MICROBIAL INHIBITION OF SULFATE REDUCTION USING NITRATE AND NITRITE ADDITION: A LABORATORY STUDY OF RESERVOIR SOURING CONTROL

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A Thesis submitted to the School of Graduate Studies
in partial fulfillment of the requirements for the degree of:

Masters of Science

Department of Earth Sciences
Memorial University of Newfoundland

March, 2017

Abstract

Microbial sulfate reduction produces unwanted H_2S in oil and gas reservoirs (i.e., reservoir souring) when seawater is used in secondary oil recovery. Previous studies have shown that the addition of nitrate and/or nitrite can inhibit sulfate reduction, but the effects are site-specific. In this thesis, seawater and produced waters were sampled offshore Newfoundland and Labrador (NL) for three incubation experiments to determine the affect of nitrate and/or nitrite addition on microbial sulfate reduction. Of the three amendments tested (i.e. just nitrate, just nitrite, and a combination of the two) all were successful at inhibiting sulfate reduction. No one type of amendment inhibited microbial sulfate reduction better than the others; however, nitrate showed potential of having a longer residence time, and therefore nitrate maybe an ideal choice for the inhibition of microbial sulfate reduction for offshore NL.

Acknowledgements

I would like to thank my supervisor Dr. Penny Morrill for her continuous and reliable support. Dr. Morrill's immense knowledge, motivation, patience, professionalism, and unprecedented enthusiasm has been a beacon of light throughout my graduate degree. I would also like to thank my co-supervisor Helen Zhang for her encouraging words and dependable assistance. I would also like to extend my gratitude to the rest of the sour gas research group for stimulating discussions and sharing of knowledge.

I feel honoured to be a part of Dr. Morrill's research group and I would like to acknowledge the support, fruitful conversation and companionship from the crew: Lukas Kohl, Melissa Cook, Emily Cummings, Leanne Fisher-Power, Amanda Reitz, Robert Bazely, Heidi Kavanagh and Liam Morrissey. I would also like to extend thanks to Dr. Susan Zeigler and her research group, most notably Jamie Warren and Dr. Geert Van Biesen for their patience and willingness to answer any of my questions. It was also a pleasure of mine to work beside and share a laboratory space with Sherri Strong, Pam King, Amanda Langille, and Leigha Nolan, they always looked out for me and provided a boost in morale. Furthermore, I would like to extend a thank you to the Department of Earth Sciences main office staff for their help along the way, especially Keir Hiscock, Michelle Miskell, Danielle Drodge, Jillian Kean, and Diane Guzzwell.

This thesis would not be possible without the love and support of my family and close friends. To my parents: thank you for keeping me disciplined, for giving me tough love when I need it, and for showing me what strength and humility is. To Simon

Bonisteel: thank you for challenging me intellectually, for being a constant source of motivation, and for accompanying me on our many hikes in the woods. I would also like to make a special thank you to my office mate turned best friend Shannon Broughm: I am forever grateful to have shared this journey with you, and especially grateful to have eaten your food and drank your coffee.

This research was funded by Suncor Energy Inc. as a part of their research and development (R&D) and education and training (E&T) expenditures to support Multidisciplinary Research in Offshore Reservoir Souring at Memorial University. I would like to acknowledge the assistance and consultation provided by the people of Suncor, most notably from Philip Stemler, Katrina Herritt, and the offshore laboratory technicians.

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List of abbreviations and symbols

± 1σ	Standard deviation
1SLC	Experiment 1 Live Control treatment in seawater
$1SKCN_{32}$	Experiment 1 Killed Control treatment in seawater with nitrate and nitrite addition
$1SLN_{32}$	Experiment 1 Live Experimental treatment in seawater with nitrate and nitrite addition
2SLC	Experiment 2 Live Control treatment in seawater
$2SKCN_{32}$	Experiment 2 Killed Control treatment in seawater with nitrate and nitrite addition
$2SLN_{32}$	Experiment 2 Live Experimental treatment in seawater with nitrate and nitrite addition
2PLC	Experiment 2 Live Control treatment in produced water
$2PKCN_{32}$	Experiment 2 Killed Control treatment in produced water with nitrate and nitrite addition
$2PLN_{32}$	Experiment 2 Live Experimental treatment in produced water with nitrate and nitrite addition
3SLC	Experiment 3 Live Control treatment in seawater

$3SKCN_3$	Experiment 3 Killed Control treatment in seawater with nitrate addition
$3SLN_3$	Experiment 3 Live Experimental treatment in seawater with nitrate addition
$3SKCN_2$	Experiment 3 Killed Control treatment in seawater with nitrite addition
$3SLN_2$	Experiment 3 Live Experimental treatment in seawater with nitrite addition
D.I water	Deionized water
DIC	Dissolved inorganic carbon
DIN	Dissolved inorganic nitrogen
D.L.	Detection limit
DOC	Dissolved organic carbon
DON	Dissolved organic nitrogen
Dsr	Dissimilatory sulfite reductase
EDTA	Ethylenediaminetetraacetic acid
E_h	Redox potential
$\Delta G°$	Change in Gibbs free energy

GFF filter	Glass fibre filter
IC	Ion chromatograph
MCE filters	Mixed cellulose ester filters
NADPH	Enzyme nitrite reductase
NAD(P)H	enzyme nitrate reductase
NRB	Nitrate reducing bacteria
NR-SOB	Nitrate reducing sulfur oxidizing bacteria
pH	Hydrogen potential
PLFA	Phospholipid-derived fatty acids
P/N	Part number
SCBA	Self-contained breathing apparatus
SOR	Secondary oil recovery
SRB	Sulfate reducing bacteria
TDN	Total dissolved nitrogen
TIC	Total inorganic carbon
TIN	Total inorganic nitrogen
tNRB	Thermophilic nitrate reducing bacteria

tSRB	Thermophilic sulfate reducing bacteria
VOA vials	Volatile organic analysis vials
WHMIS	Workplace hazardous material information system

Chapter 1: Introduction

1.1 Study significance

Dihydrogen sulfide gas (H_2S) is an issue for oil and gas operations, especially when seawater injection is used to maintain reservoir pressure (Reinsel et al., 1996). Seawater contains up to 28 mmoles/L of sulfate (Barton, 1995) which can be bacterially reduced to sulfide under reducing conditions such as those found in reservoir fluids (Bastin et al., 1926). The production of sulfide causes many operational problems. H_2S causes an increase in corrosion (Kaster et al., 2007), oil field plugging (Gieg et al., 2011), precipitation of metal sulphides (Reinsel et al., 1996) which reduces the permeability of the formation (Rosnes et al., 1991), lowers the quality of the oil (Gieg et al., 2011), raises sulphur content (Reinsel et al., 1996) and therefore increases the cost of refinement (Kumaraswamy et al., 2011). Most notably H_2S is extremely toxic (Hendrickson et al., 2004).

Microbial sulfate reduction produces unwanted H_2S in oil and gas reservoirs (i.e., reservoir souring) when seawater is used in secondary oil recovery. An oil and gas well is deemed "sour" if H_2S concentrations exceed 3 ppmv, conversely a well is considered "sweet" if H_2S concentrations are below 3 ppmv (Eden et al., 1993). Potential solutions to reservoir souring include aerated injection wells, addition of sulfide scavengers, early prevention (e.g., the use of sterilized or naturally low in SO_4^{2-} injection water) (Bader, 2007; Gieg et al., 2011), biocides (Kumaraswamy et al., 2011; Reinsel et al., 1996) and nitrate and nitrite injection (Jenneman et al., 1986). This thesis aimed to mitigate the

production of bacterially produced H_2S in seawater and produced water sampled from offshore Newfoundland and Labrador

1.2 Literature review

1.2.1 Reservoir souring

Reservoir souring is a term used to describe the increase of H_2S gas in oil and gas wells (Eden et al., 1993). H_2S can be generated by the respiration of anaerobic sulfate reducing bacteria (SRB), whereby sulfate is reduced to sulfide (Bastin et al., 1926). Souring is widespread amongst the oil and gas industry and can affect terrestrial and offshore operations, and reservoir or topside facilities (Gieg et al., 2011). Reservoir souring is a problem in Canada and elsewhere. Approximately 85% of sour gas production in Canada occurs in Alberta and there is roughly 6,000 sour gas wells in Alberta (VanderKlippe, 2011).

One of the reasons reservoir souring is a problem globally is because seawater is often used for primary and secondary oil recovery (SOR) in oil and gas wells. In SOR water is injected into the reservoir in order to maintain pressure in the well, thus helping to extract the oil from the reservoir (Figure 1.1). If pressure is not maintained in a well, then production rates would slow as oil extraction progresses (Eden et al., 1993). Seawater injection is the most common reason for souring in SOR, and ~70% of fields using seawater eventually encounter reservoir souring issues (Kuijvenhoven et al., 2006). Some operations have the capacity to inject over a million barrels of seawater per day (Bader, 2007). SOR has the potential to generate large amounts of H_2S because seawater has a relatively high concentration of sulfate (28 mmoles/L) (Barton, 1995), which is the

source of sulfur in biogenic sulfide generation. In severe cases of reservoir souring retrofitting may be required for field, transportation, and processing equipment (Barton, 1995). The specialized metallurgy, materials, and operating procedures needed for soured wells increases the overall cost of operation and production of an oil and gas well (Barton, 1995). H_2S is detrimental to production, because it is very corrosive, explosive, dangerous to life, and reduces the quality of oil.

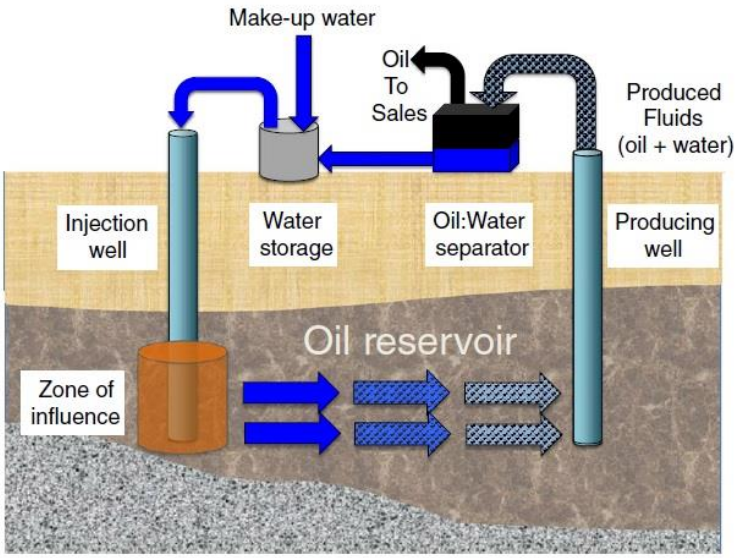

Figure 1.1: Diagram of reservoir re-pressurization using water injection (and re-injection) for the purpose of secondary oil recovery. The "zone of influence" is highly affected by the injection water and is cooled near the injection well, enabling bacterial growth in high temperature wells [image taken from (Gieg et al., 2011)]. It is in this zone of influence that most of the bacterial sulfate reduction via SRB takes place (Sunde et al., 2005).

H_2S is very toxic and is a workplace hazard. According to the US Bureau of Labor Statistics H_2S is one of the leading causes of workplace gas inhalation deaths in the US,

and has caused 52 deaths between 1993 and 1999 (Hendrickson et al., 2004). At concentrations of 0.02 ppm the characteristic H_2S odor is present. Fifty ppm H_2S can cause headaches, nausea, coughing and conjunctivitis. One hundred ppm H_2S causes loss of smell, vertigo, light-headedness, and may cause permanent brain damage and or fluid formation in the lungs. Five hundred to seven hundred ppm H_2S will cause immediate unconsciousness and is fatal (Hendrickson et al., 2004). H_2S is not only a problem in the USA, but it also occurs in Canada. In 2011 three workers were rendered unconscious by an H_2S leak in Alberta and one of the workers died on the scene (VanderKlippe, 2011). The British Columbia Workers Compensation Board has said 4 to 5 people are rendered unconscious every year due to the inhalation of H_2S (VanderKlippe, 2011).

1.2.2 Sulfate reducing bacteria (SRB)

Sulfate reducing bacteria (SRB) are widely distributed on earth in terrestrial and aquatic environments such as wetlands, wastewaters, freshwater, seawater and oil and gas reservoirs; however, SRB proliferate specifically in anaerobic environments. SRB are unique as they have the ability to utilize inorganic sulfate as an electron acceptor during anaerobic respiration and produce H_2S as a product (Barton, 1995). While SRB use SO_4^{2-} as an electron acceptor, they also use a range of electron donors such as organic acids, short chain fatty acids, or petroleum by-products to gain energy (Eq. 1.1) (Eckford et al., 2002; Liamleam et al., 2007).

$$\text{(Eq. 1.1)} \qquad CH_3CO_2^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^-$$

Therefore SRB are heterotrophic. SRB reduce SO_4^{2-} to sulfide and oxidize organic matter to bicarbonate. Sulfate reduction is more prominent in seawater than compared to freshwater because seawater has more sulfate (e.g. seawater has 28 mmoles/L of sulfate and freshwater has 10-200 nmoles/L of sulfate). The optimal environment for SRB activity is a pH range between 7.0 – 7.8 (Barton, 1995), and salinities below 10% (Gieg et al., 2011). There are two optimal temperature ranges for SRB. Mesophilic SRB grow between 28-38 °C, and thermophilic SRB grow between 55-85 °C (Barton, 1995).

In oil and gas wells where seawater injection is used for reservoir pressurization (also known as secondary oil recovery, Figure 1.1) SRB grow near the injection wellbore region (Sunde et al., 2005). The injection wellbore is an ideal location for SRB growth because injected seawater cools the surrounding area (Gieg et al., 2011). Extensive flooding of the injection wellbore changes the chemical environment surrounding the well creating a mixture of carbon from the reservoir and nutrients (including sulfate) from seawater, which provides a habitable environment for SRB (Sunde et al., 2005).

1.2.3 Oxidation-reduction potential

Oxidation - reduction potential, otherwise known as redox potential (E_h), is a measure of the tendency of an aqueous environment to gain or release electrons when a new species is added to the environment. If electrons are being released, then the environment is considered reducing. Conversely, if electrons are being accepted, then the environment it is considered oxidizing (Bier, 2009). Redox potential can be used as an indicator for a reducing environment, which is favorable for nitrate and sulfate reduction. Sulfate reduction occurs under more reducing conditions compared to nitrate reduction.

Figure 1.2 also illustrates the relationship between reduction potential, energy yield, and the change in Gibbs free energy.

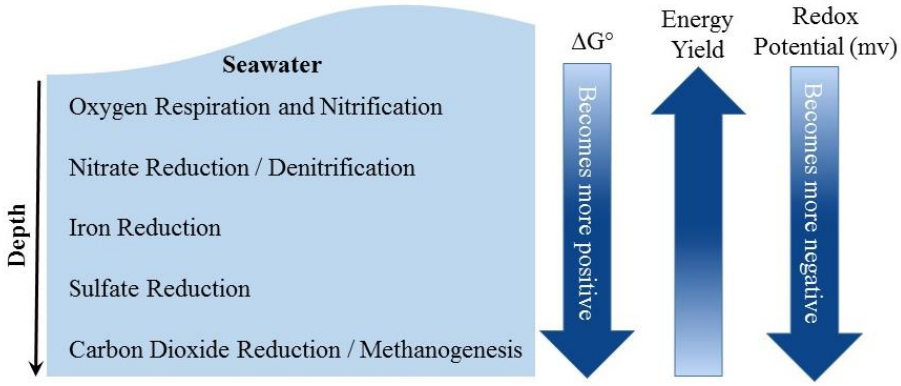

Figure 1.2. Schematic of the "redox ladder" showing the relative relationship between the change in Gibbs free energy (ΔG°), energy yield, and redox potential on reactions below sea level. With increasing depth there is a decrease in oxygen and thus an increasingly negative redox potential value. Gibbs free energy becomes less negative with increasing depth. Energy yields for redox reaction decrease with depth. Image modified from Albarede (2011).

Nitrate reduction has a larger negative change in Gibbs Free Energy (-495 KJ/mol NO_3^-) compared to microbial sulfate reduction (-47 KJ/mol SO_4^{2-}). Since there is an inverse relationship between ΔG° and energy yield (as seen in Figure 1.2), nitrate reduction produces more energy than sulfate reduction. Once all of the nitrate is consumed in a system, then sulfate reduction will become dominant and the redox potential of the system will become more negative. This is why redox potential is

indicative of where a system sits on the "redox ladder". In this thesis, E_h was used as an indicator for nitrate and sulfate reduction.

1.2.4 Nitrate reducing bacteria (NRB)

Nitrate reducing bacteria are anaerobic organisms that utilize nitrate as an electron acceptor for the purpose of respiration. Nitrate is reduced to ammonia via intermediate nitrite using the enzymes nitrate reductase [NAD(P)H] (Eq. 1.2) and nitrite reductase (NADPH)(Eq. 1.3).

(Eq. 1.2) $NO_3^- + NAD(P)H + H^+ \rightarrow NO_2^- + NAD(P)^+ + H_2O$

(Eq. 1.3) $NO_2^- + 3NADPH + H^+ \rightarrow NH_3 + 3NADP^+ + H_2O + OH^-$

In seawater however, ammonia is usually present as its ion ammonium (NH_4^+) (Wada et al., 1991). Nitrate reducing bacteria (NRB) produce many chemical species during anaerobic respiration (also known as denitrification) including: nitrite (NO_2^-), nitrogen gas (N_2) (Eq. 1.4), ammonia (NH_3), and nitrous oxide (N_2O).

(Eq. 1.4) $5CH_3CO_2^- + 8NO_3^- + 3H^+ \rightarrow 10HCO_3^- + 4N_2 + 4H_2O$

$\Delta G = -495$ KJ (mol NO_3^-) $^{-1}$

Nutrients, organic acids and nitrate are consumed metabolically by NRB. Myhr et al. (2000) showed that a NRB strain ($N2460^T$) isolated from an oil reservoir lived between 4 – 40 °C (optimal at 35-37 °C), a salinity of 6% NaCl (w/v), and growth was observed between pH 6.5 and 8.6. Oxygen inhibited the growth of $N2460^T$, therefore exhibiting the bacteria's anaerobic nature.

The addition of nitrate has been attributed to the inhibition of microbial sulfate reducers due to a rise in redox potential via biological nitrate reduction (Reinsel et al., 1996), the toxic action of nitrite and nitrous oxide (products of NRB respiration), substrate competition, and sulfide oxidation (Jenneman et al., 1986). NRB and SRB utilize, and compete for, the same carbon substrate and nutrients (Sunde et al., 2005). Nitrate is a better electron acceptor for growth than sulfate due to the much more favorable change in Gibbs Free Energy for nitrate reduction than for sulfate reduction (Eckford et al., 2002; Reinsel et al., 1996). Moreover, the energy released during nitrate reduction is greater than the energy released during the reduction of sulfate (Eckford et al., 2002), which means NRB can outcompete SRB and yields higher growth rates for NRB when competing for the same carbon substrates and electron donors. Therefore, as long as there is nitrate or nitrite present in the system, NRB should inhibit SRB's production of H_2S via bio-competitive exclusion.

Another means by which nitrate addition can remediate reservoir souring is by the stimulation of nitrate reducing sulfur oxidizing bacteria (NR-SOB). The NR-SOB gain energy by oxidizing reduced sulfur compounds to sulfate and elemental sulfur, and in doing so suppresses sulfide production (Eckford et al., 2002). NR-SOB are also capable of nitrate reduction with the primary end product being N_2 gas (Eq. 1.4). If NR-SOB have the same level of activity as SRB, then in theory the net rate of sulfide generation would be zero (Haghshenas et al., 2011).

Inorganic nitrogen has been added to various oil and gas wells in efforts to reduce microbial H_2S production. To illustrate this, two case studies from the North Sea (Bodtker et al. (2008) and Kaster et al. (2007) have been summarized below.

1.2.5 Case Study 1: Bødtker et al. (2008): The effect of long-term nitrate treatment on SRB activity, corrosion rate and bacterial community composition in offshore water injection systems.

Bødtker et al. (2008) studied the long-term effects of nitrate injection on SRB activity, corrosion rates, and bacterial community composition of the Veslefrikk and Gullfacks fields in the North Sea. Biocides were traditionally used at this site to mitigate sulfide production, but nitrate injection was tested because it was considered more effective and environmentally safe. Nitrate injection enriches NRB, which outcompete SRB due to the more favorable energy potential of nitrate reduction compared to sulphate reduction. Overall, SRB were inhibited and corrosion rates decrease.

In January of 1999 nitrate injection commenced with [$Ca(NO_3)_2$] being injected continuously at 0.25 mM NO_3^-. In October 2001 the nitrate dosage was increased to 0.33 mM NO_3^-. SRB/NRB incubation experiments were performed in this study. Incubations were amended with a sulfate free mineral medium, 20 mM of acetate, 5 mM of butyrate, 5 mM of caproate, and 18.2 mM of lactate.

The Veslefrikk field study observed a 50–fold reduction in H_2S production (SRB activity) and a reduction in the corrosion rate immediately following continuous nitrate injection (1999-2001). NR-SOB formed major populations, and three of the four major

populations that were observed before biocide treatment were no longer observed after 1 year of nitrate amendment. Sulphate reduction rates remained at ≤0.3 μg H_2S/cm^2/day during nitrate treatment. A decrease in corrosion rate was also observed with a reduction of up to 40%. The Gullfaks field observed an initial decrease in number and activity of SRB, and an increase in the numbers of NRB. The SRB activity remained low during the 8 years of nitrate injection at ≤0.9 μg H_2S/cm^2/day.

SRB activity and corrosion rate increased in the water injection systems at Veslefikk and Gullfaks during biocide treatment. However, the long-term nitrate injection treatment inhibited SRB activity and decreased corrosion rates. This enabled the development of a stable NRB dominated biofilm. Nitrate injection was proven to be an effective and safe way to mitigate biogenic sulfide production in offshore oil wells practicing secondary oil recovery.

This study is an example of a successful nitrate injection treatment program that has been used in the Norwegian Sea. It highlighted the importance of adding electron donors to incubations to provide bacteria with an ideal growing medium. This experiment showed that nitrate was effective at reducing H_2S activities and there was an observed reduction of corrosion.

1.2.6 Case Study 2: Kaster et al. (2007): Effect of nitrate and nitrite on sulfide production by two thermophilic, sulfate-reducing enrichments from an oil field in the North Sea.

Nitrate, nitrite and the combination of the two have been tested in numerous studies (Eckford et al., 2002; Jenneman et al., 1986; Reinsel et al., 1996; Voordouw et al., 2009). However unlike most other studies, Kaster et al. (2007) found that nitrate alone was ineffective at inhibiting thermophilic SRB, but conversely nitrite proved very effective. This suggests that nitrite may be a better option for high temperature reservoirs. Although, it is important to recognize that nitrite is a by-product of NRB respiration, and thus nitrate could also be effective as long as it is oxidized. Nitrate/nitrite injection can potentially stimulate the growth of NRB, NR-SOB, or both organisms. Both oxidized forms of nitrogen can aid in the mitigation of H_2S gas by biocompetitive exclusion of SRB and direct oxidation of sulfide respectively. Unlike biocides, nitrate/nitrite injection is economically feasible and is therefore an attractive method for H_2S mitigation.

Kaster et al. (2007) studied the effects of nitrate/nitrite injection on sulfide production in the Ekofisk field of the Norwegian sector in the North Sea. The field was a deep reservoir where thermophilic sulfate reducing bacteria (tSRB) contributed to most of the sulfide production. The field was 80-90 °C, but was cooled to 60 °C near injection wells due to the mixture of cold seawater with reservoir water, and thus experiments were run at this temperature. It was hypothesized that nitrite may be preferable for some high-temperature oil fields as it reacts directly with sulfide. Nitrite inhibits dissimilatory sulfite reductase (Dsr), which is an enzyme that catalyzes the reduction of sulfite to sulfide. Dsr

has a strong affinity for nitrite by reducing it to ammonia, thus inhibiting Dsr usage by SRB (Kaster et al., 2007).

Experiments were conducted in an upflow bioreactor filled with Ekofisk Chalk inoculated with 0.1 ml/min of produced waters (liquids that come out of the reservoir) that had 0.1 mM of phosphate added to it. Phosphate is a limiting nutrient in many ecosystems and was thus included to ensure bacterial growth. Two enrichments of bacteria (NS-tSRB1 and NS-tSRB2) were studied with differing additions of organic acids (Table 1.1).

Table 1.1. Organic acids added to bacterial enrichments.

Bacterial Enrichment	**Electron donor**
NS - tSRB1	12 mM Acetate
	1.2 mM Propionate
	0.6 mM Butyrate
NS - tSRB2	28 mM Lactate

The experiments were enriched with 1mL of Ethylenediaminetetraacetic acid (EDTA) chelated trace elements solution, 1 mL of selenate-tungstate solution, 30 mL of 1M $NaHCO_3$, and 1 mL of vitamin B12 solution. One mL of 1M Na_2S was added to reduce the medium, and pH was adjusted to 7.2 in order to mimic that of the produced water. Nitrate/nitrite injection did not take place until the bioreactor was stabilized with a sulfide concentration of 4-6 mM.

It was observed that 2 and 10 mM of nitrate addition had little to no effect on sulfide production for both NS – tSRB1 and NS – tSRB2. It is also noted that no nitrite was detected after nitrate addition (i.e. nitrate was not oxidized to nitrite). Conversely, nitrite addition strongly inhibited sulfate reduction. For example, 0.25 mM of nitrite inhibited sulfate reduction for the duration of the experiment for NS – tSRB1 , and inhibited sulfide production for 1100 hrs for NS – tSRB2. A decrease in sulfide production was observed immediately after the nitrite addition. As seen in Table 1.1, the only difference between the two bacterial enrichments was the type of organic acid added, suggesting that the organic acid used as an electron donor had an influence on the survival of SRB during nitrate/nitrite injection.

Although these results suggest that nitrite addition is the most optimal way to inhibit H_2S production in high temperature reservoirs, nitrate has proven effective for two other high-temperature oil fields in the Norwegian and Danish sectors of the North Sea; Veslefikk and Halfdan fields respectively (Kaster et al., 2007). It was hypothesized that thermophilic nitrate reducing bacteria (tNRB) likely converted nitrate into nitrite, thus inhibiting tSRB of organics derived from oil in these cases. This was not observed in Ekofisk, suggesting that tNRB were not present, or they could have been lost during enrichment with sulfate. It also highlights the variability of bacterial sulfate and nitrate reduction in oil and gas reservoirs (i.e. individual reservoirs will respond differently to the addition of nitrogen amendments for the purpose of H_2S control). In conclusion, this study showed that 0.25 mM of nitrite effectively controlled souring for the bioreactor experiments.

This study showed that nitrite alone was an effective inhibitor of souring in some high-temperature oil fields. It also showed that the produced water was important to characterize as it provided clues of the biogeochemical reactions taking place within the reservoir. This highlights the importance of testing sea water before (injected) and after (produced) it has travelled through the reservoir.

1.3 Thesis hypothesis and objectives

This thesis aimed to determine the affect of nitrate addition, nitrite addition, and a combination of the two on the production of bacterially produced H_2S in seawater and produced water sampled from offshore Newfoundland and Labrador. Three sets of laboratory incubation experiments were performed in closed microcosm serum bottles using seawater and produced water. Each experiment received different nitrate and/or nitrite amendments. The experiments were monitored over time to determine the amendment effects on the microbial production of H_2S. It was hypothesized that both nitrate and nitrite would have an inhibitory affect on biological sulfide production. The optimal concentrations of nitrate and nitrite, and the combination of the two, on the remediation of reservoir souring are unknown as highlighted in the case studies. The case studies have shown that there is variability on the bacterial response among different oil and gas reservoirs, thus the optimal dosage of each amendment, both separately and together were tested for this location.

Chapter 2: Methods

2.1 Geochemical characterization of waters

Seawater and produced waters were sampled from offshore Newfoundland and Labrador on March 13, 2015 and were received by the Department of Earth Sciences on March 25th, 2015. In situ measurements were taken during all water sampling periods. In-situ measurements included redox potential, dissolved oxygen, and hydrogen potential (pH). Waters were sampled and stored for later analysis. These samples include: dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), total dissolved nitrogen (TDN), organic acids, nutrients including sulfate, ammonia, and dissolved inorganic nitrogen (DIN). Bulk water for Experiment 1 was received on May 26, 2015. Bulk water for Experiment 2 was sampled on January 10th and 12th, 2016 and was received on January 25th. Water for Experiment 3 was sampled on May 13th 2016 and were received on May 27th, 2016. Table 2.1 describes the geochemical parameters that were analysed during each experiment and initial water characterizations.

Table 2.1. Geochemical parameters sampled and analyzed in water characterization and experiments.

Parameter	Characterization	Experiment 1	Experiment 2	Experiment 3
DOC	✓	✓	✓	✓
DIC	✓	✓	✓	✓
TDN		✓	✓	✓
Nitrate	✓	✓	✓	✓
Sulfate	✓	✓	✓	✓
Ammonium		✓	✓	✓

2.2 Experimental setup

Three incubation experiments were performed to determine the impact of nitrate and nitrite additions on bacterially produced H_2S in seawater. See Figure 2.1 for an explanation of experimental labelling. Experiment 1 (Figure 2.2) tested a mixture of nitrate and nitrite with no other amendments and consisted of three treatments (1SLC, $1SKCN_{32}$, $1SLN_{32}$) involving just seawater and 9 incubation bottles. Experiment 2 (Figure 2.3) tested the same mixture of nitrate and nitrite with the addition of reducing amendments. This experiment consisted of six treatments (2SLC, $2SKCN_{32}$, $2SLN_{32}$, 2PLC, $2PKCN_{32}$, $2PLN_{32}$) and included seawater and produced water in 18 incubation bottles. Experiment 3 (Figure 2.4) tested the impact of nitrate and nitrate additions independently on the production of bacterially produced H_2S. This third experiment consisted of five treatments (3SLC, $3SKCN_3$, $3SLN_3$, $3SKCN_2$, $3SLN_2$) and included just seawater and 15 incubation bottles.

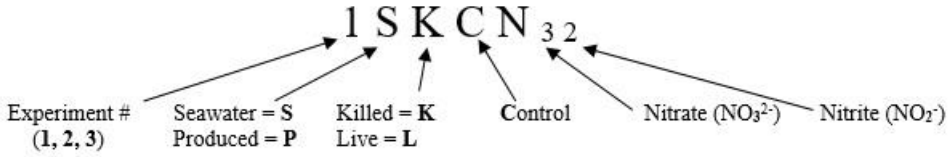

Figure 2.1. Experimental nomenclature explanation for all three experiments.

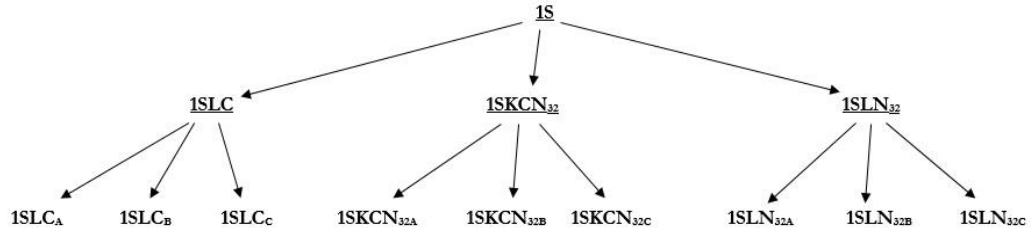

Figure 2.2. Experimental flow chart for Experiment 1 (1S). The experiment consisted of three treatments in seawater (1SLC, $1SKCN_{32}$, $1SLN_{32}$) and tested a mixture of nitrate and nitrite with no other amendments.

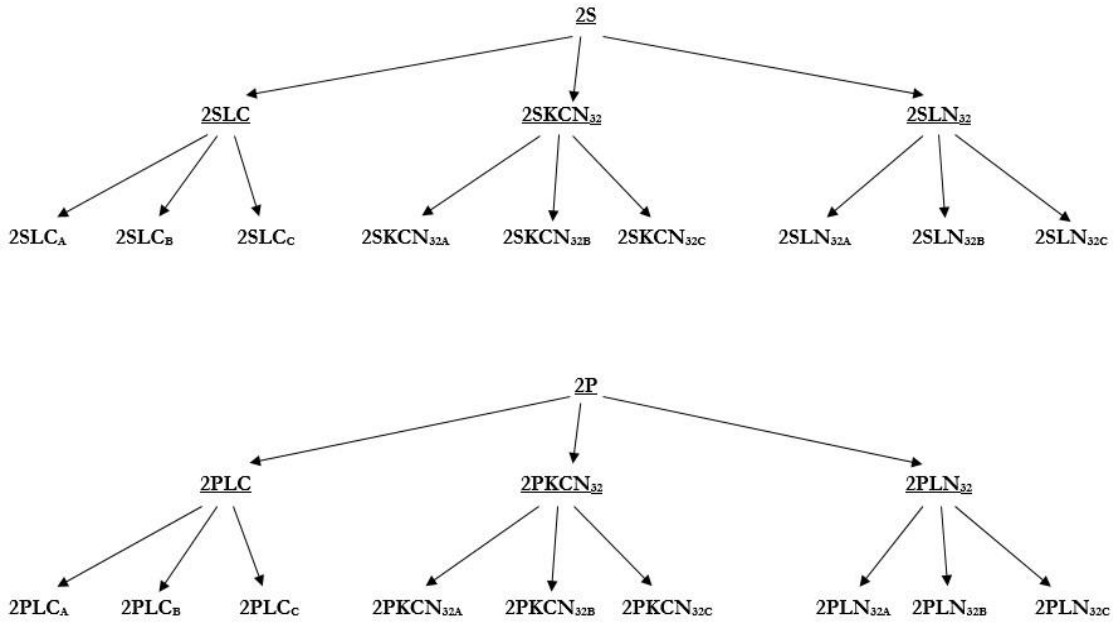

Figure 2.3. Experimental flow chart for Experiment 2 (2S & 2P). The experiment consisted of six treatments in seawater and produced water (2SLC, $2SKCN_{32}$, $2SLN_{32}$, 2PLC, $2PKCN_{32}$, $2PLN_{32}$) and tested a mixture of nitrate and nitrite with reducing amendments.

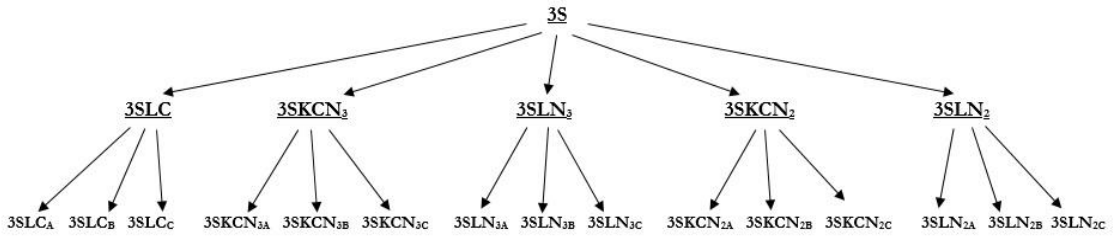

Figure 2.4. Experimental flow chart for Experiment 3 (3S). The experiment consisted of five treatments (3SLC, $3SKCN_3$, $3SLN_3$, $3SKCN_2$, $3SLN_2$) and tested nitrate and nitrate additions independently with reducing amendment

2.2.1 Experiment 1: Nitrate & Nitrite additions to seawater

The first incubation experiments were setup in an anaerobic environment with seawater on August 4th, 2015. The experiment had three treatments including Live Control (1SLC), Killed Control with nitrate and nitrite addition ($1SKCN_{32}$), and Experimental Live treatment nitrate and nitrite addition ($1SLN_{32}$). All experiments were constructed in triplicates totalling nine incubation bottles (Figure 2.2). Each 250 mL glass serum bottle (KG-35 borosilicate glass, Kimble Chase Life Science) contained equal volumes of seawater (220 mL) and were sealed with blue butyl septa and aluminum crimp seals. Contamination by volatile organic compounds from the blue butyl septa was minimized by pre-conditioning [boiling in 0.1 N NaOH followed by immersion in distilled water overnight, Oremland et al. (1987)]. The incubation experiments were stored in an anaerobic chamber (Plas Labs Inc, Model 855-AC) with an atmosphere of 4% H_2 in He. Experiments were shaken at 160 rotations per minute. Treatment 1SLC was a Live Control and had no additional amendments. Treatment $1SKCN_{32}$ consisted of

nitrate and nitrite addition at 0.00161 mol/L and 0.02174 mol/L respectively and contained 2.5 mL of 0.0276 mol/L $HgCl_2$. The $HgCl_2$ was added as a poison to stop all bacterial activity. Treatment $1SLN_{32}$ consisted of just nitrate and nitrite addition at the same concentrations as $1SKCN_{32}$, but were not poisoned. It was hypothesized that treatment 1SLC would promote SRB growth and potentially yield H_2S. Treatment $1SKCN_{32}$, the killed control, was hypothesized to yield no bacterial growth, and therefore no H_2S production. While treatment $1SLN_{32}$ would stimulate NRB growth and inhibit H_2S production by SRB.

Within the anaerobic chamber 5 mL aliquots of water were periodically extracted from the experimental bottles with BD syringes and needles for routine in-situ measurements (i.e., redox potential and acidity). Redox potential (E_h) and pH were measured on days 22, 29, 45, 69, and 109 of the experiment. Detailed sampling and termination dates were determined based upon the in-situ E_h and pH measurements. Dates of the experimental modifications are noted in Table 2.2. On day 17, 7 mL of He was added to over pressurize each serum bottle. On days 41 and 51, 15 mL of 4% H_2 in He was added. A catalyst heater with a palladium canister (PLAS LABS, P/N: 800-HEATER) (used to remove O_2 from the atmosphere of the anaerobic chamber) was activated inside the glove box at a temperature of 25°C on day 51. Lastly, on day 78, 20 mL of 4% H_2 in He was added along with acetate at a final concentration of 0.0002 mol/L. On day 109 the incubations were terminated.

Table 2.2. Modifications to Experiment 1: Nitrate & nitrite incubation in seawater

Day	Date	Amendment
1	04-Aug-15	Start of incubations
17	21-Aug-15	7 mL addition of He
41	05-Oct-15	15 mL addition of 4% H_2 in He
51	15-Oct-15	Heater switched on at 25°C and 15 mL addition of 4% H_2 in He
78	11-Dec-15	20 mL addition of 4% H_2 in He and 0.0002 mol/L acetate
109	11-Jan-16	Incubations terminated

2.2.2 Experiment 2: Nitrate & Nitrite additions to seawater and produced water

The second incubation experiment was constructed and stored in an anaerobic chamber in an environment of 4% H_2 in He on February 11, 2016. The experiment had 6 treatments and included seawater and produced water. Treatments consisted of a Live Control with seawater (2SLC), Killed Control with nitrate & nitrite addition in seawater ($2SKCN_{32}$), Live Experimental treatment with nitrate & nitrite addition in seawater ($2SLN_{32}$), Live Control with produced waters (2PLC), a Killed Control with nitrate and nitrite addition in produced water ($2PKCN_{32}$) and a Live Experimental treatment with nitrate & nitrite addition in produced water ($2PLN_{32}$). All experiments were constructed in triplicates with 15 bottles in total (Figure 2.3). Similar bottles, volume of water, septa, and rotation speed that were used in Experiment 1 were also used in Experiment 2. The catalyst heater was activated inside the glove box on day 1 at a temperature of 30 °C. To stimulate bacterial growth all bottles at the time of construction received a selenium

tungstate, trace element, and B12 solution modified from Widdle et al. (1992). As a reducing agent 660 ppb of Na_2S was also added. Moreover, organic substrates were also added using concentrations similar to Kaster et al. (2007) at 0.012 mol/L acetate, 0.012 mol/L lactate, 0.0012 mol/L propionate, and 0.0006 mol/L butyrate.

Treatments 2SLC and 2PLC were Live Controls in seawater and produced water respectively and had no additional amendments. Treatments $2SKCN_{32}$ and $2PKCN_{32}$ were killed controls in seawater and produced water respectively and consisted of nitrate and nitrite addition at 0.00161 mol/L and 0.02174 mol/L respectively and contained 2.5 mL of 0.0276 mol/L $HgCl_2$. Treatments $2SLN_{32}$ and $2PLN_{32}$ consisted of just nitrate and nitrite additions in seawater and produced water respectively, and also had 0.00161 mol/L and 0.02174 mol/L of nitrate and nitrite respectively. Similar to Experiment 1, it was hypothesized that for Experiment 2 the Live Controls would stimulate SRB growth and yield H_2S. The killed controls were hypothesized to yield no bacterial growth and therefore no H_2S. Lastly, it was hypothesized that $2SLN_{32}$ and $2PLN_{32}$ would stimulate NRB growth and inherently inhibit SRB growth and the production of H_2S.

Similar to Experiment 1, 5 mL aliquots of water were periodically extracted from the Experiment 2 bottles for E_h and pH measurements. These measurements occurred on days 1, 37, 51, and 97. Sampling and termination dates were determined based upon the in-situ E_h and pH measurements. On day 97 the incubation experiments were terminated.

2.2.3 Experiment 3: Nitrate or Nitrite additions to seawater

Similar to Experiments 1 and 2, the third incubation experiment was setup and stored in the anaerobic chamber in an environment of 4% H_2 in He on June 1, 2016. This experiment had 5 treatments in seawater. Treatments included a Live Control (3SLC), a Killed Control with nitrate addition ($3SKCN_3$), a Live Experimental treatment with nitrate addition ($3SLN_3$), a Killed Control nitrite addition ($3SKCN_2$) and a Live Experimental treatment with nitrite addition ($3SLN_2$). All experiments were constructed in triplicates with 15 bottles in total (Figure 2.4). With the exception of the addition of only nitrate or nitrite, all other experimental conditions remained the same as the seawater treatments in Experiment 2.

Treatment 3SLC was a Live Control in seawater and had no additional amendments. Treatments $3SKCN_3$ and $3SKCN_2$ were killed controls and consisted of nitrate and nitrite addition independently at 0.02174 mol/L respectively and also contained 2.5 mL of 0.0276 mol/L $HgCl_2$. Treatments $3SLN_3$ and $3SLN_2$ consisted of just nitrate and nitrite addition independently at 0.02174 mol/L respectively. The purpose of treatments $3SLN_3$ and $3SLN_2$ was to determine the individual ability of nitrate and nitrite to inhibit H_2S production by SRB.

Similar to Experiments 1 and 2, 5 mL aliquots of water were periodically extracted from the Experiment 3 bottles for E_h and pH measurements. These measurements occurred on days 1, 28, and 59. Sampling and termination dates were determined based upon the in-situ E_h and pH measurements. On day 59 the incubation experiments were terminated.

2.3 Sampling methods

In-situ measurements were taken at each sampling period. During water characterization, sample water was measured before, during and after the sampling period. In the experiments, 5 mL aliquots of water were sampled from the incubations and the redox potential was measured inside the anaerobic chamber and pH was measured immediately thereafter outside of the chamber.

In the field, seawater and produced waters were sampled separately for dissolved organic carbon (DOC) and total inorganic carbon (TIC). However, due to limited amounts of water in the laboratory experiments, organic and inorganic carbon were sampled together and filtered such that the dissolved forms of organic carbon and inorganic carbon (DOC/DIC) were measured for the experiments. All DOC and TIC/DIC samples were refrigerated in darkness until analysis. During water characterization and for Experiment 1, 20 mL DOC samples were collected in acid rinsed clear 24 mL volatile organic analysis (VOA) vials pre-spiked with 0.2 mL of 20% H_3PO_4 and filtered through 0.7 um glass fibre (GFF) filters (GE Healthcare Life Services, Whatman, Cat #: 1825-025). Furthermore, DIC samples were collected with no headspace in clear acid rinsed 24 mL VOA vials equipped with a black butyl rubber septa spiked with 0.1 mL of 0.0276 mol/L of $HgCl_2$. For Experiments 2 and 3, DOC and DIC was sampled for with no headspace in acid rinsed 24 mL VOA vials equipped with 20 mm fluoropolymer resin silicone septa (VWR Cat# 11311-628) and filtered through 0.7 um GFF filters. Experiments 2 and 3 DIC samples were not spiked with $HgCl_2$ because a black precipitate formed in the DIC

sample bottles after adding water samples that contained H_2S. As an alternative to this fixing agent, DOC and DIC samples were analyzed less than 24 hour after sampling.

During water characterization, 30 mL of sample water was collected for organic acid analyses in triplicate in 50 mL falcon tubes. All organic acid samples were sterilized by filtering the fluid through a 0.22 um mixed cellulose ester (MCE) filter (Thermo Fisher P/N: 09-720-004) and kept frozen until analysis. Six mL of sample water was collected for organic acids analysis in 15 mL falcon tubes for Experiment 1 and acid rinsed 24 mL VOA vials for Experiment 2 and 3.

Total dissolved nitrogen (TDN) was sampled for during the experiments in acid rinsed 24 mL VOA vials. Twelve mL of sample water were filtered through 0.7 um GFF filters and were refrigerated in darkness until analysis. Experiment 1 TDN samples were preserved with $HgCL_2$; however, Experiment 2 and 3 samples were not preserved with $HgCL_2$ as it reacted with the sulfide in the sample water. Therefore, Experiment 2 and 3 samples were not preserved with $HgCL_2$ and were instead analysed within 2 days of sampling. Sulfate, ammonia, and nitrate/nitrite were also sampled for in 15 mL falcon tubes. Ten mL of sample water were filtered through 0.22 MCE filters and were frozen and stored in the dark until analysis.

To monitor the changes in the headspace of the incubation experiments, 2 mL of headspace from each bottle was removed using a 5 mL BD syringes and injected into 5 mL serum bottles (Supelco P/N: 33102-Y). The serum bottles were flushed and filled with helium gas and crimp sealed with aluminum seals and conditioned blue butyl septa.

2.4 Analytical methods

Redox potential was measured using a water proof ORPTestr 10 with a platinum band electrode. The redox meter was stabilized by soaking in water for 24 hours before measurement in the field and for Experiment 1. During Experiment 2 and 3 the redox meter was calibrated using pH 4 and pH 7 solutions saturated with quinhydrone (CAS # 106-34-3, Sigma Aldrich P/N: 282960) (Reinsel et al., 1996) (http://www.astisensor.com/Alternate_ORP_Calibration_Procedure.pdf, 2016). Dissolved oxygen was measured in the field using CHEMets Self-Filling Ampoules for Colorimetric Analyses. pH was measured using a probe during bulk water sampling. In the laboratory an H-Series H280G benchtop pH meter was used with an ion-sensitive field-effect transistor (ISFET probe, HACH P/N: PHW17-SS) calibrated with Oakton pH 4, 7, and 10 calibration solutions (P/N: 00654-00, 00654-04, 00654-08).

DOC, DIC and TDN were analyzed using a high temperature combustion total organic carbon analyzer [Shimadzu Total Organic Carbon Analyzer (TOC-V)]. DOC and DIC were measured using a nondispersive infrared sensor (NDIR). DOC was measured from a calibration curve of diluted Potassium hydrogen phthalate ranging in concentration from 20 – 1000 mg C/L (D.L 8 ppb, 0.3% analytical error). DIC was measured from a calibration curve of diluted sodium carbonate and sodium bicarbonate ranging concentration from 4 – 200 mg C/L (D.L 8 ppb, 0.7% analytical error). TDN was measured by a Shimadzu equipped with a thermal decomposition catalyst that combusts the sample at 720°C, whereby the total nitrogen is thermally decomposed to nitrogen monoxide and is then detected with a chemiluminescent NO_x detector (TNM-1;

Shimadzu, Japan). TDN was measured from a calibration curve of diluted glutamic acid ranging in concentration from 0.4 – 20 mg N/L (D.L 8 ppb, 3.5% analytical error). Dissolved organic nitrogen (DON) was calculated from the difference between the measured TDN and measured TIN (NH_4^+, NO_2^-, and NO_3^-, described below).

Organic acids were measured using a Thermo Scientific DIONEX (ICS-2100) ion chromatograph (I.C.) with an AC11 HC (high capacity column) and concentrator column. Samples were run at 1.5 mL/min with 1 mM KOH, 223 mA, and 2650 psi. Sigma-Aldrich standards for the I.C. were used including formate, acetate, propionate, butyrate, lactate, and succinate (P/N in order as listed: 44293, 51716, 51716, 08089, 07096, and 43057). Samples were filtered through Dionex OnGuard II Ba/Ag/H Cartridges (P/N 063955). The cartridges removed halides by precipitation with silver, sulfate by precipitation with barium, and earth metals and cationic transition metals by cation-exchange with H^+. The maximum amount of water that could be filtered through the cartridge before Cl^- breakthrough was determined to be 2 mL. Deionized water and seawater were spiked with organic acids at concentrations ranging from ~ 400 ppb to ~13 ppb and were filtered through the Ba/Ag/H cartridges and diluted to 5.5 mL (minimum amount of water needed for analysis on the I.C). Due to the large variability of organic acid recovery, this method was determined to have very poor reproducibility and was not used for sample analysis.

Sulfate concentrations were measured using a HACH DR 2700 portable spectrophotometer (P/N: DR-2700-01) equipped with a 1” square glass 10 mL sample cell (HACH P/N: 2495402). A stock standard solution of 1000 mg/L of SO_4^{2-} (Na_2SO_4) and 0.0239 g/mL NaCl (to reflect the salt content of seawater) was used for calibration curves.

Three point calibration curves were made daily by a series of dilutions of the stock standard solution at concentrations of 10, 30 and 60 mg/L. The HACH Method 8051 (USEPA SulfaVer 4 Method) was used (detection limits of 2-70 mg/L, 3.2% error), whereby barium reacts with sulfate in the sample to form a precipitate and turbidity was measured with a wavelength of 450 nm (the amount of turbidity formed was proportional to the sulfate concentration).

Ammonia was measured using the same spectrophotometer and sample cell as sulfate. Daily three point calibration curves (0.1, 0.3, and 0,5 mg/L) were made by a series of dilutions of the HACH nitrogen-ammonia 10 mg/L standard solution (HACH P/N: 15349). The HACH Method 8155 (Salicylate Method) was used (detection limits of 0.1 – 0.5 mg/L, 3.1% error) whereby ammonia compounds combined with chorine to form monochloramine which then reacted with salicylate to form 5-aminosalicylate. The sample was then oxidized by the addition of a sodium nitroprusside catalyst to form a blue colored compound and was measured at a wavelength of 655 nm.

Nitrate was measured by a Lachat FIA 8500 inorganic nutrient analyzer (HACH, Loveland, CO, USA) using the QuickChem Method 10-107-01-1-A (detection limits of 0.2 – 20 mg N/L as NO_3^- or NO_2^-). In summary, nitrate was reduced to nitrite by a copperized cadmium column, the resulting nitrite (reduced nitrite plus original nitrite) was then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl)ethylenediamine dichloride. The resulting sample was magenta in color and was measured at a wavelength 520 nm (4.3% analytical error).

N_2O concentrations were measured using a gas chromatograph with an electron capture detector (ECD). A HayeSep D column with a helium carrier gas and a temperature program of 80°C hold 7 minutes, ramp 20°C/minute to 200°C, hold 2 minutes was used. Daily calibration curves were created for N_2O by injecting varying volumes (0.3-1 mL) of a standard containing 2.1 ppm by volume of N_2O using a 50-microliter gas tight locking Hamilton syringe.

2.5 Safety Procedures

H_2S gas is extremely toxic to human life and thus safety was the main priority when conducting these experiments. All participants received H_2S Alive, compressed gas training, WHMIS, and lab safety training where we learned of the many dangers of H_2S and of the laboratory. Lessons included the prevention of an accident from happening, the materials and devices needed to ensure safety, and also the protocols of what should be done if H_2S concentrations in the laboratory reach a dangerous level.

Firstly, we limited the amount of water in the anaerobic chamber such that if the entire amount of sulfate in the seawater was reduced to sulfide and if all that sulfide was released into the atmosphere of the lab, then the resulting concentrations of sulfide in laboratory air would be below dangerous levels. All incubation bottles were completely sealed upon setup and were stored within a sealed anaerobic chamber. This chamber was placed underneath a large vent. The anaerobic chamber was equipped with three 13X Molecular Sieve canisters (Plas Labs Inc., P/N: 800-MOLS/M) used to remove H_2O, CO_2 and H_2S. Additionally, 5.0 M NaOH scrubbers equipped with spargers were installed; one inside the chamber and one outside of the chamber to act as H_2S traps. The external

NaOH scrubber used a Master Flex Easy-Load Peristaltic Pump housed in a Master Flex Portable Sampling Drive and was only used during experimental set-up and sampling times and was leak checked before use. The internal scrubber was continuously running and used a Fluval Q.5 Air Pump. NaOH solutions were held in sealed Buchner flasks and were changed once the spargers became blocked with precipitate.

Personal and fixed H_2S detectors were used (Drager Pac 3500 P/N: 4543958, Drager PointGard II P/N: 453310, respectively). One personal detector was placed inside the glove box and all personnel inside the laboratory were required to wear one at all times. The fixed H_2S detector was constantly running and had an attached battery pack in lieu of power outages (12VDC 12Ah). The personal H_2S detectors were bump tested before laboratory work using the Drager calibration gas (58 L, 25 ppm H_2S in N_2, P/N: 4502155) and 500cc 5/8" 18 UNF regulator (P/N: 4557020). The fixed detector was bump tested every 6 months using the same calibration gas in addition to the Drager Calibration Kit (P/N: 4594620). A self-contained breathing apparatus (SCBA) was also purchased in case dangerous levels were reached and an immediate rescue was needed. During the experiments, the maximum level of H_2S reached in the anaerobic chamber was 5 ppm. Safety procedures were strictly enforced and followed by all working in laboratory.

2.6 Statistical analyses

All treatment values given in the results section are the mean of the triplicate incubations for each treatment. The standard deviations ($\pm 1\sigma$) are also calculated from the same triplicates, and are displayed as error bars in the figures below. The analytical error

for the dissolved organic nitrogen (DON) values was determined from the square root of the sum of the squares of the standard deviations associated with measurements of total dissolved nitrogen (TDN) and dissolved inorganic nitrogen (DIN). The analytical error for each method was calculated by dividing the standard deviation from the average of multiple check standards within a run, and then multiplying by 100%. Where indicated, significance tests (t-tests) were conducted with an α–value of 0.05 (2 tailed, type three), whereby p-values above 0.05 were considered non-significant, and p-values below 0.05 were considered significant.

Chapter 3: Results

3.1 Bulk water characterization

Waters were collected from offshore Newfoundland and Labrador on March 13, 2015. The geochemical characterization values for seawater (injection water) and produced water are shown in Table 3.1.

Table 3.1. Geochemical characterization of seawater (injection water) and produced water.

Seawater		**Produced Water**	
pH	7.24	pH	6.68
Dissolved Oxygen	N.a.*	Dissolved Oxygen	>40 ppb
Redox Potential	+203 mV	Redox Potential	-51.5 mV
Temperature	28 °C	Temperature	52 °C
DOC (mol C/L)	$3.2 \times 10^{-4} \pm 2.9 \times 10^{-5}$	DOC (mol C/L)	$1.02 \times 10^{-2} \pm 2.4 \times 10^{-5}$
DIC (mol C/L)	$2.1 \times 10^{-3} \pm 8.3 \times 10^{-7}$	DIC (mol C/L)	$8.0 \times 10^{-3} \pm 4.5 \times 10^{-5}$
Sulfate (mol SO_4^{2-} /L)	$3.28 \times 10^{-2} \pm 1.8 \times 10^{-3}$	Sulfate (mol SO_4^{2-} /L)	$2.77 \times 10^{-2} \pm 1.3 \times 10^{-3}$
Nitrate + Nitrite (mol N/L)	$5.35 \times 10^{-4} \pm 1.0 \times 10^{-4}$	Nitrate + Nitrite (mol N/L)	$6.13 \times 10^{-3} \pm 1.4 \times 10^{-4}$

*N.a. Not analyzed

There were many differences in the geochemical values measured for seawater and produced waters. Firstly, the redox potential (E_h) for the produced waters was reducing (negative), whereas the seawater had a positive E_h. Moreover, the produced waters were more acidic and had a higher temperature than the seawater. Additionally, there was more sulfate present in the seawater than the produced waters, as was expected with high amounts of sulfate present in seawater and the hypothesized consumption of sulfate within the reservoir. However, there was a higher concentration of nitrate + nitrite and overall carbon (DOC and DIC) in the produced waters compared to the seawater.

3.2 Experiment 1: Testing the effect of nitrate and nitrite addition on microbial consumption of sulfate in seawater

The redox potential values for Experiment 1 are shown in Figure 3.1. Initially the samples were oxic (i.e. positive redox value). However, reducing conditions are required for nitrate and sulfate reduction. To create reducing conditions biologically, the incubations were biostimulated by adding H_2 gas and acetate. Biostimulation was attempted on days 41, 51, and 78 of the experiment, as shown by the vertical lines in Figure 3.1. However, despite these additions reducing conditions were not achieved in any of the treatments. From day 1 to day 109 there was a great deal of change over time, but at each time point the redox values were relatively similar in all treatments. On day 109 the redox values were relatively similar for all treatments (148 mV for the Live Control, 152 mV for the Experimental Live, and 163 mV for the Killed Control). Conversely, on day 29 there was a larger difference in treatment redox values (235 mV for the Live Control treatments, 198 mV for Experimental Live treatments, and 160 mV for the Killed Control treatments). However, the total overall change in redox value throughout Experiment 1 was approximately only 30 mV, which was not a relatively large difference. Ultimately reducing conditions needed for nitrate and sulfate reduction were not achieved.

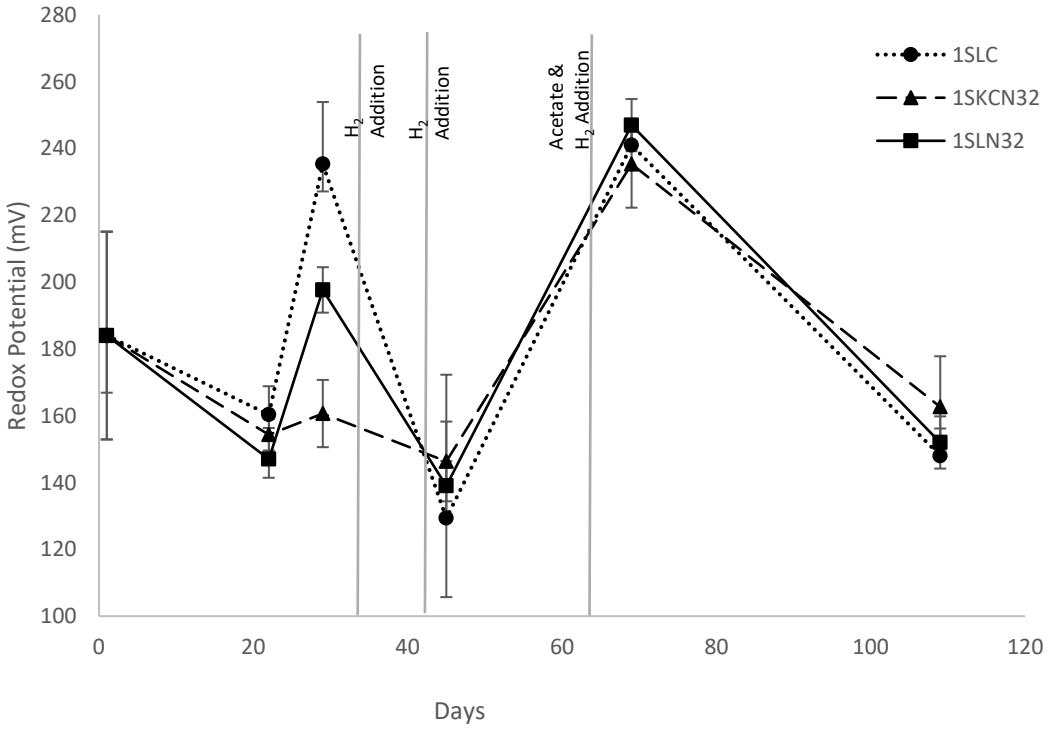

Figure 3.1. Redox potentials for Experiment 1 seawater treatments. The experiment included three treatments: Live Control (1SLC), Killed Control nitrate and nitrite addition (1SKCN32), and Live Experimental nitrate and nitrite addition (1SLN32). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment. Vertical lines represent the days when H_2 gas and acetate were added to all treatments.

Sulfate concentrations for Experiment 1 (Figure 3.2) remained relatively unchanged for the entirety of the experiment. The sulfate concentration on day 1 was 2.35 x 10^{-2} ± 1.1 x 10^{-3} mol/L. The Killed Control treatment had the lowest final concentration of sulfate at 2.31 x 10^{-2} ± 4 x 10^{-4} mol/L. The Experimental Live treatment had the highest final concentration of sulfate at 2.42 x 10^{-2} ± 1 x 10^{-3} mol/L and the Live Control had a final concentration of sulfate at 2.39 x 10^{-2} ± 4 x 10^{-4} mol/L. There was no discernable

difference in final concentrations of the three treatments within their standard deviations. This was in agreement with the redox potential values for Experiment 1(Figure 3.1), as the live treatments were not reducing and thus sulfate reduction was not expected.

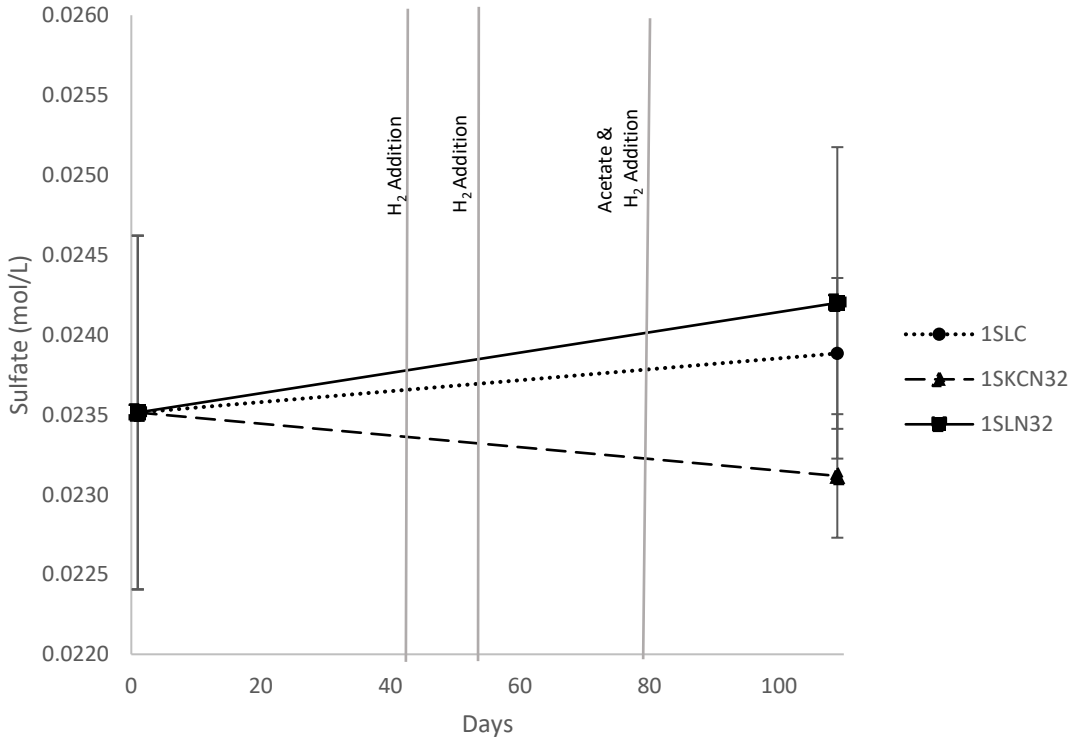

Figure 3.2. Sulfate concentrations for Experiment 1 seawater treatments. The experiment included three treatments: Live Control (1SLC), Killed Control nitrate and nitrite addition (1SKCN32), and Live Experimental nitrate and nitrite addition (1SLN32). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment. Vertical lines represent the days when H_2 gas and acetate were added to all treatments.

Dissolved inorganic nitrogen (DIN) for Experiment 1 seawater treatments was calculated from the summation of the measured nitrate, nitrite and ammonia concentrations. The starting concentrations for the Killed Control ($1SKCN_{32}$) and Experimental Live ($1SLN_{32}$) treatments consisted of 0.00161 mol/L nitrate and 0.02174 mol/L nitrite which was approximately 7.8×10^{-2} mol N/L. The Live Control (1SLC) treatment bottles had no nitrogen amendments added and had a starting DIN concentration of $1.75 \times 10^{-4} \pm 5.6 \times 10^{-6}$ mol N/L. Figure 3.3 shows that there was a decrease of ~70% DIN for the Killed Control ($1SKCN_{32}$) treatments and a decrease of ~60% DIN for the Experimental Live ($1SLN_{32}$) treatments. The DIN in the Live Control incubations stayed relatively constant for the duration of the experiment with a final concentration of $1.6 \times 10^{-4} \pm 9 \times 10^{-6}$ mol N/L. The observed decrease in DIN concentrations in the Live nitrate and nitrite addition treatments was not due to biological reactions since a similar decrease in DIN was observed in the Killed Control nitrate and nitrite addition treatments.

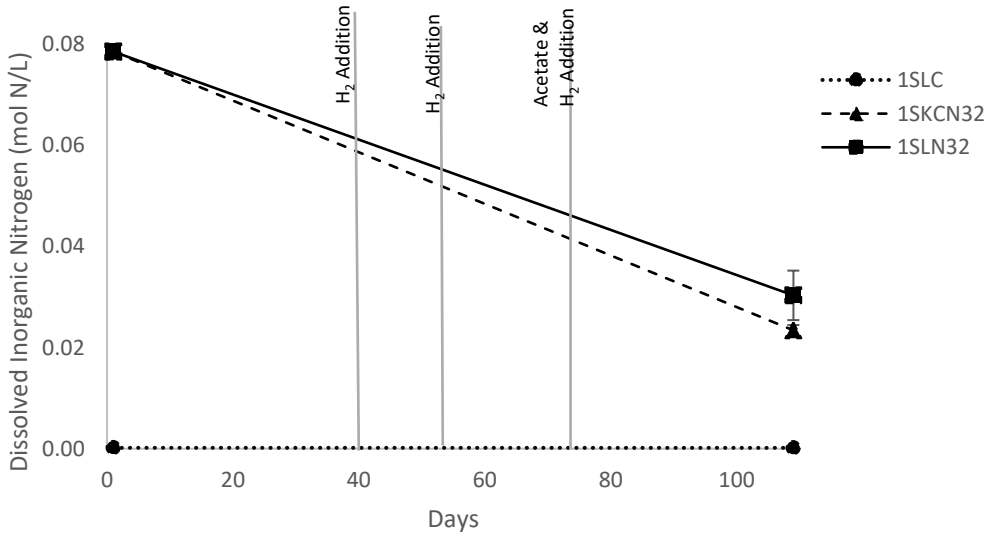

Figure 3.3. Dissolved inorganic nitrogen concentrations for Experiment 1 seawater treatments. The experiment included three treatments: Live Control (1SLC), Killed Control nitrate and nitrite addition (1SKCN32), and Live Experimental nitrate and nitrite addition (1SLN32). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment. Vertical lines represent the days when H_2 gas and acetate were added to all treatments.

Ammonia concentrations measured during Experiment 1 are plotted in Figure 3.4. Ammonia concentration increased by 154% in the Killed Control ($1SKCN_{32}$) treatments and 150% in the Experimental Live ($1SLN_{32}$) treatments. However, ammonia concentrations in the Live Control (1SLC) treatments (no nitrogen amendments added) did not increase during this experiment.

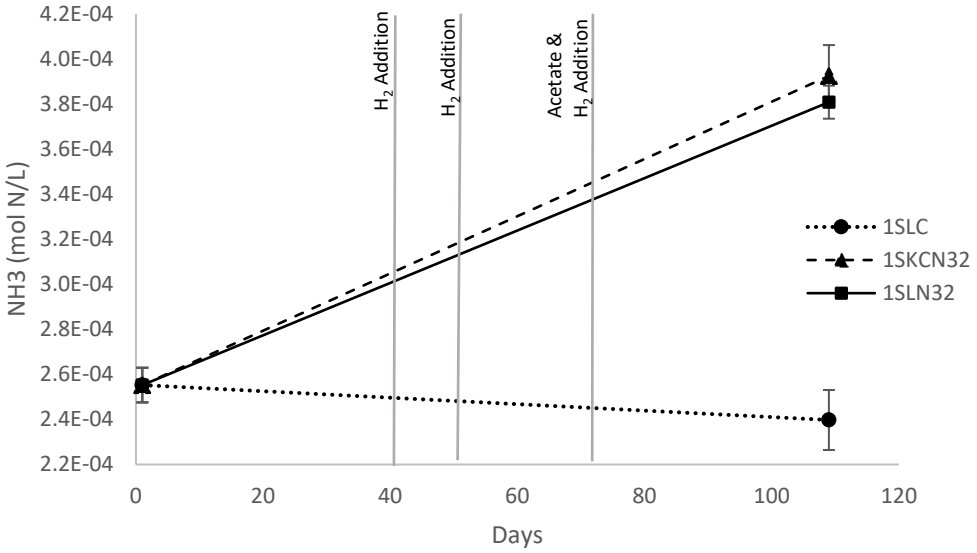

Figure 3.4. Ammonia concentrations for Experiment 1 seawater treatments. The experiment included three treatments: Live Control (1SLC), Killed Control nitrate and nitrite addition (1SKCN32), and Live Experimental nitrate and nitrite addition (1SLN32). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment. Vertical lines represent the days when H_2 gas and acetate were added to all treatments.

Dissolved organic nitrogen (DON) concentrations calculated during Experiment 1 are shown in Figure 3.5. DON was calculated by subtracting the measured total dissolved nitrogen from the calculated DIN. DON concentrations increased in the Killed Control ($1SKCN_{32}$) and Experimental Live ($1SLN_{32}$) treatments from day 1 to day 109. The average DON concentration on day 1 for all three treatments was $1.46 \times 10^{-4} \pm 1.4 \times 10^{-5}$ mol N/L. The final average concentration of DON for the Killed Control treatments was $4.85 \times 10^{-3} \pm 1 \times 10^{-3}$ mol N/L and the final average DON concentration for the Experimental Live treatments was $7.94 \times 10^{-4} \pm 1.3 \times 10^{-3}$ mol N/L. DON concentration

in the Live Control (1SLC) treatments stayed relatively constant for the duration of the experiment. The observed increase in DON concentrations in the Experimental Live ($1SLN_{32}$) treatments was not due to biological reactions since a similar increase in ammonia was observed in the Killed Control ($1SKCN_{32}$) treatments.

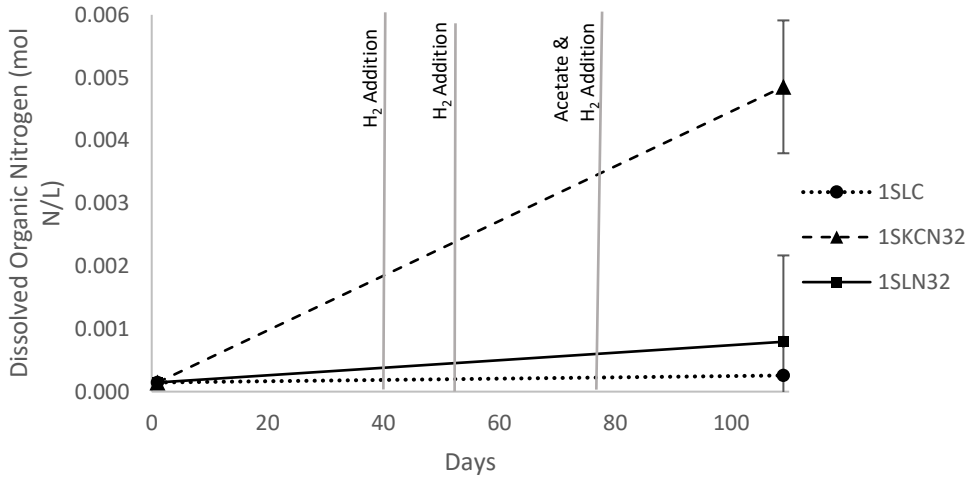

Figure 3.5. Dissolved organic nitrogen concentrations for Experiment 1 seawater treatments. The experiment included three treatments: Live Control (1SLC), Killed Control nitrate and nitrite addition (1SKCN32), and Live Experimental nitrate and nitrite addition (1SLN32). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment. Vertical lines represent the days when H_2 gas and acetate were added to all treatments.

Dissolved organic carbon (DOC) concentration decreased in all treatments in Experiment 1 (Figure 3.6). DOC decreased by ~36% for the Live and Killed Control treatments and by ~48% for the Live Experimental treatments. The decrease in DOC in all bottles occurred despite the addition of 0.0002 mol/L of acetate on day 78. The

observed decrease in DOC concentrations in the Live treatments was not due to biological reactions because a similar decrease in DOC was also observed in the Killed Control treatments.

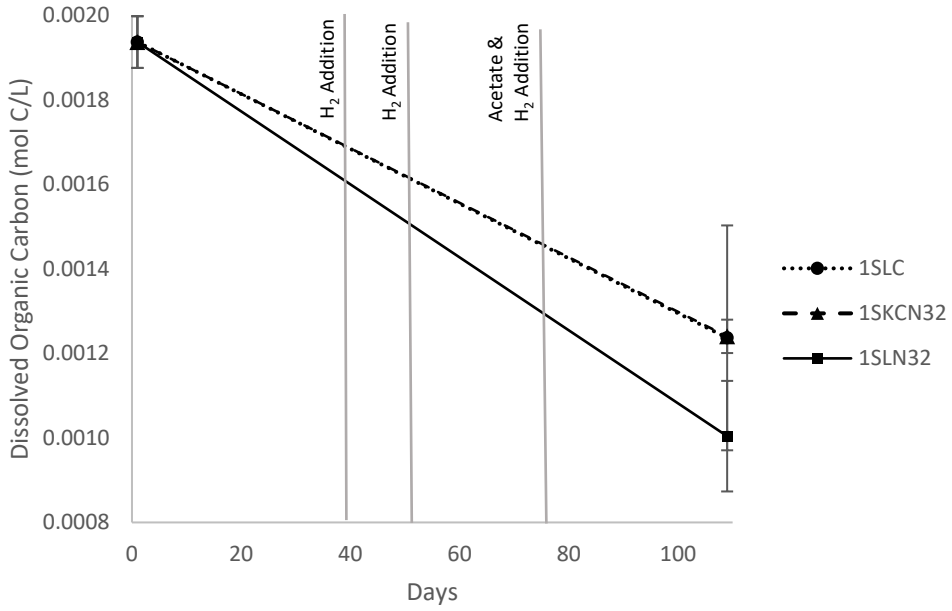

Figure 3.6. Dissolved organic carbon (DOC) concentrations for Experiment 1 seawater treatments. The experiment included three treatments: Live Control (1SLC), Killed Control nitrate and nitrite addition (1SKCN32), and Live Experimental nitrate and nitrite addition (1SLN32). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment. Vertical lines represent the days when H_2 gas and acetate were added to all treatments.

The hydrogen potential (pH) measurements for Experiment 1 are shown in Figure 3.7. On day 109, the nitrate and nitrite addition treatments ($1SLN_{32}$) were more basic (pH of 8.2) than the Live Control (pH of 7.8) and Killed Control (pH of 7.5) treatments.

Moreover, the Killed Control ($1SKCN_{32}$) treatments were more acidic than both of the Live treatments. Therefore, by day 109 the addition of nitrate and nitrite and biological reactions, created a less acidic environment in the Live treatments than the Killed treatments.

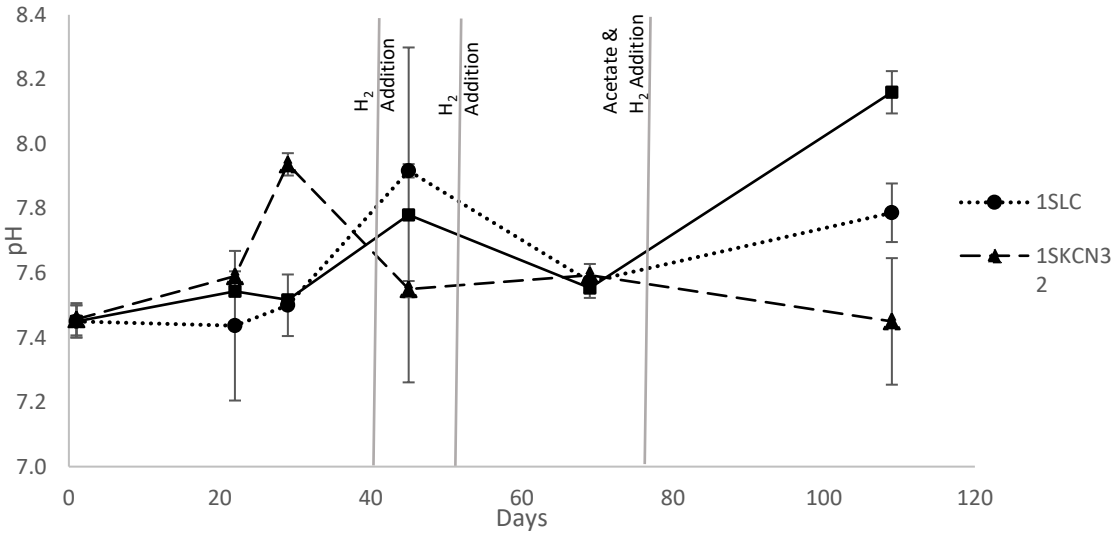

Figure 3.7. pH for Experiment 1 seawater treatments. The experiment included three treatments: Live Control (1SLC), Killed Control nitrate and nitrite addition (1SKCN32), and Live Experimental nitrate and nitrite addition (1SLN32). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment. Vertical lines represent the days when H_2 gas and acetate were added to all treatments.

3.3 Experiment 2: Testing the affect of nitrate and nitrite addition in seawater and produced waters with the addition of a reducing agent.

3.3.1 Seawater treatments

Within 37 days all Live seawater treatments of Experiment 2 became reducing (Figure 3.8). The Live Control treatments (2SLC) were reduced at the fastest rate (-17 mV/day), and remained relatively constant at approximately -370 mV after day 37. It was evident that the addition of the reducing agent (Na_2S) and mineral media was successful in stimulating anaerobic conditions. The significant difference (p-value < 0.05) between the Live Control and Killed Control treatments suggested that microbial activity influenced the redox value. Moreover, the Live Nitrate and Nitrite addition incubations ($2SLN_{32}$) were less reducing on day 97 at a value of -194 mV than the Live Control (2SLC) incubations at -386 mV. This is consistent with the nitrate reduction position on the redox ladder (Figure 1.2) whereby the SRB were active in more reducing environments than NRB. Therefore, unlike Experiment 1, in Experiment 2 reducing conditions were established that were conducive to nitrate and sulfate reduction. Additionally, it was important to add a reducing agent in order to create reducing conditions; biostimulation through the addition of H_2 and acetate (like in Experiment 1) was not enough.

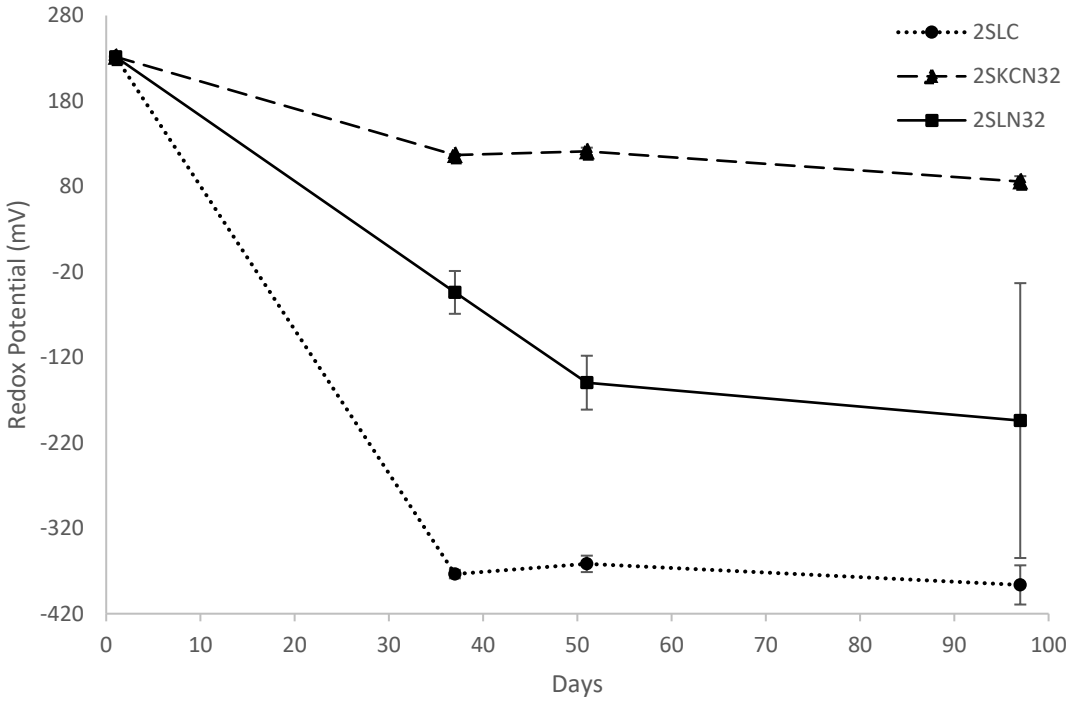

Figure 3.8. Redox potential for Experiment 2 seawater treatments. The experiment included 3 treatments consisting of a Live Control in seawater (2SLC), Nitrate & Nitrite addition Killed Control in seawater ($2SKCN_{32}$), and Nitrate & Nitrite addition Live Experimental in seawater ($2SLN_{32}$). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment.

Sulfate concentrations measured during Experiment 2 are plotted in Figure 3.9. There was a 20% decrease in sulfate concentration in the Live Control (2SLC) seawater treatments between day 1 and day 97. Moreover, sulfate concentrations did not decrease substantially over time in the nitrate and nitrite addition Live Experimental ($2SLN_{32}$) or Killed Control ($2SKCN_{32}$) treatments. The Live Experimental ($2SLN_{32}$) and Killed Control ($2SKCN_{32}$) treatments were higher in sulfate concentration on day 97 (2.78×10^{-2}

± 2.2 x 10^{-3} mol/L, and 2.53 x 10^{-2} ± 1.2 x 10^{-3} mol/L respectively) than the Live Control (2SLC) treatments (2.1 x 10^{-2} ± 5.5 x 10^{-4} mol/L). The Live Control treatments were significantly lower (p-value < 0.05) in sulfate concentration than the Killed Control and Live Experimental treatments on day 97. The lower concentration of sulfate in the Live Control treatments compared to the Killed Control and Live Experimental treatments was evidence of microbial sulfate reduction in the Live Control incubations. Particularly, microbial sulfate reduction was inhibited in the nitrate and nitrite addition experiments. In summary, the addition of nitrate and nitrite successfully inhibited microbial sulfate reduction.

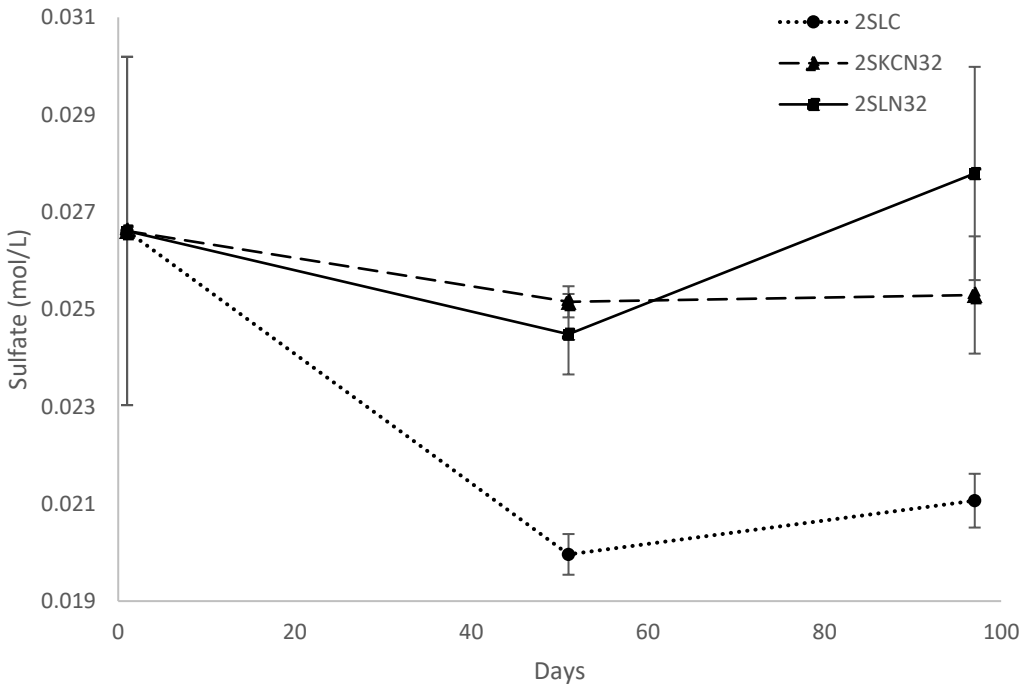

Figure 3.9. Sulfate concentrations for Experiment 2 seawater treatments. The experiment included 3 treatments consisting of a Live Control in seawater (2SLC), Nitrate & Nitrite addition Killed Control in seawater water ($2SKCN_{32}$), and Nitrate & Nitrite addition Live

Experimental in seawater ($2SLN_{32}$). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment.

Dissolved inorganic nitrogen (DIN) concentrations for Experiment 2 are plotted in Figure 3.10. DIN was calculated from the sum of the measured nitrate and nitrite concentrations and the measured ammonia concentrations. Nitrate and nitrite were added at time zero to both the Killed Control (2SKCN32) and Experimental Live treatments (2SLN32). Nitrate was added at a concentration of 0.00161 mol/L and nitrite was added at a concentration of 0.02174 mol/L. However, no nitrate or nitrite were added to the Live Control (2SLC) treatments. The addition of nitrogen species to some treatments, but not others was responsible for the different starting concentrations of dissolved inorganic nitrogen (DIN) seen in Figure 3.10. All nitrate and nitrite addition treatments decreased in DIN between days 0 and 51. The Live Nitrate and Nitrite addition ($2SLN_{32}$) treatments decreased in DIN at the highest rate. On days 51 and 97 the Killed Control treatments were significantly higher (p-value < 0.05) in DIN concentration ($32.7 \times 10^{-2} \pm \times 10^{-3}$ mol N/L) than the Live Experimental treatments ($1.6 \times 10^{-4} \pm 4 \times 10^{-5}$ mol N/L). Therefore the faster DIN consumption rate observed in the Live Experimental ($2SLN_{32}$) treatments was due to microbial activity (e.g. nitrate reducers). However, there are also abiotic reactions that consume DIN as well.

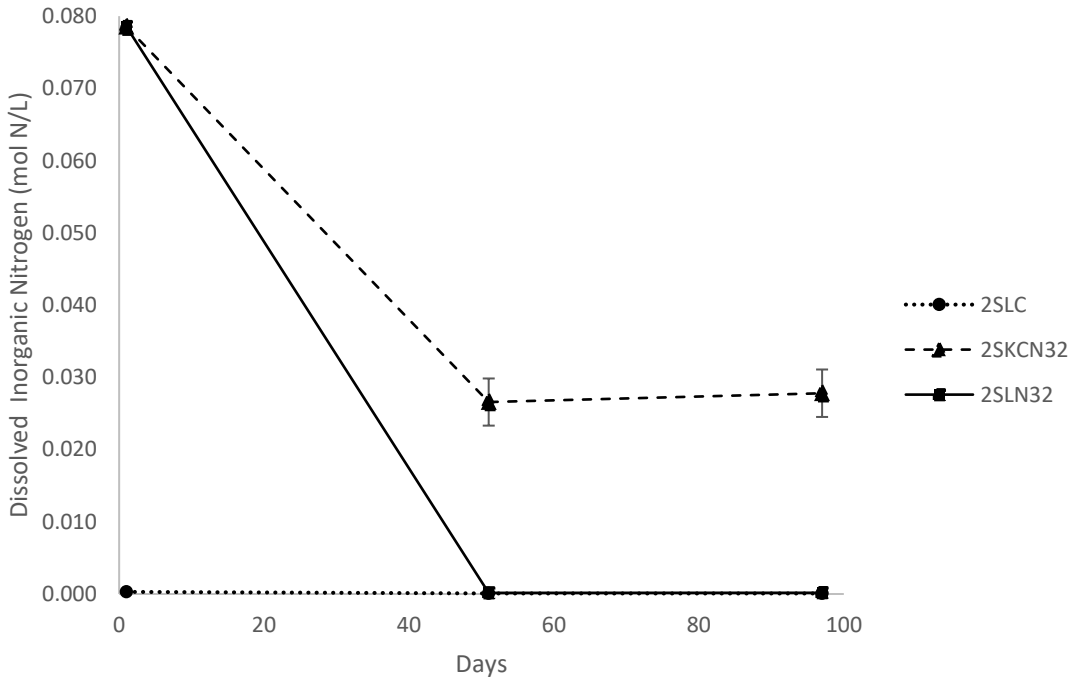

Figure 3.10. Dissolved inorganic nitrogen concentrations for Experiment 2 seawater treatments. The experiment included 3 treatments consisting of a Live Control in seawater (2SLC), Nitrate & Nitrite addition Killed Control in seawater water ($2SKCN_{32}$), and Nitrate & Nitrite addition Live Experimental in seawater ($2SLN_{32}$). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment.

Ammonia concentrations measured during Experiment 2 were plotted in Figure 3.11. Ammonia concentrations for Experiment 2 Killed Control treatments increased by ~150% between days 1 and 97 (Figure 3.4). In contrast, ammonia concentrations decreased by 75% in the Live Control treatments and 43% in the Live Experimental treatments between days 1 and 97 (Figure 3.11). Moreover, ammonia was consumed in both the Live Nitrate and Nitrite addition treatments ($2SLN_{32}$) and the Live Control treatments (2SLC). Therefore the significant difference (p-value < 0.05) in ammonia

concentration between the Live and Killed treatments suggests that the ammonia consumption in the live incubations was due to microbial activity.

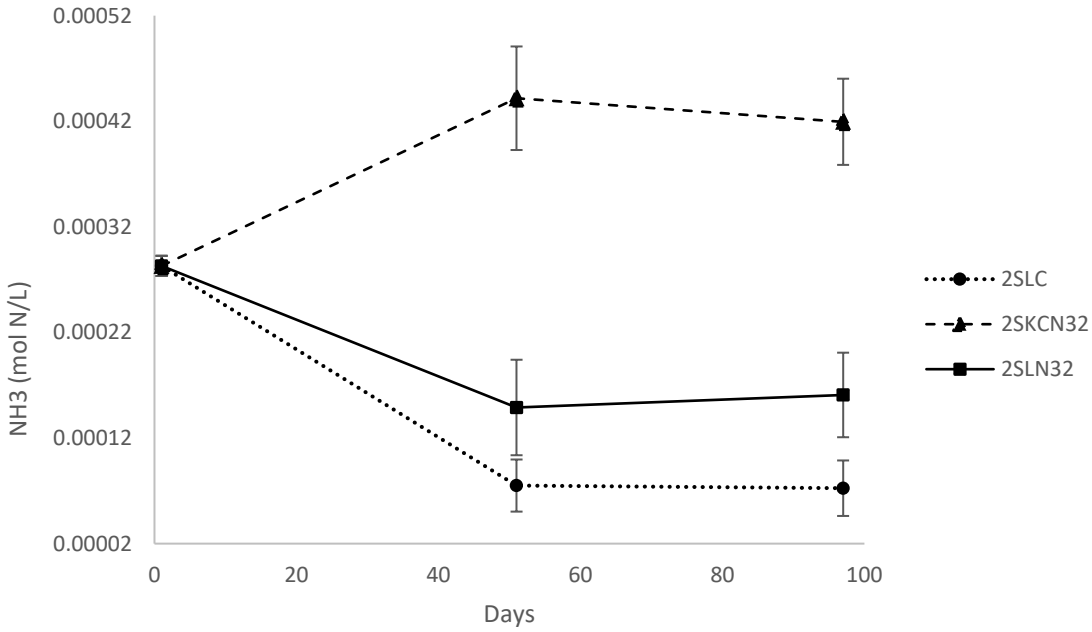

Figure 3.11. Ammonia concentrations for Experiment 2 seawater treatments. The experiment included 3 treatments consisting of a Live Control in seawater (2SLC), Nitrate & Nitrite addition Killed Control in seawater water ($2SKCN_{32}$), and Nitrate & Nitrite addition Live Experimental in seawater ($2SLN_{32}$). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment.

The dissolved organic nitrogen (DON) concentrations for Experiment 2 with seawater are plotted in Figure 3.12. Dissolved organic nitrogen (DON) was calculated from the difference between the measured total dissolved nitrogen (TDN) and the dissolved inorganic nitrogen (DIN). As seen in Figure 3.12, DON increased by 1.9×10^{-3} mol N/L within the first 50 days in the Killed Control, while the DON remained relatively

constant (and nearing zero) in the Live Control and Live nitrate and nitrite addition treatments.

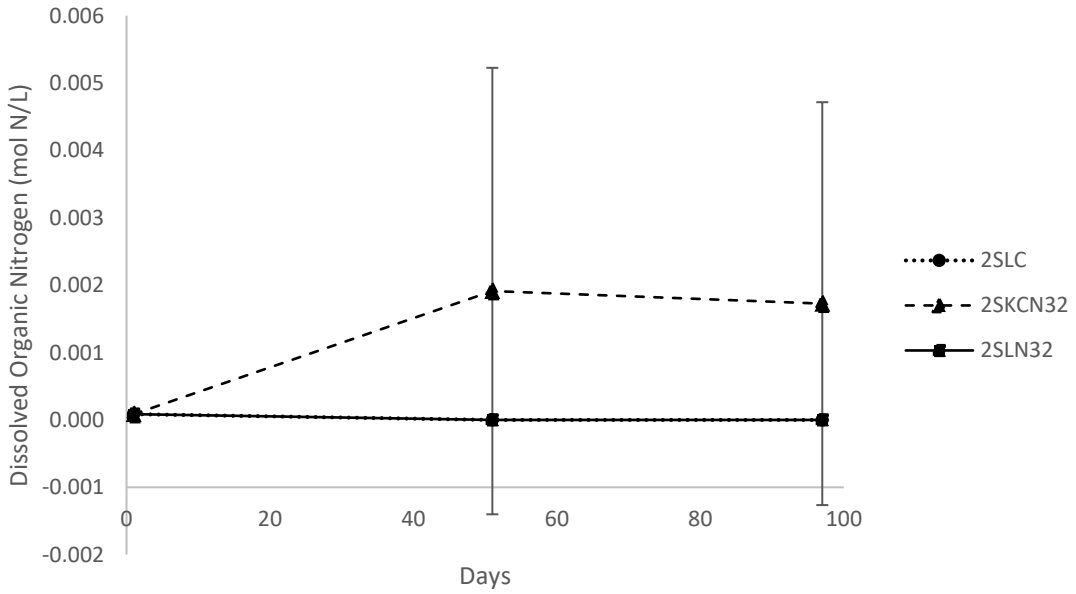

Figure 3.12. Dissolved organic nitrogen concentrations for Experiment 2 seawater treatments. The experiment included 3 treatments consisting of a Live Control in seawater (2SLC), Nitrate & Nitrite addition Killed Control in seawater water ($2SKCN_{32}$), and Nitrate & Nitrite addition Live Experimental in seawater ($2SLN_{32}$). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment.

Nitrous oxide (N_2O) gas concentrations were measured during Experiment 2 with seawater for the Killed Control and Live Experimental treatments (Figure 3.13). N_2O decreased in concentration from day 51 to day 97 in both the Live Experimental treatment ($2SLN_{32}$) (~ 32% decrease) and Killed Control treatment ($2SKCN_{32}$) (~ 38% decrease). The observed decrease in N_2O concentrations in the Live Experimental treatments was

not due to biological reactions because a similar decrease was also observed in the Killed Control treatments.

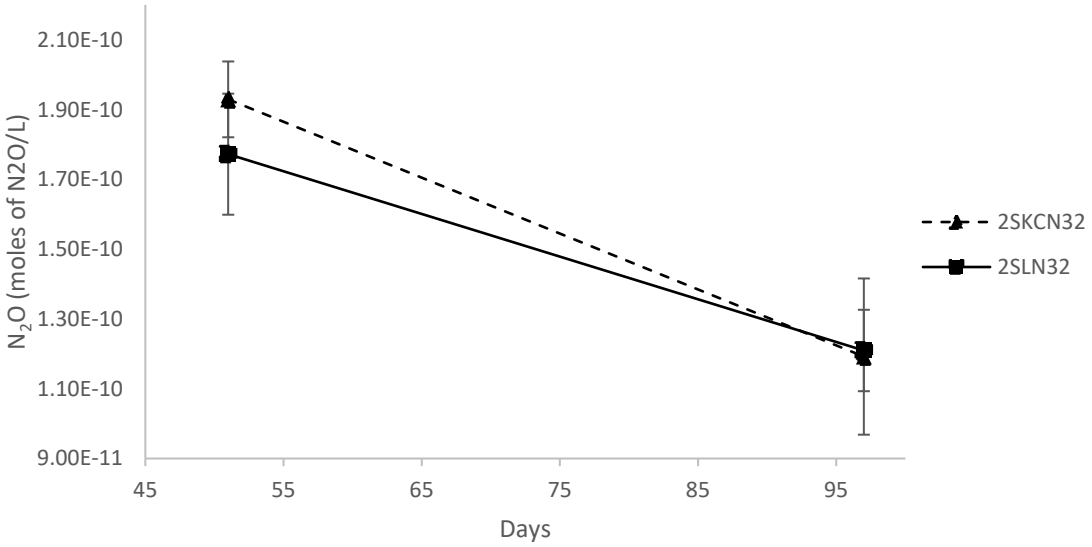

Figure 3.13. N_2O gas concentrations for Experiment 2 seawater treatments. The experiment included 3 treatments consisting of a Live Control in seawater (2SLC), Nitrate & Nitrite addition Killed Control in seawater water ($2SKCN_{32}$), and Nitrate & Nitrite addition Live Experimental in seawater ($2SLN_{32}$). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment.

Dissolved organic carbon (DOC) concentrations measured during Experiment 2 with seawater are plotted in Figure 3.14. The starting concentration of DOC for all treatments was approximately 0.160 mol C/L, which reflected the organic acids that were added to each incubation bottle during the experimental setup. During the 1st 50 days of the experiment, there was a decrease in DOC concentrations in all treatments (Figure 3.14). On day 50 the Live Control treatments had decreased to $5.18 \times 10^{-} \pm 5.5 \times 10^{-3}$ mol

C/L, the Killed Control treatments decreased to $6.7 \times 10^{-2} \pm 8.5 \times 10^{-3}$ mol C/L, and the Experimental Live treatments decreased to $4.4 \times 10^{-2} \pm 3.4 \times 10^{-3}$ mol C/L. The Live Control (2SLC) treatments consumed DOC at the highest rate of 1×10^{-3} mol/day and reached the lowest overall value of $4.8 \times 10^{-2} \pm 3 \times 10^{-3}$ mol C/L. The Killed Control ($2SKCN_{32}$) treatments had a higher concentration of DOC than the Live incubations (a difference of 1.73×10^{-2} mol C/L between the Killed Control and Live Experimental), suggesting microbial consumption of DOC in the live treatments.

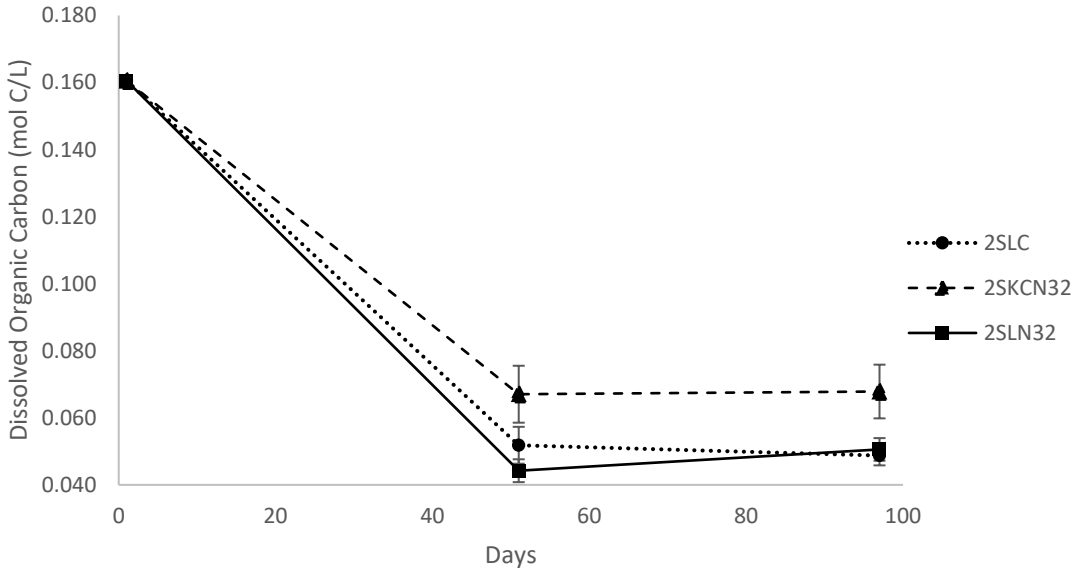

Figure 3.14. Dissolved organic carbon concentrations for Experiment 2 seawater treatments. The experiment included 3 treatments consisting of a Live Control in seawater (2SLC), Nitrate & Nitrite addition Killed Control in seawater water ($2SKCN_{32}$), and Nitrate & Nitrite addition Live Experimental in seawater ($2SLN_{32}$). Plotted points are the mean values ($\pm 1\sigma$) of triplicate incubations for each treatment.

Dissolved inorganic carbon (DIC) concentrations measured during Experiment 2 with seawater are plotted in Figure 3.15. DIC concentrations increased in all treatments between day 0 and 51: over 2200% increase for the Live Control treatments, 440% increase for the Killed Control treatments, and ~ 1700% increase for the Live Experimental. The Live Control (2SLC) incubations had the highest rate of DIC production (2×10^{-4} mol C/L/day) from day 1 to 51, and had the highest overall concentration of $1.05 \times 10^{-2} \pm 5 \times 10^{-4}$ mol C/L. The Killed Control ($2SKCN_{32}$) treatments had the overall lowest amount of DIC production. Therefore the significantly (p-value < 0.05) increased production of DIC in the Live treatments compared to the Killed Control ($2SKCN_{32}$) treatments suggest that the greater amounts of DIC produced in the Live treatments were due to microbial processes. Together the increasing trends of DIC and decreasing trends of DOC in the live treatments supported heterotrophic metabolism of the DOC being oxidized to DIC. Moreover, the Live Control (2SLC) treatments, where there is evidence for microbial sulfate reduction, had an overall higher DIC concentration than the nitrate and nitrite addition treatments on days 51 and 97 (greater than 2.7×10^{-3} and 1.5×10^{-3} mol C/L respectively). This was consistent with the observed difference in DOC concentrations between the Live Control and Live Nitrate and Nitrite addition treatments and suggests DIC was produced faster and at a higher concentration in the sulfate reducing environment, and furthermore DOC was consumed faster in a sulfate reducing environment.

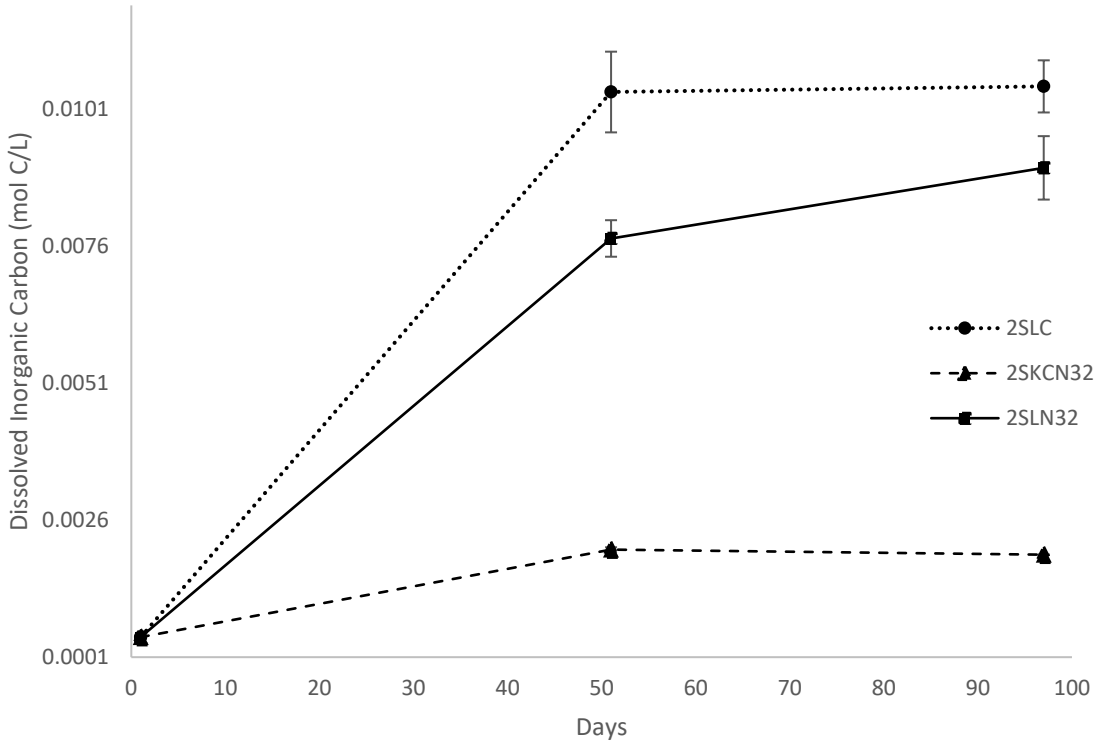

Figure 3.15. Dissolved inorganic carbon concentrations for Experiment 2 seawater treatments. The experiment included 3 treatments consisting of a Live Control in seawater (2SLC), Nitrate & Nitrite addition Killed Control in seawater water ($2SKCN_{32}$), and Nitrate & Nitrite addition Live Experimental in seawater ($2SLN_{32}$). Plotted points are the mean values ($\pm$ 1σ) of triplicate incubations for each treatment.

The hydrogen potential (pH) measurements for Experiment 2 with seawater are plotted in Figure 3.16. There was no overall change in pH for the duration of the experiment for each incubation. Furthermore, there was no observable difference in pH between each treatment.

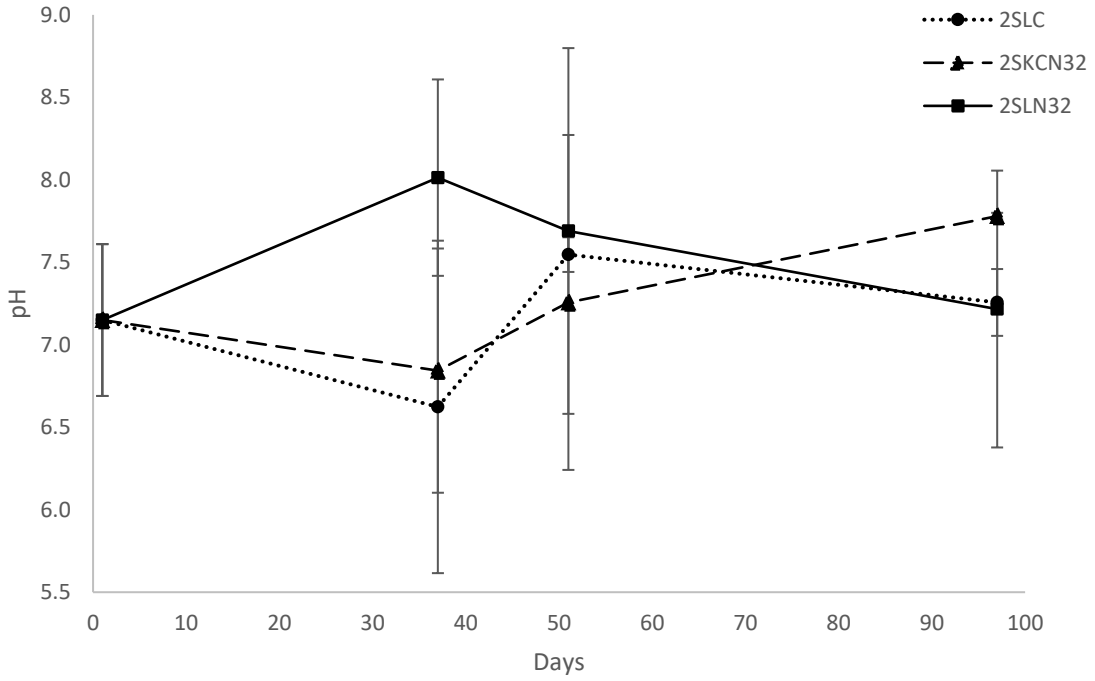

Figure 3.16. Hydrogen Potential for Experiment 2 seawater treatments. The experiment included 3 treatments consisting of a Live Control in seawater (2SLC), Nitrate & Nitrite addition Killed Control in seawater water ($2SKCN_{32}$), and Nitrate & Nitrite addition Live Experimental in seawater ($2SLN_{32}$). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment.

3.3.2 Produced water treatments

The oxidation reduction (redox) potentials for Experiment 2 with produced waters are plotted in Figure 3.17. All produced water incubations for Experiment 2 became more reducing between day 1 and day 37(i.e. a decrease of in 284 mV for the Live Control treatments, decrease of 97 mV for the Killed Control treatments, and a decrease of 198 mV for the Experimental Live treatments) (Figure 3.17). The lowering in redox value for all 3 treatments was aided by the addition of a reducing agent (i.e, Na_2S). The Live Control (2PLC) treatments were the most reduced treatment on days 37 and 51 (-63 mV and -204 mV respectively). Moreover, the Live Control incubations were more reduced than the Live Nitrate and Nitrite addition ($2PLN_{32}$) experiments (a difference of 239 mV on day 51). Likewise, the Live Nitrate and Nitrite treatments were more reduced (61 mV, day 97) than the Killed Control treatments (91 mV, day 97). The more reduced environments of the Live treatments compared to the Killed Control treatment can be attributed to microbial processes. Therefore, while the reducing agent was effective in creating a more reducing environment in all treatments, microbial activity in the Live treatments created an even more reducing environment which is reflected by the more negative redox values in the Live treatments compared to the Killed control.

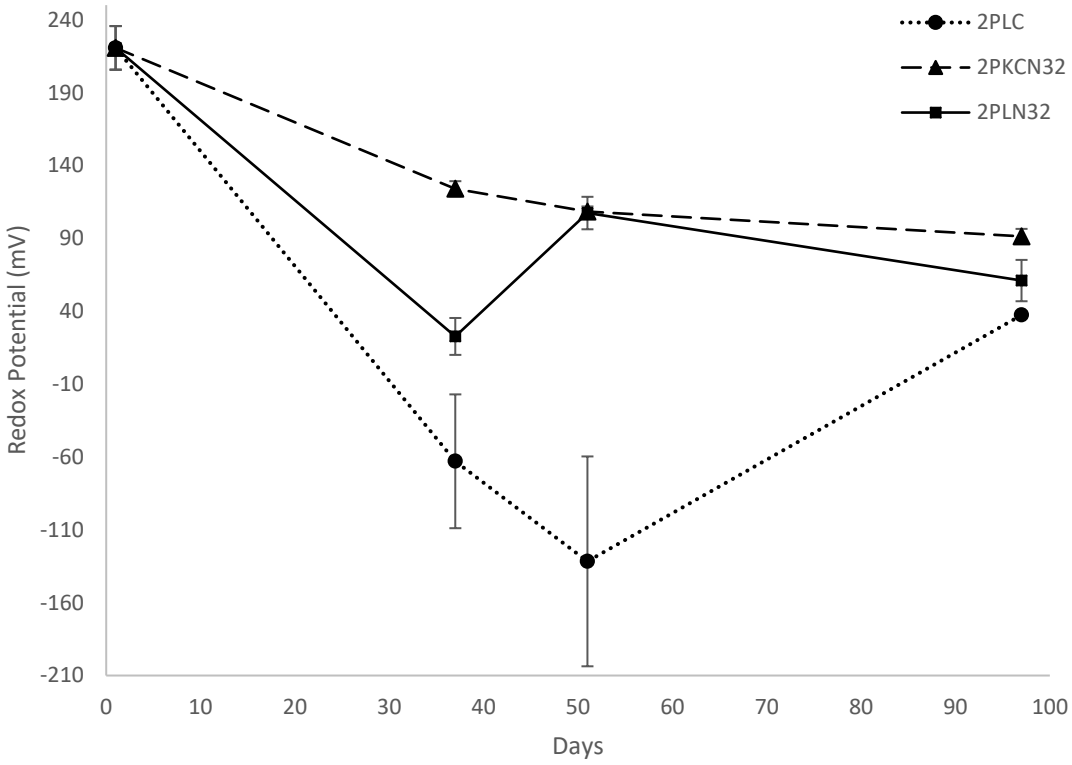

Figure 3.17. Redox potential for Experiment 2 produced water treatments. The experiment included 3 treatments consisting of a Live Control in produced water (2PLC), Nitrate & Nitrite addition Killed Control in produced water ($2PKCN_{32}$), and Nitrate & Nitrite addition Live Experimental in produced water ($2PLN_{32}$). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment.

Sulfate concentrations for Experiment 2 with produced waters are plotted in Figure 3.18. There was no significant difference (p-value > 0.05) in sulfate concentrations over the duration of the experiment and between the different treatments. Therefore, unlike Experiment 2 with seawater, Experiment 2 with produced waters showed no evidence of sulfate reduction despite the reducing conditions created.

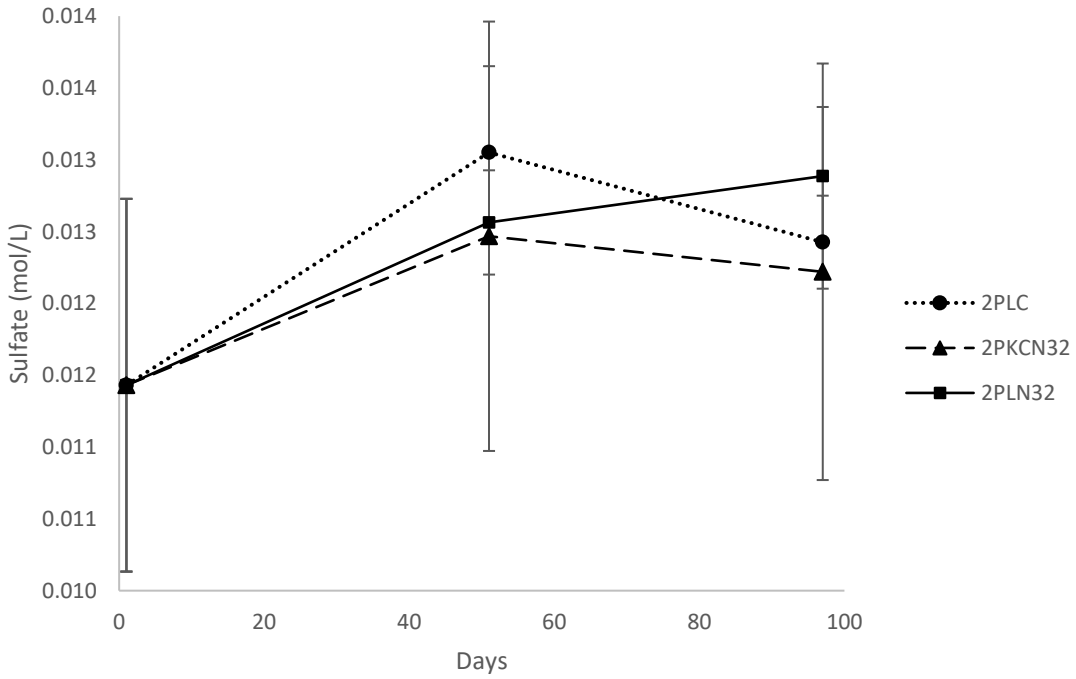

Figure 3.18. Sulfate concentrations for Experiment 2 produced water treatments. The experiment included 3 treatments consisting of a Live Control in produced water (2PLC), Nitrate & Nitrite addition Killed Control in produced water (2PKCN32), and Nitrate & Nitrite addition Live Experimental in produced water (2PLN32). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment.

Dissolved inorganic nitrogen (DIN) values for Experiment 2 with produced waters were plotted in Figure 3.19. DIN concentrations for produced water treatments of Experiment 2 were calculated from the summation of ammonia, nitrate and nitrite concentrations. During the experimental set-up, nitrate and nitrite were added to the Killed Control ($2PKCN_{32}$) and Nitrate and Nitrite addition treatments ($2PLN_{32}$) at concentrations of 0.00161 mol/L and 0.02174 mol/L respectively, totalling 7.9×10^{-2} mol N/L addition to each treatment. The DIN concentrations in the Killed Control ($2PKCN_{32}$)

and in the Live Nitrate and Nitrite ($2PLN_{32}$) addition treatments decreased from ~3 x 10^{-4} mol N/L on day 1 to 2.65 x 10^{-2} ± 9 x 10^{-3} mol N/L and 3 x 10^{-2} ± 4 x 10^{-3} mol N/L on day 51, respectively. Similar DIN results were observed in Experiment 2 with seawater (Figure 3.10). More DIN was consumed in the live nitrate and nitrite addition treatments than in the Killed Control treatments. While this data showed that there was at least one abiotic process that consumed DIN, the greater rate of DIN consumption in the Live nitrate and nitrite amended treatments suggested that there was also at least one microbial process consuming DIN.

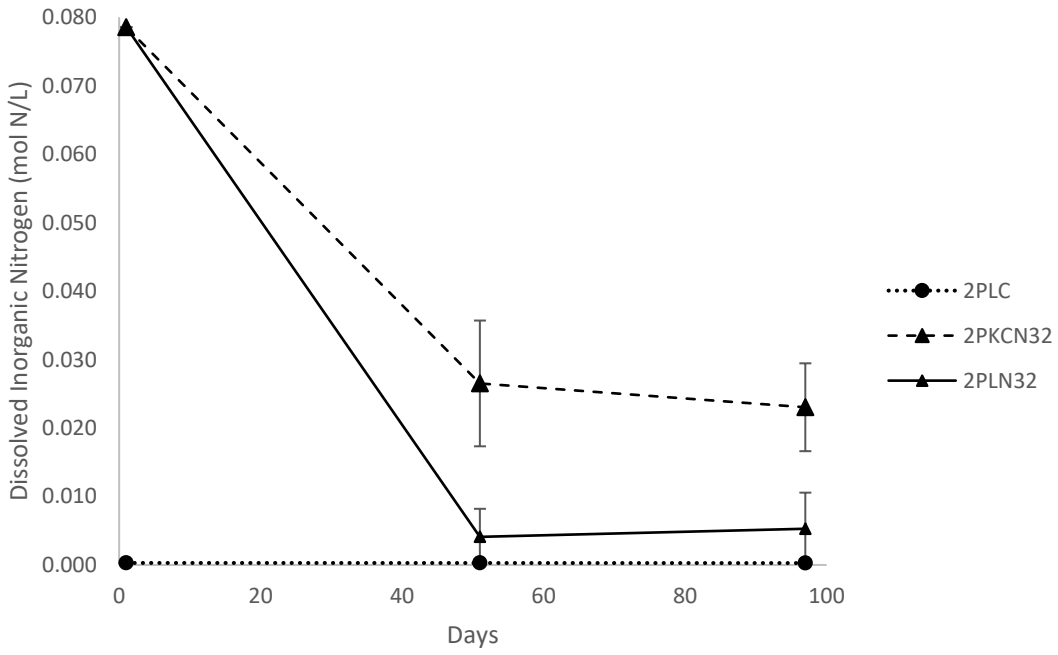

Figure 3.19. Dissolved inorganic nitrogen (DIN) concentrations for Experiment 2 with produced water treatments. The experiment included 3 treatments consisting of a Live Control in produced water (2PLC), Nitrate & Nitrite addition Killed Control in produced water ($2PKCN_{32}$), and Nitrate & Nitrite addition Live Experimental in produced water

(2PLN$_{32}$). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment.

Ammonia concentrations in produced water treatments of Experiment 2 are shown in Figure 3.20. Ammonia was produced in both the Killed Control (2PKCN$_{32}$) and Live Nitrate and Nitrite addition (2PLN$_{32}$) treatments. Ammonia concentrations in the Live Control treatments (2PLC) stayed relatively constant for the duration of the experiment. Between days 1 and 51 the Killed Control (2PKCN$_{32}$) and Live Nitrate and Nitrite addition (2PLN$_{32}$) treatments increased at a rate of ~ 3.0 x 10^{-6} mol N/L/day and 3.7 x 10^{-6} mol N/L/day, respectively, then stay at the same relative concentration for the rest of the experiment.

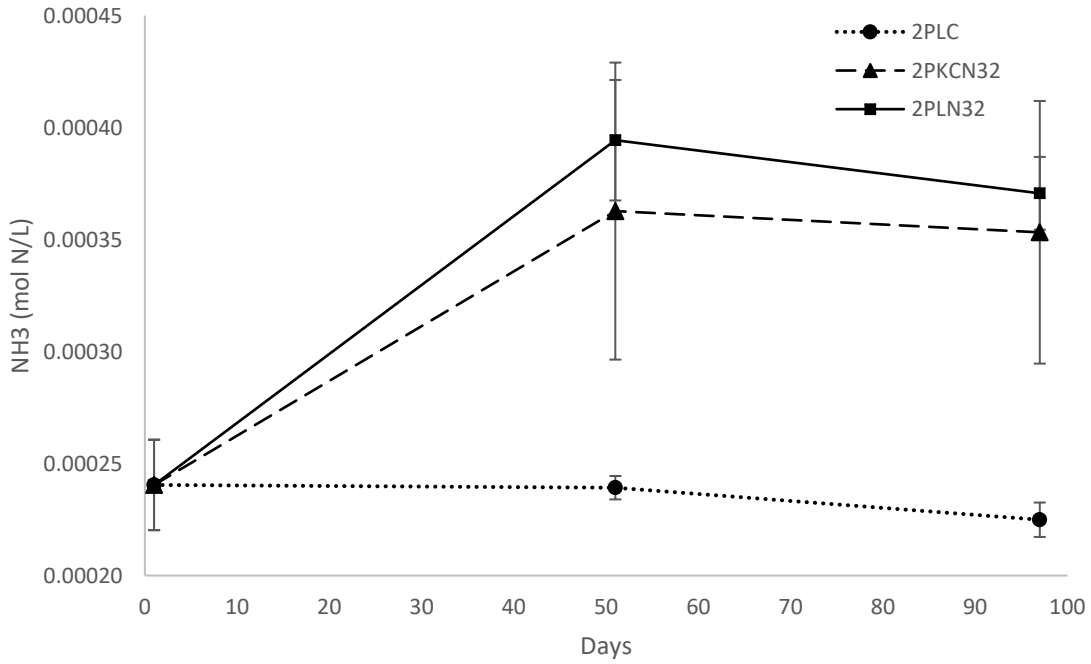

Figure 3.20. Ammonia concentrations for Experiment 2 with produced water treatments. The experiment included 3 treatments consisting of a Live Control in produced water

(2PLC), Nitrate & Nitrite addition Killed Control in produced water ($2PKCN_{32}$), and Nitrate & Nitrite addition Live Experimental in produced water ($2PLN_{32}$). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment.

Dissolved organic nitrogen (DON) concentrations in Experiment 2 with produced waters are plotted in Figure 3.21. DON concentrations for produced water experiments were calculated by subtracting the DIN from the measured TDN. DON concentration increased in the Killed Control ($2PKCN_{32}$) treatment between days 0 and 50 by an average of 2.4 x 10^{-3} mol N/L, however the standard deviation on day 50 was relatively large at ~ ± 2.2 x 10^{-3} mol N/L. The DON concentration in the Live Control (2PLC) and Live nitrate and nitrite addition ($2PLN_{32}$) treatments remained relatively unchanged for the duration of the experiment, and neared zero.

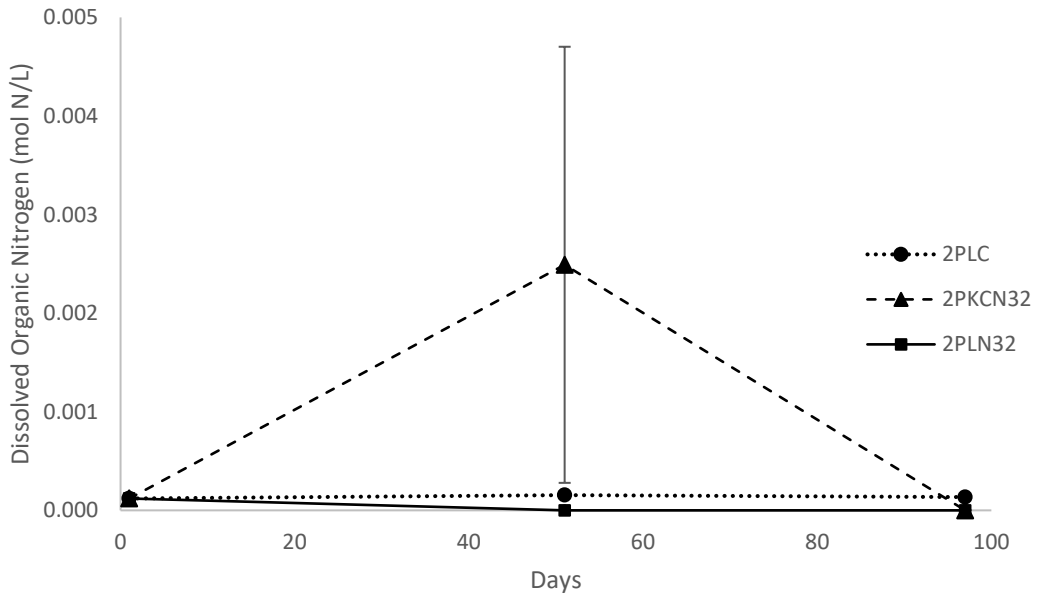

Figure 3.21. Dissolved organic nitrogen (DON) concentrations for Experiment 2 produced water treatments. The experiment included 3 treatments consisting of a Live

Control in produced water (2PLC), Nitrate & Nitrite addition Killed Control in produced water ($2PKCN_{32}$), and Nitrate & Nitrite addition Live Experimental in produced water ($2PLN_{32}$). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment.

Dissolved organic carbon (DOC) concentrations measured during Experiment 2 with produced waters are plotted in Figure 3.22. The starting DOC concentration for the produced water experiment was similar to the starting DOC concentration for the seawater experiments, at approximately ~ 0.160 mol C/L. There was a decrease in DOC in all produced water treatments of Experiment 2. For example, there was a ~ 47% decrease in the Live Control treatments, ~58% decrease in the Killed Control, and ~56% decrease in the Live Experimental. However, little to no difference in DOC concentrations were observed between the Live and Killed treatments during this experiment. Therefore abiotic processes were mostly responsible for the decrease in DOC in the Live and Killed control treatments.

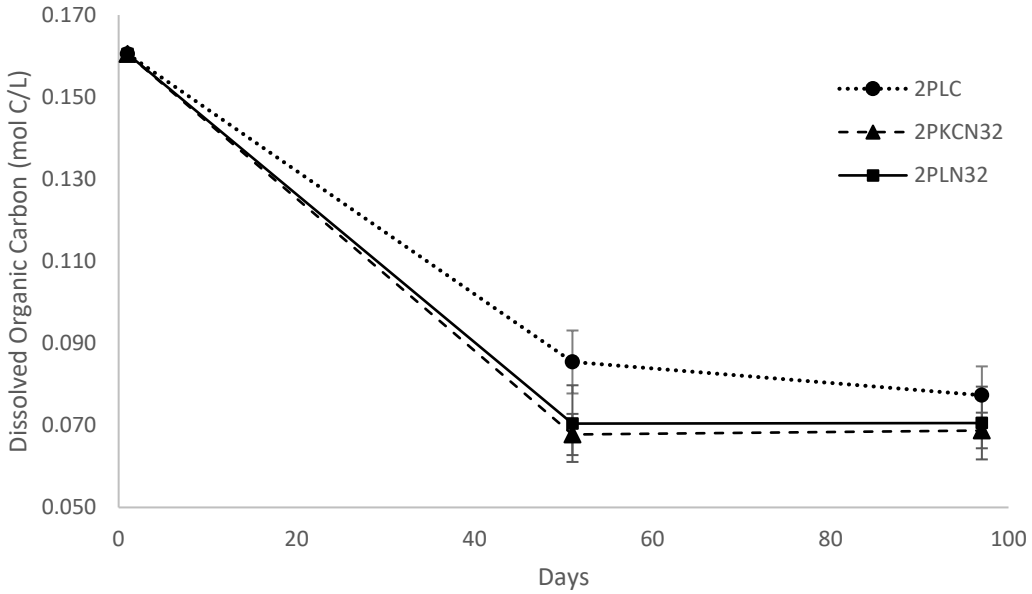

Figure 3.22. Dissolved organic carbon concentrations for Experiment 2 produced water treatments. The experiment included 3 treatments consisting of a Live Control in produced water (2PLC), Nitrate & Nitrite addition Killed Control in produced water ($2PKCN_{32}$), and Nitrate & Nitrite addition Live Experimental in produced water ($2PLN_{32}$). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment.

Dissolved inorganic carbon (DIC) concentrations measurements during Experiment 2 with produced waters are plotted in Figure 3.23. There was a decrease in DIC concentration in all produced water treatments. The starting concentration of DIC for all treatments in produced water was $9.1 \times 10^{-3} \pm 7 \times 10^{-4}$ mol C/L. Between day 1 and 51 the DIC concentration decreased by 2.6×10^{-3} mol C/L for the Live Control treatment and 2.4×10^{-3} mol C/L for the Killed Control and Live Experimental treatments. There was no discernable difference between the Killed Control treatment and Live Experimental

treatment for the duration of the experiment. However, the Live nitrate and nitrite addition treatment was significantly higher in DIC concentration (p-value < 0.05) than the Live Control treatment on day 97 with a difference in 5 x 10^{-4} mol C/L.

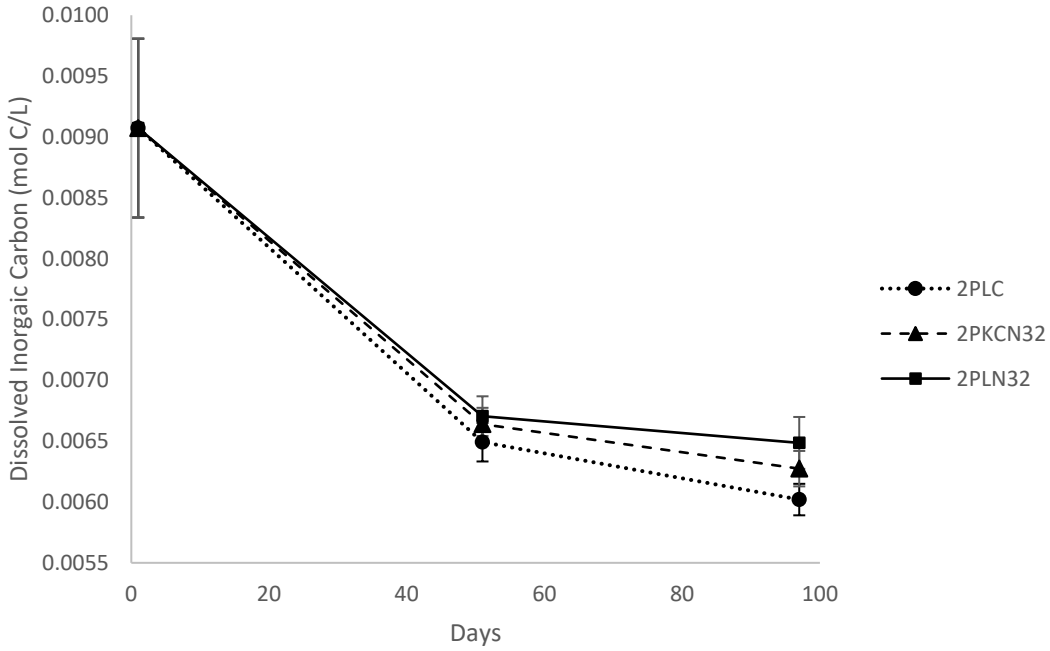

Figure 3.23. Dissolved inorganic carbon concentrations for Experiment 2 produced water treatments. The experiment included 3 treatments consisting of a Live Control in produced water (2PLC), Nitrate & Nitrite addition Killed Control in produced water ($2PKCN_{32}$), and Nitrate & Nitrite addition Live Experimental in produced water ($2PLN_{32}$). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment.

Hydrogen potential (pH) measured during Experiment 2 with produced waters are plotted in Figure 3.24. The starting pH value for the produced water treatments was 7.16 ± 0.3, and the final pH values were: 7.1 ± 0.6 for the Live Control treatment, 7.3 ± 0.03

for the Killed Control treatment, and 6.7 ± 1 for the Live nitrate and nitrite addition treatment. There was no discernable difference in pH values over time or between treatments.

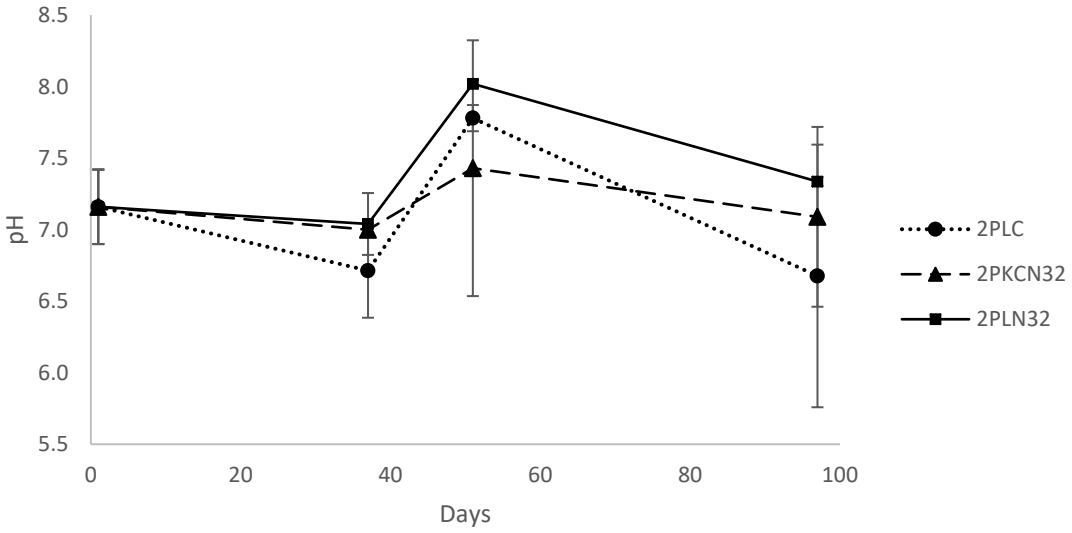

Figure 3.24. Hydrogen Potential (pH) for Experiment 2 produced water treatments. The experiment included 3 treatments consisting of a Live Control in produced water (2PLC), Nitrate & Nitrite addition Killed Control in produced water ($2PKCN_{32}$), and Nitrate & Nitrite addition Live Experimental in produced water ($2PLN_{32}$). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment.

3.4 Experiment 3: Testing the affect of independent nitrate and nitrite additions to seawater

Redox potential (E_h) measurements taken during Experiment 3 with seawater were plotted in Figure 3.25. Experiment 3 was successful in creating reducing conditions in all

live treatments. The starting redox potential was 125 mV. Treatments Live Control (3SLC), Live Nitrate addition (3SLN3), and Live Nitrite addition (3SLN2) all decreased in redox potential from day 1 to day 28. The Live Control treatment decreased by 530 mV to a value of -405 mV, the Live Nitrate decreased by 115 mV to a value to +10mV, and the Live Nitrite decreased by 60 mV to a value of 66 mV. The Live Control remained relatively constant at ~ 400 mV from day 28 to day 59. However, the Live Nitrate addition treatment (3SLN3) and Live Nitrite addition treatment (3SLN2) continued to decrease from day 28 to 59 with both treatments reaching negative redox values (-55 mV and -73 mV, respectively). On day 28 the Live Nitrate addition treatments (3SLN3) were significantly less reducing (p-value < 0.05) at +66 mV, than the Live Nitrite treatments (3SLN2) at +10 mV. The Live Nitrate and Nitrite addition treatments became more similar in redox value by day 59, however they remained significantly different (p-value < 0.05) with the Live Nitrate addition treatments being less reducing than the Live Nitrite addition treatments by 18 mV. The observed decrease in redox potential value in the live treatments was likely due to microbial reactions as the Killed Control treatments (3SKCN3 and 3SKCN2) remained relatively unchanged. Moreover, the Live Control bottles with no additional amendments reached a much lower E_h value compared to the other treatments in this experiment.

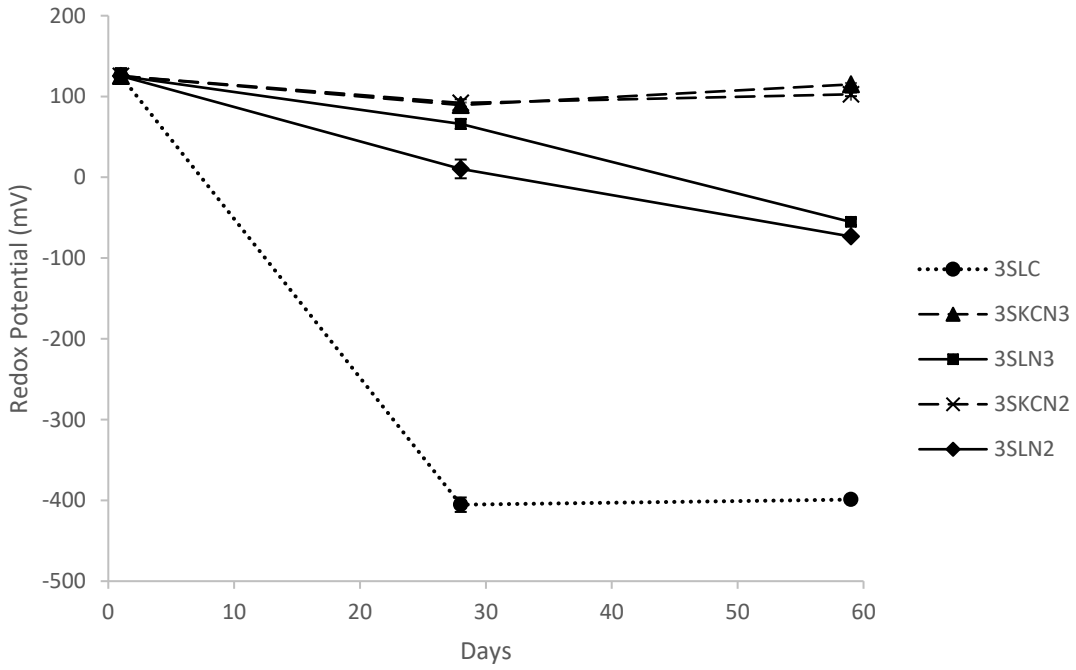

Figure 3.25. Redox potential for Experiment 3 seawater treatments. The experiment included 5 treatments in seawater consisting of a Live Control (3SLC), Nitrate addition Killed Control ($3SKCN_3$), Nitrate addition Live Experimental ($3SLN_3$), Nitrite addition Killed Control ($3SKCN_2$), and Nitrite addition Live Experimental ($3SLN_2$). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment.

Sulfate concentrations measured in Experiment 3 are plotted in Figure 3.26. Differences in sulfate concentrations between the Live and Killed treatments that had nitrate or nitrite additions were not significant (p-value > 0.05) on days 28 and 59. However, the sulfate concentrations in the Live Control (3SLC) treatments (i.e. no addition of nitrate/nitrite) were significantly less (p-value < 0.05) than all other treatments

by ~3.3 x 10^{-3} mol/L on day 28, and ~ 5.6 x 10^{-3} mol/L on day 58. This indicates that microbial sulfate reduction occurred in treatments where no nitrate or nitrite was added.

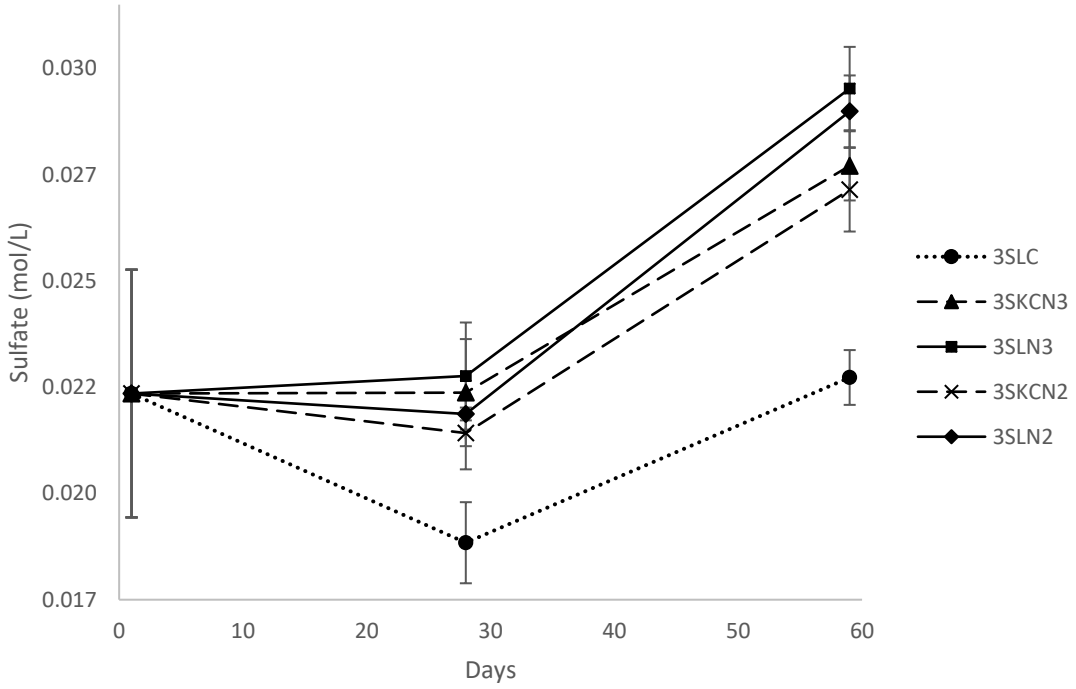

Figure 3.26. Sulfate concentrations for Experiment 3 seawater treatments. The experiment included 5 treatments in seawater consisting of a Live Control (3SLC), Nitrate addition Killed Control ($3SKCN_3$), Nitrate addition Live Experimental ($3SLN_3$), Nitrite addition Killed Control ($3SKCN_2$), and Nitrite addition Live Experimental ($3SLN_2$). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment.

Dissolved inorganic nitrogen (DIN) concentrations measured during Experiment 3 with seawater are plotted in Figure 3.27. DIN was calculated from the sum of the measured nitrate and ammonia concentrations. The Live Nitrate ($3SLN_3$) and Live Nitrite ($3SLN_2$) addition treatments, and the Killed Control treatments ($3SKCN_3$ & $3SLN_2$)

received 0.02174 mol/L of nitrate and nitrite respectively. The Live Control (3SLC) had no additional amendments, and this attributed to the different starting concentrations. As seen in Figure 3.27, both the Killed and Live nitrate and nitrite addition treatments decreased in DIN concentration from day 1 to day 28 by ~ 1.5 x 10^{-1} mol N/L for the Live and Killed Nitrate addition treatments. On day 28 the Nitrate addition treatments had ~ 1.2 x 10^{-2} mol N/L more DIN than the Nitrite addition treatments. Furthermore, on day 59 the Killed Control treatments were higher in DIN than all live treatments by 1.5 x 10^{-2} mol N/L for the Nitrate addition treatments and 2.8 x 10^{-2} mol N/L for the Nitrite addition treatments. The Live Control incubations remained relatively unchanged for the duration of the experiment.

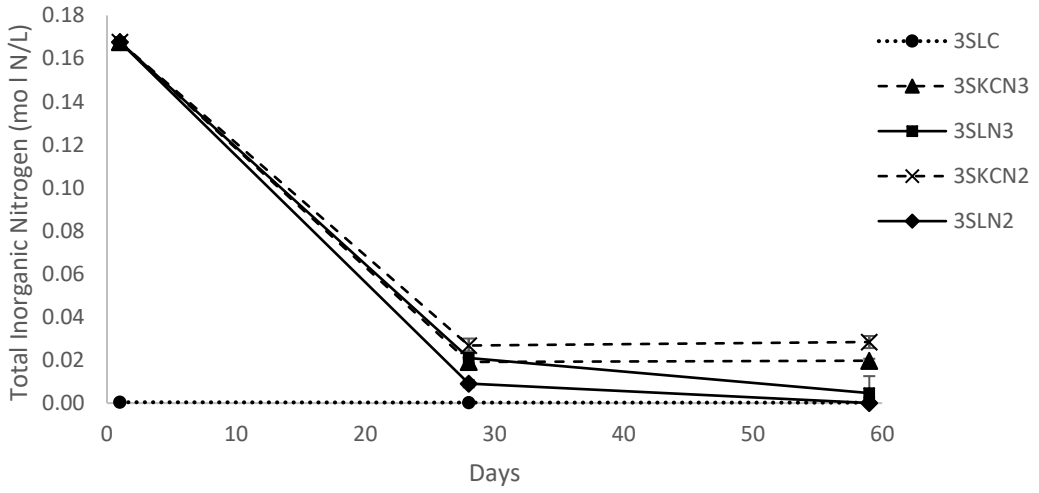

Figure 3.27. Dissolved inorganic nitrogen concentration for Experiment 3 seawater treatments. The experiment included 5 treatments in seawater consisting of a Live Control (3SLC), Nitrate addition Killed Control ($3SKCN_3$), Nitrate addition Live Experimental ($3SLN_3$), Nitrite addition Killed Control ($3SKCN_2$), and Nitrite addition Live

Experimental ($3SLN_2$). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment.

Ammonia concentrations measured during Experiment 3 with seawater are plotted in Figure 3.28. Ammonia was consumed in all live treatments for Experiment 3. In the Live Control (3SLC) incubations ammonia concentrations decreased from $4.1 \times 10^{-4} \pm 6 \times 10^{-6}$ mol N/L to $2.34 \times 10^{-4} \pm 2 \times 10^{-5}$ mol N/L between days 1 and 28, then remained relatively unchanged from day 28 to day 59. In the Live Nitrate and Nitrite addition treatments ammonia concentrations decreased by 65% and 90% respectively between day 1 and day 59. The decrease in ammonia concentrations observed in the live treatments were likely due to microbial processes because the ammonia concentrations in the Killed Control treatments remained relatively unchanged and significantly higher (p-value < 0.05) in concentration than all live treatments for the duration of the experiment.

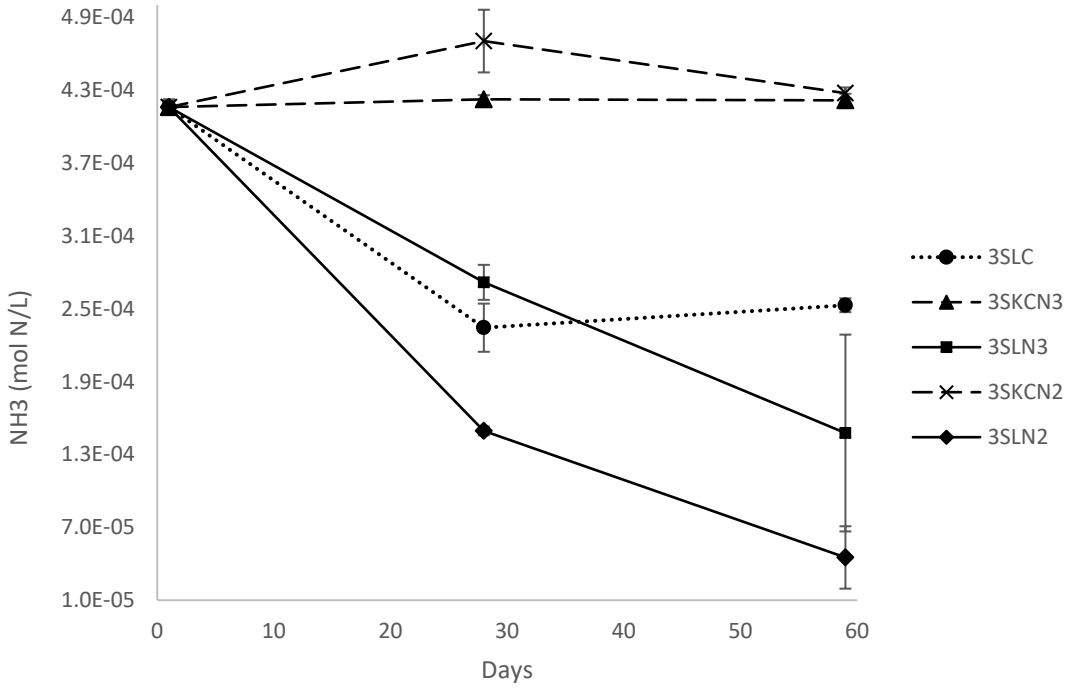

Figure 3.28. Ammonia concentrations for Experiment 3 seawater treatments. The experiment included 5 treatments in seawater consisting of a Live Control (3SLC), Nitrate addition Killed Control ($3SKCN_3$), Nitrate addition Live Experimental ($3SLN_3$), Nitrite addition Killed Control ($3SKCN_2$), and Nitrite addition Live Experimental ($3SLN_2$). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment.

Dissolved organic nitrogen (DON) concentrations for Experiment 3 with seawater were plotted in Figure 3.29. DON was calculated by subtracting the dissolved inorganic nitrogen (DIN) from the measured total dissolved nitrogen (TDN). The Live Control treatments increased slightly in DON concentration between days 28 and 59 by ~ 9.6 x 10^{-5} mol N/L. Between days 28 and 59 the Nitrate addition treatments increased in DON

concentration slightly more (~ 1.3 x 10^{-3} mol N/L) than the Nitrite addition treatments (~ 1.1 x 10^{-3} mol N/L). The Live Control and Killed Control treatments stayed relatively constant (and near zero) for the duration of the experiment.

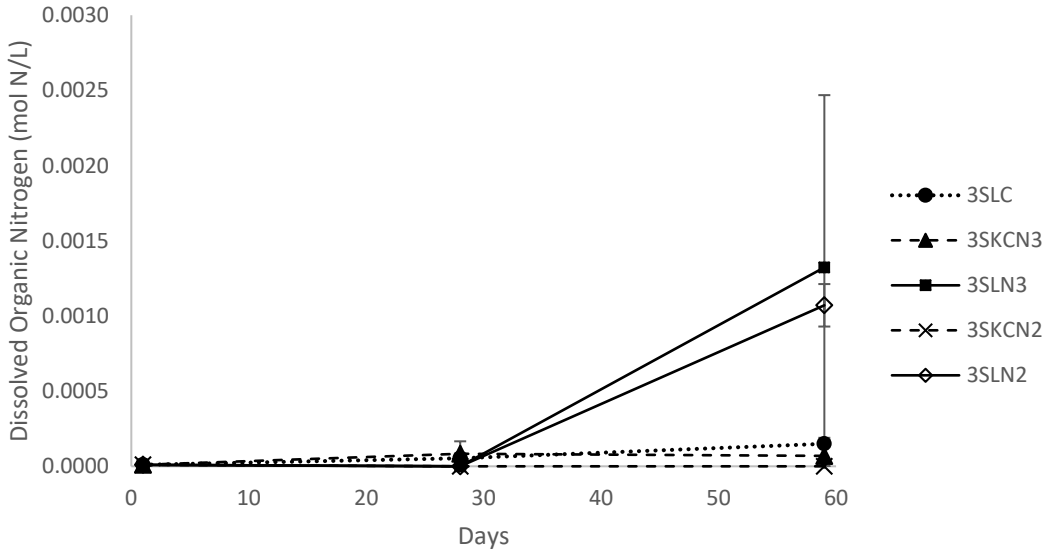

Figure 3.29. Dissolved organic nitrogen concentration for Experiment 3 seawater treatments. The experiment included 5 treatments in seawater consisting of a Live Control (3SLC), Nitrate addition Killed Control ($3SKCN_3$), Nitrate addition Live Experimental ($3SLN_3$), Nitrite addition Killed Control ($3SKCN_2$), and Nitrite addition Live Experimental ($3SLN_2$). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment.

Nitrous oxide (N_2O) concentrations measured during Experiment 3 with seawater were plotted in Figure 3.30. N_2O was measured for the Killed Control and Live Experimental treatments. No significant change (p-value > 0.05) was observed in N_2O concentration for the duration of the experiment in both the Live and Killed treatments.

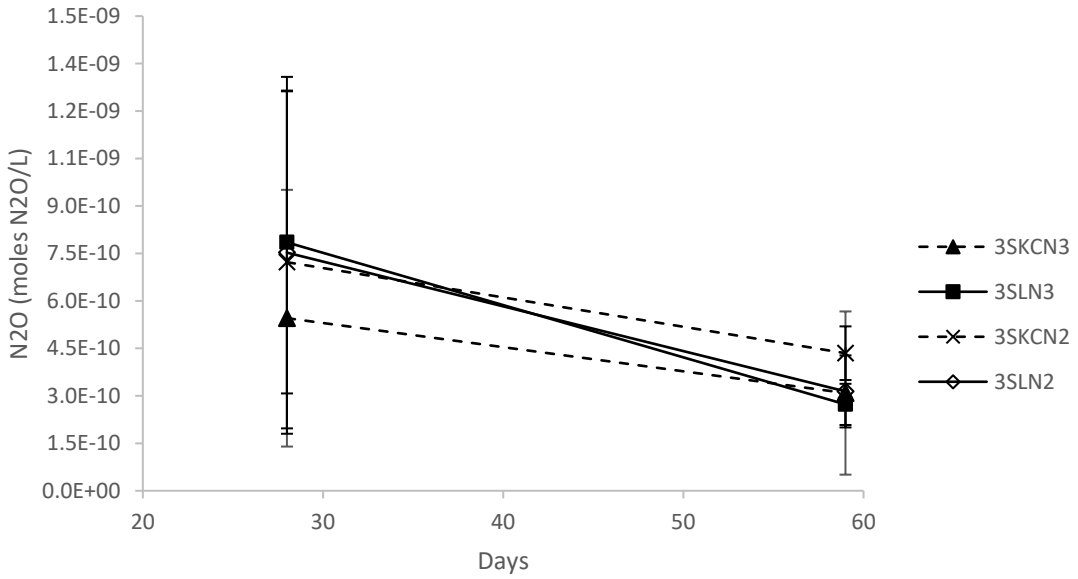

Figure 3.30. Nitrous oxide (N_2O) concentrations for Experiment 3 seawater treatments. The experiment included 5 treatments in seawater consisting of a Live Control (3SLC), Nitrate addition Killed Control ($3SKCN_3$), Nitrate addition Live Experimental ($3SLN_3$), Nitrite addition Killed Control ($3SKCN_2$), and Nitrite addition Live Experimental ($3SLN_2$). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment.

Dissolved organic carbon (DOC) concentrations measured during Experiment 3 with seawater are plotted in Figure 3.31. All treatments received approximately 0.160 mol C/L at the time of construction. The DOC concentration decreased in all treatments from day 1 to 28 by 64% for the Live Control and Live Nitrate addition treatments, 57% for the Killed Control Nitrate addition treatment, 61% for Killed Control Nitrite addition

treatment, and 66% for the the Live Nitrite addition treatment. All treatments remained relatively unchanged between days 28 and 59.

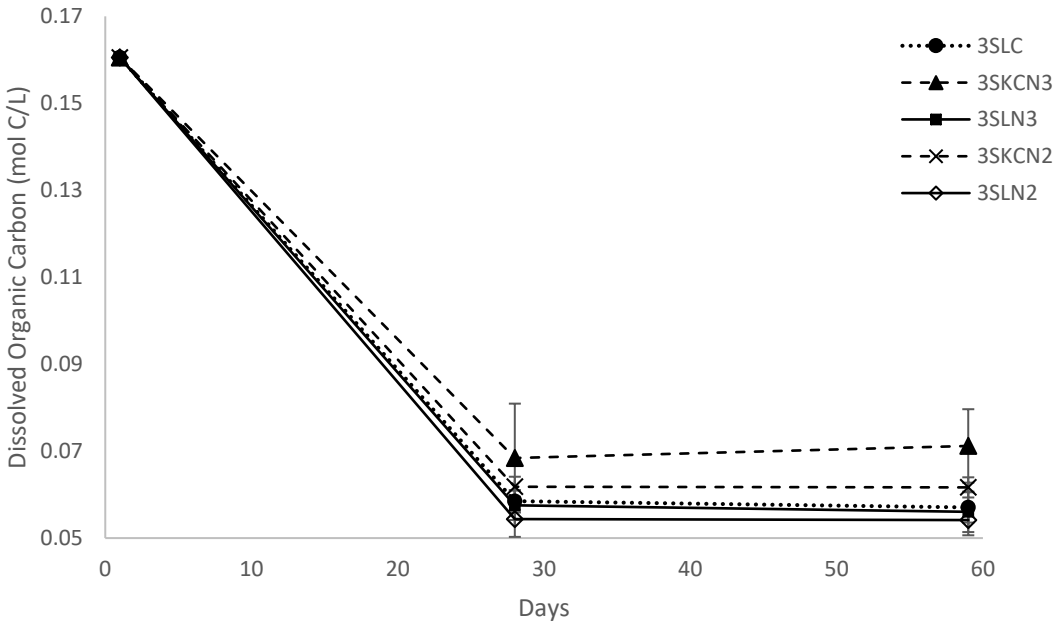

Figure 3.31. Dissolved organic carbon (DOC) concentration for Experiment 3 seawater treatments. The experiment included 5 treatments in seawater consisting of a Live Control (3SLC), Nitrate addition Killed Control ($3SKCN_3$), Nitrate addition Live Experimental ($3SLN_3$), Nitrite addition Killed Control ($3SKCN_2$), and Nitrite addition Live Experimental ($3SLN_2$). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment.

Dissolved inorganic carbon (DIC) concentrations measured during Experiment 3 with seawater are plotted in Figure 3.32. Between days 1 and 59 the Live Nitrate and Nitrite addition treatments increased in DIC concentration by the greatest amount out of all treatments at 1.88×10^{-2} mol C/L and 7.4×10^{-3} mol C/L respectively. The DIC

concentration in the Live Control treatments increased from $1.8 \times 10^{-3} \pm 1.2 \times 10^{-5}$ mol C/L to $1.15 \times 10^{-2} \pm 6.8 \times 10^{-4}$ mol C/L between days 1 and 28 then remained relatively unchanged between days 28 to 59. There was a significant difference (p-value < 0.05) in DIC concentration between the Live and Killed treatments with both Killed Control treatments remaining unchanged for the duration of the experiment at a concentration of ~ 1.87×10^{-3} mol C/L.

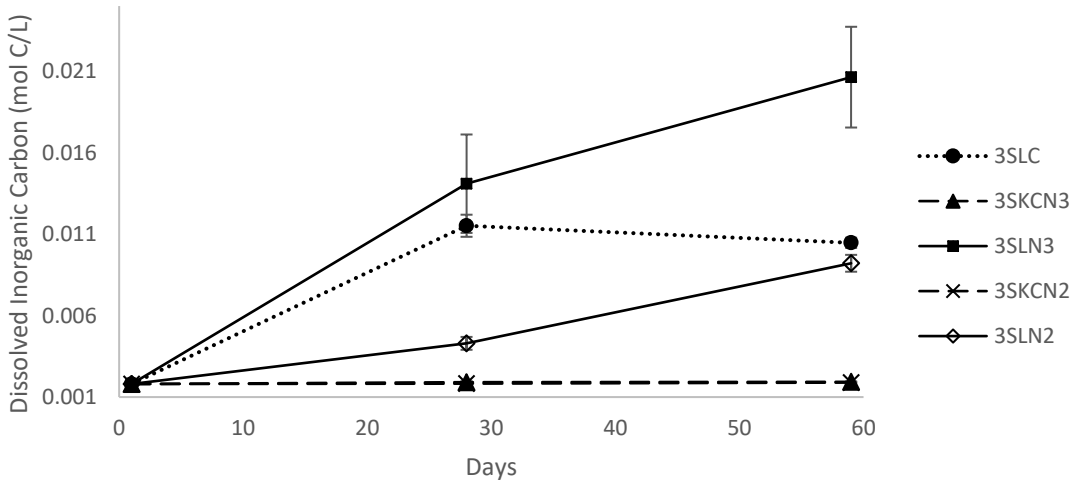

Figure 3.32. Dissolved inorganic carbon (DIC) concentration for Experiment 3 seawater treatments. The experiment included 5 treatments in seawater consisting of a Live Control (3SLC), Nitrate addition Killed Control ($3SKCN_3$), Nitrate addition Live Experimental ($3SLN_3$), Nitrite addition Killed Control ($3SKCN_2$), and Nitrite addition Live Experimental ($3SLN_2$). Plotted points are the mean values ($\pm 1\sigma$) of triplicate incubations for each treatment.

Hydrogen potential (pH) measurements during Experiment 3 with seawater are plotted in Figure 3.33. As seen in Figure 3.33, the pH increased in all experimental

treatments. The Live Nitrate and Nitrite addition incubations had the highest pH on day 59 at 7.8 and 8.3, respectively. The increase in pH in the Nitrate and Nitrite addition incubations was likely due to microbial processes, because on day 58 the pH of the Killed Control treatments were lower at a value of 7.5. Moreover, the Nitrite addition treatments were more basic on day 58 at a pH of 8.3 than the Nitrate addition treatments at a pH of 7.8.

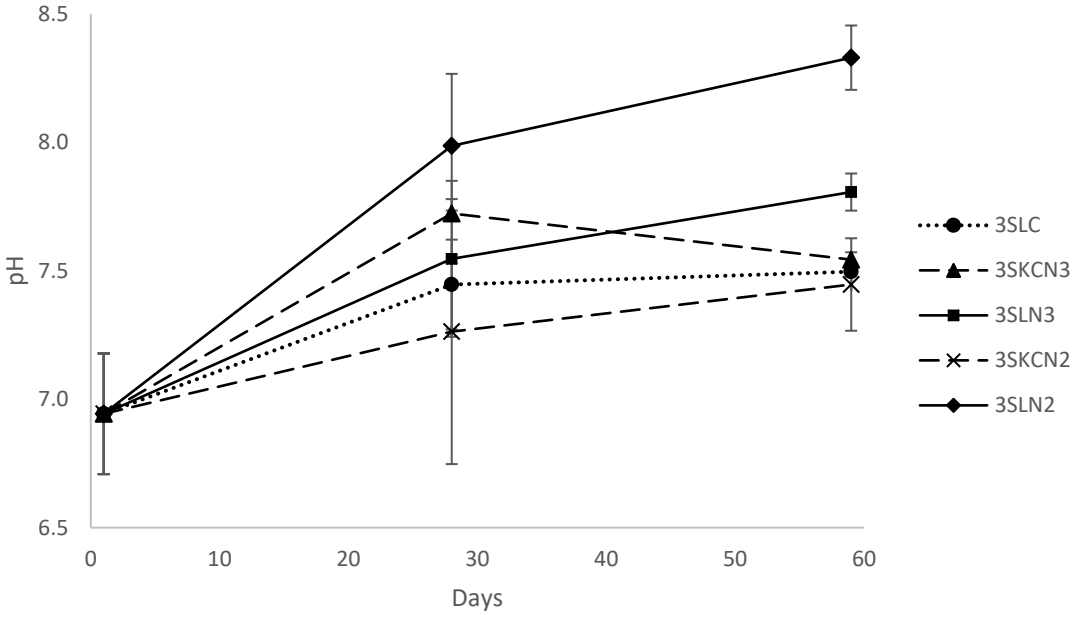

Figure 3.33. Hydrogen potential (pH) for Experiment 3 seawater treatments. The experiment included 5 treatments in seawater consisting of a Live Control (3SLC), Nitrate addition Killed Control ($3SKCN_3$), Nitrate addition Live Experimental ($3SLN_3$), Nitrite addition Killed Control ($3SKCN_2$), and Nitrite addition Live Experimental ($3SLN_2$). Plotted points are the mean values (± 1σ) of triplicate incubations for each treatment.

Chapter 4: Discussion and Conclusion

4.1 The need for reducing environments

Experiment 1 remained oxidizing despite the addition of H_2 and acetic acid as biostimulants. The redox potential remained relatively unchanged at a maximum range of 30 mV difference for the duration of the experiment. Similarly, the sulfate concentrations also remained unchanged. Sulfate reduction was not expected in oxic conditions (positive redox value), and this is consistent with the redox ladder in Figure 1.2. Additionally, biological sulfate reduction does not occur when the redox potential is greater than -100 mV (Postgate, 1979). Moreover, there was no evidence of sulfate reduction in Experiment 1. DIN decreased for the nitrate and nitrite addition Killed Control and Live Experimental treatments of Experiment 1, and conversely the DON increased for both of the nitrogen amended treatments. The NH_3 concentrations increased in the nitrate and nitrite addition Killed and Live treatments, however, since the DIN, NH_3, and DON concentrations changed in both the Live and Killed nitrogen amended treatments, then the change observed was not due to biological reactions.

The reducing agent Na_2S was added to Experiments 2 and 3. The redox values in live experiments became more reducing than the redox values in the Killed controls (Figures 3.8, 3.17, 3.25). This indicated the need to stimulate anoxic conditions in incubations for SRB/NRB growth. In experiments 2 and 3 the Live controls (i.e., no nitrate or nitrite amendments) were constituently more reducing than the amended treatments. This is consistent with the nitrate reduction position on the redox ladder (Figure 1.2) whereby the SRB were active in more reducing environments than NRB.

Therefore, unlike Experiment 1, in Experiment 2 reducing conditions were established that were conducive to nitrate and sulfate reduction.

4.2 Inhibition of sulfate reduction

The Experiment 2 and 3 Live Control treatments in seawater both showed a decrease in sulfate concentration (Figure 3.9 and 3.26). This decrease was not observed in the Killed Controls nor in the amended treatments. This indicates microbial sulfate reduction was occurring only in the Live Controls. Microbial sulfate reduction was also observed by Kaster et al. (2007), and they showed that sulfide increased inversely to sulfate. Sulfide was not measured in this study; alternatively the decrease in sulfate concentration was used as an indicator of sulfate reduction to sulfide. The decrease in sulfate concentration in the Live Control treatments (i.e., no nitrogen amendment) in seawater supports the hypothesis that sulfate reduction would occur under reducing conditions without the addition of nitrate or nitrite. The difference between average sulfate concentrations in the Live nitrogen addition treatments and the Live Control treatments (i.e., $\triangle SO_4^{2-} = [SO_4^{2-}]_{LN} - [SO_4^{2-}]_{LC}$) is shown in Figure 4.1, such that the larger the $\triangle SO_4^{2-}$ value the more the nitrate/nitrite addition inhibited sulfate consumption (and by inference, H_2S production). Sulfate consumption was more inhibited in the seawater treatments of Experiments 2 & 3 than the produced water treatment of Experiment 2 (i.e., $\triangle SO_4^{2-}$ EX 2S & 3S > $\triangle SO_4^{2-}$ EX 2P). The more positive redox value in the produced waters supports the hypothesis that sulfate reduction would occur under more reducing conditions. Jenneman et al. (1986) also showed that sulfate reduction was inhibited at the same time as redox values increased. Interestingly, there was no

significant difference (p-value > 0.05) found in the $\triangle SO_4^{2-}$ for the treatments amended with just nitrate, just nitrite, or a combination of nitrate and nitrite. Therefore, the nitrogen additions resulted in similar inhibition in sulfate reduction for the duration of the experiments presented in this thesis.

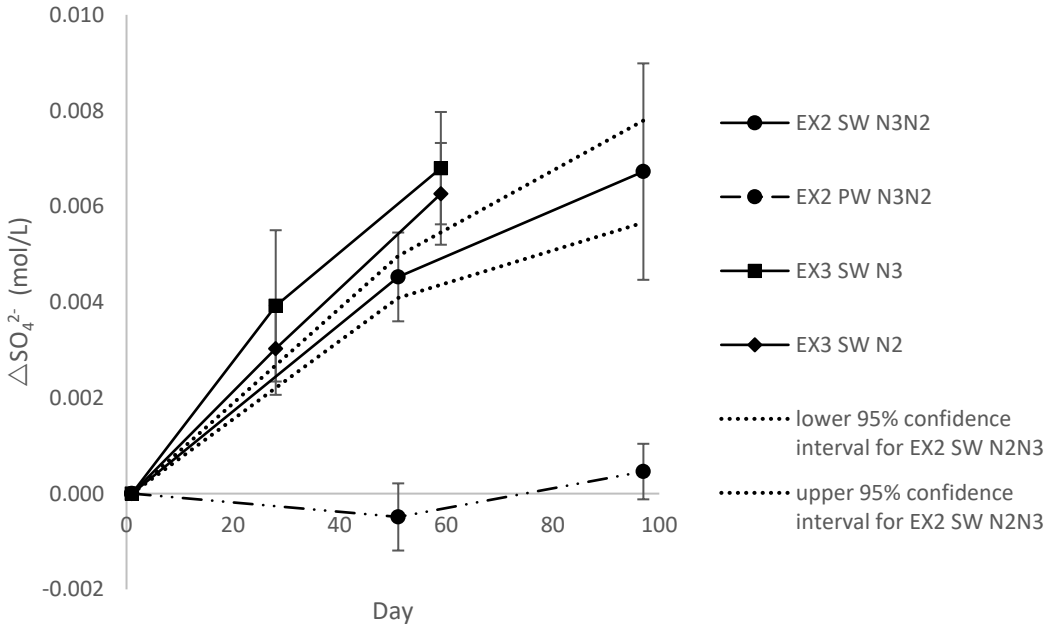

Figure 4.1. Difference between average sulfate concentrations in the Live nitrogen addition treatments and the Live Control treatments (i.e. $\triangle SO_4^{2-} = [SO_4^{2-}]_{LN} - [SO_4^{2-}]_{LC}$). Values shown are the nitrate/nitrite addition treatments in seawater and produced water (EX2 SW N3N2 and EX2 PW N3N2, respectively), and Experiment 3 nitrate and nitrite addition treatments in seawater (EX3 SW N3 and EX3 SW N2, respectively). Error bars are the square root of the sum of the squares of the standard deviations of the mean measured sulfate concentrations. The dotted line represents the 95% confidence intervals of Experiment 2 seawater nitrate and nitrite addition.

4.3 Nitrogen transformation and fate

Dissolved inorganic nitrogen (DIN) concentrations decreased in all Live and Killed nitrogen amended treatments for Experiment 2 and 3 (Figures 3.10, 3.19, 3.27, and 4.2 A, B, C). The Killed Control nitrogen amended treatments in seawater had a higher concentration of DIN than the nitrogen amended Live treatments on the middle and final sampling days, and this suggests microbial consumption of DIN. Nitrate and/or nitrite were added to the Killed Control and Experimental Live treatments of all three experiments and accounted for 99% of the nitrogen at the start of each experiment (Figure 4.2 A). However, by the middle and final sampling periods (Figure 4.2 B & C) less than 50% of the nitrogen could be accounted for. It is possible that the unaccounted nitrogen may be N_2 gas, biomass or another form of particulate matter. Both N_2 gas and biomass can be products of NRB respiration which can be seen in Equation 1.4. To better establish the nitrogen budget in similar incubation experiments it is recommended that future studies determine the complete composition of gas phase nitrogen and the makeup of particulate matter (e.g., microbial membrane lipids such as phospholipid-derived fatty acids (PLFA's)). The Live treatments of the seawater experiments had a much lower concentration in nitrate + nitrite than the Killed Control treatments by the end of the experiments (Figure 4.2. C), which showed possible nitrate/nitrite consumption by NRB. Additionally, the Killed Control treatments of Experiment 1 had a larger amount of DON than the nitrogen amended Live treatments throughout the experiment. Conversely, on the final sampling period of Experiment 3, the Live Control treatments exhibited a larger concentration of DON compared to the Killed Control treatments (Figure 3.29 and 4.2 C).

Since this concentration difference was not observed in Experiment 2, the differences must be due to the separate additions of nitrate and nitrite and this should be studied in further investigations. Interestingly, the produced water experiments did not exhibit microbial nitrate/nitrite consumption and moreover the Live nitrogen amended treatments had a higher concentration of nitrate and nitrite than the Killed Control treatments. The difference in nitrate + nitrite consumption between the seawater and produced waters may be indicative of different microbial communities existing in the two waters. Eckford et al. (2002) also found differences in the amount of nitrate consumed by microorganisms of differing origins.

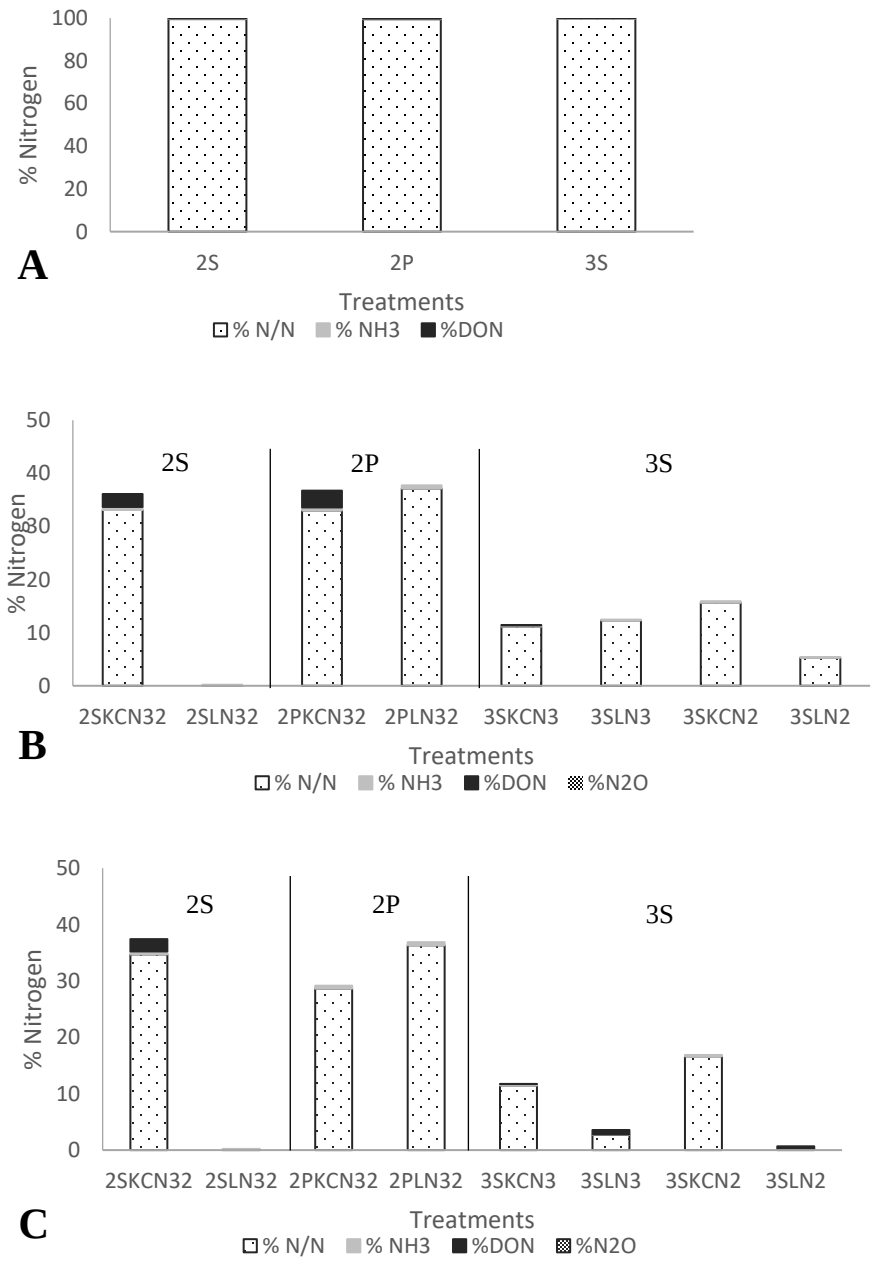

Figure 4.2. The nitrogen budget for experiments 2 and 3 at the (A) beginning, (B) middle, (C) and final sampling periods. The percentages of nitrate + nitrite (% N/N), ammonia (% NH_3), total organic nitrogen (% DON), and N_2O (% N_2O) were calculated by dividing the respecting average concentrations by the total amount of nitrogen at the start of the

experiment (i.e. the added nitrogen amendments plus the measured starting concentrations).

The fact that more nitrate/nitrite remained in the $3SLN_3$ treatments on the middle and final sampling days compared to the $3SLN_2$ treatments (Figure 4.2 C) suggests that nitrate has a longer residence time in the incubations, compared to nitrite. Also, it is likely that there was a two-step nitrate reduction in the $3SLN_3$ treatments whereby nitrate was first reduced to nitrite (Figure 4.2 B) then over time was further reduced to other products of nitrate respiration (Figure 4.2 C). Since the inhibition in sulfate reduction was similar in $3SLN_3$ and $3SLN_2$ (Figure 4.1), it may be hypothesized that nitrate is a more effective inhibitor of sulfate reduction.

Interestingly, when the trends in ammonia concentrations in the produced water experiments are compared with those from Experiment 2 and 3 in seawater (Figures 3.11, 3.20, and 3.28), different patterns were observed. The Killed Control treatments in the seawater experiments showed an increase in ammonia over time, and the Live treatments showed a decrease over time. Resinel et al. (1996) listed NH_3 as a product of microbial nitrate reduction (also seen in Equation 1.3), however NH_3 was not observed as a product of nitrate/nitrite reduction in these seawater experiments. Moreover, ammonia was consumed microbially in Experiment 2 and 3 with seawater, but was produced abiotically in produced waters. While ammonia was (interestingly) consumed microbially in Experiment 2 and 3 with seawater, the produced waters showed no evidence of microbial ammonia consumption (or microbial sulfate reduction for that matter). The Live Nitrate and Nitrite treatments along with the Killed Control treatments in produced waters

showed a production of NH_3 over the duration of the experiment. The observed production of NH_3 for the Live treatments of 2P was not due to biological reactions because a similar increase in NH_3 was also observed in the Killed Control treatments. In summary, microbial ammonia consumption and microbial sulfate reduction were only observed in the Live nitrogen amended treatments and Live Control treatments respectively in experiments constructed in seawater with reducing agents.

N_2O was measured in Experiments 2 and 3 in seawater nitrogen amended treatments to try and further determine the nitrogen budget for the incubations. There was no considerable difference or change in the concentration of N_2O over time. Although N_2O is a product of NRB respiration, there was no evidence of microbial N_2O production. This contrasts with the findings of Jenneman et al. (1986) as they found a buildup of N_2O in their experiments. The discrepancies in the nitrogen budget between this thesis and other studies highlights the importance of better understanding site-specific nitrate utilizing bacteria in anaerobic environments, especially for the purpose of sulfide production control. Further site-specific biogeochemical characterization and the respecting amendment responses is an important subject of question for future research on reservoir souring control.

4.4 Carbon transformations and fate

DOC was microbially consumed in the Live treatments for Experiment 2 and 3 in seawater. DOC and DIC concentrations were plotted in Figure 4.3 A, B and C. The data in Figure 4.3 were normalized to the total starting concentration of carbon (i.e. the measured concentration of DOC and DIC of the seawater and produced waters plus the

added organic acid concentrations). Between the start of the experiment and the middle sampling point, DOC was likely converted to particulate carbon, as seen in the <50% carbon accounted for in the dissolved phase (Figures 4.3 B and C). In other words, 100% of the initial dissolved carbon cannot be account for in the dissolved carbon measurements taken in the middle (Figure 4.3 B) and at the end (Figure 4.3 C) of the experiments, which is similar to unaccounted nitrogen in the experiments. This highlights the importance of expanding the amount of parameters measured in future incubation experiments, including the makeup of particulate matter (e.g., PLFA).

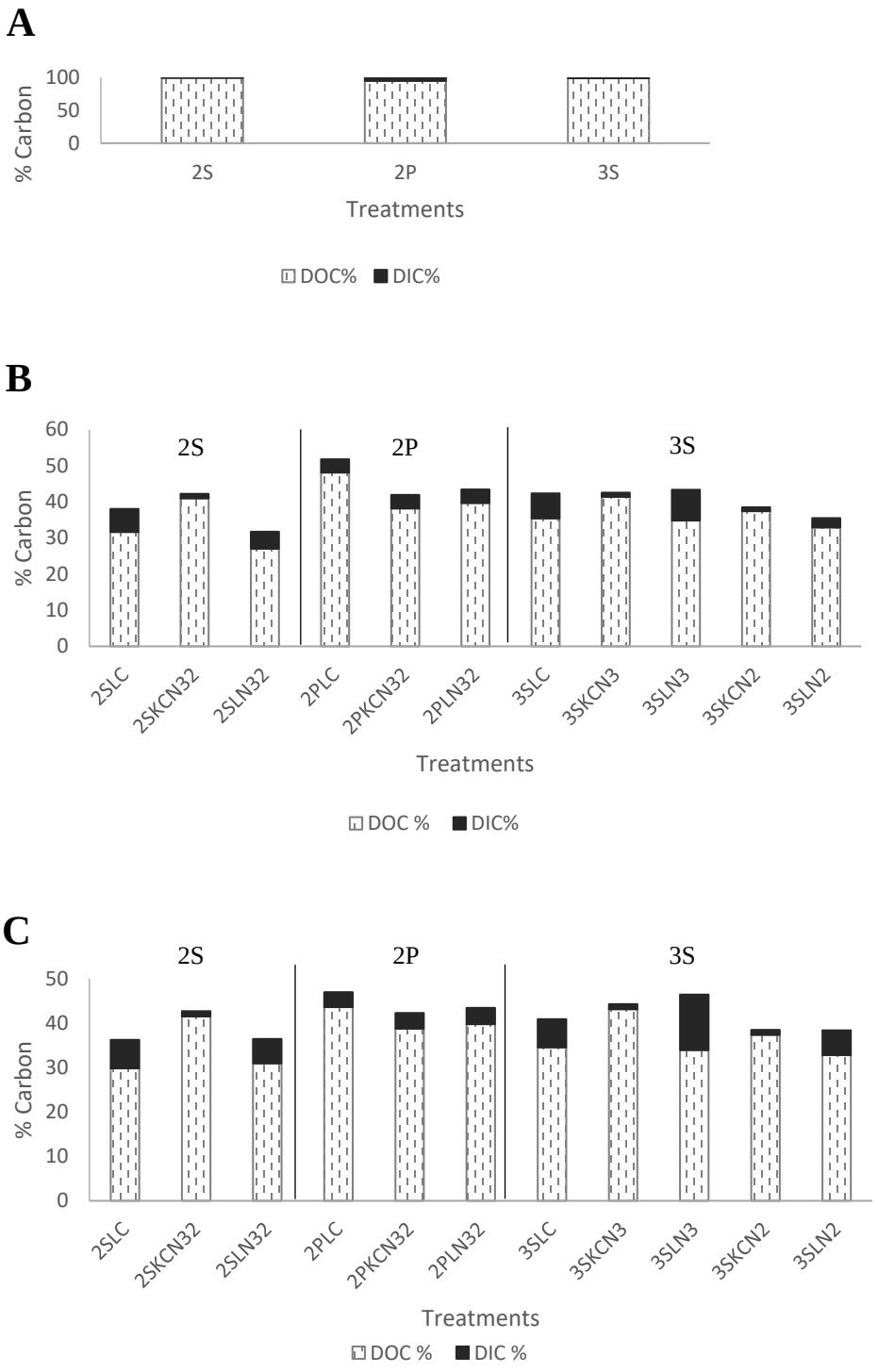

Figure 4.3. DOC and DIC percentages of the total carbon in treatments of Experiment 2 and 3 at (A) time zero, (B) the middle sampling period and (C) the final sampling period.

The data were normalized to the total starting concentration of carbon (i.e. the measured concentration of DOC and DIC of the seawater and produced waters plus the added organic acid concentrations).

The DOC concentrations in the live treatments of Experiments 2 and 3 in seawater continued to decrease throughout the duration of the experiments as seen Figure 4.3 B and C. The largest decrease (and most obvious in Figure 4.3 C) in DOC concentration was in the Live Nitrate addition treatment of Experiment 3 (3SLN3). Conversely, the Killed Control treatments had a larger DOC concentration than the live treatments, and a lower concentration of DIC than the live treatments. As seen in Equations 1.1 and 1.4, organic carbon ($CH_3CO_2^-$) is a reactant in microbial sulfate and nitrate reduction, and inorganic carbon (HCO_3^-) is a product of SRB and NRB heterotrophic respiration. Thus, the lower concentrations of DIC (and the higher concentrations of DOC) in the Killed Control treatments suggests microbial respiration was not taking place, whereas the live treatments did exhibit microbial respiration with their larger DIC concentrations and smaller DOC concentrations.

DIC for produced water experiments had a much higher initial concentration than the DIC for the seawater experiments (Figures 3.15, 3.23, 3.32, and 4.3 A). In the produced water treatments of Experiment 2, DOC was not consumed microbially (i.e. no major difference in live vs. killed treatments). Furthermore, in Experiment 2 with produced waters there is no evidence of microbial sulfate reduction, ammonia consumption, or DOC respiration. As seen in the photographs below, the produced water Live Control incubation (Figure 4.4 B) had no biomass visible, whereas the Live Control

of Experiment 2 in seawater (Figure 4.4 A) and the Live Control of Experiment 3 (4.4 C) had very visible black biomass. Figure 4.4 D shows a Live Nitrate addition treatment of Experiment 3, and biomass was also visible in this incubation but was white to beige/yellow in color (in contrast to the SRB treatments) and was only present in the nitrogen amended treatments in seawater with reducing agents. More photographs of the incubations can be found in Appendix B

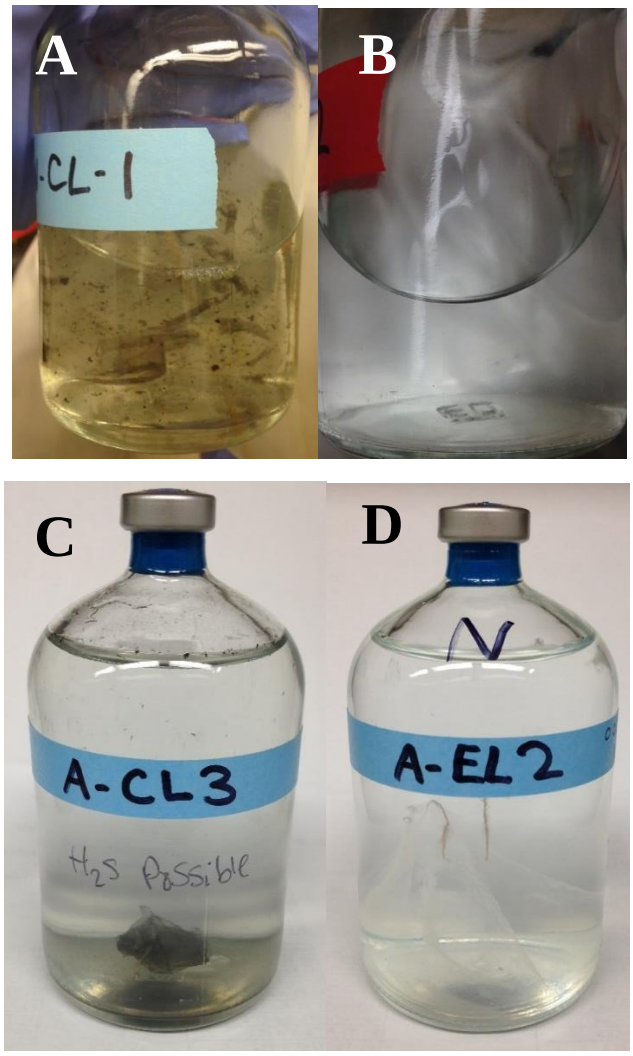

Figure 4.4. Photographs of incubation experiments. (A) Control Live treatment of Experiment 2 in seawater. (B) Control Live treatments of Experiment 2 in produced

water. (C) Control Live treatment of Experiment 3 in seawater. (D) Nitrate addition treatmennt of Experiment 3 in seawater.

4.5 Conclusion

In conclusion, Experiments 2 and 3 showed that nitrate and nitrite addition can suppress microbial sulfate reduction (and thus H_2S gas production) which is in agreement with previous studies: (Bødtker et al., 2008; Eckford et al., 2002; Hubert et al., 2007; Jenneman et al., 1986; Kaster et al., 2007; Reinsel et al., 1996; Voordouw et al., 2009). There was no considerable difference between using just nitrate, just nitrite, or the combination of the two for suppressing sulfate reduction; however, adding just nitrate or just nitrite resulted in less reducing conditions compared to adding a combination of the two. Less reducing conditions are favorable for suppressing sulfate reduction in these environments. Additionally, nitrate appeared to have a longer retention time in the experiments than nitrite, but had a similar effect on the inhibition of sulfate reduction. A better understanding of the microbial community in the waters of this study is needed to better understand the reactions observed such as, why was NH_3 and N_2O not produced in the seawater experiments?

The next suggested step for this research is conducting similar amendment experiments but with a site-specific engineered flow through bioreactor system (i.e. site-specific sediment, water, and microbial community). Voordouw et al. (2009) highlights the importance of mineral interactions with sulfide (i.e. sulfide is reactive with iron minerals and can convert sulfide to S°) and thus future experiments should explore how sulfate, sulfide, nitrate and nitrite react with site specific sediment and rock. Future

research on the biotic/abiotic relationships in experiment and reservoirs is suggested by measuring sulfur isotopes (Hubert et al., 2009). Identification and classification of the site specific particulate matter and microbial community though PLFA's would also be beneficial in understanding how NRB and SRB influence one another. In order to optimize the mitigation of H_2S with nitrate and nitrite addition, there needs to be a greater understanding of the overall nitrogen systematics in anaerobic environments.

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Appendix A: Raw Data

A1. Raw Data for the Bulk Water Characterization

Table A1.1. Dissolved organic carbon (DOC) concentrations for seawater (I-DOC) and produced water (P-DOC).

Sample	DOC (mol C/L)	Average	STDEV (± 1σ)	% RSD
I-DOC-1	0.000352			
I-DOC-2	0.000318	0.000322	0.000028	8.8
I-DOC-3	0.000296			
P-DOC-1	0.010191			
P-DOC-2	0.010149	0.010163	0.000024	0.236490
P-DOC-3	0.010149			

Table A1.2. Dissolved inorganic carbon (DIC) concentrations for seawater (I-DOC) and produced water (P-DOC).

Sample	DIC (mol C/L)	Average	STDEV (± 1σ)	% RSD
I-DIC-1	0.002069			
I-DIC-2	0.002070	0.002069	0.000001	0.04
I-DIC-3	0.002068			
P-DIC-1	0.008013			
P-DIC-2	0.008084	0.008033	0.000045	0.557599
P-DIC-3	0.008002			

Table A1.3. Sulfate concentrations for seawater (I-S) and produced water (P-S).

Sample	SO_4^{2-} (mol/L)	Average	STDEV (± 1σ)	% RSD
I-S1	0.0325			
I-S2	0.0311	0.0328	0.0018	5.6
I-S3	0.0348			
P-S1	0.0275			
P-S2	0.0290	0.0277	0.0013	4.5
P-S3	0.0265			

Table A1.4. Nitrate plus nitrite concentrations (mol N/L) for seawater (I-S) and produced water (P-S).

Sample	**NO_3^- + NO_2^- (mol N/L)**	**Average**	**STDEV (± 1σ)**
I-S1	0.0004705		
I-S2	0.0004819	5.35E-04	1.01E-04
I-S3	0.0006511		
P-S1	0.00599		
P-S2	0.0062756	6.13E-03	1.43E-04
P-S3	0.0061256		

A2. Redox potential data for the incubation experiments

Table A2.1. Redox Potential for Experiment 1 in seawater.

Day 1	**Redox Potential (mV)**	**Average**	**STDEV (± 1σ)**
Before	211		
During	191	184	31
After	150		
Day 22	**Redox Potential (mV)**	**Average**	**STDEV (± 1σ)**
1SLC-A	170		
1SLC-B	157	160	8.5
1SLC-C	154		
$1SKCN_{32}$-A	158		
$1SKCN_{32}$-B	149	154	4.7
$1SKCN_{32}$-C	156		
$1SLN_{32}$-A	153		
$1SLN_{32}$-B	146	147	5.6
$1SLN_{32}$-C	142		
Day 29	**Redox Potential (mV)**	**Average**	**STDEV (± 1σ)**
1SLC-A	256	235	18.58
1SLC-B	230		
1SLC-C	220		
$1SKCN_{32}$-A	170	161	10.07
$1SKCN_{32}$-B	162		
$1SKCN_{32}$-C	150		
$1SLN_{32}$-A	200	198	6.81
$1SLN_{32}$-B	203		
$1SLN_{32}$-C	190		
Day 45	**Redox Potential (mV)**	**Average**	**STDEV (± 1σ)**
1SLC-A	149		
1SLC-B	118	129	17
1SLC-C	121		

1SKCN$_{32}$-A	138		
1SKCN$_{32}$-B	141	146	12
1SKCN$_{32}$-C	160		
1SLN$_{32}$-A	125		
1SLN$_{32}$-B	115	139	33
1SLN$_{32}$-C	177		
Day 69	**Redox Potential (mV)**	**Average**	**STDEV (± 1σ)**
1SLC-A	245		
1SLC-B	237	241	4
1SLC-C	241		
1SKCN$_{32}$-A	249		
1SKCN$_{32}$-B	234	235	13
1SKCN$_{32}$-C	223		
1SLN$_{32}$-A	242		
1SLN$_{32}$-B	243	247	8
1SLN$_{32}$-C	256		
Day 109	**Redox Potential (mV)**	**Average**	**STDEV (± 1σ)**
1SLC-A	150		
1SLC-B	139	148	8
1SLC-C	155		
1SKCN$_{32}$-A	180		
1SKCN$_{32}$-B	152	163	15
1SKCN$_{32}$-C	156		
1SLN$_{32}$-A	148		
1SLN$_{32}$-B	161	152	8
1SLN$_{32}$-C	147		

Table A2.2. Redox Potential for Experiment 2 in seawater.

Day 1	**Redox Potential (mV)**	**Average**	**STDEV (± 1σ)**
Before	236		
During	230	232	4
After	229		
Day 37	**Redox Potential (mV)**	**Average**	**STDEV (± 1σ)**
2SLC-A	-377		
2SLC-B	-368	-374	5
2SLC-C	-376		
$2SKCN_{32}$-A	116		
$2SKCN_{32}$-B	118	117	1
$2SKCN_{32}$-C	116		
$2SLN_{32}$-A	-70		
$2SLN_{32}$-B	-20	-44	25
$2SLN_{32}$-C	-42		
Day 51	**Redox Potential (mV)**	**Average**	**STDEV (± 1σ)**
2SLC-A	-360		
2SLC-B	-372	-362	10
2SLC-C	-353		
$2SKCN_{32}$-A	116		
$2SKCN_{32}$-B	123	121	4
$2SKCN_{32}$-C	124		
$2SLN_{32}$-A	-184		
$2SLN_{32}$-B	-122	-150	32
$2SLN_{32}$-C	-143		
Day 97	**Redox Potential (mV)**	**Average**	**STDEV (± 1σ)**
2SLC-A	-402		
2SLC-B	-397	-386	23
2SLC-C	-360		
$2SKCN_{32}$-A	82	86	6

2SKCN$_{32}$-B	93		
2SKCN$_{32}$-C	82		
2SLN$_{32}$-A	80		
2SLN$_{32}$-B	-165	-194	161
2SLN$_{32}$-C	-223		

Table A2.3. Redox Potential for Experiment 2 in produced water.

Day 1	**Redox Potential (mV)**	**Average**	**STDEV (± 1σ)**
Before	233		
During	225	221	15
After	204		
Day 37	**Redox Potential (mV)**	**Average**	**STDEV (± 1σ)**
2PLC-A	-10		
2PLC-B	-89	-63	46
2PLC-C	-90		
2PKCN$_{32}$-A	121		
2PKCN$_{32}$-B	121	124	5
2PKCN$_{32}$-C	130		
2PLN$_{32}$-A	9		
2PLN$_{32}$-B	25	23	13
2PLN$_{32}$-C	34		
Day 51	**Redox Potential (mV)**	**Average**	**STDEV (± 1σ)**
2PLC-A	-60		
2PLC-B	-131	-132	72
2PLC-C	-204		
2PKCN$_{32}$-A	112		
2PKCN$_{32}$-B	108	108	4
2PKCN$_{32}$-C	105		
2PLN$_{32}$-A	120	107	11
2PLN$_{32}$-B	103		

$2PLN_{32}$-C	99		
Day 97	**Redox Potential (mV)**	**Average**	**STDEV (± 1σ)**
2PLC-A	37		
2PLC-B	37	37	1
2PLC-C	38		
$2PKCN_{32}$-A	87		
$2PKCN_{32}$-B	90	91	5
$2PKCN_{32}$-C	97		
$2PLN_{32}$-A	72		
$2PLN_{32}$-B	66	61	14
$2PLN_{32}$-C	45		

Table A2.4. Redox Potential for Experiment 3 in seawater.

Day 1	**Redox Potential (mV)**	**Average**	**STDEV (± 1σ)**
Before	136		
During	120	125	9
After	120		
Day 28	**Redox Potential (mV)**	**Average**	**STDEV (± 1σ)**
3SLC-A	-410		
3SLC-B	-411	-405	9
3SLC-C	-395		
$3SKCN_3$-A	88		
$3SKCN_3$-B	93	89	3
$3SKCN_3$-C	87		
$3SLN_3$-A	71		
$3SLN_3$-B	68	66	6
$3SLN_3$-C	59		
$3SKCN_2$-A	87		
$3SKCN_2$-B	93	92	5
$3SKCN_2$-C	96		

$3SLN_2$-A	16		
$3SLN_2$-B	18	10	12
$3SLN_2$-C	-3		
Day 59	**Redox Potential (mV)**	**Average**	**STDEV (± 1σ)**
3SLC-A	-395		
3SLC-B	-402	-399	4
3SLC-C	-400		
$3SKCN_3$-A	116		
$3SKCN_3$-B	113	115	2
$3SKCN_3$-C	116		
$3SLN_3$-A	-57		
$3SLN_3$-B	-58	-55	4
$3SLN_3$-C	-51		
$3SKCN_2$-A	100		
$3SKCN_2$-B	104	103	2
$3SKCN_2$-C	104		
$3SLN_2$-A	-75		
$3SLN_2$-B	-73	-73	2
$3SLN_2$-C	-72		

A3. Sulfate data for the incubation experiments

Table A3.1. Sulfate concentrations for Experiment 1 in seawater

Day 1	**Sulfate (mol/L)**	**Average**	**STDEV (± 1σ)**
T0-1	2.39E-02		
T0-2	2.44E-02	2.35E-02	1.11E-03
T0-3	2.23E-02		
Day 109	**Sulfate (mol/L)**	**Average**	**STDEV (± 1σ)**
1SLC-A	2.42E-02		
1SLC-B	2.41E-02	2.39E-02	4.72E-04
1SLC-C	2.33E-02		
$1SKCN_{32}$-A	2.33E-02		
$1SKCN_{32}$-B	2.27E-02	2.31E-02	3.87E-04
$1SKCN_{32}$-C	2.34E-02		
$1SLN_{32}$-A	2.53E-02		
$1SLN_{32}$-B	2.35E-02	2.42E-02	9.75E-04
$1SLN_{32}$-C	2.38E-02		

Table A3.2. Sulfate concentrations for Experiment 2 in seawater

Day 1	**Sulfate (mol/L)**	**Average**	**STDEV (± 1σ)**
T0-1	3.07E-02		
T0-2	2.52E-02	2.66E-02	3.58E-03
T0-3	2.40E-02		
Day 51	**Sulfate (mol/L)**	**Average**	**STDEV (± 1σ)**
2SLC-A	1.98E-02		
2SLC-B	1.97E-02	2.00E-02	4.18E-04
2SLC-C	2.04E-02		
$2SKCN_{32}$-A	2.51E-02	2.52E-02	3.20E-04
$2SKCN_{32}$-B	2.55E-02		

2SKCN$_{32}$-C	2.49E-02		
2SLN$_{32}$-A	2.35E-02		
2SLN$_{32}$-B	2.49E-02	2.45E-02	8.27E-04
2SLN$_{32}$-C	2.50E-02		
Day 97	**Sulfate (mol/L)**	**Average**	**STDEV (± 1σ)**
2SLC-A	2.06E-02		
2SLC-B	2.17E-02	2.11E-02	5.53E-04
2SLC-C	2.09E-02		
2SKCN$_{32}$-A	2.44E-02		
2SKCN$_{32}$-B	2.49E-02	2.53E-02	1.21E-03
2SKCN$_{32}$-C	2.67E-02		
2SLN$_{32}$-A	2.93E-02		
2SLN$_{32}$-B	2.53E-02	2.78E-02	2.19E-03
2SLN$_{32}$-C	2.88E-02		

Table A3.3 Sulfate concentrations for Experiment 2 in produced water

Day 1	**Sulfate (mol/L)**	**Average**	**STDEV (± 1σ)**
T0-1	1.03E-02		
T0-2	1.11E-02	1.14E-02	1.30E-03
T0-3	1.29E-02		
Day 51	**Sulfate (mol/L)**	**Average**	**STDEV (± 1σ)**
2PLC-A	1.35E-02		
2PLC-B	1.33E-02	1.31E-02	6.00E-04
2PLC-C	1.24E-02		
2PKCN$_{32}$-A	1.29E-02		
2PKCN$_{32}$-B	1.08E-02	1.25E-02	1.49E-03
2PKCN$_{32}$-C	1.37E-02		
2PLN$_{32}$-A	1.26E-02		
2PLN$_{32}$-B	1.29E-02	1.26E-02	3.63E-04
2PLN$_{32}$-C	1.22E-02		

Day 97	Sulfate (mol/L)	Average	STDEV (± 1σ)
2PLC-A	1.21E-02		
2PLC-B	1.27E-02	1.24E-02	3.24E-04
2PLC-C	1.26E-02		
$2PKCN_{32}$-A	1.06E-02		
$2PKCN_{32}$-B	1.35E-02	1.22E-02	1.45E-03
$2PKCN_{32}$-C	1.25E-02		
$2PLN_{32}$-A	1.24E-02		
$2PLN_{32}$-B	1.33E-02	1.29E-02	4.81E-04
$2PLN_{32}$-C	1.30E-02		

Table A3.4. Sulfate concentrations for Experiment 3 in seawater

Day 1	Sulfate (mol/L)	Average	STDEV (± 1σ)
T0-1	2.51E-02		
T0-2	2.11E-02	2.19E-02	2.91E-03
T0-3	1.94E-02		
Day 28	**Sulfate (mol/L)**	**Average**	**STDEV (± 1σ)**
3SLC-A	1.94E-02		
3SLC-B	1.79E-02	1.83E-02	9.54E-04
3SLC-C	1.77E-02		
$3SKCN_3$-A	2.30E-02		
$3SKCN_3$-B	2.05E-02	2.19E-02	1.26E-03
$3SKCN_3$-C	2.22E-02		
$3SLN_3$-A	2.36E-02		
$3SLN_3$-B	2.12E-02	2.23E-02	1.26E-03
$3SLN_3$-C	2.20E-02		
$3SKCN_2$-A	2.19E-02	2.09E-02	8.53E-04
$3SKCN_2$-B	2.02E-02		

$3SKCN_2$-C	2.07E-02		
$3SLN_2$-A	2.12E-02		
$3SLN_2$-B	2.15E-02	2.14E-02	1.51E-04
$3SLN_2$-C	2.14E-02		
Day 59	**Sulfate (mol/L)**	**Average**	**STDEV (± 1σ)**
3SLC-A	2.23E-02		
3SLC-B	2.15E-02	2.22E-02	6.45E-04
3SLC-C	2.28E-02		
$3SKCN_3$-A	2.63E-02		
$3SKCN_3$-B	2.76E-02	2.72E-02	8.14E-04
$3SKCN_3$-C	2.78E-02		
$3SLN_3$-A	2.82E-02		
$3SLN_3$-B	2.87E-02	2.90E-02	9.80E-04
$3SLN_3$-C	3.01E-02		
$3SKCN_2$-A	2.59E-02		
$3SKCN_2$-B	2.63E-02	2.66E-02	9.84E-04
$3SKCN_2$-C	2.78E-02		
$3SLN_2$-A	2.93E-02		
$3SLN_2$-B	2.76E-02	2.85E-02	8.45E-04
$3SLN_2$-C	2.86E-02		

A4. Dissolved inorganic carbon (DIN) data for the incubation experiments

Table A4.1. Dissolved inorganic nitrogen (DIN) concentrations for Experiment 1 in seawater

Day 1	**DIN (mol N/L)**	**Average**	**STDEV (± 1σ)**
T0-1	1.80E-04		
T0-2	1.69E-04	1.75E-04	5.58E-06
T0-3	1.77E-04		
Day 109	**DIN (mol N/L)**	**Average**	**STDEV (± 1σ)**
1SLC-A	1.65E-04		
1SLC-B	1.73E-04	1.64E-04	9.12E-06
1SLC-C	1.55E-04		
$1SKCN_{32}$-A	2.36E-02		
$1SKCN_{32}$-B	2.44E-02	2.34E-02	1.04E-03
$1SKCN_{32}$-C	2.23E-02		
$1SLN_{32}$-A	2.47E-02		
$1SLN_{32}$-B	3.31E-02	3.03E-02	4.90E-03
$1SLN_{32}$-C	3.32E-02		

Table A4.2. Dissolved inorganic nitrogen (DIN) concentrations for Experiment 2 in seawater

Day 1	**DIN (mol N/L)**	**Average**	**STDEV (± 1σ)**
T0-1	2.87E-04		
T0-2	3.02E-04	2.91E-04	9.05E-06
T0-3	2.85E-04		
Day 51	**DIN (mol N/L)**	**Average**	**STDEV (± 1σ)**
2SLC-A	1.02E-04		
2SLC-B	6.96E-05	7.50E-05	2.47E-05
2SLC-C	5.35E-05		

2SKCN$_{32}$-A	2.35E-02		
2SKCN$_{32}$-B	2.62E-02	2.66E-02	3.26E-03
2SKCN$_{32}$-C	3.00E-02		
2SLN$_{32}$-A	1.64E-04		
2SLN$_{32}$-B	1.95E-04	1.53E-04	4.82E-05
2SLN$_{32}$-C	1.01E-04		
Day 97	**DIN (mol N/L)**	**Average**	**STDEV (± 1σ)**
2SLC-A	1.03E-04		
2SLC-B	6.08E-05	7.25E-05	2.63E-05
2SLC-C	5.41E-05		
2SKCN$_{32}$-A	3.06E-02		
2SKCN$_{32}$-B	2.42E-02	2.78E-02	3.29E-03
2SKCN$_{32}$-C	2.86E-02		
2SLN$_{32}$-A	1.75E-04		
2SLN$_{32}$-B	2.05E-04	1.70E-04	3.79E-05
2SLN$_{32}$-C	1.30E-04		

Table A4.3. Dissolved inorganic nitrogen (DIN) concentrations for Experiment 2 in produced water

Day 1	**DIN (mol N/L)**	**Average**	**STDEV (± 1σ)**
T0-1	2.66E-04		
T0-2	2.97E-04	2.92E-04	2.45E-05
T0-3	3.14E-04		
Day 51	**DIN (mol N/L)**	**Average**	**STDEV (± 1σ)**
2PLC-A	2.96E-04		
2PLC-B	2.84E-04	2.91E-04	6.36E-06
2PLC-C	2.92E-04		
2PKCN$_{32}$-A	1.96E-02		
2PKCN$_{32}$-B	2.29E-02	2.65E-02	9.18E-03
2PKCN$_{32}$-C	3.69E-02		

2PLN$_{32}$-A	3.17E-02		
2PLN$_{32}$-B	2.51E-02	2.98E-02	4.10E-03
2PLN$_{32}$-C	3.26E-02		
Day 97	**DIN (mol N/L)**	**Average**	**STDEV (± 1σ)**
2PLC-A	2.63E-04		
2PLC-B	2.75E-04	2.73E-04	9.34E-06
2PLC-C	2.82E-04		
2PKCN$_{32}$-A	2.42E-02		
2PKCN$_{32}$-B	2.88E-02	2.30E-02	6.42E-03
2PKCN$_{32}$-C	1.61E-02		
2PLN$_{32}$-A	3.35E-02		
2PLN$_{32}$-B	2.32E-02	2.91E-02	5.28E-03
2PLN$_{32}$-C	3.06E-02		

Table A4.4. Dissolved inorganic nitrogen (DIN) concentrations for Experiment 3 in seawater

Day 1	**DIN (mol N/L)**	**Average**	**STDEV (± 1σ)**
T0-1	4.09E-04		
T0-2	4.19E-04	4.16E-04	6.03E-06
T0-3	4.20E-04		
Day 28	**DIN (mol N/L)**	**Average**	**STDEV (± 1σ)**
3SLC-A	2.57E-04		
3SLC-B	2.27E-04	2.34E-04	1.98E-05
3SLC-C	2.19E-04		
3SKCN$_{3}$-A	1.79E-02		
3SKCN$_{3}$-B	1.87E-02	1.91E-02	1.43E-03
3SKCN$_{3}$-C	2.07E-02		
3SLN$_{3}$-A	2.45E-02		
3SLN$_{3}$-B	1.92E-02	2.10E-02	3.10E-03
3SLN$_{3}$-C	1.92E-02		

$3SKCN_2$-A	2.47E-02		
$3SKCN_2$-B	3.05E-02	2.67E-02	3.24E-03
$3SKCN_2$-C	2.51E-02		
$3SLN_2$-A	1.03E-02		
$3SLN_2$-B	9.70E-03	9.05E-03	1.72E-03
$3SLN_2$-C	7.09E-03		
Day 59	**DIN (mol N/L)**	**Average**	**STDEV (± 1σ)**
3SLC-A	2.51E-04		
3SLC-B	2.49E-04	2.53E-04	5.67E-06
3SLC-C	2.59E-04		
$3SKCN_3$-A	1.86E-02		
$3SKCN_3$-B	1.99E-02	1.97E-02	9.34E-04
$3SKCN_3$-C	2.04E-02		
$3SLN_3$-A	1.00E-04		
$3SLN_3$-B	1.21E-04	4.66E-03	7.88E-03
$3SLN_3$-C	1.38E-02		
$3SKCN_2$-A	2.94E-02		
$3SKCN_2$-B	3.05E-02	2.84E-02	2.81E-03
$3SKCN_2$-C	2.52E-02		
$3SLN_2$-A	3.40E-05		
$3SLN_2$-B	8.37E-05	5.65E-05	2.52E-05
$3SLN_2$-C	5.19E-05		

A5. Ammonia data for the incubation experiments

Table A5.1. Ammonia concentrations for Experiment 1 in seawater

Day 1	**Ammonia (mol N/L)**	**Average**	**STDEV (± 1σ)**
T0-1	2.61E-04		
T0-2	2.47E-04	2.55E-04	7.69E-06
T0-3	2.58E-04		
Day 109	**Ammonia (mol N/L)**	**Average**	**STDEV (± 1σ)**
1SLC-A	2.43E-04		
1SLC-B	2.51E-04	2.40E-04	1.33E-05
1SLC-C	2.25E-04		
$1SKCN_{32}$-A	4.08E-04		
$1SKCN_{32}$-B	3.85E-04	3.92E-04	1.37E-05
$1SKCN_{32}$-C	3.84E-04		
$1SLN_{32}$-A	3.84E-04		
$1SLN_{32}$-B	3.86E-04	3.81E-04	7.33E-06
$1SLN_{32}$-C	3.72E-04		

Table A5.2. Ammonia concentrations for Experiment 2 in seawater

Day 1	**Ammonia (mol N/L)**	**Average**	**STDEV (± 1σ)**
T0-1	2.78E-04		
T0-2	2.94E-04	2.83E-04	9.55E-06
T0-3	2.77E-04		
Day 51	**Ammonia (mol N/L)**	**Average**	**STDEV (± 1σ)**
2SLC-A	1.02E-04		
2SLC-B	6.96E-05	7.50E-05	2.47E-05
2SLC-C	5.35E-05		
$2SKCN_{32}$-A	4.64E-04		
$2SKCN_{32}$-B	4.76E-04	4.42E-04	4.90E-05
$2SKCN_{32}$-C	3.86E-04		

$2SLN_{32}$-A	1.56E-04		
$2SLN_{32}$-B	1.90E-04	1.49E-04	4.52E-05
$2SLN_{32}$-C	1.01E-04		
Day 97	**Ammonia (mol N/L)**	**Average**	**STDEV (± 1σ)**
2SLC-A	1.03E-04		
2SLC-B	6.08E-05	7.25E-05	2.63E-05
2SLC-C	5.41E-05		
$2SKCN_{32}$-A	4.24E-04		
$2SKCN_{32}$-B	4.58E-04	4.19E-04	4.08E-05
$2SKCN_{32}$-C	3.77E-04		
$2SLN_{32}$-A	1.66E-04		
$2SLN_{32}$-B	1.98E-04	1.61E-04	4.00E-05
$2SLN_{32}$-C	1.18E-04		

Table A5.3 Ammonia concentrations for Experiment 2 in produced water

Day 1	**Ammonia (mol N/L)**	**Average**	**STDEV (± 1σ)**
T0-1	2.66E-04		
T0-2	2.97E-04	2.92E-04	2.45E-05
T0-3	3.14E-04		
Day 51	**Ammonia (mol N/L)**	**Average**	**STDEV (± 1σ)**
2PLC-A	2.96E-04		
2PLC-B	2.84E-04	2.91E-04	6.36E-06
2PLC-C	2.92E-04		
$2PKCN_{32}$-A	5.16E-04		
$2PKCN_{32}$-B	4.51E-04	4.41E-04	8.06E-05
$2PKCN_{32}$-C	3.56E-04		
$2PLN_{32}$-A	5.17E-04		
$2PLN_{32}$-B	4.65E-04	4.80E-04	3.27E-05
$2PLN_{32}$-C	4.57E-04		
Day 97	**Ammonia (mol N/L)**	**Average**	**STDEV (± 1σ)**

2PLC-A	2.63E-04		
2PLC-B	2.75E-04	2.73E-04	9.34E-06
2PLC-C	2.82E-04		
$2PKCN_{32}$-A	4.80E-04		
$2PKCN_{32}$-B	4.61E-04	4.30E-04	7.13E-05
$2PKCN_{32}$-C	3.48E-04		
$2PLN_{32}$-A	4.69E-04		
$2PLN_{32}$-B	4.53E-04	4.51E-04	1.97E-05
$2PLN_{32}$-C	4.30E-04		

Table A5.4. Ammonia concentrations for Experiment 3 in seawater

Day 1	**Ammonia (mol N/L)**	**Average**	**STDEV (± 1σ)**
T0-1	4.09E-04		
T0-2	4.19E-04	4.16E-04	6.03E-06
T0-3	4.20E-04		
Day 28	**Ammonia (mol N/L)**	**Average**	**STDEV (± 1σ)**
3SLC-A	2.57E-04		
3SLC-B	2.27E-04	2.34E-04	1.98E-05
3SLC-C	2.19E-04		
$3SKCN_3$-A	4.26E-04		
$3SKCN_3$-B	4.22E-04	4.22E-04	3.41E-06
$3SKCN_3$-C	4.19E-04		
$3SLN_3$-A	2.63E-04		
$3SLN_3$-B	2.64E-04	2.72E-04	1.44E-05
$3SLN_3$-C	2.88E-04		
$3SKCN_2$-A	4.56E-04		
$3SKCN_2$-B	5.00E-04	4.70E-04	2.58E-05
$3SKCN_2$-C	4.56E-04		
$3SLN_2$-A	1.46E-04	1.49E-04	3.66E-06
$3SLN_2$-B	1.53E-04		

$3SLN_2$-C	1.49E-04

Day 59	**Ammonia (mol N/L)**	**Average**	**STDEV (± 1σ)**
3SLC-A	2.51E-04		
3SLC-B	2.49E-04	2.53E-04	5.67E-06
3SLC-C	2.59E-04		
$3SKCN_3$-A	4.16E-04		
$3SKCN_3$-B	4.23E-04	4.22E-04	5.35E-06
$3SKCN_3$-C	4.26E-04		
$3SLN_3$-A	9.00E-05		
$3SLN_3$-B	1.13E-04	1.48E-04	8.10E-05
$3SLN_3$-C	2.40E-04		
$3SKCN_2$-A	4.29E-04		
$3SKCN_2$-B	4.32E-04	4.28E-04	4.68E-06
$3SKCN_2$-C	4.22E-04		
$3SLN_2$-A	2.18E-05		
$3SLN_2$-B	7.27E-05	4.52E-05	2.57E-05
$3SLN_2$-C	4.10E-05		

A6. Dissolved organic nitrogen data for the incubation experiments

Table A6.1. Dissolved organic nitrogen (DON) concentrations for Experiment 1 in seawater

Day 1	**DON (mol N/L)**	**Average**	**STDEV (± 1σ)**
T0-1	1.47E-04		
T0-2	1.59E-04	1.46E-04	1.39E-05
T0-3	1.32E-04		
Day 109	**DON (mol N/L)**	**Average**	**STDEV (± 1σ)**
1SLC-A	2.39E-04		
1SLC-B	2.54E-04	2.57E-04	1.95E-05
1SLC-C	2.78E-04		
$1SKCN_{32}$-A	5.26E-03		
$1SKCN_{32}$-B	3.65E-03	4.85E-03	1.06E-03
$1SKCN_{32}$-C	5.64E-03		
$1SLN_{32}$-A	2.38E-03		
$1SLN_{32}$-B	0	7.94E-04	1.38E-03
$1SLN_{32}$-C	0		

Table A6.2. Dissolved organic nitrogen (DON) concentrations for Experiment 2 in seawater

Day 1	**DON (mol N/L)**	**Average**	**STDEV (± 1σ)**
T0-1	1.07E-04		
T0-2	9.27E-05	8.58E-05	2.55E-05
T0-3	5.76E-05		
Day 51	**DON (mol N/L)**	**Average**	**STDEV (± 1σ)**
2SLC-A	0		
2SLC-B	0	0	0
2SLC-C	0		

2SKCN$_{32}$-A	5.74E-03		
2SKCN$_{32}$-B	0	1.91E-03	3.31E-03
2SKCN$_{32}$-C	0		
2SLN$_{32}$-A	0		
2SLN$_{32}$-B	0	0	0
2SLN$_{32}$-C	0		
Day 97	**DON (mol N/L)**	**Average**	**STDEV (± 1σ)**
2SLC-A	0		
2SLC-B	0	0	0
2SLC-C	0		
2SKCN$_{32}$-A	0		
2SKCN$_{32}$-B	0	1.73E-03	2.99E-03
2SKCN$_{32}$-C	5.18E-03		
2SLN$_{32}$-A	0		
2SLN$_{32}$-B	0	0	0
2SLN$_{32}$-C	0		

Table A6.3. Dissolved organic nitrogen (DON) concentrations for Experiment 2 in produced water

Day 1	**DON (mol N/L)**	**Average**	**STDEV (± 1σ)**
T0-1	1.54E-04		
T0-2	1.24E-04	1.20E-04	3.60E-05
T0-3	8.27E-05		
Day 51	**DON (mol N/L)**	**Average**	**STDEV (± 1σ)**
2PLC-A	1.50E-04		
2PLC-B	1.83E-04	1.54E-04	2.71E-05
2PLC-C	1.29E-04		
2PKCN$_{32}$-A	4.22E-03		
2PKCN$_{32}$-B	3.25E-03	2.49E-03	2.21E-03
2PKCN$_{32}$-C	0		

2PLN$_{32}$-A	0		
2PLN$_{32}$-B	0	0	0
2PLN$_{32}$-C	0		
Day 97	**DON (mol N/L)**	**Average**	**STDEV (± 1σ)**
2PLC-A	1.46E-04		
2PLC-B	1.35E-04	1.35E-04	1.13E-05
2PLC-C	1.23E-04		
2PKCN$_{32}$-A	0		
2PKCN$_{32}$-B	0	0	0
2PKCN$_{32}$-C	0		
2PLN$_{32}$-A	0		
2PLN$_{32}$-B	0	0	0
2PLN$_{32}$-C	0		

Table A6.4. Dissolved organic nitrogen (DON) concentrations for Experiment 3 in seawater.

Day 1	**DON (mol N/L)**	**Average**	**STDEV (± 1σ)**
T0-1	1.04E-05		
T0-2	5.64E-06	1.01E-05	4.26E-06
T0-3	1.41E-05		
Day 28	**DON (mol N/L)**	**Average**	**STDEV (± 1σ)**
3SLC-A	1.74E-05		
3SLC-B	6.61E-05	5.39E-05	3.22E-05
3SLC-C	7.82E-05		
3SKCN$_3$-A	1.66E-04		
3SKCN$_3$-B	8.43E-05	8.33E-05	8.28E-05
3SKCN$_3$-C	0		
3SLN$_3$-A	0		
3SLN$_3$-B	0	0	0
3SLN$_3$-C	0		

$3SKCN_2$-A	0		
$3SKCN_2$-B	0	0	0
$3SKCN_2$-C	0		
$3SLN_2$-A	0		
$3SLN_2$-B	0	0	0
$3SLN_2$-C	0		
Day 59	**DON (mol N/L)**	**Average**	**STDEV (± 1σ)**
3SLC-A	1.49E-04		
3SLC-B	1.36E-04	1.49E-04	1.34E-05
3SLC-C	1.63E-04		
$3SKCN_3$-A	2.08E-04		
$3SKCN_3$-B	0	6.93E-05	1.20E-04
$3SKCN_3$-C	0		
$3SLN_3$-A	1.95E-03		
$3SLN_3$-B	2.01E-03	1.32E-03	1.15E-03
$3SLN_3$-C	0		
$3SKCN_2$-A	0		
$3SKCN_2$-B	0	0	0
$3SKCN_2$-C	0		
$3SLN_2$-A	9.71E-04		
$3SLN_2$-B	1.01E-03	1.07E-03	1.41E-04
$3SLN_2$-C	1.23E-03		

A7. N_2O data for the incubation experiments

Table A7.1. N_2O concentrations for Experiment 2 in seawater

Day 51	**Moles N_2O/L**	**Average**	**STDEV (± 1σ)**
$2SKCN_{32}$-A	2.05E-10		
$2SKCN_{32}$-B	1.86E-10	1.93E-10	1.09E-11
$2SKCN_{32}$-C	1.88E-10		
$2SLN_{32}$-A	1.58E-10		
$2SLN_{32}$-B	1.91E-10	1.77E-10	1.73E-11
$2SLN_{32}$-C	1.83E-10		
Day 97	**Moles N_2O/L**	**Average**	**STDEV (± 1σ)**
$2SKCN_{32}$-A	1.08E-10		
$2SKCN_{32}$-B	1.45E-10	1.19E-10	2.24E-11
$2SKCN_{32}$-C	1.04E-10		
$2SLN_{32}$-A	1.15E-10		
$2SLN_{32}$-B	1.34E-10	1.21E-10	1.17E-11
$2SLN_{32}$-C	1.14E-10		

Table A7.2. N_2O concentrations for Experiment 3 in seawater

Day 28	**Moles N_2O/L**	**Average**	**STDEV (± 1σ)**
$3SKCN_3$-A	2.78E-10		
$3SKCN_3$-B	1.01E-09	5.45E-10	4.06E-10
$3SKCN_3$-C	3.46E-10		
$3SLN_3$-A	2.35E-10		
$3SLN_3$-B	1.03E-09	7.85E-10	4.77E-10
$3SLN_3$-C	1.09E-09		
$3SKCN_2$-A	1.05E-09		
$3SKCN_2$-B	9.66E-11	7.22E-10	5.42E-10
$3SKCN_2$-C	1.02E-09		
$3SLN_2$-A	1.15E-09	7.53E-10	5.55E-10

$3SLN_2$-B	1.19E-10		
$3SLN_2$-C	9.86E-10		
Day 59	**Moles N_2O/L**	**Average**	**STDEV (± 1σ)**
$3SKCN_3$-A	1.82E-11		
$3SKCN_3$-B	3.98E-10	3.09E-10	2.58E-10
$3SKCN_3$-C	5.10E-10		
$3SLN_3$-A	3.00E-10		
$3SLN_3$-B	1.99E-10	2.73E-10	6.51E-11
$3SLN_3$-C	3.20E-10		
$3SKCN_2$-A	5.31E-10		
$3SKCN_2$-B	4.02E-10	4.35E-10	8.48E-11
$3SKCN_2$-C	3.71E-10		
$3SLN_2$-A	3.56E-10		
$3SLN_2$-B	1.85E-10	3.14E-10	1.14E-10
$3SLN_2$-C	4.01E-10		

A8. Dissolved organic carbon data for the incubation experiments

Table A8.1. Dissolved organic carbon (DOC) concentrations for Experiment 1 in seawater.

Day 1	**DOC (mol C/L)**	**Average**	**STDEV (± 1σ)**
T0-1	1.98E-03		
T0-2	N/A	1.94E-03	6.12E-05
T0-3	1.89E-03		
Day 109	**DOC (mol C/L)**	**Average**	**STDEV (± 1σ)**
1SLC-A	1.41E-03		
1SLC-B	1.36E-03	1.24E-03	2.66E-04
1SLC-C	9.31E-04		
$1SKCN_{32}$-A	1.26E-03		
$1SKCN_{32}$-B	1.19E-03	1.24E-03	3.95E-05
$1SKCN_{32}$-C	1.27E-03		
$1SLN_{32}$-A	1.15E-03		
$1SLN_{32}$-B	9.51E-04	1.00E-03	1.31E-04
$1SLN_{32}$-C	9.08E-04		

Table A8.2. Dissolved organic carbon (DOC) concentrations for Experiment 2 in seawater.

Day 1	**DOC (mol C/L)**	**Average**	**STDEV (± 1σ)**
T0-1	2.17E-03		
T0-2	2.17E-03	2.18E-03	2.68E-06
T0-3	2.18E-03		
Day 51	**DOC (mol C/L)**	**Average**	**STDEV (± 1σ)**
2SLC-A	5.16E-02		
2SLC-B	5.74E-02	5.18E-02	5.50E-03
2SLC-C	4.64E-02		

2SKCN$_{32}$-A	6.08E-02		
2SKCN$_{32}$-B	7.67E-02	6.70E-02	8.49E-03
2SKCN$_{32}$-C	6.35E-02		
2SLN$_{32}$-A	4.20E-02		
2SLN$_{32}$-B	4.25E-02	4.42E-02	3.41E-03
2SLN$_{32}$-C	4.81E-02		
Day 97	**DOC (mol C/L)**	**Average**	**STDEV (± 1σ)**
2SLC-A	5.21E-02		
2SLC-B	4.70E-02	4.87E-02	2.93E-03
2SLC-C	4.71E-02		
2SKCN$_{32}$-A	6.19E-02		
2SKCN$_{32}$-B	7.69E-02	6.78E-02	8.01E-03
2SKCN$_{32}$-C	6.47E-02		
2SLN$_{32}$-A	4.91E-02		
2SLN$_{32}$-B	4.82E-02	5.06E-02	3.37E-03
2SLN$_{32}$-C	5.44E-02		

Table A8.3. Dissolved organic carbon (DOC) concentrations for Experiment 2 in produced water.

Day 1	**DOC (mol C/L)**	**Average**	**STDEV (± 1σ)**
T0-1	7.53E-03		
T0-2	7.52E-03	7.52E-03	6.36E-06
T0-3	7.52E-03		
Day 51	**DOC (mol C/L)**	**Average**	**STDEV (± 1σ)**
2PLC-A	8.65E-02		
2PLC-B	9.25E-02	8.54E-02	7.67E-03
2PLC-C	7.73E-02		
2PKCN$_{32}$-A	7.10E-02		
2PKCN$_{32}$-B	7.04E-02	6.78E-02	5.02E-03
2PKCN$_{32}$-C	6.20E-02		

2PLN$_{32}$-A	6.19E-02		
2PLN$_{32}$-B	6.89E-02	7.04E-02	9.34E-03
2PLN$_{32}$-C	8.04E-02		
Day 97	**DOC (mol C/L)**	**Average**	**STDEV (± 1σ)**
2PLC-A	7.91E-02		
2PLC-B	8.33E-02	7.73E-02	7.04E-03
2PLC-C	6.96E-02		
2PKCN$_{32}$-A	6.97E-02		
2PKCN$_{32}$-B	7.26E-02	6.88E-02	4.34E-03
2PKCN$_{32}$-C	6.40E-02		
2PLN$_{32}$-A	6.27E-02		
2PLN$_{32}$-B	6.88E-02	7.06E-02	8.87E-03
2PLN$_{32}$-C	8.02E-02		

Table A8.4. Dissolved organic carbon (DOC) concentrations for Experiment 3 in seawater.

Day 1	**DOC (mol C/L)**	**Average**	**STDEV (± 1σ)**
T0-1	2.43E-03		
T0-2	2.44E-03	2.46E-03	3.73E-05
T0-3	2.50E-03		
Day 28	**DOC (mol C/L)**	**Average**	**STDEV (± 1σ)**
3SLC-A	6.14E-02		
3SLC-B	5.66E-02	5.85E-02	2.55E-03
3SLC-C	5.74E-02		
3SKCN$_3$-A	5.89E-02		
3SKCN$_3$-B	8.26E-02	6.84E-02	1.25E-02
3SKCN$_3$-C	6.38E-02		
3SLN$_3$-A	5.40E-02		
3SLN$_3$-B	5.80E-02	5.75E-02	3.39E-03
3SLN$_3$-C	6.07E-02		

3SKCN$_2$-A	5.93E-02		
3SKCN$_2$-B	6.24E-02	6.18E-02	2.29E-03
3SKCN$_2$-C	6.38E-02		
3SLN$_2$-A	4.99E-02		
3SLN$_2$-B	5.80E-02	5.44E-02	4.09E-03
3SLN$_2$-C	5.52E-02		
Day 59	**DOC (mol C/L)**	**Average**	**STDEV (± 1σ)**
3SLC-A	5.53E-02		
3SLC-B	5.48E-02	5.71E-02	3.49E-03
3SLC-C	6.11E-02		
3SKCN$_3$-A	6.38E-02		
3SKCN$_3$-B	8.04E-02	7.12E-02	8.45E-03
3SKCN$_3$-C	6.93E-02		
3SLN$_3$-A	5.78E-02		
3SLN$_3$-B	5.08E-02	5.60E-02	4.65E-03
3SLN$_3$-C	5.95E-02		
3SKCN$_2$-A	6.03E-02		
3SKCN$_2$-B	6.03E-02	6.17E-02	2.32E-03
3SKCN$_2$-C	6.43E-02		
3SLN$_2$-A	5.01E-02		
3SLN$_2$-B	5.60E-02	5.41E-02	3.50E-03
3SLN$_2$-C	5.63E-02		

A9. Dissolved inorganic carbon data for the incubation experiments

Table A9.1. DIC concentrations for Experiment 2 in seawater.

Day 1	**DIC (mol C/L)**	**Average**	**STDEV (± 1σ)**
T0-1	4.82E-04		
T0-2	4.79E-04	4.67E-04	2.42E-05
T0-3	4.39E-04		
Day 51	**DIC (mol C/L)**	**Average**	**STDEV (± 1σ)**
2SLC-A	1.12E-02		
2SLC-B	1.02E-02	1.04E-02	7.37E-04
2SLC-C	9.82E-03		
$2SKCN_{32}$-A	2.07E-03		
$2SKCN_{32}$-B	2.10E-03	2.06E-03	4.29E-05
$2SKCN_{32}$-C	2.02E-03		
$2SLN_{32}$-A	8.13E-03		
$2SLN_{32}$-B	7.56E-03	7.74E-03	3.33E-04
$2SLN_{32}$-C	7.54E-03		
Day 97	**DIC (mol C/L)**	**Average**	**STDEV (± 1σ)**
2SLC-A	1.06E-02		
2SLC-B	1.09E-02	1.05E-02	4.76E-04
2SLC-C	1.00E-02		
$2SKCN_{32}$-A	1.97E-03		
$2SKCN_{32}$-B	1.97E-03	1.97E-03	2.92E-06
$2SKCN_{32}$-C	1.97E-03		
$2SLN_{32}$-A	9.69E-03		
$2SLN_{32}$-B	8.60E-03	9.03E-03	5.79E-04
$2SLN_{32}$-C	8.81E-03		

Table A9.2. DIC concentrations for Experiment 2 in produced water.

Day 1	**DIC (mol C/L)**	**Average**	**STDEV (± 1σ)**
T0-1	9.91E-03		
T0-2	8.78E-03	9.07E-03	7.35E-04
T0-3	8.53E-03		
Day 51	**DIC (mol C/L)**	**Average**	**STDEV (± 1σ)**
2PLC-A	6.32E-03		
2PLC-B	6.63E-03	6.49E-03	1.59E-04
2PLC-C	6.52E-03		
$2PKCN_{32}$-A	6.70E-03		
$2PKCN_{32}$-B	6.49E-03	6.64E-03	1.34E-04
$2PKCN_{32}$-C	6.73E-03		
$2PLN_{32}$-A	6.88E-03		
$2PLN_{32}$-B	6.56E-03	6.71E-03	1.63E-04
$2PLN_{32}$-C	6.68E-03		
Day 97	**DIC (mol C/L)**	**Average**	**STDEV (± 1σ)**
2PLC-A	5.87E-03		
2PLC-B	6.10E-03	6.02E-03	1.29E-04
2PLC-C	6.09E-03		
$2PKCN_{32}$-A	6.21E-03		
$2PKCN_{32}$-B	6.17E-03	6.27E-03	1.46E-04
$2PKCN_{32}$-C	6.44E-03		
$2PLN_{32}$-A	6.66E-03		
$2PLN_{32}$-B	6.55E-03	6.49E-03	2.13E-04
$2PLN_{32}$-C	6.25E-03		

Table A9.3. DIC concentrations for Experiment 3 in seawater.

Day 1	**DIC (mol C/L)**	**Average**	**STDEV (± 1σ)**
T0-1	1.79E-03		
T0-2	1.81E-03	1.80E-03	1.17E-05
T0-3	1.81E-03		
Day 28	**DIC (mol C/L)**	**Average**	**STDEV (± 1σ)**
3SLC-A	1.08E-02		
3SLC-B	1.21E-02	1.15E-02	6.79E-04
3SLC-C	1.17E-02		
$3SKCN_3$-A	1.88E-03		
$3SKCN_3$-B	1.86E-03	1.91E-03	7.09E-05
$3SKCN_3$-C	1.99E-03		
$3SLN_3$-A	1.13E-02		
$3SLN_3$-B	1.73E-02	1.41E-02	3.02E-03
$3SLN_3$-C	1.37E-02		
$3SKCN_2$-A	1.83E-03		
$3SKCN_2$-B	1.84E-03	1.83E-03	9.36E-06
$3SKCN_2$-C	1.82E-03		
$3SLN_2$-A	4.01E-03		
$3SLN_2$-B	4.14E-03	4.30E-03	3.95E-04
$3SLN_2$-C	4.75E-03		
Day 59	**DIC (mol C/L)**	**Average**	**STDEV (± 1σ)**
3SLC-A	1.02E-02		
3SLC-B	1.08E-02	1.05E-02	3.13E-04
3SLC-C	1.03E-02		
$3SKCN_3$-A	1.92E-03		
$3SKCN_3$-B	1.96E-03	1.94E-03	2.50E-05
$3SKCN_3$-C	1.92E-03		
$3SLN_3$-A	2.26E-02	2.06E-02	3.09E-03
$3SLN_3$-B	2.23E-02		

$3SLN_3$-C	1.71E-02		
$3SKCN_2$-A	1.91E-03		
$3SKCN_2$-B	1.84E-03	1.89E-03	4.17E-05
$3SKCN_2$-C	1.92E-03		
$3SLN_2$-A	9.45E-03		
$3SLN_2$-B	8.62E-03	9.21E-03	5.18E-04
$3SLN_2$-C	9.57E-03		

A10. Hydrogen potential data for the incubation experiments

Table A10.1. Hydrogen potential for Experiment 1 in seawater.

Day 1	**pH**	**Average**	**STDEV (± 1σ)**
Before	7.45		
During	7.40	7.45	0.05
After	7.50		
Day 22	**pH**	**Average**	**STDEV (± 1σ)**
1SLC-A	7.17		
1SLC-B	7.59	7.44	0.23
1SLC-C	7.55		
$1SKCN_{32}$-A	7.59		
$1SKCN_{32}$-B	7.61	7.59	0.02
$1SKCN_{32}$-C	7.58		
$1SLN_{32}$-A	7.55		
$1SLN_{32}$-B	7.53	7.54	0.01
$1SLN_{32}$-C	7.55		
Day 29	**pH**	**Average**	**STDEV (± 1σ)**
1SLC-A	7.60		
1SLC-B	7.41	7.50	0.10
1SLC-C	7.49		
$1SKCN_{32}$-A	7.59		
$1SKCN_{32}$-B	7.53	7.55	0.03
$1SKCN_{32}$-C	7.53		
$1SLN_{32}$-A	7.52		
$1SLN_{32}$-B	7.52	7.52	0.01
$1SLN_{32}$-C	7.51		
Day 45	**pH**	**Average**	**STDEV (± 1σ)**
1SLC-A	7.94		
1SLC-B	7.90	7.92	0.02
1SLC-C	7.91		

1SKCN$_{32}$-A	7.96		
1SKCN$_{32}$-B	7.91	7.94	0.03
1SKCN$_{32}$-C	7.94		
1SLN$_{32}$-A	7.20		
1SLN$_{32}$-B	8.20	7.78	0.52
1SLN$_{32}$-C	7.94		
Day 69	**pH**	**Average**	**STDEV (± 1σ)**
1SLC-A	7.56		
1SLC-B	7.59	7.57	0.02
1SLC-C	7.57		
1SKCN$_{32}$-A	7.61		
1SKCN$_{32}$-B	7.61	7.59	0.03
1SKCN$_{32}$-C	7.55		
1SLN$_{32}$-A	7.52		
1SLN$_{32}$-B	7.58	7.55	0.03
1SLN$_{32}$-C	7.56		
Day 109	**pH**	**Average**	**STDEV (± 1σ)**
1SLC-A	7.75		
1SLC-B	7.72	7.79	0.09
1SLC-C	7.89		
1SKCN$_{32}$-A	7.25		
1SKCN$_{32}$-B	7.64	7.46	0.20
1SKCN$_{32}$-C	7.48		
1SLN$_{32}$-A	8.09		
1SLN$_{32}$-B	8.22	8.16	0.07
1SLN$_{32}$-C	8.17		

Table A10.2. Hydrogen potential for Experiment 2 in seawater.

Day 1	**pH**	**Average**	**STDEV (± 1σ)**
Before	7.38		
During	7.45	7.15	0.46
After	6.62		
Day 37	**pH**	**Average**	**STDEV (± 1σ)**
2SLC-A	7.23		
2SLC-B	5.46	6.62	1.01
2SLC-C	7.18		
$2SKCN_{32}$-A	6.87		
$2SKCN_{32}$-B	7.57	6.84	0.74
$2SKCN_{32}$-C	6.09		
$2SLN_{32}$-A	8.29		
$2SLN_{32}$-B	7.33	8.01	0.60
$2SLN_{32}$-C	8.42		
Day 51	**pH**	**Average**	**STDEV (± 1σ)**
2SLC-A	7.55		
2SLC-B	7.65	7.55	0.11
2SLC-C	7.44		
$2SKCN_{32}$-A	7.74		
$2SKCN_{32}$-B	6.09	7.26	1.02
$2SKCN_{32}$-C	7.94		
$2SLN_{32}$-A	8.31		
$2SLN_{32}$-B	8.35	7.69	1.11
$2SLN_{32}$-C	6.41		
Day 97	**pH**	**Average**	**STDEV (± 1σ)**
2SLC-A	7.03	7.26	0.20
2SLC-B	7.32		

2SLC-C	7.42		
2SKCN$_{32}$-A	7.80		
2SKCN$_{32}$-B	7.78	7.78	0.02
2SKCN$_{32}$-C	7.76		
2SLN$_{32}$-A	7.90		
2SLN$_{32}$-B	6.28	7.22	0.84
2SLN$_{32}$-C	7.47		

Table A11.3. Hydrogen potential for Experiment 2 in produced water.

Day 1	**pH**	**Average**	**STDEV (± 1σ)**
Before	7.18		
During	6.89	7.16	0.26
After	7.41		
Day 37	**pH**	**Average**	**STDEV (± 1σ)**
2PLC-A	6.34		
2PLC-B	6.84	6.71	0.33
2PLC-C	6.96		
2PKCN$_{32}$-A	7.05		
2PKCN$_{32}$-B	6.99	7.00	0.05
2PKCN$_{32}$-C	6.96		
2PLN$_{32}$-A	7.22		
2PLN$_{32}$-B	6.80	7.04	0.22
2PLN$_{32}$-C	7.10		
Day 51	**pH**	**Average**	**STDEV (± 1σ)**
2PLC-A	7.86		
2PLC-B	7.68	7.78	0.09
2PLC-C	7.80		
2PKCN$_{32}$-A	8.01		
2PKCN$_{32}$-B	6.40	7.43	0.89
2PKCN$_{32}$-C	7.88		

2PLN$_{32}$-A	8.01		
2PLN$_{32}$-B	8.00	8.02	0.03
2PLN$_{32}$-C	8.05		
Day 97	**pH**	**Average**	**STDEV (± 1σ)**
2PLC-A	7.28		
2PLC-B	5.62	6.68	0.92
2PLC-C	7.13		
2PKCN$_{32}$-A	7.53		
2PKCN$_{32}$-B	7.37	7.09	0.63
2PKCN$_{32}$-C	6.37		
2PLN$_{32}$-A	7.31		
2PLN$_{32}$-B	7.33	7.34	0.03
2PLN$_{32}$-C	7.37		

Table A10.4. Hydrogen potential for Experiment 3 in seawater.

Day 1	**pH**	**Average**	**STDEV (± 1σ)**
Before	6.71		
During	6.94	6.94	0.24
After	7.18		
Day 28	**pH**	**Average**	**STDEV (± 1σ)**
3SLC-A	7.40		
3SLC-B	7.30	7.45	0.17
3SLC-C	7.64		
3SKCN$_3$-A	7.73		
3SKCN$_3$-B	7.71	7.72	0.01
3SKCN$_3$-C	7.73		
3SLN$_3$-A	7.21		
3SLN$_3$-B	7.80	7.55	0.30
3SLN$_3$-C	7.63		
3SKCN$_2$-A	7.45	7.26	0.52

3SKCN$_2$-B	7.66		
3SKCN$_2$-C	6.68		
3SLN$_2$-A	8.23		
3SLN$_2$-B	7.68	7.99	0.28
3SLN$_2$-C	8.05		
Day 59	**pH**	**Average**	**STDEV (± 1σ)**
3SLC-A	7.48		
3SLC-B	7.51	7.50	0.02
3SLC-C	7.50		
3SKCN$_3$-A	7.56		
3SKCN$_3$-B	7.51	7.54	0.03
3SKCN$_3$-C	7.56		
3SLN$_3$-A	7.76		
3SLN$_3$-B	7.89	7.81	0.07
3SLN$_3$-C	7.77		
3SKCN$_2$-A	7.57		
3SKCN$_2$-B	7.53	7.45	0.18
3SKCN$_2$-C	7.24		
3SLN$_2$-A	8.20		
3SLN$_2$-B	8.45	8.33	0.13
3SLN$_2$-C	8.34		

Appendix B: Photographs of Incubation Experiments

B1. Experiment 1: Nitrate and Nitrite incubations in seawater

Figure B1.1. Incubation bottles for Experiment 1 at time zero. The experiment had three treatments including Live Control (1SLC, seen in the back row), Killed Control with nitrate and nitrite addition (1SKCN$_{32}$, seen in the front row), and Experimental Live treatment nitrate and nitrite addition (1SLN$_{32}$, seen in the middle row). The experiments were constructed in triplicates totalling nine incubation bottles. As seen above, all incubations were clear, colorless and transparent at the time of construction.

B2. Experiment 2: Nitrate & nitrite incubation in seawater and produced water

Figure B2.1. Incubation bottles for Experiment 2 at time zero. The experiment had 6 treatments and included seawater and produced water. Treatments consisted of a Live Control with seawater (2SLC, seen in last row), Killed Control with nitrate & nitrite addition in seawater (2SKCN32, not photographed), Live Experimental treatment with nitrate & nitrite addition in seawater (2SLN32, not photographed), Live Control with produced waters (2PLC, second row from the back), a Killed Control with nitrate and nitrite addition in produced water (2PKCN32, first row) and a Live Experimental

treatment with nitrate & nitrite addition in produced water (2PLN32, second row from the front). All experiments were constructed in triplicates with 18 bottles in total. The seawater incubations were clear, colorless and translucent, similarly to Experiment 1 seawater incubations. The produced water incubations were yellow in color, had small orange “floaty bits”, and were translucent (i.e. more cloudy than the seawater incubations).

Figure B2.2. Experiment 2 Live Control treatments with seawater (2SLC) photographed on day 52. Drager Pac 3500 used for scale. Distinguished black biomass and/or precipitate evident in the Live Control incubations with seawater.

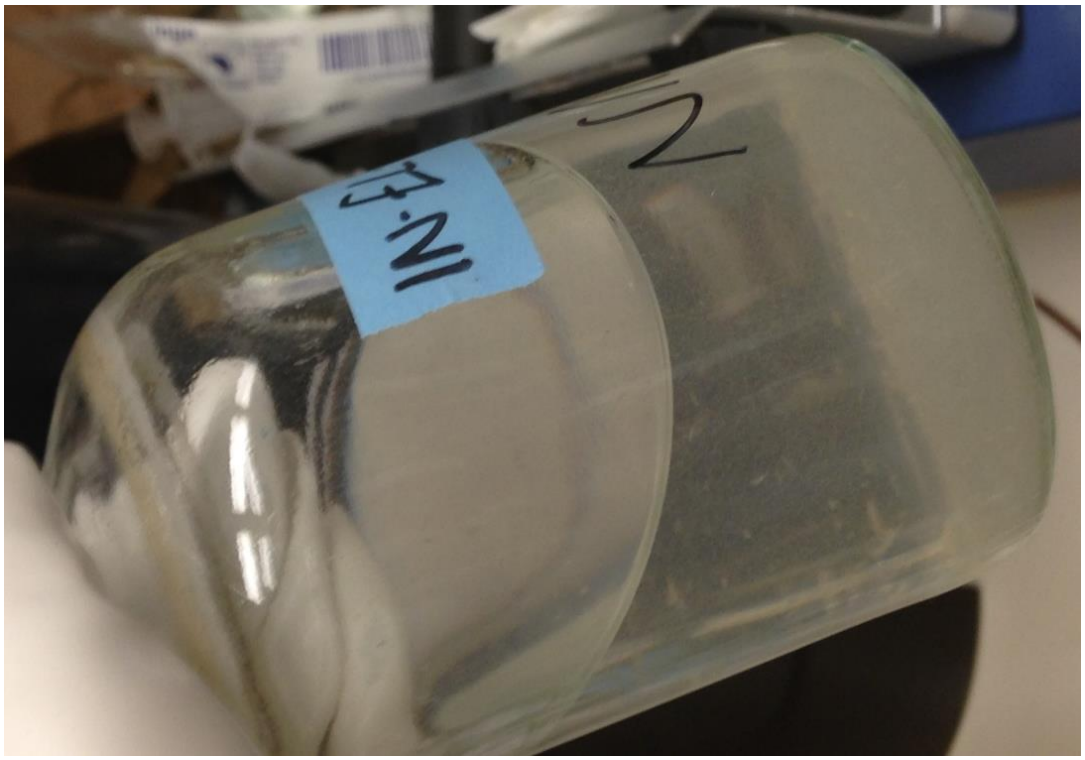

Figure B2.3. Experiment 2 Live Experimental treatment with nitrate & nitrite addition in seawater ($2SLN_{32}$) photographed on day 52. The Live Experimental treatments consisted of nitrate and nitrite addition at 0.00161 mol/L and 0.02174 mol/L respectively. White "stringy" biomass and-or precipitate can be seen visually. The water is translucent, clear in color, and slightly clouded.

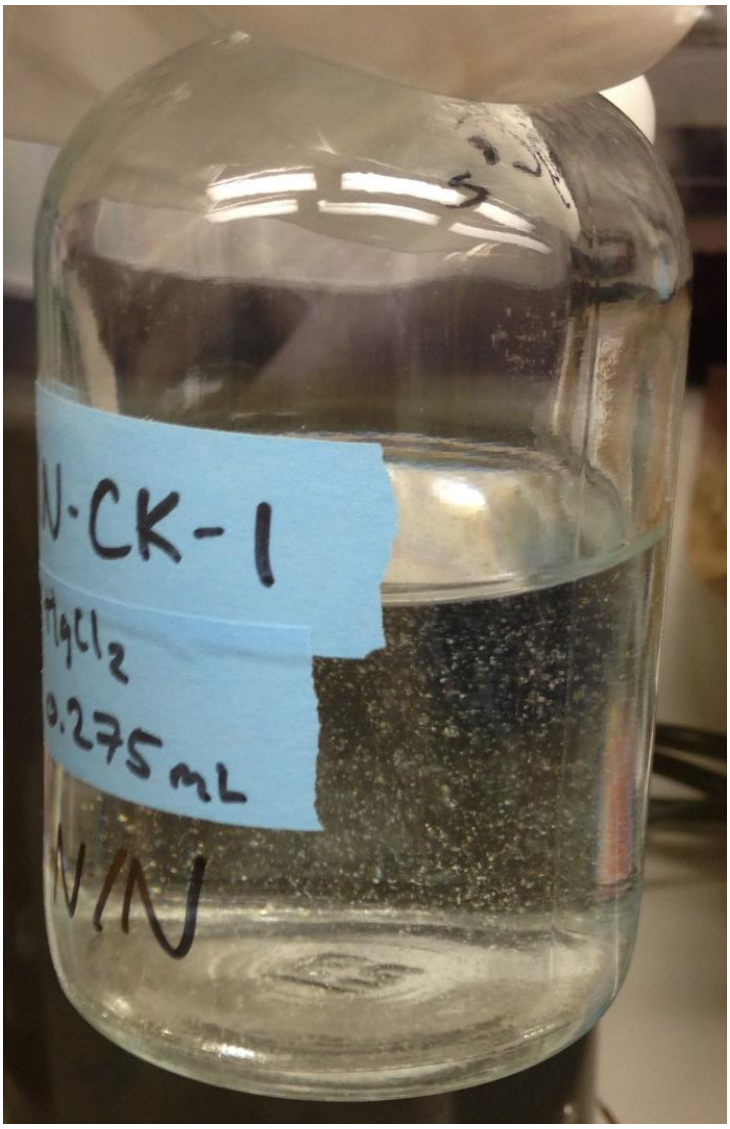

Figure B2.4. Experiment 2 Killed Control ($2SKCN_{32}$) treatment with nitrate & nitrite addition in seawater. The Killed Control treatment consisted of nitrate and nitrite addition at 0.00161 mol/L and 0.02174 mol/L respectively and contained 2.5 mL of 0.0276 mol/L $HgCl_2$. The liquid is clear and colorless with small circular precipitate. This is consistent with other observations in the laboratory where incubations with $HgCl_2$ addition often show white bits of precipitate.

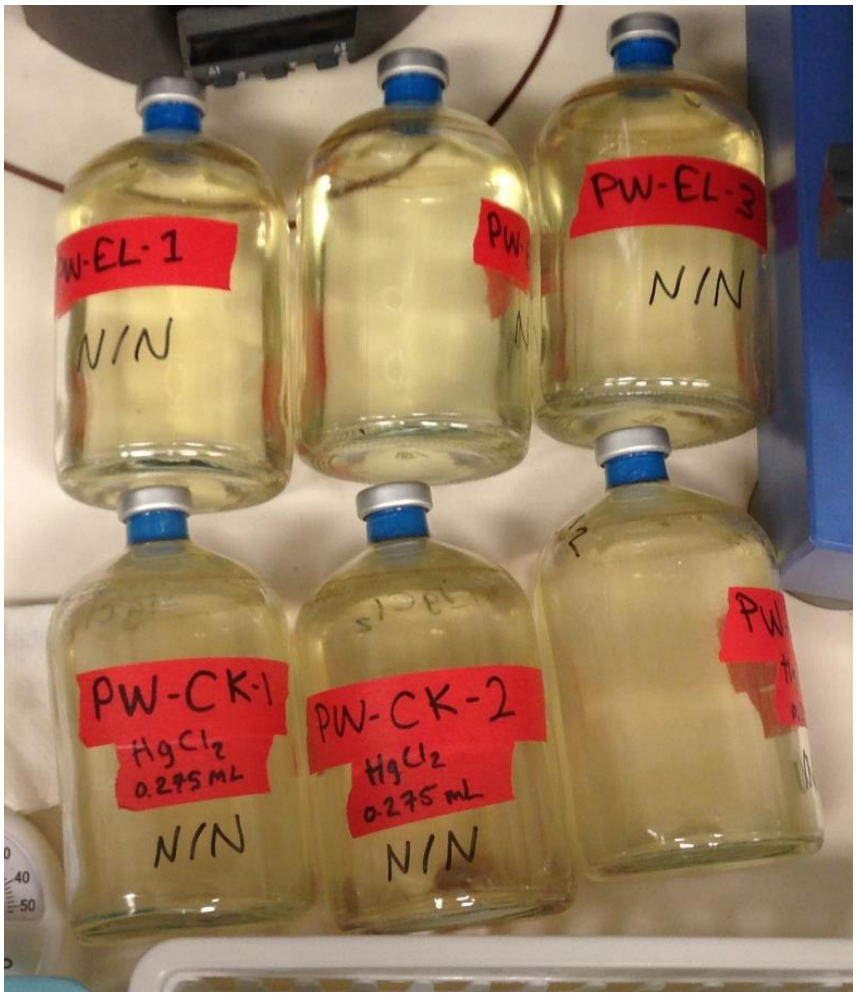

Figure B2.5. Experiment 2 Experimental Live ($2PLN_{32}$, seen on top row) and Killed Control ($2PKCN_{32}$, seen on bottom row) treatments with produced waters photographed on day 97. Both treatments for the duration of the experiment remained yellow in color. There is no visual biomass present. In the Killed Control treatment there was a small amount of white precipitate present, similarly to the Killed Control treatments with seawater in Figure B2.4.

Figure B2.6. Experiment 2 Live Control (2PLC) treatments in produced waters photographed on day 97. The incubations are clear and colorless. The yellow coloring of the waters that was present on day 1 is no longer present. There is a very small orange ring present at the water's surface. No biomass or precipitate was visually evident.

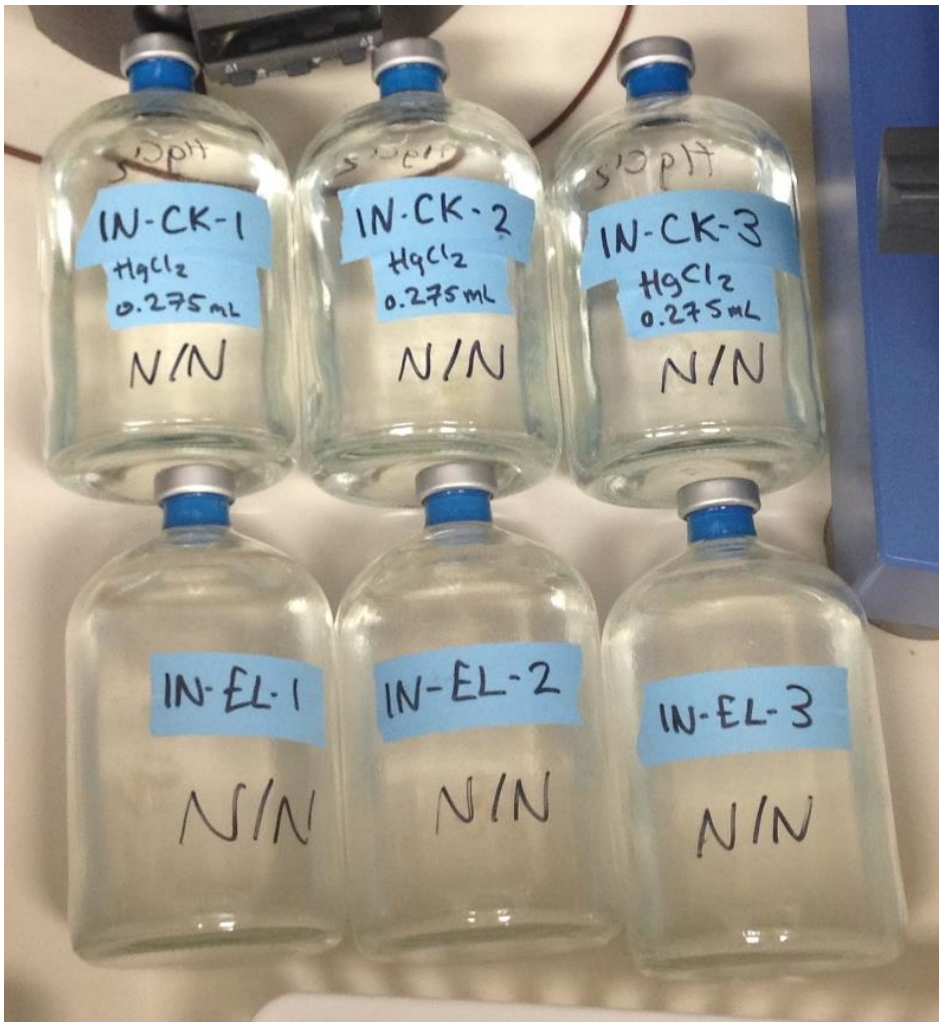

Figure B2.7. Experiment 2 Control Killed ($2SKCN_{32}$, top row) and Experimental Live ($2SLN_{32}$, bottom row) incubations with seawater photographed on day 97. Both treatments are clear and mostly colorless. The Control Killed incubations had a small white precipitate present that is typical of incubations "killed" with $HgCl_2$. The Experimental Live treatments (although not obvious in this photograph) had off white to yellow colored stringy/globular biomass present.

Figure B2.8. Experiment 2 Live Control (2SLC) treatments with seawater photographed on day 97. The Live Control treatments had black biomass present. One of the incubation bottles had a very large round/globular piece of biomass and the other two incubations had small round pieces of biomass.

B3: Experiment 3: Nitrate/Nitrite incubation in seawater

Figure B3.1. Experiment 3 incubation treatments with seawater. This experiment had 5 treatments in seawater. Treatments included a Live Control (3SLC), a Killed Control with nitrate addition ($3SKCN_3$), a Live Experimental treatment with nitrate addition ($3SLN_3$), a Killed Control nitrite addition ($3SKCN_2$) and a Live Experimental treatment with nitrite addition ($3SLN_2$). All experiments were constructed in triplicates with 15 bottles in total. With the exception of the addition of only nitrate or nitrite, all other experimental conditions remained the same as the seawater treatments in Experiment 2. The incubations on day 1 were clear, colorless and transparent with no particulate matter.

Figure B3.2. Experiment 3 Killed Control ($3SKCN_3$) treatments with nitrate addition photographed on day 59. The treatments had additions of 0.02174 mol/L of nitrate and 0.0276 mol/L $HgCl_2$. Small white precipitate was present in these incubations. Incubation water was clear and colorless.

Figure B3.3. Experiment 3 Killed Control ($3SKCN_2$) treatment with seawater and nitrite addition photographed on day 59. The treatments had additions of 0.02174 mol/L of nitrite and 0.0276 mol/L $HgCl_2$. Small white precipitate was present in these incubations. Incubation water was clear and colorless.

Figure B3.4. Experiment 3 Live Experimental ($3SLN_3$) treatment with nitrate addition photographed on Day 59. The Live Experimental treatments were amended with 0.02174 mol/L of nitrate. White-yellow stringy pieces of biomass can be seen floating in these incubations. The incubation water remains clear and colorless with the exception of the yellow tinted biomass.

Figure B3.5. Experiment 3 Live Experimental ($3SLN_2$) treatments with nitrite addition photographed on Day 59. The Live Experimental treatments were amended with 0.02174 mol/L of nitrite. Beige biomass was present in these incubations and can be seen settled onto the bottom of the serum bottle. The incubation water is translucent and slightly yellow in color due to biomass and particulate matter.

Figure B3.6. Experiment 3 Live Control (3SLC) treatments photographed on Day 59. Large globular black clumps of biomass were very distinguishable in these incubations, along with some smaller particulate matter that is settled onto the bottom of the serum bottles.

Figure B3.7. Experiment 3 Live Control (3SLC) treatments photographed on Day 59. Both of these serum bottles are the Live Control incubations of experiment 3, although they are labelled differently. By comparison, these two photographs show that some of the biomass clumps are shaped differently among the serum bottles, however the characteristic black “clumps” and smaller bits of settled particular matter remains constant in all Live Control treatments.