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Among the top impacts of hydraulic fracturing include the disposal of the highly-polluted produced waters. Biological and hybrid alternatives are a potential approach to improve upon standard physical treatment. Although promising, these technologies face challenges, including the effect of the organic composition on biodegradation—for which further investigation is needed in order for decision-makers to better identify the most feasible treatment alternatives. This study investigated the relationship between the biodegradation and the organic speciation of produced water samples from the Utica and Bakken Shales through an engineered biofilm approach. Results demonstrate that there is substantial variation in biodegradability both between and within shales with implications for the biological treatability of the produced waters – we observed TOC removal varied between 1% and 87% for different produced waters normalized to the same salinity. We performed statistical data-reduction analyses that yielded positive correlations between the biodegradation rate and the presence of polyethylene and polypropylene glycols and nonylphenol ethoxylates, and negative correlations between the biodegradation rate and the presence of heteroatoms containing Br, S, I, and Cl and long-chained fatty acids. These results contribute to the improved understanding of the organic composition and the biological treatment of produced waters.

Characterization and Biological Removal of Organic Compounds from Hydraulic Fracturing Produced Water

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ABSTRACT

Hydraulic fracturing generates large volumes of produced water, and treatment of produced water may be necessary for disposal or reuse. Biological treatment of produced water is a potential approach to remove organic constituents and reduce fouling, in conjunction with other treatment processes. This study investigates the biological treatability of produced water samples from the Utica and Bakken Shales using engineered biofilms. Observed total dissolved organic carbon (DOC) removal varied between 1-87% at normalized total dissolved solids concentrations, suggesting that the composition of produced water, including organic constituents and trace elements such as nutrients and metals, is an important driver of biological treatment performance. Mass spectrometric analyses of the DOC composition revealed various alkanes in all samples, but differences in non-ionic surfactant, halogenated, and acidic compound content. Statistical data reduction approaches suggest that the latter two groups are correlated with reduced biodegradation kinetics. These results demonstrate that the combination of biodegradation performance and organic speciation can guide the assessment of the biological treatment of produced water.

INTRODUCTION

During hydraulic fracturing to recover gas and oil from unconventional formations, large volumes of fracturing fluid, composed of 98-99% water and sand and 1-2% additive chemicals, are injected into the target formation[1, 2]. Much of this fluid ultimately returns to the surface and the fluid that is returned once the well is put into production is referred to as produced water [1]. Large volumes of produced water are generated following the fracturing process (up to 4 million gallons per well)[3]. Surface disposal of produced water is problematic due to high salt and

radionuclide concentrations as well as the presence of potentially toxic compounds, including organic compounds. Furthermore, regulations may not allow municipal wastewater treatment plants to accept produced waters in some areas (e.g., Pennsylvania)[4]. Currently, the most common disposal option for produced waters is deep well injection. However, this method induces seismicity in regions with high injection rates[5, 6].

Hydraulic fracturing fluids include chemical additives to facilitate the fracturing process. Some of these chemicals also create concerns upon their release to environment due to fracturing operations or incidental spills. A previous study demonstrated higher estrogenic, anti-estrogenic, or anti-androgenic activities in surface and ground water samples from high-density drilling regions of Colorado compared to reference sites with limited proximity to drilling locations. Their results suggested that endocrine-disrupting chemical activity in surface and groundwater can occur due to natural gas extraction operations.[7] Therefore, an effective produced water treatment approach is essential for the final surface disposal of produced water. Residual organic content in produced waters may contribute to fouling during physical-chemical treatment. Reusing produced water can reduce the disposal volumes and decrease water demand during the opening of new wells; however, produced water may require treatment before reuse, including treatment to remove organic compounds that serve as electron donors for microorganisms that contribute to well fouling or souring. Biological produced water treatment may be used to remove organic compounds prior to reuse or final disposal. Biological treatment systems can decrease chemical oxygen demand (COD) in produced waters[8, 9] and this decrease in COD increases subsequent membrane fluxes[9].

The organic content of produced water can vary by well-site depending on the additives in the fracturing fluid, as well as on the subsurface chemistry. Observed organic carbon

concentrations in produced waters from shale plays range between 1 – >5,000 mg/L total organic carbon[10, 11]. In this study, total organic carbon (DOC) concentrations of 177 – 3,990 mg/L were measured in seven samples collected from two different shale plays (Bakken and Utica). It was identified in a previous study that biofilm treatment performance significantly decreased above 100,000 mg/L TDS [8]. Due to the high salinity concentration of the samples (at or above 170,000 mg/L total dissolved solids, TDS), the biological treatability of these samples using mixed-culture biofilms was evaluated after dilution to 50,000 and 100,000 mg/L TDS. Furthermore, an organic constituent analysis was performed to better understand the chemical signature of each sample and their effect on biological treatment performance.

MATERIALS AND METHODS

Sample Collection and Analysis

Safety coated glass containers (Qorpak, 2L, Fisher Scientific) with PTFE caps were pre-cleaned for organic analysis using methanol, acetone, and hexane solutions (rinsed three times in this order and dried) and then shipped for sampling. A total of seven produced water samples were collected from separators of the wells located in Bakken (1) and Utica Shale (6) regions and then either shipped overnight on ice or picked up following collection. Once received, the samples were stored at -20^{o}C.

Table 1 presents the characteristics of the collected samples. Samples were analyzed for total dissolved solids (TDS) and total dissolved organic carbon content. Biocide information for these samples was collected using the FracFocus Chemical Disclosure Registry (www.fracfocus.org) for the individual wells.

Biofilm Preparation

Biofilms were grown aerobically on freshly-cut grass silage using 25 g/L Luria Bertani (LB) broth in deionized water (Synergy-R purification system with 18.2 MΩ resistance) amended with 50,000 mg/L TDS (35 g/L NaCl, 15 g/L $CaCl_2$).[8] Grass silage was used as the biofilm scaffold that could also provide initial nutrients[12]. The salt concentration of 50,000 mg/L was selected for growth to both provide salt adaptation for microorganisms while still allowing for a relatively greater microbial community diversity than higher salt concentrations. The growth media was seeded with 10% (v/v) of a mixed stock of produced water and activated sludge from municipal wastewater.[8] After three weeks of biofilm growth, 5x9 inch aluminum net screens were used to encase 32.5 ± 7.0 wet grams of grass silage biofilms. The biofilms were then placed individually into 500 mL Erlenmeyer flasks. Before use, all biofilms were rinsed three times for 30 minutes in a 50,000 mg/L TDS (35 g/L NaCl, 15 g/L CaCl2, at 170 rpm) solution to remove any cultivation media carryover.

Experimental Procedure

Biodegradation experiments were conducted with seven produced water samples from the Utica (6) and Bakken (1) Shales. The microbial loading due to the biofilms is expected to be orders of magnitude above the microbial loading due to native microorganisms in the produced water;[13-15] thus, the native microbial community was not removed prior to treatment. The TDS concentrations of the samples were equal to or above 170,000 mg/L. Earlier results[8] showed no biodegradation for TDS concentrations at 200,000 mg/L and, therefore, the produced water samples were diluted to 50,000 and 100,000 mg/L TDS for the biological treatability experiments. Since the dilution of TDS would also dilute the DOC, the samples diluted to 50,000 mg/L TDS were selected from the ones with the highest DOC concentrations: Utica1, Utica2,

Utica3. All seven samples were diluted to 100,000 mg/L TDS. Additionally, a blank (no biofilm in produced water) was performed for produced water samples at their respective dilutions in the biodegradation experiments. Among those, the blank Utica5 experiment showed 83% and 86% reduction in DOC concentration at the end of the experiments at 50,000 and 100,000 mg/L, respectively (Figure S1), suggesting that the organic content in the Utica5 sample was likely comprised of a significant fraction of organic compounds that were removed without biofilm treatment. Subsequently, the biological treatment data of Utica5 was not included here.

Total Dissolved Organic Carbon (DOC) Analysis

Appropriate dilutions were performed for DOC analysis, and the samples were filtered through 0.45 μm filter paper (Millipore MF-EMD, Billerica MA) into pre-baked 40 mL amber glass vials (Thermo Scientific, VOA glass vials). The DOC of the samples was analyzed using a TOC analyzer (Shimadzu TOC-L) immediately after sampling. Triplicate injections were performed at 720^{o}C during measurements.

Organic Constituent Analysis

All glass equipment was pre-combusted at 500^{o}C for 4 hours. All metal equipment was washed with methanol-acetate-hexane and dried before contacting the sample. Samples were filtered through glass fiber filter GF/B coarse and GF/F fine filters (pre-muffled in a furnace at 450^{o}C for 4 hours) using all-glass filtration apparatus. Samples were then stored in screw-capped (the Teflon caps were washed with methanol-acetate-hexane) glass bottles in 4^{o}C if the organic liquid extraction was possible the following day. If not, they were stored at -20^{o}C for up to one week before extraction. Dichloromethane (DCM) was used to extract organic compounds for gas

chromatography analysis as the columns used are incompatible with water. 500 mL volume samples, including a Milli-Q water blank, were washed four times with 60 mL DCM, resulting in 240 mL of combined extract for each sample. Pre-combusted (at 450°C for 6 hours) $NaSO_4$ was used to remove any moisture in the liquid extracted samples. The DCM was then evaporated using a rotary evaporator at 35°C, 70 rpm until ~2-4 mL of the sample remained. Samples were transferred to labeled, 4 mL glass sample vials whose weight was previously measured. Samples were dried under N_2 gas and stored at 4°C for GC/MS measurement.

(Semi-)volatile organics in Utica1 and Utica2 DCM extracts were analyzed by a ThermoScientific Trace 1310 gas chromatograph coupled to a ThermoScientific ISQ LT Single Quadrupole mass spectrometer (GC/MS). The carrier gas was ultra-high purity helium at a constant flow rate of 1 mL/min. The following oven temperature program was applied at 1 µL injection volume: 50°C (held for 2 min), then increased at 10°C/min to 70°C and immediately at 4°C/min to 310°C (held for 15 min). A DB-5 column (30 m long, 320 micron inner diameter, 0.25 micron film thickness, Agilent) was used at a GC-MS transfer line temperature of 300°C, and an ion source temperature of 275°C. All remaining DCM extracts were analyzed for (semi-) volatile organics by an Agilent 6890 gas chromatograph equipped with an Agilent 5973N Mass Selective Detector using a TG-SQC TraceGold GC column (15 m length, 0.25 mm internal diameter, 0.25 µm film thickness, ThermoFisher) and the following oven temperature program: 80°C (held for 2 min), then increased to 15°C/min to 315°C (held for 5 min). The carrier gas was ultra-high purity helium at a constant flow rate of 1 mL/min. Sample injections were 2 µL at an injector temperature of 285°C. The GC-MS transfer line temperature was 320°C, and the ion source temperature was 230°C. The mass spectrometer was in electron ionization mode (70 eV), and mass spectra recorded in full-scan mode (*m/z* 45-600). For samples Utica2-6 and Bakken,

compound identification was achieved using mass spectra and retention time of analytical standards, including Gasoline Range Organics (Restek, Bellefonte, PA), Diesel Range Organics (Restek, Bellefonte, PA), 1-methylnapthalene, 1-iodohexadecane, 1-chlorohexadecane, 2-butoxyethanol, benzyl chloride, and decanoic acid (Sigma Aldrich, Saint Louis, MO). All other identified species were determined by comparison with spectra in the NIST library, 2011 edition (Gaithersburg, MD), with a match factor above 800. Detection was only reported if abundances were higher than in the extraction blank by at least a factor of 20.

Solid phase extraction (SPE) was used to concentrate surfactants and reduce the salt concentrations in the samples. Before extraction, high-purity hydrochloric acid was added to samples, including a Milli-Q water blank, to adjust to pH 3 to increase extraction efficiency. Supel Select HLB cartridges (200mg/6mL, Supelco, Bellefonte, PA) were conditioned with methanol (HPLC grade, Fisher) and rinsed with Milli-Q water and adjusted to pH 3 using hydrochloric acid. A volume of 100 mL of sample was applied to the cartridges (5-10 mL/min). Cartridges were dried under vacuum for 15 minutes and then rinsed with 100 mL of 5% methanol solution. Surfactants were eluted from the cartridge using 10 mL of methanol and stored at -20°C. Recoveries from the extraction were less than 100%.

Solid phase extracts were analyzed using an Agilent 1290 Infinity Series Liquid Chromatograph (LC) coupled with an Agilent 6530 Quadrupole Time-of-Flight mass spectrometer (Q-ToF), using the method described in Thurman et al. (2014)[16] with the following exceptions. Mobile phases were A (0.1% formic acid) and B (acetonitrile). A gradient elution method was developed with 0-2 minutes, 20% B; 2-15 min, 20-95% B; 15-22 min, 95% B; 22-25 min, 20% B. The flow rate was 0.6 mL/min, the injection volume was 20 μL, and the temperature of the drying gas was 325°C. Peaks were identified by accurate mass and possible

chemical formulae, which were then verified with polyethylene glycol 400 (Alfa Aesar, Haverhill, MA), polypropylene glycol (Alfa Aesar, Haverhill, MA), and 4-nonylphenol-polyethylene glycol standards (Sigma Aldrich, Saint Louis, MO). An exact concentration of each surfactant series could not be determined due to a lack of commercial standards with known ethoxymer distribution. Detection was only reported if abundances were higher than in the extraction blank by at least a factor of 20.

GC/MS (10.6084/m9.figshare.7215095) and LC/QToF (10.6084/m9.figshare.7209905) data have been deposited on figshare. Screenshots of chromatograms, including major peak identifications, are provided in the Supporting Information (Figure S2-S17).

Rate Kinetics and Data Analysis

DOC removal was reported as DOC_t/DOC_0, where DOC_t represents the DOC concentration measured at time t, DOC_0 is the initial DOC concentration of the sample after biofilm addition followed by a 30-minute homogenization (mixing) period. First-order degradation rates were calculated using Eqn.1 and Eqn.2:

$$-\frac{dC}{dt} = k_{obs} * C \quad \text{Eqn.1}$$

$$ln\left(\frac{C_t}{C_0}\right) = -k_{obs} * t \quad \text{Eqn.2}$$

where C_o is the initial DOC concentration and C_t is the DOC concentration at time t. The (negative) slope of the linear fit of Eqn.2 was reported as the observed rate constant (k_{obs}). The first order rate kinetics were calculated between time 0 and the time when DOC concentration no longer decreased in the solution for the following 48 hours (i.e., $DOC_{(t)} \leq DOC_{(t+24hour)}$ and $DOC_{(t+48hour)}$). Final percent DOC removals were calculated using Eqn.3:

$$\text{Final DOC removal (\%)} = \left[1 - \left(\frac{DOC_t}{DOC_0}\right)\right] * 100 \quad \text{Eqn.3}$$

A principal component analysis (PCA) was performed on the sample organic constituents using Minitab v17. These characteristics include the presence versus absence results from GC/MS (e.g., branched, linear, cyclic hydrocarbons) and LC/Q-ToF (e.g., PPG, PEG), as well as the DOC and biodegradation rates of each sample. Detailed information on these analyses is provided in Table S5 and Table S6 in Supplementary Information.

RESULTS

Seven produced water samples were evaluated from the Utica and Bakken Shales. The characteristics of these samples are presented in Table 1. The DOC concentration of the samples ranged from 177 mg/L to 3,990 mg/L. The biocide use information was also collected from each well's FracFocus report as biocides can affect biological degradation[17, 18]. Only the Bakken sample had 2,2-dibromo-3-nitrilopropionamide (DBNPA) as the choice of biocide as opposed to glutaraldehyde-based biocides in all the Utica samples.

The biodegradation experiments were conducted with produced waters diluted to 50,000 (Utica1, Utica2, and Utica3) and 100,000 mg/L TDS (Utica1, Utica2, Utica3, Utica4, Utica6, and Bakken). Figures 1 and 2 show the DOC removal as a function of time at 50,000 and 100,000 mg/L TDS, respectively. Utica3 showed an increase in DOC concentration following a 40% (at 48 hours) and 30% (at 96 hours) DOC removal at 50,000 and 100,000 mg/L TDS, respectively; while Utica1 and Utica2 biodegradation curves stabilized at 24 and 144 hours at 50,000 mg/L TDS and at 96 and 408 hours at 100,000 mg/L TDS, respectively.

The increase in DOC for Utica3 is potentially due to biomass die-off, although this was not directly measured. Three potential causes that could lead to the biomass loss are i) the lack of nutrients and trace minerals, ii) presence of toxic compounds and biotic generation of toxic

intermediates, iii) starvation due to consumption of biodegradable DOC. Since the DOC increase occurred later in the less diluted Utica3 (96 hours at 100,000 mg/L TDS) compared to the more diluted Utica3 (48 hours at 50,000 mg/L TDS), the presence of toxic compounds is an unlikely reason for the biomass die-off, suggesting a lack of nutrients or starvation as the likely cause of biomass die-off.

First order biodegradation constants are presented in Table 2. These results do not show a strong correlation of the first order biodegradation rates with the initial DOC concentration (R^2 = 0.51 at 50,000 mg/L TDS, R^2 = 0.06 at 100,000 mg/L TDS) or total percent DOC removed (R^2 = 0.59 at 50,000 mg/L, R^2 = 0.55 at 100,000 mg/L). The biodegradation rates at 100,000 mg/L TDS (k_{obs} = 0 to 0.0063 h^{-1}) are slower compared to those at 50,000 mg/L TDS (k_{obs} = 0.0111-0.0338 h^{-1}).

GC and LC/Q-ToF mass spectrometer measurements were performed to understand the effect of the organic compound composition on the biodegradability of produced water samples. The compounds identified in each sample are shown in Table 3 with references to previous studies reporting their presence in hydraulic fracturing produced water. Mid-chain alkanes were present in all samples, such as tetradecane, pentadecane, hexadecane, heptadecane (except Utica6), octadecane (except Utica5 and Utica6), docosane, and eicosane (except Utica6). LC/Q-ToF measurement results are presented in Table 4 and Table S3. Polyethylene glycols (PEGs) were detected in all samples. Also, PEG carboxylates and polypropylene glycols (PPGs) were found in all Utica samples. Alkyl ethoxylates (AEOs) were detected in Utica3 and Utica4, while nonylphenol ethoxylates (NPEOs) were detected in the Utica1 and Utica4 samples.

Finally, a principal component analysis (PCA) with four principal components was performed to determine the relationships between the chemical characteristics of the samples and

the biodegradation performance. These characteristics include statistics of the organic chemicals identified through GC/MS and LC/QToF measurements, such as the average number of carbon atoms and the counts of hydrocarbon structures (i.e., linear, branched, and cyclic), and are included in Table S6. All these factors were normalized to zero mean and unit variance beforehand, and the results of the PCA are presented in a biplot of the first two components shown in Figure 3. The eigenvectors are shown in Table S5. The first and second components explain 38% and 30% of the variability, respectively. Figure 3 shows a positive correlation between the first order reaction rate and the presence of NPEOs, PEGs, PPGs, and branched hydrocarbons. On the other hand, there is a negative correlation between the first order reaction rate and the presence of cyclic hydrocarbons, other heteroatoms (i.e., heteroatoms containing bromine (2-bromododecane), sulfur (*N*-[thiophene-2-carbonyl]piperazine, *N*-butyl-benzenesulfonamide), iodine (1-iodohexane, 1-iodohexadecane), chlorine (1-chlorohexadecane, benzyl chloride), and long-chain fatty acids. Taking advantage of the availability of duplicates for each sample, a linear regression was fitted to estimate the reaction rate from the chemical characteristics ($R^2 = 0.61$, Table S1). Moreover, an analysis of variance was performed to determine the effect of the chemical characteristics on the reaction rate (Table S2). The residuals were distributed normally, thus validating the assumptions of the analysis. Neither of these analyses yielded a statistically significant relationship ($p \geq 0.07$ for all). Furthermore, the PCA biplot shows two clear clusters of samples, labeled Group I and Group II in Figure 3. The samples in Group I (Utica2-50, Utica2-100, and Utica6-100) show high counts of cyclic hydrocarbons, nitrogen- and oxygen-containing heteroatoms. Those in Group II (Utica1-50, Utica1-100, Utica3-50, Utica3-100, and Utica4-100) display high counts of linear and branched hydrocarbons, AEOs, NPEOs, and higher average carbon counts. The Bakken sample did not

cluster into any of the defined groups; however, most of its differentiation is explained by the second component—which is heavily weighted for long-chain fatty acids and the other heteroatoms (containing bromine, iodine, chlorine, and sulfur atoms).

Qualitative organic analyses showed that Utica1 and Utica3 were predominantly composed of linear and branched hydrocarbons, whereas Utica2, Utica5, Utica6 and Bakken samples contained mostly linear and cyclic compounds. Moreover, Utica3 demonstrated the highest number of compounds in the range of C_{26}– C_{32} (23.5% of the total number of the compounds detected) compared to all other samples. The highest count of halogenated compounds was observed in the Utica5 and Bakken samples, which had 1-chlorohexadecane and 1-iodohexadecane in common. Utica5 also included *N*-butyl-benzenesulfonamide, whereas benzyl chloride was detected in the Bakken sample.

DISCUSSION

Variability in organic compound composition of examined produced waters

In this study GC/MS and LC/Q-ToF mass spectrometer analyses were performed to understand the effect of organic composition on biodegradability of produced waters using mixed-culture biofilms (Tables 3 and 4). Substantial variability in organic compound composition was observed between samples. Previously, differences in the organic compound composition of produced water samples were documented[10, 19-21]. For instance, higher low-molecular-weight organic acids were found in the Burket Shale compared to those in the Marcellus Shale[21]. Maguire and Boyle reported that $C_6 - C_{16}$ hydrocarbons dominated produced waters from Marcellus and Barnett but Eagle Ford produced waters were dominated by $C_{17} - C_{30}$ hydrocarbons[19]. Furthermore, Orem et al. (2007) found variability in the organic content of the

produced waters from the wells of the same basin (Powder River Basin, WY)[20]. These results also demonstrate variability in the composition of organic compounds from produced waters even from the same play, with substantial implications for the treatability of the produced water.

Non-ionic ethoxylated surfactants (e.g., ethoxylated glycols and alcohol ethoxylates) were previously listed in FracFocus reports as additives in fracturing fluids[22] (also in Table S3). These compounds are used to control viscosity, surface tension, and fluid recovery[23], and have been suggested as tracers of hydraulic fracturing fluids[16]. PEGs and linear alkyl ethoxylates (AEOs) were previously detected in fracturing fluids[16, 17, 23] using non-targeted chemical analysis based on accurate mass. In this study, PEGs and PPGs were detected via the same technique in the six fracturing fluid samples from the Utica Shale. On the other hand, only PEGs were observed in the Bakken Shale sample (Table 4). Additionally, NPEOs were detected only in the Utica1 sample. NPEOs were not previously detected in recovered hydraulic fracturing fluids, although they were reported in the FracFocus database (Table S3), have been successfully measured in spiked produced water samples,[24] and have been found in produced water-impacted river and lake sediments [25]. Detailed surfactant accurate-mass tables are presented in Table S4. PEGs, AEOs, and NPEOs, were previously shown to be readily biodegradable[17, 26]; however, PPGs demonstrated lower biodegradability compared to AEOs and NPEOs. Due to being readily biodegradable, these compounds could be significant contributors to the observed DOC removal.

Impact of organic composition on the biological treatability of produced water

The first-order biodegradation rates (Table 2) were lower in all samples at 100,000 mg/L TDS compared to those at 50,000 mg/L TDS. These results support the previous findings demonstrating that salinity is an obstacle for the biological treatment of produced water. Additionally, substantial variation in DOC biodegradation was observed even at the same TDS

concentration. For instance, this variation ranged from 1% DOC removal (Bakken) to 87% DOC removal (Utica2) at 100,000 mg/L TDS, suggesting that DOC composition is likely also a driver of biodegradation performance.

The reaction rate (first order) had a higher contribution in the second PCA component compared to the first, separating the Group I and II Utica samples from the Bakken sample where 1% DOC removal was observed. The second component was dominated by long-chain fatty acids and heteroatoms containing bromine, sulfur, iodine, and chlorine found primarily in the Bakken sample. These compounds included benzyl chloride (C_7H_7Cl), 1-chlorohexadecane ($C_{16}H_{33}Cl$), 1-iodohexadecane ($C_{16}H_{33}I$), and long-chain fatty acids such as stearic acid ($C_{18}H_{36}O_2$), nonadecanoic acid ($C_{19}H_{38}O_2$), and docosanoic acid ($C_{22}H_{44}O_2$). Commercial standards confirmed all species reported using LC/Q-ToF except the three long-chain fatty acids reported in the Bakken sample (i.e., stearic, nonadecanoic, and docosanoic acids). Stearic acid is an occasionally detected methanol impurity (methanol was used to elute SPE cartridges for LC/Q-ToF analysis); however, it was not detected in any of the blanks or Utica samples, which had been extracted with methanol as well. 1-Chlorohexadecane was previously demonstrated to be metabolized by *Mycobacterium vaccae* JOB5 and *Mycobacterium convolutum* R22, if used as the sole carbon source[27]. Benzyl chloride reached 71% removal following a 28-day ready-biodegradation test[28], therefore making it unlikely to have a role in the decreased biodegradation in the Bakken sample. Further literature searches did not return any reports on the biodegradability of 1-iodohexadecane. Given limited biodegradability information, it is difficult to relate the low biodegradation performance in the Bakken sample to specific compounds. The GC/MS and LC/Q-ToF data input to the PCA model is based on the relative abundance and presence versus absence of the compounds in untreated and undiluted produced water samples

and not of the initial and final treated values. The qualitative nature of the analysis is unlikely to provide a greater connection than it has. However, future studies evaluating the quantitative responses of the treatment approach used here could further the understanding of the relationships between organic constituents and the biodegradation rates.

Halogenated compound abundance was negatively correlated with the biodegradation rate in this study. Possible sources of halogenated compounds in hydraulic fracturing wastewater include chemical additives, the shale, and biotic and abiotic reactions[29]. Interactions of oxides, peroxides, persulfates and other oxidants with halide ions such as bromide may result in disinfection by-product (DBP) formation. Increased concentrations of brominated and iodinated THM compounds were reported in publicly owned wastewater treatment plants following disinfection [30]. Moreover, it has been shown that persulfates could react with chloride and bromide ions and dissolved organic matter to form chlorinated and brominated DBPs[29, 31, 32]. Ammonium persulfate was used as a breaker in all Utica samples (according to FracFocus reports, Table S3) and could result in the formation of the brominated (2-bromo dodecane) and chlorinated (1-chlorohexadecane) heteroatoms in Utica1 and Utica5. The Bakken sample had the highest relative number of oxides and peroxides disclosed in its FracFocus report compared to the Utica samples, and it also contained the highest number of halogenated compounds, supporting the abiotic generation of these compounds.

Impact of biocides in produced water

Another reason for the variation in DOC removal in produced waters could be residual biocide. Biocides can impact biodegradation depending on the type and the concentration present in the solution[17, 18]. In this study, the biocide use information collected from each well's

FracFocus report showed that Bakken was the only well in which 2,2-dibromo-3-nitrilopropionamide (DBNPA) was injected as the biocide, whereas glutaraldehyde was used in all Utica wells. Both glutaraldehyde and DBNPA are electrophilic biocides acting via their reactive electron accepting functional groups[33] and are the most commonly used biocides in hydraulic fracturing [34]. While glutaraldehyde acts as a cross-linker for amino and nucleic acids, resulting in cell damage, DBNPA's mode of action includes reacting with sulfur-containing nucleophiles—leading to the destruction of cell components and biological functions[34, 35]. Electrophilic biocides are often degraded through hydrolysis and the resulting degradation products may be more toxic than the parent compound. The hydrolysis of DBNPA generates dibromoacetic acid and dibromoacetonitrile[34, 36]. Dibromoacetic acid is significantly more recalcitrant to biological degration than DBNPA[36, 37]. Another reason for higher biodegradation rates in Utica samples could be the potential downhole transformation of glutaraldehyde and subsequent loss of its biocidal effect. It has been shown that glutaraldehyde can go through a transformation in shale formations such as autopolymerization and precipitation depending on the temperature of the shale, pH, salt, and glutaraldehyde concentration[38].

Factors not quantified in this study, such as inorganic ions, metals, and residual biocides, may also have an impact on the observed biodegradation rates. Inorganic ions such as boron, ammonium, and sulfate in Bakken Shale could be as much as one order of magnitude higher than those of Marcellus Shale, whereas barium is one order of magnitude more abundant in Marcellus Shale[39]. Moreover, a previous study[40] showed a synergistic biocidal effect of quaternary ammonium compounds (QACs) and copper on biofilms. In this study, QACs were used in Utica1 and Utica6 in conjunction with the primary biocidal compound and Utica1 and Utica6 showed above 45% DOC removal in the experiments performed at both 50,000 and 100,000 mg/L TDS

concentration. Quantifying additional factors that may influence biological treatment performance would be of high future interest. Finally, future evaluation of biodegradation rates in produced water mixtures could be beneficial as those waters may be collected, transported and treated in central treatment facilities.

CONCLUSION

The biological treatability of seven produced water samples from the Bakken and Utica Shales was investigated using mixed-culture biofilms. A biofilm approach instead of suspended culture treatment was used in this study to provide greater tolerance to high salt concentrations and complex organic compounds present in produced waters. Variability in the biodegradation rates of the produced water samples at the same salinity concentration was observed, suggesting that the organic composition of these produced waters affected biodegradation rate. Organic analyses of the produced water samples further demonstrated a difference in organic composition even within the same shale formation, although mid-chain alkanes (e.g., $C_{11} - C_{25}$) were observed in all samples. These analyses helped identify factors that affect biodegradation by using a principal component analysis and other statistical methods. The qualitative nature of our observations here provides an initial framework for future quantitative approaches that will build on the presented results. Our results show that the first order biodegradation rate positively correlated with the higher relative presence of PEG, PPG, NPEO, whereas, it negatively correlated with the presence of long-chain fatty acids and other heteroatoms containing bromine, sulfur, iodine, or chlorine. This information can be valuable for operators to adequately select treatment processes in cases where there are variations in the characteristics of the source water. Future studies can build on the conclusions presented here and help further unravel the complexity of produced water biodegradation using both qualitative and quantitative organic characterization approaches.

Conflicts of Interest

There are no conflicts of interest to declare.

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Table 1. Chemical characteristics of produced water samples.

Sample Name	Formation	Year of Fracking	True Vertical Depth (ft)	Days after Fracturing	Biocide used	TDS (ppm)	DOC (ppm)
Bakken	Bakken	2013	11,075	1,122	2,2-Dibromo-3-nitrilopropionamide, (20%), polyethylene glycol (55%), sodium bromide (4%), dibromoacetonitrile (3%).	285,000	353
Utica1	Utica	2012	7,773	1,549	Glutaraldehyde (30%), didecyl dimethyl ammonium chloride (10%), quaternary ammonium compound (7%), ethanol (5%).	267,000	275
Utica2	Utica	2013	7,646	1,271	Glutaraldehyde	238,000	1,038
Utica3	Utica	2014	7,214	1,027	Glutaraldehyde, methanol	256,000	483
Utica4	Utica	2014	8,038	748	Glutaraldehyde, methanol	220,000	177
Utica5	Utica	2013	7,646	1,092	Glutaraldehyde	251,000	3,990
Utica6	Utica	2012	8,103	1,346	Glutaraldehyde (30-60%), alkyl (C12-16) dimethylbenzylammonium chloride (5-10%), ethanol (1%).	170,000	206

Table 2. First order biodegradation kinetics for produced water samples using biofilms. Errors (±) represent standard error.

Sample	TDS (mg/L)	Initial DOC (mg/L)	k_{obs} (hr^{-1})	R^2	Final DOC Removal (%)
Utica1	50,000	41 ± 4	0.0338	1.00	56 ± 2
Utica2	50,000	216 ± 11	0.0117	0.98	79 ± 0
Utica3	50,000	93 ± 5	0.0111	0.95	41 ± 2
Utica1	100,000	103 ± 7	0.0063	0.96	45 ± 1
Utica2	100,000	456 ± 25	0.0050	0.98	87 ± 1
Utica3	100,000	207 ± 26	0.0037	0.82	34 ± 12
Utica4	100,000	62 ± 0	0.0036	0.74	41 ± 6
Utica6	100,000	77 ± 0	0.0035	0.77	50 ± 2
Bakken	100,000	128 ± 2	0.0000	0.00	1 ± 0

Table 3. GC-compatible fraction in Utica (U) and Bakken (Ba) produced water samples. Previous studies identifying the presence of individual compounds in hydraulic fracturing produced water are noted by compounds.

Compound	CASRNs	Molecular Formula	Molecular Weight	U1	U2	U3	U4	U5	U6	Ba	Ref.
1-(2-Butoxyethoxy) ethanol	54446-78-5	$C_8H_{18}O_3$	162.2					X			
1-(Methoxymethyl)-4-methylnaphthalene	71235-76-2	$C_{13}H_{14}O$	186.3							X	
1,1'-[1,2-Propanediylbis(oxy)]di(2-propanol)	1638-16-0	$C_9H_{20}O_4$	192.3				X				
1,1'-Bi(3-cyclopenten-1-yl)	n.a.	$C_{10}H_{14}$	134.2						X		
1,2,3,4-Tetrahydro-1-phenanthrenol	7508-20-5	$C_{14}H_{14}O$	198.3				X				
1,2,4-Trimethylbenzene	95-63-6	C_9H_{12}	120.2	X				X	X	X	
1,4:3,6-Dianhydro-D-glucitol	652-67-5	$C_6H_{10}O_4$	146.1				X				
1,7-Dimethylnaphthalene	575-37-1	$C_{12}H_{12}$	156.2							X	
10-Methylnonadecane	56862-62-5	$C_{20}H_{42}$	282.6	X							[19]
1-Butoxy-2-propanol	5131-66-8	$C_7H_{16}O_2$	132.2	X							
1-Chlorohexadecane	4860-03-1	$C_{16}H_{33}Cl$	260.9					X		X	
1-Iodohexadecane	544-74-4	$C_{16}H_{33}I$	352.3					X		X	
1-Iodohexane	638-45-9	$C_6H_{13}I$	212.1				X				
1-Methyl-4-*n*-pentylcyclohexane	n.a.	n.a.	n.a.			X					
1-Methylnaphthalene	90-12-0	$C_{11}H_{10}$	142.2							X	
1-Piperazinyl(2-thienyl)methanone	52063-83-9	$C_9H_{12}N_2OS$	196.3		X						
2-(2-Butoxyethoxy)ethanol	112-34-5	$C_8H_{18}O_3$	162.2				X				
2-(2-Phenoxyethoxy)ethanol	104-68-7	$C_{10}H_{14}O_3$	182.2						X		
2-(Octadecyloxy)ethanol	9005-00-9	$C_{20}H_{42}O_2$	314.6					X	X		
2,11-Dodecane-dione	7029-09-6	$C_{12}H_{22}O_2$	198.3	X							
2,2'-(1,12-Dodecanediyl)dioxirane	n.a.	$C_{16}H_{30}O_2$	254.4		X						
2,4-Bis(2-methyl-2-propanyl)phenol	96-76-4	$C_{14}H_{22}O$	206.3		X						
2,4-Dimethylphenol	105-67-9	$C_8H_{10}O$	122.2						X		
2,6,10,14-Tetramethylheptadecane	18344-37-1	$C_{21}H_{44}$	296.6		X			X	X		
2,6,10,14-Tetramethylhexadecane	18435-22-8	$C_{15}H_{32}$	212.4	X							
2,6,10,15-Tetramethylheptadecane	54833-48-6	$C_{21}H_{44}$	296.6			X					
2,6,10-Trimethyldodecane	90622-46-1	$C_{14}H_{30}$	198.4	X							
2,6,10-Trimethylhexadecane	55000-52-7	$C_{19}H_{40}$	268.5			X				X	
2,6,10-Trimethyltetradecane	14905-56-7	$C_{17}H_{36}$	240.5			X					
2,6,19,14-Tetramethylhexadecane	n.a.	n.a.	n.a.	X	X		X			X	
2,6-Dimethylheptadecane	n.a.	n.a.	n.a.	X							
2,6-Dimethylheptadecane	54105-67-8	$C_{19}H_{40}$	268.5			X					
2,6-Dimethylnaphthalene	581-42-0	$C_{12}H_{12}$	156.2							X	
2,6-Dimethylundecane	17301-23-4	$C_{13}H_{28}$	184.4			X				X	

Compound	CASRNs	Molecular Formula	Molecular Weight	U1	U2	U3	U4	U5	U6	Ba	Ref.
2-Bromododecane	13187-99-0	$C_{12}H_{25}Br$	249.2	X							
2-Butoxyethanol	111-76-2	$C_6H_{14}O_2$	118.2	X	X			X	X		41
2-Ethyl-3-methylnaphthalene	31032-94-7	$C_{13}H_{14}$	170.3							X	
2-Ethyl-4-(3-pyridyl)-1(2*H*)-phthalazinone	137381-66-9	$C_{15}H_{13}N_3O$	251.3		X						
2-Hexyl-1-decanol	2425-77-6	$C_{16}H_{34}O$	242.4	X							
2-Hydroxy-3-hexanone	54073-43-7	$C_6H_{12}O_2$	116.2						X		
2-Methyl-1-hexadecanol	2490-48-4	$C_{17}H_{36}O$	256.5	X	X						
2-Methyldodecane	68551-19-9	$C_{13}H_{28}$	184.4			X					
2-Methylphenol	95-48-7	C_7H_8O	108.1	X	X						41
2-Methylundecane	31807-55-3	$C_{12}H_{26}$	170.3			X					
2-Phenoxyethanol	122-99-6	$C_8H_{10}O_2$	138.2		X			X	X		
2-Tetradecanyl methoxyacetate	n.a.	$C_{17}H_{34}O_3$	286.5	X							
2-Tridecanyl methoxyacetate	n.a.	$C_{16}H_{32}O_3$	272.4	X							
3-(2-Methyl-propenyl)-1*H*-indene	819871-70-0	$C_{13}H_{14}$	170.3							X	
3,6,9,12,15-Pentaoxanonadecan-1-ol	1786-94-3	$C_{14}H_{30}O_6$	294.4						X		
3-Benzyl-2,3,6,7-tetrahydropyrrolo [1,2-a]pyrazine-1,4-dione	14705-60-3	$C_{14}H_{16}N$	244.3						X		
3-Ethyl-1,2-cyclopentanedione	21835-01-8	$C_7H_{10}O_2$	126.2						X		
3-Methylhexadecane	6418-43-5	$C_{17}H_{36}$	240.5	X							
3-Methylphenol	108-39-4	C_7H_8O	108.1	X	X			X	X		41
3-Methyltetradecane	3891-98-3	$C_{15}H_{32}$	212.4			X					
4-Methylphenol	106-44-5	C_7H_8O	108.1		X			X	X		41
4-Methyltetradecane	25117-24-2	$C_{15}H_{32}$	212.4	X							
6,9-Dimethyltetradecane	55045-13-1	$C_{16}H_{34}$	226.4			X					
6-Methyloctadecane	10544-96-4	$C_{19}H_{40}$	268.5	X							
7-Methylhexadecane	26730-20-1	$C_{17}H_{36}$	240.5	X							
7-Methylpentadecane	6165-40-8	$C_{16}H_{34}$	226.4	X							
9-Methylnonadecane	13287-24-6	$C_{20}H_{42}$	282.6			X					
Benzyl chloride	100-44-7	C_7H_7Cl	126.6							X	
Cyclo(L-leucyl-L-prolyl)	2873-36-1	$C_{11}H_{18}N_2O_2$	210.3		X			X	X		
Decanoic Acid	334-48-5	$C_{10}H_{20}O_2$	172.3			X					
Docosane	629-97-0	$C_{22}H_{46}$	310.6	X	X	X	X	X	X	X	19, 20
Dodecane	112-40-3	$C_{12}H_{26}$	170.3	X		X		X	X	X	19
Dotriacontane	544-85-4	$C_{32}H_{66}$	450.9			X					19
Eicosane	112-95-8	$C_{20}H_{42}$	282.6	X	X	X					19
Heneicosane	629-94-7	$C_{21}H_{44}$	296.6	X	X	X	X			X	19
Hentriacontane	8006-44-8	$C_{31}H_{64}$	436.8			X					
Heptacosane	593-49-7	$C_{27}H_{56}$	380.7			X					19, 20

Compound	CASRNs	Molecular Formula	Molecular Weight	U1	U2	U3	U4	U5	U6	Ba	Ref.
Heptadecane	629-78-7	$C_{17}H_{36}$	240.5	X	X	X	X	X		X	[19]
Heptylcyclohexane	5617-41-4	$C_{13}H_{26}$	182.4	X							
Hexacosane	630-01-3	$C_{26}H_{54}$	366.7			X				X	[19, 20]
Hexadecane	544-76-3	$C_{16}H_{34}$	226.4	X	X	X	X	X	X	X	[19]
Hexylcyclohexane	4292-75-5	$C_{12}H_{24}$	168.3			X					
Icosylcyclohexane	4443-55-4	$C_{26}H_{52}$	364.7			X					
Methylnapthalene	90-12-0	$C_{11}H_{10}$	142.2			X					
m-Xylene	108-38-3	C_8H_{10}	106.2						X		
N,*N*-Dimethyl-1-dodecanamine	112-18-5	$C_{14}H_{31}N$	213.4						X		
N,*N*-Dimethyloctylamine	7378-99-6	$C_{10}H_{23}N$	157.3						X		
N-Butylbenzenesulfonamide	3622-84-2	$C_{10}H_{15}NO_2S$	213.3					X			
Nonacosane	630-03-5	$C_{29}H_{60}$	408.8			X					[19]
Nonadecane	629-92-5	$C_{19}H_{40}$	268.5	X	X	X	X			X	[19]
Octacosane	630-02-4	$C_{28}H_{58}$	394.8			X					[19]
Octadecane	593-45-3	$C_{18}H_{38}$	254.5	X	X	X	X	X	X	X	[19]
o-Xylene	106-42-3	C_8H_{10}	106.2	X					X		
Pentacosane	629-99-2	$C_{25}H_{52}$	352.7			X	X				[19, 20]
Pentadecane	629-62-9	$C_{15}H_{32}$	212.4	X	X	X	X	X	X	X	[19]
Phenol	108-95-2	C_6H_6O	94.1		X			X	X	X	[41]
Phenylmethanol	100-51-6	C_7H_8O	108.1						X		[41]
Tetracosane	646-31-1	$C_{24}H_{50}$	338.7	X	X	X	X	X		X	[19, 20]
Tetradecane	90622-46-1	$C_{14}H_{30}$	198.4	X		X		X	X	X	[19, 20]
Triacontane	638-68-6	$C_{30}H_{62}$	422.8			X					[19]
Tricosane	638-67-5	$C_{23}H_{48}$	324.6	X	X	X					[19, 20]
Tridecane	129813-67-8	$C_{13}H_{28}$	184.4	X		X				X	[19]
Undecane	1120-21-4	$C_{11}H_{24}$	156.3							X	[19]

Table 4. Surfactant groups identified in Utica (U) and Bakken (Ba) produced water samples.

Products	U1	U2	U3	U4	U5	U6	Ba*
*Polyethylene Glycol Carboxylate Derivatives (*PEG-	X	X	X	X	X	X	

COOH)							
Polyethylene Glycols (PEGs)	X	X	X	X	X	X	X
Polypropylene Glycols (PPGs)	X	X	X	X	X	X	
C_{10} ethoxylates (C10-EOs)			X	X			
C_{12} ethoxylates (C12-EOs)			X	X			
C_{13} ethoxylates (C13-EOs)			X	X			
C_{14} ethoxylates (C14-EOs)				X			
Nonylphenol ethoxylates (NPEOs)	X			X			
Long-Chain Fatty Acids							X**

*Unidentified surfactants present – possibly ethoxylated alcohols

**Stearic acid, nonadecanoic acid, docosanoic acid

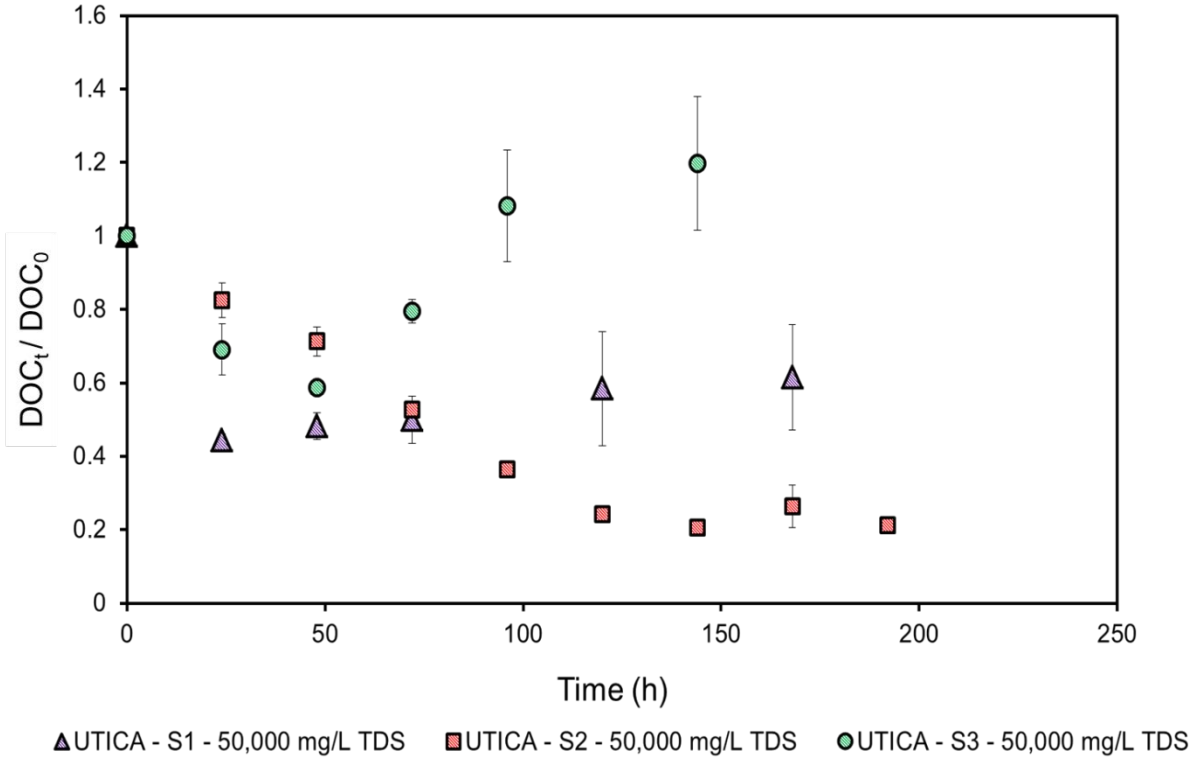

Figure 1. Removal of DOC as a function of time at 50,000 mg/L TDS produced waters using biofilms. Error bars represent ±1 standard error.

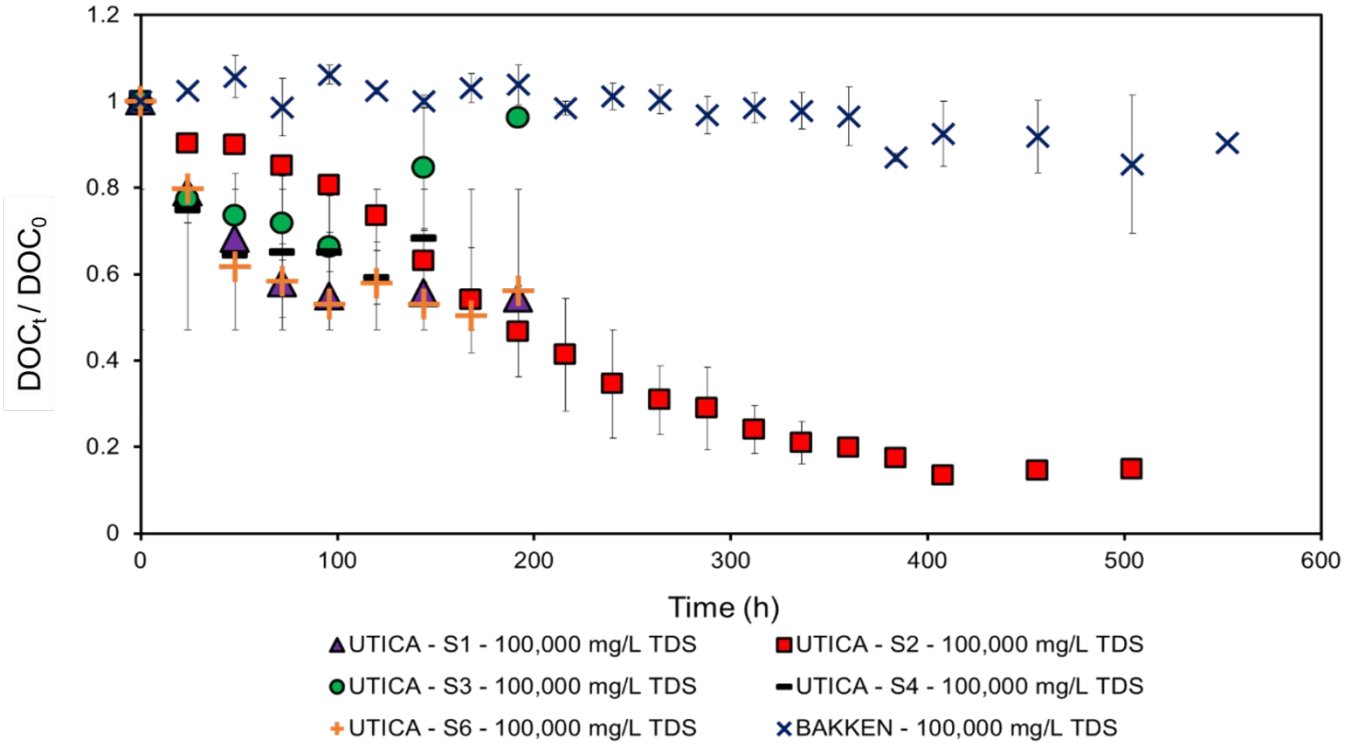

Figure 2. Removal of DOC as a function of time at 100,000 mg/L TDS produced waters using biofilms. Error bars represent ±1 standard error.

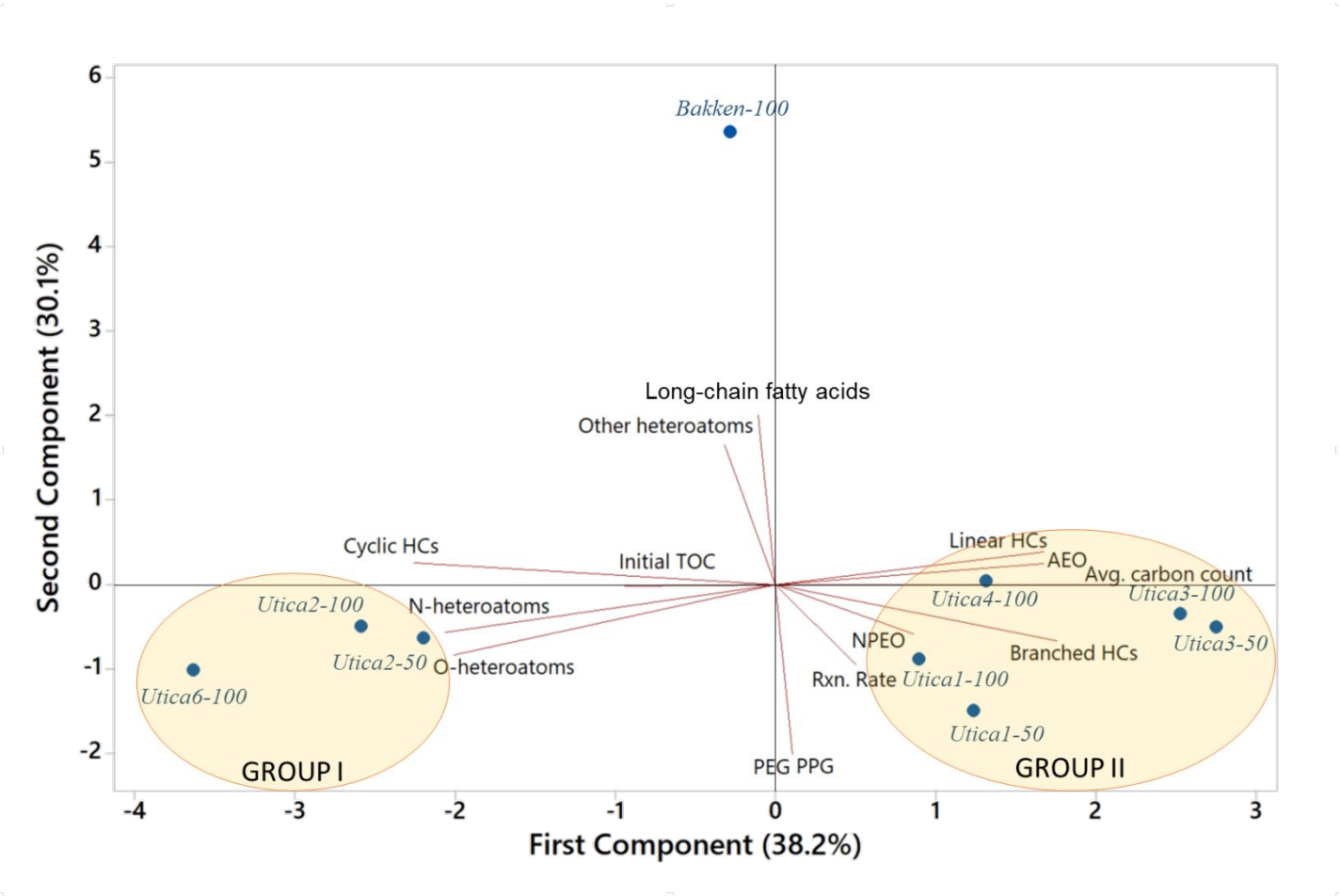

Figure 3. Principle component analysis of the Bakken and Utica Shale produced water samples. "Avg.": Average; "AEO": Alkyl ethoxylates; "HCs": Hydrocarbons; "Long-Chain Fatty Acids": stearic acid, nonadecanoic acid, docosanoic acid; "N-, O-Heteroatoms": heteroatoms containing nitrogen and oxygen; "Other heteroatoms": organics containing bromine, sulfur, iodine, or chlorine; "Rxn Rate": first order biodegradation rate.

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