect against biocides and reduce their efficacy. Additionally, biofilms have been shown to be an integral part of GA biodegradation, whereas planktonic cells are not.[51] It is likely that this substance was biofilm, however, further analysis needs to be conducted to confirm this.

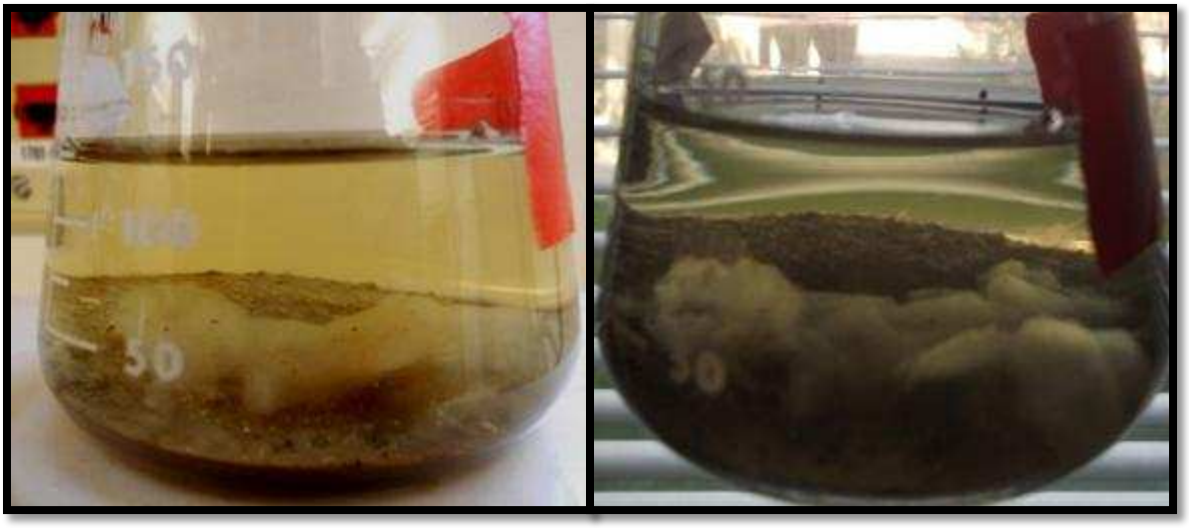


**Figure 12.** Pictures showing a substance that formed, lightly attached to the soil in all biotic reactors containing glutaraldehyde (GA). It is believed that this substance is a biofilm, but that was not confirmed over the course of the experiment.

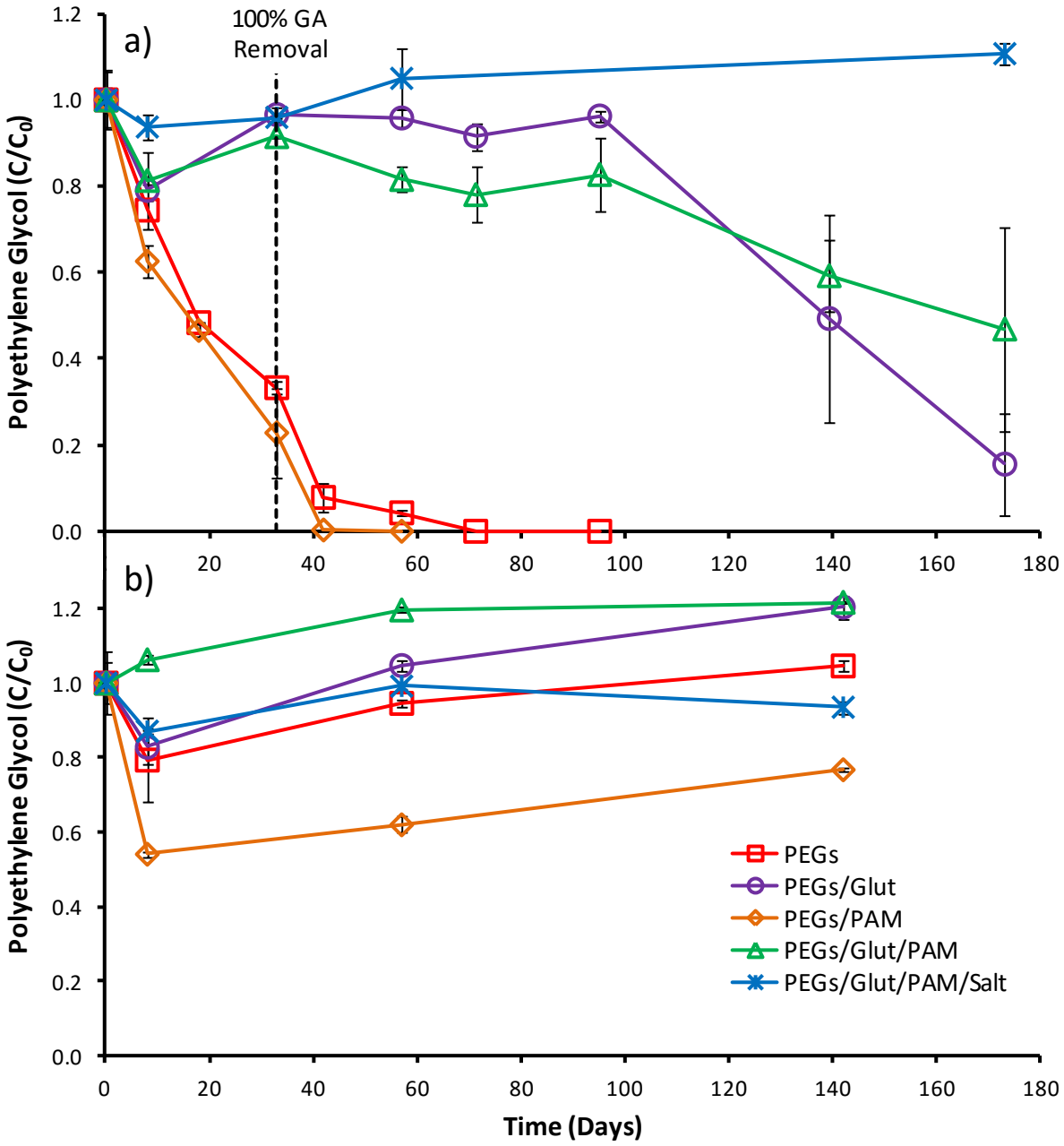



**Figure 13.** Total polyethylene glycol (PEG) removal from the aqueous phase over time in a) biotic and b) abiotic reactors containing agricultural topsoil, synthetic surface water, varying additives and salt or no salt. The dashed line in (a) shows the point in time when GA had been completely removed from biotic reactors without salt, and reduced by more than 90 % in the biotic reactor containing salt.

### 3.2 Polyethylene 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 13). The observed pseudo-first order rate constants in these reactors were 0.0339 $d^{-1}$ and 0.0435 $d^{-1}$, respectively, as measured between the beginning of the experiment and day 33 (Table 4, Figure 14). Additionally, the concentration of ATP in the PEGs/PAM reactor was consistently higher (up to one order of magnitude) than in the PEGs only reactor (Figure 18c). 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 endpoint) once most of the GA had been removed by day 25 (Figure 16a, b). On day 95, degradation rates increased in both reactors containing GA. Between 95 and 173 days, the observed pseudo-first order rate constants in the PEGs/GA and PEGs/GA/PAM reactors were 0.0230 $d^{-1}$ and 0.0073 $d^{-1}$, respectively (Figure 14). The PEGs/GA/PAM/Salt reactor showed no signs of biodegradation by day 173 but did show signs of initial sorption to the soil. At the end of the experiment, ATP concentrations were lowest in this reactor as compared to all other reactors except the control with salt. Aldehyde- and carboxylate-derivatives of PEGs were observed as oxidation intermediates in all biotic reactors where degradation occurred (Figure 15, Table A1 - Table A4), and were obtained as described in the Experimental Methods section.

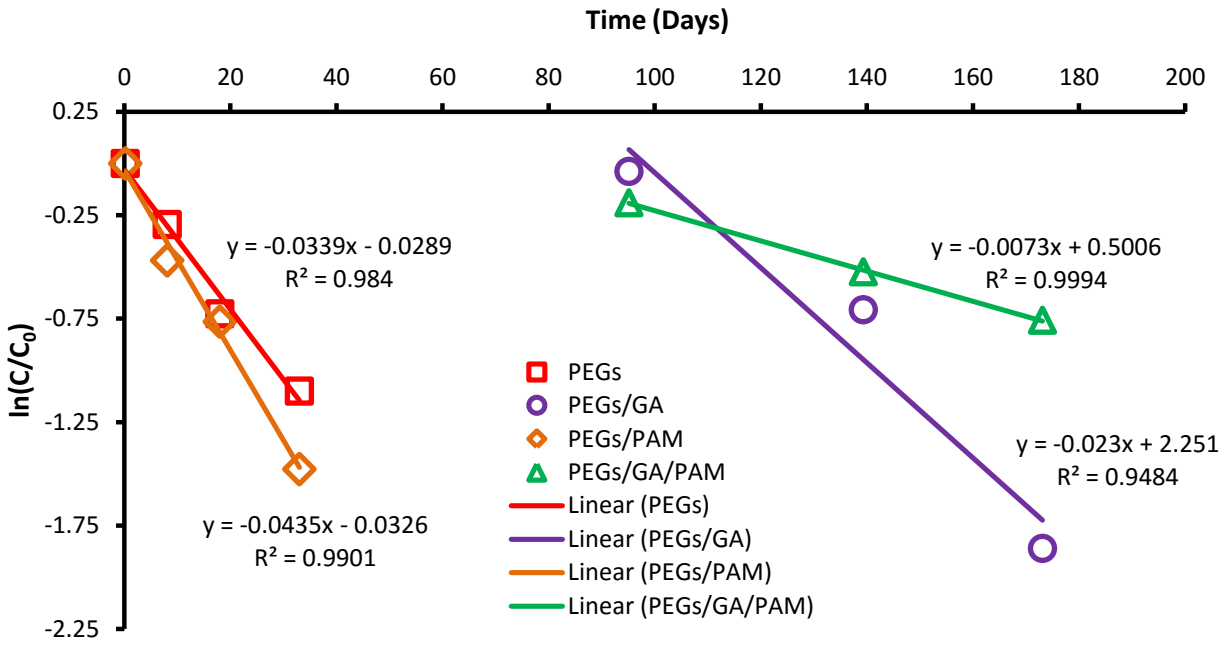



**Figure 14.** Pseudo-first order reaction rates for biotic PEGs in all reactors in which PEG degradation was observed.

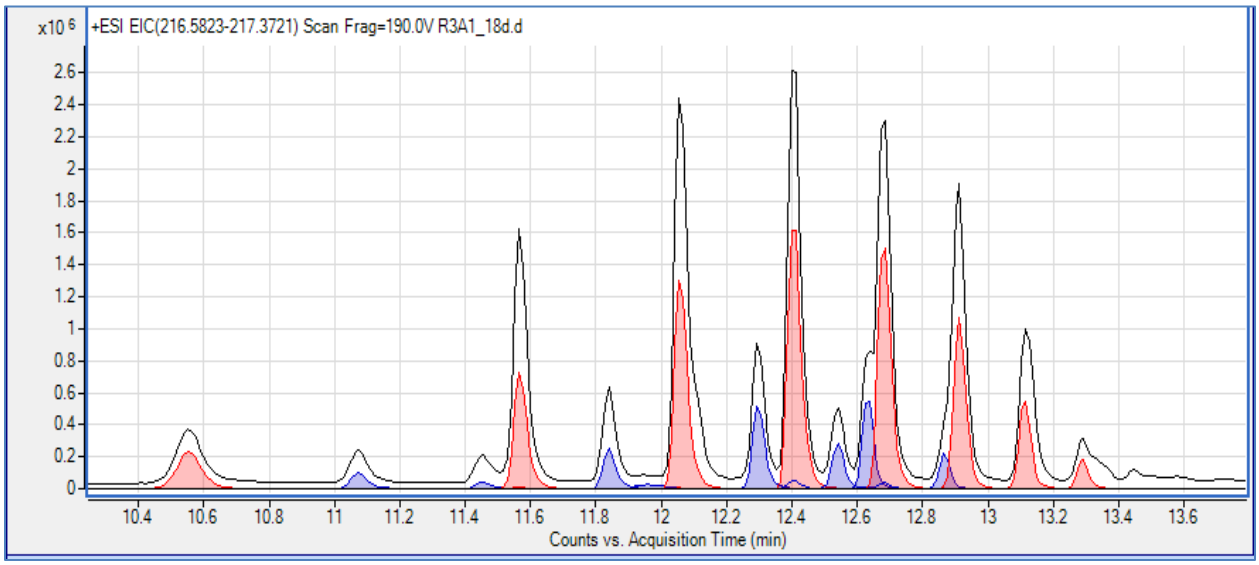



**Figure 15.** Chromatogram showing total ion count (black line) as well as ion chromatograms for polyethylene glycols (red) and carboxylate-derivatives of polyethylene glycols (blue). This chromatogram is from the biotic PEGs only reactor on day 18 of the experiment. At this time, 52% of the PEGs had been removed due to biodegradation.

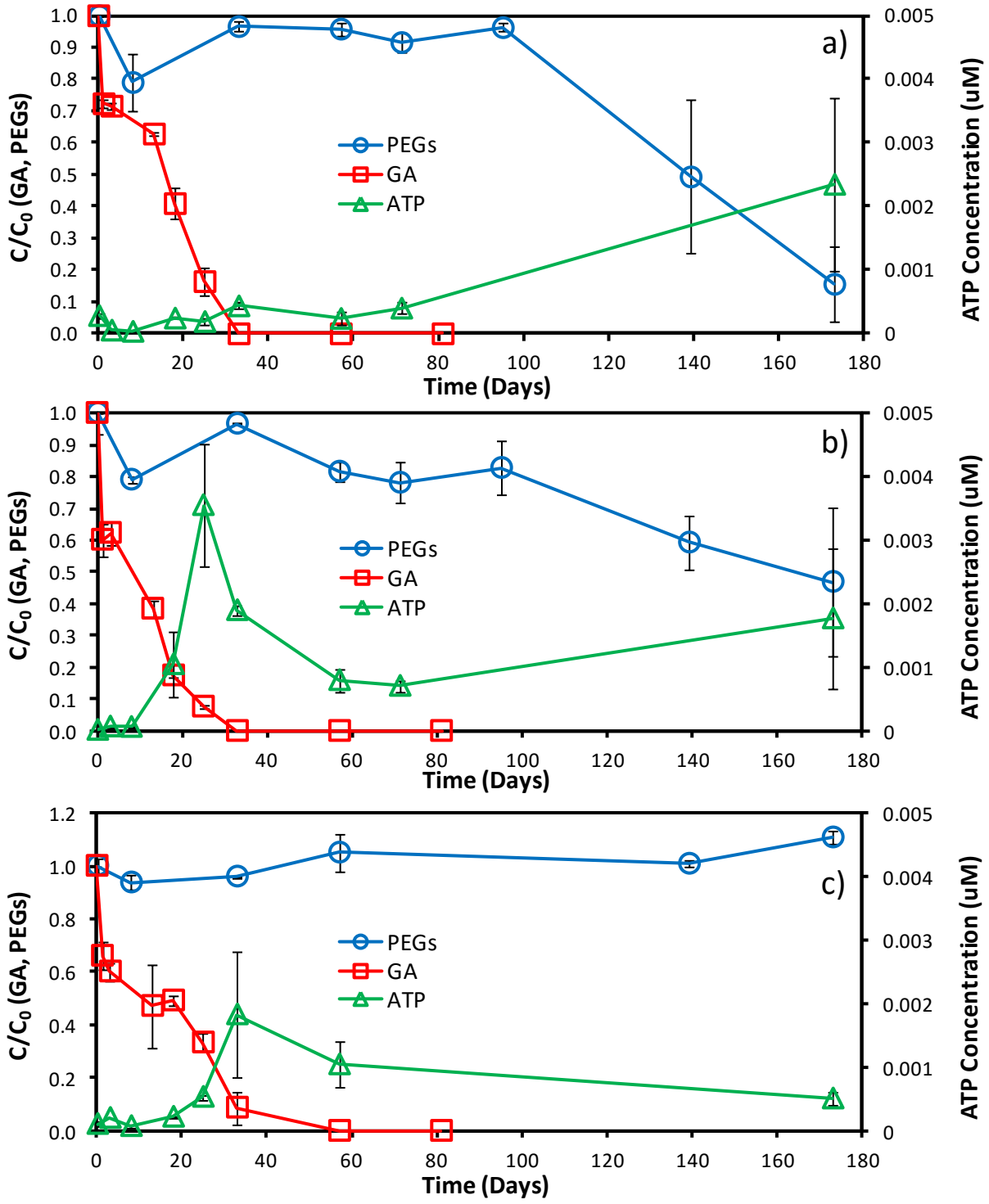



**Figure 16.** Normalized GA concentration (red squares), normalized PEG concentration (blue circles) and ATP concentration (green triangles) in the biotic reactors containing a) PEG/GA, b) PEG/GA/PAM and c) PEG/GA/PAM/Salt to highlight relationship between GA, ATP and resulting PEG degradation.

In the abiotic reactors, no degradation intermediates or other signs of PEG degradation were detected over a period of 140 days. Initial decreases in aqueous PEGs concentrations in all reactors except the one containing PEGs/GA/PAM appeared to indicate initial sorption (Figure 13b). This was also observed in the biotic reactor containing salt (where no biological activity was observed), although not to the same degree. This trend in the abiotic reactors was likely biased by outlying initial concentration measurements due to an unusually high inter-day variation. As a result, the extent of initial sorption is likely exaggerated in these plots. ATP concentrations remained relatively constant with time in the abiotic PEGs reactors, and were consistently lower than in the biotic reactors (by at least one order of magnitude as compared to biotic reactors without GA).

### 3.3 Polyethylene Glycol Speciation

Polyethylene glycols are polymeric surfactants 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 weight of 400 g/mol, enabling assessment of the individual polymeric species after chromatographic separation and analysis.[35]

The initial PEG distribution was typical for a polydisperse mixture where the majority of PEGs was present in the mid-range of molecular weights as EO10, and the minority was present at the highest and lowest detectable molecular weights. As shown in Figure 13, PEGs were removed from the biotic PEGs only reactor over time, with full removal occurring by day 71. Figure 17a shows how the distribution of PEG species in this reactor shifted towards a higher average molecular weight over time as PEGs were removed from the aqueous phase. The initial PEG distribution was observed in the 4 hour and 8 day samples. On day 33, when about 65% of the

PEGs had been removed, this distribution had clearly begun to shift towards 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 trend was observed in all biotic reactors where degradation occurred except the PEGs/PAM/GA reactor, as shown in the Appendix (Figure A2 - Figure A4).

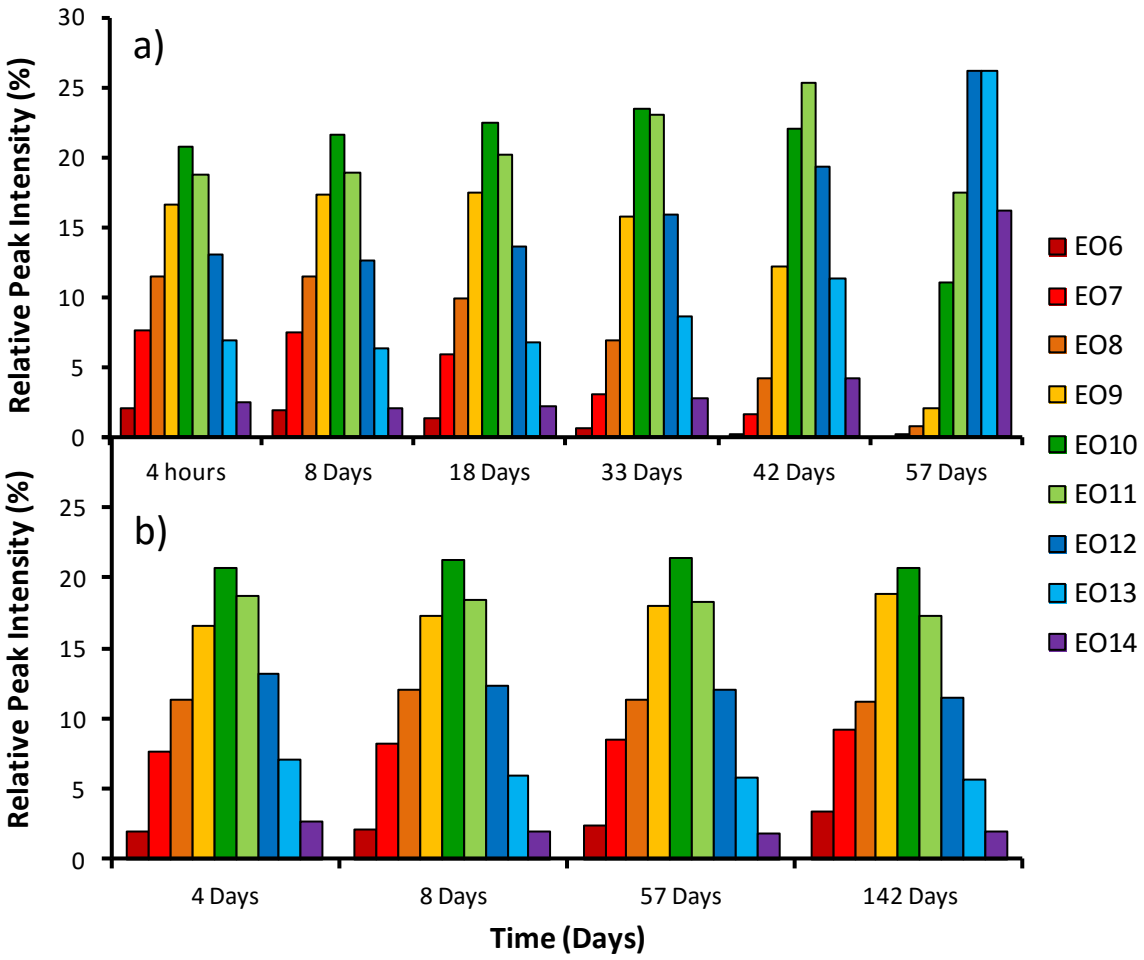



**Figure 17.** Changes in PEGs relative peak intensity over time in a) biotic and b) abiotic reactors containing PEGs. A shift towards higher molecular weight PEG species (higher EO) was observed in the biotic reactors only. All PEGs were removed from the biotic reactor by day 71. Peak intensities are set in relation to the total peak area for the respective sample.

In the abiotic reactors and the biotic PEGs/PAM/GA/Salt reactor, only small variations in PEG composition were detected. As shown in Figure 17b for the abiotic PEGs only reactor, there was a slight, yet statistically significant shift (at the 0.05 level) towards 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, as shown in the Appendix (Figure A5 - Figure A9).

### 3.4 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 (Figure A12 and Figure A13). ATP concentrations were consistently higher (up to one order of magnitude) in reactors with PAM as compared to reactors without PAM (Figure 18).

There were complications associated with the PAM analysis and the results were somewhat inconclusive (Figure A14 and Figure A15). This is likely due to the fact that that size exclusion chromatography method was developed using aqueous PAM-based friction reducer, without soil. Because PAM is a flocculant and associates so strongly with soil, the addition of soil made this analysis much more complicated. Additionally, because soil organic matter is composed of high molecular weight compounds, these compounds influenced the analysis and resulted in additional peaks in the PAM.[52] Additional method development would need to be conducted to determine what species are associated with the non-PAM peaks in these chromatograms.

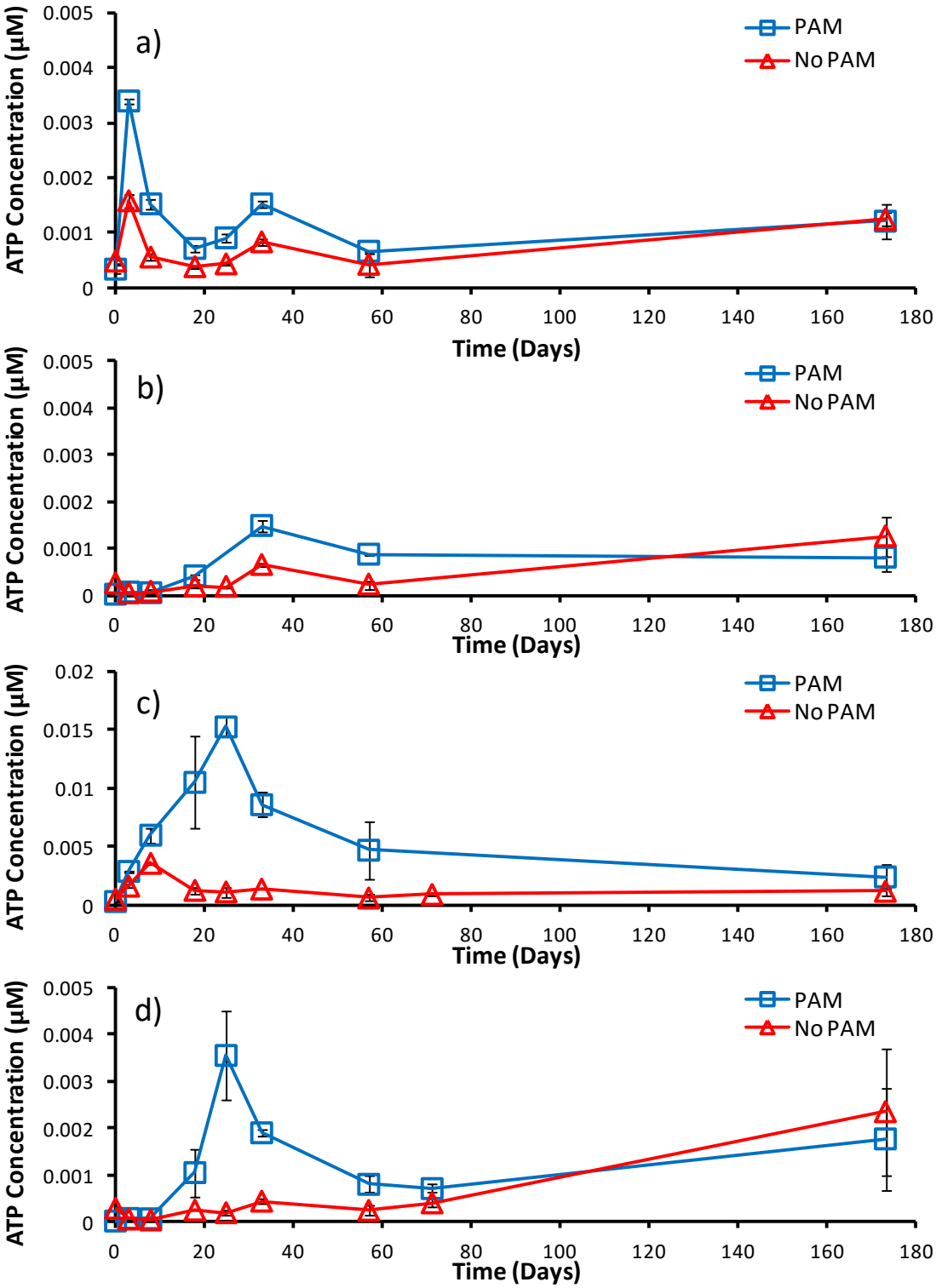



**Figure 18.** ATP concentration in biotic reactors with PAM (blue squares) versus ATP concentration in biotic reactors without PAM (red triangles). Plots show a) PAM only vs. Control, b) GA/PAM vs. GA only, c) PEG/PAM vs. PEG only and d.) PEG/GA/PAM vs. PEG/GA. ATP concentration is consistently higher in reactors containing PAM, except for the last data point in a, b and d. Note that the x-axis in c) is larger than the others.

### 3.5 Ammonium Sulfate and Glutaraldehyde Studies

Because the friction reducer (FR) contains both ammonium sulfate (AS) and PAM, and ammonia ($NH_3$, i.e., the deprotonated form of ammonium ion) also has the potential to cross-link with GA, additional batch studies were conducted to determine if chemical GA removal was dominated by interactions with PAM, ammonia or both. Results showed that GA was fully removed in all reactors by day 45 except for the GA/FR reactors (Figure 19). The only data points collected for GA were at the start of the experiment and after GA had been fully removed. While GA and ammonia cross-link based on the findings presented in Figure 19a, the data do not reveal whether GA and PAM are cross-linking due to the absence of an AS-free PAM reactor or due to the fact that the experiments do not allow for comparison of non-zero data points between the GA/AS and the GA/ASP900 batches. As a result, it is not possible to conclude whether cross-linking with PAM or ammonia is the dominant mechanism.

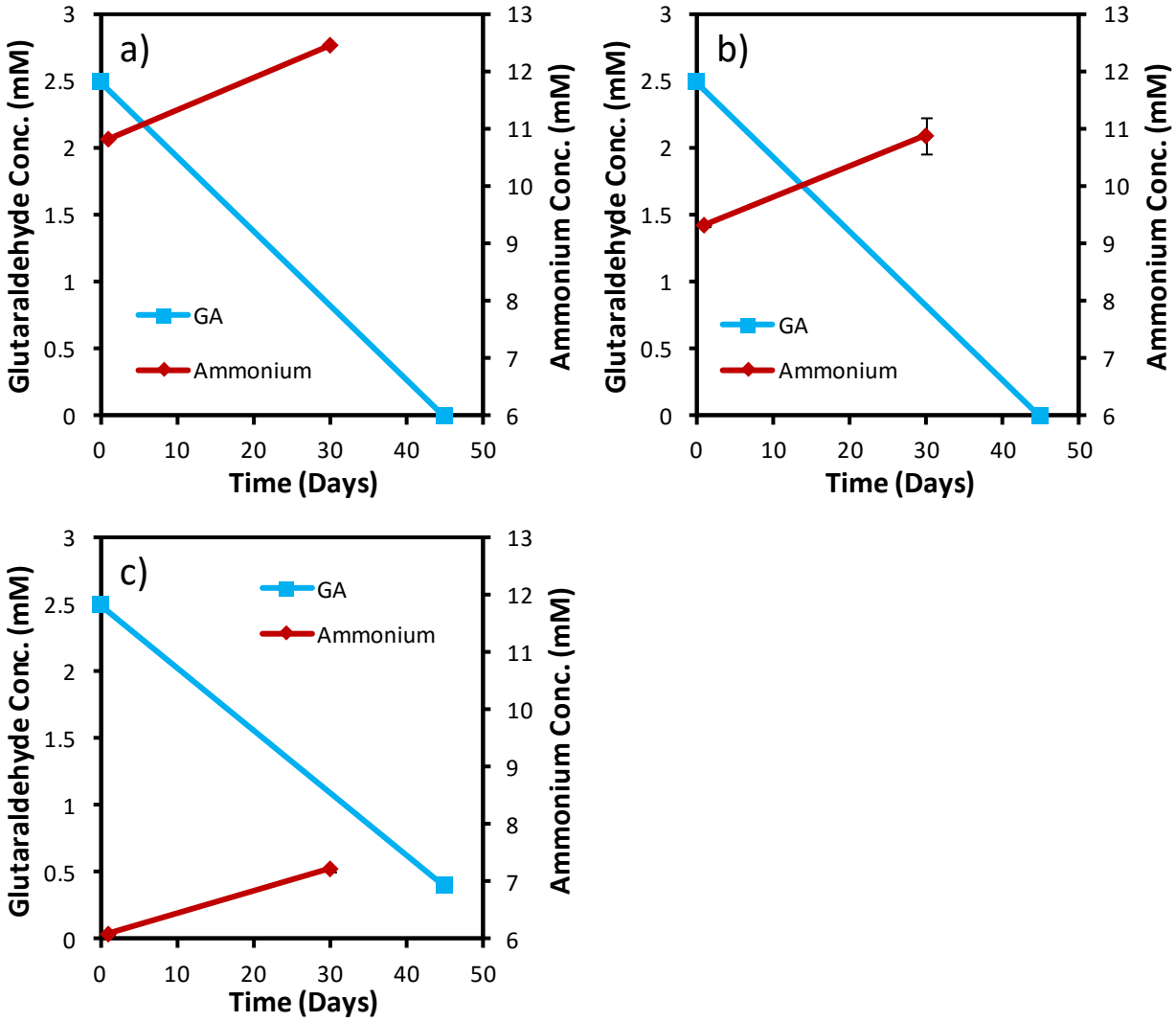



**Figure 19**. Glutaraldehyde vs. ammonium concentration in reactors containing a) GA/AS, b) GA/AS/Soil and c) GA/ASP900.

# 4. 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] Microbial degradation of a biocide becomes possible, however, when concentrations are reduced to sub-lethal levels (due to dilution, sorption, etc.) or when biocide-adapted bacteria proliferate.[24-25] Our results show that in the presence of soil, rapid sorption lowered the aqueous GA concentrations by as much as 40% within the first 3 days of the experiment (Figure 9). 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.

The observed biological GA removal can be attributed to two different mechanisms, namely metabolization by organisms and covalent binding to microbial 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.[32] In this study, ATP concentrations in the GA-containing reactors did not increase substantially during biotic GA removal (Figure 11), suggesting that non-metabolic 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 (Figure 9b). 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 13a).

The overall rate of GA removal was about 50% higher in the biotic reactors that contained PAM (Table 4), and complete abiotic GA removal was only observed in reactors containing PAM, suggesting that chemical interactions with PAM actively reduced the concentration of GA. GA has been shown to be a potent cross-linker with amine groups.[53, 54] 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 20), which is extremely stable under both acidic and basic conditions.[54]

The friction reducer that contained PAM (ASP900) also contained ammonium sulfate. Because ammonia ($NH_3$, i.e., the deprotonated form of ammonium ion) also has the potential to cross-link with GA, additional batch studies were conducted to determine which species, if either, dominated chemical removal of GA. Because the reactors containing ammonium sulfate contained a higher concentration of ammonium ion (and therefore ammonia) as compared to the reactor containing GA/ASP900, the degree to which each of these mechanisms contributed to overall removal cannot be accurately determined. It is clear, however, that the increase in GA removal can be attributed to cross-linking with both PAM and ammonia. A previous industry study showed that

GA removed increased substantially (8-fold) in the presence of ammonium chloride, providing further support for a cross-linking reaction between ammonia and GA.[30]

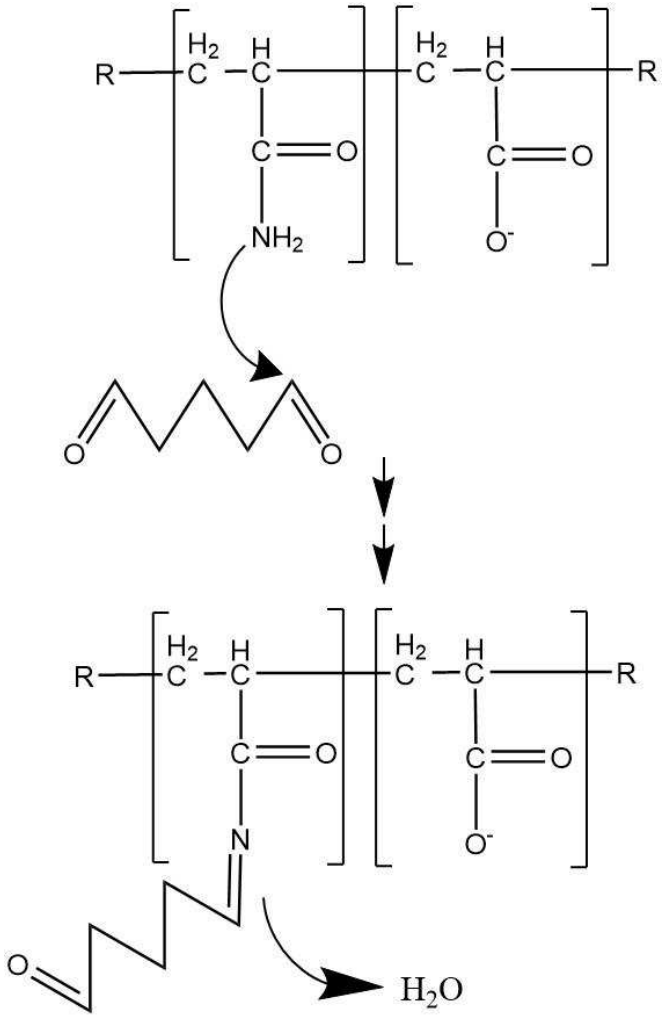



**Figure 20.** Cross-linking mechanism between the aldehyde group on glutaraldehyde and the amide group on anionic polyacrylamide to form a C=N double bond. The cross-linking reaction between GA and ammonia proceeds by a similar mechanism.

The 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 4) imply that about one third of the GA removal in the PAM-containing biotic reactors can be attributed to GA crosslinking 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.

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 start of the experiment. The detection of aldehyde- and carboxylate-derivatives (Table A1 - Table A4) during biodegradation matches previous reports of aerobic microbial polyether transformation via oxidation at the terminal ethoxylate units with subsequent release of glyoxylate (Figure 21), producing homologues of lower molecular weight.[23] The presence of GA, however, substantially delayed biodegradation of PEGs (Figure 13a). 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 $d^{-1}$ vs. 0.0339 $d^{-1}$, respectively; Table 4). 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 $d^{-1}$ vs. 0.0435 $d^{-1}$ vs. 0.0230 $d^{-1}$, respectively). This suggests that the product formed by the crosslinking reaction between GA and PAM or ammonia 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 amino groups, nucleic acids, etc.[55] Finally, the rate of PEG removal in the PEGs/PAM reactor (0.0435 $d^{-1}$) proceeded slightly faster than in the PEGs only reactor (0.0339 $d^{-1}$). 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 once less than 50 % of the original PEG concentration remained.

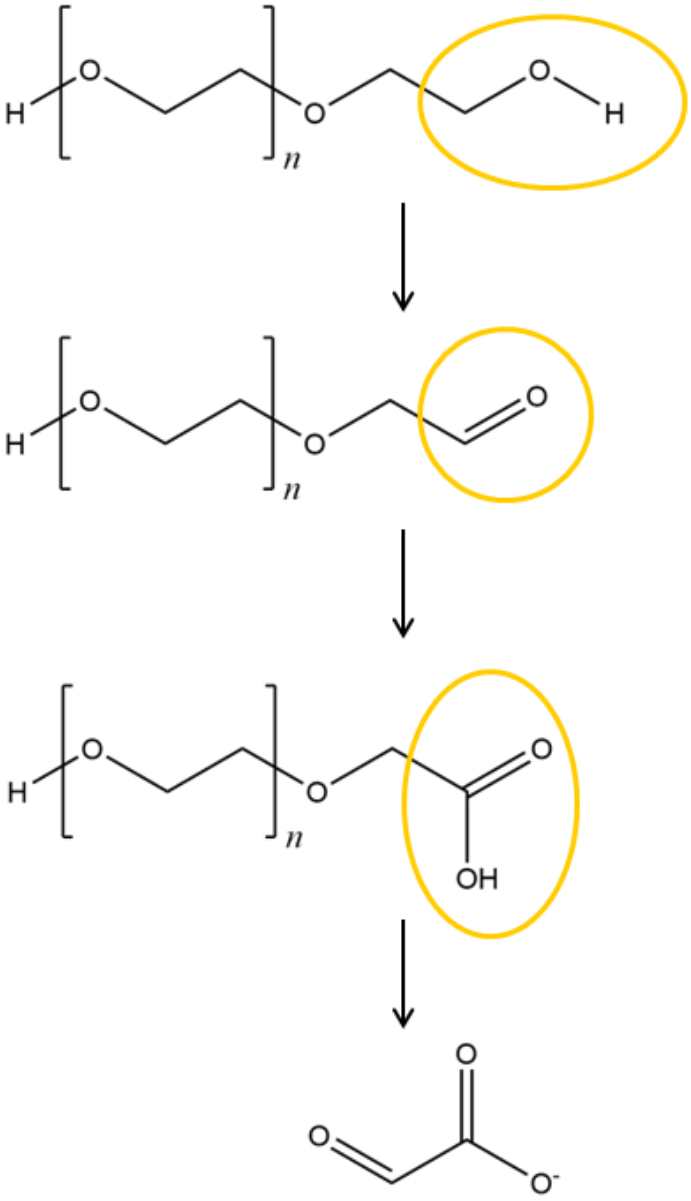



**Figure 21.** Aerobic polyethylene glycol biodegradation pathway. Transformation occurs via oxidation at the terminal ethoxylate units, resulting in aldehyde- and carboxylate-derivatives of PEGs with subsequent (sequential) release of glyoxylate, producing homologues of lower molecular weight.

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 7,400 Da (initial concentration 20 mg/L) were fully degraded within 130 days.[37] In contrast, GA was still removed in the presence of salt, even though slowed by a factor of 1.7 (Table 4), comparable to previous observations made for GA biodegradation in seawater.[32] 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 towards homologues of lower molecular weight were observed in the abiotic reactors (Figure 17b). 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 ethylene oxide (EO) units ($CH_2CH_2O$).[36, 56] This finding is also supported by the initial sorption that was observed all abiotic reactors except the one containing PEGs/GA/PAM (Figure 13b), as well as the biotic PEGs/PAM/GA/Salt reactor (Figure 13a).

In the biotic reactors, shifts in speciation over time towards homologues of higher molecular weight were much more pronounced due to preferential biodegradation of shorter-chain species (Figure 17a). 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 only became apparent 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 towards lower molecular weight homologues with time.[37, 57]

PAM analyses via size-exclusion chromatography did not provide any evidence for substantial PAM transformation other than the observed cross-linking to GA (Figure A12, Figure A13). PAM is generally regarded as stable in soil and water at temperatures up to 200°C[20], although one study found two bacterial strains capable of degrading PAM and using it as their sole source of carbon. These bacteria, however, had been subjected to PAM contamination in an oil field for an extended period of time prior to the study.[44] The presence of the friction reducer, however, did result in an increased ATP concentration (Figure 18), 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.

# 5. SUMMARY AND CONCLUSIONS

The results of this study 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 purposes such as crop irrigation. To fully assess the impacts of frac fluid releases, models need to be designed that consider numerous factors including depth to water table, soil type, weather patterns and accurate kinetic data for both biotic and abiotic processes. As shown in this study, kinetic data for individual chemicals are not sufficient and mixture interactions must be considered. The chemical and biological transformation mechanisms and products determined here for only a fraction of the many hundred HF chemical products used today reveal a complex picture of co-contaminant fate and toxicity. The major results of this study are summarized below.

## 5.1 Glutaraldehyde

In the first 3 days of the experiment, aqueous GA concentrations decreased by 23 - 40% as a result of sorption to soil. Complete GA biodegradation occurred within 33 to 57 days and degradation rates were increased by ~50% in the presence of the PAM-based friction reducer. This increased removal can be attributed to the cross-linking reaction between GA and both PAM and $NH_3$, both of which were constituents of the friction reducer product. Finally, in the presence of salt, full GA removal was achieved; however, the removal rate was slightly lower than in the other reactors.

## 5.2 Polyethylene Glycol

In the absence of GA and salt, PEG surfactants were completely biodegraded in agricultural topsoil within 42 to 71 days. In the presence of GA, however, PEG transformation was impeded and significant degradation was not observed until 95 days into the experiment. At this point,

degradation proceeded in the PEGs/GA reactor at a similar rate as seen in the reactors without GA. In the PEG/GA/PAM, reactor, however, degradation occurred more slowly (by one order of magnitude) possibly because GA cross-linked with friction reducer ingredients retained some of its toxicity. Finally, PEG transformation was completely inhibited by salt at concentrations typical for oil and gas wastewater.

### 5.3 Polyacrylamide

No aqueous removal of PAM friction reducer was observed over a period of six months. However, as stated previously, both PAM and ammonia cross-linked with GA, further lowering the biocide's aqueous concentration. Additionally, ATP concentrations were consistently higher in the presence of PAM friction reducer, suggesting that microorganisms possibly used ammonium ion or the amide groups in PAM as a nitrogen source.

Because there are so many different chemicals used in HF, it is important that the results of this study can be extrapolated to other chemicals besides those used in this experiment. As shown in this study, 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. Salt concentrations (25-180 g/L in flowback)[14] as well as the presence of heavy metals and naturally occurring radioactive materials (e.g. arsenic, selenium, uranium, radium,) vary by formation, providing yet another incentive to fully investigate chemical interactions. In addition, surface-active agents such as surfactants (including PEGs) may increase the mobility of other organic HF additives or produced water constituents (e.g. heavy metals, radioactive materials, etc.) through co-solvent effects, and possibly solubilize otherwise immobile metals in the soil.[36] If chemicals, such as surfactants, are removed at a slower rate due to the presence of salt or biocides,

their impact on solubility, or other aspects of the soil environment, will last longer than expected. This also infers the need for considering mixture toxicity rather than relying on data for individual compounds.

Considering chemical interactions, however, does not always lead to adverse results. The results of this study also showed that sorption to soil and biodegradation, even of biocides at toxic levels,[15] are operative retardation and/or removal mechanisms. Furthermore, chemicals may be transported at different rates, thereby separating the mixtures and allowing for degradation.

### 5.4 Environmental Implications

In the case of accidental frac fluid releases, it is extremely important to understand the fate and transport of frac fluid chemicals in order to assess the severity of the situation and design a proper remediation strategy. A spill can contaminate soil, groundwater, or surface water, or come in contact with nearby livestock or agriculture, all of which have the potential to effect environmental and human health. 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. The potential for contamination is also clearly affected by the chemicals contained in the frac fluid since some chemicals may increase contaminant transport or react to form toxic intermediates, while other chemicals may immediately sorb to soil and have little to no influence. As a consequence, monitoring and remediation strategies that go beyond current standard parameters[36] and target site-specific HF fluid additives are critically needed. Additionally, this and future studies will help determine if a certain chemical or chemical mixture should be phased out of use due to the negative impacts caused by that chemical or mixture.

Conditions that do not favor degradation or transport, however, may result in accumulation of HF additives in (agricultural) topsoil layers, with potential for uptake in crops or negative impacts on plant growth. Recently, as drought conditions have worsened in California and much of the West, farmers have begun irrigating their crops with treated wastewater from hydraulic fracturing. The results from this study - and future studies expanding on its findings - will be a critical contribution in understanding the potential for plant uptake. Clearly, as this practice becomes more common, it is important to understand the chemical interactions between frac fluid additives as well as their transformation products. Although some chemicals may pose no threat to plant uptake or leeching on their own, there may be the potential for problems when combined with other chemicals. It is also important to consider that in water-logged soils, contaminants may break down more slowly or not mineralize completely as a result of the anaerobic conditions. Water-logged soils are common in the eastern United States and therefore the Marcellus Shale, which is the largest shale play in the country.

Overall, the findings of this study 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.

### 5.5 Future Work

In regards to hydraulic fracturing, it was recently stated that, "Now is the time to work on these environmental issues to avoid an adverse environmental legacy similar to that from abandoned coal mine discharges in Pennsylvania".[2] Although natural gas has the potential to improve our energy security, it also has the potential to cause major environmental and health problems if not

researched and regulated properly. As such, there are many opportunities for future work on this subject.

Analyzing soil and groundwater from a field site where a frac fluid spill has occurred is an extremely important next step for this research. For these studies, extractions would need to be conducted to remove any frac additives from the soil for analysis. Similar analytical techniques could be used as were used in this study. Additionally, a non-targeted approach using high-resolution mass spectrometry should be employed. It is recommended that two types of extractions be conducted on each soil sample. First, a simple shake flask extraction using water as an extractant would be useful for determining which chemicals in the soil, if any, are readily bioavailable to microorganisms or livestock. Secondly, a harsher extraction technique, such as accelerated solvent extraction (ASE), would be helpful to maximize contaminant recovery. Because of the wide range of chemicals used in HF, it would be helpful to have multiple field sites to characterize a wider range of chemical combinations.

Additionally, in light of the fact that farmers are using frac fluid to irrigate their crops, a crop uptake study is another important next step for this research. In these studies, various plants (e.g., lettuce, carrots and tomatoes) would be watered with a frac fluid mixture. Ideally, some plants would be watered with a synthetic frac fluid, containing the additives that go downhole, while other plants would be watered with treated produced water from an actual well. Plants will be allowed to grow to maturity, at which point they will be harvested. Various parts of the plant (root, shoot and fruit) will be analyzed for chemical uptake by homogenizing that portion of the plant and extracting the chemicals from the plant. Once again, similar analytical techniques will be used as were used in this study.

For both of these studies, it would be extremely helpful to further characterize the constituents contained in flowback and produced water. This includes, but is not limited to, characterizing the chemical transformation products that may resurface after frac fluid additives have been subjected to downhole conditions.

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APPENDIX

## A.1 Glutaraldehyde Analysis

As a result of the two DNPH configurations (*cis* and *trans*), three peaks are formed by the glutaraldehyde-2,4-dinitrophenylhydrazone product during this analysis. The first, second and third peak account for 1%, 23% and 76% of the total peak area, respectively. As a result, the area beneath the second and third peaks must be summed in order to create a calibration curve and analyze samples. A sample chromatogram showing the two peaks is shown in Figure A1.

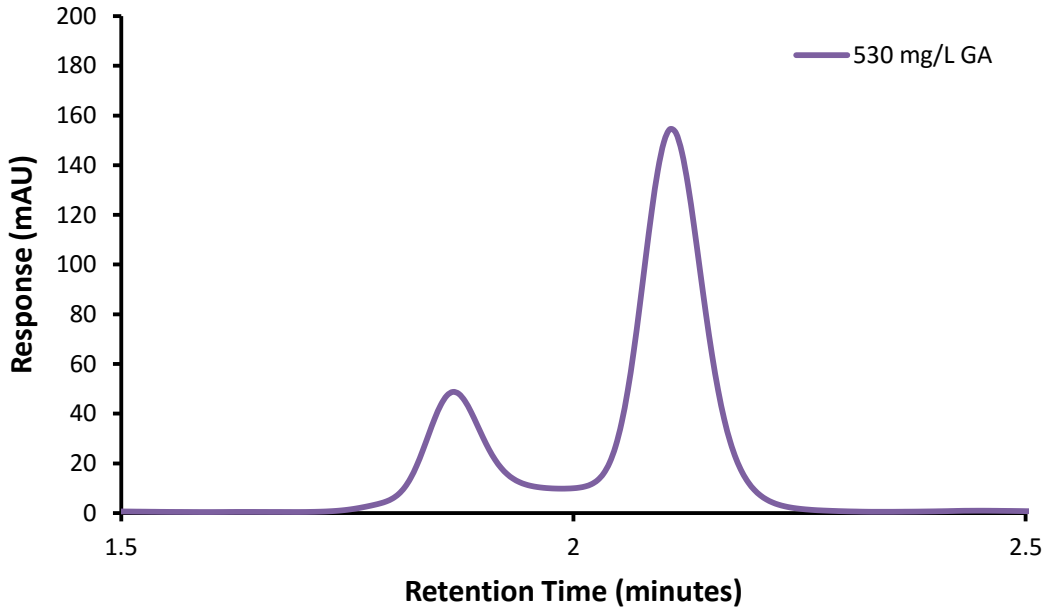


**Figure A1.** UV-DAD chromatogram (λ = 358 nm) showing the two major peaks associated with glutaraldehyde analysis.

## A.2 Polyethylene Glycol Parent and Transformation Products

**Table A1.** Chromatographic retention times and ToF-MS data for polyethylene glycols 400. The base peak and formula correspond to the most prominent adduct that was observed.

| Surfactant Species | Retention Time (min) | Base Peak | Base Peak Formula | Observed *m/z* | Theoretical *m/z* | Error (ppm) |
|---|---|---|---|---|---|---|
| PEG-EO4 | 4.15 | $[M+Na]^+$ | $C_8H_{18}O_5Na^+$ | 217.1046 | 217.1046 | 0.0 |
| PEG-EO5 | 5.46 | $[M+Na]^+$ | $C_{10}H_{22}O_6Na^+$ | 261.1330 | 261.1309 | 8.0 |
| PEG-EO6 | 7.51 | $[M+H]^+$ | $C_{12}H_{26}O_7H^+$ | 283.1764 | 283.1751 | 4.6 |
| PEG-EO7 | 10.54 | $[M+H]^+$ | $C_{14}H_{30}O_8H^+$ | 327.2023 | 327.2013 | 3.1 |
| PEG-EO8 | 11.57 | $[M+H]^+$ | $C_{16}H_{34}O_9H^+$ | 371.2284 | 371.2276 | 2.2 |
| PEG-EO9 | 12.06 | $[M+NH_4]^+$ | $C_{18}H_{38}O_{10}NH_4^+$ | 432.2812 | 432.2803 | 2.1 |
| PEG-EO10 | 12.40 | $[M+NH_4]^+$ | $C_{20}H_{42}O_{11}NH_4^+$ | 476.3070 | 476.3065 | 1.0 |
| PEG-EO11 | 12.68 | $[M+NH_4]^+$ | $C_{22}H_{46}O_{12}NH_4^+$ | 520.3330 | 520.3328 | 0.4 |
| PEG-EO12 | 12.90 | $[M+NH_4]^+$ | $C_{24}H_{50}O_{13}NH_4^+$ | 564.3588 | 564.3590 | 0.4 |
| PEG-EO13 | 13.11 | $[M+NH_4]^+$ | $C_{26}H_{54}O_{14}NH_4^+$ | 608.3850 | 608.3852 | 0.3 |
| PEG-EO14 | 13.27 | $[M+NH_4]^+$ | $C_{28}H_{58}O_{15}NH_4^+$ | 652.4114 | 652.4114 | 0.0 |

**Table A2.** Chromatographic retention times and ToF-MS data for the carboxylate-derivative PEG degradation products detected in the biotic reactors. The base peak and formula correspond to the most prominent adduct that was observed.

| Product Species | Retention Time (min) | Base Peak | Base Peak Formula | Observed *m/z* | Theoretical *m/z* | Error (ppm) |
|---|---|---|---|---|---|---|
| Double COOH of PEG EO5 | 6.81 | $[M+Na]^+$ | $C_{10}H_{18}O_8Na^+$ | 289.0915 | 289.0894 | 7.3 |
| COOH of PEG EO6 | 8.45 | $[M+H]^+$ | $C_{12}H_{24}O_8H^+$ | 297.1562 | 297.1544 | 6.1 |
| Double COOH of PEG EO6 | 9.71 | $[M+H]^+$ | $C_{12}H_{22}O_9H^+$ | 311.1356 | 311.1337 | 6.1 |
| COOH of PEG EO7 | 11.10 | $[M+H]^+$ | $C_{14}H_{28}O_9H^+$ | 341.1820 | 341.1806 | 4.1 |
| Double COOH of PEG EO7 | 11.55 | $[M+H]^+$ | $C_{14}H_{26}O_{10}H^+$ | 355.1608 | 355.1599 | 2.5 |
| COOH of PEG EO8 | 11.85 | $[M+H]^+$ | $C_{16}H_{32}O_{10}H^+$ | 385.2076 | 385.2068 | 2.1 |
| Double-COOH of PEG EO8 | 12.17 | $[M+H]^+$ | $C_{16}H_{30}O_{11}H^+$ | 399.1869 | 399.1861 | 2.0 |
| COOH of PEG EO9 | 12.35 | $[M+NH_4]^+$ | $C_{18}H_{36}O_{11}NH_4^+$ | 446.2598 | 446.2596 | 0.4 |
| Double COOH of PEG EO9 | 11.50 | $[M+NH_4]^+$ | $C_{18}H_{34}O_{12}NH_4^+$ | 460.2391 | 460.2389 | 0.4 |
| COOH of PEG EO10 | 12.70 | $[M+NH_4]^+$ | $C_{20}H_{40}O_{12}NH_4^+$ | 490.2855 | 490.2858 | -0.6 |
| Double COOH of PEG EO10 | 12.92 | $[M+NH_4]^+$ | $C_{20}H_{38}O_{13}NH_4^+$ | 504.265 | 504.2651 | -0.2 |
| COOH of PEG EO11 | 12.90 | $[M+NH_4]^+$ | $C_{22}H_{44}O_{13}NH_4^+$ | 534.3117 | 534.3120 | -0.6 |
| Double COOH of PEG EO11 | 13.18 | $[M+NH_4]^+$ | $C_{22}H_{42}O_{14}NH_4^+$ | 548.291 | 548.2913 | -0.5 |
| COOH of PEG EO12 | 13.17 | $[M+NH_4]^+$ | $C_{24}H_{48}O_{14}NH_4^+$ | 578.3375 | 578.3382 | -1.2 |
| Double COOH of PEG EO12 | 13.40 | $[M+NH_4]^+$ | $C_{24}H_{46}O_{15}NH_4^+$ | 592.3166 | 592.3175 | -1.5 |
| COOH of PEG EO13 | 13.36 | $[M+NH_4]^+$ | $C_{26}H_{52}O_{15}NH_4^+$ | 622.3637 | 622.3644 | -1.1 |
| Double COOH of PEG EO13 | 13.58 | $[M+NH_4]^+$ | $C_{26}H_{50}O_{16}NH_4^+$ | 636.3427 | 636.3437 | -1.6 |
| COOH of PEG EO14 | 13.52 | $[M+NH_4]^+$ | $C_{28}H_{56}O_{16}NH_4^+$ | 666.3909 | 666.3907 | 0.3 |
| Double COOH of PEG EO14 | 13.75 | $[M+NH_4]^+$ | $C_{28}H_{54}O_{17}NH_4^+$ | 680.3687 | 680.3699 | -1.8 |

**Table A3.** Chromatographic retention times and ToF-MS data for the aldehyde-derivative PEG degradation products detected in the biotic reactors. The base peak and formula correspond to the most prominent adduct that was observed.

| Product Species | Retention Time (min) | Base Peak | Base Peak Formula | Observed *m/z* | Theoretical *m/z* | Error (ppm) |
|---|---|---|---|---|---|---|
| Aldehyde of PEG EO7 | 7.50 | $[M+H]^+$ | $C_{14}H_{28}O_8H^+$ | 325.1857 | 325.1857 | 0.0 |
| Double Aldehyde of PEG EO7 | 11.13 | $[M+H]^+$ | $C_{14}H_{26}O_8H^+$ | 323.1719 | 323.1700 | 5.9 |
| Double Aldehyde of PEG EO8 | 11.85 | $[M+H]^+$ | $C_{16}H_{30}O_9H^+$ | 367.1963 | 367.1963 | 0.0 |
| Aldehyde of PEG EO9 | 11.56 | $[M+NH_4]^+$ | $C_{18}H_{36}O_{10}NH_4^+$ | 430.2647 | 430.2647 | 0.0 |
| Aldehyde of PEG EO10 | 12.01 | $[M+NH_4]^+$ | $C_{20}H_{40}O_{11}NH_4^+$ | 474.2909 | 474.2909 | 0.0 |
| Aldehyde of PEG EO11 | 12.31 | $[M+NH_4]^+$ | $C_{22}H_{44}O_{12}NH_4^+$ | 518.3171 | 518.3171 | 0.0 |
| Aldehyde of PEG EO12 | 12.58 | $[M+NH_4]^+$ | $C_{24}H_{48}O_{13}NH_4^+$ | 562.3433 | 562.3433 | 0.0 |
| Aldehyde of PEG EO13 | 12.81 | $[M+NH_4]^+$ | $C_{26}H_{52}O_{14}NH_4^+$ | 606.3695 | 606.3695 | 0.0 |
| Aldehyde of PEG EO14 | 13.00 | $[M+NH_4]^+$ | $C_{28}H_{56}O_{15}NH_4^+$ | 650.3957 | 650.3957 | 0.0 |

**Table A4.** Chromatographic retention times and ToF-MS data for the mixed aldehyde- and carboxylate-derivative PEG degradation products detected in the biotic reactors. The base peak and formula correspond to the most prominent adduct that was observed.

| Product Species | Retention Time (min) | Base Peak | Base Peak Formula | Observed *m/z* | Theoretical *m/z* | Error (ppm) |
|---|---|---|---|---|---|---|
| Aldehyde and COOH of PEG EO5 | 4.61 | $[M+Na]^+$ | $C_{10}H_{18}O_7Na^+$ | 273.0945 | 273.0945 | 0.0 |
| Aldehyde and COOH of PEG EO6 | 6.17 | $[M+H]^+$ | $C_{12}H_{22}O_8H^+$ | 295.1421 | 295.1387 | 11.5 |
| Aldehyde and COOH of PEG EO7 | 8.65 | $[M+H]^+$ | $C_{14}H_{26}O_9H^+$ | 339.1667 | 339.1650 | 5.0 |
| Aldehyde and COOH of PEG EO8 | 11.15 | $[M+H]^+$ | $C_{16}H_{30}O_{10}H^+$ | 383.1903 | 383.1912 | -2.3 |
| Aldehyde and COOH of PEG EO9 | 11.82 | $[M+NH_4]^+$ | $C_{18}H_{34}O_{11}NH_4^+$ | 444.2455 | 444.2439 | 3.6 |
| Aldehyde and COOH of PEG EO10 | 12.24 | $[M+NH_4]^+$ | $C_{20}H_{38}O_{12}NH_4^+$ | 488.2713 | 488.2702 | 2.3 |
| Aldehyde and COOH of PEG EO11 | 12.55 | $[M+NH_4]^+$ | $C_{22}H_{42}O_{13}NH_4^+$ | 532.2982 | 532.2964 | 3.4 |
| Aldehyde and COOH of PEG EO12 | 12.80 | $[M+NH_4]^+$ | $C_{24}H_{46}O_{14}NH_4^+$ | 576.3244 | 576.3226 | 3.1 |
| Aldehyde and COOH of PEG EO13 | 13.05 | $[M+NH_4]^+$ | $C_{26}H_{50}O_{15}NH_4^+$ | 620.3507 | 620.3488 | 3.1 |
| Aldehyde and COOH of PEG EO14 | 13.21 | $[M+NH_4]^+$ | $C_{28}H_{54}O_{16}NH_4^+$ | 664.3731 | 664.3750 | -2.9 |

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### A.3 Polyethylene Glycol Speciation Graphs

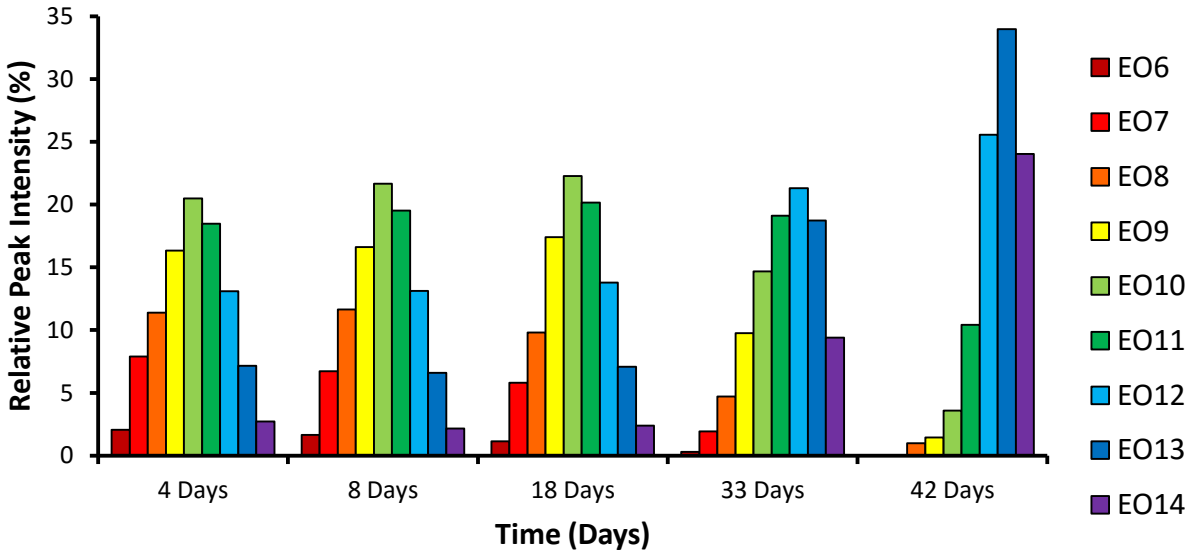


**Figure A2.** Speciation plot for the biotic PEGs/PAM reactor. PEGs were 78% removed on day 33 and 99% removed on day 42. A comparison of the 33 and 42 day samples clearly shows the shift towards higher molecular weight species. The majority of PEGs are present as EO10 in the 4 hour, 8 day and 18 days samples.

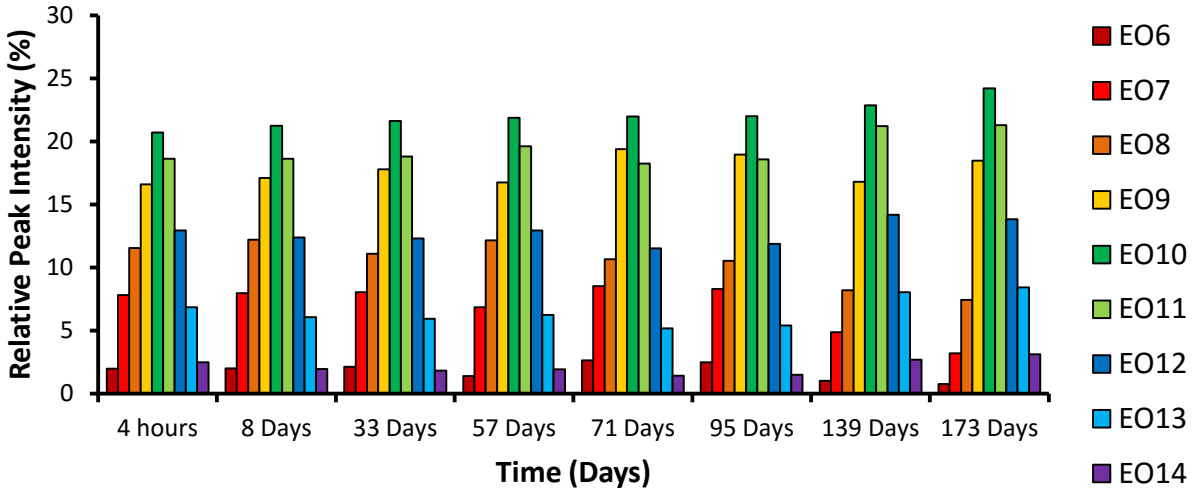


**Figure A3.** Speciation plot for the biotic PEGs/GA reactor. PEGs were 50% removed on day 139 and 85% removed by day 173. A comparison of the 4 hour and 173 day samples most clearly shows the preferential degradation of the smaller PEGs species. As compared to the previous samples, the EO6, EO7 and EO8 species are all present at their lowest levels while the EO13 and EO14 are present at their highest relative peak intensity.

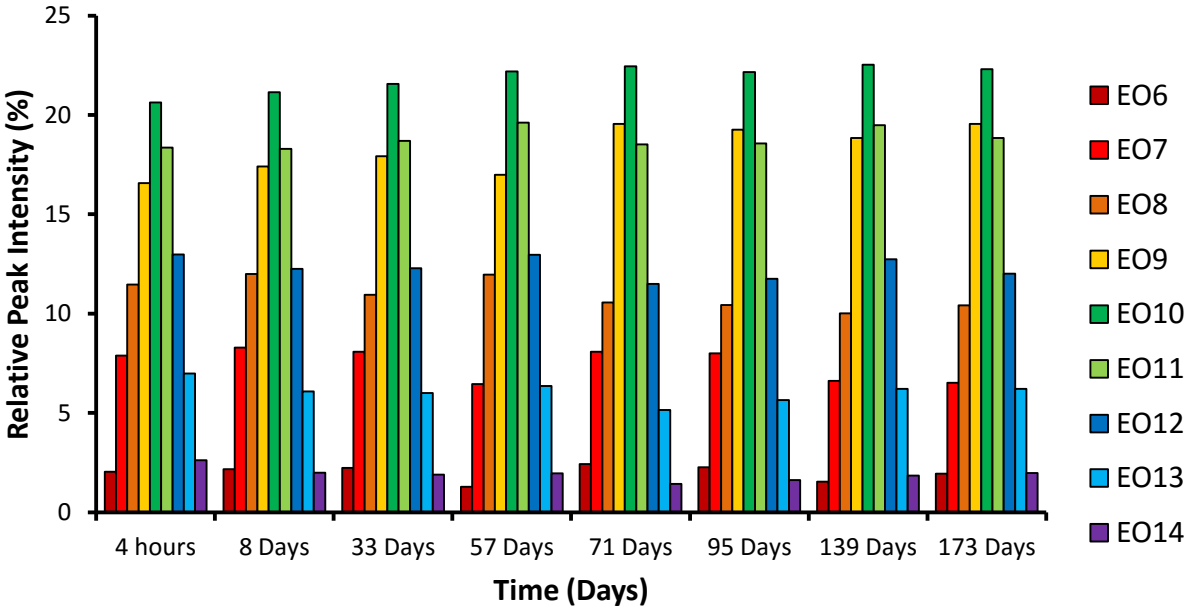


**Figure A4.** Speciation plot for biotic PEGs/PAM/GA reactor. PEGs were only 55% degraded by the 173 day sample, so there is no clear trend showing preferential degradation in this reactor.

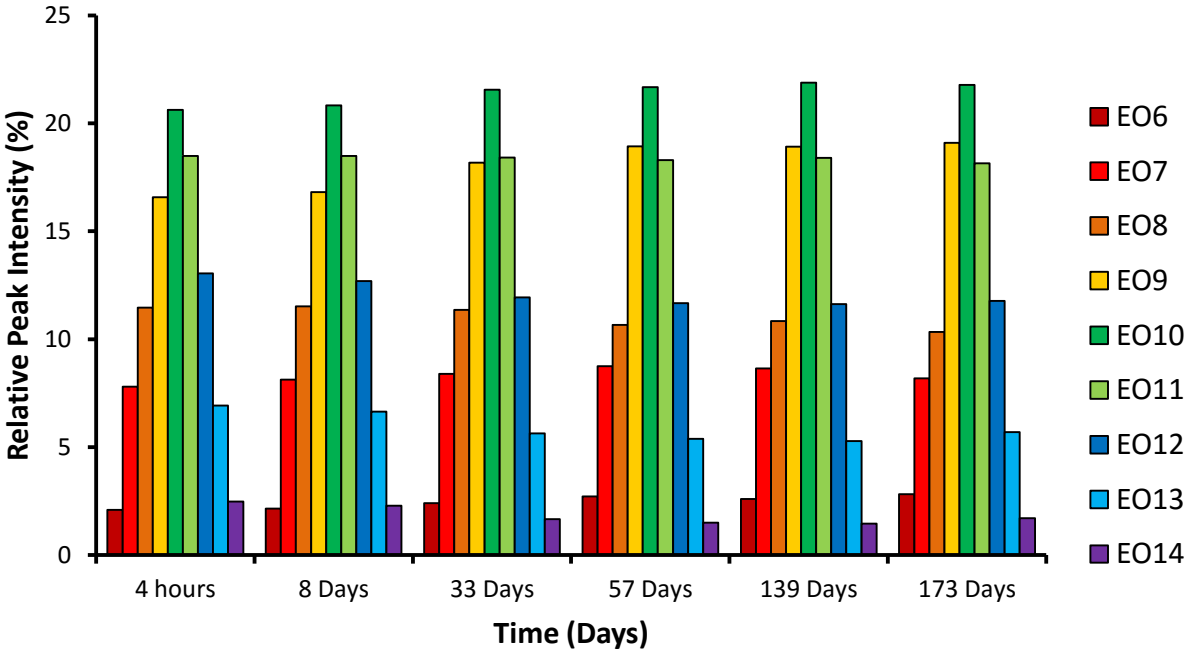


**Figure A5.** Speciation plot for biotic PEGs/PAM/GA/Salt reactor. No PEG degradation occurred in this reactor and the speciation does not follow the same trend as seen in the other biotic reactors. There is a slight shift towards lower molecular weight species as can be seen by comparing the EO13 species over time.

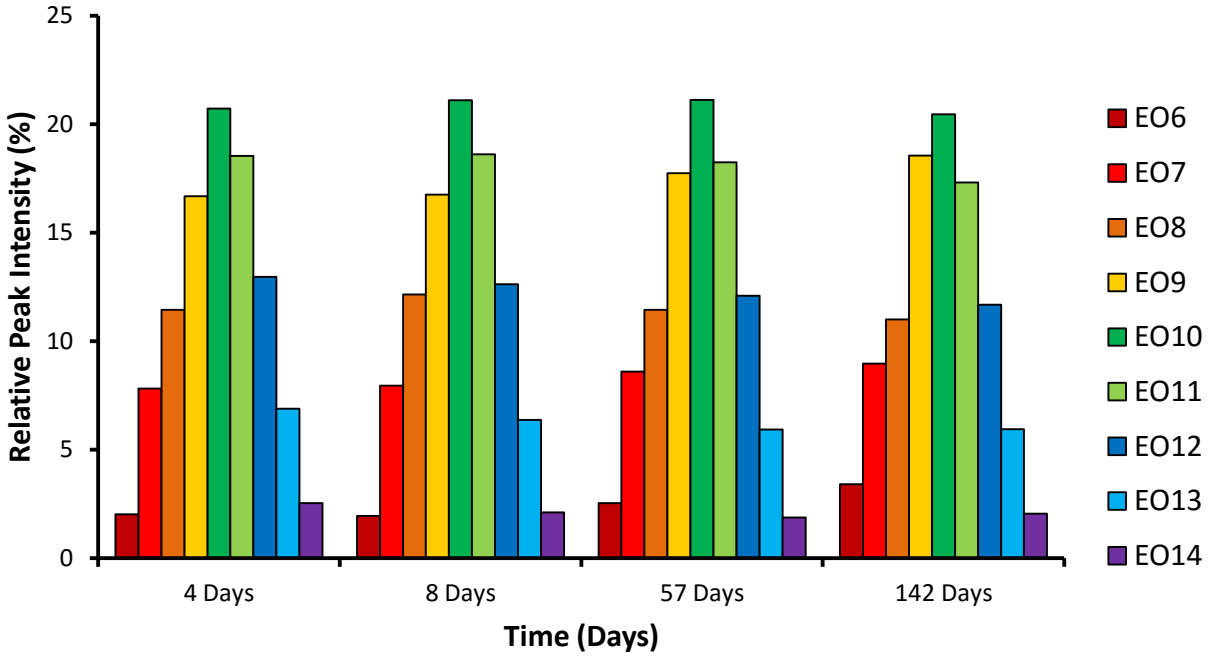


**Figure A6.** Speciation plot for abiotic PEGs/GA reactor. There is a slight shift towards lower molecular weight PEG species over time as can be seen by looking at the slight increase in EO9 relative peak intensity over time.

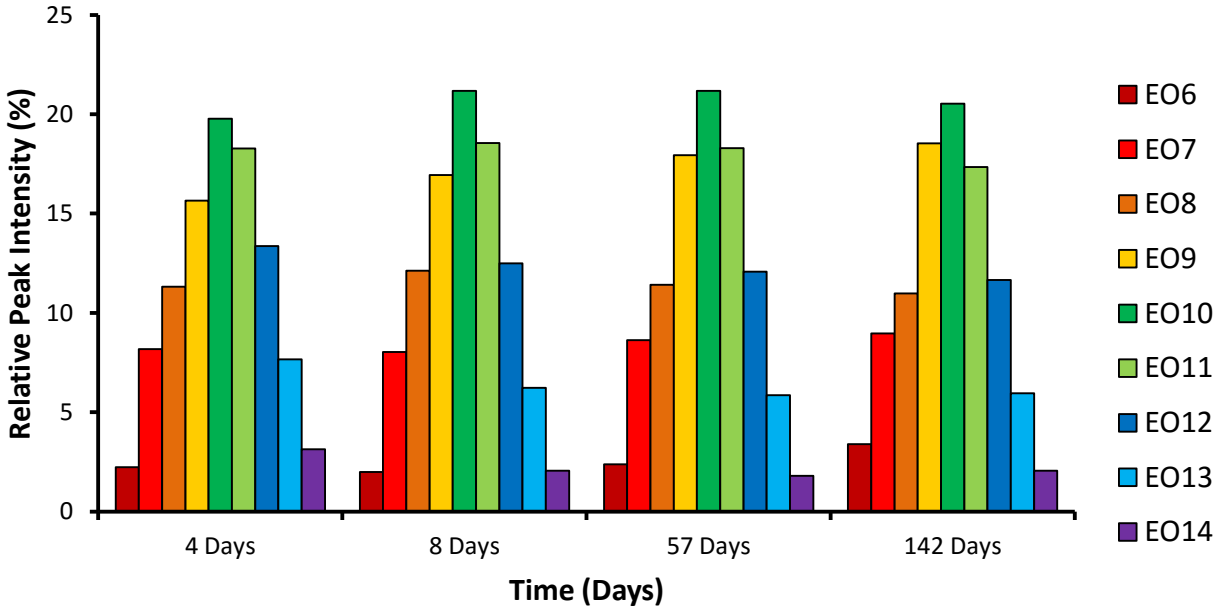


**Figure A7.** Speciation plot for abiotic PEGs/PAM reactor. There is a slight shift towards lower molecular weight PEG species over time as can be seen by looking at the slight increase in EO9 relative peak intensity over time.

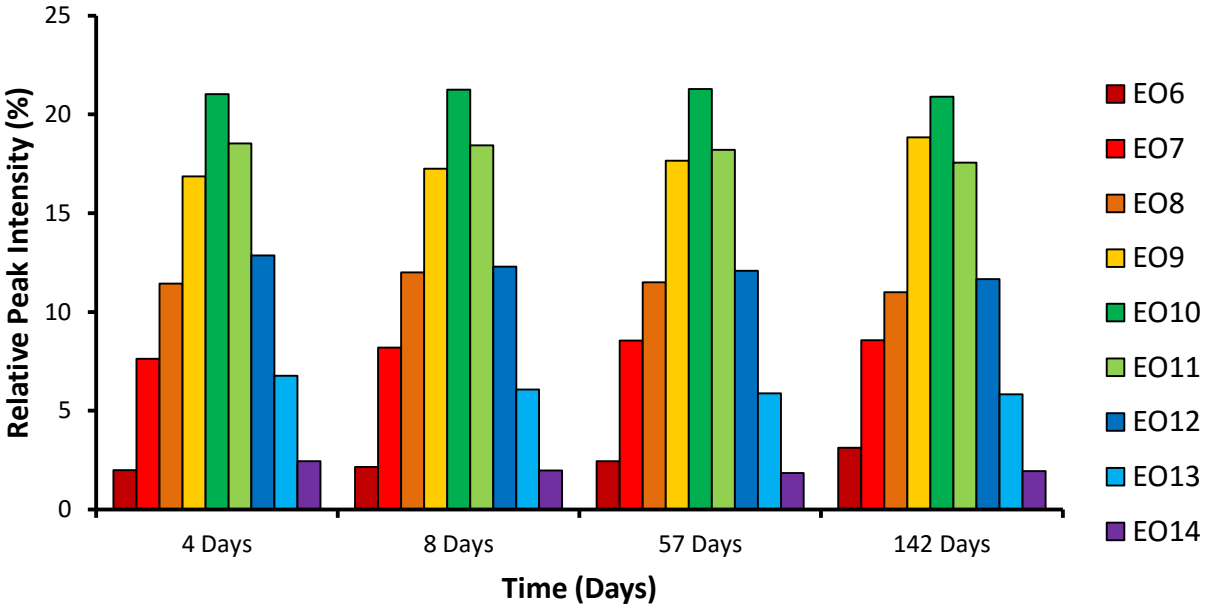


**Figure A8.** Speciation plot for abiotic PEGs/PAM/GA reactor. There is a slight shift towards lower molecular weight PEG species over time as can be seen by looking at the slight increase in EO9 relative peak intensity over time.

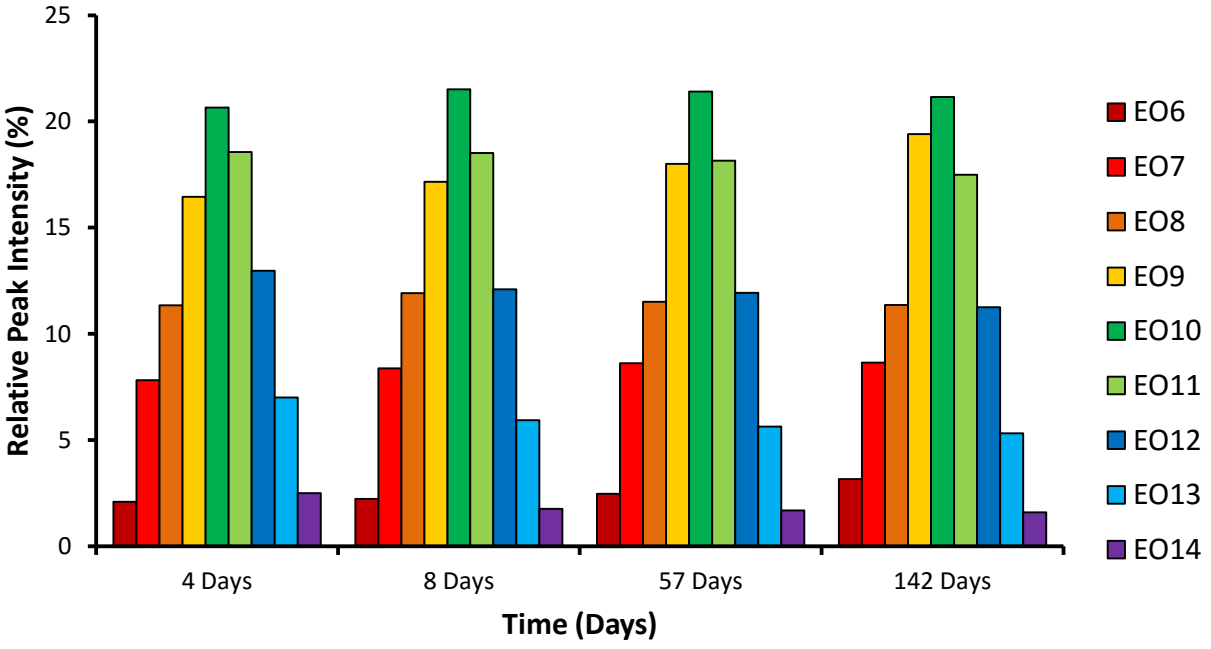


**Figure A9.** Speciation plot for abiotic PEGs/PAM/GA/Salt reactor. There is a slight shift towards lower molecular weight PEG species over time as can be seen by looking at the slight increase in EO9 relative peak intensity over time.

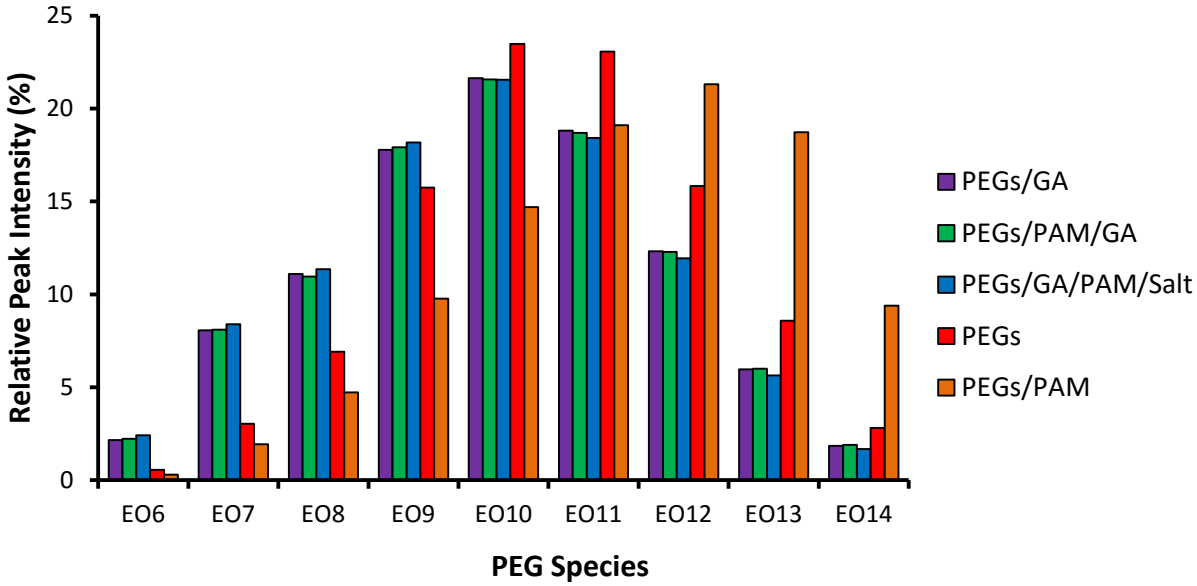


**Figure A10.** Speciation data for all 5 PEGs reactors on day 33. At this point, there was 0% degradation in the PEGs/GA, PEGs/GA/PAM and PEGs/GA/PAM/Salt reactors. There was 70% degradation in the PEGs reactor and 80% degradation in the PEGs/PAM reactor. The original PEGs distribution is present in the reactors where degradation has not occurred while the shift to higher molecular weight species increases in the PEGs/PAM vs. PEGs reactor as would be expected because more of the PEGs have been degraded in the PEGs/PAM reactor at this point.

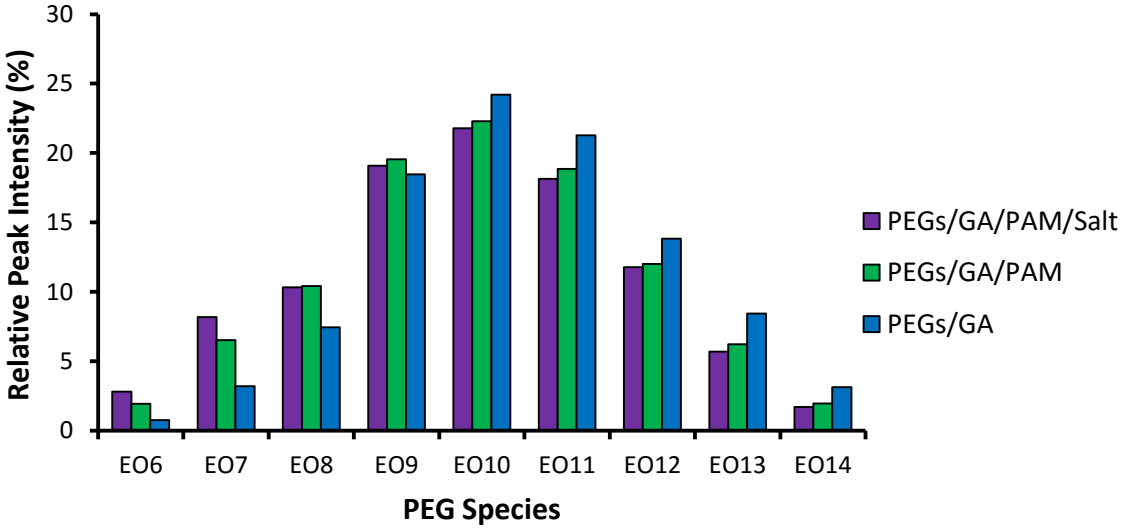


**Figure A11.** Speciation data for the PEGs/GA, PEGs/GA/PAM and PEGs/GA/PAM/Salt reactors on day 173. The PEGs and PEGs/PAM reactors are not included because PEGs were fully removed from those reactors by this point. There is 0% removal in the PEGs/GA/PAM/Salt reactor, 55% removal in the PEGs/GA/PAM reactor and 85% removal in the PEGs/GA reactor at this point. As degree of removal increases in these reactors, there is a shift towards higher molecular weight PEGs species.

## A.4 Polyacrylamide Chromatograms

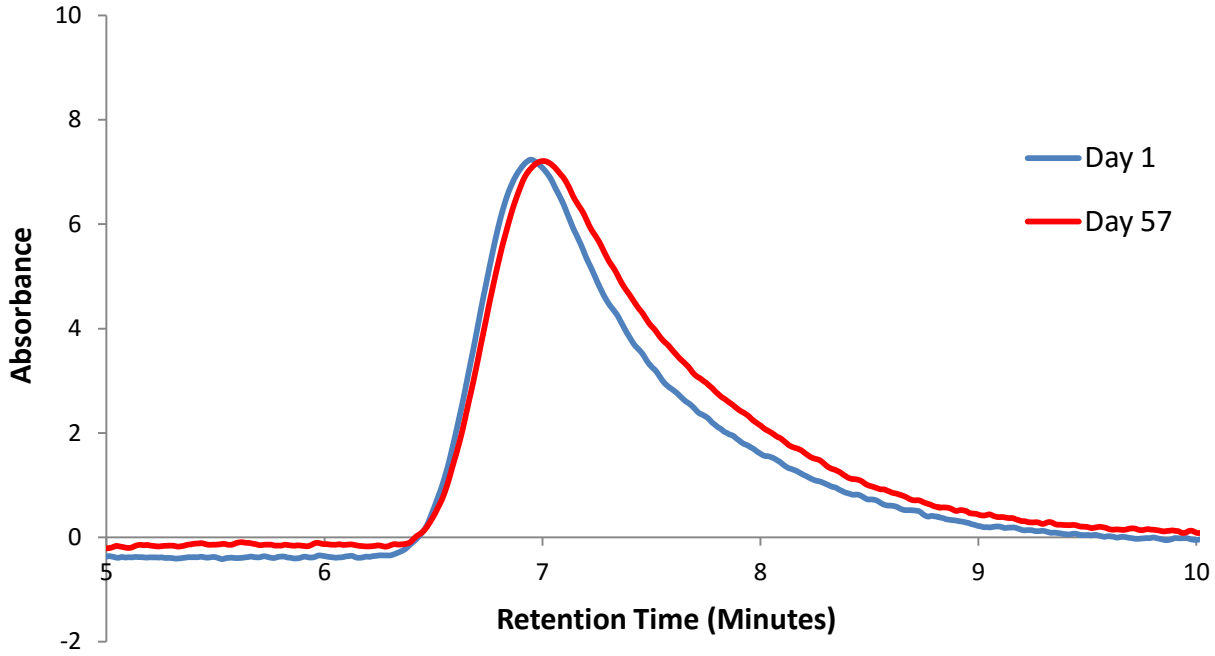


**Figure A12.** Biotic Polyacrylamide Chromatogram for PAM only reactor. Polyacrylamide peak is shown at time = 7 minutes. No change is seen in this peak over the first 57 days of the experiment.

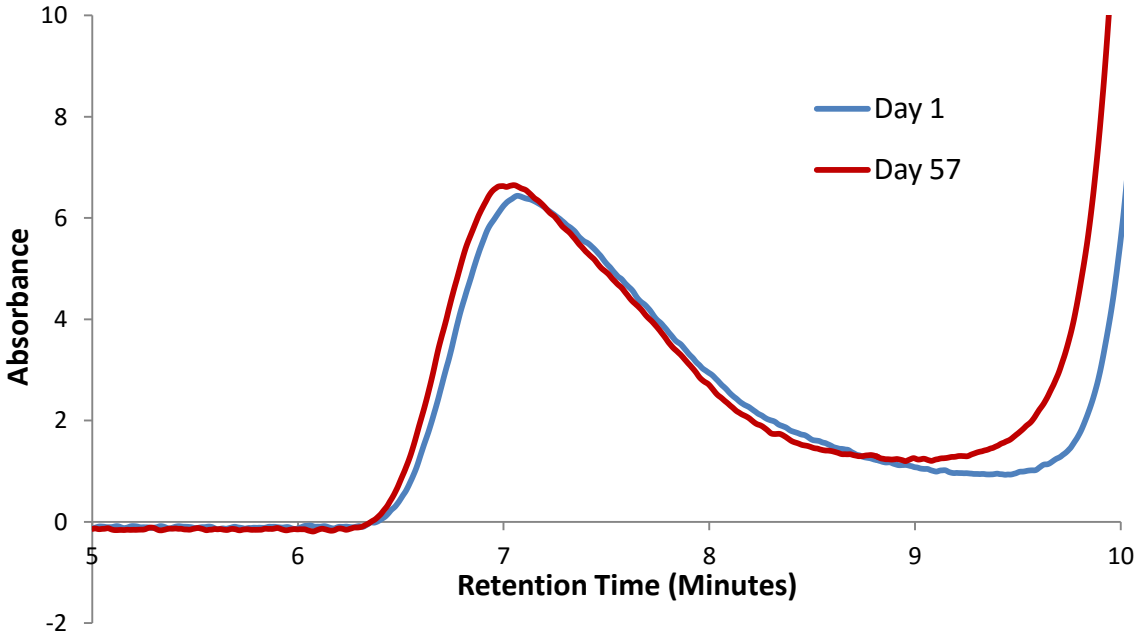


**Figure A13.** Abiotic Polyacrylamide Chromatogram for PAM only reactor. Polyacrylamide peak is shown at time = 7 minutes. No change is seen in this peak over the first 57 days of the experiment.

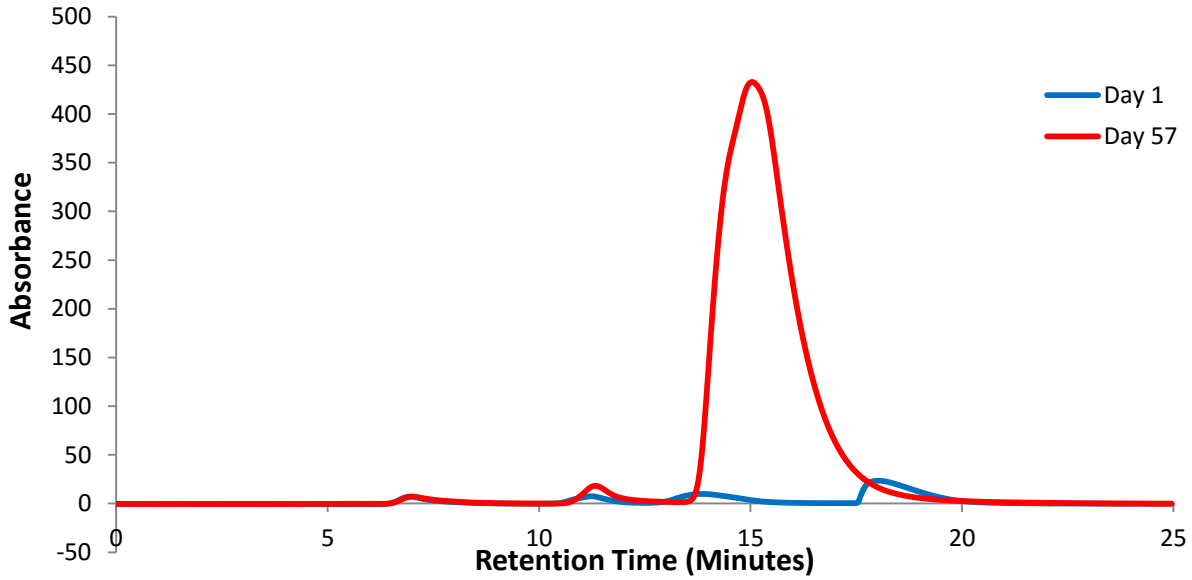


**Figure A14.** Complete biotic polyacrylamide chromatogram for PAM only reactor. By Day 57, an additional peak has formed around 15 minutes, even though the original PAM peak area has not changed. This is likely a soil organic matter interference.

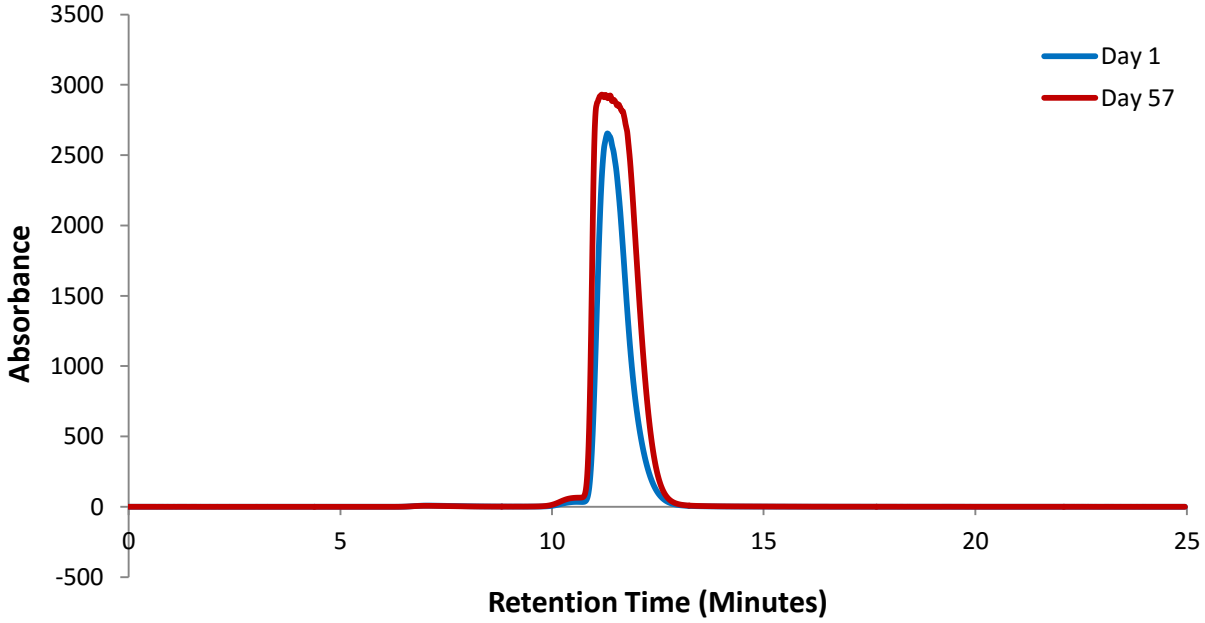


**Figure A15.** Complete abiotic polyacrylamide chromatogram for PAM only reactor. A large peak is present around 12 minutes on Day 1, but this peak increases over time. This may be due to an interference with soil organic matter as well as sodium azide.