BIOLOGICAL TREATMENT OF HYDRAULIC FRACTURING PRODUCED WATER

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Hydraulic fracturing enables the enhanced recovery of hydrocarbons from shale formations while generating large volumes of produced water, i.e. wastewater from hydraulic fracturing. Treatment of produced water for reuse or final disposal is challenged by both high salinity and the presence of organic compounds. This dissertation is focused on the biological treatment of produced water using a mixed-culture biofilm approach to remove the available electron donors and therefore, potentially limit microbial growth, biocide use, and fouling of wells (during reuse) and membranes (during treatment prior to final disposal). Conventional activated sludge treatments are intolerant of high salinity, thus a biofilm approach was proposed to provide a more robust treatment method for high salinity produced waters. First, a preliminary evaluation on COD biodegradation (as acetate and guar gum) in synthetic and real produced waters was performed. Biodegradation was mainly driven by salinity; however, microbial activity was observed at salt concentrations as high as 100,000 mg/L TDS. Next, the effect of glutaraldehyde (GA), a commonly used biocide in hydraulic fracturing, on biodegradation of organic chemicals that are commonly used in fracturing fluids, is investigated. Results demonstrated that glutaraldehyde can affect the observed lag period and half-lives of the compounds, depending on the compound. Finally, the biodegradation of produced waters were evaluated in seven samples from different wells. Results showed a negative correlation between salinity and biodegradation rates. Moreover, variable biodegradation rates were observed at the same salt concentration.

Finally, a Ra-226 biosorption was evaluated in synthetic and real produced waters to determine the efficacy of Ra-226 removal by a halophilic microalga *D. salina*.

This study contributes to the understanding of biological treatment applicability in produced water management. The proposed biofilm approach could further encourage the use of similar approaches in produced water treatment and possibly in other industrial wastewaters containing high salinity and toxic chemicals. The evaluation of the biocide effect on biodegradation can enhance the understanding and accuracy of environmental model predictions for bio-treatment, bio-remediation, and pollution transport. Ultimately, this dissertation will contribute to more sustainable and economical produced water management strategies.

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PREFACE

My special thanks to my mother Ayse, my father Bekir Sitki, and my grandmother Nazife. They raised me bravely.

1.0 INTRODUCTION

This dissertation investigates the challenges and opportunities of produced water management with a special focus on biological treatment of produced water using a mixed-culture biofilm approach. Additionally, radium-226 (Ra-226) biosorption in produced waters using halotolerant microalgae *Dunaliella salina* was investigated.

Advances in high-volume hydraulic fracturing and horizontal drilling techniques require the injection of 10-20 million liters (2-5 million gallons) of fluids at high pressure to fracture the target formation and thus stimulate reservoir permeability.[2-4] Fracture fluid, that is typically composed 98-99% water and sand, 1-2% fracturing chemicals,[2, 5, 6] mixes with the subsurface brine and returns to the surface as produced water.[5, 6] Produced water typically has an elevated total dissolved solids (TDS) concentration that ranges from 5,000 to 300,000 mg/L,[7-9] including high concentrations of sodium, calcium, barium, strontium, chloride, bromide, and naturally occurring radioactive material (NORM). Among the NORM, radium is the most common due to its soluble nature in a large spectrum of pH conditions.[9-11] Moreover, the organic content of produced water can vary greatly by wellsite depending on the additives in the fracturing fluid. Previous results show that organic concentration in produced waters from shale plays can range between 1.2 – 5,804 mg/L TOC.[12, 13] Thus, both fracture fluid and produced water characteristics are unique to each geological formation.[10] In the following sections, the problems associated with produced water management and the research objectives of this dissertation will be stated.

1.1 PROBLEM IDENTIFICATION

Water management challenges associated with hydraulic fracturing, including produced water disposal and water sourcing for fracturing, have emerged at the forefront of the public and regulatory discussion regarding hydraulic fracturing. Due to large volumes, high salt and radionuclide concentrations, and organic content, disposal and treatment options for produced water are limited. Currently, disposal via deep well injection is among the most frequently used produced water management strategies and will likely continue to be in the future, since concentrated brine produced after desalination processes can be disposed in regulated underground wells.[14] EPA regulates the injection of fluids associated with oil and gas production (e.g., class II wells) under the Underground Injection Control program and necessary permits need to be obtained from the authorities.[15] However, this method has been linked to induced seismicity[16] and, hence, high volumes of produced water injection is not likely to be sustainable in the long run. For instance, wastewater disposal volumes in central Oklahoma nearly doubled from 2004 to 2008 and seismicity in that area was reported to increase 40-fold during 2008-2013 compared to the period from 1976 to 2007. This amount is comprising 45% of the seismicity with magnitude (M)3 or larger in the central and eastern U.S between 2008 and 2013.[16] Moreover, treatment of produced water in municipal wastewater treatment plants for surface disposal is no longer a viable alternative with new regulations in effect.[2] Finally, in some regions with suitable disposal capacity, there are concerns about the environmental impacts of fresh water sourcing.[17, 18] Therefore, an on-site, low cost, low maintenance produced water treatment approach can prove to be both environmentally and economically beneficial for either reuse or final disposal.

Biological treatment is a promising and underexplored treatment technology to remove organic compounds in high-salinity produced water. Biological treatment approaches may be used in conjunction with physical-chemical treatment to limit energy costs and membrane fouling for both produced water reuse in future hydraulic fracturing operations and final disposal. Additionally, reduced concentrations of organic compounds due to biological treatment would limit heterotrophic microbial growth during produced water holding, and thus decrease the need for biocide use. Furthermore, there is evidence that biocides in produced water can alter the contamination transport durations and distances.[19] Additionally, biosorption could prove useful for the removal of inorganic produced water constituents that cannot be degraded biologically and further help to decrease NORM concentration, potential odor production, toxicity, and corrosion by sulfates.

1.2 RESEARCH OBJECTIVES

This dissertation investigates the biological treatment of produced water using a mixed-culture biofilm approach, evaluating co-contaminant interactions of organics in the presence of a biocide. Additionally, radium-226 (Ra-226) biosorption using the halotolerant microalgae *Dunaliella salina* was evaluated.

The first research objective was to develop and use a mixed-culture engineered biofilm approach to treat synthetic and real produced waters from hydraulic fracturing with an external COD source. Biological treatment to remove available electron donors has the potential to decrease heterotrophic microbial growth and the necessity for biocide use. The development of on-site biological removal processes will encourage produced water reuse in future hydraulic

fracturing operations, reducing the environmental impacts of fresh water sourcing, the costs related to produced water transportation and disposal, and excess biocide use.

The second research objective was to evaluate the effect of glutaraldehyde (a commonly used biocide) on biodegradation of organics used in hydraulic fracturing. Evidence has shown that the presence of toxic compounds such as biocides can alter the biodegradation potential of organics in produced water[19], creating concerns in case of an environmental exposure.[20]

The third research objective was to use the mixed-culture biofilms to treat produced waters with no external organic addition. This approach can potentially create a more applicable knowledge for this treatment method to be implemented in real-world scenarios.

The final research objective was to evaluate the removal Ra-226 from produced waters via biosorption. Limited earlier studies[21, 22] showed that Ra-226 biosorption could be promising to be used in produced water to help removing Ra-226 using a low-cost treatment approach.

This dissertation is composed of seven chapters in which five journal manuscripts are presented. Chapter 1 consists of an introduction, problem identification, and the research objectives. The main goal of Chapter 2 is to review the current challenges and opportunities associated with produced water management and the current biological treatment approaches applied to produced waters. An additional goal is to propose a decision-making approach for real world applications of produced water treatment. In Chapter 3, the research objective is to evaluate the chemical oxygen demand (COD) removal in synthetic and real produced water using engineered biofilms at various salinity concentrations (0 – 200,000 mg/L TDS). The research objective in Chapter 4 is to evaluate the effect of glutaraldehyde (a frequently used biocide in hydraulic fracturing) concentration (0 – 300 mg/L) on the readily biodegradable organic compounds commonly reported in produced waters at 50,000 mg/L TDS. The research

objective in Chapter 5 is to investigate the biodegradation of organics in produced water samples from unconventional shale plays using the biofilm approach developed in Chapter 3 and 4. In Chapter 6, I used *D. salina* to evaluate Ra-226 biosorption at changing salt, pH, and biomass concentrations and finally biosorption in Marcellus Shale Produced Water. Finally, in Chapter 7 an overall summary and conclusions of the findings reported in this dissertation and suggestions future research goals and possible environmental implications for real-world scenarios were provided.

2.0 A REVIEW OF BIOLOGICAL TREATMENT FOR PRODUCED WATER FROM HYDRAULIC FRACTURING: CHALLENGES AND OPPORTUNITIES

2.1 INTRODUCTION

Energy production in the United States is highly dependent on natural gas, coal, and oil (85% of the nation's supply). High-volume hydraulic fracturing combined with horizontal drilling, known colloquially as 'fracking' has become an important part of the United State's (and World's) energy mix. Horizontal drilling was first used in Barnett Shale and created great interest for enhancing oil and gas production.[23] Further improvements in horizontal drilling and hydraulic fracturing led to the expansion of unconventional natural gas and oil generation. Shale oil production in U.S. started to increase exponentially in 2004, and has increased linearly since 2012.[24] Eagle Ford and Bakken Shales are among the most productive shale oil plays. Shale oil production has a great impact in the overall oil production in the U.S. In 2014, shale oil (3.6. million barrels/day – mbd) comprised half of the total crude oil production (8.2 mbd), and met one quarter of the total oil demand (15.5 mbd – a total of produced and imported crude oil) in the U.S.[24] Total crude oil production in the U.S. continues to increase and forecasted to reach 9.7 mbd in 2018.[25] The high energy content of natural gas (about 30 kj/m^3), decreased CO_2 emissions, and criteria pollutants make it a desirable energy source.[8, 26] The gas reserves of the major shale formations in the U.S. (e.g., Barnett, Haynesville/Bossier, Antrim, Fayetteville, New Albany, and Marcellus) have been estimated to be able to supply the country for at least 90

years.[8] The Marcellus Shale, one of the top five unconventional gas reservoir in the U.S., is a black, organic rich shale in the Northern Appalachian basin with an estimated production capacity of 489 trillion cubic feet natural gas.[8, 26, 27]

Hydraulic fracturing is the process of injecting high volumes of fracturing fluid into shale formations under high pressure (480 – 680 bar) to increase permeability to recover gas and oil trapped in the formation.[10] The composition of the fracturing fluid varies depending on the shale formation and the preferences of the industry. Fracturing fluid generally includes 98-99% water and sand, and 1-2% chemical additives such as gelling agents, friction reducers, scale inhibitors, biocides, and gel breakers.[6, 28] The amount of water used to open a new well can range between 2 to 8 million gallons depending on the well length and is generally supplied locally, either using available surface water or groundwater.[23] Water usage raises concerns and requires effective local management especially in locations with a limited water supply.[8, 23] After fracturing a well, a fraction of the water injected (varies depending on the wellsite) returns over a period of 30 days and this water is called flowback water.[5] Once the well is in production, the returning water is then referred to as produced water.[6, 29]

Produced water management strategies vary according to regional regulations. Some common disposal methods include deep well injection, reuse, open pits for evaporation, and processing in wastewater treatment plants. In the United States, the U.S. EPA regulates the injection of fluids associated with oil and gas production (i.e., class II wells) under the Underground Injection Control program and necessary permits need to be obtained from the authorities.[15] However, disposal wells have been linked to induced seismicity[16] challenging the sustainability of this approach. For instance, wastewater disposal volumes in central Oklahoma had nearly doubled from 2004 to 2008 and seismicity in that area was reported to increase 40-

fold during 2008-2013 compared to the period from 1976 to 2007. Disposal well injection has been linked to 45% of the seismicity with magnitude (M)3 or larger in the central and eastern U.S between 2008 and 2013.[16]

Alternative approaches for produced water management include reuse (internal – to open new wells – or external – for livestock watering and irrigation) or treatment for disposal to surface water. Reuse of produced water to fracture new wells has become a common practice in Pennsylvania. Reports from 831 wells in Pennsylvania show that 10% of the water used for the fracturing of a new well comes from flowback water and the rest consists of fresh water coming either from surface waters or purchased from public supplies.[29] In the following sections, the challenges and opportunities associated with produced water management and biological treatment were discussed, and a decision-making approach for determination of appropriate treatment technology for individual wells was proposed.

2.2 CHALLENGES IN PRODUCED WATER MANAGEMENT AND BIOLOGICAL TREATMENT

Extraction of hydrocarbons from shale formations results in large volumes of produced waters that require treatment and disposal; however, development of treatment approaches has lagged behind compared to the development of hydrocarbon extraction methodologies. Current methods for produced water management primarily include deep well injection, reuse, and treatment. In the Marcellus Shale, approximately 60% of the produced water is treated and reused, and the rest is disposed of via deep-well injection.[30] Reusing produced water requires removal of scale-forming constituents such as Ba, Ca, Fe, Mg, Mn, and Sr to minimize scaling and fouling.[8]

Moreover, biocides are used to inhibit microbial growth in wells and in holding ponds as microorganisms can cause fouling and souring. Disposal of produced waters to surface water requires treatment to reduce salinity (TDS < 500 mg/L in Pennsylvania) and municipal wastewater treatment plants are not permitted to accept produced waters in some states (such as Pennsylvania).[2] The unique composition of produced water can affect physical, chemical, and biological treatment methods. For instance, high salinity in produced waters increases the energy requirements, and the organic and microbiological content increase fouling potential of membranes—leading to increased cost and maintenance issues.

Biological treatment methods have been widely employed in other wastewaters to remove dissolved organic contaminants. Thus, biological treatment alternatives can increase physical-chemical process performance of produced water treatment by removing fouling organics and organic constituents poorly removed by these processes. Nevertheless, there are certain challenges that need to be addressed for the effective use of biological treatment of produced waters. The basic challenges for both final disposal and biological treatment are salinity, the presence of biocides, and varying organic carbon content and concentration. In addition, final disposal is also challenged by high radionuclide content and toxic compounds present in the produced water. In this section, the produced water management challenges were discussed in detail, from the perspective of both final disposal and biological treatment.

2.2.1 Salinity

Following the hydraulic fracturing of a well, produced water begins to return mixed with gas, oil, and, potentially, formation water from the shale. This flow continues as a decreased volume until the hydrocarbon production of the well ends. The concentration of total dissolved solids (TDS)

in produced water increases as time passes following the fracturing.[10] TDS concentration in produced water can range from 5,000 to greater than 300,000 mg/L.[7-9] The most common inorganic constituents in Marcellus Shale produced waters are Na, Ca, Cl, Ba, Ra, Sr, Mg, Br, Fe, and Mn.[10, 31] The source of salinity in produced water is largely believed to be from concentrated seawater. Barbot et al. investigated 160 flowback and produced water samples from the Marcellus Shale.[10] Their results showed that produced water from conventional wells and high salinity produced water samples (from late in the production period) exhibit trends similar to those of concentrated seawater. However, there were no clear similarities between seawater evaporation and less concentrated produced water samples from early in the production period.[10] According to Haluszczak et al., the Br/Cl ratio of produced waters indicates that mixing with the formation waters has an important role in the high salinity of produced waters and that dissolution of salt and minerals from the rock units is not the main mechanism for high salt concentration in produced waters.[31]

Recent regulations in Pennsylvania (2010) require effluent concentrations of 500 ppm (mg/L), 250 ppm, 10 ppm for TDS, Cl, Ba, and Sr (based on monthly averages), respectively, for the facilities treating natural gas wastewaters for surface disposal.[29] Hence, the removal of salts is a prerequisite for surface discharge of produced waters. Municipal wastewater treatment facilities do not remove high TDS concentrations that decrease the efficiency of biological treatment units, and produced water dilution with municipal wastewater is not sufficient in some places due to the high volumes (in Pennsylvania regulations do not allow gas drilling operators to send produced water to publicly owned treatment plants).[29] Therefore, produced waters either must be sent to brine treatment facilities or be disposed of by deep well injection. The

concentrated brine produced after the desalination processes can be disposed in regulated disposal wells.[14]

The influence of salinity on biological treatment has been studied previously.[32, 33] Recent studies showed that salt concentration in produced water is the main factor affecting the rate of biodegradation[34, 35] and, therefore, salinity is one of the primary challenges that must be considered in biological treatment strategies. It has been previously shown that activated sludge treatment systems (suspended growth) have limited tolerance to salinity concentrations above 10,000 mg/L.[32, 33] Thus, more robust biological treatment methods, such as attached growth systems (e.g., biofilms) or produced water dilution, may be considered if applicable.

2.2.2 Radionuclides

Elevated concentrations of Naturally Occurring Radioactive Material (NORM) originate from the subsurface formations.[31] Geochemical properties of the shale formation can affect the dissolution of NORM. For instance, high ionic strength and low SO_4^{2-} concentrations can increase radium-226 solubility.[36] Among NORM, the alkaline earth metal radium-226 (Ra-226) is especially concerning due to its high concentration in produced water, radioactive properties, and half-life of 1,620 years. Ionizing radiation caused by the decay of radioactive materials during long-term exposure can increase the risk of cancer.[37] Moreover, Nelson et al. (2015) estimated that in a closed system where Ra-226 decays, the radioactivity level increases by a factor of 8 in 100 years.[36] The industrial discharge limit for radium in the U.S. is 60 pCi/L[9] and the maximum contaminant level (MCL) in the National Primary Drinking Water Regulations for combined concentration of Ra-226 and Ra-228 is 5 pCi/L.[38] On the other hand, the radium concentrations reported in produced waters can be alarmingly high, reaching total radium

activities up to 10,000 pCi/L in produced waters from the Marcellus Shale.[39] Median radium activities reported in Marcellus and non-Marcellus reservoirs (including Medina, Theresa, Queenstone, Rochester, Bass Islands, Onondaga, Oriskany, Helderberg, and more) are 2,460 pCi/L and 734 pCi/L, respectively.[39] Previously, 13 produced water samples were analyzed by New York's Department of Environmental Conservation and elevated Ra-226 concentrations were found (up to 267 times the limit for safe discharge).[40]

There are two main issues related to Ra-226 management in produced waters. One of the challenges is its detection. The most commonly used approaches for radium detection are often time and labor intensive, such as alpha spectrometry (which takes 3 weeks of radon-222 equilibrium period)[41], liquid scintillation (which involves tedious sample preparation procedures), and gamma spectrometry (with 24-48 hours counting times).[39, 42] Moreover, the recovery of Ra-226 can be challenged by high TDS concentration due to ionic interferences[43] and the current wet chemical purification techniques performed prior to measurement suffers from high concentration of divalent cations such as Ba, Ca, and Sr.[42] However, a recent study proposed a rapid Ra-226 analysis using ICP-MS and their results showed accurate detection of Ra-226 even at 415,000 mg/L TDS concentration and an average of 97% recovery by ICP-MS.[42]

The second issue related to Ra-226 in produced water is that Ra-226 concentration tends to increase during reuse of produced water and as the sediments in holding ponds age, starting from less than 10 pCi/g in fresh sediments to several hundred pCi/g in aged sediments.[44] The Ra-226 accumulation in the sediments occurs due to co-precipitation with $BaSO_4$, hence, aged sludge could require to be handled as radioactive solid waste since it could exceed the disposal limits of municipal landfills.[44]

A potential treatment to remove radium from produced water is biosorption. Biosorption of Ra-226 has been studied in the literature[21, 22, 45] and in Chapter 6. Tsezos et al. used biomass (return sludge from a municipal wastewater treatment plant) as a biosorption medium to remove Ra-226 in aqueous solutions and in wastewaters from uranium mining operations. Their results showed that acidic pH (pH = 2) limited the Ra-226 adsoption.[21] Satvanamesh et al. used bacterial strains isolated from hot springs and soil from areas of elevated radiation in Ramsar, Iran to evaluate Ra-226 biosorption in aqueous solutions. They reported a wide variation in the Ra-226 biosorption from 53 $Bq.g^{-1}$ to 202 $Bq.g^{-1}$ depending on the bacterial strain used.[45] One of the greatest challenges for removing Ra-226 from produced waters using biomass is the high salinity concentration. Rowan et al. reported that Ra-226 activity is correlated with the salt concentration in produced waters[39] and studies (in the literature[46, 47] and in Chapter 6) showed decreased ion uptake with increasing ionic strength. In Chapter 6, Ra-226 removal in synthetic produced water using a halophilic alga at changing salinity concentrations (0 – 200,000 mg/L NaCl) was evaluated. Our results demonstrated a positive correlation between the remaining Ra-226 activity at equilibrium and salt concentration. It was suggested previously that divalent alkaline earth metal ions (e.g., Ca, Sr, Ba, Ra) can form surface complexes that bond weakly with the hydroxide surfaces, and that increased ionic strength can lead to the competition of ions for the available active surfaces.[47, 48]

2.2.3 Biocides

A wide variety of biocides are used in hydraulic fracturing operations to limit the growth of bacteria (i.e. sulfate reducing bacteria, acid producing bacteria) that can cause clogging, corrosion, and souring of the wells.[49] Among those glutaraldehyde (GA), dibromo-

nitrilopropionamide, tetrakis hydroxymethyl, dodecyl dimethyl ammonium chloride, and chlorine dioxide are the biocides that are most commonly used.[50]

Biocides are used aboveground, for produced water storage, and in pipelines. They are also often used in the fracturing process and for well maintenance.[50] The selection criteria of biocides include the properties of the geological formation, the compatibility with other chemicals and the environment, the cost, and the desired bacterial control.[50, 51] However, current practices on selecting biocides can also depend on historical usage and may not be specialized for individual wells.[50]

The use of biocides may affect biological treatment systems and result in biomass loss. Biocides can also transform into more toxic compounds and react with other chemicals during use, possibly altering the biodegradation rates of the compounds present in produced waters during biological treatment or in case of an accidental release.[19] For instance, a recent study showed that biodegradation of polyethylene glycol surfactants used in hydraulic fracturing can be limited by the presence of biocides (i.e., glutaraldehyde was used as the model biocide in their experiments).[19] Moreover, there is evidence that increased salt concentration tends to decrease GA transformation,[52] demonstrating that biocide degradation can be further impeded by the high salt concentration in produced waters, increasing the longevity of its toxic effects. Current research suggests that contaminant interaction effects need to be evaluated to determine the environmental persistence of hydraulic fracturing chemicals and biocides. In Chapter 4, the effect of GA on biodegradation of the most commonly reported fracturing fluid chemicals using a biofilm approach was evaluated. The results demonstrated varying observed lag periods and half-lives at changing GA concentrations, depending on the compound and suggest that co-

contaminant interactions should be taken into consideration for the determination of the environmental toxicity of mixtures.

2.2.4 Toxicity

A desirable outcome of biological produced water treatment would be removal of toxic compounds for subsequent reuse or disposal. The chemicals present in the produced waters are a combination of introduced chemicals during fracturing and formation chemicals. Toxic metals, salts, and radionuclides can be released from the formations and mixed in the fracturing fluid that flows back to the surface. Chemicals used in fracturing fluids have certain functions in the fracturing process such as regulation of viscosity, pH, microbial growth inhibition, friction reduction, and scale inhibition. Among the ingredients reported in fracturing fluids, there are compounds with high acute toxicity (Globally Harmonized System of Classification and Labeling of Chemicals – GHS – defines acute toxicity categories from 1-4, 1 being the highest - https://www.osha.gov/dsg/hazcom/appendix_a.pdf) such as glutaraldehyde (GHS Category 1), thiourea, and 2-butoxyethanol (GHS Category 2).[20] Based on rat inhalation and oral toxicity data, of 81 components, Stringfellow et al. identified 13 (16%) hydraulic fracturing chemicals to have low to moderate toxicity, and 25 (31%) chemicals lacking toxicity information. The remaining 43 (53%) chemicals were considered as non-toxic.[20] A previous study predicted 19 compounds in fracturing fluids to have "elevated exposure potential" via groundwater, meaning these compounds were identified in more than 50 FracFocus reports and were predicted to have more than 10% of their concentration remaining at 94m setback distance.[1] It is important to note that this study did not account for downhole transformation products, mixture toxicity, and potential

co-contaminant interactions that may result in different biodegradation, mobility, and solubility values.[1]

Evaluation of the toxicity caused by unconventional oil and gas production is challenging due to incomplete disclosure of the compound identity and the concentrations used in fracturing chemicals, the wide spectrum of chemical structures (e.g., organic, inorganic, radioactive), the variation in the compound use depending on the geographical properties of the shale formation, physicochemical properties of fracturing chemicals (e.g., octanol-water partition), temporal variations in emissions and exposure, and, finally, a lack of measurements of health-relevant compounds.[53] Although the chemicals introduced into fracturing fluids only account for 1-2% of the total volume, this amount could reach from 150,000 to 600,000 liters of chemicals to open a new well.[53, 54] A recent study performed detailed toxicity evaluations for hydraulic fracturing chemicals[53] and, according to their investigations, 781 (76%) out of 1021 identified hydraulic fracturing chemicals did not have reproductive and developmental toxicity information in the investigated toxicity data bases (e.g., REPROTOX). Of the 240 chemicals with available toxicity data, 126 chemicals had reproductive and 192 had developmental toxicity information available. A total of 67 chemicals with current drinking water standards were possibly associated with reproductive or developmental toxicity.[53] Therefore, toxic compounds in produced water create a challenge for final disposal and proper treatment is required to eliminate environmental and human health concerns.

2.2.5 Variation in Organic Content and Concentration

Total organic carbon (TOC) concentration of produced water can vary significantly between and within shale formations.[12, 55] Table 2.1 presents the ranges of TOC concentrations of produced waters sampled from various shale plays. TOC concentrations ranged from as low as 1.2 mg/L to as high as 43,550 mg/L.[13, 56] Moreover, TOC concentration can change in the same well over its production lifetime. Orem et al. observed that, in a Marcellus Shale gas well, TOC concentration was decreased from over 200 mg/L at day 0 to 55 mg/L at day 20 and remained relatively stable until 240 days after production.[12]

High TOC values can be associated with the presence of miscible oil residuals in produced water as well as with organic chemicals used during fracturing.[12] Organic analyses of produced waters from the Marcellus and New Albany shale formations identified PAHs (e.g., naphthalene, pyrene), heterocyclic compounds (e.g., benzothiazole, quinolone), aliphatic alcohols (e.g., ethylene glycol and derivatives), aromatics, fatty acids (i.e., long chain fatty acids such as C_5-C_{18} in Marcellus Shale), phthalates, and nonaromatic compounds (e.g., C_{11}-C_{37} alkanes/alkenes).[12]

Table 2.1: Chemical produced water characteristics of various shale plays.

Location	TDS (mg/L)	TOC* (mg/L)	Study
Antrim Shale	n.a.	4.3 – 12.75	[57]
Bakken Shale	262,000-287,000	50.2 - 353	Chapter 5
Barnett Shale	n.a.	43,550 ± 730	[56]
Denver-Julesburg	22,500	590	[58]
Eagle Ford	n.a.	6,095 ± 300	[56]
Marcellus Shale	n.a.	23.7-5,804	[12]
Marcellus Shale	20,000 -140,000	12 - 551	[59]
Marcellus Shale	3,010 – 261,000	1.2 - 509	[13]
Marcellus Shale	48,000	720	[60]
Marcellus Shale	n.a.	2,348 ± 22	[56]
Utica Shale	170,013-267,000	176.6 - 3,990	Chapter 5

*TOC as total dissolved organic carbon
n.a. : Not available

2.3 OPPORTUNITIES FOR BIOLOGICAL PRODUCED WATER TREATMENT

Biological produced water treatment has potential applications in produced water treatment for reuse and disposal. In the Marcellus Shale, flowback water is treated to remove divalent cations, reused, and/or disposed via deep-well injection.[5, 30] Reports from 831 wells in Pennsylvania show that 10% of the water used in fracturing fluids is produced water and the rest is fresh water either from surface sources or the public supplies.[29] To reuse the produced water, removal of scale forming constituents such as Ba, Ca, Fe, Mg, Mn, Sr is necessary.[5, 8] Physical-chemical treatment technologies proposed for produced waters include reverse osmosis, thermal distillation and crystallization, ion exchange, and capacitive deionization. These methods often suffer from high energy requirements due to high TDS and from maintenance issues due to the presence of organic compounds that may foul the membranes.[5, 35] Recently, biological treatment of produced water has been proposed as a treatment approach for removing organics to decrease

the costs of membrane replacement and maintenance.[35] Various biological treatment approaches have been proposed to overcome the challenge of high salt concentration of produced water. Some of the proposed treatment systems use salt-acclimatized suspended biomass, biofilms, microbial fuel cells, and membrane biofilm systems.[34, 58, 61-63] This area of research has been studied only recently and there is substantial opportunity for more lab-scale and pilot application studies. In this section, the current attempts at biological treatment methods used in produced waters from shale formations were evaluated.

2.3.1 Biodegradable Compounds

Previously, 55 (68%) out of 81 commonly used hydraulic fracturing chemicals were reported to be organics and 27 (50%) of these are known to be either readily or inherently biodegradable.[20] A separate study found 10 out of 14 of the most frequently reported organic compounds in Fracfocus disclosure reports were considered readily biodegradable (e.g., methanol, isopropanol, ethylene glycol, guar gum, ethanol, glutaraldehyde).[1, 20] The median maximum concentrations of commonly reported compounds in hydraulic fracturing fluids differ in oil and gas disclosures and range from 0.00007 to 0.17 (% by mass) depending on the compound.[64] These compounds are alcohols, diols, butyl ethers, polysaccharides, aldehydes, and carboxylic acids, and are present in the additives that can be used for various purposes such as corrosion inhibitors, surfactant, scale inhibitor, non-emulsifier, friction reducer, iron control, biocide, and gelling agent. Petroleum distillates and heavy aromatic petroleum naphta are other frequently reported compounds in FracFocus reports[1] and these are the mixtures of C_{10}-C_{14} naphtenes, iso- and n-paraffins, and C_9-C_{16} aromatic hydrocarbons mixtures, respectively. The biodegradability of these mixtures varies according to the types of hydrocarbons present.[65]

The most common chemical analyses for determining biodegradability of produced waters are total organic carbon (TOC) measurement and the BOD_5/COD ratio.[66] Table 2.1 presents the ranges of total organic carbon (TOC) concentrations of produced waters sampled from various shale plays. TOC concentrations in produced waters can be as high as 5,804 mg/L. High organic loads in produced waters can create potential for effective biological treatment to remove organic content for both reuse and disposal. Especially if combined with membrane systems, biological treatment can increase the efficiency of membrane treatment via decreasing the fouling frequencies[35] and maintenance costs of the membranes.

2.3.2 Saline Tolerant Bio-Treatment Approaches

Biological treatment approaches are well recognized to be negatively affected by salinity.[32, 33, 67, 68] The major reasons for low biological treatment performance at high salinity include cell disruptions due to ionic strength changes, limited adaptation of conventional cultures (only up to 3-5% (w/v) salinities), decreased biodegradation rates (low food/microorganism ratio (F/M) required), and high effluent turbidity (decreased protozoa and filamentous organism populations also decreases sedimentation efficiency).[32] Hence, high salt content in produced waters is the main challenge for biological treatment approaches. The proposed solutions to create salt-tolerant biological treatment systems include using acclimatized biomass, salt tolerant microorganisms, and native microbes from high salinity environments (Table 2.2).

Table 2.2 demonstrates the biological treatment evaluations performed for produced waters from various oil and gas production shale plays including the Denver-Julesburg, Barnett, Marcellus, and Piceance basins using biological treatment approaches. The TDS concentration range of the produced waters treated in these studies ranges from 16,000 to 91,350 mg/L and the

removal percentages are reported in terms of total/dissolved organic carbon (TOC, DOC) and chemical oxygen demand (COD). From Table 2.2, a substantial variation in the treated produced waters (e.g., influent TDS and TOC/DOC/COD concentrations) and the biodegradation results have been reported.

Previously, a COD reduction in synthetic produced waters was demonstrated in a sequencing batch reactor by Lester et al.[35] Their results demonstrated decreased COD removal (60%) at 45,000 mg/L TDS concentration compared to that of at 1,500 mg/L (90%) using a sequencing batch reactor (SBR) and they observed increased turbidity in the effluent.[35]

Akyon et al. studied the removal of COD in both synthetic and real produced waters from the Marcellus Shale using a mixed-culture biofilm approach (Chapter 3 in this dissertation).[34] This study showed that salinity is the main driver in produced water biodegradation and that there is microbial activity at salt concentrations as high as 100,000 mg/L TDS (1.45 mg COD removed/$gram_{wet}$-day at 91 g/L TDS). No biodegradation was observed at 200,000 mg/L TDS. Moreover, microbial community and metagenomic analyses demonstrated an adaptive community shift driven by the salinity.[34]

In another study, Lester et al. performed an on-site biological treatment for DJ basin flowback water using a SBR together with RO and an advanced oxidation process. They observed 50% TOC removal at a TDS concentration of 22,500 mg/L (initial TOC = 590 mgC/L). Moreover they suggested a combination of biological, aeration/filtration, RO, and, finally, UV/H_2O_2 treatment train for DJ basin flowback waters.[58]

Riley et al. proposed the use of biologically active filtration (BAF) prior to membrane filtration (ultrafiltration and nanofiltration). They used granular activated carbon (GAC) as the medium for both microbial biofilm formation and adsorption using the native bacteria from

produced waters as the seed. The salt concentration studied in these experiments ranged between 12,600 mg/L and 31,200 mg/L TDS. A TOC removal of 79% was achieved at 72 h HRT (influent TOC = 732 mg/L) and 31,200 mg/L TDS in the BAF system that TOC removal was a combination of contaminant adsorption and biodegradation. They showed that the system can adapt to changing salt and organic concentrations. However, the hydraulic retention time (HRT) needed to be positively correlated with the TDS and TOC concentration of the samples to achieve a steady state biodegradation.[63]

Monzon et al. used microbial fuel cells (MFC) to treat produced water from the Barnett Shale while simultaneously generating electricity. Their results provided sufficient electricity for a capacitive deionization (CDI) desalination process and reported a COD removal efficiency of 68% (influent COD = 10,520 mg/L). The power generated by the MFC was 47 mW/m^2.[62] In another study, Stoll et al. used microbial capacitive desalination cells to treat the effluent of a wastewater treatment plant (WWTP) that receives produced water from the Piceance Basin in Colorado.[69] The main focus of this study was to provide a self-sustaining (no external energy input) desalination system for produced water while removing the organic content. They reported 20% TOC removal at a 16,000 mg/L TDS concentration (Influent TOC = 230 mg/L) in one hour. Average TDS removal was reported as 36 mg TDS/$g_{\text{activated carbon}}$. On the other hand, they pointed out that the desalination efficiency depends on the amount of substrate in the produced water.[69] Therefore, the limitation of organics in produced water as well as the fluctuations in the concentration of the organic matter will be the primary challenges of this technology.

Table 2.2: Biological treatment technologies proposed for produced waters from hydraulic fracturing.

Treatment Technology	Water Treated	Formation	Biomass source	Influent TDS (mg/L)	Influent TOC[a], DOC[b], COD[c] (mg/L)	HRT (h)	TOC[a], DOC[b], COD[c] (mg/L) Removal(%)	Source
Biologically active and membrane filtration	Oil and gas produced water	Piceance and Denver-Julesburg	Denver-Julesburg produced water	31,200	732[b]	72	79[b]	[63]
Microbial fuel cell	Produced water	Barnett	Barnett Shale produced water	85,700	10,520[c]	n.a.	68[c]	[62]
Sequencing batch reactor	Oil and gas produced water	Denver-Julesburg	Acclimated sludge from WWTP	22,500	590[b]	6	50[b]	[58]
Biofilm on grass silage	Produced water	Marcellus	Marcellus Shale produced water + activated sludge	91,350	2,500[c] (as guar gum)	72	58[c]	[34]
Microbial capacitative desalination cell	Produced water receiving WWTP effluent	Piceance	Activated sludge from WWTP	16,000	230[b]	1	20[b]	[69]

WWTP: Wastewater treatment plant
n.a: Not available

2.4 DECISION-MAKING IN PRODUCED WATER TREATMENT

Large volumes of produced waters have been generated during oil and gas extraction depending on the type of hydrocarbon produced, the geographic location of the well, and the production method used.[15] As the component concentrations can vary substantially, treatment methods need to be tailored according to the relative role of those components in produced water. To address similar management challenges for coalbed methane produced waters, an online tool was proposed previously (http://aqwatec.mines.edu/produced_water/index.htm).[70]

It has been suggested that the best technologies to treat produced water should be chosen based on the chemical composition of the produced water, the cost of treatment, and reuse and discharge requirements.[14, 30] Furthermore, a combination of various treatment approaches (physical, chemical, and biological removal), rather than a single process, is more likely to achieve the goals for the intended use of produced waters such as internal or external reuse (e.g., livestock watering, irrigation) and final disposal.

In order to provide a decision-making strategy for the best treatment combination for produced waters, a tailored design approach based on the produced water quality was proposed in Figure 2.1. In this approach, three stages were considered: (1) Initial Strategy, (2) Evaluation, and (3) Final Assessment. During the *initial strategy* phase, the first step is to perform chemical and organic analyses. The main chemical analyses that can help selecting the most suitable treatment alternatives are TDS, TOC, BOD, and COD analyses. TDS is one of the main decision parameters for the various treatment options since it drives the *evaluation* phase in aspects such as the determination of the energy, chemical, and dilution requirements for physical, chemical,

and biological treatment approaches. Similarly, organic composition (TOC, BOD/COD) will shape the treatment strategy and evaluation step. The necessity of biological treatment can emerge from the high organic loads that can cause membrane fouling, well fouling/souring, odor, and effluent requirements for reuse and disposal. In Figure 2.1, the double-sided arrow between *dilution* and *biodegradability potential* suggests considering these two factors simultaneously for evaluating the treatment requirements for biological approaches. This simultaneous analysis is important since any dilution performed to decrease the salt concentration of the produced water for greater biodegradation potential will also decrease the amount of organics available for microorganisms—possibly decreasing the necessity for biological organic removal.

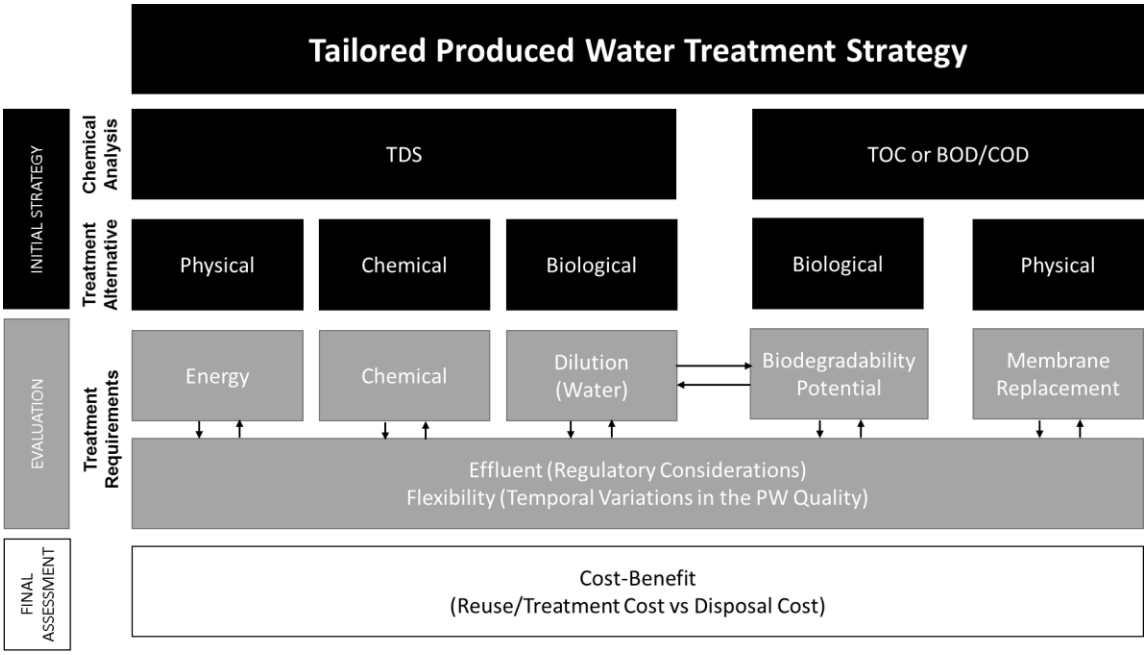

Figure 2.1: Decision-making scheme for tailored produced water treatment strategy.

Moreover, regulatory effluent requirements and flexibility of the treatment system (to the temporal variations in the produced water quality) are also affected by the initial strategy and other treatment requirements (e.g., energy, chemical, membrane replacement requirements). In the *evaluation* step, all treatment requirements need to be optimized simultaneously (represented with double-sided arrows in Figure 2.1) for the selected treatment alternatives. For instance, if a chemical treatment is selected as the initial strategy, an increase in the TDS concentration could require the use of more chemicals to provide system flexibility and to satisfy the required effluent quality. If the selected treatment strategy cannot satisfy the desired effluent quality for the high and low TDS concentrations expected in a certain well, then a different treatment alternative may be selected.

The final stage of the proposed tailored treatment design approach is the *final assessment*. Produced water management is ultimately an economic, regulatory, and liability decision and all the factors in the initial strategy and evaluation phases have their associated costs and benefits. For example, in the final assessment step, the cost and benefit of the treatment that satisfies the reuse and/or final disposal requirements (surface disposal or deep well injection) can be compared with the cost and benefit of the deep well injection disposal with no treatment. For some shale plays, such as the Marcellus,[4] the availability of the injection wells close to the oil and gas production sites could also be limited, hence increasing the costs of transportation. These factors can all be implemented in this final assessment phase.

Finally, following the cost-benefit assessment, if a treatment strategy is selected successfully, a pilot test needs to be performed to determine the design parameters and possible system issues that may arise (e.g., poor biodegradability due to toxic organics, low hydraulic retention periods, poor effluent quality due to limited sedimentation, infrastructure failures, etc.).

2.5 SUMMARY AND CONCLUSION

In this chapter, the challenges and opportunities associated with produced water management was evaluated from a biological treatment perspective. Limited studies on this area have shown that the main biological treatment challenges rise from the high salt concentration and variation in the organic content. Therefore, more research on robust and flexible biological treatment systems that can tolerate high salinity and changes in the organic content will improve the knowledge towards more efficient produced water treatment strategies, especially if those treatment approaches can be combined with physical-chemical methods. Proven technologies in the lab-scale can be implemented in real world using the proposed decision-making approach for individual wells or a combination of well sites with comparable produced water quality.

3.0 BIOFILMS AS A BIOLOGICAL TREATMENT APPROACH FOR PRODUCED WATER FROM HYDRAULIC FRACTURING

This work has been published as:

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Treatment of produced water, i.e. wastewater from hydraulic fracturing, for reuse or final disposal is challenged by both high salinity and the presence of organic compounds. Organic compounds in produced water may foul physical-chemical treatment processes, or support microbial corrosion, fouling, and sulfide release. Biological approaches have potential applications in produced water treatment, including reducing fouling of physical-chemical treatment processes and decreasing biological activity during produced water holding; however, conventional activated sludge treatments are intolerant of high salinity. In this study, a biofilm treatment approach using constructed biofilms was evaluated for biodegradation performance in both simulated and real produced water. Results demonstrated that engineered biofilms are active at total dissolved solids (TDS) concentrations up to at least 100,000 mg/L, and experiments in real produced water showed a biodegradation capacity of 1.45 mg COD/gram$_{wet}$-day at a TDS concentration of 91,351 mg/L.

3.1 INTRODUCTION

Advances in high-volume hydraulic fracturing and horizontal drilling techniques have enabled oil and gas production from unconventional reservoirs and have altered the current and future energy landscape. In 2012, more than 34% of U.S. natural gas was produced from unconventional resources,[71] and that percentage is expected to increase.[72] Additionally, shale gas resources are globally distributed,[73] with worldwide exploration expected to begin in the coming decades. High-volume hydraulic fracturing involves the injection of 10-20 million liters of fracture fluid at high pressure to fracture the target formation and stimulate reservoir permeability.[2] Following well completion, a portion (5% to greater than 100%) of the fracture fluid mixed with subsurface brine returns to the surface as produced water.[5, 6] Fracture fluid is typically 99% water and sand, with the remaining 1% comprised of chemicals to regulate pH, viscosity, and reduce friction, precipitation, scaling and biological fouling.[2, 5] Produced water typically has an elevated total dissolved solids (TDS) concentration that ranges from 5,000 to 300,000 mg/L,[7-9] including high concentrations of sodium, calcium, barium, strontium, chloride, bromide and naturally occurring radioactive material (NORM).[9, 10] Produced waters also typically contain a large suite of poorly defined organic compounds.[58] A large volume of this water is produced upon well completion and is known as 'flowback water'; however, wells continue to produce water during their entire operation. Here, the term 'produced water' is used to refer to all wastewater generated during unconventional well operation. Produced water characteristics are unique to each geological formation.[10]

Water management challenges associated with hydraulic fracturing, including produced water disposal and water sourcing for fracturing, have emerged at the forefront of the public and regulatory discussion regarding hydraulic fracturing. Due to large volumes and high salt

concentrations, disposal and treatment options for produced water are limited. Treatment of produced water in municipal wastewater treatment plants for surface disposal is no longer a viable alternative with new regulations in effect.[2] Deep well injection is one of the most common methods for produced water disposal; however, some regions (e.g. Pennsylvania) have limited deep well disposal capacity.[2] Additionally, induced seismic activity has been associated with deep well injection,[16] suggesting the potential for future regulatory limitations to this disposal approach. Finally, in some regions with suitable disposal capacity, there are concerns about the environmental impacts of fresh water sourcing.[5]

Biological treatment is a promising and underexplored treatment technology to remove organic compounds in high-salinity produced water. Biological treatment approaches may be used in conjunction with physical-chemical treatment to limit energy costs and membrane fouling for both produced water reuse in future hydraulic fracturing operations and final disposal. Additionally, reduced concentrations of organic compounds due to biological treatment would limit heterotrophic microbial growth during produced water holding, decreasing the need for biocide use. Recently, the effect of dissolved solids on chemical oxygen demand (COD) biodegradation in sequencing batch reactors was examined.[35] While the biological removal of COD decreased the membrane fouling potential, a significant decrease in COD degradation with increasing salt concentration was observed,[35] prompting further investigation into the suitability and potential of biological treatment approaches for produced water. These results suggest the need for more halo-tolerant biological treatment approaches.

Biofilms, clustered biofilms of mixed microbial communities,[74, 75] occur naturally in hypersaline habitats, with abundant microbial diversity.[16, 20] Biofilms have been used as a bioremediation technique for more than twenty years, and are tolerant of high salinity as well as

metal and metalloid toxicity.[76] An early study demonstrated oil biodegradation by biofilms developing in an oil-contaminated area.[77, 78] Hypersaline biofilms are capable of removing 25-85% of model petroleum compounds, with performance decreasing as salinity increases.[78-80] Various approaches have been attempted to cultivate biofilms,[75, 76, 81-83] including using glass wool,[76] coconut mesh,[81] polyester fiber,[82] silica particles,[83] and grass silage[75] as a growth scaffold. Among these, grass silage has been found to perform well due to stimulation of rapid microbial growth by providing a scaffold surface to support microbial growth and an initial supply of nutrients including lactic acid, amino acids and various minerals.[75]

In the current study, biofilms were constructed using grass silage, and the degradation of model compounds at various TDS concentrations was tested in both simulated and real produced water, and biodegradation rates were evaluated.

3.2 MATERIALS AND METHODS

3.2.1 Construction of engineered biofilms

To prepare biofilms, window screen was cut in approximately 2.5 centimeter diameter circles, filled with grass silage (1 gram), and sewn together. Growth medium for the biofilms was comprised of 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 $CaCl_2$). The growth media was seeded with 10% (v/v) of a mixed stock of produced water and activated sludge from municipal wastewater. Prepared mats were then placed in the growth medium in a 2

L plastic beaker and mixed continuously for 21 days to maintain aerobic conditions. The same growth batch of biofilms was used for each set of experiments.

3.2.2 Preparation of test media

Biofilms guar gum degradation capacity was tested in both synthetic and real hydraulic fracturing produced water; acetate degradation was tested only in synthetic produced water. Acetate was utilized as a model simple organic molecule, as a fermentation and breakdown product from other complex organic molecules in fracturing fluid, such as guar gum, and has previously been identified in produced water.[30] In experiments conducted with synthetic produced water media, NaCl and $CaCl_2$ were added at a Na/Ca mass ratio of 3.5^{10} to supply TDS concentrations of 0, 50,000, 100,000, or 200,000 mg/L. For the synthetic produced water acetate degradation experiment, a 5,000 mg/L acetate stock solution was prepared with 6.94 g/L sodium acetate in deionized water. The stock solution was used to provide a 2,500 mg/L acetate concentration to all test conditions. In the synthetic produced water guar gum degradation experiment, guar gum solution was prepared using a modified approach of Lester et al.[35] Guar gum is a commonly used chemical in fracturing fluid to increase viscosity[35] and was used here as a representative complex organic COD source. Typical guar gum dosage in fracturing fluid ranges from 600-4,800 mg/L.[84] Briefly, 3,000 mg/L guar gum was prepared with deionized water, the supernatant was withdrawn after an 18-hour settling period and filtered through glass fiber filters (Fisher Scientific, Pittsburgh, PA). The resulting filtrate had a COD value of approximately 2,500 mg/L. Real produced water experiments were conducted with two different produced water samples, Sample A and Sample B, together with each sample diluted one half, Sample $A^{1/2}$ and Sample $B^{1/2}$. Sample characteristics are shown in Table 3.1. Sample A (182,702

mg/L TDS) was produced water from a well in southwest PA, and the biocide used in the fracturing fluid was glutaraldehyde. Sample B (18,400 mg/L TDS) was from an open produced water holding pond containing water from multiple wells in southwest PA that was maintained with chlorine dioxide. All test conditions were supplemented with 2,500 mg COD/L guar gum as described above. As the produced water samples used had low biodegradable COD concentrations, guar gum addition was utilized to more accurately provide a degradation rate estimate. Chemical details of all test media are included in Table 3.1.

Table 3.1 Chemical characteristics of synthetic produced water, Sample A, and Sample B.

Constituent	**Sample A**	**Sample B**	**Synthetic produced water[a]**
Na (mg/L)	47,107	5,272	17,500
Ca (mg/L)	16,509	1,691	5,000
Mg (mg/L)	1,820	193	0
Ba (mg/L)	328	14.6	0
Sr (mg/L)	1,888	1,051	0
Fe (mg/L)	19	4.22	0
Cl (mg/L)	115,277.6	13,867	27,500
COD (mg/L)	1,865	440	2,500
TDS (mg/L)	182,702	18,400	50,000
pH	5.9	7.35	6.66
Days after Fracture	20	N/A	N/A

[a]: Example synthetic media given for 50,000 mg/L TDS condition.

3.2.3 Experimental procedure

Prior to use, biofilms were rinsed for 1.5 hours at 70 rpm in 50,000 mg/L TDS synthetic produced water to limit the carryover of the cultivation media to the test medium. Following rinsing, the wet weight of the mats was recorded and they were placed into 10 mL of test media in 6-well plates (Corning Costar, Tewksbury, MA). For an initial homogenization period, 6-well plates were placed on a shaker table at 110 rpm for 15 minutes and time zero sampling was performed. Biofilms were continuously shaken at 110 rpm throughout each experiment. All conditions were sampled at 0, 6, 24, 48, and 72 hours. Successive loadings of test media were performed, with each loading lasting 72 hours. The 72 hour loading period was chosen based upon preliminary tests demonstrating limited substrate removal following this time period. Three biological replicates were conducted for each test condition. Surface samples of the mats were taken at the beginning and at the end of each cycle and stored at -20°C for later microbial analysis.

High performance liquid chromatography (HPLC, Agilent Technologies 1200 Series) and HACH (Loveland, CO) COD kits were used to quantify acetate and guar gum, respectively. In acetate experiments with synthetic produced water, 0.5 mL of liquid was withdrawn from each well and centrifuged (Fisher Scientific, Accuspin Micro 17) at 4000xg for 5 minutes. Supernatants were transferred to slip syringes (Fisher Scientific, Luer-Slip Syringes, 3 mL capacity) and filtered through syringe filters (Fisher Scientific, Cellulose Syringe Filter, 0.2µm) into microcentrifuge tubes (Fisher Scientific, 1.5 mL) and 0.2 mL from each filtered sample was analyzed by HPLC. The HPLC was equipped with a refractive index detector and a Rezex ROA-Organic Acid H+ (8%) column (Phenomenex Inc., Torrance, CA). The column was eluted with 0.005 N H_2SO_4 as a mobile phase at a flow rate of 0.6 mL/min at 50°C with a quaternary pump.

In guar gum experiments, 0.5 mL of liquid was withdrawn from each well, diluted 20 times with deionized water, and filtered through 0.45 µm filter paper (Millipore MF-EMD, Billerica MA). Filtered samples were stored at -20°C prior to analysis. The COD of the filtered samples were measured with HACH LR (low range) COD vials and a DR850 HACH Colorimeter. Due to the high salt content of samples, 0.5 g of mercuric sulfate (Acros Organics, Geel, Belgium) was added to COD vials to decrease chloride interference.[85] Standard errors were calculated with biological triplicates of each test condition. Technical replicates of COD measurements were performed for each time point.

3.2.4 Rate analysis and kinetics

Rate analysis was performed on all experiments to analyze COD removal. The assumed theoretical COD value from acetate was 0.92 g acetate/g COD (64 g COD/mole acetate). The second and third loadings of each experiment were utilized to determine the removal rates. Reaction rate constants are shown in Table A1 in Appendix. The highest and lowest kinetic constants were selected (denoted as highest and lowest performance, respectively) for each TDS condition.

3.3 RESULTS AND DISCUSSION

Results from synthetic produced water experiments with acetate and guar gum are provided in Figure 3.1 and 3.2, respectively. The first loading cycle demonstrated significant performance variability for all conditions, potentially due to growth media carryover or microbial community

adaptation. For acetate, the removal performance of the 0 and 50,000 mg/L total dissolved solids (TDS) conditions were statistically indistinguishable ($p=0.735$), while the 100,000 mg/L TDS condition demonstrated statistically significant decreased performance ($p<0.05$). For guar gum, the 0 and 50,000 and 0 and 100,000 mg/L TDS conditions were statistically indistinguishable ($p=0.186$ and $p=0.137$, respectively), while the 100,000 mg/L condition showed a decreased performance compared to the 50,000 mg/L condition ($p=0.004$). The 200,000 mg/L TDS condition did not demonstrate any removal throughout either the acetate or guar gum experiment and was thus not considered for further analysis. All conditions trended towards decreasing performance with each loading after the second, with a more dramatic decrease in the 100,000 mg/L TDS condition.

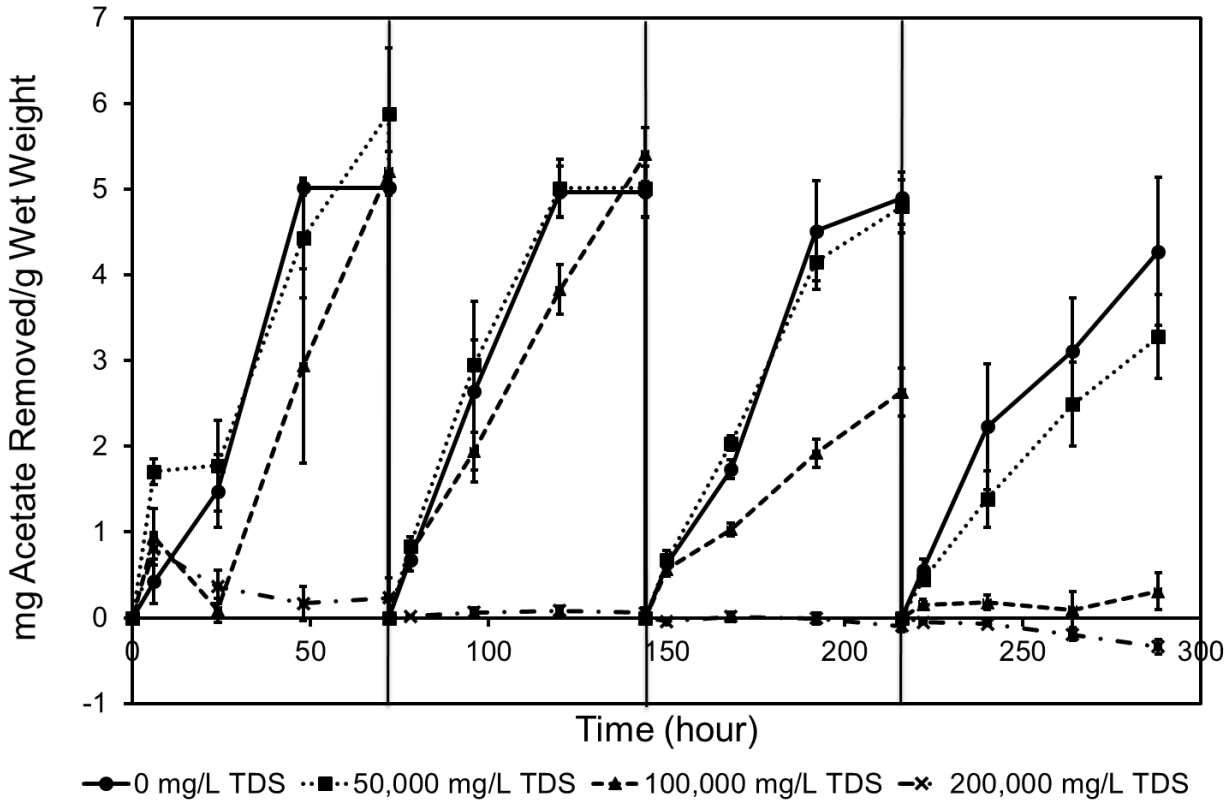

Figure 3.1: Acetate removed in synthetic produced water as a function of time. Each 72-hour loading is denoted by a dark vertical line. Data shown is the average of biological triplicates for each condition. Error bars represent +/- 1 standard error.

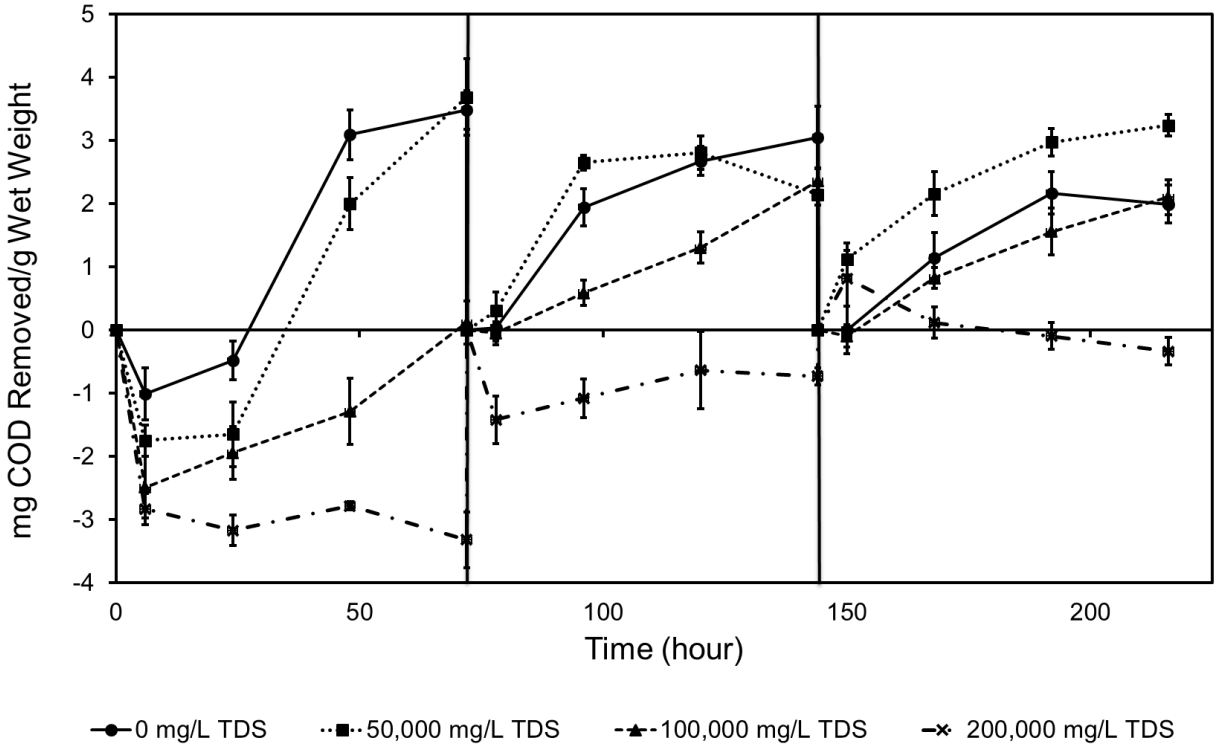

Figure 3.2: Guar gum removed in synthetic produced water as a function of time. Each 72-hour loading is denoted by a dark vertical line. Data shown is the average of biological triplicates for each condition. Error bars represent +/- 1 standard error.

Figure 3.3 shows the removal of COD from guar gum amended produced water. Produced water characteristics are shown in Table 3.1. Sample A was produced water sampled from an actively producing well in southwestern PA with a TDS concentration of 182,702 mg/L. Sample B was sampled from a produced water holding pond containing water from many wells from southwest PA with a TDS concentration of 18,400 mg/L. In order to expand the number of samples evaluated, while maintaining the relative composition of real produced water, a one half dilution of each sample was included, denoted as Sample $A^{1/2}$ and Sample $B^{1/2}$.

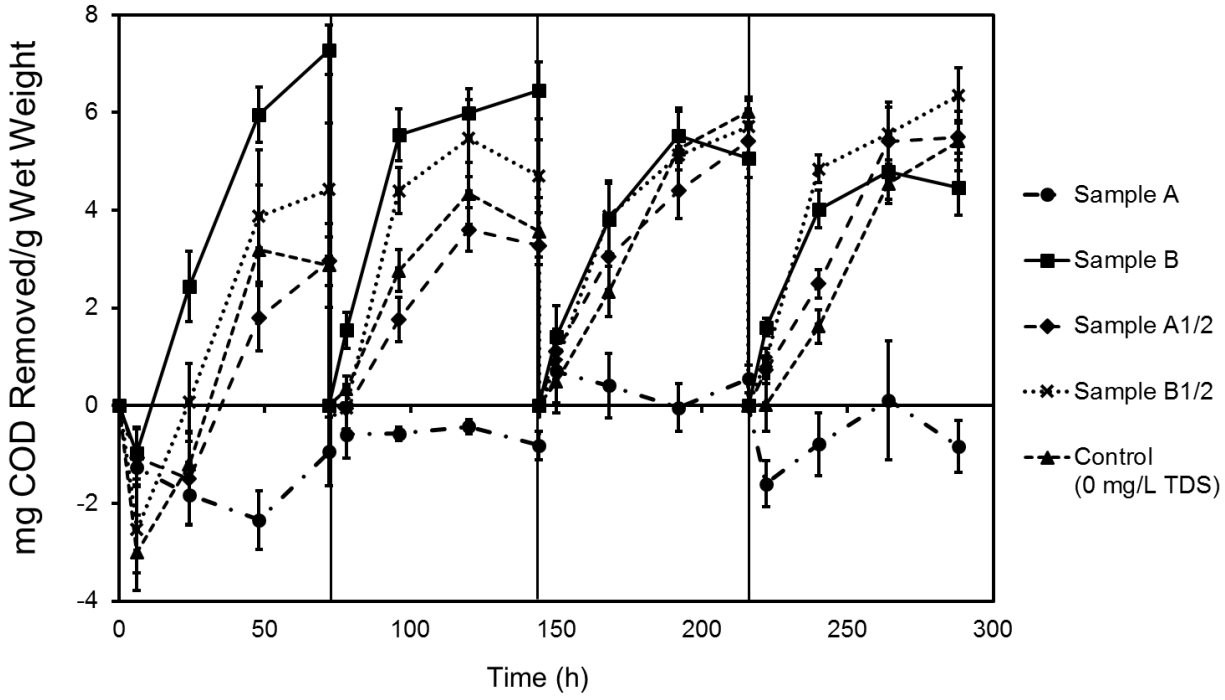

Figure 3.3: Guar gum removed in real produced water as a function of time. Each 72-hour loading is denoted by a dark vertical line. Data shown is the average of biological triplicates for each condition. Error bars represent +/- 1 standard error.

Consistent with experiments in synthetic produced water, the first loading cycle demonstrated significant variability. In the last three loadings, all experimental conditions except Sample A (182,702 mg/L TDS), showed similar removal performance ($p \geq 0.266$), demonstrating microbial activity in a TDS range from at least 0 - 91,351 mg/L. Mats in undiluted Sample A did not demonstrate COD removal.

Microbial mat treatment experiments conducted for two different substrates in synthetic and real produced water demonstrated similar trends. For all experiments, the first sample loading showed significant performance variability. This is likely due to the acclimation period

of the microorganisms[86] or growth medium leaching from the mats to the test medium. Our approach was to treat the first cycle as a wash and adaption step and exclude it from further rate evaluation. After several successive loadings, the biodegradation performance decreased with a steeper decrease at higher TDS concentrations. This behavior could be a result of a reduction in mat activity due to nutrient limitation, salinity stresses, or starvation between cycles. The highest biodegradation rates were observed at 0 and 50,000 mg/L TDS conditions for both acetate and guar gum. The 100,000 mg/L TDS condition exhibited a decreased rate, and no biological substrate removal was observed in the 200,000 mg/L TDS condition. Tests in real produced water reflected trends observed in synthetic produced water. A similar trend was seen in real produced water experiments, where the performance of mats decreased in Sample $A^{1/2}$ (91,351 mg/L TDS) and no biodegradation occurred in Sample A (182,702 mg/L TDS), similar to the test conditions with salt concentrations of 100,000 and 200,000 mg/L TDS, respectively. Improved COD removal in real produced water is likely due to constituents in the produced water, such as trace minerals, organics, nutrients, or salts. Additionally, it has been shown that trace minerals (e.g. Mg, Fe) have a significant effect on cell viability during starvation conditions.[87] As demonstrated by biofilms treating synthetic produced water, salinity had a strong role in driving the performance of the mats. In real produced water, uncharacterized compounds, including biocides, may have influenced the performance; however, these results demonstrate the ability of biofilms to perform in actual produced water samples.

Acetate degradation rates in synthetic produced water were used to calculate removal rates and reaction kinetics. Reaction kinetics for acetate degradation in synthetic produced water are shown in Table A1 in Appendix. Zero order reaction kinetics dominated under all conditions, including cycles where biofilms demonstrated the highest removals ($R^2 > 0.99$ in all conditions)

and lowest removals (R^2 > 0.96 for 0 and 50,000 mg/L and R^2 = 0.53 for 100,000 mg/L TDS) within a 72-hour period. The second and third produced water loading cycles of each experiment were evaluated to determine removal rates (Figure 3.4). Comparable removal rates were observed in 0 and 50,000 mg/L TDS conditions for degradation of each substrate, with reduced rates at 100,000 mg/L TDS. No degradation was observed at 200,000 mg/L TDS. Biofilms demonstrated higher guar gum degradation rates in real produced water than in synthetic produced water (p = 0.008).

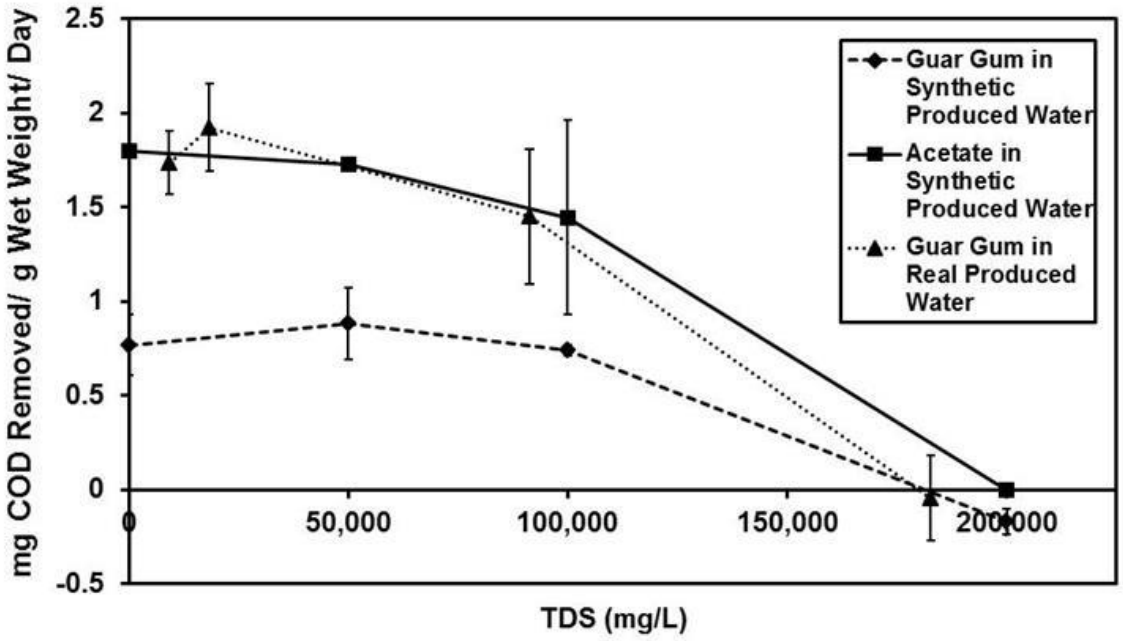

Figure 3.4 Microbial mat substrate removal rates in synthetic and real produced water. Data shown is the average of biological triplicates for each condition. Error bars represent +/- 1 standard deviation.

The influence of salinity on microbiological treatment performance has long been recognized.[88] Previous studies have demonstrated that activated sludge treatment systems experience a sharp decrease in COD removal efficiency above TDS concentrations of 10,000 mg/L,[32, 33] and a decrease in the COD removal efficiency from 85% to 59% when the TDS concentration increased from zero to 50,000 mg/L.[32] A recent study evaluating the efficiency of activated sludge to treat synthetic produced water demonstrated a 60% removal efficiency in 31 hours at a salt concentration of 45,000 mg/L.[35] The current study demonstrates guar gum COD removals of 66% and 45% COD at 50,000 and 100,000 mg/L TDS concentrations, respectively. A rate analysis (Figure 3.4) demonstrated that the acetate degradation rate by biofilms was higher than the guar gum degradation rate, likely due to the simpler chemical structure of acetate. Both acetate and guar gum showed similar removal rate trends with increasing salt concentration.

3.4 SUMMARY AND CONCLUSION

This study demonstrates the ability of engineered biofilms to treat saline hydraulic fracturing produced water. Several concerns (e.g. fouling, souring, and corrosion) regarding produced water holding and reuse have emerged, generally requiring some level of treatment before reuse, and biocide application during holding. Currently, nearly all produced water treatment is physical-chemical and generally involves transportation to a centralized facility. Biological produced water treatment approaches may be combined with physical-chemical treatment to reduce process fouling, or applied prior to produced water holding to reduce biological activity. The development of on-site and low-cost treatment options, including biofilm processes such as biofilms, will encourage produced water reuse in future hydraulic fracturing operations, reducing

the environmental impacts of fresh water sourcing, produced water transportation and disposal, and excess chemical usage. Biological treatment to remove available electron donors has the potential to decrease heterotrophic microbial growth and the necessity of biocide use. It is envisioned that biofilms may be used as either an on-site technology during produced water holding or coupled with physical-chemical treatment to reduce process fouling, although additional evaluation is necessary to determine the best application of this technology.

In the current study, the biodegradation performance of engineered biofilms was investigated in both synthetic and real produced water, with acetate and guar gum utilized to simulate simple and complex substrates. Our experiments demonstrate that engineered biofilms are capable of degrading COD in a broad range of salt concentrations. Results suggest the potential applicability of biofilms for produced water treatment within a wide range of salinity concentrations, and rates were found to be zero-order. While further work is necessary to understand the scale-up requirements of biofilms for produced water treatment, biofilms represent an emerging biological treatment technology to encourage produced water reuse, improve the performance of physical-chemical treatment approaches, remove organic constituents, and reduce biocide application. Biological produced water treatment approaches, including biofilms, have the potential to decrease the operational costs and improve the efficiency of treating produced water from hydraulic fracturing. Ultimately, improved produced water treatment will serve to address a significant source of public and regulatory concern surrounding the environmental impacts of the hydraulic fracturing process.

4.0 EFFECT OF GLUTARALDEHYDE ON BIOFILM TREATMENT PERFORMANCE IN HYDRAULIC FRACTURING PRODUCED WATER

Hydraulic fracturing requires injection of high volumes of fracturing fluid into shale formations to collect gas and oil trapped in the pores. Fracturing fluid is comprised of 98-99% water and sand, with 1-2% chemical additives that include inorganic and organic compounds together with biocides.[5, 6, 28] Biocides are often added to fracturing fluids or to the holding ponds to limit microbial growth to prevent fouling and souring of the wells, odor production in the holding ponds, and fouling of membranes during physical-chemical treatment. Glutaraldehyde (GA) is the most commonly reported biocide in hydraulic fracturing. Previously, the effects of GA on biodegradation of organic compounds were studied in agricultural top soil[19] and the results showed that there is a knowledge gap in co-contaminant interactions of GA in produced waters from hydraulic fracturing. In this study, we evaluated the co-contaminant interactions of GA with five of the most commonly reported organic compounds[1] in fracturing fluids, namely, acetate, guar gum, ethylene glycol, ethanol, and isopropanol. Our results demonstrate that GA concentration affects the biodegradation rate of these compounds, but the effect is variable for differing compounds. Moreover, the observed effects appear to be due to altered microbial activity rather than altered microbial abundance. These results will inform modeling of biological treatment and contaminant release to soil and water sources.

4.1 INTRODUCTION

Advancements in hydraulic fracturing have increased oil and gas recovery volumes from unconventional reservoirs, leading to a significant growth in the industry. Increased hydraulic fracturing has also resulted in the use of higher volumes of water and wastewater. In shale plays, drilling and completion of wells require an average 4 million gallons of water, and these amounts could create issues especially in arid regions such as Colorado, California, Texas, and Oklahoma.[89] Even in areas where water scarcity is not an issue, the surface disposal of large volumes of produced water (wastewater generated during hydraulic fracturing) is a problem due to high salt and radionuclide concentrations. As such, recent regulations have limited the disposal of produced waters via municipal wastewater treatment plants.[2] The most common disposal approach for produced waters is deep-well injection; however, in some regions, such as the Marcellus Shale, the geological formations considerably limit deep well injection.[4] As a result, states and industry have turned their attention to on-site treatment and/or reuse of produced water in the fracturing of new wells, which both decreases the water demand and the volume of produced water generated.

Final disposal and reuse of produced water typically require some level of treatment to meet discharge criteria. For reuse, pretreatment is performed to remove problematic compounds during fracturing, such as divalent cations (e.g., Ca, Ba, Sr, etc.), to prevent precipitation and scaling in the wells and to limit microbial activity that cause souring and fouling of the wells. The use of biocides is the most common approach to control biological growth. A wide array of commercial biocides are used aboveground in storage ponds (for odor prevention) and in pipelines (to limit bacterial growth).[50]

Glutaraldehyde (GA) is the most commonly-used biocide in the hydraulic fracturing industry, accounting for 27% of reported biocide use.[50] It is an electrophilic biocide that works by reacting with the thiol (-SH) and secondary amine groups (-NH) in membrane proteins,[90] cross-linking the proteins, altering the cellular permeability, and causing inhibition of the outer membrane functions such as nutrient transport and waste release, leading to cell damage.[50] Although GA is readily biodegradable under both aerobic and anaerobic conditions,[91] it is among the most toxic chemicals reported in the fracturing fluids, meeting GHS Category 1 standards for chemical toxicity based on rat inhalation studies.[20] A recent study by McLaughlin et al. demonstrated decreased biodegradation of certain fracturing fluid chemicals when they are mixed with GA in soil.[19] Their study showed inhibited polyethylene glycol transformation in the presence of GA and high salt concentration, demonstrating that there is a knowledge gap on co-contaminant effects of hydraulic fracturing chemicals. In this chapter, the effect of GA on the biodegradation of five frequently reported hydraulic fracturing chemicals, namely, acetate, guar gum, ethylene glycol, isopropanol, and ethanol, were investigated using engineered biofilms.

4.2 MATERIALS AND METHODS

4.2.1 Biofilm preparation

Biofilms were grown aerobically on freshly-cut grass silage using a growth medium comprised of 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 $CaCl_2$). The growth media was seeded with 10% (v/v) of a mixed stock of produced water and activated sludge from

municipal wastewater. Following three weeks of biofilm growth, 3x6 inch aluminum net screens were used to encase 11.3 ± 1.5 g_{wet} grass silage biofilms. The weight of the biofilms was recorded and then they were placed individually into 250 mL Erlenmeyer flasks. Prior to use, all biofilms were rinsed three times for 30 minutes in 50,000 mg/L TDS (35 g/L NaCl, 15 g/L $CaCl_2$, at 170 rpm) solution to limit cultivation media carryover.

4.2.2 Preparation of the test media

The five most frequently-reported compounds in FracFocus reports[1] (excluding methanol due to potential toxicity at the necessary concentrations), acetate (sodium salt of acetic acid), guar gum, ethylene glycol, ethanol, and isopropanol, were evaluated individually for the effect of GA on biodegradation in synthetic produced water using engineered hypersaline biofilms. The synthetic produced water media contained NaCl and $CaCl_2$ at a Na/Ca mass ratio of 3.5[10] to supply 50,000 mg/L TDS. All organic compounds to be tested were supplied at a TOC of 1,000 mg/L. Acetate (as $C_2H_3NaO_2$), guar gum, ethylene glycol, ethanol, and isopropanol were directly added to the test media at the target concentrations. The guar gum solution was prepared using a modified approach of Lester et al.[35] Briefly, 3.8 g/L of guar gum were prepared with deionized water, the supernatant was withdrawn after an 18-hour settling period, and the solution was then filtered through glass fiber filters (Fisher Scientific, Pittsburgh, PA). The resulting filtrate was further diluted to reach a final TOC concentration of 1,000 mg/L. The selected TOC concentration falls within range of previously-reported concentrations in produced water (23.7 – 5,804 mg/L).[12] We used GA as the biocide in these experiments.

4.2.3 Experimental Procedure

50 mL of test media were added to the 250 mL glass Erlenmeyer flasks containing the grass silage biofilms. Following an initial homogenization period of 30 minutes, time zero sampling was performed. The biofilms were shaken continuously at 200 rpm throughout the experiment. Three biological replicates and duplicate controls with no biofilm were performed. Moreover, controls with autoclaved (heat killed) biofilms were performed to determine abiotic removal of compounds. The pH of the samples was monitored to be below pH=8 throughout the experiment to limit rapid polymerization of GA.[92] The compounds were sampled at 24-hour increments until the TOC in the solution was depleted or no more removal was observed. Appropriate dilutions of the samples were performed for TOC analysis and the samples were filtered through 0.45 µm filter paper (Millipore MF-EMD, Billerica MA).

Biofilm samples were taken at the beginning and end of each experiment to determine culturable microbial community concentrations (colony forming unit per biofilm wet weight - $CFU/g_{biofilm}$). The colonies were counted at the beginning and end of the experiments using the pour-plate technique. Pour-plates were prepared using 5g NaCl, 2g Luria Broth, and 1.5g bacteriological agar in 100 mL deionized water. Immobilized biofilms were weighed and washed twice (vortex mixed at 3000rpm for 20 seconds) in 50 mL Falcon tubes using sterilized 1xPBS solution. The supernatant was transferred to a new tube and centrifuged at 3000 rpm for 15 minutes. Afterwards, the pelleted biomass was re-suspended in 1mL 1xPBS solution. Dilutions were performed and plated. The colonies were counted after an incubation period of 48-hour at room temperature.

4.2.4 Total Dissolved Organic Carbon (TOC) Analysis

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

4.2.5 Data Analysis and Rate Kinetics

In our experiments, measured TOC concentration was comprised of the TOC from both the compound of concern (e.g., acetate, guar gum, etc.) and GA. Therefore, normalization of the samples was performed by subtracting the TOC amount supplied by GA from the total TOC concentration measured at each data point. This normalization assumes GA was conserved during the experimental period to better compare the compound biodegradation between different conditions. To confirm this assumption, we measured the ratio of TOC_{GA}/TOC_{Total} to verify that this ratio is low enough to demonstrate that the compound of interest (e.g., acetate, guar gum, etc.) was removed. The TOC amount supplied by GA was 33.7 ± 1.2, 102.3 ± 0.9, 202.5 ± 1.4 mg/L for 50, 150, and 300 mg/L GA, respectively. According to these measurements, and GA represented 3.8 ± 0.3%, 10.2 ± 0.6% and 18.0 ± 1.3 % of the total TOC content at 50, 150, and 300 mg/L GA, respectively.

TOC removal was reported as TOC_t/TOC_0 where TOC_t represents the TOC concentration measured at time *t*, TOC_0 is the initial TOC concentration of the sample after biofilm addition

followed by a 30-minutes homogenization (mixing) period. An observed lag period was defined here as the time necessary to measure 2% removal as previously used.[93] First order degradation rates were calculated using Eqn. 4.1 and Eqn. 4.2:

$$\frac{dC}{dt} = k_{obs} * C \qquad \text{Eqn. 4.1}$$

$$ln\left(\frac{C_t}{C_0}\right) = k_{obs} * t \qquad \text{Eqn. 4.2}$$

where C is the TOC concentration and C_t is the TOC concentration at time t. The slope of the linear fit of Eqn. 4.2 was reported as the observed rate constant (k_{obs}). The variable t_L is the time when the lag period ends and the variable t_s is the time when TOC concentration no longer decreased in the solution, the first order rate kinetics were calculated between time t_L, and time t_s (i.e., the lag period was excluded from the first order biodegradation rate calculation). Let the observed lag period be L_{obs}, the observed half-lives ($t_{1/2}$) were calculated using Eqn. 4.3:

$$t_{1/2} = L_{obs} + \frac{ln\left(\frac{C_{t_{1/2}}}{C_0}\right)}{k_{obs}} \qquad \text{Eqn. 4.3}$$

Statistical analyses were conducted using Minitab 7.00 software (two sample t-test with a 95% confidence interval, equal variances were not assumed).

4.3 RESULTS AND DISCUSSION

4.3.1 Biodegradation Results

Biodegradation experiments were conducted with acetate, guar gum, ethylene glycol, ethanol, and isopropanol at 50,000 mg/L TDS (Table 4.1). Results were normalized in terms of GA concentration in each test condition for comparison purposes as described in detail in Section 4.2.5.

Table 4.1: The five most frequently-reported[1] biodegradable organic chemicals evaluated in this study.

Chemical Name	**Chemical Formula**	**Frac Focus Frequency(%)[1]**	**Biodegradability[20, 94]**	**Additive purposes [64]**
Isopropanol	C_3H_8O	50.1	RB	Corrosion inhibitor, non-emulsifier, surfactant
Ethylene glycol	$C_2H_6O_2$	49.7	RB	Cross-linker, scale and corrosion inhibitor, friction reducer
Guar gum	various	45.2	RB	Gelling agent
Ethanol	C_2H_6O	34.2	RB	Surfactant, biocide
Glutaraldehyde*	C_5H_8O	33.3	RB	Biocide
Acetic acid**	CH_3COO^-	31.7	RB	Buffer, iron control

*Used as the biocide in our experiments, RB stands for "readily biodegradable".
**Used in the sodium acetate (CH_3COONa) form in this experiment.

Figure 4.1 shows acetate removal at 0, 50, 150, and 300 mg/L GA concentrations. First order rate kinetic constants (k_{obs}) presented in Table 4.2 shows a negative correlation between the reaction rate and the GA concentration. Moreover, observed lag period (described here as the time necessary to measure 2% removal) was increased with increasing GA concentration, starting from 1.67 hours at no GA and reaching up to 72 hours at 300 mg/L GA for acetate. Figure 4.2 demonstrates a positive correlation between the observed half-lives and the GA concentration. Table 4.3 presents p-values from two-sample t-tests for the observed half-lives for acetate. The observed half-lives at 150 and 300 mg/L GA were significantly higher than those at 0 and 50 mg/L GA. These results show that the removal of acetate was inhibited by GA concentrations at 150 and 300 mg/L, significantly increasing the observed half-lives.

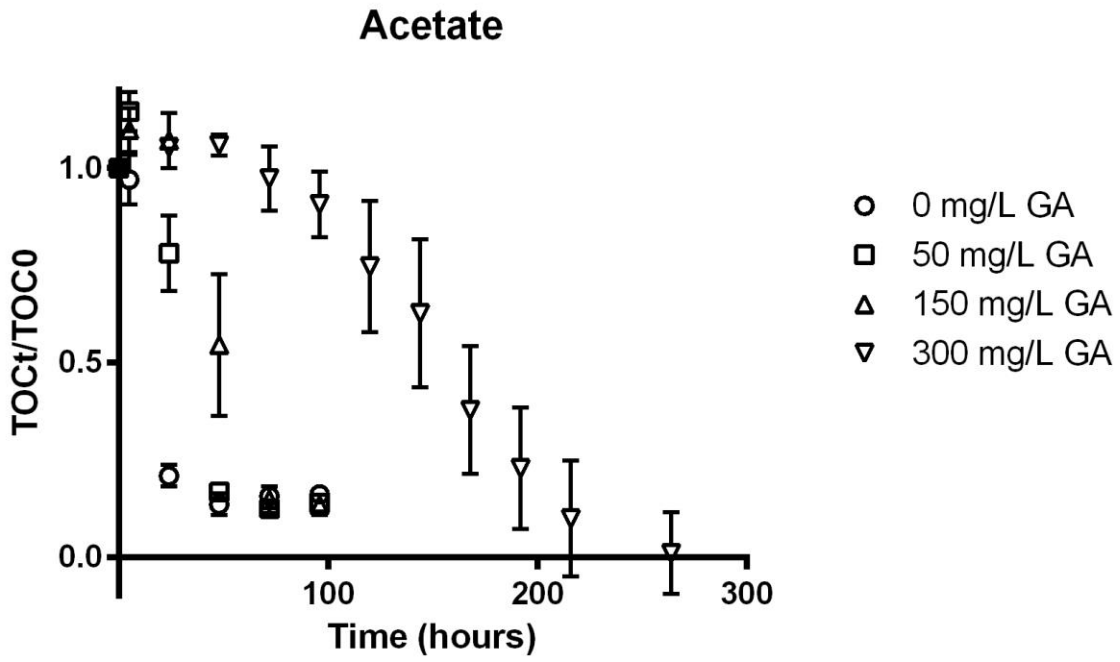

Figure 4.1: Acetate removal at 0, 50, 150, and 300 mg/L GA concentrations at 50,000 mg/L TDS. Error bars represent ±1 standard deviation.

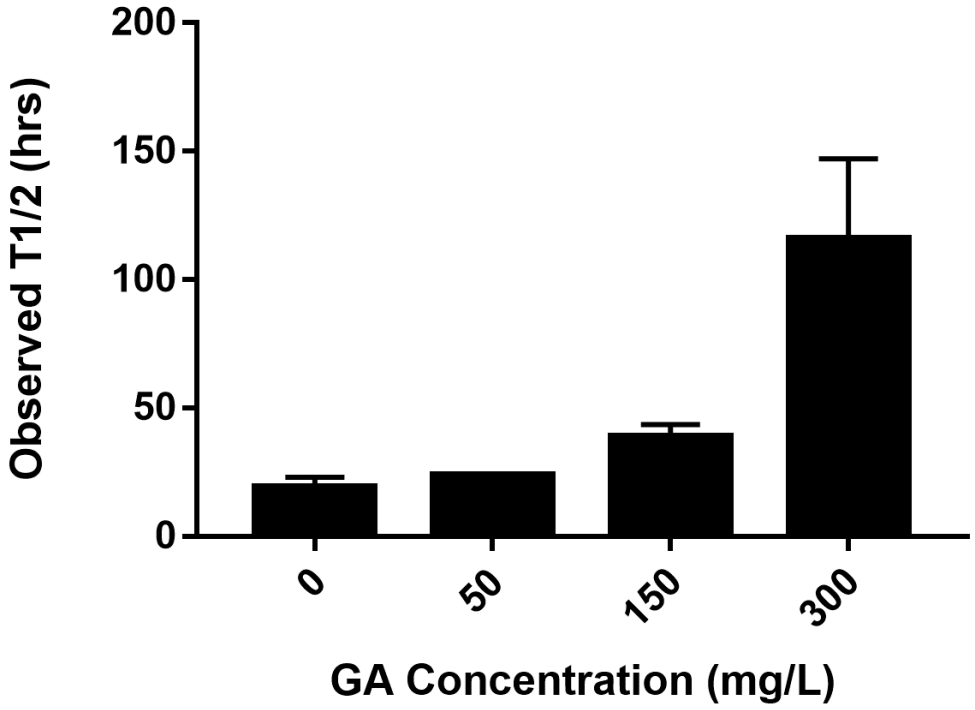

Figure 4.2: Observed half-lives for acetate at 0, 50, 150, and 300 mg/L GA concentrations at 50,000 mg/L TDS. Error bars represent ±1 standard deviation.

Table 4.2: First order rate constants and half-lives for compounds at changing GA concentration at 50,000 mg/L TDS.

Compound	Glutaraldehyde Concentration (mg/L)	Observed Lag (h)	K_{obs} (1/h)	Observed $t_{1/2}$ (h)
Acetate	0	1.67	0.0418	19.2 ± 3.8
	50	5	0.0365	24.0 ± 0.3
	150	17.67	0.0353	38.8 ± 4.8
	300	72	0.0162	116.1 ± 30.9
Guar Gum	0	0	0.0082	85.4 ± 8.7
	50	8	0.0109	71.7 ± 7.8
	150	40	0.0098	111.1 ± 29.0
	300	72	0.0175	126.5 ±48.2
Ethylene Glycol	0	24	0.0145	71.9 ± 0.7
	50	24	0.0143	73.2 ± 24.3
	150	48	0.0167	91.9 ± 34.7
	300	120	0.0110	203.9 ± 65.3
Ethanol	0	0	0.0101	68.9 ± 4.4
	50	0	0.0105	79.3 ± 41.8
	150	16	0.0043	181.0 ± 41.0
	300	48	0.0036	263.5 ± 115.8
Isopropanol	0	0	0.0027	262.5 ± 44.3
	50	8	0.0024	299.5 ± 32.7
	150	16	0.0067	233.7 ± 145.9
	300	40	0.0030	293.5 ± 70.8

Table 4.3: P-values from two-sample t-test for the observed half-lives at different GA concentrations of tested compounds at 50,000 mg/L TDS. 95% confidence intervals were used and equal variances were not assumed.

GA Conc. (mg/L)	Acetate	Guar Gum	Ethylene Glycol	Ethanol	Isopropanol
0-50	0.163	0.137	0.937	0.711	0.329
0-150	0.012	0.279	0.424	0.042	0.774
0-300	0.033	0.283	0.073	0.101	0.567
50-150	0.034	0.151	0.500	0.051	0.525
50-300	0.036	0.192	0.083	0.122	0.905
150-300	0.050	0.668	0.079	0.133	0.588

The removal results for guar gum are presented in Figure 4.3. First order rate kinetic constants (k_{obs}) presented in Table 4.2 do not show correlation between the guar gum biodegradation rate and the GA concentration; however, the observed lag period was increased with increasing GA concentration, starting from 0 hours at no GA and reaching up to 72 hours at 300 mg/L GA for acetate. Figure 4.4 demonstrates an increasing trend of the observed half-lives as GA concentration increases; however, observed trends were not significant (Table 4.3).

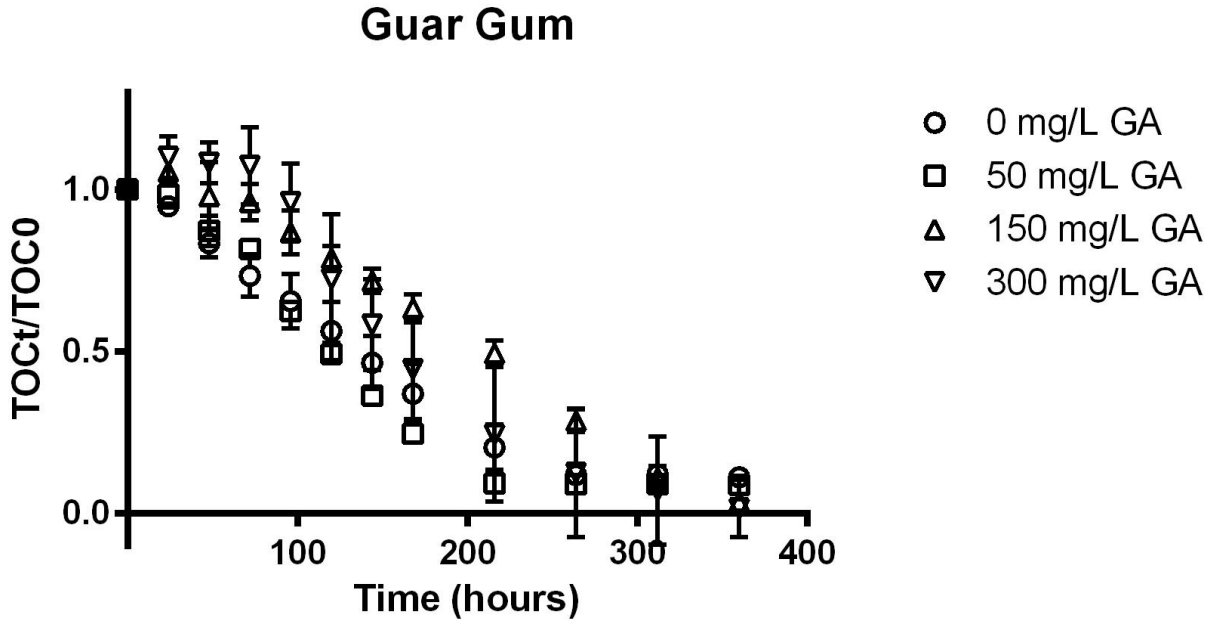

Figure 4.3: Guar gum removal at 0, 50, 150, and 300 mg/L GA concentrations at 50,000 mg/L TDS. Error bars represent ±1 standard deviation.

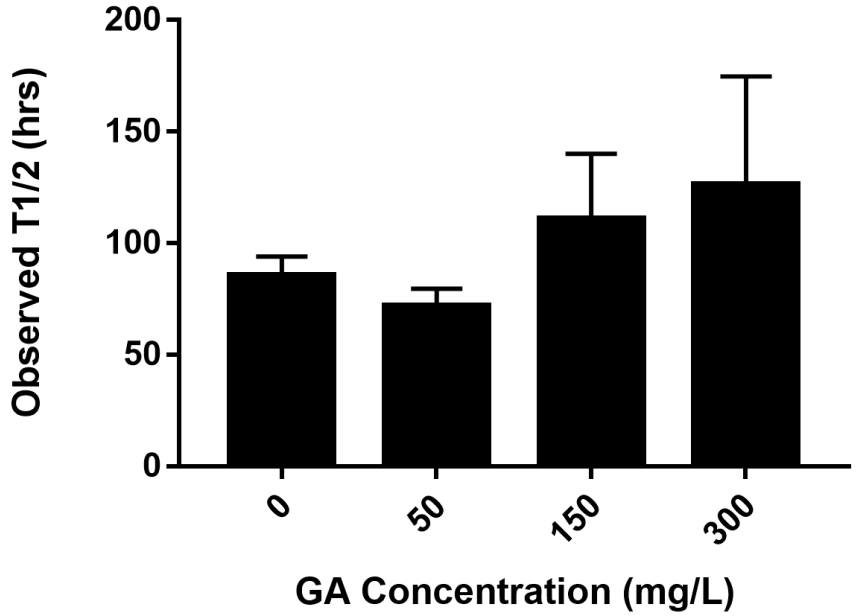

Figure 4.4: Observed half-lives for guar gum at 0, 50, 150, and 300 mg/L GA concentrations at 50,000 mg/L TDS. Error bars represent ±1 standard deviation.

Figure 4.5 presents the results of ethylene glycol removal. The observed lag period tends to increase with GA concentration, starting from 24 hours at 0 and 50 mg/L GA and reaching up to 120 hours at 300 mg/L GA (Table 4.2). Figure 4.6 demonstrates an increasing trend of the observed half-lives as GA concentration increases, almost tripling at 300 mg/L GA with 203.9 ± 65.3 hours compared to 0 and 50 mg/L GA (71.9 ± 0.7 and 73.2 ± 24.3 hours, respectively); however, trends were not significant (Table 4.3). Similar to guar gum, these results show that there is an increasing trend in the lag period and observed half-lives of ethylene glycol biodegradation in the presence of GA.

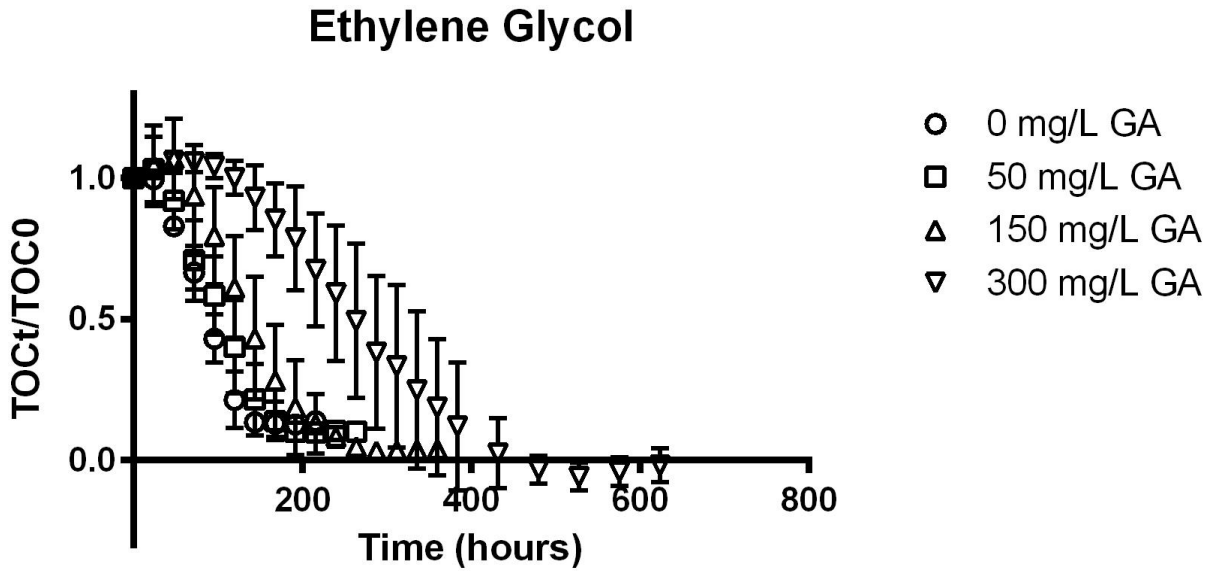

Figure 4.5: Ethylene glycol removal at 0, 50, 150, and 300 mg/L GA concentrations at 50,000 mg/L TDS. Error bars represent ±1 standard deviation.

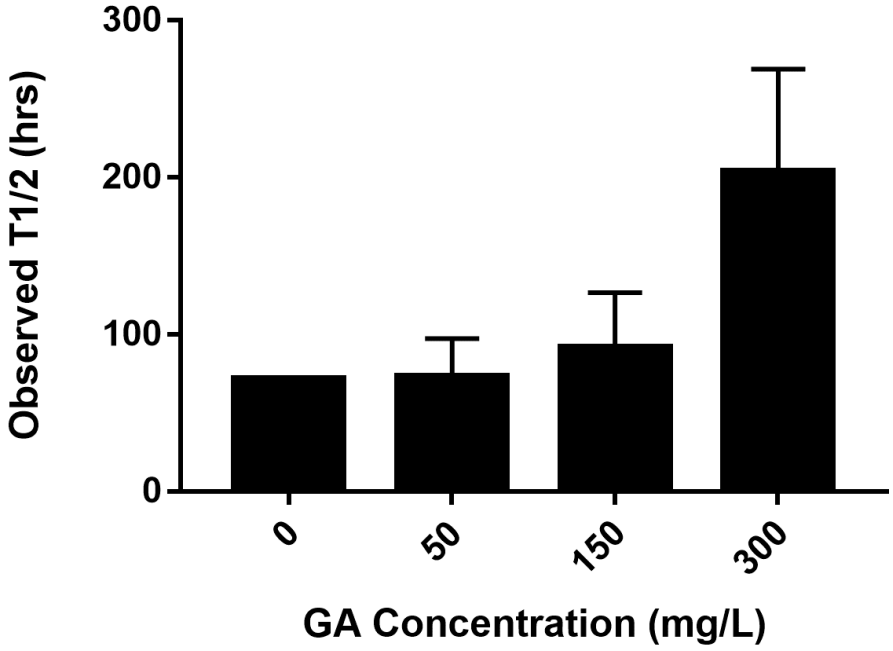

Figure 4.6: Observed half-lives for ethylene glycol at 0, 50, 150, and 300 mg/L GA concentrations at 50,000 mg/L TDS. Error bars represent ±1 standard deviation.

Figure 4.7 presents the removal of ethanol. The observed lag period tends to increase with GA concentration, starting from 0 hours at 0 and 50 mg/L GA and reaching to 48 hours at 300 mg/L GA (Table 4.2). A decreasing trend was observed in the first order biodegradation rates with GA concentration (Table 4.2). Figure 4.8 demonstrates an increasing trend of the observed half-lives as GA concentration increases, specifically a significant increase was observed at 150 mg/L (181.0 ± 41.0 hours) reaching more than two times the half-life at 0 mg/L (68.9 ± 4.4) (Table 4.3). These results show that there is an increasing trend in the observed lag period and half-lives of ethanol biodegradation in the presence of GA.

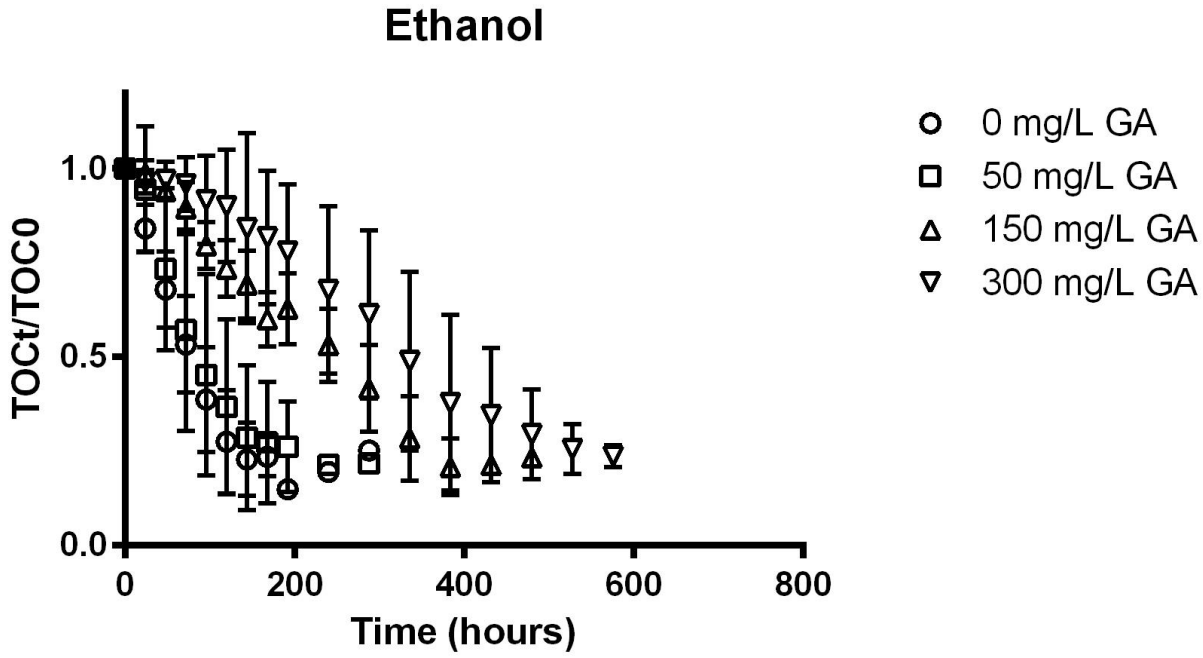

Figure 4.7: Ethanol removal at 0, 50, 150, and 300 mg/L GA concentrations at 50,000 mg/L TDS. Error bars represent ±1 standard deviation.

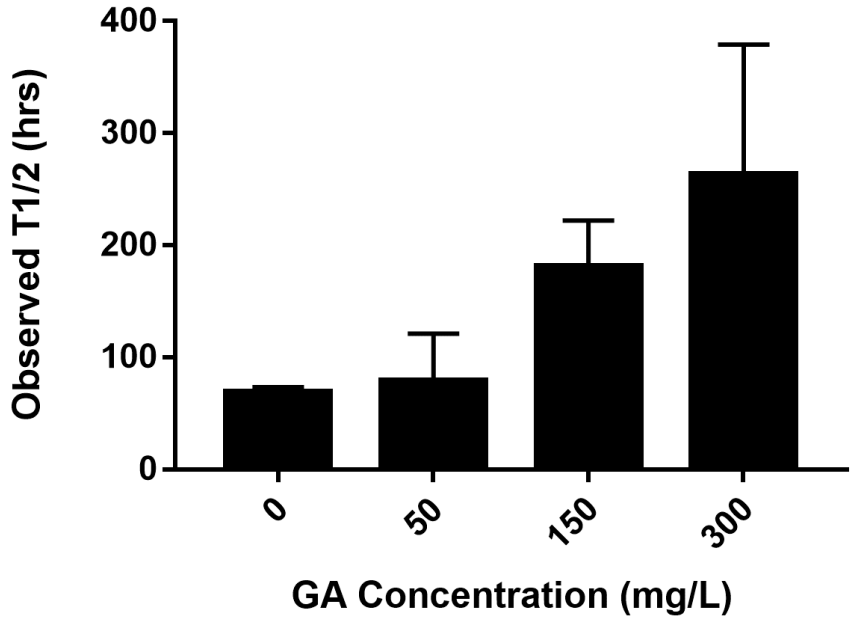

Figure 4.8: Observed half-lives for ethanol at 0, 50, 150, and 300 mg/L GA concentrations at 50,000 mg/L TDS. Error bars represent ±1 standard deviation.

Figure 4.9 demonstrates the removal of isopropanol. Table 4.2 shows that isopropanol removal rates were lower at all GA concentrations compared to that of acetate, guar gum, and ethylene glycol (removal rates of isopropanol $\leq 0.0067\ h^{-1}$). Moreover, the observed lag period increased with GA concentration; however, the observed half-lives did not show a trend (Figure 4.10) and did not change significantly with GA concentration (Table 4.3). These results show that isopropanol was more slowly removed compared to acetate, guar gum, ethylene glycol, and ethanol (for ethanol, only at 0 and 50 mg/L GA concentrations) regardless the GA concentration in the solution, suggesting that for compounds that are more slowly removed at 0 mg/L GA, GA concentration does not have any effect at the concentration range studied here. A previous study that used mixed pesticides enriched culture reported that complete biodegradation of isopropanol (13 days) was slower compared to ethanol (9 days) at 10,000 mg/L initial substrate

concentration.[95] Moreover, ethanol showed a higher maximum specific growth rate compared to isopropanol as predicted by a Haldane inhibition model, 0.0415 and 0.0393 h^{-1}, respectively.[95] A major intermediate of aerobic biodegradation of isopropanol is acetone.[95-97] Isopropanol conversion to acetone is rapid; however, bioconversion of acetone is relatively slower. The same study reported maximum bacterial specific growth rate for acetone to be 0.0320 h^{-1}, that is lower compared to that of isopropanol (0.0393 h^{-1}). [95]

Additionally, glutaraldehyde was previously shown to cross-link alcohol dehydrogenase (an enzyme responsible for the oxidative metabolism of alcohols), resulting in complete inactivation of this enzyme in *L. brevis*.[98] However, this is unlikely to be an important mechanism in describing the lower biodegradation rates observed in alcohols studied in this experiment (i.e., ethylene glycol, ethanol, isopropanol; 0.0030 – 0.0110 h^{-1}) compared to the biodegradation rate of acetate at all GA concentrations (0-300 mg/L GA).

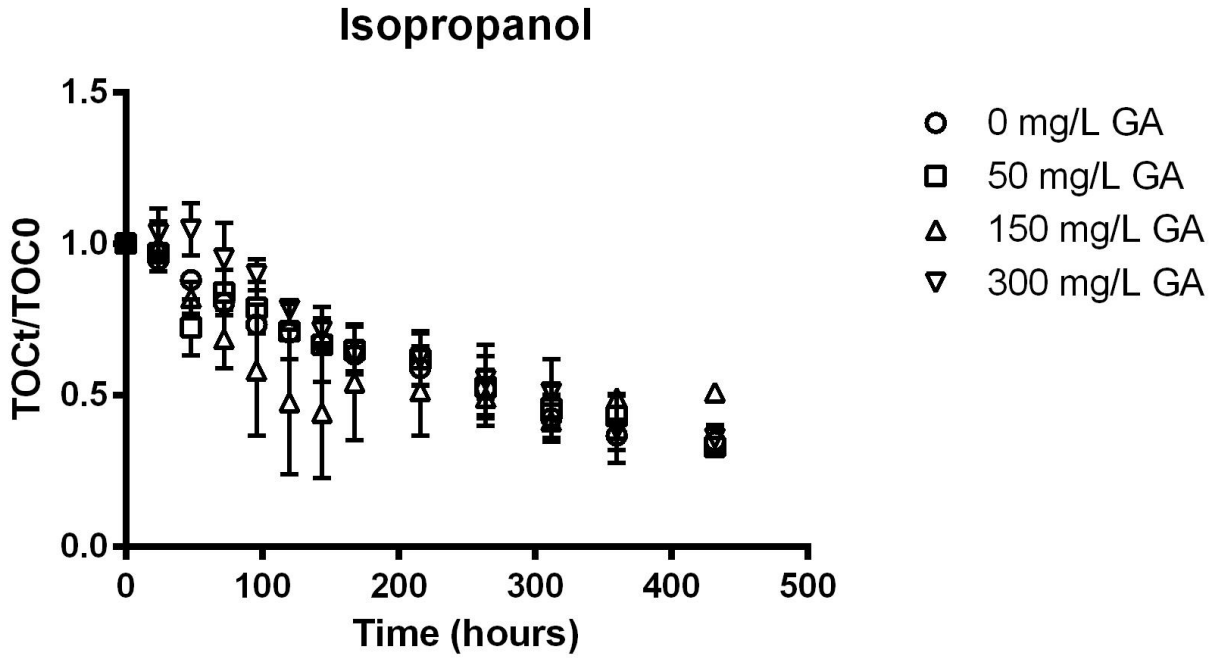

Figure 4.9: Isopropanol removal at 0, 50, 150, and 300 mg/L GA concentrations at 50,000 mg/L TDS. Error bars represent ±1 standard deviation.

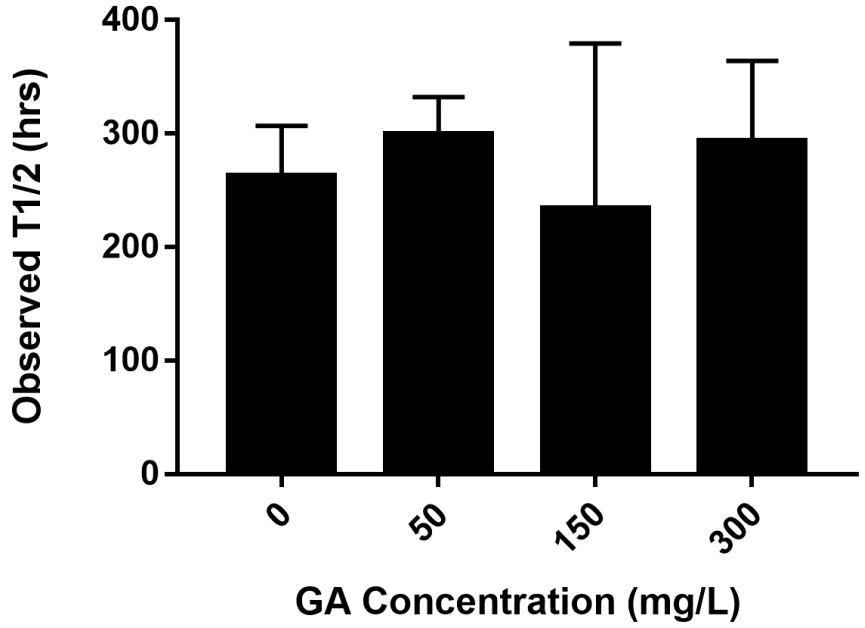

Figure 4.10: Observed half-lives for isopropanol at 0, 50, 150, and 300 mg/L GA concentrations at 50,000 mg/L TDS. Error bars represent ±1 standard deviation.

4.3.2 Plate Colony Counts

Plate counts of each test condition were performed before and after the biodegradation experiments to investigate the effect of changing GA concentration on the viability of the biofilm. Start (starting biofilms before experimental media addition) and end of treatment plate counts ($CFU/g_{biofilm\ weight}$) are presented in Figure 4.11. The results demonstrate that viable microbial counts increased at the end of the treatment compared to the start, regardless of the GA concentration of the test media and organic compound used. The statistical analysis of the results is presented in Table 4.4. For acetate and isopropanol, there was no significant difference between the plate counts at 0, 50, 150, and 300 mg/L GA concentrations. A significantly

decreased amount of colony counts was observed in ethylene glycol at 300 mg/L GA concentration compared to 0, 50, and 150 mg/L GA.

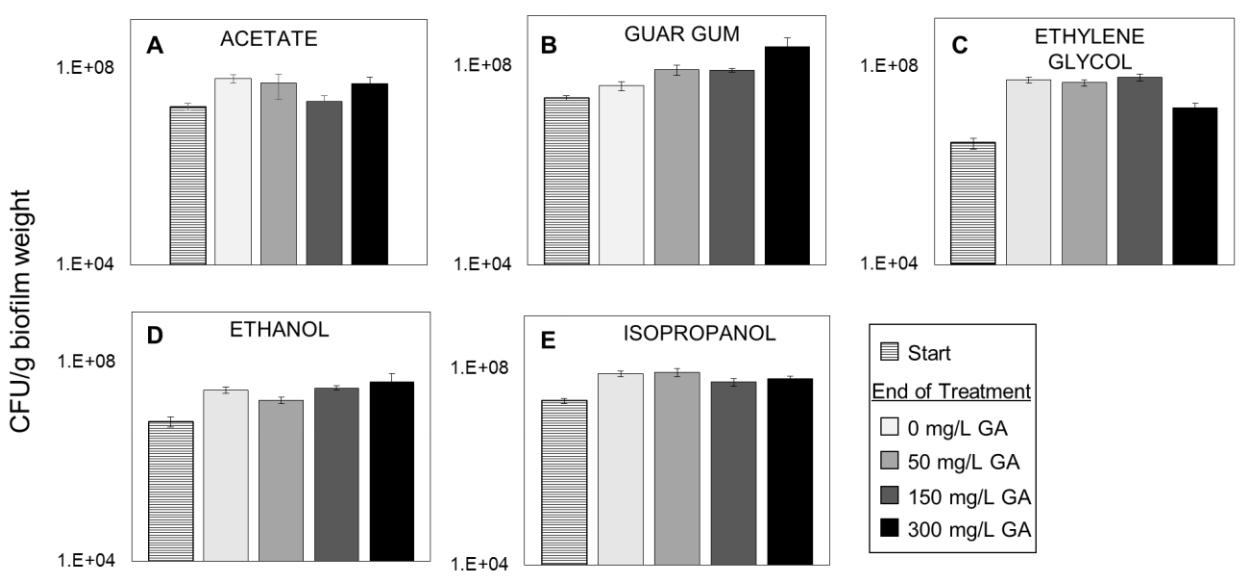

Figure 4.11: Colony counts per mL of sample per gram of biofilm weight at the beginning and end of the experiments at GA concentrations ranging from 0-300 mg/L and 50,000 mg/L TDS. Error bars represent ±1 standard error.

Table 4.4: Two-sample t-test p-values for colony counts at changing GA concentration. 95% confidence interval was used and equal variances were not assumed.

GA Conc. (mg/L)	Acetate	Guar Gum	Ethylene Glycol	Ethanol	Isopropanol
0-50	0.728	0.060	0.532	0.043	0.765
0-150	0.094	0.001	0.616	0.592	0.072
0-300	0.546	0.577	0.002	0.543	0.229
50-150	0.406	0.960	0.303	0.008	0.126
50-300	0.981	0.204	0.001	0.285	0.268
150-300	0.135	0.098	0.002	0.628	0.452

Biofilm treatment approaches were previously shown to be tolerant to high salinity concentrations and toxicity,[76] and were used successfully in treating guar gum in real produced waters with external COD addition (Chapter 3).[34] A recent study investigated simulated spills of hydraulic fracturing fluid additives on agricultural topsoil to determine the environmental fate, toxicity, and co-contaminant interactions of widely used compounds such as glutaraldehyde, polyethylene glycol, and polyacrylamide.[19] They demonstrated decreased polyethylene glycol degradation rate and longer lag phases when polyethylene glycol was mixed with GA (250 mg/L), suggesting that GA was present at inhibitory levels for microorganisms in the tested conditions.[19]

Mathematical models estimating biodegradation of substrate mixtures can be divided into non-interactive and interactive models.[95] Non-interactive models assume that growth rate of microorganisms is only governed by one limiting substrate at a time; on the other hand, interactive models assume growth is dependent on multiple limiting substrates. In a biological system, the interactions between substrates can be beneficial or detrimental to microorganisms. Beneficial interaction can occur as a result of increased growth of biomass due to presence of multiple substrates or due to generation of essential enzymes for biodegradation. On the other

hand, detrimental interactions may occur due to inhibition effect of one substrate on the utilization of another by the microorganism. Therefore, substrate interactions can enhance or decrease the biodegradation of individual compounds in a mixture compared to its single-substrate biodegradation.[95] A previous study investigated the co-contaminant interaction of aromatic hydrocarbon mixtures using pure and mixed bacterial cultures (*P. putida* F1 and *Burkholderia sp.* JS150) and compared their results to existing bio-treatment modeling methods.[93] Their results showed that simplified mathematical models assuming pure culture/single-substrate and competitive inhibition kinetics were inadequate for predicting both the growth of pure cultures and mixed cultures in hydrocarbon mixtures (pure cultures grown in toluene, benzene, phenol, and mixed cultures grown in 1-butanol, 2-butoxyethanol, N,N-dimethylethanolamine).[93]

Building on the results from the aforementioned two studies,[19, 93] co-contaminant effects in observed half-lives and lag times during biodegradation when the organic compounds are mixed with GA was observed in this study. The effect of GA concentration on biodegradation changed depending on the organic substrate used. An increasing trend in the observed lag periods and half-lives was observed in all compounds (except for isopropanol), suggesting that microbial inhibition in wastewater mixtures containing biocides and other toxic compounds should be taken into account when biological treatment approaches, biocide use, and environmental models are concerned.

4.4 SUMMARY AND CONCLUSION

In this study, the effect of glutaraldehyde (GA) on the biodegradation of commonly reported fracturing fluid chemicals (i.e., acetate, guar gum, ethylene glycol, ethanol, and isopropanol) was investigated at 0, 50, 150, and 300 mg/L GA concentrations at 50,000 mg/L TDS using an engineered biofilm. Results demonstrated varying removal rates and lag periods at changing GA concentrations for each compound tested at 50,000 mg/L salt concentration. The biodegradation rates of acetate decreased significantly at 150 and 300 mg/L GA concentration. Furthermore, the effect of GA concentration was positively correlated with the observed lag period and half-lives for all compounds (except for isopropanol). Thus, environmental models dealing with the effects of wastewater treatment, reuse, disposal, and incidental spillage should consider the effects caused by contaminant interactions. Otherwise, model predictions based on either no interaction or competitive inhibition kinetics could significantly underestimate the environmental persistence, toxicity, concentration, and destination of the compounds.

Our results also demonstrate that isopropanol biodegradation rates were slower compared to acetate, guar gum, and ethylene glycol at all GA concentrations, suggesting that GA inhibition is more evident when compounds are more rapidly biodegraded. Further co-contaminant interaction studies (e.g., using different biocides or mixtures reported in fracturing fluids), could provide better understanding of microbial inhibition and biodegradation kinetics in produced waters that can lead to a more conscious use of biocides and better implications of biological treatment options.

5.0 REMOVAL OF ORGANICS FROM HYDRAULIC FRACTURING PRODUCED WATER USING AN ENGINEERED BIOFILM APPROACH

High volumes of produced water are generated during hydraulic fracturing, and disposal of this wastewater is a current environmental concern due to high salinity and radionuclide concentrations and the potential to contain toxic organics. The treatment of produced water is vital for disposal or reuse; however, physical chemical treatment approaches to remove salts and radionuclides suffer from fouling due to microorganisms and high organic content. Moreover, the reuse of produced waters in the opening of new wells is challenged by the fouling and scaling of the wells due to electron donor for microbial growth (organics) and divalent cation presence in the produced water. Therefore, a biological treatment approach that removes available electron donors from produced water would prove useful for reuse and disposal as a complementary treatment method for physical-chemical treatment techniques. This study investigates the biodegradation potential in produced water samples from Utica and Bakken Shale. Our results show high variability in TOC concentration and TOC removal efficiency of the different produced water samples. Moreover, the salt concentration is found to be a main driver of the organic removal in produced waters, consistent with previous results to degrade model compounds (Chapter 3). These results demonstrate that a biological treatment approach would be most effective for produced waters with low TDS and high TOC concentration.

5.1 INTRODUCTION

Hydraulic fracturing is a process to recover gas and oil from unconventional (shale) formations. During hydraulic fracturing, large volumes of fracturing fluid, composed of 98-99 % water and sand and 1-2 % additive chemicals, are injected into the target formation.[6, 28] Much of this fluid ultimately returns to the surface, and the returning fluid is referred to as produced water.[6] High volumes of produced water are generated (up to 4 million gallons per well)[89] during well opening. Disposal of this wastewater is problematic due to high salt and radionuclide concentrations as well as the presence of potentially toxic compounds. Currently, the main disposal option for produced waters is deep well injection. However, this method was shown to induce seismicity in the regions with high injection rates.[16] Furthermore, regulations may not allow municipal wastewater treatment plants to accept produced waters in some areas such as Pennsylvania.[2] Therefore, an effective produced water treatment approach is essential for final disposal to water bodies. Nevertheless, the high salt and residual organic content in produced water creates issues during physical chemical treatment such as high energy requirements and fouling of the membranes. Reusing produced water can serve as an alternative to reduce the produced water disposal volumes and to decrease the water demand during the opening of new wells; however, produced water still needs to be treated prior to reuse. Specifically, it is necessary to remove organics that serve as electron donor for microorganisms that cause fouling, souring of the wells, and divalent cations that lead to precipitation and scaling. Biological treatment of produced water could be effective for reuse or by removing organics prior to physical chemical treatment for final disposal, therefore decreasing membrane fouling and the potential costs related to frequent membrane replacement. Previously, it was demonstrated that

biological treatment systems can decrease the COD content in produced waters[34, 35] (Chapter 3) and a decrease in COD was shown to increase subsequent membrane fluxes.[35]

The organic content of produced water can vary by wellsite depending on the additives in the fracturing fluid to regulate the pH, viscosity, scaling, microbial inhibition, etc. Previous results show that organic concentration in produced waters from shale plays can range between 1.2 – 5,804 mg/L TOC.[12, 13] In this study, TOC concentrations of 177 – 3,990 mg/L were measured in seven samples from two different shale plays (Bakken and Utica). Recent studies reported the most frequently used compounds in fracturing fluid to include methanol, isopropanol, ethylene glycol, ethanol, and glutaraldehyde.[1, 12] However, the overall composition of the organics in produced waters can change depending on the conditions in the shale play and fracturing fluid preferences of the industry. Here, the biodegradation potential in seven produced water samples from two shale plays, namely, Utica and Bakken, was investigated as measured by the fraction of Total Organic Carbon (TOC) removed. As a result of the high salinity concentration (at or above 170, 000 mg/L TDS), the biodegradation of these samples were evaluated after dilutions to 50,000 and 100,000 mg/L TDS using engineered biofilms.

5.2 MATERIALS AND METHODS

5.2.1 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 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 5.1 presents the characteristics of the collected samples. Samples were analyzed for total dissolved solids (TDS) and total dissolved organic carbon (TOC) content. Biocide information for these samples was collected using the FracFocus Chemical Disclosure Registry (www.fracfocus.org) for the individual wells.

5.2.2 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 $CaCl_2$). The growth media was seeded with 10% (v/v) of a mixed stock of produced water and activated sludge from municipal wastewater. After three weeks of biofilm growth, a 5x9 inch aluminum net screens were used to encase 32.5 ± 7.0 g_{wet} grass silage biofilms. The weight of the biofilms was recorded and biofilms were placed individually into 500 mL Erlenmeyer flasks. Prior to use, all biofilms were rinsed three times for 30 minutes in 50,000 mg/L TDS (35 g/L NaCl, 15 g/L $CaCl_2$, at 170 rpm) solution to remove any cultivation media carryover.

5.2.3 Experimental Procedure

Biodegradation experiments were conducted with seven produced water samples from Utica (6 samples) and Bakken (1 sample) Shales. The TDS concentrations of the samples were equal to or

above 170,000 mg/L. Previous experiments[34] (Chapter 3 in this dissertation) showed no biodegradation for TDS concentrations above 200,000 mg/L, therefore produced water samples were diluted to 50,000 and 100,000 mg/L TDS concentrations for biodegradation experiments. Since the dilution of TDS will also cause a dilution of TOC content, samples to be diluted to 50,000 mg/L TDS were selected from the ones with highest TOC concentrations: Utica S1, S2, S3, and S5 were selected to be diluted to 50,000 mg/L and 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 Blank-Utica S5 showed 83% and 86% reduction in TOC concentrations at the end of the experiments at 50,000 and 100,000 mg/L, respectively, suggesting that Utica S5 organic content is comprised of a large fraction of volatile organic compounds and hence the TOC content can volatilize without any biological treatment. Subsequently, the biodegradation data of Utica S5 was not included here.

5.2.4 Total Dissolved Organic Carbon (TOC) Analysis

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

5.2.5 Data Analysis and Rate Kinetics

TOC removal was reported as TOC_t/TOC_0, where TOC_t represents the TOC concentration measured at time *t*, TOC_0 is the initial TOC concentration of the sample after biofilm addition followed by a 30-minutes homogenization (mixing) period. First order degradation rates were calculated using Eqn. 5.1 and Eqn. 5.2:

$$\frac{dC}{dt} = k_{obs} * C \qquad \text{Eqn. 5.1}$$

$$ln\left(\frac{C_t}{C_0}\right) = k_{obs} * t \qquad \text{Eqn. 5.2}$$

Where C is the TOC concentration and C_t is the TOC concentration at time *t*. The slope of the linear fit of Eqn. 5.2 was reported as the observed rate constant (k_{obs}). The variable t_s was the time when TOC concentration no longer decreased in the solution for the following 48 hours (i.e., $TOC_{(ts)} \leq TOC_{(ts+24hour)}$ and $TOC_{(ts+48hour)}$), the first order rate kinetics were calculated between time 0 and time t_s. Final percent TOC removals were calculated using Eqn. 5.3:

$$Final\ TOC\ removal\ (\%) = \left[1 - \left(\frac{TOC_{ts}}{TOC_0}\right)\right] * 100 \qquad \text{Eqn. 5.3}$$

5.3 RESULTS AND DISCUSSION

A total of seven produced water samples were evaluated from Utica and Bakken Shale formations. The characteristics of these samples are presented in Table 5.1. Moreover, the biocide use information in these samples (using FracFocus reports) was collected as biocides can have an effect on biological degradation as demonstrated in Chapter 4 of this dissertation. The biodegradation experiments were conducted with produced waters diluted to 50,000 (Utica S1, S2, and S3) and 100,000 mg/L TDS (Utica S1, S2, S3, S4, S6, and Bakken). The Utica S5 sample was excluded due to volatilization of the available TOC. Dilution was necessary as a result of the inhibitory salinity of the produced water samples (TDS ≥ 170,000 mg/L in all samples) (Chapter 3).[34]

Table 5.1 Chemical characteristics of produced water samples.

Sample Name	Formation	Days after Fracturing	Biocide used	TDS (ppm)	TOC (ppm)
Bakken	Bakken	1122	2,2-dibromo-3-nitrilopropionamide[a]	285,000	353
Utica-S1	Utica	1549	Glutaraldehyde[b]	267,000	275
Utica-S2	Utica	1271	Glutaraldehyde	238,000	1,038
Utica-S3	Utica	1027	Glutaraldehyde[c]	256,000	483
Utica-S4	Utica	748	Glutaraldehyde[c]	220,000	177
Utica-S5	Utica	1092	Glutaraldehyde	251,000	3,990
Utica-S6	Utica	1346	Glutaraldehyde[d]	170,000	206

[*]**Solution 1:** 2,2-dibromo-3-nitrilopropionamide (20%), polyethylene glycol (55%), sodium bromide (4%), dibromoacetonitrile (3%).
[b]**Solution 2:** Glutaraldehyde (30%), didecyl dimethyl ammonium chloride (10%), quaternary ammonium compound (7%), ethanol (5%).
[c]**Solution 3:** Glutaraldehyde and methanol
[d]**Solution 4:** Glutaraldehyde (30-60%), alkyl (C12-16) dimethylbenzylammonium chloride (5-10%), ethanol (1%)

Figure 5.1 shows the TOC_t/TOC_0 as a function of time for Utica S1, S2, and S3 at 50,000 mg/L TDS. These three samples were selected for dilution up to 50,000 mg/L TDS due to their relatively high TOC concentration compared to other samples. First order biodegradation rates were calculated and are presented in Table 5.2 as described in Section 5.2.5. Our results did not show a strong correlation of the biodegradation rate with the initial TOC concentration (R^2 = 0.51) and total percent TOC removed (R^2 = 0.59).

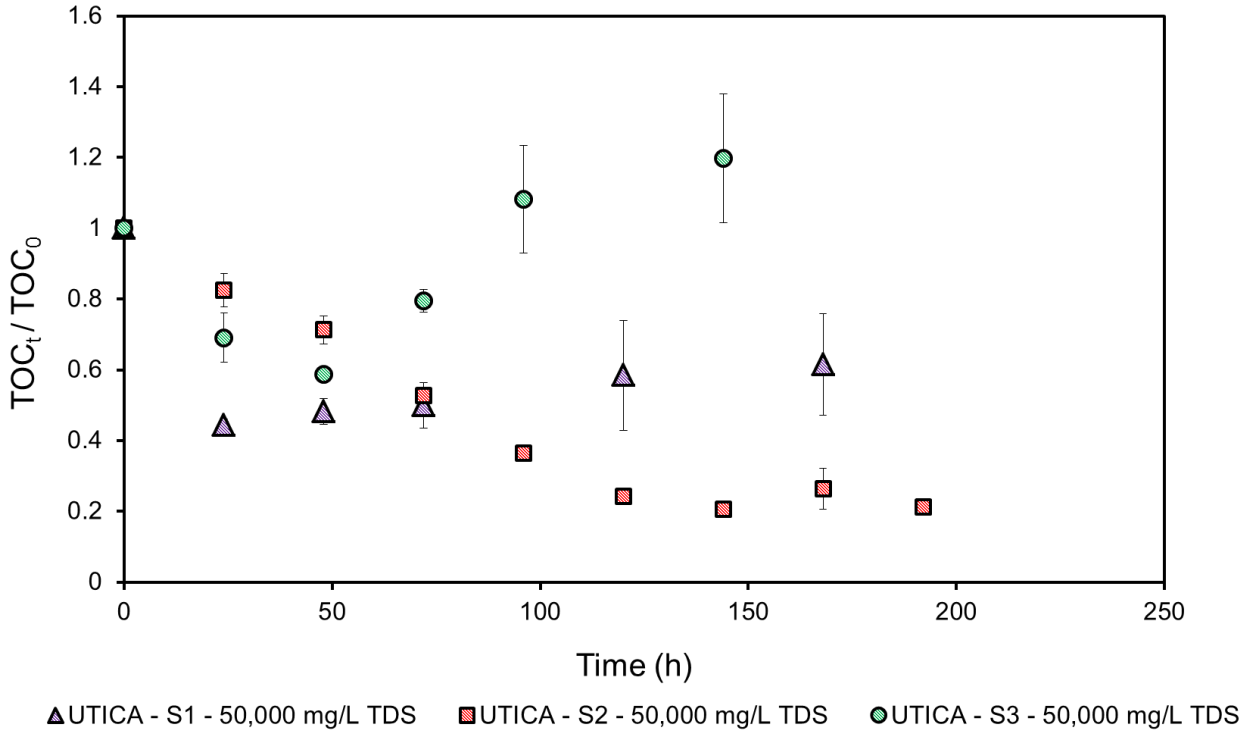

Figure 5.1 Removal of TOC as a function of time at 50,000 mg/L TDS produced waters. Error bars represent ±1 standard error.

The biodegradation results at 100,000 mg/L TDS are presented in Figure 5.2. The first order biodegradation rates are shown in Table 5.2. The biodegradation rates at 100,000 mg/L TDS decreased compared to the rates at 50,000 mg/L TDS, demonstrating that the salt concentration is a primary driver of produced water biodegradation. Moreover, variability in the biodegradation rates ranged between 0 and 0.0063 h^{-1} at 100,000 mg/L TDS, suggesting that there could be factors affecting biodegradation rates in addition to the TDS concentration, such as the composition of TOC, or presence of biocides (with changing concentration and composition) and other toxic compounds, and their co-contaminant interactions. The biodegradation rates did not show a strong correlation with the final TOC removed (%) or with the initial TOC concentration of these samples ($R^2 = 0.55$, $R^2 = 0.06$, respectively).

At both 50,000 and 100,000 mg/L TDS an increase in the remaining TOC fraction in the solution was observed in Utica S3 after 48 and 96 hours, respectively. This increase may suggest loss of biomass—that contributes to the total organic carbon in the Utica S3. Two potential causes that lead to the biomass loss could be lack of nutrients and trace minerals and/or the presence of toxic compounds. However, since the TOC increase occurred later in the more concentrated Utica S3 sample (i.e., 100,000 mg/L TDS Utica S3 is two times more concentrated than 50,000 mg/L TDS Utica S3), the presence of toxic compounds is not likely to be the reason for the loss of biomass in Utica S3.

Table 5.2 First order degradation kinetics for produced water samples. Errors (±) represent standard error.

Sample	TDS (mg/L)	Initial TOC (mg/L)	K_{obs}	R^2	Final TOC Removal (%)
Utica - S1	50,000	41 ± 4	0.0338	1	56 ± 2
Utica - S2	50,000	216 ± 11	0.0117	0.98	79 ± 0
Utica - S3	50,000	93 ± 5	0.0111	0.95	41 ± 2
Utica - S1	100,000	103 ± 7	0.0063	0.96	45 ± 1
Utica - S2	100,000	456 ± 25	0.0050	0.98	87 ± 1
Utica - S3	100,000	207 ± 26	0.0037	0.82	34 ± 12
Utica - S4	100,000	62 ± 0	0.0036	0.74	41 ± 6
Utica - S6	100,000	77 ± 0	0.0035	0.77	50 ± 2
Bakken	100,000	128 ± 2	0.0000	0.00	1 ± 0

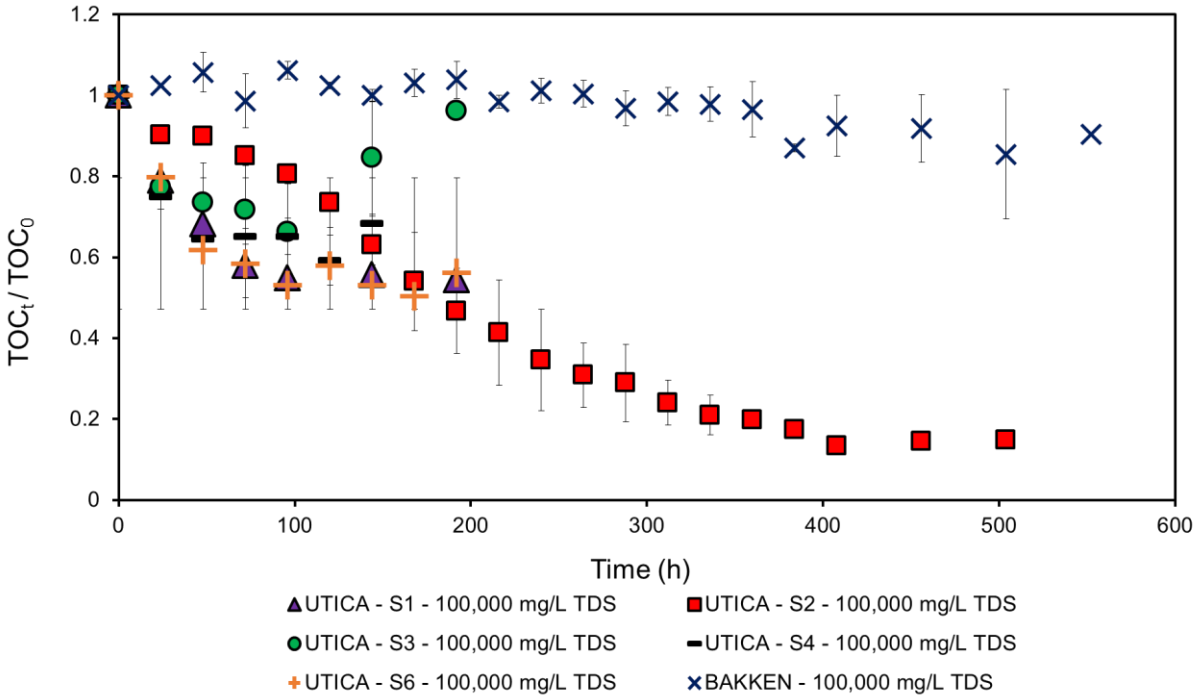

Figure 5.2 Removal of TOC as a function of time at 100,000 mg/L TDS produced waters. Error bars represent ±1 standard error.

The removal of organic matter from produced waters may be beneficial for reducing the cost of physical-chemical treatment (by decreasing membrane fouling)[35, 99] for final disposal and external reuse of the produced waters (e.g., livestock watering or irrigation[14]). A recent study investigated the biological treatment of produced water from Denver-Julesburg Basin using a sequencing batch reactor (SBR). Their results showed more than 50% DOC (dissolved organic carbon) removal after 6 hours in produced water with initial DOC concentration of 590 mg/L at 22,500 mg/L TDS.[58] High salinity is the biggest challenge in biological produced water treatment. A study reported results from a pilot scale trial of SBR treatment for produced water at 200,000 mg/L TDS and 130 mg/L TOC concentration.[100] Their results showed deteriorated TOC removal following several injections due to a loss of biomass in the effluent as a result of

poor settling. Our previous experiments with synthetic produced waters showed no COD removal at 200,000 mg/L TDS concentration using engineered biofilms (Chapter 3). The current study shows a negative correlation between salt concentration and biodegradation rate in produced waters from Utica and Bakken shale formations. To the best of our knowledge, this study examined the highest number of produced water samples in terms of biodegradation potential with a biofilm process.

For an effective biological treatment of produced waters, the process evaluation should include understanding the chemical characteristics of the produced water to be treated, such as salt and TOC concentrations or the BOD_5/COD ratio[66] as a basis for a tailored biological treatment design. Our results can help to understand the potential of biological treatment for produced waters with varying chemical characteristics (e.g., salt concentration), and serve as a starting point during pilot-scale biological treatment tests using biofilm processes.

5.4 SUMMARY AND CONCLUSION

In this chapter, the biodegradation potential of seven produced water samples from the Bakken and Utica Shale was investigated. Our results show a negative correlation between the first order biodegradation rates and the salt concentration of the produced waters. We did not observe a strong correlation of the biodegradation rates with either the initial TOC concentration or the final percent TOC removal of the samples. Moreover, it should be noted that there was a substantial variability in the biodegradation rates of the produced water samples at the same salinity concentration, suggesting that there are other parameters affecting the biodegradation rate. One of these parameters can potentially be the organic content of these produced waters. It

is known that varying chemical formulations are used in fracturing fluids depending on the formation characteristics. A previous study[19] and Chapter 4 in this dissertation showed that the presence of biocides can significantly affect the biodegradation rates of different compounds. The next step in this area of research could be the combination of possible biological and physical-chemical treatment methods for a number of produced waters from different wells with changing salt and organic concentrations to potentially create a framework for preliminary treatment design concepts.

6.0 RADIUM-226 BIOSORPTION BY THE HALOPHILIC MICROALGAE *DUNALIELLA SALINA* IN PRODUCED WATER FROM THE MARCELLUS SHALE

In this study we evaluated the growth of the halophilic microalgae *Dunaliella salina* in produced water from the Marcellus Shale and the application of *D. salina* biomass for the removal of radium-226 (Ra-226) from produced water. Growth of *D. salina* in produced water was significantly lower than in optimized growth media. Ra-226 removal using *D. salina* biomass was highest at alkaline pH and low salinity. Autoclaved biomass was found to out-perform viable biomass, suggesting biosorption, rather than bioaccumulation, to be the primary Ra-226 removal mechanism. Tests in Marcellus Shale produced water demonstrated Ra-226 biosorption of 3,935 – 5,764 $pCi/gram_{dry\ weight}$ with a trend of decreasing biosorption with increasing salinity. Results from this study suggest that biosorption for Ra-226 removal holds the greatest potential in lower salinity waste streams with high Ra-226 levels.

6.1 INTRODUCTION

Developments in horizontal drilling and hydraulic fracturing have increased production of shale gas and oil from unconventional reservoirs.[10, 101] The fluid that returns to the surface during well completion and operation is referred to as "produced water".[6] Produced water typically contains high concentration of salts originating from underground sediments or formation brine,[31]

resulting in treatment and disposal challenges. Currently, disposal options for produced water rely heavily on deep well injection; however, this approach has been linked to induced seismicity [16] and more sustainable alternatives are required in the long term.

Produced water may contain Naturally Occurring Radioactive Material (NORM) originating from the subsurface formations.[31] Among NORM, the alkaline earth metal radium has raised concerns due to its high concentration in produced water, radioactive properties, and half-life of 1,620 years. Marcellus Shale produced waters have been previously demonstrated to have high total radium activity, reaching up to 10,000 pCi/L.[39] The reported median radium activities in Marcellus and non-Marcellus shales are 2,460 pCi/L and 734 pCi/L, respectively.[39] The industrial discharge limit for radium in the U.S. is 60 pCi/L[102] and the maximum contaminant level (MCL) reported in the National Primary Drinking Water Regulations for combined concentration of Ra-226 and Ra-228 is 5 pCi/L.[38] The two isotopes primarily contributing to the total radium activity in produced waters are Ra-226 and Ra-228. The ratio of Ra-228/Ra-226 in Marcellus Shale produced water is less than 0.3,[39] emphasizing the importance of Ra-226 in NORM management. Additionally, accumulation of Ra-226 in the produced water may occur through reuse of produced waters to fracture new wells.[44] For instance, the Ra-226 concentration in holding pond sludges has been reported to increase over the pond's lifetime.[44] Moreover, Ra-226 accumulates in sediments at the discharge points of centralized waste treatment facilities treating produced water, with levels exceeding disposal limits for municipal landfills.[9] Therefore, efficient removal of Ra-226 is desirable for either reuse or surface disposal of produced water.

Biosorption is a treatment technology to remove undesirable aqueous constituents via passive binding onto a cell surface.[103-105] Various biomass types, such as fungi, bacteria, algae,

and seaweed have been used for removal of metals and radioactive materials.[21, 103, 106-112] Ra-226 biosorption has not been as widely studied as heavy metals or other radionuclides such as uranium; however, previous studies have shown promising results for further evaluation of Ra-226 biosorption.[21, 45, 113, 114]

Dunaliella salina is a halophilic unicellular alga capable of tolerating salinity concentrations up to 5 M.[115, 116] Here, *D. salina* was evaluated due to its salinity tolerance, suggesting the potential for growth in hypersaline produced water. Additionally, ~40% of *D. salina*'s dry weight is composed of proteins[117] which could play an important role in biosorption.[114, 118, 119] Finally, *D. salina* can produce extracellular polymeric substances (EPS) containing amine groups[120, 121] which can serve as sites for biosorption together with carboxyl groups in amino acids.[105, 114, 118, 119]

In the current study, first the potential for *D. salina* growth was examined in produced water. Next, *D. salina* biomass for Ra-226 biosorption in synthetic produced water was evaluated, and the impact of solution chemistry, such as pH and salinity, on Ra-226 biosorption was determined. Observed trends were then confirmed by evaluating radium biosorption by *D. salina* biomass in real produced water samples. Finally, adsorption isotherm models were proposed for Ra-226 removal by *D. salina* biomass.

6.2 MATERIALS AND METHODS

6.2.1 Culture Growth and Biomass Preparation

Dunaliella salina (UTEX # LB 1644) was obtained from the University of Texas culture collection (UTEX Culture Collection, TX) and cultured in 2XErdschreiber's growth medium (UTEX Culture Collection, https://utex.org/products/2x-erdschreibers-medium) in a 2L bioreactor (UTEX Culture Collection, TX). Algae cells were exposed to an 8:16 hour light:dark cycle with a light intensity of 2000 lux and continuous air flow. For biosorption experiments, algae suspension at an optical density (OD_{680nm}) of 8 was centrifuged (Sorvall Legend X1R, Thermo Scientific) at 4^{o}C and 10,000 rpm for 15 minutes. Harvested biomass was washed three times with deionized water (Synergy-R purification system with 18.2 MΩ resistance) and either stored or autoclaved and kept at 4^{o}C to be used in future biosorption experiments.

Growth rate (μ) calculated as the slope of the natural logarithm of the optical density (lnX) as a function of time (t) using Eq. 6.1.

$$lnX = lnX_0 + \mu t \tag{6.1}$$

6.2.2 *D. salina* Growth in Produced Water

Initially, two produced water samples (S1 and S2) and their 50% dilutions ($S1_{1:2dilution}$ and $S2_{1:2dilution}$) were used to culture *D. salina*. Chemical composition of these samples is provided in Table 6.1. Produced water samples were supplemented with alkalinity (5 mg/L as $CaCO_3$) and

nutrients (0.67 mL/200 mL from stock solutions of 0.7 M $NaNO_3$ and 0.02 M $Na_2HPO_4.7H_2O$) based on the chemical concentrations in 2XErdschreiber's growth medium. Samples were initially inoculated with algae suspension to reach an initial OD_{680nm} of 0.02 in 250 mL autoclaved flasks and mixed continuously at 150 rpm while exposed to 8:16 hours light:dark cycle with a light intensity of 2000 lux for 18 days. The pH of the samples was maintained at 7.8±0.2. OD_{680nm} measurements and cell counts (Hausser Bright Line, PA) were performed throughout the inoculation period. 2XErdschreiber's growth medium was used as the control growth condition. All experiments were completed in triplicate.

Table 6.1: Chemical characteristics of produced water samples S1 and S2 used for *D. salina* cultivation.

Component	**S1**	**S2**
Na^+ (mg/L)	17,060	47,107
Ca^{2+} (mg/L)	3,688	16,509
Ba^{2+} (mg/L)	1,386	328
Sr^{2+} (mg/L)	680	1,888
Mg^{2+} (mg/L)	373	1,820
Fe^{2+} (mg/L)	ND	19
SO_4^{2-} (mg/L)	ND	7.7
Cl^- (mg/L)	33,000	115,277
pH	6.5	5.9
Alkalinity (mg/L as $CaCO_3$)	245	46
Days after fracturing	7	20

*ND: not detected

6.2.3 Biosorption in Synthetic Media

Ra-226 removal with fresh and autoclaved *D. salina* biomass was evaluated with varying pH, salt, and biomass concentrations. An adsorption time of six hours was chosen based upon preliminary experiments (Figure B1, Appendix). 50 mL polypropylene falcon tubes were used to minimize Ra-226 sorption on the container surfaces.[21] A $RaCl_2$ stock solution with Ra-226 activity of 1,155 ± 167 pCi/mL was used to provide an initial Ra-226 concentration of 3,000 pCi/L and the sample pH after the addition of $RaCl_2$ was 5 ± 0.3.

Samples were centrifuged (10,000xg for 5 minutes) prior to biomass addition to remove any impurities. Unless otherwise stated, biomass concentrations of 0.04 $g_{dry\ weight}$/L were utilized. Each experimental condition was run in triplicate with duplicate measures made for Ra-226 activity, as well as controls without biomass for each set of experimental conditions. Following adsorption, 10.5 mL was withdrawn from each sample and centrifuged (4^{o}C, 10,000 rpm for 7 minutes) to remove the biomass. The Ra-226 concentration of the supernatant was measured using a liquid scintillation counting (LSC).

A modified approach of Ba-Ra-SO_4 precipitation method[42] was used for LSC sample preparation. Briefly, following biomass removal by centrifugation, samples were put into 50 mL Teflon beakers (Chemglass Life Sciences, Vineland, NJ) and 1.82 mL of 20 mM $BaCl_2$ were added to provide a 5 mg Ba^{2+} carrier in the solution.[122] After, 20 mL of 1 M H_2SO_4 were added and the samples were heated at 50^{o}C for 1 hour to complete Ba-Ra-SO_4 precipitation. Samples were filtered through 0.45 µm filter paper (Millipore MF-EMD, Billerica, MA). The filtrate was transferred into glass vials (Econo Glass Vials, Perkin Elmer, 20 mL) using 2 mL of 0.25 M EDTA solution (pH was previously adjusted to 9). Vials were heated at 50^{o}C until the solution became clear and allowed to cool to room temperature. Finally, 14 mL of Ultima Gold universal

LSC cocktail (PerkinElmer) was added for the analysis in LSC. Ra-226 activity was analyzed with a Beckman LS 6500 Liquid Scintillation Counter. Measurements were completed within 6 hours from the addition of the LSC cocktail to prevent any interference that might occur due to longer exposure of the samples to the cocktail. The counting time was set to 40 minutes in the specific energy range of 170-230 keV to avoid the interferences caused by other elements.[42, 122]

6.2.4 Biosorption in Produced Water

Four produced water samples from three fracturing sites (Site 1, Site 2, and Site 3) were used. Sample characteristics and chemical composition are provided in Table 6.2. Biosorption experiments were conducted as detailed above. Atomic absorption spectrometry (AAS) was used to measure cation concentrations in the produced water samples. An AAS background solution of 2.3% HNO_3 and 1.5 g/L KCl was used for dilutions.[10] Ion chromatography (Dionex ICS-1100) with the inorganic anion column Dionex Ion-Pac A22 was used for the detection of anions.

Gamma spectrometry was used to determine Ra-226 activity. For gamma spectrometry, liquid samples were centrifuged at 4^{o}C, 10,000 rpm for 7 minutes to remove biomass, then transferred into Teflon bottles and dried in the oven. The dry residuals were transferred into 46 mm diameter petri dishes and grounded to achieve a homogeneous 1 mm thickness (equal to 2 gram dry residual) to eliminate potential impact of sample geometry on gamma spectrometry. Ra-226 content in produced water samples was analyzed using a high resolution Apex Gamma Spectrometry System (Ortec) with a High-Purity Germanium (HPGe) detector. Ra-226 activity was measured by detecting gamma ray emissions at 186 KeV peak.

Table 6.2 Produced water composition for Ra-226 biosorption experiments.

Sample	**Site 1**		**Site 2**	**Site 3**
	Day 5	**Day 15**	**n.a.**	**Day 7**
TDS (mg/L)	54,915	94,005	308,334	92,800
Cl^- (mg/L)	29,653	52,640	188,728	63,588
Ba^{2+} (mg/L)	1,405	2,687	6,256	3,743
Sr^{2+} (mg/L)	651	1,127	11,910	1,620
Ca^{2+} (mg/L)	3938	6,292	32,901	6,523
Na^+ (mg/L)	33,200	56,230	81,442	26,427
Mg^{2+} (mg/L)	381	630	2,664	675
pH	7.4	7.5	3.09	7.29
Ra-226 (pCi/L)	889.5 ± 84.8	1,968 ± 114	14,407 ± 968	1,413 ± 131
Ra-226 Removal ($pCi/g_{dry\ weight}$)	3,935	5,764	No uptake	5,372

n.a.: Not available

6.2.5 Data Analysis

Ra-226 removal in the biosorption experiments was calculated using Eqn. 6.2:

$$q = \frac{(C_0 - C_s) * V}{M * 1000} \qquad \text{Eqn. 6.2}$$

where q ($nCi/g_{dry\ wt}$) is Ra-226 removal per dry weight of *D. salina*, C_0 (pCi/L), and C_s (pCi/L) are the Ra-226 concentrations at equilibrium in control (no biomass) and in sample, respectively, V (L) is sampling volume, and M ($g_{dry\ wt}$) is dry biomass weight. Statistical analyses were performed using Minitab 7 Statistical Software. A two-sample t-test (not assuming equal variances) and one-way ANOVA analysis (equal variances assumed) were used with a 95% confidence interval. Experimental isotherm results were fitted with Freundlich and Langmuir adsorption isotherm models.

Linearized Langmuir and Freundlich model equations were used for the determination of isotherm constants, Eqns. 6.3 and 6.4, respectively.

$$\frac{Ce}{qe} = \frac{1}{q_{max} * b} + \frac{1}{q_{max}} * Ce \qquad \text{Eqn. 6.3}$$

where q_{max} represents the maximum adsorption capacity and b is described as the ratio of adsorption/desorption rate.[123]

$$log(qe) = log(K_F) + \frac{1}{n} * log(Ce) \qquad \text{Eqn. 6.4}$$

where K_F and n are the Freundlich constants associated with the adsorption capacity and intensity of the adsorption, respectively.

6.3 RESULTS AND DISCUSSION

Radium is the primary source of NORM in Marcellus Shale produced water.[39] The need for on-site, cost-effective strategies for treatment, disposal, and reuse of produced water is well recognized.[34] Our study investigated the growth of *D. salina* in produced water from hydraulic fracturing and the removal of Ra-226 from produced water using biosorption on *D. salina* biomass as potential mechanisms of biological produced water treatment.

Initial growth experiments were conducted with *D. salina* to evaluate the growth potential in two produced water samples (Table 6.1) along with 50% dilutions of each sample. The samples were amended with nutrients and growth was only observed in half diluted produced water sample S2 ($S2_{1:2dilution}$) (Statistical analysis is presented in Table B1, Appendix).

Figure 6.1 shows the growth of *D. salina* in control media and $S2_{1:2dilution}$ over 18 days. *D. salina* growth was measured using optical density and cell counts. Correlation coefficients (r^2) between OD_{680nm} and cell counts were 0.97 and 0.69 for control and $S2_{1:2dilution}$, respectively (Figure 6.2). It was observed that the cells grown in $S2_{1:2dilution}$ aggregated into cell clusters but no clustering occurred in the control condition. This likely resulted in the observed difference in the correlation of OD_{680nm} and cell count for the control and $S2_{1:2dilution}$ conditions. Results showed significantly lower growth in $S2_{1:2dilution}$ compared to the control ($p < 0.001$). The specific growth rate for control and $S2_{1:2dilution}$ (~90,000 mg/L TDS) was calculated to be 0.163 and 0.115 day^{-1}, respectively (Figure 6.3). Repeated attempts to improve algal growth in produced water were unsuccessful. Our findings suggest limited potential for *D. salina* growth in Marcellus Shale produced water. In agreement with our findings, a recent study showed the growth rate of *D. salina* in produced water to be the lowest among the three microalgal strains evaluated at a TDS concentration of 40,000 mg/L, regardless of the nutrient concentration.[124] Earlier studies have reported the optimum salt concentration for *D. salina* growth to be 12% NaCl;[125, 126] however, as previous studies reported salt concentrations in terms of NaCl only, they fail to explain the impeded *D. salina* growth in produced waters containing multiple ionic species. Both the presence of potentially toxic ions and residual additives from the fracturing process, including biocides, may have inhibited algal growth.[6, 50, 127]

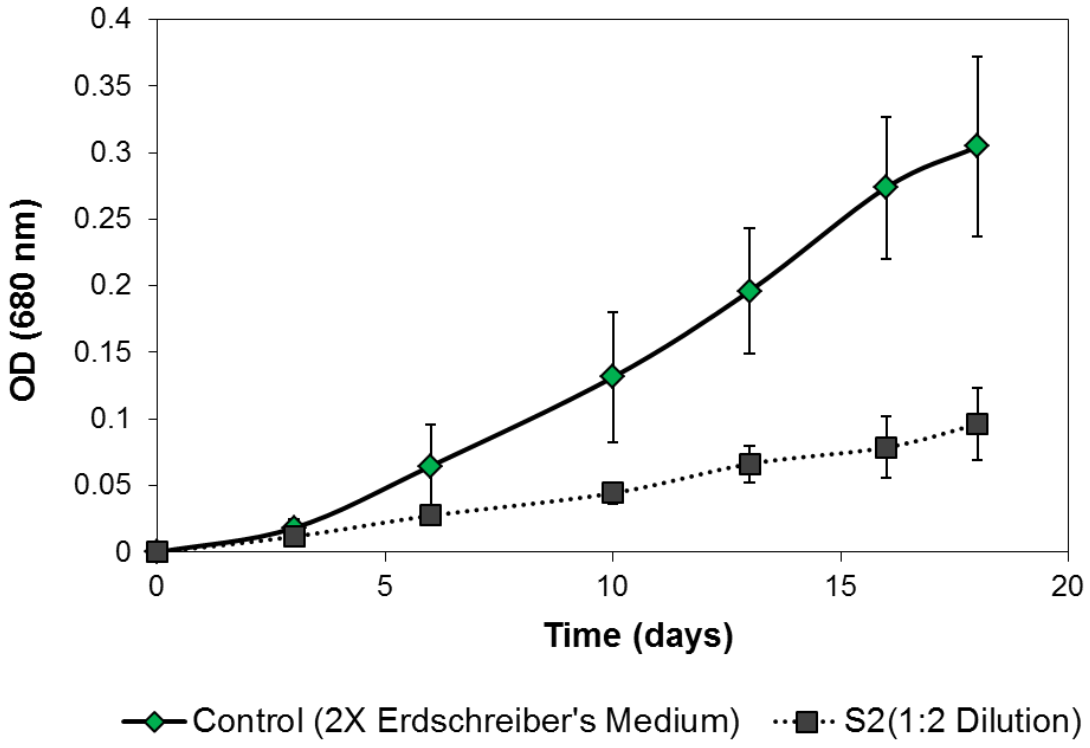

Figure 6.1: *D. salina* growth in 2X Erdschreiber's medium and a one-half dilution of produced water sample S2. Error bars represent ±1 standard deviation.

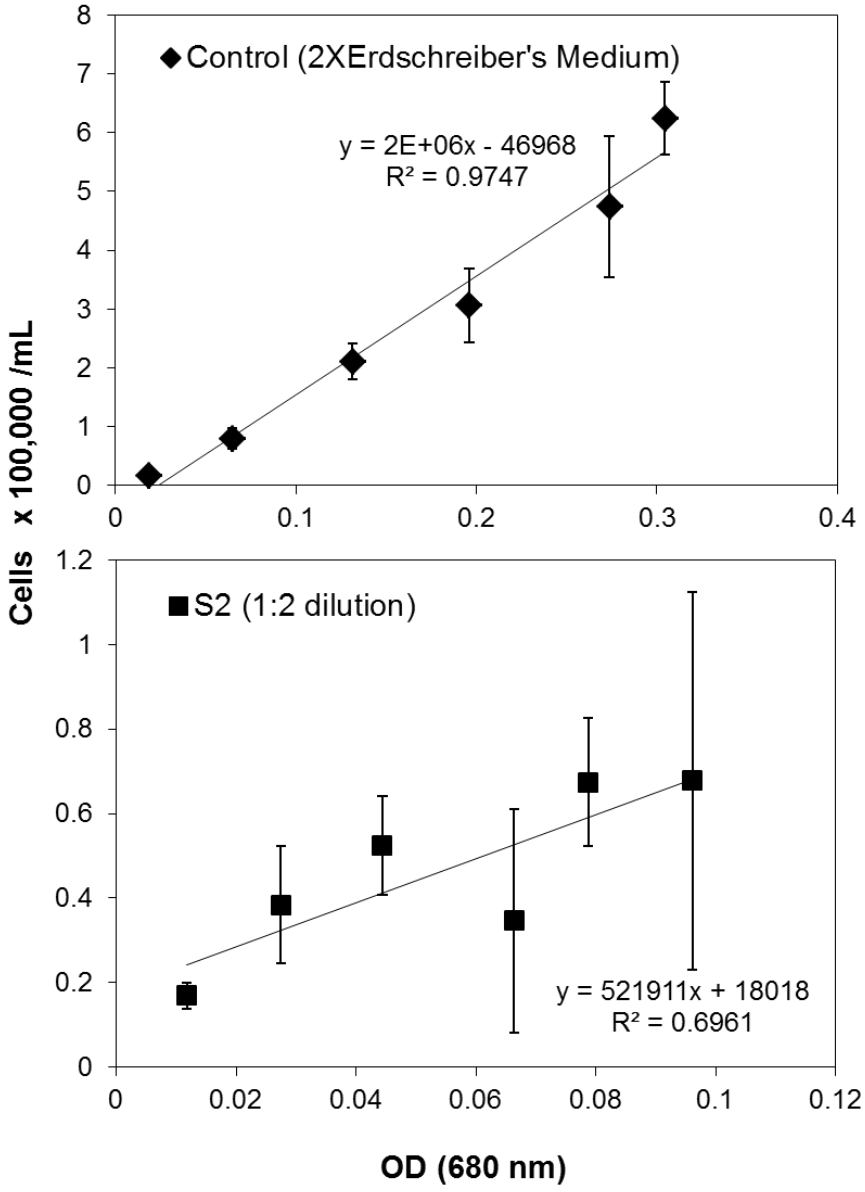

Figure 6.2: Cell counts as a function of optical density at 680 nm. Error bars represent ±1 standard deviation.

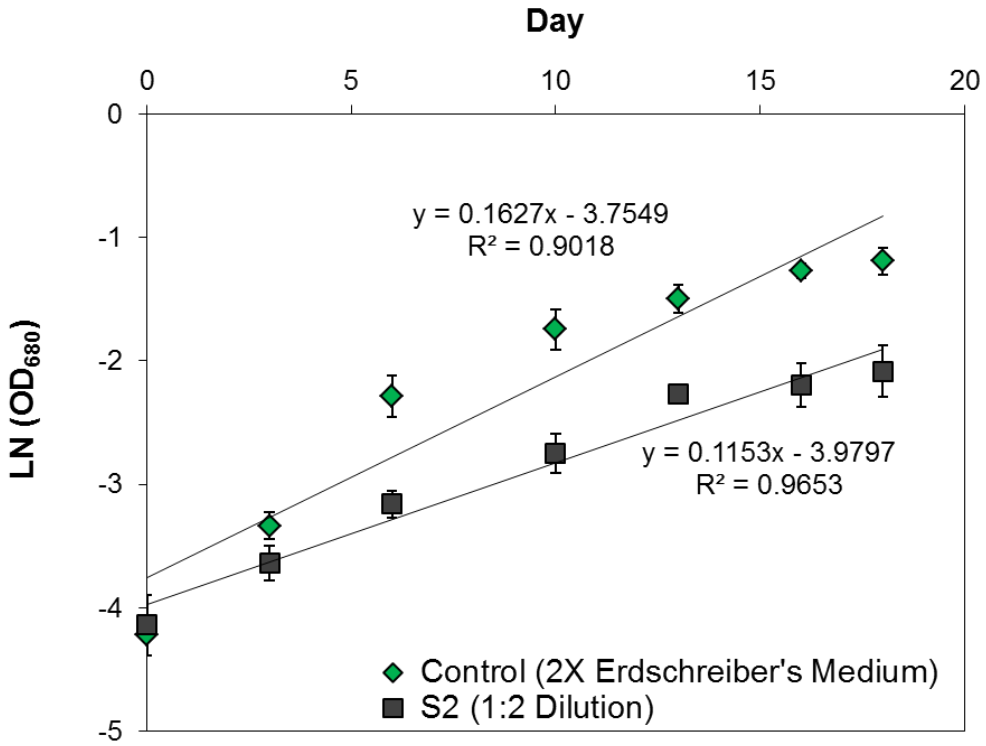

Figure 6.3: Specific growth rate of control and $S2_{1:2dilution}$. Error bars represent ±1 standard deviation.

Ra-226 biosorption on *D. salina* was first evaluated in synthetic produced water. Ra-226 removal was tested at pH 3, 5, 9, and 11, with both fresh and autoclaved biomass. Controls were performed at pH 5 with no biomass (Figure B2, Appendix). Remaining Ra-226 activity was negatively correlated with pH for both fresh and autoclaved biomass (Figure 6.4). For fresh biomass, at an initial Ra-226 activity of 3,000 pCi/L, the highest remaining Ra-226 activity in the solution was observed at pH 3 (2,913 ± 242 pCi/L) and the lowest remaining Ra-226 activity was observed at pH 9 (1,577 ± 171 pCi/L). For autoclaved biomass, at an initial Ra-226 activity of 3,000 pCi/L, remaining Ra-226 activities of 2,228 ± 455 pCi/L and 373 ± 162 pCi/L were observed at pH 3 and 11, respectively. Increased Ra-226 biosorption at alkaline pH has been

reported previously.[21, 114] Negatively charged carboxyl groups are protonated at low pH conditions, decreasing their metal binding capacity.[118, 119] A slightly acidic pH of 5 was used in the following experiments, as neutral and alkaline pH can increase Ra-226 binding on the container walls.[21]

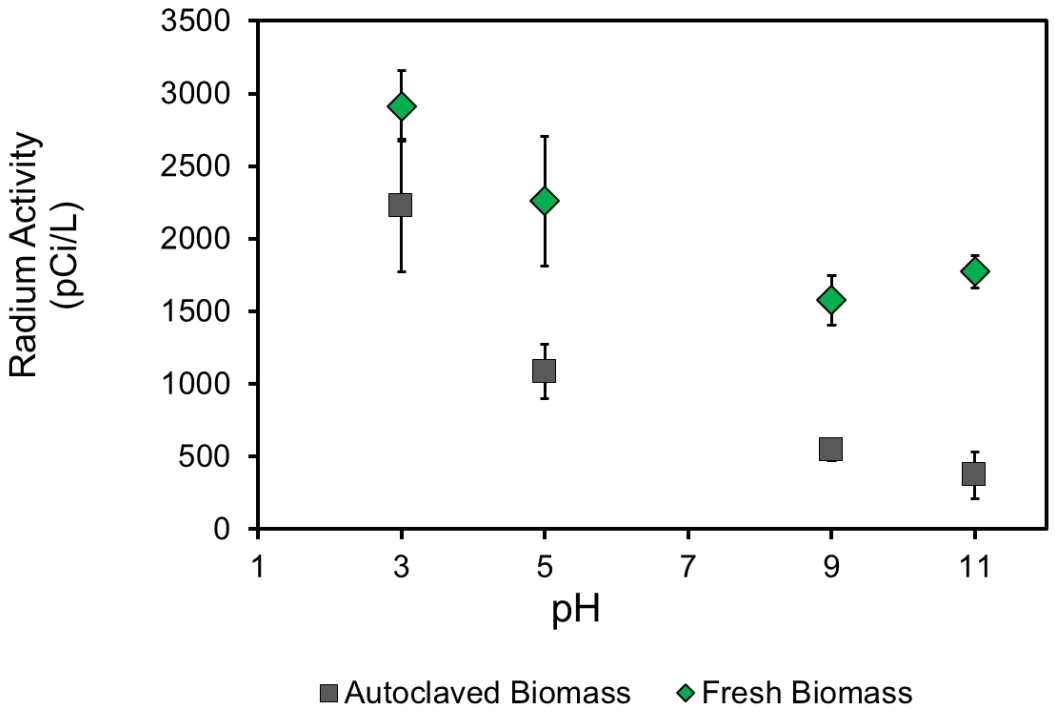

Figure 6.4: Remaining Ra-226 activity (Cs) as a function of pH at equilibrium**.** Error bars represent ±1 standard deviation. (Biomass concentration = 0.04 $g_{dryweight}$/L, initial Ra-226 concentration = 3,000 pCi/L, TDS = 0 mg/L).

Ra-226 biosorption was evaluated at 0, 20,000, 50,000, 100,000, and 200,000 mg/L NaCl concentrations at an initial Ra-226 concentration of 3,000 pCi/L (Figure 6.5). The salt concentration range was selected to be comparable to salinity ranges in produced water from

different shale formations.[39] Controls were performed at 0 mg/L salt concentration with no biomass (Figure B3, Appendix). A positive correlation was observed between remaining Ra-226 activity and salinity concentration. In fresh biomass, the remaining Ra-226 activity increased from 1,041 ± 62 pCi/L at 0 mg/L TDS to 2,524 ± 141 pCi/L at 20,000 mg/L TDS. In autoclaved biomass, the remaining Ra-226 activity at 0 mg/L TDS (890 ± 86 pCi/L) was increased more than two times at 20,000 mg/L, reaching 2,098 ± 210 pCi/L. No Ra-226 removal was observed at 200,000 mg/L TDS (Ra-226 = 3,109 ± 282 pCi/L).

Previous studies suggested that divalent alkaline earth metal ions form surface complexes that bond weakly with the hydroxide surfaces[47] and therefore can be replaced by Na^+ ions.[48] Na^+ competition with alkaline earth metals for the active adsorption sites was previously investigated with Ba^{2+} adsorption on montmorillonite.[48] This study identified a negative correlation between Ba^{2+} adsorption and ionic strength.[48] This prior work suggests that Na^+ ions may compete with Ra^{2+} for the active adsorption sites.

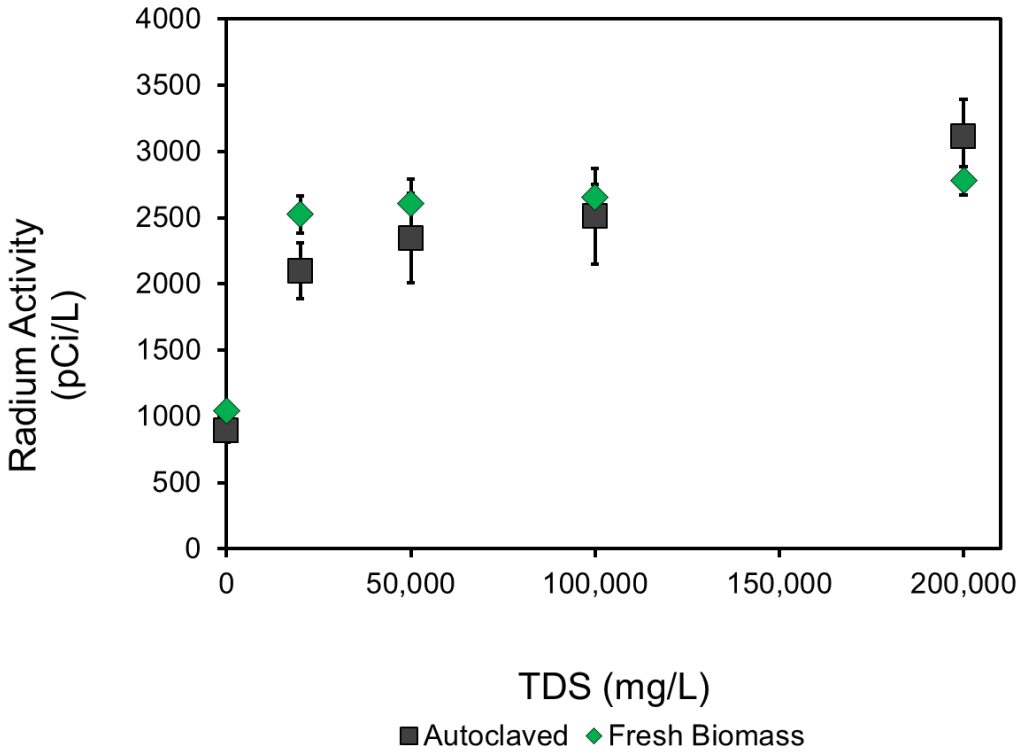

Figure 6.5: Remaining Ra-226 activity (Cs) as a function of TDS at equilibrium**.** Error bars represent ±1 standard deviation. (Biomass concentration = 0.04 $g_{dryweight}$/L, initial Ra-226 concentration = 3,000 pCi/L, pH = 5 ± 0.3).

Our experiments demonstrated a higher Ra-226 removal by autoclaved (non-viable) biomass than viable biomass under multiple test conditions (Figure 6.4 and Figure 6.5, $p < 0.001$ for pH and $p = 0.583$ for salinity). Autoclaving has been previously shown to be an effective method for cell disruption that increases available biomass surface area.[128] Previous studies, in general, have shown viable biomass to be more effective than non-viable biomass for biological metals removal. The effectiveness of biomass depends on the type of adsorbate, growth medium, and biomass pre-treatment methods (e.g. chemical treatment, oven drying, autoclaving)[129] as well as the role of bioaccumulation with viable biomass.[130, 131] Bioaccumulation is dependent on

the metabolic activity of cells, whereas biosorption is a physicochemical process.[130] Our results with higher radium removal by autoclaved biomass suggest biosorption, rather than bioaccumulation, to be the predominant Ra-226 removal mechanism with *D. salina* biomass. While the effectiveness and feasibility of various biomass treatment methods for large-scale applications could benefit from further investigation, we continued using only autoclaved biomass to evaluate Ra-226 biosorption.

Next, the effect of biomass concentration in the range from 0.02 to 0.16 g_{drywt}/L on Ra-226 biosorption was investigated (Figure 6.6). Remaining Ra-226 activity at equilibrium decreased from 2,081 ± 145 pCi/L to 444 ± 254 pCi/L when the biomass concentration increased from 0.02 g_{drywt}/L to 0.16 g_{drywt}/L. This result is consistent with previous studies.[111, 132-135] Ra-226 removal increased from 44% at 0.02 g_{drywt}/L biomass concentration to 81% at 0.16 g_{drywt}/L biomass concentration. Similar findings have been reported in previous biosorption studies.[111, 132, 133, 136, 137]

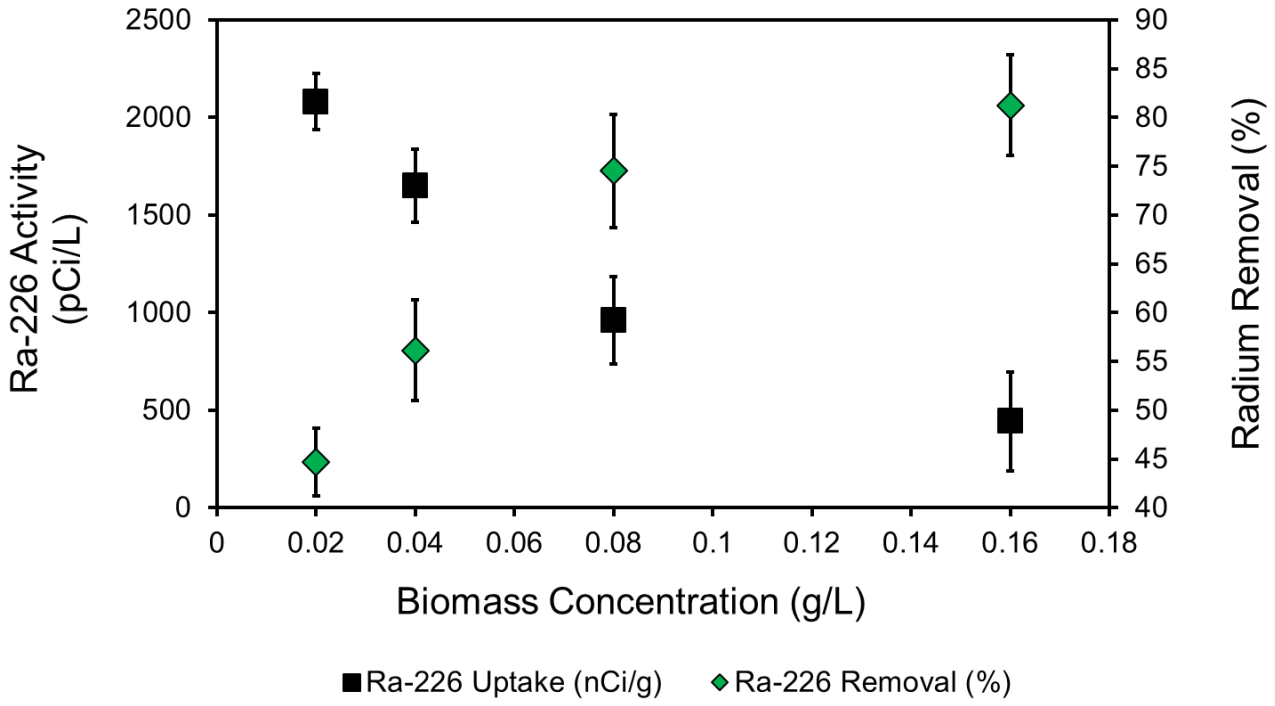

Figure 6.6: Remaining Ra-226 activity and % Ra-226 removal at equilibrium as a function of autoclaved *D. salina* biomass concentration. Error bars represent ±1 standard deviation (Initial Ra-226 concentration = 3000 pCi/L, pH= 5.0 ±0.3, TDS = 0 mg/L).

Two parameter isotherm models (Freundlich and Langmuir) were evaluated for Ra-226 removal. Figure 6.7 shows the non-linear equilibrium isotherms for Ra-226 biosorption with autoclaved *D. salina* biomass. The isotherm constants are presented in Table 6.3. In Figure 6.7, non-linear Freundlich and Langmuir models demonstrated similar fits to the experimental data. Moreover, it should be noted that the residual Ra-226 concentration range evaluated in this study is likely to be located in the initial rise of the biosorption isotherm curves; therefore we advise caution when using these isotherm constants for concentration values above the experimental range.

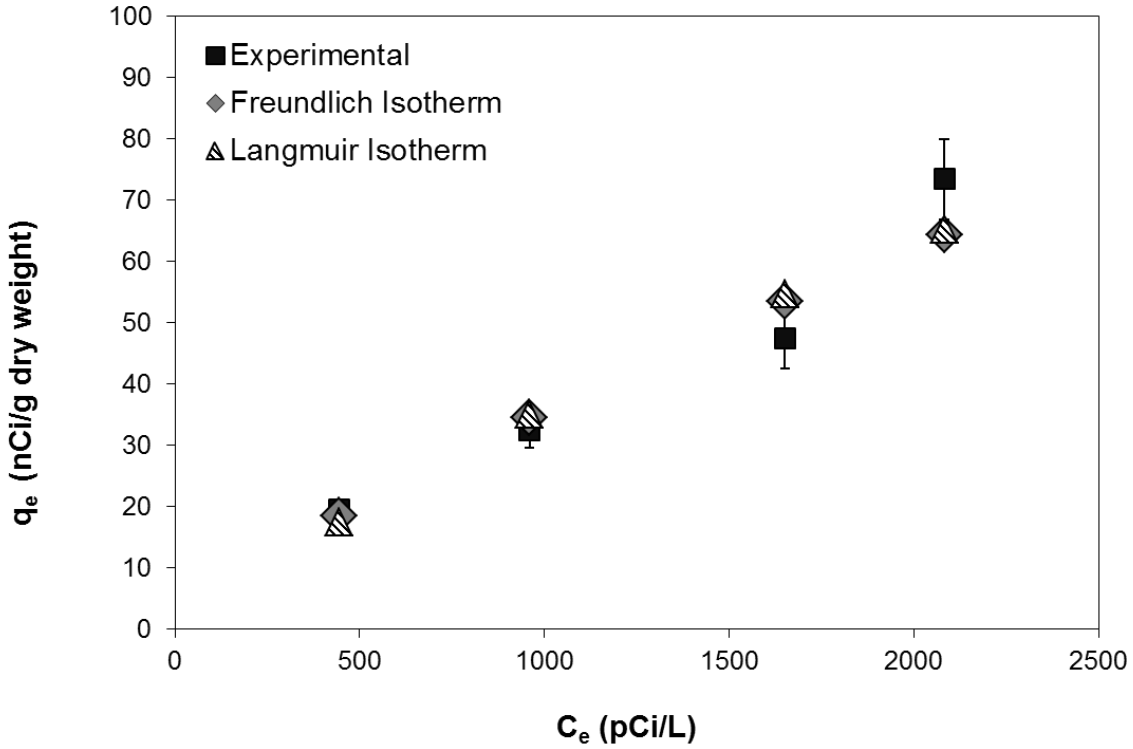

Figure 6.7: Equilibrium isotherms for Ra-226 biosorption with autoclaved *D. salina* biomass. Error bars represent ±1 standard deviation (Initial Ra-226 concentration = 3,000 pCi/L, pH= 5.0 ±0.3, 0.02-0.16 g/L biomass concentration).

Table 6.3: Biosorption isotherm model parameters for Ra-226 biosorption on *D. salina* biomass (Initial Ra-226 concentration = 3000 pCi/L, pH = 5 ± 0.3).

Model	**Parameters**	**Value**
Freundlich Isotherm	K_F (pCi/g)(pCi/L)^n	135
	n	1.239
Langmuir Isotherm	q_{max} (pCi/g)	2.50×10^5
	b (L/pCi)	1.69×10^{-4}

Ra-226 biosorption with autoclaved biomass was investigated with four produced water samples, two of which were taken from the same well located in southwest Pennsylvania (Site 1). Site 1 was sampled at day 5 (Site1-D5) and day 15 (Site1-D15) following fracturing. Site 2 and Site 3 samples were from north and southwest Pennsylvania, respectively. Site 3 was sampled at day 7 following fracturing while information on well age was not available for Site 2. Table 6.2 presents the chemical characteristics and Ra-226 removal in the samples. Site 3 and Site1-D15, having similar TDS and Ra-226 concentrations, showed Ra-226 biosorption of 5,372 and 5,764 pCi/g $_{\text{dry weight}}$, respectively (Table 6.2). Site1-D5 with a lower TDS and Ra concentration compared to Site1-D15 and Site 3 demonstrated lower Ra-226 biosorption of 3,935 pCi/g $_{\text{dry weight}}$; however, the difference was not statistically significant ($p = 0.944$). Figure 6.8 shows the initial and final Ra-226 activity and percent Ra-226 removal in the produced water samples from three sampling sites. Ra-226 removal of four samples was in the range of 0-18%. The differences in the percent Ra-226 removal in Site1-D5, Site1-D15, and Site 3 were not statistically significant ($p = 0.925$). Moreover, Site 2 had a salinity concentration of 308,334 mg/L TDS, which is much higher than the previously tested 200,000 mg/L TDS where no Ra-226 removal was observed.

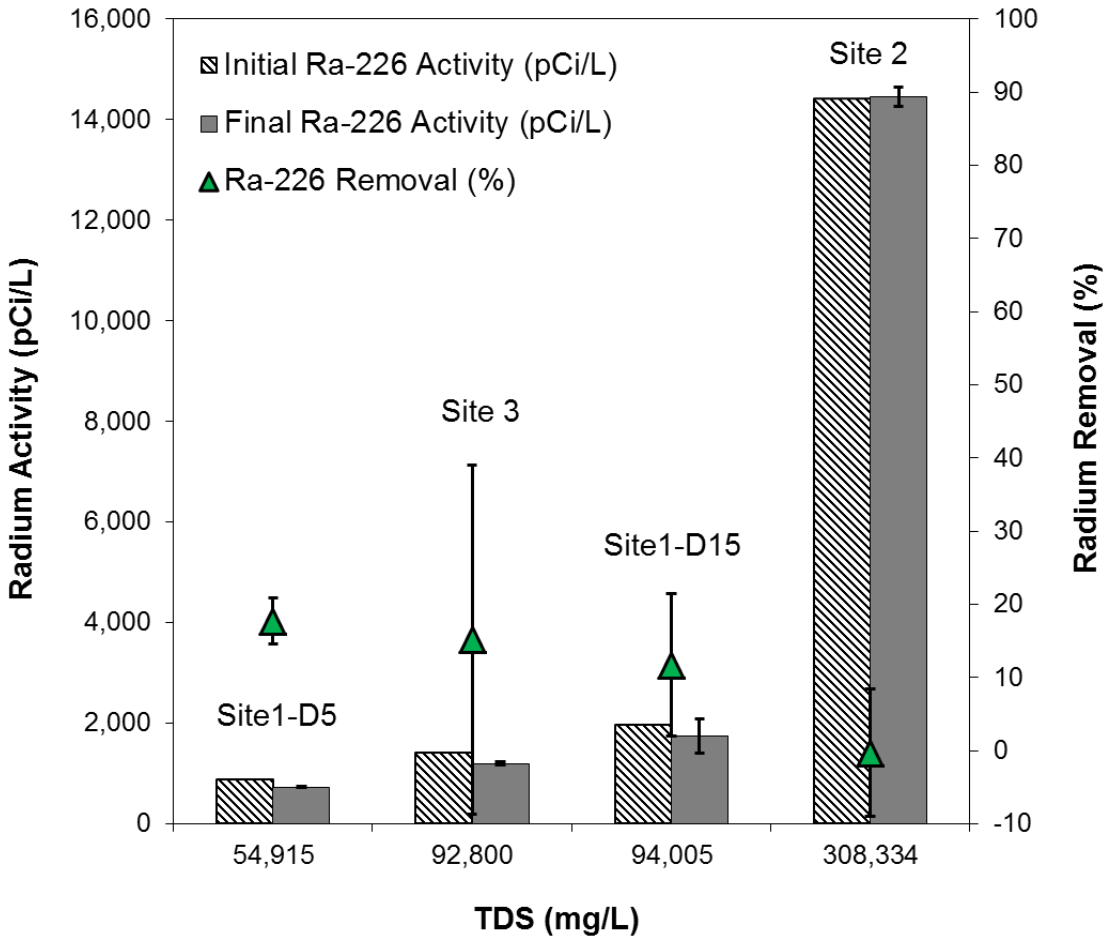

Figure 6.8 Initial and final Ra-226 activity and Ra-226 removal (%) as a function of TDS in produced water samples. Error bars represent ±1 standard deviation. Autoclaved biomass concentration = 0.04 $g_{dryweight}$/L. Sample characteristics provided in Table 6.2.

The results of this study showed that both the initial Ra-226 concentration and salinity are determiners of Ra-226 biosorption in produced water. Comparisons between the Ra-226 biosorption in synthetic and real produced water were conducted using the synthetic solution with TDS of 50,000 mg/L and Site1-D5 sample (TDS = 54,915 mg/L). Our results showed that Ra-226 biosorption in synthetic solution (Initial Ra-226 activity = 3,000 pCi/L, pH = 5.0 ± 0.3, and biomass concentration = 0.04 g_{drywt} /L) was significantly higher than observed in the Site1-

D5 sample (Initial Ra-226 activity = 890 pCi/L, pH = 7.4, and biomass concentration = 0.04 g_{drywt} /L): 14,466 pCi/g $_{dry\ weight}$ versus 3,935 pCi/g $_{dry\ weight}$, respectively ($p = 0.002$). One reason for this result is the higher initial Ra-226 activity in the synthetic solution compared to that of Site1-D5, since the uptake of Ra-226 is influenced by the initial concentration for the same mass of adsorbent. Second, the presence of competing ions in the Site1-D5 sample (i.e., barium, strontium, calcium) could potentially decrease the Ra-226 biosorption.[113, 138, 139]

There have been limited studies on Ra-226 biosorption and there is difficulty in comparing adsorption studies due to the variations in the experimental setup, analysis methods, and experimental conditions used in each study. One study, performed with inactivated municipal return sludge, reported Ra-226 adsorption capacity (K_F) to be 0.013 $(nCi/g)(L/pCi)^{1/n}$ with an R^2 of 0.887 using a Freundlich isotherm.[22] The adsorption capacity in our study was 0.135 $(nCi/g)(L/pCi)^{1/n}$ with an R^2 of 0.96. Another study showed both Freundlich and Langmuir isotherm models to accurately represent the biosorption of Ra-226 on *Serratia* sp. with R^2 values of 0.980 and 0.993, respectively, and the adsorption capacity (K_F) was 1.87 $(KBq/g)(L/KBq)^{1/n}$ [114] For comparison, we calculated the K_F value for our experiments was 0.027 $(KBq/g)(L/KBq)^{1/n}$.

The results of this study suggest that the application of algae biosorption for Ra-226 removal necessitates salinities below 100,000 mg/L. Thus, biosorption is likely to have minimal benefits in the Marcellus, Haynesville, and Bakken shale plays where the TDS of produced water ranges between 120,000-300,000 mg/L.[140, 141] The Fayetteville, Woodford, and Eagle Ford shale plays have produced water TDS concentrations ranging from 13,000-55,000 mg/L,[7] suggesting the potential applicability of biosorption; however, previous studies have identified a linear correlation between Ra-226 and TDS concentration in unconventional shale plays.[39] Our results

demonstrated that Ra-226 biosorption could have potential application in waters with high Ra-226 and low TDS concentrations. A previous study showed that Ra-226 accumulates in the downstream sediments of a brine treatment facility, likely due to the dilution of salts.[9] To reduce NORM pollution in sediments, Ra-226 biosorption could perhaps be applied as a post-treatment following chemical precipitation to capture the remaining Ra-226 prior to discharge.

6.4 SUMMARY AND CONCLUSION

D. salina growth in produced water was shown to be limited. Ra-226 biosorption by *D. salina* biomass was influenced by the pH, TDS concentration, the amount of biomass applied, and initial Ra-226 concentration. Biosorption experiments in Marcellus Shale produced water samples demonstrated Ra-226 removal between 0-18%. The substantial decrease in Ra-226 removal with increasing salinity challenges the use of this technology in treatment of saline produced waters. Therefore, the best application of radium biosorption may be for wastewaters containing high radium and low salinity concentrations, for example produced waters following initial treatment.

7.0 IMPLICATIONS AND CONCLUSION

A review of the produced water management practices, challenges, and opportunities associated with biological treatment of produced water showed that biological treatment is an underexplored area of study in hydraulic fracturing treatment research (Chapter 2). The limited available studies showed that the main challenges rise due to high salinity and variation in the organic content of produced waters. Current knowledge shows that a combination of the treatment methods (i.e., physical, chemical, and biological) is likely to remove the components of concern more effectively compared to single-process treatments. Tailored treatment approaches need to be evaluated for individual well sites producing water with comparable quality. Moreover, a decision-making approach for treatment of hydraulic fracturing produced waters was proposed, which can be used as a first step to select a treatment strategy.

Biological treatment to remove available electron donors has the potential to decrease heterotrophic microbial growth and the necessity of biocide use in produced water management. In this dissertation, the biological treatment of produced water using a mixed culture biofilm process was investigated. The preliminary study evaluating COD removal in synthetic and real produced waters shows the potential of salt tolerant biofilms to treat high salinity hydraulic fracturing produced water, even at 100,000 mg/L salt concentrations (Chapter 3).

In Chapter 4, the effect of biocide glutaraldehyde on the biodegradability of most commonly reported hydraulic fracturing chemicals such as acetate, guar gum, ethylene glycol,

ethanol, and isopropanol was evaluated. The results from this study demonstrate that the observed lag period and half-lives for all compounds (except for isopropanol) were positively correlated with GA concentration, suggesting that environmental models should consider contaminant interactions for more accurate predictions for environmental persistence, toxicity, and destination of the chemical mixtures following an intentional or incidental release to the environment.

Furthermore, to test the use of mixed culture biofilms in real produced waters, seven produced water samples from Utica and Bakken Shale for TOC removal at 50,000 and 100,000 mg/L TDS were evaluated (Chapter 5). Results show negative correlation between the biodegradation rates (first order) and the salinity of produced waters; however, no strong correlation was observed between the removal rates and either the initial TOC concentration or the final percent TOC removal. Moreover, the variation observed in TOC removal for different samples at the same salt concentration suggests that there should be other parameters affecting the biodegradation rates. Future studies investigating the potential factors affecting biodegradation rates in produced water can be useful for better understanding and applying biological treatment technologies in real world scenarios.

NORM content is another challenge for the final disposal of produced waters to surface waters. Ra-226 removal using a salt tolerant microalgae *D. salina* was evaluated in Chapter 6. Results of this study demonstrate a negative correlation of Ra-226 biosorption with salinity, therefore limiting the use of this approach in radium reduction in produced waters.

APPENDIX A

CHAPTER 3 - SUPPLEMENTARY INFORMATION

Table A1: Rate constants in highest and lowest performances observed in the successive loadings for acetate in synthetic produced water.

	Zero Order Reaction Kinetics				**First Order Reaction Kinetics**			
	Highest Performance (mg acetate removed/g wet mass*h)	R^2	Lowest Performance (mg acetate removed/g wet mass*h)	R^2	Highest Performance (h^{-1})	R^2	Lowest Performance (h^{-1})	R^2
0 mg/L TDS	0.1035	0.99	0.058	0.96	0.0425	0.79	0.0252	0.81
50,000 mg/L TDS	0.1038	0.99	0.0454	0.98	0.0381	0.91	0.0233	0.78
100,000 mg/L TDS	0.0740	0.99	0.0027	0.52	0.0291	0.31	-0.0101	0.12

APPENDIX B

CHAPTER 6 – SUPPLEMENTARY INFORMATION

Table B1: One-way ANOVA test results for *D. salina* growth normalized according to OD_{680nm} reading at Time 0 for two produced water samples S1 and S2 together with their 50% dilutions ($S1_{1:2dilution}$ and $S2_{1:2dilution}$), and control.

Factor	**Mean***	**St.Dev.**	**95% C.I.**
Control (2XErdschreiber's medium)	0.03444	0.01716	(0.01727, 0.05160)
S1	-0.00256	0.02893	(-0.01973, 0.01460)
$S1_{1:2dilution}$	-0.08210	0.04810	(-0.0993, -0.0649)
S2	-0.04506	0.02384	(-0.06223, -0.02790)
$S2_{1:2dilution}$	0.02032	0.01962	(0.00316, 0.03749)

* The mean represents the average of the normalized OD_{680nm} values

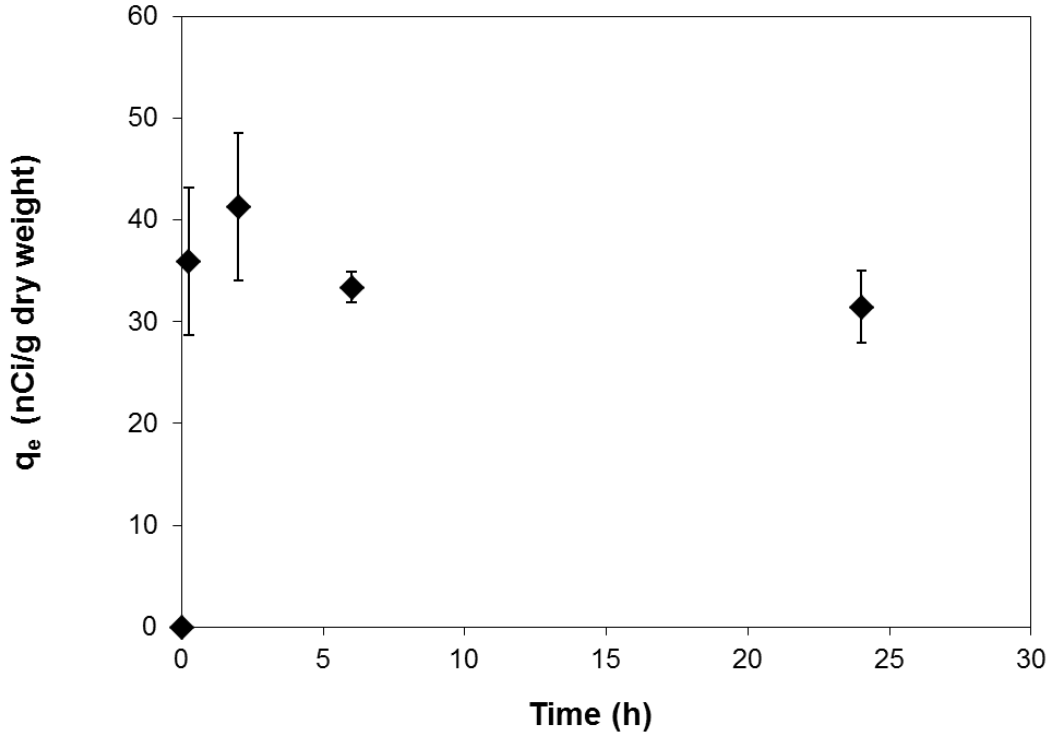

Figure B1: Ra-226 uptake as a function of time. Error bars represent ±1 standard deviation (Biomass concentration = 0.04 $g_{dryweight}$/L, initial Ra-226 concentration = 3000 pCi/L, pH = 5 ± 0.3, TDS = 0 mg/L).

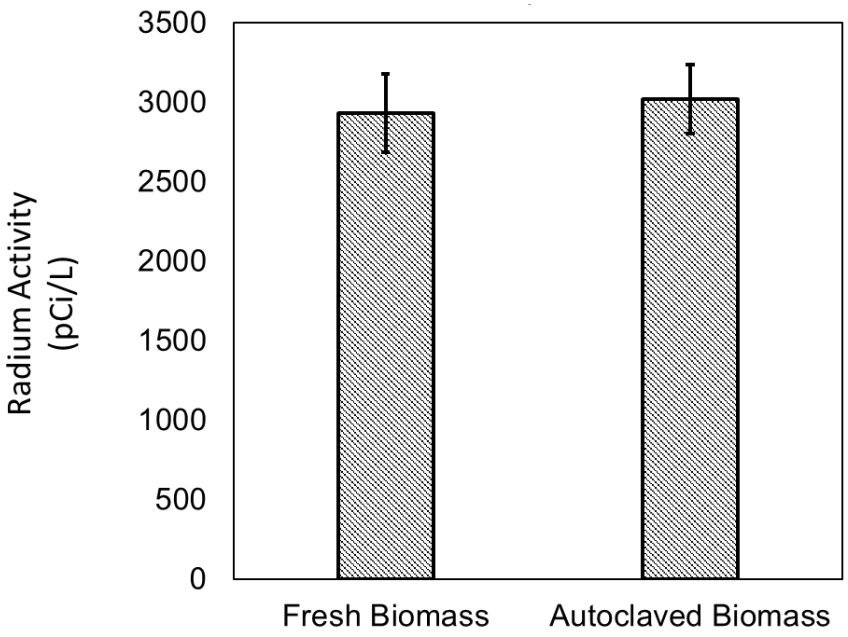

Figure B2: Average remaining Ra-226 activity in the solution for control conditions (triplicates performed at pH = 5.0 ±0.3, no biomass) for the experiments conducted for fresh and autoclaved biomass at changing pH. Error bars represent ±1 standard deviation. (No biomass, initial Ra-226 concentration = 3000 pCi/L, pH= 5.0 ±0.3).

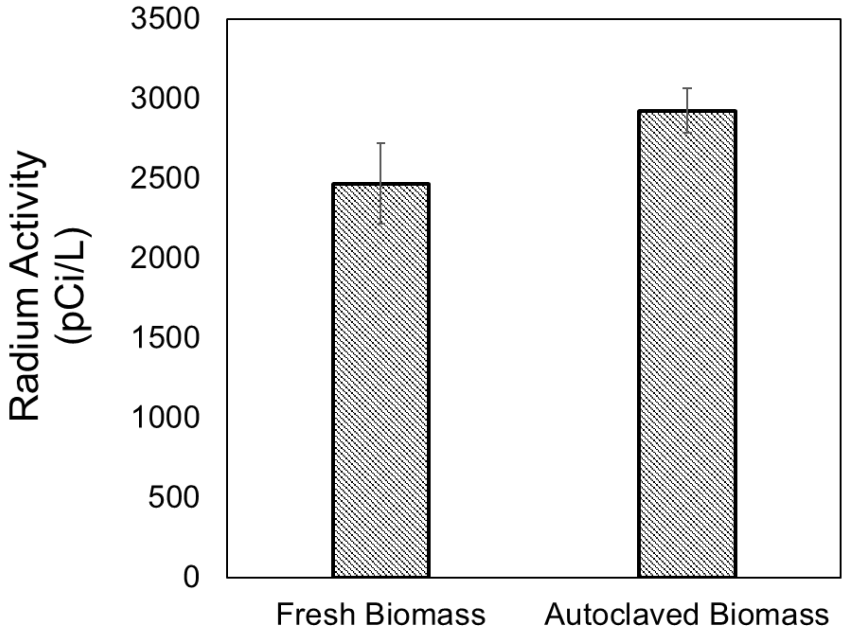

Figure B3: Average remaining Ra-226 activity in the solution for control conditions (triplicates performed at 0 mg/L TDS, no biomass) for the experiments conducted for fresh and autoclaved biomass at changing TDS concentrations. Error bars represent ±1 standard deviation. (No biomass, initial Ra-226 concentration = 3000 pCi/L, pH= 5.0 ±0.3, TDS = 0 mg/L).

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