Science of the Total Environment 668 (2019) 1094–1103

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

High total dissolved solids in shale gas wastewater inhibit biodegradation of alkyl and nonylphenol ethoxylate surfactants

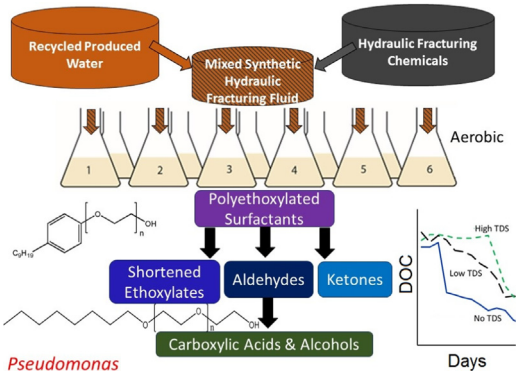
Andrea J. Hanson [a,b], Jenna L. Luek [b], Shantal S. Tummings [c], Molly C. McLaughlin [a], Jens Blotevogel [a], Paula J. Mouser [b,*]

[a] Department of Civil and Environmental Engineering, Colorado State University, Fort Collins, CO 80523, United States
[b] Department of Civil and Environmental Engineering, University of New Hampshire, Durham, NH 03824, United States
[c] Department of Civil, Environmental, and Geodetic Engineering, The Ohio State University, Columbus, OH 43210, United States

HIGHLIGHTS

- High TDS resulted in lower biodegradation rates for alkyl ethoxylates.
- Carboxylate and aldehyde species were identified as intermediate metabolites.
- Microbial community dominated by *Pseudomonas* spp.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 11 January 2019
Received in revised form 1 March 2019
Accepted 3 March 2019
Available online xxxx

Editor: Jay Gan

Keywords:
Marcellus shale
Ethoxylated surfactants
Aerobic biodegradation
TDS
Hydraulic fracturing

ABSTRACT

Hydraulic fracturing fluids are injected into unconventional oil and gas systems to stimulate hydrocarbon production, returning to the surface in flowback and produced waters containing a complex mixture of xenobiotic additives and geogenic compounds. Nonionic polyethoxylates are commonly added surfactants that act as weatherizers, emulsifiers, wetting agents, and corrosion inhibitors in hydraulic fracturing fluid formulations. Understanding the biodegradability of these ubiquitous additives is critical for produced water pre-treatment prior to reuse and for improving treatment trains for external beneficial reuse. The objective of this study was to determine the effect of produced water total dissolved solids (TDS) from an unconventional natural gas well on the aerobic biodegradation of alkyl ethoxylate and nonylphenol ethoxylate surfactants. Changes in surfactant concentrations, speciation and metabolites, as well as microbial community composition and activity were quantified over a 75-day aerobic incubation period. Alkyl ethoxylates (AEOs) were degraded faster than nonylphenol ethoxylates (NPEOs), and both compound classes and bulk organic carbon biodegraded slower in TDS treatments (10 g L^{-1}, 40 g L^{-1}) as compared to controls. Short-chain ethoxylates were more rapidly biodegraded than longer-chain ethoxylates, and changes in the relative abundance of metabolites including acetone, alcohols, and carboxylate and aldehyde intermediates of alkyl units indicated metabolic pathways may shift in the

* Corresponding author at: University of New Hampshire, 35 Colovos Road, Durham, NH 03824, United States.
E-mail address: Paula.Mouser@unh.edu (P.J. Mouser).

https://doi.org/10.1016/j.scitotenv.2019.03.041

presence of higher produced water TDS. Our key finding that polyethoxylated alcohol surfactant additives are less labile at high TDS has important implications for produced water management, as these fluids are increasingly recycled for beneficial reuse in hydraulic fracturing fluids and other purposes.

1. Introduction

Recovering oil and natural gas resources from unconventional systems such as hydrocarbon-bearing shales requires the injection of large volumes of chemically optimized fluid during the hydraulic fracturing process (Kondash and Vengosh, 2015; Kondash et al., 2017; Nicot and Scanlon, 2012). Once an unconventional oil or natural gas well is completed, a portion of injected fluids and chemical additives return to the surface along with water containing salts, metals, radioactive materials, and organic matter naturally present in the geologic formation (Chapman et al., 2012; Haluszczak et al., 2013; Hayes, 2009; Oetjen et al., 2018; Rosenblum et al., 2017a). The geochemical complexity and highly saline matrix (e.g., 0.5–400 g L^{-1} TDS (Horner et al., 2016)) of shale oil and gas produced water limits reuse options, therefore, wastewater management remains an ongoing challenge for hydraulic fracturing operations (Akyon et al., 2019; Estrada and Bhamidimarri, 2016; Gregory et al., 2011; Jiang et al., 2014). Understanding the fate of specific hydraulic fracturing fluid additives in produced water derived from unconventional hydrocarbon systems has implications for formulating optimal hydraulic fracturing fluid mixtures, produced water management (e.g., treatment and beneficial reuse), and accidental fluid releases.

Cationic, anionic, and nonionic surfactants are a major chemical component of hydraulic fracturing fluids. Surfactants are used to reduce surface tension and promote fluid recovery from the well (Stringfellow et al., 2017, 2014) and are often utilized as commercial mixtures consisting of homologous series of polyethoxylates including glycols, nonylphenol ethoxylates (NPEOs), and alkyl ethoxylates (AEOs) (Chen and Carter, 2017; Thurman et al., 2014). These substituted polyethoxylates have been detected in produced water (Akyon et al., 2019), centralized wastewater treatment facility effluent (Getzinger et al., 2015), and in stream sediments (Burgos et al., 2017); consequently, polyethoxylates have been suggested as tracer compounds for hydraulic fracturing fluids (Luek et al., 2018a; Rosenblum et al., 2017b; Thurman et al., 2014). The environmental fate of these substituted polyethoxylates varies based on structure (e.g., substituent type, ethoxylate chain length) and environmental conditions (e.g., oxic versus anoxic). Under oxic conditions, AEOs and NPEOs may be degraded through central ether scission followed by ω- and β-oxidation, or via sequential ethoxylate chain shortening (John and White, 1998; Liu et al., 2006; Tidswell et al., 1996). As a result of NPEO degradation via ethoxylate chain shortening, nonylphenol can be produced as a more stable yet toxic metabolite (Ying, 2006; Ying et al., 2002), further emphasizing the need to understand the environmental fate of these compounds.

Despite biocidal applications to fracturing fluids prior to injection and during the storage of waste fluids, microbial communities persist and alter fluid geochemistry (Cluff et al., 2014; Murali Mohan et al., 2013a, 2013b). Additionally, produced waters from hydraulically fractured oil and natural gas wells frequently contain high total dissolved solids (TDS) that can reduce or inhibit biological degradation of dissolved organic carbon (DOC) (Kekacs et al., 2015; Lester et al., 2015, 2014; McLaughlin et al., 2016). Indeed, several studies have observed reduced DOC biodegradation rates with increasing TDS in a number of different aerobic environments. In engineered biofilms, Akyon and colleagues observed decreased DOC biodegradation rates and overall bulk removal in produced water with 50 g L^{-1} TDS from Utica formation natural gas wells; at 100 g L^{-1} TDS, DOC biodegradation rates were ~1.5 to 9.5-fold lower, suggesting the increase in TDS further inhibited microbial removal of organic compounds (Akyon et al., 2019). Under aerobic conditions in microcosms containing fresh water microbial consortia, activated sludge, and 40 g L^{-1} TDS, synthetic hydraulic fracturing fluid DOC biodegradation was completely inhibited for nearly one week despite the microbial communities being pre-acclimated to the elevated salinity (Kekacs et al., 2015). Similarly, in aerobic microcosms containing agricultural topsoil, the biodegradation of poly(ethylene glycol) surfactants was completely inhibited when the TDS was increased to 30 g L^{-1} (McLaughlin et al., 2016), suggesting a similar effect may be observed in produced water reuse scenarios. In a wastewater treatment application, guar gum (a hydraulic fracturing fluid additive contributing to DOC) was degraded at a rate ~10-fold slower in an activated sludge mixture with 45 g L^{-1} TDS than a sludge mixture with 1.5 g L^{-1} TDS (Lester et al., 2014). Although DOC biodegradation rates may be reduced at high TDS, halotolerant microbial communities that thrive in produced water are likely to degrade chemical additives including alkyl polyethoxylates as well as the biocides applied to inhibit biological activity (Campa et al., 2018; Cluff et al., 2014; Heyob et al., 2017; Murali Mohan et al., 2013a).

Although substituted polyethoxylates are consistently used in hydraulic fracturing fluid mixtures and contribute substantially to the total DOC (Rosenblum et al., 2017b), their biodegradability in high TDS produced water is poorly understood. Considering the increase in produced water recycling for future unconventional energy well completion practices (Lebas et al., 2013; Lutz et al., 2013), investigating the fate of these ubiquitous fluid additives in high TDS mixed fluids is important for informing this and other produced water management practices including treatment and beneficial reuse. Considering the functional importance of surfactants in unconventional energy extraction, biodegradation of these chemicals in blended fluids formulated with recycled produced water would be undesirable. Specifically, examination of the bulk rates of surfactant biodegradation and the microbial communities and metabolic pathways involved are needed, particularly with respect to produced water reuse for hydraulic fracturing operations. Microbial communities present in typical surface soils may be capable of degrading mixtures of alkyl polyethoxylates (Heyob et al., 2017), although previous research indicates high TDS will reduce alkyl polyethoxylate biodegradation rates (Potter et al., 1999). Therefore, this study sought to address the following hypotheses: i) AEOs and NPEOs would degrade more slowly as TDS increased and ii) pathways for their degradation would be altered as a result of a change in microbial community structure, specifically an increase in the abundance of halotolerant taxa.

To test these hypotheses, aerobic batch reactors containing a synthetic hydraulic fracturing fluid made up with an increasing portion of high TDS produced water obtained from a Marcellus Shale natural gas well were established and evaluated over 2.5 months. In an effort to simulate potential produced water reuse for hydraulic fracturing operations in the region, we selected produced water TDS concentrations of 10 and 40 g L^{-1} relative to a freshwater control (0 g L^{-1} TDS). The extent and rates of both AEO and NPEO biodegradation was assessed using liquid chromatography paired with high resolution mass spectrometry (LC-MS) for these bioreactors containing varying TDS. Microbial activity, microbial community composition, and intermediate metabolites were also evaluated over the degradation experiments to infer changes in metabolism. Our results show reduced DOC removal and a shift in surfactant degradation pathways with increasing TDS,

suggesting halotolerant taxa degrade AEOs and NPEOs using a different enzymatic mechanism, and to a reduced extent, as compared to mesotolerant taxa.

2. Materials and methods

2.1. Sample sources and experimental setup

Produced water (20 L) was collected in an autoclaved HDPE carboy from the gas-fluid separator tank of a natural gas-producing Marcellus Shale well near Morgantown, West Virginia, U.S.A. [Well MIP 3H, 20 months after hydraulic fracturing], and stored for 2 days at ambient temperature before use in experiments. Additional well information including drilling, production, geophysical and geochemical data can be found at https://mseel.org. For source water, Monongahela River water was collected in an autoclaved HDPE carboy and stored for 2 days at ambient temperature. First, Monongahela River water (0 g L^{-1} TDS) was combined with the Marcellus Shale produced water (108 g L^{-1} TDS) to obtain two elevated TDS levels (10 g L^{-1}, 40 g L^{-1} TDS). These three fluid mixtures (0, 10, 40 g L^{-1} produced water TDS) were then used as a base fluid for a synthetic fracturing fluid (SFF) containing alkyl ethoxylates and nonylphenol ethoxylates (Table S1) as described previously (Kekacs et al., 2015). A background (ambient) control included 10 g L^{-1} produced water TDS with no SFF amendment; a substrate control included 10 g L^{-1} produced water TDS with an acetate amendment, and an abiotic control contained 10 g L^{-1} produced fluid TDS, the SFF amendment, and sodium azide as a biocide.

The three treatment batch reactors and three control batch reactors were prepared in duplicate in autoclaved 1 L flasks with metal lids. A nutrient mixture (OECD method 306) was added to prevent nutrient limitation during degradation experiments (Table S2). Reactor flasks were capped loosely and kept in the dark, rotated at 150 rpm, and subsampled over 75 days (0, 1, 2, 3, 4, 5, 7, 11, 14, 21, 30, 45, 75) for bulk fluid assays, microbial analyses, and chemical analyses. Dissolved oxygen, conductivity and pH were measured using an Orion 5 Star Meter (Thermo Scientific) at each sampling event.

2.2. Bulk fluid assays

Dissolved organic carbon (DOC; as non-purgeable organic carbon (NPOC)) was measured using a Shimadzu TOC/TN analyzer and quantified using potassium hydrogen phthalate standards. According to method OECD 306, DOC values from the background (ambient) control were subtracted from all other treatments before presentation. ATP concentrations were determined using BacTiterGlo Microbial Cell Viability Assay (Promega) using 100 μL samples in a 96 well plate format. All luminescence measurements were performed using a BioTek Plate Reader within 1 h of sampling.

2.3. Microbial community analysis

Aerobic batch reactors were subsampled on Day 0, 7, 21, and 45 in duplicate for DNA extraction and sequencing analysis. The produced water microbial community composition had been under investigation for nearly two years in separate parallel investigations; *Halanaerobium* was the predominant member in the produced water used in this study (data not shown). Fluid subsamples were centrifuged at 4000 ×*g* for 20 min, and the pellet (~1.5 mL) was stored at −80 °C until extraction. DNA extraction was performed on 500 μL aliquots using a PowerSoil DNA Isolation kit (MoBio, Carlsbad, CA). Briefly, bead beating was used for rapid homogenization and cells lysed both mechanically and chemically. Total genomic DNA was captured, rinsed, eluted through a silica membrane, and stored at −80 °C until sequencing. Sequencing of the V4 region of the 16S rRNA gene was performed on an Illumina HiSeq2500 with Rapid Run chemistries at the Hubbard Center for Genome Studies at the University of New Hampshire (Durham, NH). Sequences were analyzed using the QIIME pipeline (Caporaso et al., 2010b) adapted with the PhyloToAST toolset (Dabdoub et al., 2016) to generate an OTU table; QIIME parameters were set at 97% similarity. OTUs were selected using BLAST (Altschul et al., 1990) and aligned with PyNAST against Greengenes, a chimera-checked 16S rRNA database (Caporaso et al., 2010a; DeSantis et al., 2006). In five instances, poor extraction of a duplicate was evident; therefore, a duplicate was removed when the total OTU count was <10% of the other duplicate. Data were normalized by dividing individual OTU number by the total OTU, with duplicate abundances averaged for downstream analysis. Further data processing was performed using R (v 3.4.1) and the package ampvis2 (Albertsen et al., 2015). Microbial 16S rRNA gene data is available through NCBI under BioProject ID PRJNA521770.

2.4. Surfactant analysis via LC-MS

Polyethoxylated alcohols were analyzed using non-targeted accurate mass analysis. A Bruker MAXIS II quadrupole time-of-flight (Q-TOF) MS coupled to a Waters ACQUITY Class-H UPLC was used for non-targeted accurate mass analysis in positive electrospray ionization mode with a Waters ACQUITY UPLC BEH C8 column (1.7 um × 2.1 mm × 100 mm). The flow rate was 0.3 mL min^{-1}, mobile phases were A (0.1% ammonium acetate) and B (acetonitrile), and a gradient elution method was developed containing 0–2 min 30% B; 2–10 min 30–90% B; 10–13 min 90% B; and 13–16 min 30% B. Data analysis was performed with Compass Data Analysis software (Bruker) using extracted ion chromatograms; octyl ethoxylates (C_8EO_n), decyl ethoxylates ($C_{10}EO_n$), nonylphenol ethoxylates ($NPEO_n$), polyethylene glycols (PEGs) and polypropylene glycols (PPGs) were manually quantified on the most abundant adduct $[M+NH_4]^+$ (Thurman et al., 2014). Sodium and proton adducts were also identified for some ions but were not consistently detected across ethoxylates. Additionally, putative carboxylate and aldehyde metabolites of C_8EO_6, C_8EO_{10}, $C_{10}EO_6$, $C_{10}EO_{10}$, $NPEO_6$, and $NPEO_{10}$ were also quantified as $[M+NH_4]^+$ adducts; these metabolites were selected to encompass both short and long alkyl and carbon chain lengths (Table S3). Ethoxylates could not be definitively confirmed as branched or linear due to a lack of commercial standards. No AEOs or NPEOs were detected in the mineral media, Monongahela River source water, or acetate biotic control solution.

2.5. Low molecular weight metabolite analysis

Small organic alcohols and aldehydes were analyzed using a Thermo TRACE 1300 Gas Chromatograph with flame ionization detection (FID). Separation was performed using a Zebron ZB-WAX_{PLUS} column (Phenomenex) (30 m, 0.25 mm ID, 0.25 μM film thickness). Using an AI1310 autosampler, 1 μL of sample was injected to the inlet (225 °C, 1:10 split) with a column flow rate of 1.5 mL min^{-1} He and the following oven parameters applied: 30 °C for 5 min, ramp to 200 °C at 10 °C min^{-1}, hold 2 min, ramp to 250 °C at 25 °C min^{-1}, hold 2 min, for a total run time of 30.8 min. The FID temperature was set to 225 °C. Blanks were injected between samples to limit carryover and a check standard was analyzed every ~12 samples to confirm retention time. Significant overlap was observed between ethanol and propanol; these compounds are therefore reported as ethanol + propanol.

Acetate, propionate, and glyoxylic acid were analyzed using a Thermo Integrion High Pressure Ion Chromatography system using a Dionex IonPac analytical column (4 × 250 mm) with a Dionex IonPac guard column (4 × 50 mm) and a Dionex IonPac MFC-1 trap column (3 × 27 mm). Using a 25 μL sample loop, samples were separated using a 30 mM potassium hydroxide eluent at 1.5 mL min^{-1} and detected with a conductivity detector. Samples were diluted (1:5, 1:10) with high purity distilled deionized water to reduce high salinity interferences and spiked samples were analyzed to confirm retention time of metabolites in the high salinity matrix. Data for alcohols, aldehydes, and

organic acids was processed using Chromeleon 7 software (Thermo Scientific) and all peaks checked for proper alignment and integration.

3. Results and discussion

Over the 75-day experiment, clear changes consistent with microbial biodegradation of organic additives in the synthetic fracturing fluid were observed in the aerobic biotic batch reactors. In higher TDS treatments, we observed slower microbial growth and consequently slower metabolism and removal of DOC. Proteobacteria dominated the microbial community composition after Day 0, with up to 78% of the community consisting of taxa within the genus *Pseudomonas*. AEOs were degraded faster than NPEOs, with short-chain ethoxylates more rapidly degraded than longer-chain ethoxylates in both classes. Aerobic alkyl polyethoxylate metabolites including carboxylate and aldehyde ethoxylates and low molecular weight metabolites (e.g., acetone) were detected.

3.1. High TDS delays DOC removal and microbial activity

Carbon removal in batch reactors was tracked as DOC (Fig. 1a). The lowest DOC removal rate was observed in the abiotic control, indicating minimal microbial activity remained in the control killed with sodium azide, and more generally a minimal degree of DOC removal would be expected from volatilization or sorption across all reactors. As anticipated, DOC in the acetate control was removed most rapidly, confirming the base fluid was biologically active and substrate could be consumed despite the 10 g L^{-1} produced water TDS. DOC removal in the SFF treatment with no added TDS was consistent with previously reported aerobic degradation rates for sludge microbial communities over the first 7 days (53% versus 56% DOC removed, respectively) (Kekacs et al., 2015). DOC was removed more rapidly and to a greater extent in the SFF 0 g L^{-1} TDS treatment than the higher TDS treatments (Fig. 1a). The 10 g L^{-1} and 40 g L^{-1} TDS treatments had similar DOC removal after 75 days, but an extended lag period of 4 and 14 days was observed, respectively, from TDS addition. Although all acetate-derived DOC was removed with 10 g L^{-1} TDS, 42% of the DOC remained in the 10 g L^{-1} TDS treatment containing SFF as substrate. At 40 g L^{-1} TDS, 44% of the SFF DOC remained after 75 days (Fig. 1a).

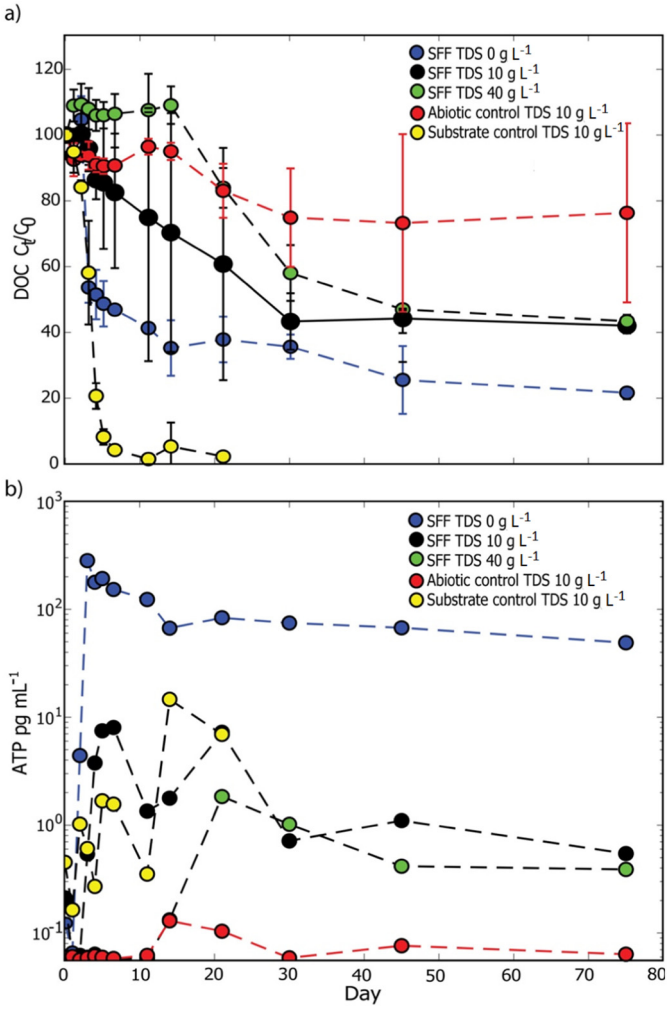


Fig. 1. Changes in relative DOC concentration relative to time 0 (C_t/C_0) (a) and ATP concentration (b) in aerobic batch reactors over a 75 day period. Error bars in (a) show range of duplicate experimental treatments.

ATP was measured as an indicator of microbial activity in batch reactors in tandem with DOC measurements (Fig. 1b). The highest ATP concentrations were observed in the SFF TDS 0 g L^{-1} treatment (~10^2 pg L^{-1}), while the lowest ATP concentrations were observed in the abiotic control (maximum ~10^{-1} pg L^{-1}). Although acetate was removed in the substrate control reactor after 21 days, ATP remained an order of magnitude lower than observed in the 0 g L^{-1} TDS treatment near values of the 10 g L^{-1} TDS treatment (~10 pg L^{-1}), indicating microbial growth was suppressed by higher TDS rather than available substrate (Fig. 1b). A 14-day lag in ATP concentration increase was observed in the 40 g L^{-1} TDS treatment, mirroring the lag observed in DOC removal. As DOC concentration in both SFF 10 and 40 g L^{-1} TDS treatments approached an asymptote, this finding suggests that minimal carbon mineralization would occur after 2.5 months through aerobic biodegradation. The results suggested that microbial community exposure to the produced water-derived TDS required an acclimation period, likely accompanied by suppressed growth and metabolism, as evidenced by the observed decrease in ATP and lower DOC removal. Considering the high complexity of metals, inorganic, organic compounds in produced water TDS (Hayes, 2009; Luek et al., 2018b; Oetjen et al., 2018; Rosenblum et al., 2017a; Ziemkiewicz and He, 2015), a wide range of compounds may contribute to the inhibition of DOC biodegradation, acting as a pseudo-biocide.

3.2. Pseudomonas and other Proteobacteria dominate microbial community composition across all TDS levels

The microbial community composition at the start of the experiment (Day 0) in all biotic reactors was similar to the river water consortium used to formulate the base fluid. The river water contained taxa from diverse phyla including Proteobacteria (36–45%), Firmicutes (20–25%), Actinobacteria (14–17%), Bacteroidetes (4–12%), and Cyanobacteria (2–7%) (Table S4, Fig. 2). Stramenopiles, *Halanaerobiaceae*, *Staphylococcus*, *Lactobacillus*, and *Corynebacterium* were among the most abundant taxa on Day 0. The microbial community also included common produced water anaerobes, *Halanaerobiales* and *Bacillales*, which likely derived from the use of Marcellus Shale produced water within TDS treatments (Borton et al., 2018; Daly et al., 2016; Lipus et al., 2017). Shannon and Simpson indices (Table S5) indicated the highest microbial diversity was contained in the river water and background control (containing no SFF), followed by Day 0 treatments containing SFF. As all treatments were performed in the dark, the absence of light would be expected to result in the reduced abundance of taxa requiring light such as the predominantly algal taxa Stramenopiles.

Microbial diversity was lowest on Day 7 for the 0 and 10 g L^{-1} TDS treatments and lowest on Day 21 for the 40 g L^{-1} TDS treatment, indicating a few taxa initially dominated these systems that were capable of utilizing readily biodegradable substrates under aerobic environmental conditions. Taxa within Proteobacteria completely dominated the communities present between day 7 and 21 in the 0 and 10 g L^{-1} TDS treatments (>99.9%); in the 40 g L^{-1} TDS treatment >99.9% of identified OTUs also belonged to Proteobacteria after day 21 (Fig. 2a and Table S4).

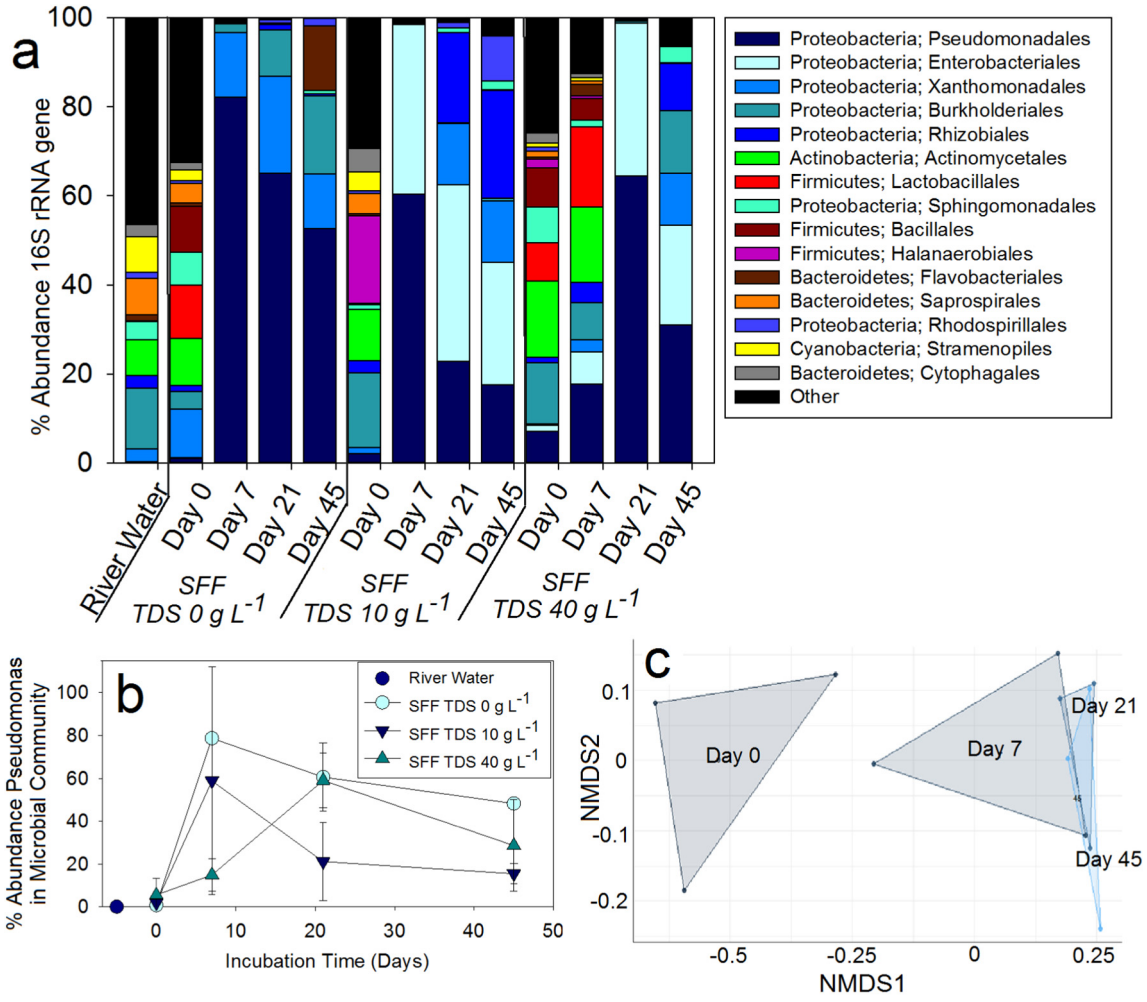


Fig. 2. Microbial 16S rRNA abundance in aerobic batch reactors as a function of time and treatment and in river water; a) percent abundance by order; b) percent *Pseudomonas* based on 16S rRNA gene sequences; c) non-metric multidimensional scaling highlighting time.

Among the Proteobacteria, Pseudomonadales was the most dominant order; others included Enterobacteriales, Xanthomonadales, and Burkholderiales. We tracked changes in *Pseudomonas* (order Pseudomonadales) through time (Fig. 2b), observing this genus represented up to 78% of detected sequences in 0 g L^{-1} TDS treatment and 59% in TDS treatments. Pseudomonadales and *Pseudomonas* peaked on Day 7 for the 0 and 10 g L^{-1} TDS treatments versus Day 21 in the 40 g L^{-1} TDS treatment, consistent with the lags observed in ATP concentration and carbon removal at higher TDS. In the following weeks, *Pseudomonas* remained dominant but competed with a handful of other Proteobacteria orders. These results clearly indicate the ability of *Pseudomonas* and related taxa to utilize the available substrate to dominate the microbial community under these environmental conditions including a range of TDS concentrations encompassing both inorganic and organic components.

Non-metric multidimensional scaling (NMDS) analysis showed a clear separation of the microbial community by incubation time, with the composition of Day 0 considerably different from later sample times across all TDS treatments (Fig. 2c). When a relatively diverse microbial community present in the Monongahela River (aerobic freshwater taxa) was mixed with the produced water (anaerobic halotolerant taxa) under aerobic conditions, *Pseudomonas* initially dominated and was likely responsible for the mineralization of readily biodegradable carbon substrates. *Pseudomonas* spp. have been isolated from sewage sludge to grow solely on AEOs and NPEOs (John and White, 1998; Tidswell et al., 1996), indicating that alkyl and alkylphenol polyethoxylates may serve as a carbon substrate for this genus. Additionally, *Pseudomonas* have also been previously identified in highly saline aerobic produced waters and during the biodegradation of hydraulic fracturing fluids, indicating their tolerance of a wide range of salinities (Kekacs et al., 2015; Lipus et al., 2018, 2017; Murali Mohan et al., 2013a, 2013b).

3.3. Alkyl ethoxylates are degraded more slowly in high TDS treatments

C_8EO and C_{10}EO peak intensities decreased through time in all biotic treatments, with longer alkyl groups (C_{10}EOs) more readily degraded than shorter ones (C_8EOs) (Figs. 3, S1). This finding is consistent with previous aerobic biodegradation results, indicating faster degradation of long alkyl chain ethoxylates (Itrich and Federle, 2004). No AEO removal was observed in the abiotic treatment, indicating observed losses in the biotic treatments were enzymatically mediated. Consistent with DOC trends, higher TDS concentrations resulted in an initial lag in AEO removal and a lower extent of AEO removal after 75 days (Fig. 3). However, the lag in removal for both the SFF 10 g L^{-1} and 40 g L^{-1} TDS treatments was longer than observed for DOC (4 vs. 21 days, 14 vs. 45 days, respectively). This longer lag in surfactant removal compared to DOC removal indicates that the more labile substrates in the SFF amendment were preferentially consumed, followed by the alkyl polyethoxylates. A lag in AEO biodegradation was also previously reported in seawater (35 g L^{-1}) (Pérez-Carrera et al., 2010), further supporting the inhibition of AEO-degrading taxa and/or metabolisms under elevated salinity.

The relative intensity of individual ethoxymers generally centered on EO8 for both C_8EO and C_{10}EOs. Shorter-chain ethoxylates were degraded more rapidly than longer ethoxylates in both the C_8EO and

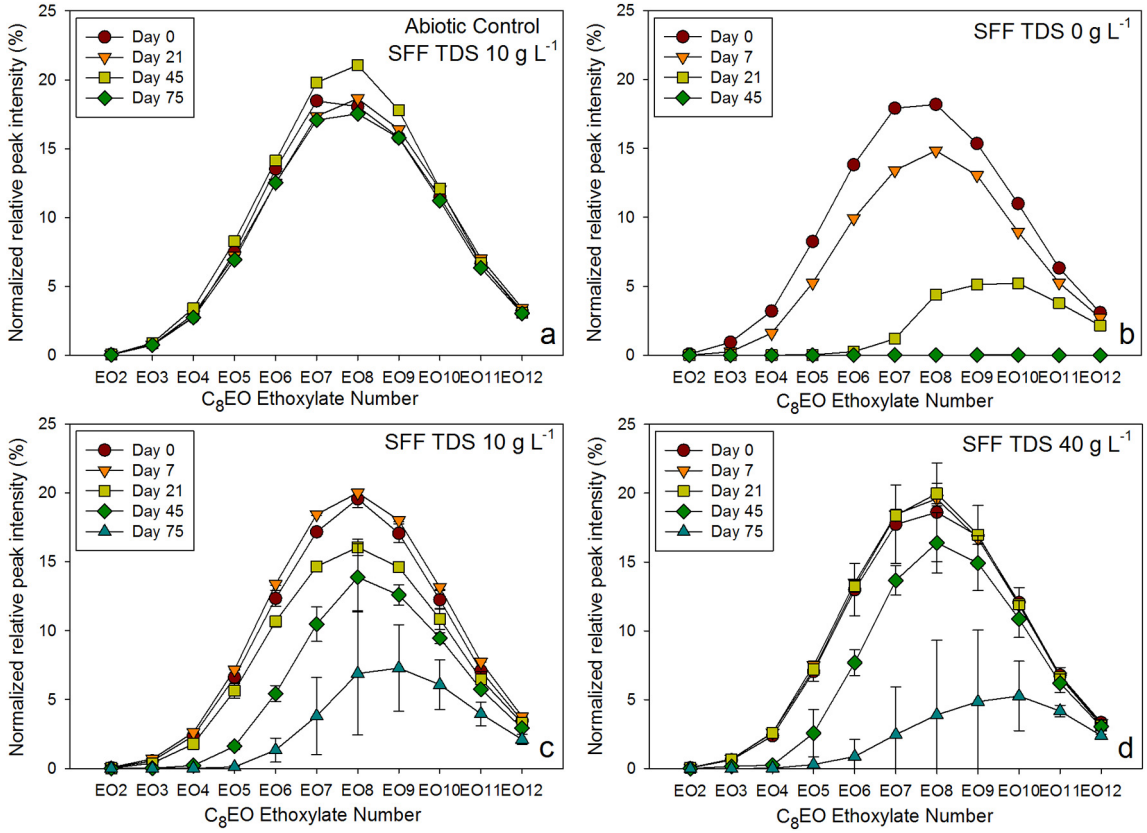


Fig. 3. Changes in C_8EO peak intensity in aerobic batch reactors as normalized to Day 0 summed EO2-EO12 ethoxylate peaks. a) Abiotic control SFF TDS 10 g L^{-1} (summed Day 0 = 1.1×10^7) b) SFF TDS 0 g L^{-1} (summed Day 0 = 1.4×10^7) c) SFF TDS 10 g L^{-1} (summed Day 0 = 9.2×10^6) d) SFF TDS 40 g L^{-1} (summed Day 0 = 9.7×10^6).

$C_{10}EO$ series (Fig. 3), consistent with previous AEO biodegradation studies (Federle and Itrich, 2006; Itrich and Federle, 2004). The removal of the shortest ethoxymers (EO2 and EO3) to levels below the quantitation limit was observed before day 21 with no added TDS. In the SFF 10 g L^{-1} and 40 g L^{-1} TDS treatments, complete removal was also observed for these low molecular weight ethoxymers after 21 or 45 days, respectively.

Average first order degradation rates were calculated for individual ethoxymers to compare removal kinetics across treatment, class, and ethoxylate number (Fig. 4). Appropriateness of fit for the first order kinetic curve was calculated as χ^2. The best fit (lowest χ^2) was observed for kinetic rates in the 0 g L^{-1} TDS treatment, where no lag was observed prior to AEO degradation. An alternative kinetic model was not fit to the severe lag data in order to allow comparison across all treatments as first order kinetic rates. Kinetic curves could not be fit to data containing only two points, so degradation rates for the short-chain ethoxymers below detection after 7 days (e.g., EO2, EO3) are given as linear rates rather than first-order rates (indicated by * in Fig. 4). Had additional samples been analyzed during the first week, the calculated first order degradation rate would likely be the highest for these short-chain ethoxymers ($C_{10}EO_2$, $C_{10}EO_3$), consistent with previous studies (Itrich and Federle, 2004).

The fastest first order kinetic rates for AEOs were observed when no TDS was added. In the 0 g L^{-1} TDS treatment, $C_{10}EO$ degradation rates (Fig. 4b) were approximately three times higher for the shortest quantified ethoxymer (EO_4, $k = 0.21 \pm 0.01$ day^{-1}) than for the longest quantified ethoxymer (EO_{11}, $k = 0.07 \pm 0.01$ day^{-1}). $C_{10}EO$ kinetic rates in the SFF 10 g L^{-1} TDS treatment were approximately a quarter of these ($k = 0.007$–0.06 day^{-1}), and were even slower in the 40 g L^{-1} TDS treatment ($k = 0.004$–0.02 day^{-1}). At 40 g L^{-1} TDS, the short and long chain $C_{10}EO$ removal rates were similar. Calculated kinetic rates for shorter-chain ethoxylates are expected to be underestimated as long-chain ethoxylates generate shorter ethoxylate homologs during biodegradation via the chain-shortening pathway (Liu et al., 2006). The measured degradation rates for $C_{10}EO$s and C_8EOs were much slower than aerobic rates measured previously in activated sludge and wastewaters (Federle and Itrich, 2006; Itrich and Federle, 2004; Schreiner et al., 1999), where near complete removal of most AEOs was typically obtained within 24–48 h. The lower first-order AEO degradation rates observed in SFF samples relative to other wastewater systems are likely a function of (1) the high TDS concentration, (2) preferential consumption of highly labile substrates, (3) the microbial inoculum, and/or (4) biocide additives in the synthetic fracturing fluid.

3.4. NPEOs are removed more slowly than AEOs in TDS treatments

Similar to AEOs, NPEOs were degraded in biotic but not abiotic treatments (Fig. 5), further supporting that the observed losses were microbially and enzymatically mediated. The dominant NPEOs were centered on EO14 and EO16 prior to degradation, with the distribution shifting to longer chain ethoxylates after 45 days in the 0 g L^{-1} TDS treatment as shorter chain ethoxylates were degraded. No change in NPEO distribution was observed in the treatments with added TDS; this finding is consistent with a previous study examining aerobic NPEO degradation in river water (Jonkers et al., 2001). Together, these findings suggest that NPEO ethoxymer chains degrade at similarly slow rates under higher salinities. Differences in salinity may have influenced LC-MS quantification due to matrix interferences across ethoxymer chain length (Nell and Helbling, 2018); however, considering rates were calculated within bioreactors containing the same TDS levels, such interference was not likely an important factor in this study. First order kinetic rates for NPEO degradation were calculated for each ethoxymer that could be quantified at more than two time points (Fig. 4c). The highest NPEO degradation rate quantified was for $NPEO_{12}$ ($k = 0.07 \pm 0.02$ day^{-1}), with rates in TDS-added treatments

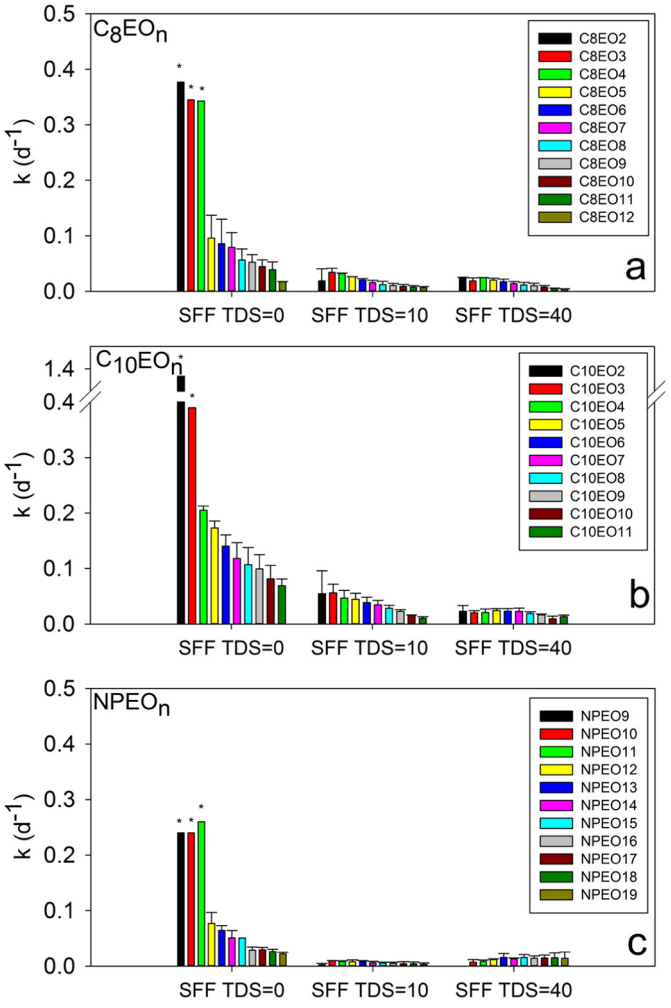


Fig. 4. First order degradation rates of a) C_8EO b) $C_{10}EO$ and c) NPEOs in aerobic batch reactors. * indicates rates given as linear due to their absence after Day 7 and inadequate time points to calculate first order rates.

remaining below 0.02 day^{-1} for all ethoxylates, and no clear trend across ethoxymer chain length. The observed NPEO removal rates were more than an order of magnitude slower than those observed in a freshwater aerobic biodegradation experiment (NPEO removal in <4 days) (Jonkers et al., 2001), but closer in magnitude to degradation rates observed in high salinity estuarine water where lags in removal (up to 12 days) were also observed (Potter et al., 1999). Like AEOs, the slower NPEO removal rates were likely a function of slowed metabolisms and substrate specificity under high TDS, among other inhibitors highlighted in Section 3.3.

3.5. Polyethoxylated alcohol biodegradation metabolites vary as a function of TDS level

Aldehyde and carboxylate species, formed during chain shortening (Liu et al., 2006; Thurman et al., 2017), have been previously identified as intermediate metabolites of polyethoxylated alcohols. In this study, both aldehyde (ALD) and carboxylate (COOH) intermediate peaks identified in MS spectra could be (semi-)quantified based on abundance, but no commercial standards were available to allow quantification of analyte concentration, as often encountered with these types of compounds. ALD and COOH intermediates of C_8EO_6, C_8EO_{10}, $C_{10}EO_6$, and $C_{10}EO_{10}$ were consistently detected after 7 days, including abiotic samples at low levels. As ALD and COOH intermediates are not assumed to have the same ionization efficiency, treatments were compared as the relative proportion of COOH to ALD (Table S6). Whereas AEO-COOH peaks were higher relative to AEO-ALD in the SFF TDS 0 g L^{-1} treatments, AEO-ALD intermediates were in higher relative abundance to carboxylate intermediates in SFF 10 g L^{-1} TDS and 40 g L^{-1} TDS treatments. No NPEO-ALD species were identified but small $NPEO_6$-COOH and substantial $NPEO_{10}$-COOH peaks were detected. NPEO-COOH are known to be a dominant metabolite of NPEO biodegradation under aerobic conditions (Jonkers et al., 2001; Potter et al., 1999), further supporting microbial-mediated removal. These results indicate that increased TDS may alter the AEO degradation pathway to direct cleavage of an aldehyde/ketone group (e.g., acetaldehyde, glycoaldehyde, acetone), rather than conversion to a carboxylic moiety before cleavage of glyoxylate. Under this enzymatic pathway, an ethoxy shift occurs on the terminal end of the ALD intermediate, after which an aldehyde is cleaved. Typically, this ethoxymer chain cleavage pathway occurs under suboxic conditions (Heyob et al., 2017), which was not the case in these experiments (DO ranged from 2.23 to 7.9 mg/L). Nevertheless, the apparent prevalence of an ALD pathway may be indicative of a metabolic shift potentially caused by bioenergetic constraints imparted by the elevated TDS and indicates these biotransformations continue even after DOC concentrations have approached an asymptote (i.e., without complete mineralization of carbon). Additionally, within the microbial community, genome-level differences in chain-shortening enzymes among dominant taxa may account for the apparent shift towards an ALD pathway.

Low molecular weight organic aldehydes, alcohols, and acids associated with microbial metabolisms were analyzed using GC-FID. Acetone, ethanol + propanol, and propionate were the most abundant and frequently detected analytes (Table S7). However, the high dilutions required for analysis of these highly saline samples limited our ability to detect small metabolites at microbially-produced concentrations. Ethanol + propanol were initially detected, but were absent at later time points; these alcohols persisted longer in high TDS treatments compared to low TDS treatments. Acetone was initially low (<100 μM), peaking in samples collected on day 7 (0, 10 g L^{-1} TDS) or 21 (40 g L^{-1} TDS) (maximum 712 μM), consistent with delayed ethoxylate degradation in high salinity treatments. Propionate was most abundant in samples from the 10 g L^{-1} TDS treatment, increasing throughout the 75 day test (18–30 mg/L); propionate was detected but not quantifiable in the 0 g L^{-1} and 40 g L^{-1} TDS treatments. The detection of these metabolites is consistent with alcohol dehydrogenase (Kekacs et al., 2015) or diol dehydratase (Heyob et al., 2017) enzymatic conversion of alcohols (e.g., isopropanol), alkyl ethoxylates (e.g., AEOs), or polyglycols (e.g., polypropylene glycol) to ketones, aldehydes, and carboxylic acids under aerobic and suboxic conditions. Further coupled genomic and mechanistic investigation of AEO and NPEO removal under high salinity is required to confirm the biodegradation pathways of halotolerant taxa.

3.6. Industrial and environmental implications

The TDS concentrations investigated in this study represent low to moderate salinities encountered during produced water reuse in the oil and natural gas industry. Increasing TDS acted as a pseudo-biocide, altering the microbial community, delaying microbial growth, and consequently limiting biodegradation of AEOs, NPEOs, and other DOC. Although the dominant components of produced water TDS used in this study were inorganic salts (e.g., chloride, sodium, calcium), produced waters also contain an array of other inorganic and organic components in low levels (e.g., Ra, BTEX) that may contribute to the observed effects to an unknown degree. The reuse of high TDS produced water will likely inhibit freshwater microbes when blended with source water, perhaps more effectively than applied chemical biocides. Moreover, the presence of *Pseudomonas* in aerobic produced fluids in our study and others (Kekacs et al., 2015; Lipus et al., 2018, 2017; Murali Mohan et al., 2013a, 2013b) support slow biodegradation of ethoxylated surfactants common in these stored waste fluids. These results indicate that when

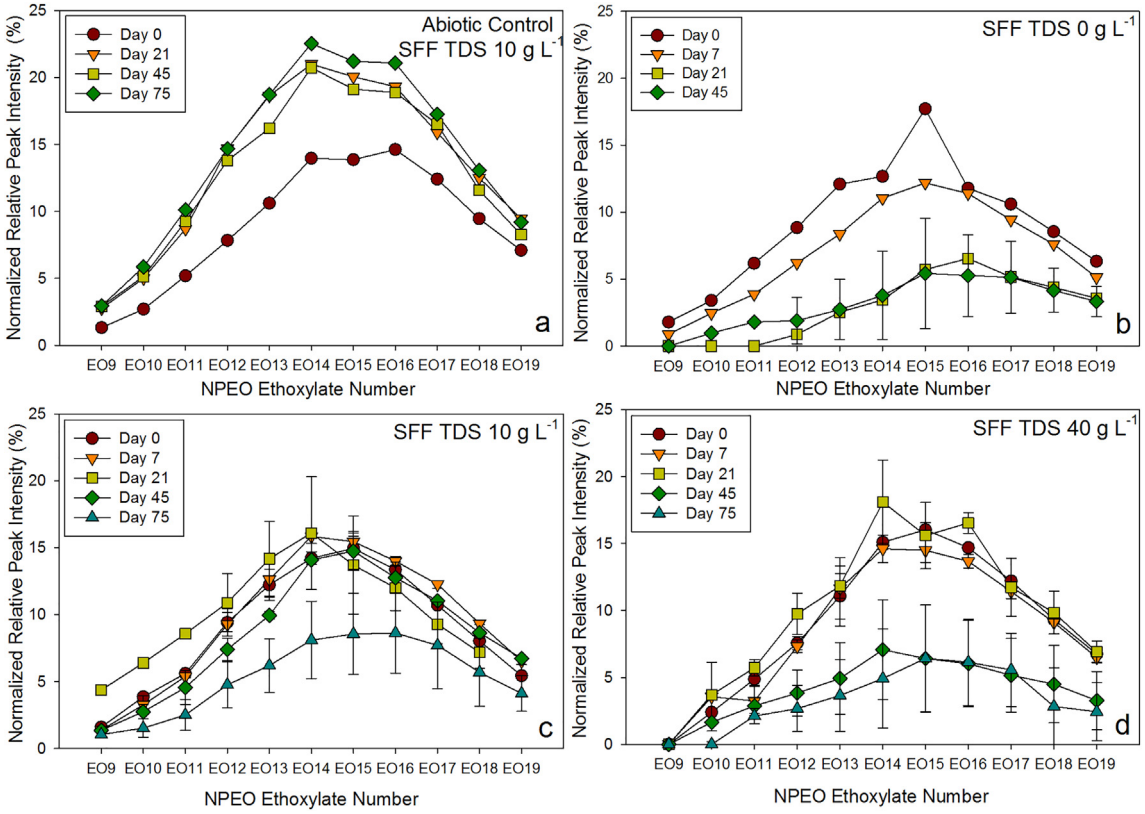


Fig. 5. Changes in NPEO peak intensity in aerobic batch reactors as normalized to Day 0 summed EO9-EO19 ethoxylate peaks. a) Abiotic SFF TDS 10 g L^{-1} (summed Day 0 = 2.4×10^5) b) SFF TDS 0 g L^{-1} (summed Day 0 = 3.1×10^5) c) SFF TDS 10 g L^{-1} (summed Day 0 = 2.8×10^5) d) SFF TDS 40 g L^{-1} (summed Day 0 = 2.7×10^5).

high TDS produced water is blended with freshwater and fracturing fluid and used within 3 weeks (as often done), minimal loss and/or biotransformation of AEO and NPEO surfactants due to microbial activity would be expected.

Although minor transformations in produced fluid water quality (including AEOs, NPEOs and DOC in general) could reduce chemical use by the oil and gas industry in a hydraulic fracturing reuse scenario, this delayed biodegradation is not ideal for potentially impacted environments. In light of the results of this study, intentional beneficial reuse (Guerra et al., 2011) and accidental releases of produced water-hydraulic fracturing fluid blends are likely to result in environmental persistence of AEOs and NPEOs, particularly at TDS concentrations above 10 g L^{-1}. A pronounced lag combined with the slower attenuation of DOC, including specific AEOs and NPEOs as a result of higher TDS, would be unfavorable in a number of shallow ecosystems, such as groundwater aquifers, ephemeral streams with minimal dilution (McDevitt et al., 2019), and/or agricultural fields (Heyob et al., 2017; Kekacs et al., 2015; Mouser et al., 2016). AEO-ALD and -COOH metabolite abundances suggest the primary biotransformation pathway shifts to aldehyde intermediates, which are undesirable from a human health perspective in the event of accidental fluid releases due to their volatility and toxicity (LoPachin and Gavin, 2014). In addition, the major aqueous removal mechanism for persistent hydrophobic species such as NPEOs may shift from biodegradation to sedimentation, potentially accumulating to form a long-term source that may affect ecosystem health (Burgos et al., 2017). Lastly, the presence of higher TDS appeared to exert an appreciable, long-standing effect on microbial community composition within one week of exposure to increased salinity, suggesting an accidental release of recycled produced water may upset naturally-occurring microbial communities.

The results of this study have implications for the treatment of oil and natural gas produced water, whether for reuse or prior to disposal such as deep-well injection. Given the high salinity of produced water, ion removal via membrane filtration or other mechanisms is crucial. However, elevated DOC derived from surfactants and other polymers (e.g., polyacrylamide) can clog membranes resulting in flux decline or compromised membranes (Xiong et al., 2018, 2016). Therefore, pre-treatment via biological processes can improve desalination technologies and extend the life of membranes (Lester et al., 2014). Like other studies, we observed the biological removal of DOC including hydraulic fracturing chemical additives from flowback and produced waters (e.g., via biologically active filtration (Freedman et al., 2017; Riley et al., 2016)) is most effective when the microbial community has sufficient time to adjust to salt concentrations and is constrained to a narrow range of diluted salinity (e.g. 10 g L^{-1}). Thus, produced water reuse strategies employing biological pre-treatment and biofilm treatment approaches (Akyon et al., 2019) may require higher retention times and/or larger basins to accommodate slower-growing halotolerant taxa and fluid recycle rates to achieve appropriate dilutions.

4. Conclusions

The reuse of oil and natural gas produced water for hydraulic fracturing operations provides a dual benefit in lessening the need for freshwater supplies and reducing both economic and environmental costs of disposing these wastewaters. Recycling produced water for future shale well completion practices is increasing (Lebas et al., 2013; Lutz et al., 2013), thereby necessitating investigation into the fate of chemical additives in mixed fluids. This study sought to simulate a produced water reuse scenario wherein high TDS produced water was blended (without prior treatment) with fresh water to formulate hydraulic fracturing fluid. If produced water is blended with fresh water to 10–40 g L^{-1} TDS and reused within 3 weeks, the results of this study indicate that minimal loss of alkyl polyethoxylate surfactant functionality due to microbial degradation would be expected. However, in the incidence of recycled fluid environmental release, the slow AEO and NPEO

degradation rates and potential accumulation of metabolites indicates natural attenuation would be delayed unless the fluids are highly diluted.

Acknowledgements

We thank Karolien Denef and MJ Riches at the Colorado State University Central Instrument Facility for assistance with LC/Q-TOF-MS analyses. Many thanks to Michael Brooker for assistance with sequence analysis. Financial assistance was obtained from NSF CBET Award #1823069 to PJM.

Competing interests statement

The authors have no competing interests to declare.

Author contributions

PJM, AJH, and SST designed and performed the experiment, AJH, SST, JLL, MM, and JB performed chemical and microbial analyses, PJM, AJH, JLL and SST analyzed data, and all authors assisted in the writing and review of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.03.041.

References

Akyon, B., McLaughlin, M., Hernández, F., Blotevogel, J., Bibby, K., 2019. Characterization and biological removal of organic compounds from hydraulic fracturing produced water. Environ. Sci. Process. Impacts. https://doi.org/10.1039/C8EM00354H.

Albertsen, M., Karst, S.M., Ziegler, A.S., Kirkegaard, R.H., Nielsen, P.H., 2015. Back to basics–the influence of DNA extraction and primer choice on phylogenetic analysis of activated sludge communities. PLoS One 10, e0132783. https://doi.org/10.1371/journal.pone.0132783.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.

Borton, M.A., Hoyt, D.W., Roux, S., Daly, R.A., Welch, S.A., Nicora, C.D., Purvine, S., Eder, E.K., Hanson, A.J., Sheets, J.M., Morgan, D.M., Wolfe, R.A., Sharma, S., Carr, T.R., Cole, D.R., Mouser, P.J., Lipton, M.S., Wilkins, M.J., Wrighton, K.C., 2018. Coupled laboratory and field investigations resolve microbial interactions that underpin persistence in hydraulically fractured shales. Proc. Natl. Acad. Sci. 115, E6585–E6594. https://doi.org/10.1073/pnas.1800155115.

Burgos, W.D., Castillo-Meza, L., Tasker, T.L., Geeza, T.J., Drohan, P.J., Liu, X., Landis, J.D., Blotevogel, J., McLaughlin, M., Borch, T., Warner, N.R., 2017. Watershed-scale impacts from surface water disposal of oil and gas wastewater in western Pennsylvania. Environ. Sci. Technol. 51, 8851–8860. https://doi.org/10.1021/acs.est.7b01696.

Campa, M.F., Techtmann, S.M., Gibson, C.M., Zhu, X., Patterson, M., Garcia de Matos Amaral, A., Ulrich, N., Campagna, S.R., Grant, C.J., Lamendella, R., Hazen, T.C., 2018. Impacts of glutaraldehyde on microbial community structure and degradation potential in streams impacted by hydraulic fracturing. Environ. Sci. Technol. 52, 5989–5999. https://doi.org/10.1021/acs.est.8b00239.

Caporaso, J.G., Bittinger, K., Bushman, F.D., DeSantis, T.Z., Andersen, G.L., Knight, R., 2010a. PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics 26, 266–267. https://doi.org/10.1093/bioinformatics/btp636.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010b. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335.

Chapman, E.C., Capo, R.C., Stewart, B.W., Kirby, C.S., Hammack, R.W., Schroeder, K.T., Edenborn, H.M., 2012. Geochemical and strontium isotope characterization of produced waters from Marcellus Shale natural gas extraction. Environ. Sci. Technol. 46, 3545–3553. https://doi.org/10.1021/es204005g.

Chen, H., Carter, K.E., 2017. Characterization of the chemicals used in hydraulic fracturing fluids for wells located in the Marcellus Shale Play. J. Environ. Manag. 200, 312–324. https://doi.org/10.1016/j.jenvman.2017.05.069.

Cluff, M.A., Hartsock, A., MacRae, J.D., Carter, K., Mouser, P.J., 2014. Temporal changes in microbial ecology and geochemistry in produced water from hydraulically fractured Marcellus Shale gas wells. Environ. Sci. Technol. 48, 6508–6517. https://doi.org/10.1021/es501173p.

Dabdoub, S.M., Fellows, M.L., Paropkari, A.D., Mason, M.R., Huja, S.S., Tsigarida, A.A., Kumar, P.S., 2016. PhyloToAST: bioinformatics tools for species-level analysis and visualization of complex microbial datasets. Sci. Rep. 6, 29123.

Daly, R.A., Borton, M.A., Wilkins, M.J., Hoyt, D.W., Kountz, D.J., Wolfe, R.A., Welch, S.A., Marcus, D.N., Trexler, R.V., MacRae, J.D., Krzycki, J.A., Cole, D.R., Mouser, P.J., Wrighton, K.C., 2016. Microbial metabolisms in a 2.5-km-deep ecosystem created by hydraulic fracturing in shales. Nat. Microbiol. 1, 16146. https://doi.org/10.1038/nmicrobiol.2016.146.

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microbiol. 72, 5069–5072. https://doi.org/10.1128/AEM.03006-05.

Estrada, J.M., Bhamidimarri, R., 2016. A review of the issues and treatment options for wastewater from shale gas extraction by hydraulic fracturing. Fuel 182, 292–303. https://doi.org/10.1016/j.fuel.2016.05.051.

Federle, T.W., Itrich, N.R., 2006. Fate of free and linear alcohol-ethoxylate-derived fatty alcohols in activated sludge. Ecotoxicol. Environ. Saf. 64, 30–41. https://doi.org/10.1016/j.ecoenv.2005.05.008.

Freedman, D.E., Riley, S.M., Jones, Z.L., Rosenblum, J.S., Sharp, J.O., Spear, J.R., Cath, T.Y., 2017. Biologically active filtration for fracturing flowback and produced water treatment. J. Water. Process. Eng. 18, 29–40. https://doi.org/10.1016/j.jwpe.2017.05.008.

Getzinger, G.J., O'Connor, M.P., Hoelzer, K., Drollette, B.D., Karatum, O., Deshusses, M.A., Ferguson, P.L., Elsner, M., Plata, D.L., 2015. Natural gas residual fluids: sources, endpoints, and organic chemical composition after centralized waste treatment in Pennsylvania. Environ. Sci. Technol. 49, 8347–8355. https://doi.org/10.1021/acs.est.5b00471.

Gregory, K.B., Vidic, R.D., Dzombak, D.A., 2011. Water management challenges associated with the production of shale gas by hydraulic fracturing. Elements 7, 181–186. https://doi.org/10.2113/gselements.7.3.181.

Guerra, K., Dahm, K., Dundorf, S., 2011. Oil and gas produced water management and beneficial use in the western United States (No. Science and Technology Program Report No. 157). U.S. Department of the Interior, Bureau of Reclamation.

Haluszczak, L.O., Rose, A.W., Kump, L.R., 2013. Geochemical evaluation of flowback brine from Marcellus gas wells in Pennsylvania, USA. Appl. Geochem. 28, 55–61. https://doi.org/10.1016/j.apgeochem.2012.10.002.

Hayes, T., 2009. Sampling and Analysis of Water Streams Associated With the Development of Marcellus Shale Gas (Final Report) (Gas Technology Institute).

Heyob, K.M., Blotevogel, J., Brooker, M., Evans, M.V., Lenhart, J.J., Wright, J., Lamendella, R., Borch, T., Mouser, P.J., 2017. Natural attenuation of nonionic surfactants used in hydraulic fracturing fluids: degradation rates, pathways. and Mechanisms. Environ. Sci. Technol. 51, 13985–13994. https://doi.org/10.1021/acs.est.7b01539.

Horner, R.M., Harto, C.B., Jackson, R.B., Lowry, E.R., Brandt, A.R., Yeskoo, T.W., Murphy, D.J., Clark, C.E., 2016. Water use and management in the Bakken shale oil play in North Dakota. Environ. Sci. Technol. 50, 3275–3282. https://doi.org/10.1021/acs.est.5b04079.

Itrich, N.R., Federle, T.W., 2004. Effect of ethoxylate number and alkyl chain length on the pathway and kinetics of linear alcohol ethoxylate biodegradation in activated sludge. Environ. Toxicol. Chem. 23, 2790–2798.

Jiang, M., Hendrickson, C.T., VanBriesen, J.M., 2014. Life cycle water consumption and wastewater generation impacts of a Marcellus shale gas well. Environ. Sci. Technol. 48, 1911–1920. https://doi.org/10.1021/es4047654.

John, D.M., White, G.F., 1998. Mechanism for biotransformation of nonylphenol polyethoxylates to xenoestrogens in Pseudomonas putida. J. Bacteriol. 180, 4332–4338.

Jonkers, N., Knepper, T.P., de Voogt, P., 2001. Aerobic biodegradation studies of nonylphenol ethoxylates in river water using liquid chromatography−electrospray tandem mass spectrometry. Environ. Sci. Technol. 35, 335–340. https://doi.org/10.1021/es000127o.

Kekacs, D., Drollette, B.D., Brooker, M., Plata, D.L., Mouser, P.J., 2015. Aerobic biodegradation of organic compounds in hydraulic fracturing fluids. Biodegradation. https://doi.org/10.1007/s10532-015-9733-6.

Kondash, A., Vengosh, A., 2015. Water footprint of hydraulic fracturing. Environ. Sci. Technol. Lett. 150918090943001. https://doi.org/10.1021/acs.estlett.5b00211.

Kondash, A.J., Albright, E., Vengosh, A., 2017. Quantity of flowback and produced waters from unconventional oil and gas exploration. Sci. Total Environ. 574, 314–321. https://doi.org/10.1016/j.scitotenv.2016.09.069.

Lebas, R.A., Shahan, T.W., Lord, P., Luna, D., 2013. Development and use of high-TDS recycled produced water for crosslinked-gel-based hydraulic fracturing, in: SPE Hydraulic Fracturing Technology Conference (Society of Petroleum Engineers).

Lester, Y., Yacob, T., Morrissey, I., Linden, K.G., 2014. Can we treat hydraulic fracturing flowback with a conventional biological process? The case of guar gum. Environ. Sci. Technol. Lett. 1, 133–136. https://doi.org/10.1021/ez4000115.

Lester, Y., Ferrer, I., Thurman, E.M., Sitterley, K.A., Korak, J.A., Aiken, G., Linden, K.G., 2015. Characterization of hydraulic fracturing flowback water in Colorado: implications for water treatment. Sci. Total Environ. 512–513, 637–644. https://doi.org/10.1016/j.scitotenv.2015.01.043.

Lipus, D., Vikram, A., Ross, D., Bain, D., Gulliver, D., Hammack, R., Bibby, K., 2017. Predominance and metabolic potential of Halanaerobium spp. in produced water from hydraulically fractured Marcellus shale wells. Appl. Environ. Microbiol. 83, e02659-16. https://doi.org/10.1128/AEM.02659-16.

Lipus, D., Roy, D., Khan, E., Ross, D., Vikram, A., Gulliver, D., Hammack, R., Bibby, K., 2018. Microbial communities in Bakken region produced water. FEMS Microbiol. Lett. 365. https://doi.org/10.1093/femsle/fny107.

Liu, X., Tani, A., Kimbara, K., Kawai, F., 2006. Metabolic pathway of xenoestrogenic short ethoxy chain-nonylphenol to nonylphenol by aerobic bacteria, Ensifer sp. strain

AS08 and Pseudomonas sp. strain AS90. Appl. Microbiol. Biotechnol. 72, 552–559. https://doi.org/10.1007/s00253-005-0288-z.
LoPachin, R.M., Gavin, T., 2014. Molecular mechanisms of aldehyde toxicity: a chemical perspective. Chem. Res. Toxicol. 27, 1081–1091. https://doi.org/10.1021/tx5001046.
Luek, J.L., Harir, M., Schmitt-Kopplin, P., Mouser, P.J., Gonsior, M., 2018a. Organic sulfur fingerprint indicates continued injection fluid signature 10 months after hydraulic fracturing. Environ. Sci. Process. Impacts. https://doi.org/10.1039/C8EM00331A.
Luek, J.L., Harir, M., Schmitt-Kopplin, P., Mouser, P.J., Gonsior, M., 2018b. Temporal dynamics of halogenated organic compounds in Marcellus Shale flowback. Water Res. 136, 200–206. https://doi.org/10.1016/j.watres.2018.02.055.
Lutz, B.D., Lewis, A.N., Doyle, M.W., 2013. Generation, transport, and disposal of wastewater associated with Marcellus Shale gas development: rapid communication. Water Resour. Res. 49, 647–656. https://doi.org/10.1002/wrcr.20096.
McDevitt, B., McLaughlin, M., Cravotta III, C.A., Ajemigbitse, M.A., Van Sice, K.A., Blotevogel, J., Borch, T., Warner, N.R., 2019. Emerging investigator series: radium accumulation in carbonate river sediments at oil and gas produced water discharges: implications for beneficial use as disposal management. Env. Sci Process. Impacts. https://doi.org/10.1039/c8em00336j.
McLaughlin, M.C., Borch, T., Blotevogel, J., 2016. Spills of hydraulic fracturing chemicals on agricultural topsoil: biodegradation, sorption, and co-contaminant interactions. Environ. Sci. Technol. doi:https://doi.org/10.1021/acs.est.6b00240
Mouser, P.J., Liu, S., Cluff, M.A., McHugh, M., Lenhart, J.J., MacRae, J.D., 2016. Redox conditions alter biodegradation rates and microbial community dynamics of hydraulic fracturing fluid organic additives in soil–groundwater microcosms. Environ. Eng. Sci. 33, 827–838. https://doi.org/10.1089/ees.2016.0031.
Murali Mohan, A., Hartsock, A., Bibby, K.J., Hammack, R.W., Vidic, R.D., Gregory, K.B., 2013a. Microbial community changes in hydraulic fracturing fluids and produced water from shale gas extraction. Environ. Sci. Technol. 47, 13141–13150. https://doi.org/10.1021/es402928b.
Murali Mohan, A., Hartsock, A., Hammack, R.W., Vidic, R.D., Gregory, K.B., 2013b. Microbial communities in flowback water impoundments from hydraulic fracturing for recovery of shale gas. FEMS Microbiol. Ecol. 86, 567–580. https://doi.org/10.1111/1574-6941.12183.
Nell, M., Helbling, D.E., 2018. Exploring matrix effects and quantifying organic additives in hydraulic fracturing associated fluids using liquid chromatography electrospray ionization mass spectrometry. Environ. Sci. Process. Impacts. https://doi.org/10.1039/C8EM00135A.
Nicot, J.-P., Scanlon, B.R., 2012. Water use for shale-gas production in Texas, U.S. Environ. Sci. Technol. 46, 3580–3586. https://doi.org/10.1021/es204602t.
Oetjen, K., Chan, K.E., Gulmark, K., Christensen, J.H., Blotevogel, J., Borch, T., Spear, J.R., Cath, T.Y., Higgins, C.P., 2018. Temporal characterization and statistical analysis of flowback and produced waters and their potential for reuse. Sci. Total Environ. 619–620, 654–664. https://doi.org/10.1016/j.scitotenv.2017.11.078.
Pérez-Carrera, E., León, V.M., Lara-Martín, P.A., González-Mazo, E., 2010. Influence of the hydrophilic moiety of anionic and nonionic surfactants on their aerobic biodegradation in seawater. Sci. Total Environ. 408, 922–930. https://doi.org/10.1016/j.scitotenv.2009.10.003.
Potter, T.L., Simmons, K., Wu, J., Sanchez-Olvera, M., Kostecki, P., Calabrese, E., 1999. Static die-away of a nonylphenol ethoxylate surfactant in estuarine water samples. Environ. Sci. Technol. 33, 113–118. https://doi.org/10.1021/es9804464.
Riley, S.M., Oliveira, J.M.S., Regnery, J., Cath, T.Y., 2016. Hybrid membrane bio-systems for sustainable treatment of oil and gas produced water and fracturing flowback water. Sep. Purif. Technol. 171, 297–311. https://doi.org/10.1016/j.seppur.2016.07.008.
Rosenblum, J., Nelson, A.W., Ruyle, B., Schultz, M.K., Ryan, J.N., Linden, K.G., 2017a. Temporal characterization of flowback and produced water quality from a hydraulically fractured oil and gas well. Sci. Total Environ. 596–597, 369–377. https://doi.org/10.1016/j.scitotenv.2017.03.294.
Rosenblum, J., Thurman, E.M., Ferrer, I., Aiken, G., Linden, K.G., 2017b. Organic chemical characterization and mass balance of a hydraulically fractured well: from fracturing fluid to produced water over 405 days. Environ. Sci. Technol. 51, 14006–14015. https://doi.org/10.1021/acs.est.7b03362.
Schreiner, A., Asmussen, C., Wiesmann, U., Stan, H.-J., 1999. Investigation of aerobic degradation kinetics of surfactants using respirometric batch experiments. Eng. Life Sci. 19, 293–304.
Stringfellow, W.T., Domen, J.K., Camarillo, M.K., Sandelin, W.L., Borglin, S., 2014. Physical, chemical, and biological characteristics of compounds used in hydraulic fracturing. J. Hazard. Mater. 275, 37–54. https://doi.org/10.1016/j.jhazmat.2014.04.040.
Stringfellow, W.T., Camarillo, M.K., Domen, J.K., Sandelin, W.L., Varadharajan, C., Jordan, P.D., Reagan, M.T., Cooley, H., Heberger, M.G., Birkholzer, J.T., 2017. Identifying chemicals of concern in hydraulic fracturing fluids used for oil production. Environ. Pollut. 220, 413–420. https://doi.org/10.1016/j.envpol.2016.09.082.
Thurman, E.M., Ferrer, I., Blotevogel, J., Borch, T., 2014. Analysis of hydraulic fracturing flowback and produced waters using accurate mass: identification of ethoxylated surfactants. Anal. Chem. 86, 9653–9661. https://doi.org/10.1021/ac502163k.
Thurman, E.M., Ferrer, I., Rosenblum, J., Linden, K., Ryan, J.N., 2017. Identification of polypropylene glycols and polyethylene glycol carboxylates in flowback and produced water from hydraulic fracturing. J. Hazard. Mater. 323, 11–17. https://doi.org/10.1016/j.jhazmat.2016.02.041.
Tidswell, E.C., Russell, N.J., White, G.F., 1996. Ether-bond scission in the biodegradation of alcohol ethoxylate nonionic surfactants by Pseudomonas sp. strain SC25A. Microbiology 142, 1123–1131.
Xiong, B., Zydney, A.L., Kumar, M., 2016. Fouling of microfiltration membranes by flowback and produced waters from the Marcellus shale gas play. Water Res. 99, 162–170. https://doi.org/10.1016/j.watres.2016.04.049.
Xiong, B., Miller, Z., Roman-White, S., Tasker, T., Farina, B., Piechowicz, B., Burgos, W.D., Joshi, P., Zhu, L., Gorski, C.A., Zydney, A.L., Kumar, M., 2018. Chemical degradation of polyacrylamide during hydraulic fracturing. Environ. Sci. Technol. 52, 327–336. https://doi.org/10.1021/acs.est.7b00792.
Ying, G.-G., 2006. Fate, behavior and effects of surfactants and their degradation products in the environment. Environ. Int. 32, 417–431. https://doi.org/10.1016/j.envint.2005.07.004.
Ying, G.-G., Williams, B., Kookana, R., 2002. Environmental fate of alkylphenols and alkylphenol ethoxylates—a review. Environ. Int. 28, 215–226.
Ziemkiewicz, P.F., He, Y.T., 2015. Evolution of water chemistry during Marcellus Shale gas development: a case study in West Virginia. Chemosphere 134, 224–231. https://doi.org/10.1016/j.chemosphere.2015.04.040.