Effect of organic carbon substrates on denitrification rates in sediment

by

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

Nitrate (NO_3^-) is a ubiquitous groundwater contaminant in agricultural and wastewater discharge areas. The prediction of microbial mediated NO_3^- removal in subsurface environments requires an understanding of the rates at which electron donors are utilized by denitrifying microbes. This study focuses specifically on the following organic carbon compounds as electron donors: glucose, acetate, adenine, cysteine and fulvic acid. Six triplicate series of flow through reactors (FTRs) containing 35 cm^3 of natural, organic-poor sediment were supplied for 10 weeks with solutions containing nitrate and the individual carbon compounds, along with a no-carbon added control. The organic carbon compounds were selected to yield a range of different types of organic carbon (sugars, amino acids etc.) as well as a range of Gibbs Free Energy (ΔG) values when their oxidation is coupled to denitrification. The initial flow rate of the FTRs was 1 ml h^{-1}. Once steady NO_3^- concentrations were reached in the outflow, the flow rate was increased to 2 ml h^{-1} and, subsequently, 4 ml h^{-1}. Potential denitrification rates (R_D) measured for the different carbon substrates spanned a range of 0 to 114 nmol cm^{-3} h^{-1}. Fulvic acid did not induce denitrification, while acetate yielded the highest rate. The outflow solutions for FTRs supplied with adenine and cysteine contained ammonia and sulfate, respectively. These results are consistent with the molecular structure of adenine, which contains an amine group, and of cysteine, containing an amine and thiol group. The results show that the addition of C-substrates to the sediment promotes denitrification, and the rate at which it occurs are dependant on which C-substrate is provided. R_D results were used to determine if the denitrification rates imposed by the different carbon substrates could be predicted using theoretical approaches such as ΔG_R or the nominal oxidation state of carbon (NOSC). However, predictions determined by thermodynamics alone were not significantly correlated with the observed trends in denitrification rates.

Acknowledgements

This thesis would not be possible without the help of many different people. First, I would like to thank the University of Waterloo and the CERC program for making this thesis possible. Second, I would like to thank my supervisors, Raoul-Marie Couture and Philippe Van Cappellen, for their continuing support and guidance. Being a supervisor is very time consuming, so thank you for all those repetitive conversations and long hours dedicated to helping me get through this. Third, I would also like to thank Fereidoun Rezanezhad for all of his help the lab and for his continual support educationally and motivationally. I would like to thank lab technician Marianne VanderGriendt, lab manager Kristin Muller and all of the co-op students that helped me to collect and run my samples. I would also like to thank Douglas LaRowe for his feedback during the experimental planning. Thank you as well to Radmila Kovac for hours spent doing most probable number counts as well. This thesis would not have been successful without all of you. I would also like to thank Sara Coyotzi from the UW Biology department for helping me run all of the lab experiment and for running the gas analyses. Without your help and dedication in the lab, our 24 "babies" wouldn't have made through the first week. Of course, I would like to thank the people who came in on weekends to help me sample and run analyses. The free time you gave up to assist me is appreciated more than you know. Last but not least, I would like to thank my family and my husband. I will always remember how your love, support and encouragement got me through grad school. Thank you everyone.

Dedication

I would like to dedicate this thesis to the people who have helped and supported me most, my parents. I love you both very much.

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Chapter 1
Introduction

Nitrogen (N) is an essential nutrient for all life on Earth. Globally, the nitrogen cycle (Figure 1.1) has been greatly influence by anthropogenic activity including agriculture, mining, industry and wastewater (Galloway et al., 1995; Tu et al., 2013). Atmospheric deposition of reactive forms of N, including N_2O, NO_x, NH_3, and inputs directly into surface and groundwater systems has greatly increased due to these various anthropogenic activities and caused an imbalance of N in the environment (Tu et al., 2013; Meybeck, 1982). Accumulation of N in ecosystems usually leads to the build of the inorganic form of N, nitrate (NO_3^-).

NO_3^- is a ubiquitous groundwater contaminant, especially in agricultural, mining, industrial and wastewater discharge areas (Xue et al., 2009). Due to its stable and negative form, NO_3^- is very mobile in the subsurface. Groundwater nitrate plumes are difficult to remediate and often rely on denitrification in groundwater aquifers and discharge areas. Discharge zones can include lakes, reservoirs and rivers in inland areas, making discharge zones of these water bodies essential to denitrification. Denitrification is one of the main ways NO_3^- is removed from a system and requires denitrifying bacteria and low oxygen levels to be sucessful. While complete denitrifcation converts NO_3^- into inert N_2 gas, incomplete denitrifcation can produce intermediate products including nitrite (NO_2^-), nitrous oxide (N_2O) and nitric oxide (NO). NO_3^- can also be removed through anammox, DNRA and assimilatory plant and microbial uptake (Wu, 2010).

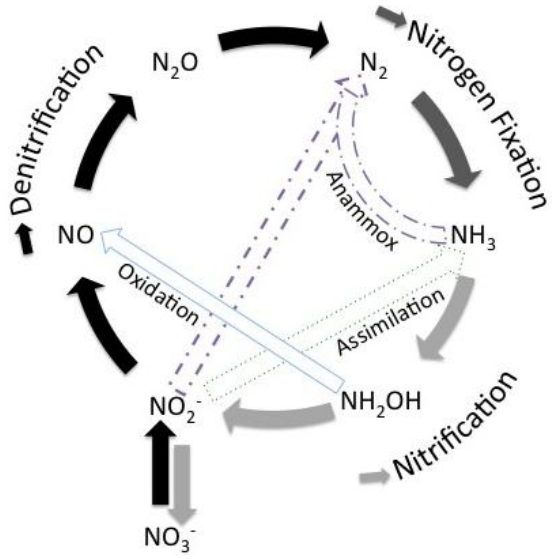


Figure 1.1: The nitrogen cycle: a summary of pathways for formation and breakdown of different N species in the environment (Redrawn from Stein and Yung, 2003).

The drinking water limit in Canada for NO_3^- is 10 mg N/L (0.7 mM) and the guideline for the protection of aquatic life is 2.9 mg N/L (0.2 mM) according to the Canadian Water Quality Guidelines (CWQG) (CCME, 2009). Although nitrate is not immediately toxic to humans or ecosystems, a build up over time can lead to serious health problems and environmental concerns. In humans, high doses can lead to serious illness in babies called methemoglobinemia or better known as "blue baby syndrome." Over time, high doses can also lead to stomach cancers in adults (Almasri and Kaluarachchi, 2004). In aquatic ecosystems facing nitrate contamination offspring of fish and amphibians present with stunted offspring and laziness, leading to lower rates of survival (CCME, 2009; Camargo et al., 2005).

Due to the importance of denitrification, there are a lot of studies on denitrification rates (Table 1.1). The studies range from simple batch experiments to full-scale field experiments, generally producing very different estimations of nitrate removal rates depending on the experiment and sediment type. The purpose of this literature review is to point out that many gaps still remain in the work that has been done on denitrification, especially when it comes to predicting rates using various electron donor substrates.

There are *in situ* field studies that have been done to look at natural denitrification rates (Blackburn and Oren, 1979; Peter et al., 2012; Joye et al., 1996; Garcia-Ruiz et al., 1998;

Laverman et al., 2011; Canavan et al., 2006) and studies that only look at potential denitrification rates for a specific site (Pfenning and McMahon, 1996; Wu, 2010; Akunna et al., 1993; Elefsiniotis and li, 2006; Calderer et al., 2010). Most of the studies focused on *in situ* denitrification rates look at how efficient the system is at removing NO_3^- without applying remediation techniques. Other studies (Pfenning and McMahon, 1996; Paul et al., 1989; Jahangir et al., 2012) focus on the capacity of a system to remove NO_3^- with the aid of remediation techniques such as adding external carbon (C) substrates. Although these studies are extremely important to help understand denitrification, certain aspects are missing for a more complete picture. A study done by Oren and Blackburn (1979) they stated that there were a few problems with reports on denitrification rates. These problems included high nitrate concentrations and long microbial incubation periods, which produce unrealistic conditions and therefore overestimates of rates. Although this study was done in 1979, upon searching the literature, this statement appears to still hold true. Experiments using flow columns, batch tests or *in situ* cores mostly use concentrations of NO_3^- much higher than typically found in natural waters. The high NO_3^- concentrations are usually used in order to prevent any NO_3^-limitations in the experiments so that maximum rates can be derived. Long incubations are often required to reach steady state conditions, which are needed in order to calculate denitrification rates.

Table 1.1: Overview of potential denitrification rates (R_D) and the NO_3^- concentration used in the experimental studies.

R_D nmol cm^{-3} h^{-1}	NO_3^- mM	Experiment Duration h	Material	Method	C-substrate Added	Reference
44.2	7.2	192-672	Local Sediment	Batch/Column/Field	Acetate	Abdelouas et al., 1999
793.8	14.3	100	Anaerobic sludge	Batch experiment	Glucose	Akunna et al., 1993
259.6	14.3	100	Anaerobic sludge	Batch experiment	Acetate	Akunna et al., 1993
n.d.	14.3	100	Anaerobic sludge	Batch experiment	Methanol	Akunna et al., 1993
110–130	14.3	2	Streamside soil	Slurries	Glucose	Ambus (1993)
3.8	1.9-2.3*	240	Subsurface Soil	Batch experiment	Acetate	Calderer et al., 2010
4.5	1.9-2.3*	240	Subsurface Soil	Batch experiment	Acetate + Glucose	Calderer et al., 2010
3.1	1.9-2.3*	240	Subsurface Soil	Batch experiment	Glucose	Calderer et al., 2010
2.7	1.9-2.3*	240	Subsurface Soil	Batch experiment	Glucose	Calderer et al., 2010
6.3	1.9-2.3*	240	Subsurface Soil	Batch experiment	Glucose	Calderer et al., 2010
60.5	1.9-2.3*	240	Subsurface Soil	Batch experiment	Glucose	Calderer et al., 2010
89.4	0.15	N/A	Lake Sediment	FTR	--	Canavan et al. (2006)
23.8	50	400	Sludge from WWTP†	Batch experiment	Acetate	Elefsiniotis and Li, 2006
23.8	50	400	Sludge from WWTP	Batch experiment	Acetate	Elefsiniotis and Li, 2006
29.6	100	400	Sludge from WWTP	Batch experiment	Acetate	Elefsiniotis and Li, 2006
29.6	100	400	Sludge from WWTP	Batch experiment	Acetate	Elefsiniotis and Li, 2006
42.5	200	400	Sludge from WWTP	Batch experiment	Acetate	Elefsiniotis and Li, 2006
42.5	200	400	Sludge from WWTP	Batch experiment	Acetate	Elefsiniotis and Li, 2006
0.4–119.4*	0-11.4	24	River sediment	Intact cores	Fresh water medium	Garcia-Ruiz et al. (1998)
300–1500	0-0.064	15	Lake sediment	Slurries	--	Hordijk et al. (1987)
1.8-5.4	6.4**	408	Grazed grassland	Intact core, incubated	Glucose	Jahangir et al. (2012)
1.6-4.0	6.4**	408	Grazed grassland	Intact core, incubated	DOC	Jahangir et al. (2012)
1–8	0.1-1	0.75	Subtidal Sediment	Intact cores	--	Joye et al. (1996)
274–933	1-10	~260	Intertidal Sediment	FTR	Acetate	Laverman et al. (2006)
662–2400	1-10	Unknown	Intertidal Sediment	Slurries	Acetate	Laverman et al. (2006)
100–325	1-10	~260	Intertidal Sediment	FTR	Acetate	Laverman et al. (2006)

98–155	1-10	~260	Subtidal Sediment	FTR	Acetate	Laverman et al. (2006)
179-233	5	~200	River Sediment	FTR	--	Laverman et al. (2011)
8.6-58.5	0.4	144	Riparian buffer zone soil	Packed Flow Column	Citric Acid	Lin Wu MSc 2010
2.8	0.4	144	Riparian buffer zone soil	Packed Flow Column	Alginic Acid	Lin Wu MSc 2010
n.d.	0.4	144	Riparian buffer zone soil	Packed Flow Column	Suwannee River DOC (1R101N)	Lin Wu MSc 2010
89	0.02-0.8	3-14	Lake sediment	Slurries	--	Messer & Brezonik (1983)
30.3	2.1	600	Subsurface Soil	Batch experiment	Acetate	Oa et al., 2006
151.5	2.1	600	Mountain soils	Batch experiment	Fumerate	Oa et al., 2006
53.0	2.1	600	Mountain soils	Batch experiment	Formate	Oa et al., 2006
49.2	2.1	600	Mountain soils	Batch experiment	Lactate	Oa et al., 2006
18.9	2.1	600	Mountain soils	Batch experiment	Propionate	Oa et al., 2006
3.8	2.1	600	Mountain soils	Batch experiment	Ethanol	Oa et al., 2006
37.9	2.1	600	Mountain soils	Batch experiment	Methanol	Oa et al., 2006
56.8	2.1	600	Mountain soils	Batch experiment	Hydrogen	Oa et al., 2006
0.16-0.24*	0-1	4-6	Intertidal Sediment	Slurries	--	Oremland et al. (1984)
18	0.05-0.5	96	Subtidal Sediment	Slurries	--	Oren & Blackburn (1979)
n.d.	35.2	72-168	Desert soil	Slurries	Dextrose	Peterjohn (1991)
7.9**	0.3-3	0-3	River bed sediment	Batch experiment	Acetate	Pfenning and McMahon (1996)
5.4**	0.4-2.1	N/A	River bed sediment	Batch experiment	Fulvic Acid (groundwater)	Pfenning and McMahon (1996)
6.0**	0.4-2.1	N/A	River bed sediment	Batch experiment	Fulvic Acid (suface water)	Pfenning and McMahon (1996)
12–25	0.3-3	0-3	Coastal marine	Cores and Slurries	Natural Sea Water	Raymond et al. (1992)
892.5	0-21	840	Methanogenic Culture	Batch experiment	Acetate	Tugtas and Pavlostathis, 2007
255	0-21	840	Methanogenic Culture	Batch experiment	Glucose	Tugtas and Pavlostathis, 2007

*mmol dm^{-3} **mmol kg^{-1} †Waste Water Treatment Plant

With so much variation in rates for published denitrification studies it is hard to assign, *a priori*, denitrification rates for a new study site. Factors including soil heterogeneity, microbial populations, carbon and nitrate concentrations, pH, redox conditions, soil moisture and temperature all play an important role in controlling denitrification rates (Pilhatie et al., 2004). There are many different types of models that attempt to use thermodynamics and kinetics to predict if a reaction is favourable and how fast it can occur. Most studies listed in Table 1.1 are solely focused on maximum potential rates of denitrification and do not consider the source of organic matter they are using. Microbial communities are highly affected by the type of organic matter present since not all C-substrates produce the same amount of energy or the same byproducts (Hunter et al., 1998; Schrenk et al., 2010; Hedges and Oades, 1997; Berner, 1980). In terms of the contaminant NO_3^-, it is important to study the effects of different types of organic matter on denitrification rates. Previous efforts have been made to remediate NO_3^- contaminated areas by adding external C-substrates to the subsurface to enhance microbial activity with varying success (King et al., 2012). Although carbon substrates are only one aspect of a very complicated system, a better understanding how C-substrates impact denitrification rates could lead to better remediation techniques.

A study by LaRowe and Van Cappellen (2011), attempted to find a general correlation amongst available thermodynamic data on C-substrates that currently exist. Although this study is not focused on denitrification, the trends that are proposed between C-substrates and the Gibbs free energy yield, ΔG, of C oxidation (ΔG_{Cox}) using the nominal oxidation state of C (NOSC) could potentially be applied to estimating denitrification rates.

NOSC for a given molecule is calculated as follows:

$$NOSC = -((-Z+4C+H-3N-2O+5P-2S)/C)+4 \qquad [1.1]$$

Where Z represents the charge of the compound and C, H, N, O, P and S represent the number of atoms of each element in the compound (carbon, hydrogen, nitrogen, oxygen, phosphorus and sulfur, respectively). In the paper, a negative trend is found between ΔG_{Cox} and NOSC indicating that the higher the NOSC, the more thermodynamically favourable a C-substrate is to be oxidized by microbes. It has not been investigated as to whether this trend hold true and what the effects of

different terminal electron acceptors (TEAs), such as NO_3^-, are. If NOSC can determine the more thermodynamically favourable terminal electron donor (TED) then it could potentially be used to predict denitrification rates based on C-substrate addition at a study site. The higher the NOSC, the more thermodynamically favourable and therefore the higher the rate of denitrification should be.

It is difficult to conduct a study that is not site specific due to the large heterogeneities in microbial communities, sub-surface environments, C-substrates, and groundwater composition in the natural environment. For these reasons, it is important to systematically investigate, at a given site, the effect of specific C substrates on denitrification rates. This work is done in the context of the current effort, in the field of biogeochemistry, to find tools to predict denitrification rates in complex sub-surface environment. Of course, this model would also have to take into account other important denitrification rate controlling factors (e.g. temp, pH, redox, etc.). Our goal is that the information presented in this thesis will contribute towards better predictive tools and remediation applications for denitrification in the sub-surface.

Consequently, the main objectives of this work are to:

1. Measure denitrification rates in intact sediments amended with a range of carbon substrates to determine the effects of different carbon substrates on denitrification rates.
2. Compare these rates with existing predictors of microbial activity, namely Gibbs free energy (ΔG) and the nominal carbon oxidation state (NOSC).

Chapter 2
Methods

2.1 Site Description

Lake Belwood (latitude: 43^{o} 43’ 56” N; Longitude: 80^{o} 19’ 54” W) is a man made reservoir just outside of Fergus, Ontario (Figure 2.1). Lake Belwood is part of the Grand River Watershed (GRW) and is impounded by the Shand Dam, which controls the water flow to the Grand River downstream and acts as a water resource for the communities in times of drought (GRCA, 2013). The reservoir is also used for recreation such as boating, swimming, fishing and cottages. Experimental studies are also conducted on the reservoir by universities and conservation authorities on water quality, geochemistry and ecosystems (Guildford, 2006; Duthie, 2011; Hamish et el., 2011; Mason, 1977; Yakobowski, 2008).

Where the samples were collected, the sediment of Lake Belwood is composed of sandy clay with some seams of organic material present. Samples were collected approximately 15m offshore in March of 2012 under approximately 0.75m of water.

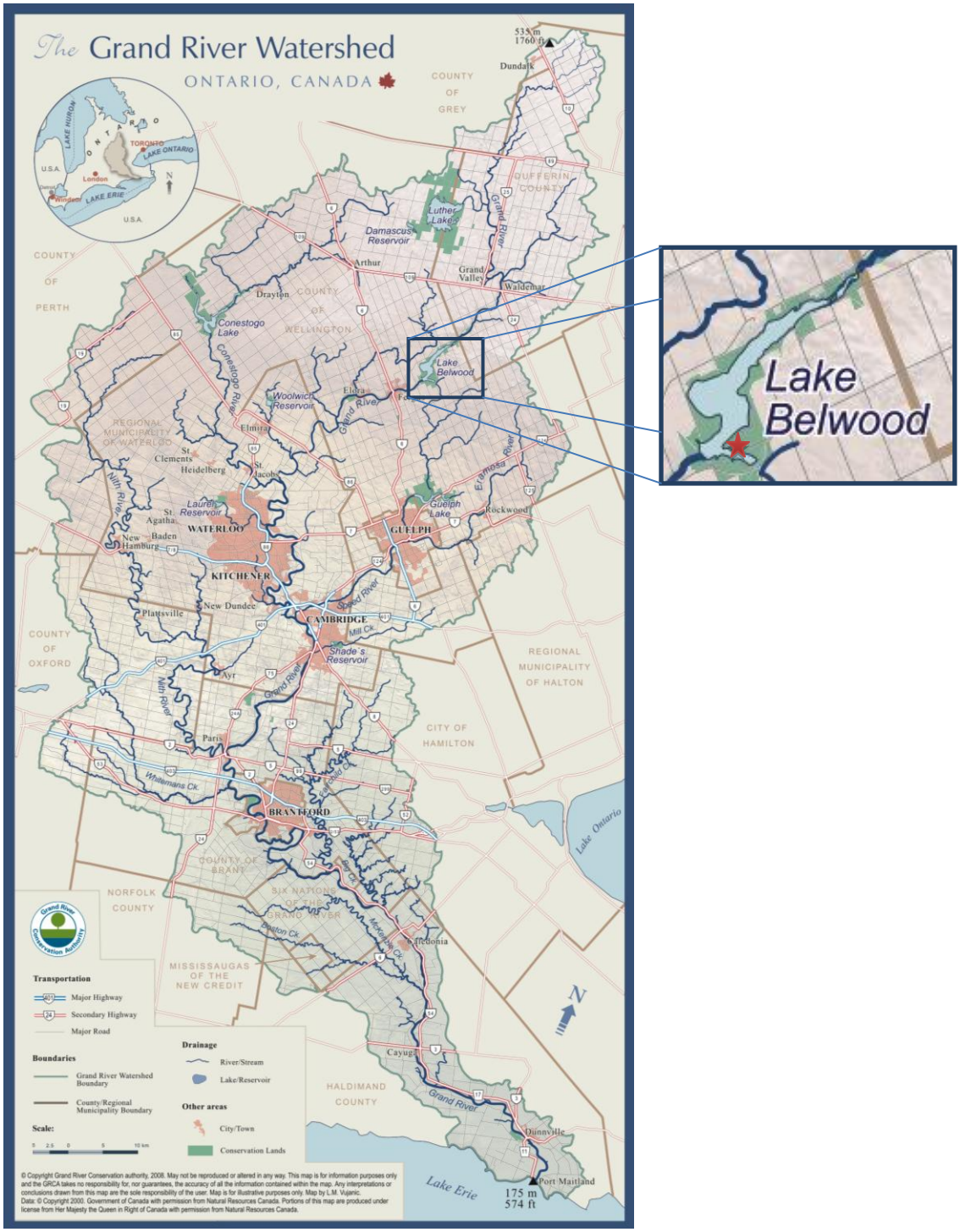


Figure 2.1: A map of the Grand River Watershed, Ontario, Canada, with Lake Belwood enlarged (GRCA, 2013). The star denotes the location of the sampling site.

The sediments of Lake Belwood sampled have low organic C content, up to 0.6% by dry weight (Chapter 3, Section 3.4.3). Low C content is important since this experiment involves the addition of external C-substrates to determine maximum potential denitrification rates of natural microbe populations in the presence of different OM. At certain times of the year, some of the reservoir bed is exposed to the atmosphere and at other times of the year it is covered by water. This causes the sediment to undergo seasonal changes in redox conditions, hence forcing the microbial community to adapt frequently to new environmental conditions.

Originally, Laurel Creek was considered as a test site for this experiment. Laurel creek runs through the University of Waterloo (UW) campus. This site was convenient and has a lot of previous data from other experiments conducted over the years through various UW departments. Laurel Creek was ruled out as a potential site for subsequent work when a preliminary experiment showed that the natural C content in the sediment was too high to produce any significant difference in denitrification rates when an external C-substrate was added. Figure 2.2 displays the difference in the calculated denitrification rates between sediment from Lake Belwood and Laurel creek, before and after the addition of an external carbon substrate (acetate).

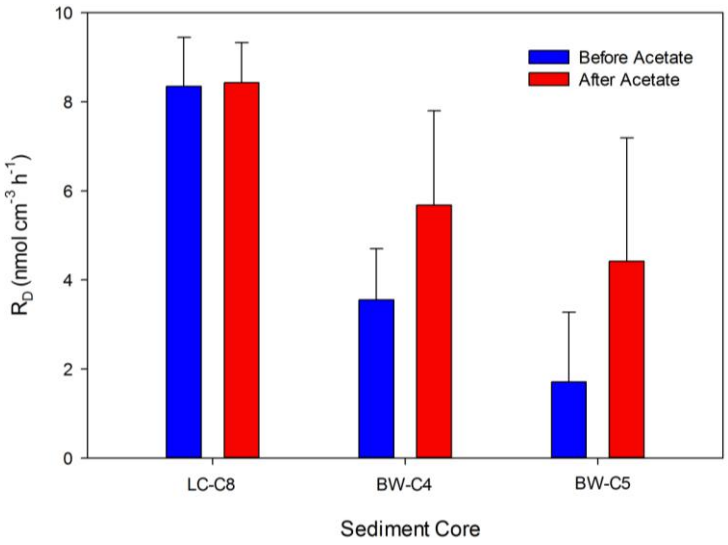


Figure 2.2: A comparison of the denitrification rates before adding an external C-substrate (acetate) and after between Laurel Creek (LC-C8 top) and Lake Belwood (BW-C4 Top and BW-C5 btm). Error bars represent the standard deviation between the averaged measurements.

The denitrification rates were calculated using equation 2.1, where R_D is the denitrification rate, ΔC is the concentration of outflow nitrate subtracted from the inflow nitrate, Q is the flow rate (1 ml h^{-1}) and V is the volume of the flow through reactor (27.7 cm^3).

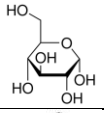
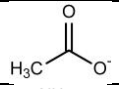
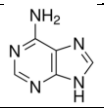
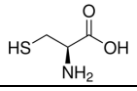
$$R_D = \frac{\Delta C \circ Q}{V} \qquad [2.1]$$

2.2 Experimental Design

2.2.1 Carbon Substrates

For the purpose of this study, 5 different C-substrates were chosen to try and span a range of different types of organic C as well as different NOSC and ΔG_R (Table 2.1). The range includes: 2 amino acids with one containing an amine group (adenine) and one containing an amine and thiol group (cysteine); a saccharide was also chosen (glucose); a simple organic compound (acetate) and a complex organic compound (fulvic acid).

Table 2.1: Chosen C-substrates and their structure, chemical formula, NOSC and ΔG_R (kJ per e^- transferred to N).

C-substrate	Structure†	Chemical Formula	NOSC	ΔG_R
Glucose		$C_6H_{12}O_6$	0	-110.1
Acetate		CH_3COO^-	0	-95.8
Adenine		$C_5H_5N_5$	2	-64.9
Cysteine		$C_3H_7NO_2S$	0.7	-109.9
Fulvic acid	Complex*	$C_{85}H_{83}O_{51}NS_{0.3}$**	0.3	56.8

†Source: Sigma-Aldrich, 2013 *Not Available **Estimated from the percent elemental composition and normalized to N (IHSS, 2013)

Gibb's Free energy (ΔG) calculations were carried out using the following equations:

$$\Delta G_R = \Delta G^o + RTlnK \quad [2.1]$$

$$\Delta G^o = \sum \Delta G^o_{fProducts} - \sum \Delta G^o_{fReactants} \quad [2.2]$$

$$K = \frac{\prod_j [Products]^{vj}_{j(t)}}{\prod_i [Reactants]^{vi}_{i(t)}} \quad [2.3]$$

Where in equation 2.1, R is the gas constant (8.314 J K^{-1} mol^{-1}), and T is temperature (295.15 K). In equation 2.2, ΔG_f is the Gibbs free energy of formation and in equation 2.3, square brackets represent concentration, *j*(t) and *i*(t) represents the concentration of products or reactants respectively at time t and v*j* and v*i* represent the stoichiometric coefficient. Calculations are based on the reaction stoichiometry found in Table 2.2 using concentrations used the experiment presented in the following sections.

Table 2.2: Complete denitrification reaction stoichiometries for the selected C-substrates

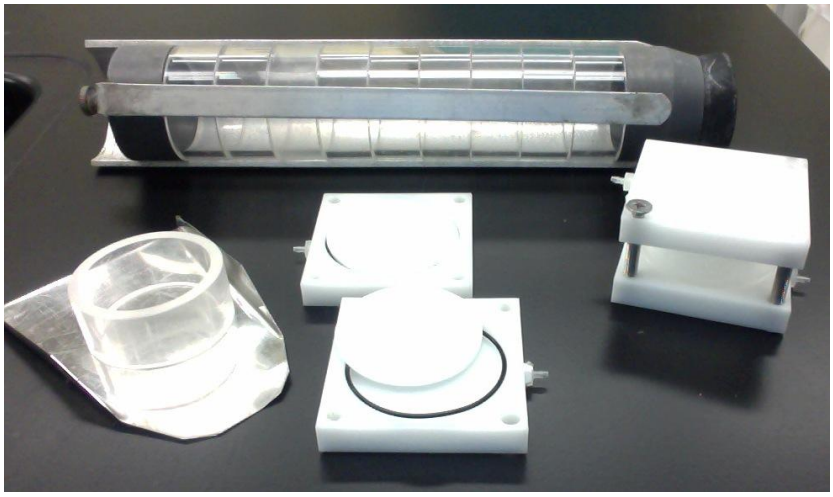
C-substrate	Denitrification Stoichiometry
Glucose	$5C_6H_{12}O_6 + 24NO_3^- \rightarrow 12N_2 + 30HCO_3^- + 6H^+ + 12H_2O$
Acetate	$5CH_3COO^- + 8NO_3^- + 3H^+ \rightarrow 4N_2 + 10HCO_3^- + 4H_2O$
Adenine	$C_5H_5N_5 + 2NO_3^- + 9H_2O + 2H^+ \rightarrow N_2 + 5HCO_3^- + 5NH_4^+$
Cysteine	$C_3H_7NO_2S + 2NO_3^- + H_2O \rightarrow N_2 + 3HCO_3^- + H^+ + NH_4^+ + HS^-$
Fulvic Acid	$C_{85}H_{83}0_{51}N_1S_{0.3} + 63.48NO_3^- + 13.56H_2O \rightarrow 31.74N_2 + 85HCO_3^- + NH_4^+ + 0.3HS^- + 20.82H^+$

2.2.2 Field Work

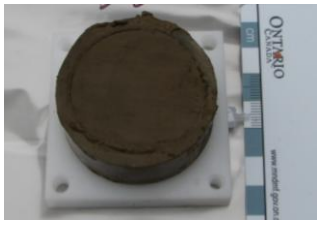
Sediment collection occurred in March 2012. All samples were collected with in approximately the same 0.6m by 0.6m section of the reservoir bed. At the time of sampling, the sample area was under approximately 0.75m of water, which ensured the collected cores would be saturated. The sampling location was shown in Figure 2.1.

The experimental design is based on Pallud and Van Cappellen (2006). Flow through reactors (FTRs) were collected and assembled through shuttle corers to obtain *in situ* sediment samples (Figure 2.3). Shuttle cores are simply half cylinders made of stainless steel with a rubber funnel shaped hole at the bottom. Plexiglass rings (inner diameter 42mm, height 20 mm), which will contain the sediment, are stacked on top of the rubber bottom and held in place with a metal rod and an adjustable top that fits in the top of the top ring. Once assembled, the corer is pushed into the sediment to the required depth and then capped on the bottom to ensure no sediment will escape before the corer is withdrawn. After the corer is withdrawn from the sediment, the metal rod is removed in order to access the plexiglass rings. A metal sheet is used to separate the different plexiglass rings one by one via slicing them to produce individual cores. By using this method to collect sediment core, it keeps samples intact in their separate rings, and can be considered an *in situ* representation. The top and bottom rims of the rings are cleaned with a cotton swab to prevent any leaks and 2 filter blocks are prepared for each side of the core (top and bottom). Each filter block contains an o-ring (47mm inner diameter) and a fiberglass filter in the center of the o-ring (47mm). These are then covered with a 0.2µm, 50mm polypropylene filter, which will be the barrier between the sediment and fiberglass filter/o-ring combination. A few drops of deionized water was added to the top of the paper filter to prevent it from falling off when the filter block is then flipped onto the plexiglass ring to seal the sediment core. Once the filter blocks are in place on either side of the core they are screwed together making sure the inflow and outflow ports were facing opposite directions. FTRs were then labeled and silicon tubing was connected to each end to prevent the sediment from drying out.

A.



B.



C.

Figure 2.3: (A) All tools used to gather sediment samples and assemble FTRs including: a shuttle corer filled with plexiglass rings to collect cores (top), a metal sheet used to slice cores (bottom left), filter blocks with o-rings and filters (bottom middle) and screws to hold the filter blocks together (bottom right). (B) A picture of a half assembled FTR after the sediment core after slicing. (C) A picture of a fully assembled FTR.

2.2.3 Experimental Design

Unless otherwise noted, the input solutions were made from stock solutions. All stock solutions were made from autoclaved Milli-Q water that was degassed with argon (Ar). Precautions were taken to avoid microbial contamination of the solutions. All solutions were prepared in autoclaved bottles, with autoclaved glassware and bottles, in an ethanol sterilized laminar flow hood. The solution was then filter sterilized as an extra precaution into a second sterile HDPE bottle. Stock solutions were kept at 4^{o}C until needed for the duration of the experiment and only opened in the laminar flow hood.

The experimental set-up (Figure 2.4) consisted of 17 FTRs containing in-situ sediment from Lake Belwood. Of the 17 FTRs, 3 were supplied with glucose, 3 with acetate, 3 with cysteine, 3 with adenine, 3 with fulvic acid and 2 had no C-substrate. The attempt was made to have 2 abiotic controls by having the FTRs gamma sterilized, however, the filter blocks became very brittle and porous after being gamma sterilized and thus became contaminated. These FTRs were not

included in analyses. Input solution flowed in through the bottom of the FTR and out through the top of the other side in an attempt to get an even distribution of water flowing through the reactor and avoid unsaturated zones. Viton tubing (Tygon) was used to connect the FTRs to the pump and to the sampling tubes to prevent oxygen diffusion in the solutions. This measured was taken after the silicon tubing, used in preliminary experiments, showed iron oxide precipitate in the tubes, suggesting oxygen diffusion.

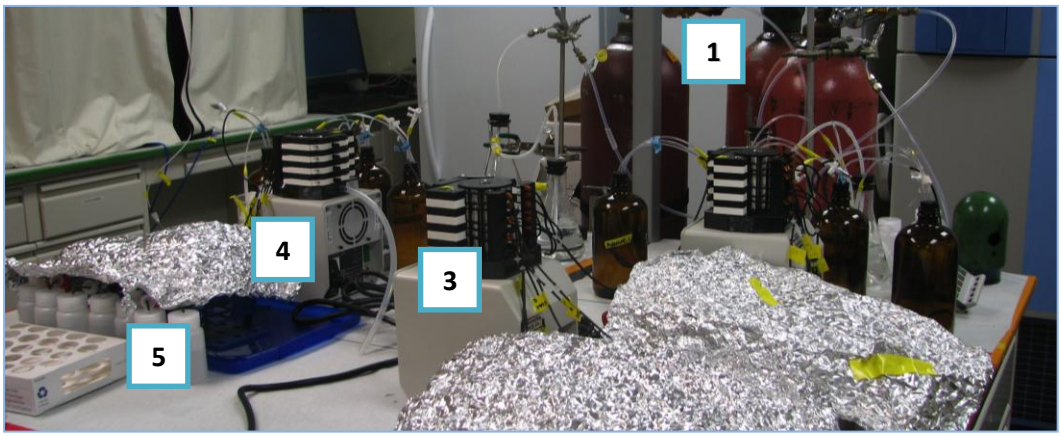


Figure 2.4: Experimental set-up: (1) argon tanks (2) input solutions (3) pumps (4) FTRs (under foil (5) sample collection area

The experiment consisted of 3 phases. The first phase lasted 1248 h (52 days) and was characterized by the supply of a non-carbon input solution to all FTRs. The initial input solution contained KNO_3 (1 mM), NaCl (8 mM) and KBr (0.05 mM, as a tracer). NaCl was added to try and raise the electronegativity of the input solution in order to help the natural microbial communities adapt to the new conditions more easily. Initial input solutions and end input

solutions were measured using an Ion Chromatograph (IC) to ensure the input solution was stable while being supplied to the FTRs. Three pumps with 8 channels on each, were used to feed FTRs with the input solution at a constant rate.

When it could be seen that the nitrate concentrations supplied to each FTR were equal to the concentrations in the outflow, phase 2 was started. Phase 2 consisted of addition of the C-substrates to the respective FTR series. Input solutions bottles were changed to a total of 8 1L amber bottles to separate the different C-substrates to each FTR series. C-substrates were added into the input solutions in concentrations calculated from the stoichiometry presented in Table 2.2 for 1 mmol NO_3^-. To ensure enough C was present in the FTRs, the calculated concentrations of each of the C-substrates were multiplied by 1.5 (Table 2.3). Cysteine, glucose and acetate were added to their respective input solutions using stock solutions. Fulvic acid and adenine were weighed and added directly into the input solution bottles due to their low solubility. Acetate was also measured on the IC to ensure C-substrate concentrations were accurately added to the input solutions and stayed consistent. Unfortunately no other C-substrates could be measured in the input.

Table 2.3: Concentrations of C-substrates used in the input solutions (mM C).

C-Substrate	**Glucose**	**Acetate**	**Cysteine**	**Adenine**	**Fulvic Acid**
Concentration	1.88	1.88	2.25	3.75	1.88

Phase 3 was started once the FTR series had reached a steady state of NO_3^- in the outflow. Phase 3 involved increasing the flow rates to see the effects of flow on the denitrification rates. Flow rates started at 1ml h^{-1} and were subsequently increased to 2 and 4 ml h^{-1} once the NO_3^- concentrations in the outflow reached a steady state at the specified flow rate.

2.2.4 Running the Experiment

The experiment was started on April 2nd, 2012 at 4pm. For the first 12 hours, outflow samples were collected every 4 hours, followed by every 2 hours for the next 12 hours and then back to 4 hours for the following 12 hours in order to achieve a Br^- breakthrough curve for the input solution and ensure all FTRs were flowing properly (See Results Section 3.1). A conductivity probe was used to measure the breakthrough of Br^-. Samples for anions (NO_3^-, NO_2^-, SO_4^{2-}, Br^-, Cl^-) cations,(Ca^{2+}, K^+, Mn^{2+}, Mg^{2+}, Na^+, Si^{4+}, total S, total P, total Fe) DOC, CO_2, N_2O and NH_4^+ were collected from the outflow on a regular basis and excess water was collected as a bulk sample and frozen (Table 2.4, Section 2.2.6). Precautions were taken to collect samples for N_2O and CO_2. They were collected in closed sample vials using needles, which was then attached to the end of the outflow tubing and inserted into the sample vial, which contained a rubber septum held on by a screw cap. A second needle was also placed in the septum to relieve pressure build up. The second needle allowed air to flow out as water flowed in.

Once the C-substrates were supplied (after 1248 hours) the experiments were run for approximately 1610 hours more. At the end of the experiment (Monday July 30th, 2012) FTRs were disassembled in a glove box and the sediment was subsampled for microbial analysis (RNA and most probable number) and elemental analyses by CHNS.

2.2.5 Sampling Protocols and Analyses

All sampling methods and collection processes are outlined in Table 2.4. Specific instrumentation information can be found in Appendix A.

Table 2.4: Outline of the sampling protocols and analyses.

Sample	Collection Interval	Sample Volume ml	Collection Time h	Sampling Bottle	Sample Processing	Storage	Detector
Anions	Every other day	4	5	4ml plastic vial	0.2µm Filtered	Freeze	Ion Chromatograph
Cations	Twice a week	10	12	10ml ICP OES tube	Acidified 2% nitric acid by volume	Fridge	Inductively Coupled Plasma Optical Emission Spectrometer
Ammonia	With anions	5	6	15ml centrifuge tube	Acidified	Freeze	Ultraviolet-Visible Spectrophotometer
Dissolved Organic Carbon	Once a week	10	12	100ml amber bottle	0.45µm Filtered Acidified with sulfuric acid	Fridge	Total Organic Carbon Analyzer
CO_2	Twice a week	1-2	2	2ml glass amber vial	Air-tight septum in lid	Run immediately	Gas Chromatograph
N_2O	Twice a week	1-2	2	2ml glass amber vial	Air-tight septum in lid	Run immediately	Gas Chromatograph
Bromide	First 48h	2	n/a	4ml plastic vial	---	Not Stored	Bromide Probe
pH	With anions	2	2-3	4ml plastic vial	---	Not stored	pH probe
Sediment	At the end	Varied	n/a	Baggies	Homogenized and separated	Freeze dried	CHNS Elemental Analyzer
Most Probable Number	At the end	1*	n/a	40 ml amber VOC vials with teflon lined septa	Homogenized	Room temperature, glove box	Visual inspection

* Measured in gram **University of Waterloo

Chapter 3
Results

The follow are the experimental results of the Lake Belwood FTRs by species. All data displayed from the FTR outflow is an average of 3 FTRs (glucose, acetate, cysteine, adenine and fulvic acid) or 2 FTRs (no carbon). Potential denitrification rates (R_D) are also determined from these averages using equation 2.1 from Chapter 2. Recall:

$$R_D = \frac{\Delta C \circ Q}{V} \qquad [2.1]$$

Where ΔC is the change in NO_3^- concentration from inflow to outflow, Q is the flow rate (1, 2 or 4 ml h^{-1}) and V is the volume of the FTR, 27.7 cm^3 (Pallud and Van Cappellen, 2006). The R_D's determined in this thesis represent denitrification in terms of NO_3^- reduction and do not represent complete denitrification to N_2. This is discussed further in Chapter 4.

3.1 Break-Through Curve

Bromide was used as a tracer in each FTR. Breakthrough curves were plotted from the data collected within the first 48 hours to ensure FTRs were flowing properly (Figure 3.1). The shape of the breakthrough curve can determine if there is a preferential flow path inside the FTR, which would affect the residence-time of the solutions in the FTRs. Measurements were made using a bromide probe for this portion of the experiment and are later measured with an ion chromatograph for more accurate results.

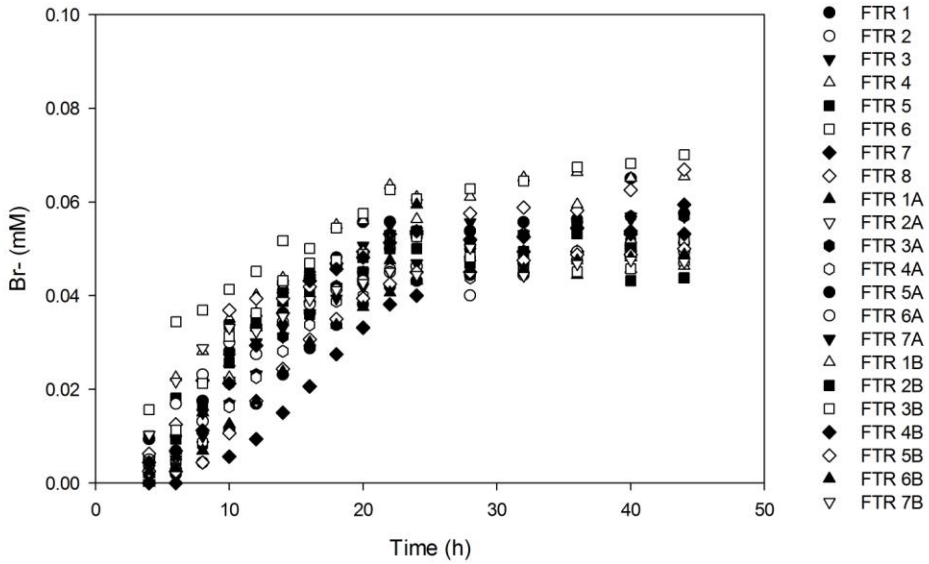


Figure 3.1: Break through curve for the bromide (Br^-) tracer in the outflow of all FTRs in the first 48h of the experiment. The flow rate at the time of the breakthrough curve was 1 ml h^{-1}.

3.2 Nitrogen Species

Table 3.1: Average N-species in the outflow and potential denitrification rate for each FTR supplied with a C-substrate. Concentrations are in mM unless otherwise specified.

C-Substrate	Flow Rate mL h^{-1}	$NO_3^-{}_{In}$	$NO_3^-{}_{out}$	$NO_2^-{}_{out}$	$NH_4^+{}_{out}$	N_2O_{out}	R_D nmol cm^{-3} h^{-1}
Glucose	1	1.0±0.2	0.14±0.10	0.30±0.15	0	D	31±13
	2	1.0±0.2	0.39±0.04	0.25±0.06	0	D	44±16
	4	1.0±0.2	0.53±0.11	0.46±0.16	0	□	67±25
Fulvic	1	1.0±0.2	0.98±0.07	0	0	□	0.9±0.3
	2	1.0±0.2	1.03±0.08	0.00±0.01	0	□	0.0±0.5
Acetate	1	1.0±0.2	0.00±0.01	0.04±0.04	0	□	36±13
	2	1.0±0.2	0.00±0.01	0.07±0.03	0	□	72±26
	4	1.0±0.2	0.21±0.06	0.37±0.22	0	□	114±41
Adenine	1	1.0±0.2	0.16±0.01	0.16±0.03	0.5±0.1	□	30±10
	2	1.0±0.2	0.36±0.08	0.31±0.15	0.62±0.16	□	46±15
Cysteine	1	1.0±0.2	0.14±0.03	0.17±0.06	0.13±0.04	D	31±10
	2	1.0±0.2	0.23±0.05	0.46±0.24	0.18±0.03	D	55±17
No Carbon	1	1.0±0.2	1.00±0.16	0	0	□	0
	2	1.0±0.2	1.04±0.20	0	0	□	0

D = Detected □ = Not Detected

3.2.1 NO_3^-

After the breakthrough curve (at 48 hours) stabilized, output concentrations of NO_3^- are approximately equal to input concentrations, 1mM (62 mg L^{-1}), until the different C sources are added (Figure 3.2). FTRs supplied with glucose had NO_3^- in the outflow decrease to around 0.14mM at a flow rate of 1mL h^{-1}, 0.39 at a flow rate of 2mL h^{-1} and 0.53mM at a flow rate of 4mL h^{-1}. No NO_3^- was measured in the outflow of the FTR supplied with acetate at 1 and 2 mL h^{-1} but increased to about 0.21 mM at 4 mL h^{-1}. Adenine and Cysteine fed reactors behaved similarly and had NO_3^- outputs of 0.14 and 0.16mM respectively at 1mL h^{-1}. The NO_3^- outflow for both carbon sources approximately doubles when the flow rate doubles to 2mL h^{-1}. FTRs supplied with Fulvic acid and those that had no carbon source supplied stayed constant at approximately equal to the input concentration and was not effected by the flow rate. NO_3^- concentrations measured for the outflow were corrected by Br^- measurements in order to correct for errors in sample injection volume within the instrument.

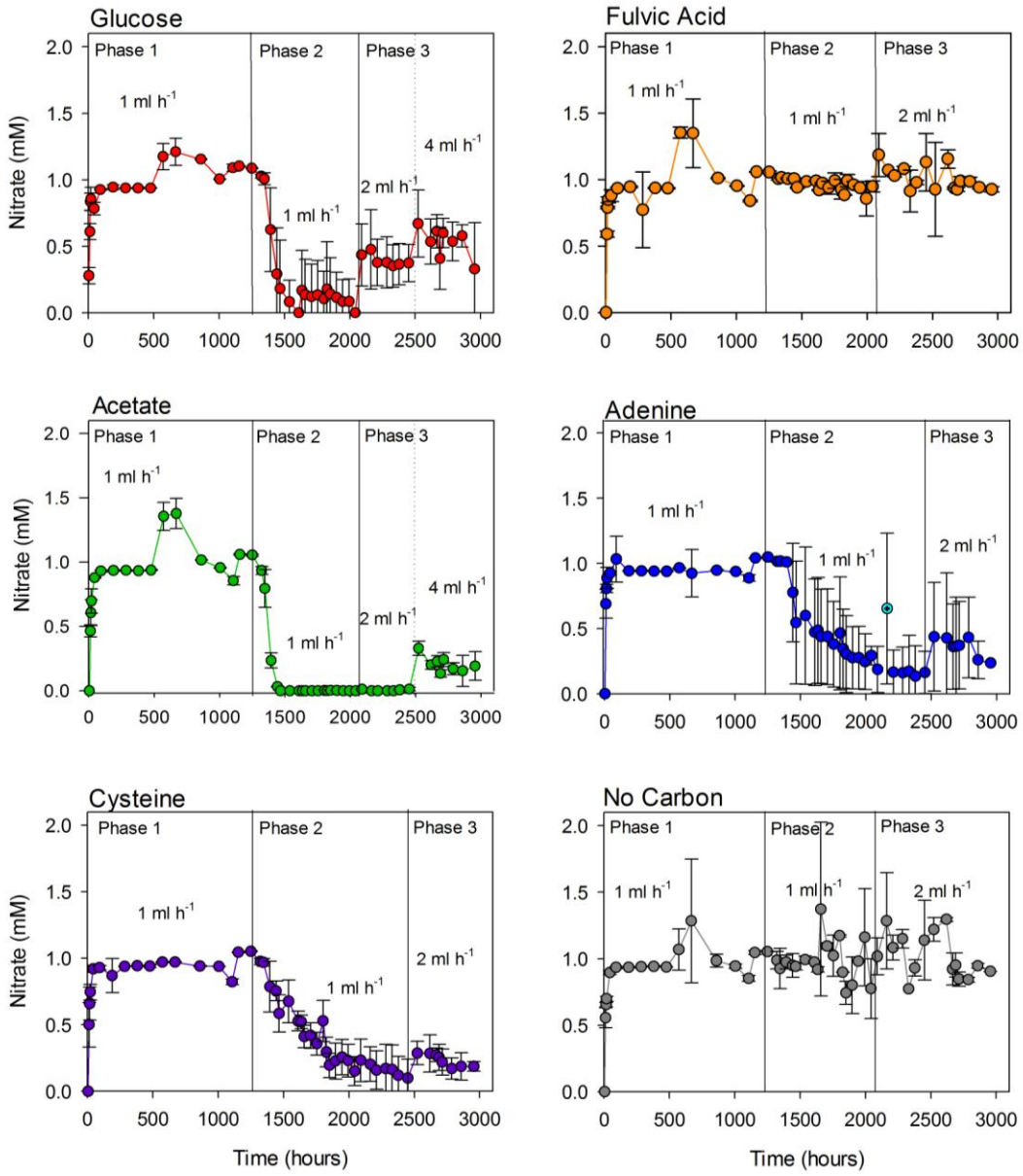


Figure 3.2: Average nitrate concentrations measured in the outflow of the FTRs for each experiment (C-substrate supplied, n = 3; no carbon supplied, n=2). Specific C-substrates were added to the FTR series at 1248h (Phase 2) as indicated on the graphs. Error bars indicate the standard deviation between FTRs in the series for that C-substrate.

3.2.2 NO_2^-

All FTRs showed no nitrite before the addition of C-substrates into the input solutions (Figure 3.3). In FTRs supplied with glucose, acetate, adenine and cysteine, some NO_3^- was converted to NO_2^- (Table 3.2). FTRs with glucose supplied measured roughly the same amount of NO_2^- at 1 and 2mL h^{-1}, approximately 0.30 mM. At 4mL h^{-1} NO_2^- output increased to 0.46mM. Outflow NO_2^- concentrations spiked shortly after supplying the FTR with acetate as a C-substrate, to values of about 0.6 mM. However, after this spike, FTRs yield very little NO_2^- at lower flow rates and increase again to about 0.6 mM when the flow is increased to 4mL h^{-1}. Adenine and Cysteine supplied reactors behave similarly to each other. At 1mL h^{-1} they produce approximately 0.16mM and increase with increased flow by about double. Fulvic acid supplied FTRs and those with no carbon supplied showed no NO_2^-.

Table 3.2: The overall NO_2^- measured in the outflow as a percentage of NO_3^- added to the FTR over the course of the experiment in the outflow, the rate of NO_2^- produced at the highest flow rate (R_N) and associated ΔG_R for NO_3^- reduction to NO_2^- (ΔG_{NO2}).

C-Substrate	Overall NO_2^- (as % of input NO_3^-)	R_N nmol cm^{-3} h^{-1}	ΔG_{NO2} kJ per e^- to N
Glucose	58	53.0	-13.5
Acetate	16	42.6	-14.0
Adenine	20	17.9	-37.8
Cysteine	23	26.5	-88.6

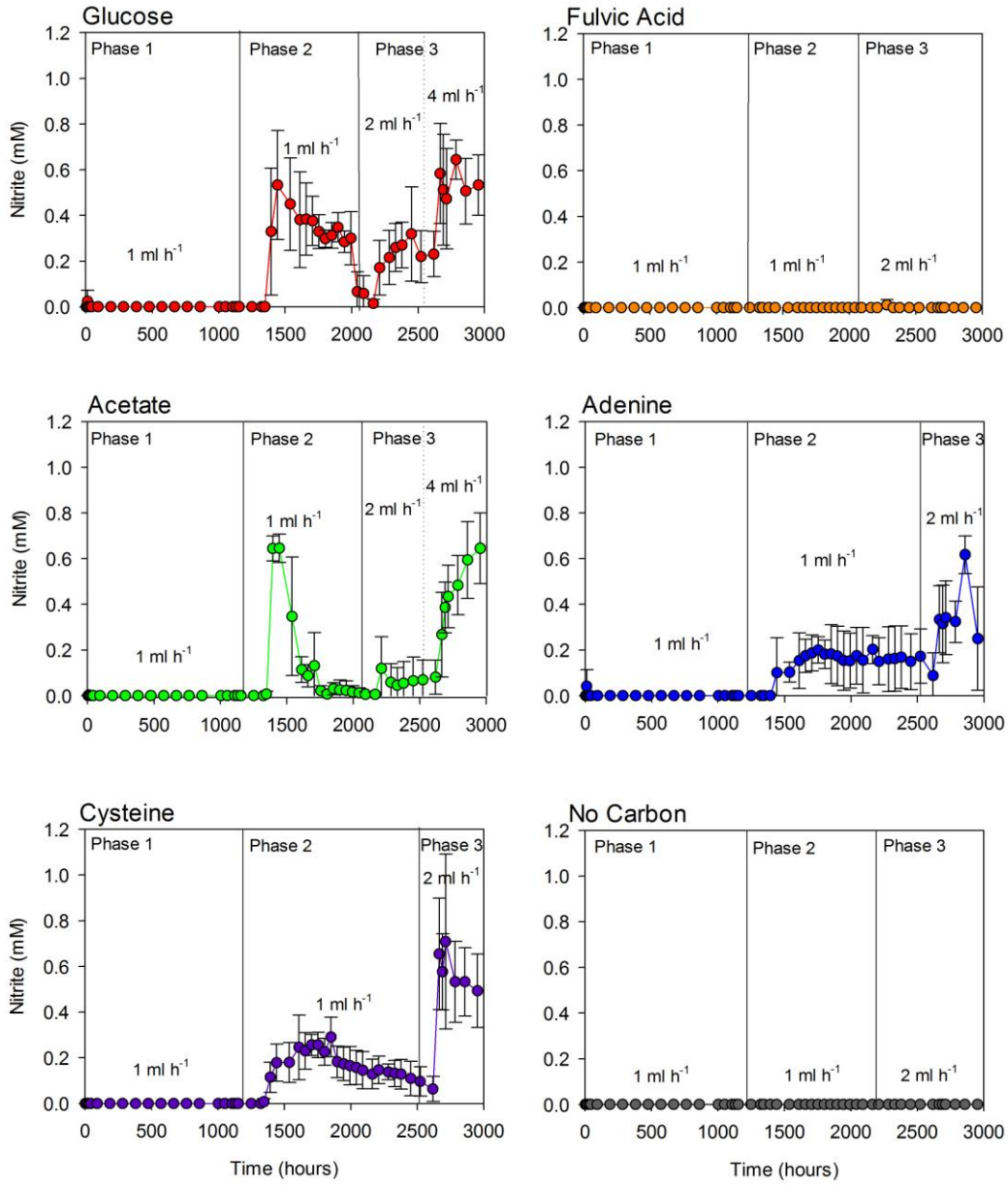


Figure 3.3: Average nitrite concentrations measured in the outflow of the FTRs for each experiment (C-substrate supplied, n = 3; no carbon supplied, n=2). Specific C-substrates were added to the FTR series at 1248h (Phase 2) as indicated on the graphs. Error bars indicate the standard deviation between FTRs in the series for that C-substrate.

3.2.3 NH_4^+

There was no NH_4^+ detected in any of the samples except for FTRs supplied with adenine and cysteine (Figure 3.4). NH_4^+ in adenine supplied FTRs increased to an average of 10.5 mM at 2mL h^{-1}. NH_4^+ was lower in the cysteine supplied FTRs and averaged out to about 3 mM.

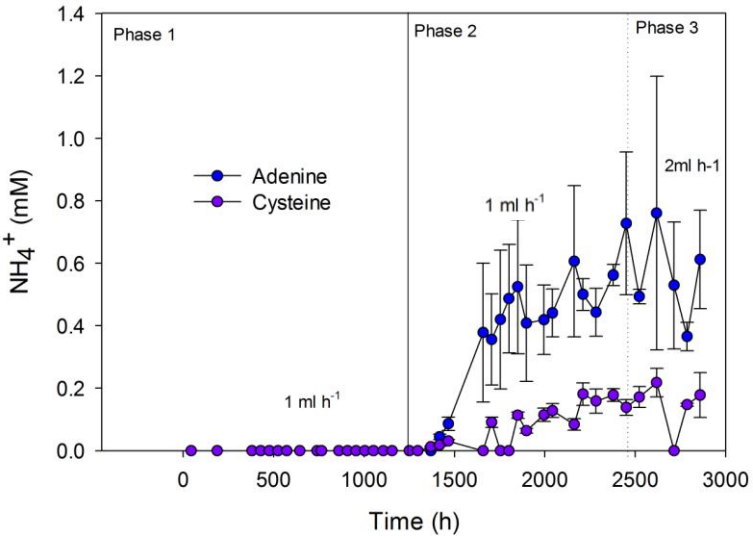


Figure 3.4: Average ammonium concentrations measured in the outflow of the FTRs supplied with cysteine and adenine (n = 3). Specific C-substrates were added to the FTR series at 1248h (Phase 2) as indicated on the graphs. Error bars indicate the standard deviation between FTRs in the series for that C-substrate.

3.2.4 N_2O

Most samples had N_2O concentrations that were below the detection limit (0.2 mM). In FTRs where N_2O was present, actual concentrations could not be quantified due problems with the equipment. Therefore, we only report on the presence (>0.2 mM) or absence (<0.2 mM) of N_2O in the outflow samples. N_2O was not detected in the reactors supplied with acetate, fulvic acid or no carbon substrate. N_2O was detected on occasion in reactors supplied with adenine, but not consistently. N_2O was detected in FTRs supplied with glucose more often then not and was always detected in reactors supplied with cysteine (Table 3.3).

Table 3.3: N_2O presence in the individual FTRs for each carbon source. Blank, grey boxes indicated no N_2O detected above 0.2 mM and + indicates N_2O was detected.

Date	Glucose				Fulvic acid			Acetate			Adenine			Cysteine			No Carbon	
	3	4	5	6	1B	2B	3B	4B	5B	6B	1A	2A	3A	4A	5A	6A	7A	7B
25-May-12					+													
29-May-12				+							+			+	+	+		
01-Jun-12	+			+										+	+	+		
05-Jun-12	+			+									+	+	+	+		
08-Jun-12	+	+		+									+	+	+	+		
12-Jun-12				+										+	+	+		
15-Jun-12	+	+	+	+		+	+	+	+	+	+	+	+		+			+
19-Jun-12	+	+	+	+									+	+	+	+		
22-Jun-12	+	+	+	+										+	+	+		
25-Jun-12				+										+	+	+		
06-Jul-12	+		+	+								+		+	+	+		
10-Jul-12	+		+															
13-Jul-12												+		+	+	+		
17-Jul-12														+	+	+		
20-Jul-12			+	+										+	+	+		
24-Jul-12			+	+					+					+	+	+		
27-Jul-12	+	+	+	+					+	+				+	+	+	+	
15-Jun-12	+	+	+	+		+	+	+	+	+	+	+	+		+			+

3.3 Sulfur Species

SO_4^{2-} and total S were measured in FTRs supplied with cysteine (Table 3.4). SO_4^{2-} increased to an average of 0.3 mM at 2 mL h^{-1} (Figure 3.5). Cysteine supplied FTRs have a plateau of S around 0.5 mM at 1mL h^{-1} and stays approximately the same when the flow is increased (Figure 3.5). Of the total S, about 61% is present as SO_4^{2-}, therefore the rest must be

other S-species, likely unused cysteine or other S species not measured in this experiment. S was not present in any FTR outflows except for FTRs supplied with cysteine.

Table 3.4: Average S-species in the outflow FTRs supplied with cysteine. Concentrations are in mM unless otherwise specified.

C-Substrate	Flow Rate ml h^{-1}	S_{in}	$SO_4^{2-}{}_{out}$	Total S_{out}
Cysteine	1	0.75	0.21±0.04	0.39±0.02
	2	0.75	0.31±0.03	0.49±0.02

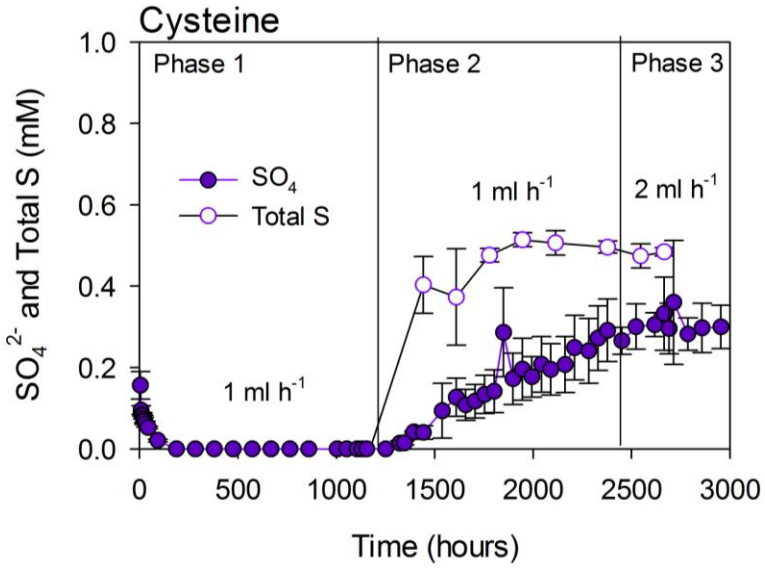


Figure 3.5: Average SO_4^{2-} and total S concentrations measured in the outflow of the FTRs supplied with cysteine (n = 3). Cysteine was added to the FTR series at 1248h (Phase 2) as indicated on the graph. Error bars indicate the standard deviation between FTRs in the series for that C-substrate.

3.4 Carbon Species

Table 3.5 show the average amount of carbon (C) supplied to all FTRs series as well as the amount of C measured in the outflow. The amount of calcium (Ca^{2+}) is also shown in Table 3.5 along with the calculated Saturation Index (SI) for calcite. SI was calculated using PHREEQCi (see section 3.4.3).

Table 3.5: Average C-species in the outflow for each FTR supplied with a C-substrate. Units are mM unless otherwise specified.

C-Substrate	Flow Rate ml h^{-1}	C_{in} mM	DOC_{out} mM	DIC_{out} mM	Sum of C_{out}	pH	$Ca^{2+}{}_{out}$ mM	SI* Calcite
Glucose	1	1.875	0.42±0.12	1.61±0.37	2.03±0.39	8.35±0.12	0.29±0.07	0.05
	2	1.875	0.58±0.13	1.02±0.13	1.60±0.18	8.26±0.04	0.34±0.05	-0.16
	4	1.875	0.90±0.02	0.87±0.17	1.77±0.17	8.19±0.18	0.30±0.05	-0.34
Fulvic	1	1.875	1.32±0.14	0.41±0.17	1.73±0.22	8.40±0.08	0.22±0.01	-0.57
	2	1.875	1.32±0.14	0.41±0.17	1.73±0.22	8.30±0.14	0.21±0.04	-0.69
Acetate	1	1.875	0.44±0.15	1.64±0.43	2.08±0.46	9.08±0.17	0.17±0.04	0.5
	2	1.875	0.52±0.03	1.56±0.15	2.08±0.15	9.38±0.09	0.11±0.02	0.53
	4	1.875	0.80±0.34	1.33±0.23	2.13±0.41	9.38±0.06	0.10±0.00	0.44
Adenine	1	2.25	2.29±0.3	0.55±0.18	2.84±0.35	8.40±0.45	0.19±0.04	-0.51
	2	2.25	1.99±0.35	0.44±0.24	2.43±0.42	8.76±0.12	0.13±0.02	-0.41
Cysteine	1	3.75	1.44±0.15	0.97±0.31	2.41±0.34	8.18±0.22	0.52±0.01	-0.08
	2	3.75	0.99±0.10	1.01±0.46	2.00±0.47	7.85±0.09	0.46±0.02	-0.45
No Carbon	1	0	0.38±0.11	0.05±0.11	0.43±0.16	8.21±0.18	0.15±0.01	-1.93
	2	0	0.38±0.11	0.05±0.11	0.43±0.16	8.49±0.20	0.14±0.01	-1.57

*Saturation Index calculated in PHREEQCi

3.4.1 Dissolved Organic Carbon

Dissolved organic carbon (DOC) was measured in the outflow of all FTRs. FTRs supplied with glucose have an increase in DOC from 0.36 mM to 0.90 mM after glucose was added to the input solution at a concentration of about 1.88 mM C-glucose. For FTRs supplied with acetate, DOC slightly increases as the flow rate increases from 0.44 at 1 ml h^{-1} to 0.80mM at

4ml h^{-1}. Acetate was added to the input solution at a concentration of 1.88 mM C-acetate. DOC peaks to 2.29 mM in FTRs when 3.75 mM C of adenine is supplied but drops again to 0.64 mM right before the flow is increased to 2 mL h^{-1} and finally increases to an average of 1.99 mM. The outflow of DOC decreases at the higher flow rate in FTRs supplied with 2.25 mM C of cysteine. DOC stabilizes in the outflow at flow rates of 1 and 2 mL h^{-1} around 1.4 and 1 mM respectively. Fulvic acid supplied FTRs have a fairly constant outflow DOC around 1.36 mM, which is lower than the input concentration of 1.88 mM C-fulvic acid. For the FTRs that were not supplied with a C-substrate, outflow DOC concentrations remained constant at about 0.4 mM. Measurements for DOC started approximately 500 hours into the experiment so it is unclear how much DOC was leeched from the FTRs initially (Figure 3.6).

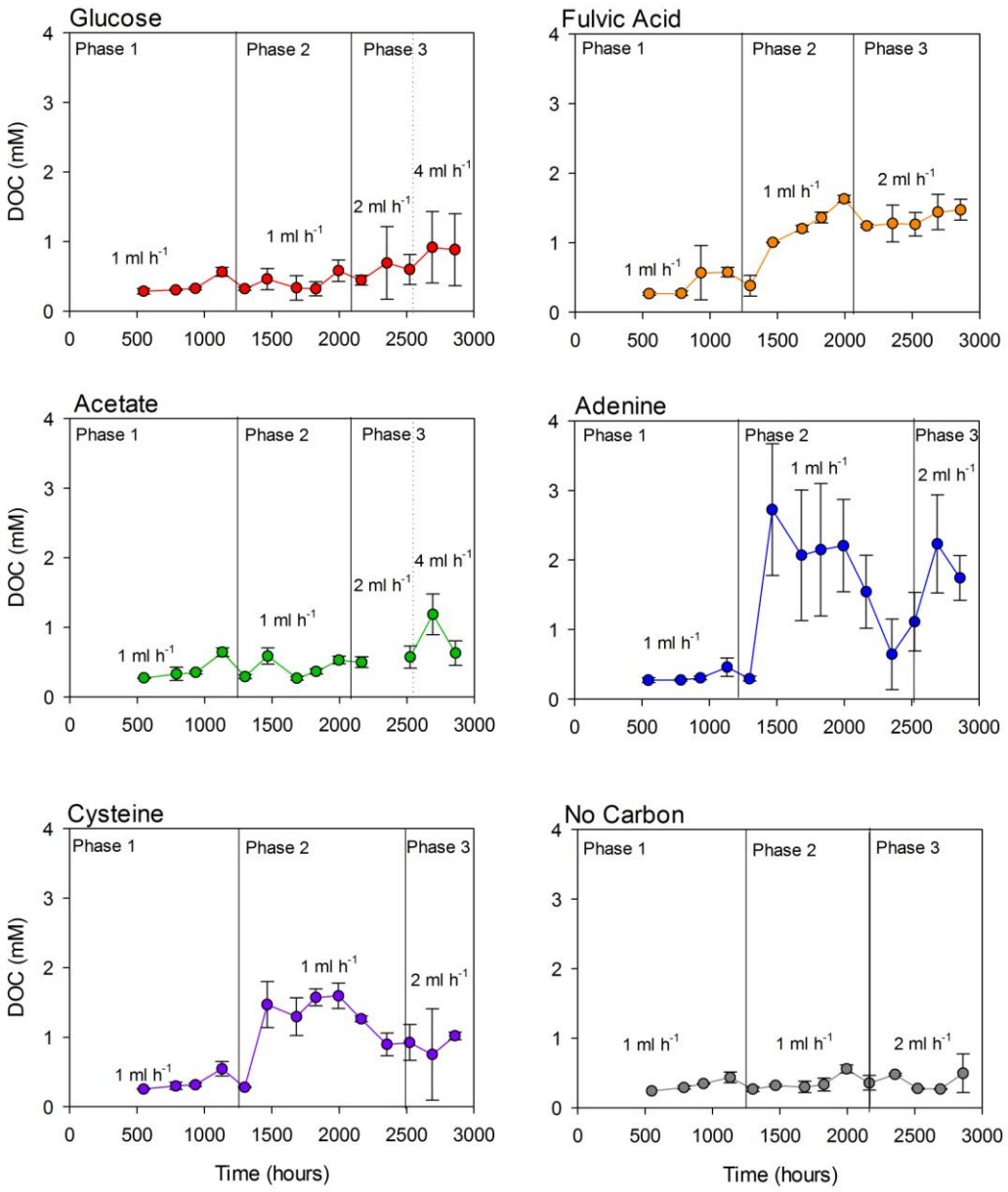


Figure 3.6: Average DOC concentrations measured in the outflow of the FTRs for each experiment (C-substrate supplied, n = 3; no carbon supplied, n=2). Specific C-substrates were added to the FTR series at 1248h (Phase 2) as indicated on the graphs. Error bars indicate the standard deviation between FTRs in the series for that C-substrate.

3.4.2 CO_2

Before the C-substrates are added to the inputs, a small amount of CO_2 is present in the outflow of each of the FTRs, approximately 0.45 mM (Figure 3.7). After the addition of glucose to the input solution, CO_2 increases to a peak of 2.30 mM at 1mL h^{-1}, then decreases and stays constant at about 0.87 mM at 2 and 4 mL h^{-1}. FTRs with acetate supplied have CO_2 increases into approximately 1.3 mM across all 3 flow rates. Adenine and cysteine fed FTR outflows gradually increase in CO_2 to approximately 1.95 mM and 0.98 mM respectively at 2 mL h^{-1}. In the outflow of FTRs where Fulvic acid is supplied, CO_2 remains more or less constant at 0.43 mM and where no carbon source is supplied, it decreases over time reaching below detection limit at about 2250 h.

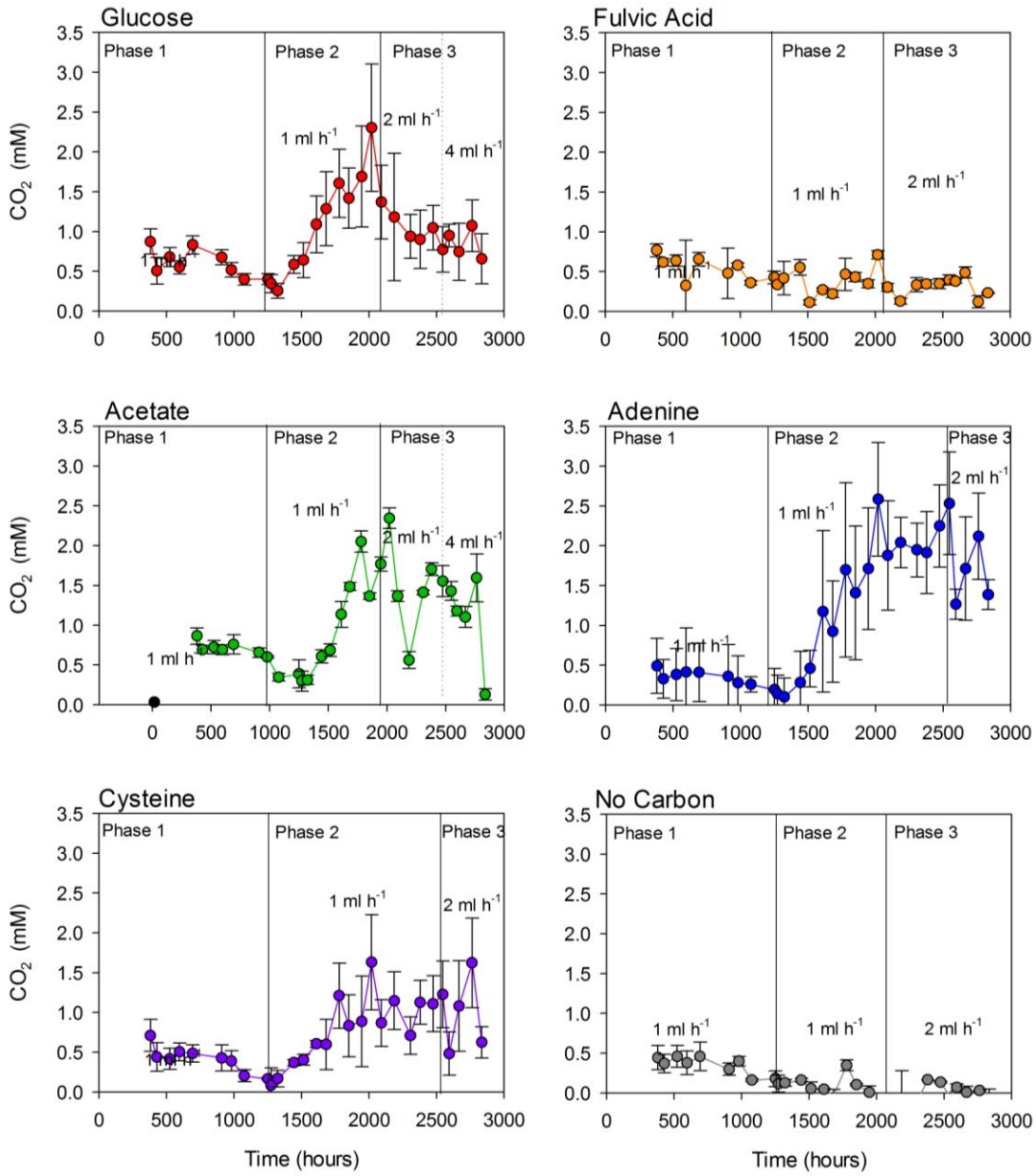


Figure 3.7: Average CO_2 concentrations measured in the outflow of the FTRs for each experiment (C-substrate supplied, n = 3; no carbon supplied, n=2). Specific C-substrates were added to the FTR series at 1248h (Phase 2) as indicated on the graphs. Error bars indicate the standard deviation between FTRs in the series for that C-substrate.

3.4.3 Organic and Inorganic C in Sediment

Varying amounts of C were found in the initial and end sediments of each FTR. Initial amounts of organic C were low and are found in the highest levels in glucose supplied FTRs. There seems to be a general decrease in inorganic C overall in all FTRs supplied with cysteine, adenine and no C-substrate compared to initial sediments (Figure 3.8) and a general increase in for those supplied with glucose, fulvic acid and acetate. Extra sediment cores were collected during sampling and used to determine the initial C in the sediment. Cores 16:A, 16:B, M1 and M4 are all initial cores and were not used in the FTRs.

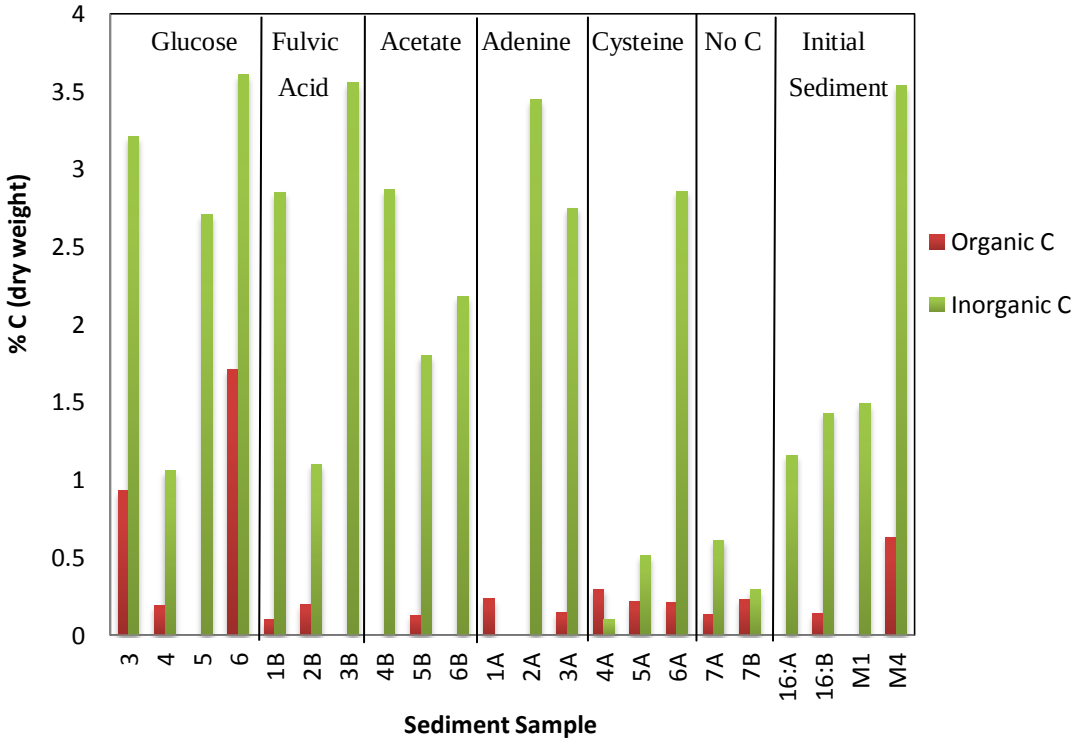


Figure 3.8: Organic and inorganic carbon measurements of the initial and end sediments from the individual FTRs for each carbon substrate.

3.5 Cations

Cations were measured in outflows from all FTRs: calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), aluminum (Al), iron (Fe), manganese (Mn), phosphorus (P), silica (Si) and sulfur (S). The results will focus on Ca, Mg, K and Na. All other cation data can be found in Appendix C. In the case of Ca, all FTRs, except those supplied with glucose and cysteine, showed Ca decreasing from >0.40mM to approximately 0.15mM. For FTRs supplied with glucose, Ca starts at >0.40mM and decreases to about 0.22mM before glucose is added. After the addition of glucose to the input solution, Ca increases again to about 0.34mM and stays constant with flow. For FTRs supplied with cysteine, Ca decreases from >0.35mM to about 0.26mM before the addition of cysteine. Once cysteine is added to the input solution, Ca increases to an average of about 0.52 mM and stays constant with flow. Mg in the FTRs showed the same patterns as Ca but at much lower concentrations. K concentrations gradually reflected those of the input solution, 1.05 mM, steadily increasing for the first few weeks of the experiment. Na concentrations were lower then the input concentration of 8 mM, but steadily increased to about 7 mM with some variation.

3.6 Most Probable Number

Most probable number (MPN) analyses were conducted for NO_3^- reducing bacteria (NRB) and S oxidizing bacteria (SOB) on the sediments from the initial samples and the once the FTR experiment was complete. FTRs supplied with adenine and glucose showed the highest count for NRB (Table 3.6). FTRs supplied with cysteine had the highest counts of SOB. SOB was also found in FTRs supplied with adenine in lower quantities than those supplied with cysteine.

Table 3.6: MPN results for NRB and SOB in each of the FTRs including initial sediment.

FTR	C-Substrate	NRB cells per g	SOB cells per g
`1A	Adenine	$1.5x10^7$	$2.3 x10^3$
2A	Adenine	$2.0 x10^7$	$2.3 x10^3$
3A	Adenine	$7.4 x10^6$	$2.3 x10^3$
4A	Cysteine	$2.8 x10^5$	$5.2 x10^4$
5A	Cysteine	$3.6 x10^5$	0
6A	Cysteine	$4.2 x10^5$	$9.2 x10^4$
1B	Fulvic	$2.3 x10^3$	N.A.*
2B	Fulvic	$4.2 x10^5$	N.A.
3B	Fulvic	$1.5 x10^4$	N.A.
4B	Acetate	$1.5 x10^6$	N.A.
5B	Acetate	$2.3 x10^7$	N.A.
6B	Acetate	$1.1 x10^4$	N.A.
7A	No C	$1.1 x10^5$	N.A.
7B	No C	$2.0 x10^6$	N.A.
3	Glucose	$2.8 x10^7$	N.A.
4	Glucose	$1.5 x10^7$	N.A.
5	Glucose	$9.2 x10^4$	N.A.
16B	Initial	$4.2 x10^5$	N.A.
16A	Initial	$1.5 x10^4$	N.A.

*N.A. = Not available

Chapter 4
Discussion

4.1 Biogeochemistry

4.1.1 Aqueous Phases

4.1.1.1 Nitrogen

As expected, there is less NO_3^- in the outflow than in the input solutions for FTRs supplied with glucose, acetate, cysteine and adenine, indicating that denitrification is occurring within the reactor. Fulvic acid did not induce denitrification, which was predicted by its positive calculated ΔG_R (52.2 kJ per e^- from C). Figure 4.1 is a representation of each of the FTRs showing all N species into and out of the FTRs. The amounts in Figure 4.1 are totals integrated over the course of the experiment starting from the addition of the C-substrates (Appendix B). Nitrite (NO_2^-), ammonium (NH_4^+), nitrous oxide (N_2O), nitric oxide (NO) and nitrogen gas (N_2) are all possible forms of N in the outflow. Unfortunately not all forms of N could be measured in the outflow, but concentrations of NO_3^-, NO_2^- and NH_4^+ are presented in Table 4.1.

Table 4.1: Average N species that could be measured into and out of each FTR for each C-substrate in mmol, integrated over the second and third phase of the experiment starting at 1248 h.

C-Substrate	N_{in} from C-substrate	Total N_{in}	NO_3^- out	NO_2^- out	NH_4^+ out	N_{out} from C-substrate	Total N_{out}
Glucose	0	4.14±0.65	2.34±0.65	1.04±0.41	0	0	3.38±1.06
Acetate	0	5.15±0.54	1.27±0.16	0.61±0.30	0	0	1.88±0.46
Adenine	7.11±1.07	10.55±1.73	1.54±0.47	0.38±0.24	3.05±0.31	2.52±1.39	7.49±2.41
Cysteine	1.42±0.21	4.89±047	1.36±0.38	0.48±0.22	0.44±0.04	0.47±0.35	2.75±0.98
Fulvic Acid	0	3.31±0.16	3.33±0.31	0	0	0	3.33±0.31
No Carbon	0	3.35±0.25	3.35±0.39	0	0	0	3.35±0.39

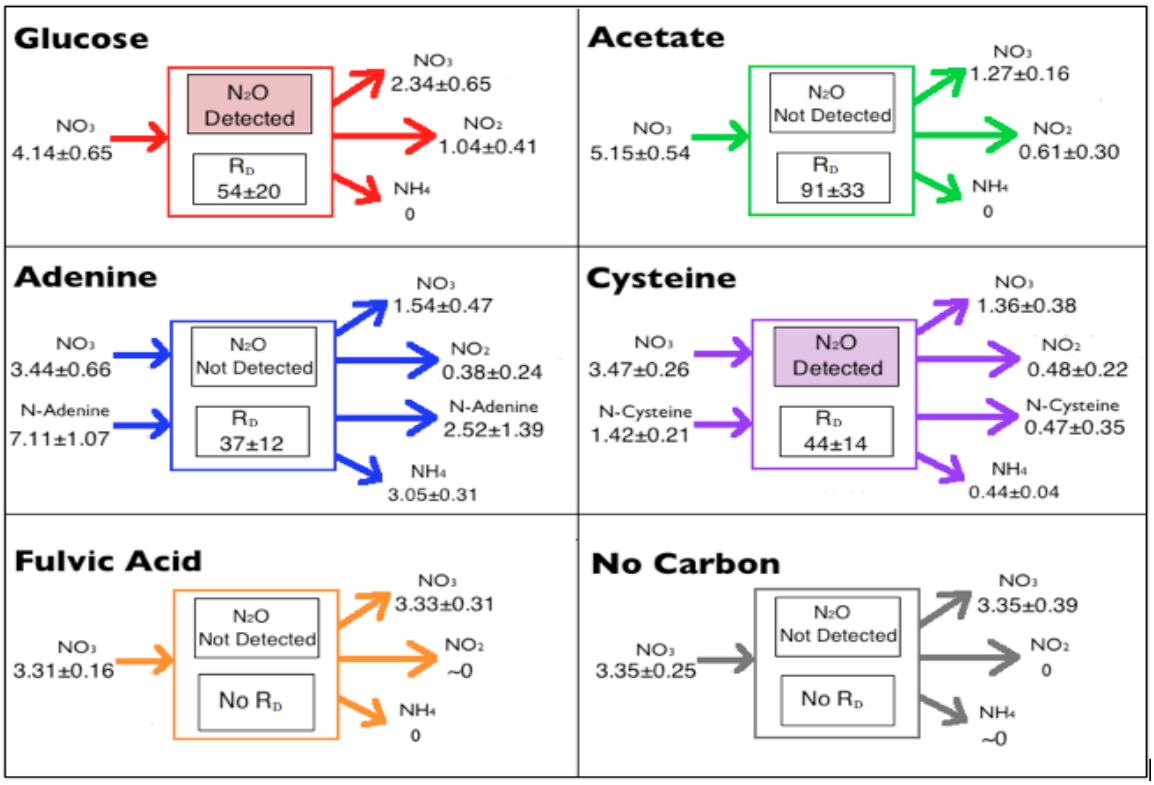


Figure 4.1: Total nitrogen (N) mass balance in mmol for each C-substrate supplied to the FTRs. Totals are calculated by integrating outflow results starting from phase 2 and averaged for each FTR series (C-substrate, n=3; no carbon n=2). Maximum potential denitrification rates (R_D; Eqn 2.1) are displayed (nmol cm^{-3} h^{-1}). The boxes represent FTRs, with arrows representing inflow and outflow concentrations.

NO_2^- is produced in all FTRs showing NO_3^- loss in the outflow, indicating complete denitrification to N_2 is not occurring (Equation 4.1). NO_2^- measured in the outflows is a significant amount (Table 3.2, Chapter 3). Changes in the outflow concentrations of NO_2^- frequently mirror those in outflow concentration of NO_3^- (Figure 3.2 and 3.3, Chapter 3). As the flow rate increases, the amount of NO_2^- also increases. Stoichiometric equations for the transformation of NO_3^- to NO_2^- with each C-substrate can be found in Table 4.2. General steps in denitrification are shown in the reaction series below:

$$2NO_3^- + CH_2O \rightarrow 2NO_2^- + CO_2 + H_2O \qquad [4.1]$$

$$4NO_2^- + CH_2O + 4H^+ \rightarrow 4NO + CO_2 + 3H_2O \quad [4.2]$$

$$4NO + 3CH_2O + H_2O \rightarrow 2N_2O + 3CO_2 + 8H^+ \quad [4.3]$$

$$2N_2O + CH_2O \rightarrow 2N_2 + CO_2 + H_2O \quad [4.4]$$

Table 4.2: NO_3^- reduction stoichiometries to NO_2^-.

C-Substrate	Stoichiometry
Glucose	$C_6H_{12}O_6 + 12NO_3^- \rightarrow 12NO_2^- + 6HCO_3^- + 6H^+$
Acetate	$CH_3COO^- + 4NO_3^- \rightarrow 4NO_2^- + 2HCO_3^- + H^+$
Adenine	$C_5H_5N_5 + 5NO_3^- + 10H_2O \rightarrow 5NO_2^- + 5HCO_3^- + 5NH_4^+$
Cysteine	$C_3H_7NO_2S + 5NO_3^- + 2H_2O \rightarrow 5NO_2^- + 3HCO_3^- + 3H^+ + NH_4^+ + HS^-$

NO_2^- could also be reduced to other forms of N, including NH_4^+, N_2, NO and N_2O. N_2O was detected in FTRs supplied with cysteine and glucose, also indicating denitrification was incomplete to N_2 in these FTRs (Paul, 2006) and reactions such as those found in Table 4.3 are also occurring.

Table 4.3: NO_3^- reduction stoichiometries to N_2O.

C-Substrate	Stoichiometry
Glucose	$C_6H_{12}O_6 + 6NO_3^- \rightarrow 3N_2O + 6HCO_3^- + 3H_2O$
Cysteine	$4C_3H_7NO_2S + 10NO_3^- + 3H_2O \rightarrow 5N_2O + 12HCO_3^- + 4NH_4^+ + 4HS^- + 2H^+$

FTRs supplied with adenine and cysteine produced NH_4^+ in their outflows. NH_4^+ can be toxic to aquatic ecosystems in high concentrations (Health Canada, 2013) and hence, the production of NH_4^+ during adenine and cysteine degradation is of concern. In the case of cysteine and adenine, the amine groups are converted to NH_4^+ in the process of amino acid degradation (Barker, 1981). Given that the total adenine-N and cysteine-N supplied were 7.11 and 1.42 mmol, respectively, FTRs supplied with adenine is expected to produce about 5 times the amount of NH_4^+ than the FTR s supplied with cysteine (Table 2.2, Chapter 2). Here, measured outflow NH_4^+

concentrations during the 2 ml h^{-1} phase were of 3.05 and 0.44 mmol, respectively, for adenine and cysteine, that is, a 7:1 ration instead of the expected 5:1. This is a relatively small difference given that different amounts of each C-substrate are consumed in the first place, and that the recovery of NH_4^+ is influenced by several concurrent NH_4^+ consuming processes, such as nitrification, anammox, and sorption.

Nitrification is excluded as a NH_3 consumption mechanism because the FTR input solutions were kept anaerobic, making this process unlikely (Canfield et al., 2005). However, under anaerobic conditions, NH_4^+ can be converted to N_2 by certain bacteria during anaerobic ammonia oxidation (anammox):

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \qquad [4.5]$$

Anammox is an important process in the environment and in wastewater treatment systems to help reduce NO_3^-, NO_2^- and NH_4^+ discharge loads to the environment (Canfield et al., 2005; Kartal et al., 2010, Nozhevnikova et al., 2011). Additionally, several other potential mechanisms can explain the discrepancy between expected and measured outflow NH_4^+ concentrations. The growing microbial populations may have assimilated some of the NH_4^+. Microbial uptake is estimated to account for 15-35% removal of NH_4^+ in different aquatic environments; however this amount can be influenced by a number of factors depending on the environment (Bunch and Bernot, 2012; Hoch and Kirchman, 1995; Fouilland et al., 2007). It is likely that NH_4^+ was sorbed to clays, abundant in the sediments used for the experiments.

Assuming that most amine groups are converted to NH_4^+ and that the difference between inflow NH_4^+ and outflow NH_4^+ is thus due to sorption, the sorption coefficients of NH_4^+ onto clay (K_d) would be 0.13 and 0.14 L g^{-1} for cysteine and adenine fed FTRs, respectively. The K_d's were calculated using the following equation:

$$\text{Mass of } NH_4^+ \text{ absorbed} = K_d \times \text{Concentration of } NH_4^+ \text{ in solution} \qquad [4.6]$$

Where the mass of NH_4^+ absorbed is in mg/g dry sediment and the concentration of NH_4^+ in solution is in mg L^{-1}. Estimates in the literature vary largely for NH_4^+ adsorption based on the pH, cation exchange capacity and titratable acidity of the soil (Schepers, 2008). Table 4.4 shows a

range of values found in the literature for ammonia adsorption to sandy and clayey soils. The values calculated for Lake Belwood sediment are within the range given in the literature.

Table 4.4: Ammonia adsorption values (K_d) for clays and sands in L g^{-1}.

Soil type	Location	K_d (L g^{-1})	Reference
Glacial outwash	Cape Cod (USA)	0.46	Böhlke et al., 2006
Fine sand	North Sea	0.3-15.3	Raaphorst and Malschaert, 1995
Vermiculite	Heibei Province (China)	5.53	Wang et al., 2011

4.1.1.2 Sulfur

Aqueous sulfur species were observed only in the outflow of the FTR fed with cysteine. Figure 4.2 displays the total S species into and out of the cysteine fed FTRs integrated throughout the experiment starting from when cysteine was added to the input. Cysteine contains a thiol group, which is released as hydrogen sulfide ions (HS^-) upon degradation. Like the degradation of amine groups to NH_4^+, degradation of thiol groups to HS^- can have serious impacts on the environment. HS^- is very toxic to aquatic ecosystems and can be fatal for fish and other aquatic species even at low doses. It is considered to have high eco-toxicity (EPA, 2011).

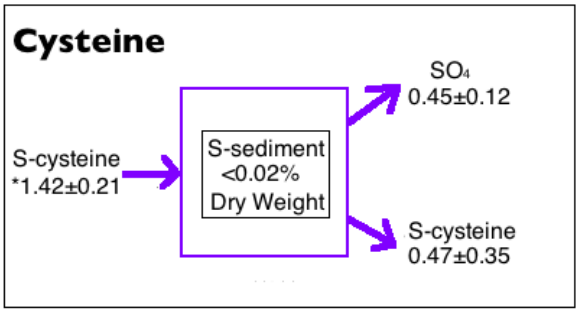


Figure 4.2: Total sulfur (S) mass balance in mmol of S for the FTRs supplied with Cysteine. Totals are calculated by integrating outflow results starting from phase 2 and averaged for the cysteine FTR series (n=3). S measured in sediment was below the detection of 0.02% by dry weight.

In the FTRs, HS^- is likely to be oxidized to SO_4^{2-} and S^0 by the microbial community under nitrate reducing conditions according to the reactions below (rebalanced to account for e- transfer from Cardoso et al., 2006):

$$NO_3^- + HS^- + H^+ \rightarrow NO_2^- + S^0 + H_2O \quad [4.7]$$

$$4NO_3^- + HS^- \rightarrow 4NO_2^- + SO_4^{2-} + H^+ \quad [4.8]$$

$$2FeOOH_{(s)} + 3HS^- \rightarrow S^0_{(s)} + 2FeS_{(s)} + H_2O + 3OH^- \quad [4.9]$$

The processes shown by Equations 4.6 and 4.7 are well studied and occur in many experiments (Krishnakumar and Manilal, 1999). Of course interactions with iron minerals are also very well studied (e.g. Equation 4.9; Appelo and Postma, 2010). Mass balance calculations suggest that not all S produced during cysteine oxidation in the FTRs is recovered as aqueous S in the outflow. The missing S was likely retained by sediment in the form of elemental sulfur (Equation 4.7 and/or 4.9). The loss of S in the outflow and production of SO_4^{2-} (measured in the outflow) support that the reactions above are occurring in the system, and potential build up in the sediment is discussed in Section 4.2.1.

SO_4^{2-} production in the FTRs supplied with cysteine is also significant in terms of HS^- removal from the system and inhibition of N_2O reduction. HS^- oxidation by NO_3^- (Equation 4.7 and 4.8) prevents the build up of H_2S, a toxic gas. Although the oxidation of HS^- during denitrification appears to be occurring, the mere presence of it is likely to have caused the increase in N_2O in cysteine fed FTRs (Pan et al., 2013). Cysteine supplied FTRs all show the presence on N_2O in the outflow and therefore agree with the findings in Pan et al. (2013) that the presence of H_2S prevents complete denitrification to N_2 due to its inhibiting effects on denitrifying bacteria. The prevention of N_2O reduction is caused by H_2S inhibiting the enzyme N_2O reductase, required by denitrifying bacteria to reduce N_2O to N_2. Nitrous oxide is a strong greenhouse gas that could potentially be produced in high quantities from wastewater treatment plants and sewer systems if H_2S is present. Transformation of H_2S to elemental S and SO_4^{2-} can help prevent the build of N_2O by lowering the concentration of H_2S in the system (Pan et al., 2013). In terms of the FTRs, is it likely that the reaction time is not long enough to convert all

sulfide into less toxic forms and the amount of H_2S present in the FTR (although not measured) is likely significant enough to reduce the conversion of N_2O to N_2.

4.1.1.3 Carbon

Overall, the C provided in the input solutions is quantitatively recovered in the FTRs outflow. Figure 4.3 shows the total C into and out of the FTRs integrated over the experiment starting from when the C-substrates were added. The totals in Figure 4.3 show that, within analytical uncertainty, the sum of DOC + DIC in the outflow of the FTR matches the concentration of C in the input. Acetate supplied FTRs, however, appear to have more C in the outflow than the inflow than can be accounted for by the error; this could potentially be caused by carbonate minerals and is discussed further in the flowing section (4.1.2). Carbonate dissolution along with assimilatory C uptake by microbes were potentially occurring in the FTRs, but the impact of those processes on the C cycling in the FTRs is unclear.

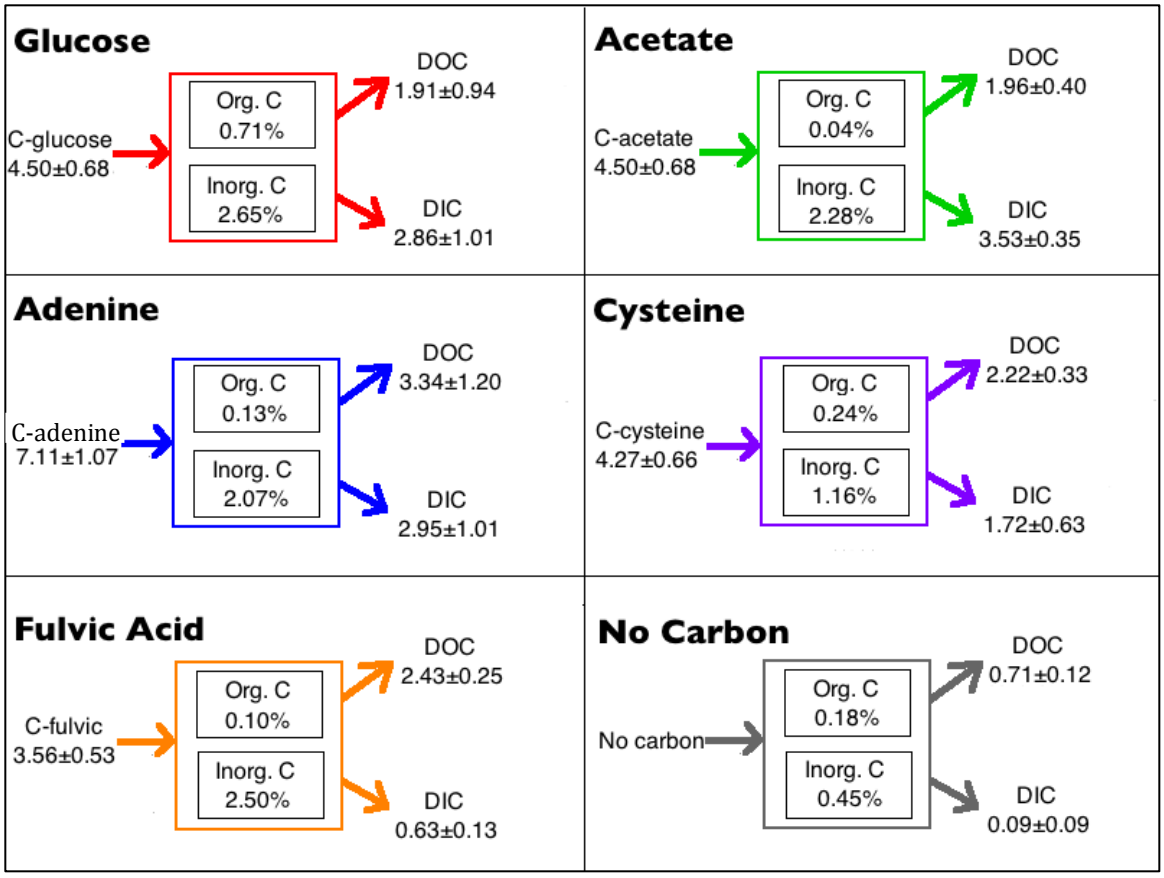


Figure 4.3: Total carbon (C) mass balance in mmol C for each C-substrate supplied to the FTRs. Totals are calculated by integrating outflow results starting from phase 2 and averaged for each FTR series (C-substrate, n=3; no carbon n=2). The amount of organic carbon (Org. C) and inorganic carbon (Inorg. C) measured in the sediment are provided in the middle of each box diagram as a percentage of dry sediment from the end of the experiment.

Fulvic acid was not quantitatively recovered as DOC, which is likely due to its sorption on the clay minerals present in the FTR. Total C input and output can be seen in Table 4.5. In FTRs supplied with no C-substrate, C is still measured in the outflow in very low concentrations compared to FTRs supplied with a C-substrate. This is likely due to C being dissolved from the sediment. Organic and inorganic C in the sediment is discussed further in section 4.1.2.2.

Table 4.5: The average total carbon (C) into and out of each FTR for each C-substrate in mmol, integrated over phase 2 and 3 of the experiment. Average organic C (C_{org}) and inorganic C (C_{inorg}) in the sediment are presented in percent dry weight and were measured at the end of the experiment.

C-Substrate	Total C_{in} mmol	DOC_{out} mmol	DIC_{out} mmol	Total C_{out} mmol	C_{org} Sediment	C_{inorg} Sediment
Glucose	4.50±0.68	1.91±0.94	2.86±1.81	4.77±2.75	0.71%	2.65%
Acetate	4.50±0.68	1.96±0.40	3.53±0.35	5.49±0.75	0.04%	2.28%
Adenine	7.11±1.07	3.34±1.20	2.95±1.01	6.29±2.21	0.13%	2.07%
Cysteine	4.27±0.66	2.22±0.33	1.72±0.63	3.92±0.96	0.24%	1.16%
Fulvic Acid	3.56±0.53	2.43±0.25	0.63±0.13	3.06±0.38	0.10%	2.50%
No Carbon	0	0.71±0.12	0.09±0.09	0.80±0.21	0.18%	0.45%

Fulvic acids play an important role in the ecosystem and are pertinent in the sequestration of C (Gaffney et al., 1996). Fulvic acid derived from surface water has been found to act as a terminal electron donor (TED) in denitrification (Pfenning and MaMahon, 1996), however, the NO_3^- mass balance in the fulvic acid FTRs indicated no significant denitrification. Since fulvic acid has been shown previously to cause denitrification, it is not unrealistic that it could react with other terminal electron acceptors via microbial activity and further break down to produce CO_2. It is possible that such reactions are occurring in the FTRs, since there is a loss of DOC in the outflow and an increase in DIC, but no NO_3^- loss.

4.1.2 Solid Phase

4.1.2.1 S-Species

As discussed in Section 4.1.2, all S added to the FTRs by supplying cysteine is not recovered in the outflow, and was likely sequestered in the solid-phase (Equation 4.7). Unfortunately, with a detection limit of 0.02% (dry weight), the CHNS measurements cannot be used to quantify the accumulation of S by the solid-phase, which is expected to be in the order of

0.001% (See Table 4.6). A potential pathway of S sequestration is through precipitation of Iron (Fe) sulfide. Fe is present in the sediment, and could potentially be reacting with S (recall Equation 4.9 below):

$$2FeOOH_{(s)} + 3HS^- \rightarrow S^0_{(s)} + 2FeS_{(s)} + H_2O + 3OH^- \quad [4.9]$$

Manganese (Mn) was detected in the outflow of cysteine supplied FTRs but not in any other outflow. It is possible that Mn oxide (MnO_2) is being reduced by H_2S in this system and causing Mn^{2+} to be present in the outflow according to Equation 4.10 (Canfield et al., 2005):

$$MnO_2 + H_2S + 2H^+ \rightarrow Mn^{2+} + S^0 + 2H_2O \quad [4.10]$$

Of all the C-substrates used in this experiment only FTRs supplied with cysteine had a decreasing pH, indicating acid producing reactions such as 4.7 are likely occurring. Elemental S will also react with any other metals present in the soil to form a metal sulfide under reducing conditions, which will also decrease pH (Equation 4.11; Wang and Chapman, 1999).

$$^2/_nMe^{n+} + HS^- \rightarrow Me_{2/n}S_{(s)} + H^+ \quad [4.11]$$

Under aerobic or S oxidizing conditions, the S built up in the sediment, along with the metals it is bound to can be released. For example if FeS was formed in anaerobic conditions, the presence of oxygen will cause the Fe(II) to oxidize to Fe(III) and produce sulphate (Sahdev, 2010):

$$4FeS + 7O_2 \rightarrow 2Fe_2O_3 + 4SO_4^{2-} \quad [4.12]$$

In reservoir sediment, these processes can fluctuate seasonally depending on the fluxes of organic carbon and on the water level. This indicates that H_2S not only can be converted to metal sulfides

under anoxic conditions but can also be produced when redox conditions change from anoxic to oxic by converting S^0 or metal sulfides (Canfield et al., 2005). It is also possible to form H_2S under reducing conditions. For example, in the presence of SO_4^{2-}, acetate can be oxidized to form HS^- (Canfield et al., 2005):

$$CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^- \qquad [4.13]$$

This shows that the fate of S is also dependent on the organic C substrate available.

Table 4.6: Summary of S build-up in the sediment and why it is not shown in CHSN analyses. Calculations can be found in Appendix B.

S_{In-aq} **mM**	S_{Out-aq} **mM**	S_{In}-S_{out} **mM**	**Total S_S g**	S_S **% dry weight**
1.42±0.21	0.92±0.47	0.50±0.68	0.016±0.03	<0.001%

4.1.2.2 C-Species

Lake Belwood, our study site, is located in an area rich in calcium carbonate. Therefore, it is likely that equilibrium with carbonate minerals such as calcite controlled C sequestration within the FTRs. To verify if calcite was likely to form or dissolve, during the experiment, its saturation index (SI) was calculated in the FTR outflow using PHREEQCi (log K = -8.475; Jacobson and Langmuir, 1974). Positive values of SI indicate that the mineral is supersaturated and can potentially precipitate, negative values of SI indicate that the mineral is under-saturated and that, if present, should dissolve. The predicted SI of the outflow relative to calcite was plotted against the quantity of inorganic C lost (ΔTIC) by each FTR series after the experiment (Figure 4.4). Although this exercise did not yield a clear relationship between SI and the quantity of TIC lost for all compounds, Figure 4.4 strongly suggest that the acetate-fed FTRs gained TIC due to calcite precipitation while the FTRs with no C-substrate added lost TIC due to calcite dissolution.

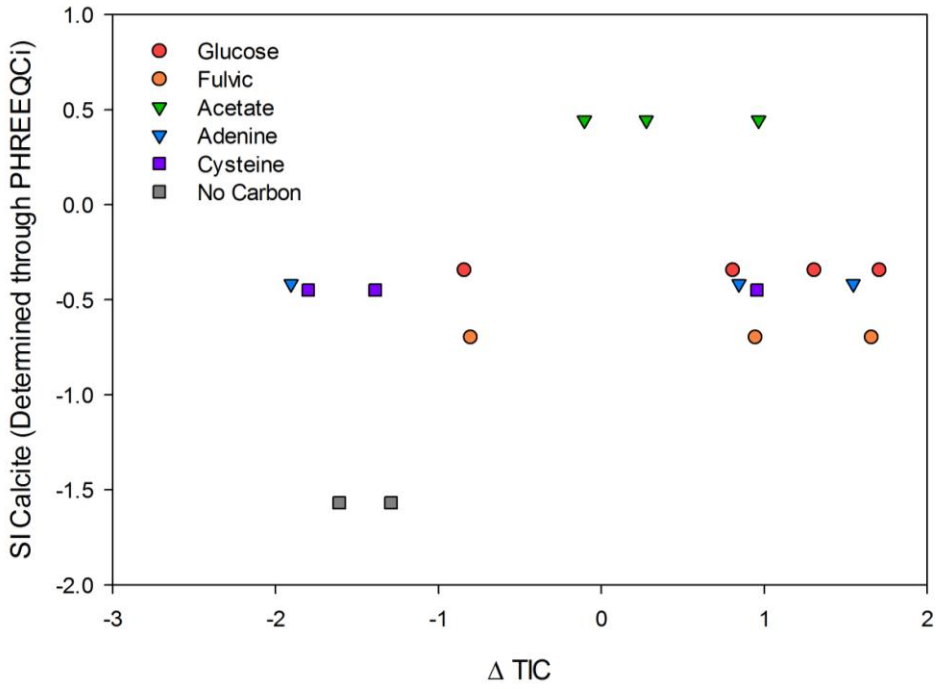


Figure 4.4: The gain or loss of total inorganic carbon (TIC) in the sediment compared to the PHREEQCi calculated saturation index of calcite (SI Calcite) using the minteq database. ΔTIC was calculated by $\Delta TIC = TIC_{end} - TIC_{initial}$.

According to Figure 4.4, there is a slight trend between the loss or gain of inorganic carbon compared to the SI. However, if calcite formation/dissolution (Equations 4.13, 4.14 and 4.15; Yarkin, 2008) were the main cause of C fluctuations in the FTRs there would be a much stronger trend in the data plotted. Figure 4.4 shows two outlying groups: the group clustered in the bottom left, which are the no carbon control FTRs showing calcite dissolution should be occurring and the group clustered in the upper right, which are FTRs supplied with acetate, showing calcite upper saturation and possible precipitation. All other FTRs supplied with various carbon substrates are located and mix across an SI of approximate -0.5, indicating slight dissolution could be occurring but does not help explain the slight gain or loss of C in those FTRs. In FTRs not supplied with C, Ca^{2+} and organic as well as inorganic C were measured in the outflow. This supports that calcite is likely dissolving as well as the organic C present in the sediment.

$$CaCO_3 + CO_2 + H_2O \rightarrow Ca^{2+} + 2HCO_3^- \quad [4.14]$$

$$HCO_3^- + H_2O \rightarrow H_2CO_3 + OH^- \quad [4.15]$$

$$H_2CO_3 \rightarrow H_2O + CO_{2(g)} \quad [4.16]$$

CHNS analyses were conducted on the sediments to see if the sediment gained or lost C during the incubations (Figure 4.5).

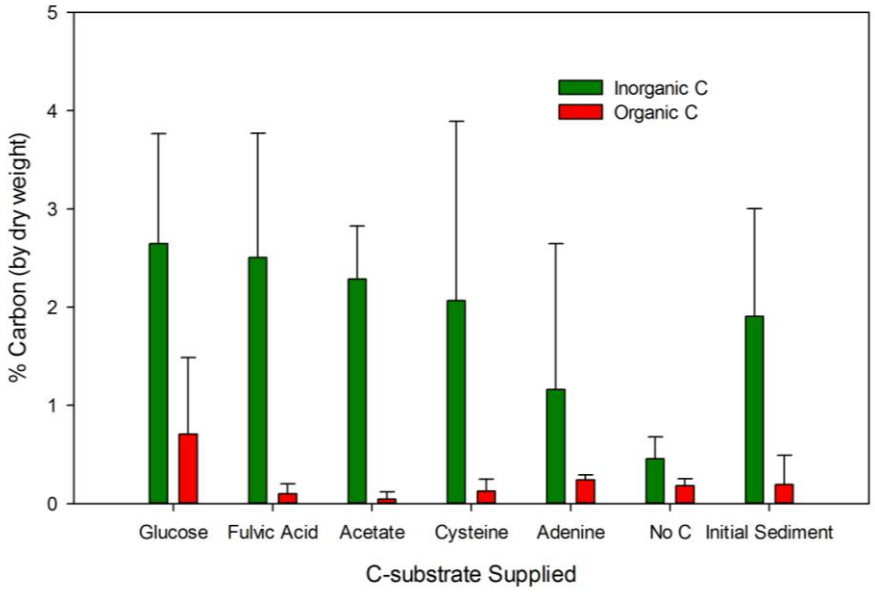


Figure 4.5: Average organic and inorganic carbon (C) measurements of the initial and end sediment from the individual FTRs for each C-substrate.

The inorganic C results are in agreement with the PHREEQCi predictions for the no carbon control FTRs and the acetate supplied FTRs. FTRs supplied with no carbon show much less inorganic carbon in the sediment than the initial sediment samples, indicating calcite could be dissolving. Acetate supplied FTRs, on the other hand, shows a higher level of inorganic carbon and there could be precipitating calcite as predicted by PHREEQCi. The rest of the samples all have approximately the same calcite SI (-0.343 to -0.695) but with varying inorganic carbon amounts compared to the initial sediment, so this again does not explain the gain or loss or carbon in the remaining cases. It can be assumed that the carbon changes in these FTRs are more

strongly linked to growth and death of the present microbial populations or adsorption of the C-substrate to the clayey soils.

Fulvic acid, according to DOC measurements, is reduced in the outflow of FTRs supplied with fulvic acid. There are a few reasons this could happen. The first reason being further degradation, discuss previously in Section 4.1.3. The second reason is the structure of fulvic acid. Fulvic acid is thought to contain several positive and negative sites (Gaffney et al., 1996). Positive sites can cause adsorption to the negative charge on the clayey sediment. It is hard to say for sure if there is a difference between the organic C in the sediment or not when compared to the initial sediment.

4.1.2.3 MPN

Since there is denitrification and C-substrate degradation occurring in the FTRs, there is likely growth in biomass as cells live and replicate or die. Dead and living cells could possibly contribute to the DOC measurements in the outflow, but the filters covering the outflow filter block will limit this contribution to the dissolved constitutions leaching from cell lysis. How much biomass contributes is also dependent on abundance and which C-substrate is present in the input. MPN was determined and shows that the most microbial growth in term of nitrate reducing bacteria (NRB) is likely occurring in FTRs supplied with acetate, adenine and glucose. Since acetate, adenine and glucose supplied FTRs show higher estimates of NRB then the initial sediment, this indicates a possible enrichment of NRB in these FTRs series. Estimated amounts of NRB present in FTRs supplied with cysteine and fulvic acid, as well as the controls (no carbon) are comparable to the estimates from the initial sediment suggesting that enrichment of the NRB did not happen in those FTRs. Cysteine and adenine FTRs also show the presence of sulfide oxidizing bacteria (SOB), mostly in the lower ranges when compared to NRB.

4.2 Rates

4.2.1 NOSC and ΔG as Predictive Tools

The fact that different denitrification rates were determined shows that C-substrates play a large role in NO_3^- reduction rates. Rates ranged from 0 to 114 nmol NO_3^- cm^{-3} h^{-1} based on the type of C-substrate supplied, and the flow rate. FTRs supplied with acetate yield the highest denitrification rate, even though glucose and cysteine were predicted to yield the highest rates according to ΔG_R and adenine was predicted by NOSC. The trends predicted at the beginning of the experiment, such as an increase in R_D as NOSC increase or as ΔG_R decrease, were either not observed or weakly observed respectively.

Table 4.7: Calculated and measured parameters for each C-substrate. ΔG_R and ΔG_{Cox} are shown in units of kJ per electron transferred from C and R_D are in nmol cm^{-3} h^{-1} calculated at a flow rate of 2 ml h^{-1}.

C-substrate	ΔG_R	ΔG_{Cox}* Estimated	ΔG_{Cox} Calculated	NOSC	R_D
Acetate	-96.4	60.3	18.0	0	71.9
Glucose	-111.7	60.3	10.1	0	44.2
Cysteine	-92.1	40.4	14.3	0.7	55.4
Adenine	-32.9	3.3	-115.3	2.0	46.2
Fulvic Acid	52.2	52.7	76.1	0.3	N/A
No carbon	N/A	N/A	N/A	N/A	N/A

*Estimated by equation: $\Delta G_{Cox}=60.3-28.5 \bullet NOSC$, where ΔG_{Cox} is the Gibbs Free Energy of the half reaction of C oxidation and NOSC is the nominal oxidation state of C (LaRowe and Van Cappellen, 2011)

The hypothesis that a high NOSC is indicative of a high R_D is rejected based on the results of this experiment. NOSC predictions do not correspond with the resulting R_D (Table 4.7). Predictions using NOSC are almost the complete opposite of what was found in this experiment. Adenine was predicted to have the fastest rate, but the results show it actually has the slowest. Fulvic acid is also predicted by NOSC to be utilized but did not induce denitrification in this experiment. When adenine degradation is coupled with denitrification, it produces the least

amount of energy of the C-substrates used according to ΔG_R calculations (except for fulvic acid). In the case of denitrification, if one was to rely on NOSC calculations to predict the more suitable C-substrate, i.e. a C-substrate that would induce a faster denitrification, it would seem that the opposite trend is more correct in this study: the more negative the NOSC, the more likely the C-substrate will be utilized and a faster rate should be produced. However, this approach still does not work for fulvic acid or for other studies found in the literature (as shown in Table 4.7) that used a variety of C-substrates. As shown in Figure 4.6, no trend was found between the rate of denitrification and NOSC ($R^2 = 0.05$; Figure 4.6 A). Comparing the rates found in the literature for denitrification with the NOSC of the C-substrates used also suggests that NOSC is a poor predictor of denitrification rates. There is a wide variety of R_D for each C-substrate but only 1 NOSC. NOSC estimate does not account for different environmental conditions, such as heterogeneity and changing redox conditions. This indicates that additional factors other than NOSC and thermodynamics are important to incorporate to develop better predictive tools.

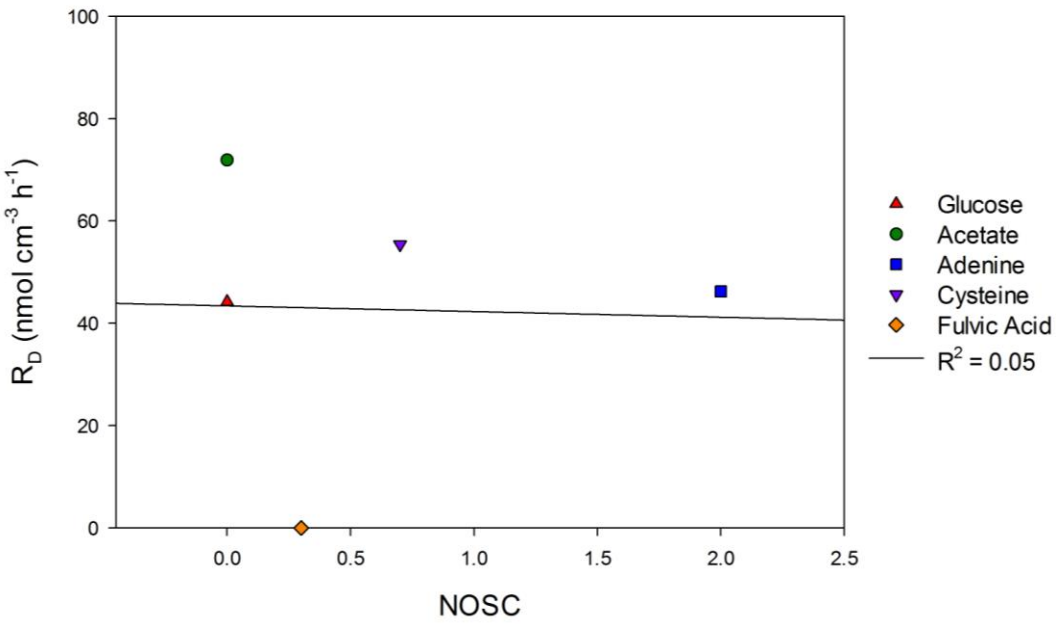


Figure 4.6: A comparison of R_D and NOSC showing no significant trend.

Table 4.8: Calculated NOSC from C-substrates found in the literature and the R_D.

NOSC	R_D nmol cm^{-3} h^{-1}	C-substrate	Chemical Formula	Reference
1	8.6-58.5	Citric Acid	$C_6H_8O_7$	Wu, 2010
0.67	2.8	Alginic Acid	$C_6H_8O_6$	Wu, 2010
0.3	0	Fulvic Acid	Similar to this experiment	Wu, 2010
1.0	151.52	Fumerate	$C_4H_4O_4$	Oa et al., 2006
2.0	53.03	Formate	CHO_2^-	Oa et al., 2006
0.0	49.24	Lactate	$C_3H_6O_3$	Oa et al., 2006
-0.3	18.94	Propionate	$C_2H_5CO_2$	Oa et al., 2006
-2.0	3.79	Ethanol	C_2H_6O	Oa et al., 2006
-2.0	37.88	Methanol	CH_4O	Oa et al., 2006
N/A	56.82	Hydrogen	H_2	Oa et al., 2006

The negative trend found in LaRowe and Van Cappellen (2011) between NOSC and ΔG_{Cox} is also found in this experiment, but predicts the C-substrate utilization in the wrong order, as well as with a much greater slope (Figure 4.7). The order predicted, from highest to lowest, is almost opposite the trend found by the R_D values: adenine, cysteine, fulvic acid, glucose and acetate. LaRowe and Van Cappellen (2011) used approximately 50 C-substrates to determine the trend with NOSC and ΔG_{Cox}; in this experiment only 5 C-substrates were used. Thus, there is the potential that the C-substrates chosen in this experiment are misrepresentation of a larger trend, such as that found in LaRowe and Van Cappellen (2011). However, without actual experimental data on all 50 C-substrates, this more general trend cannot be verified, and NOSC predicts C-substrates utilization in the wrong order for the current experiment with 5-compounds.

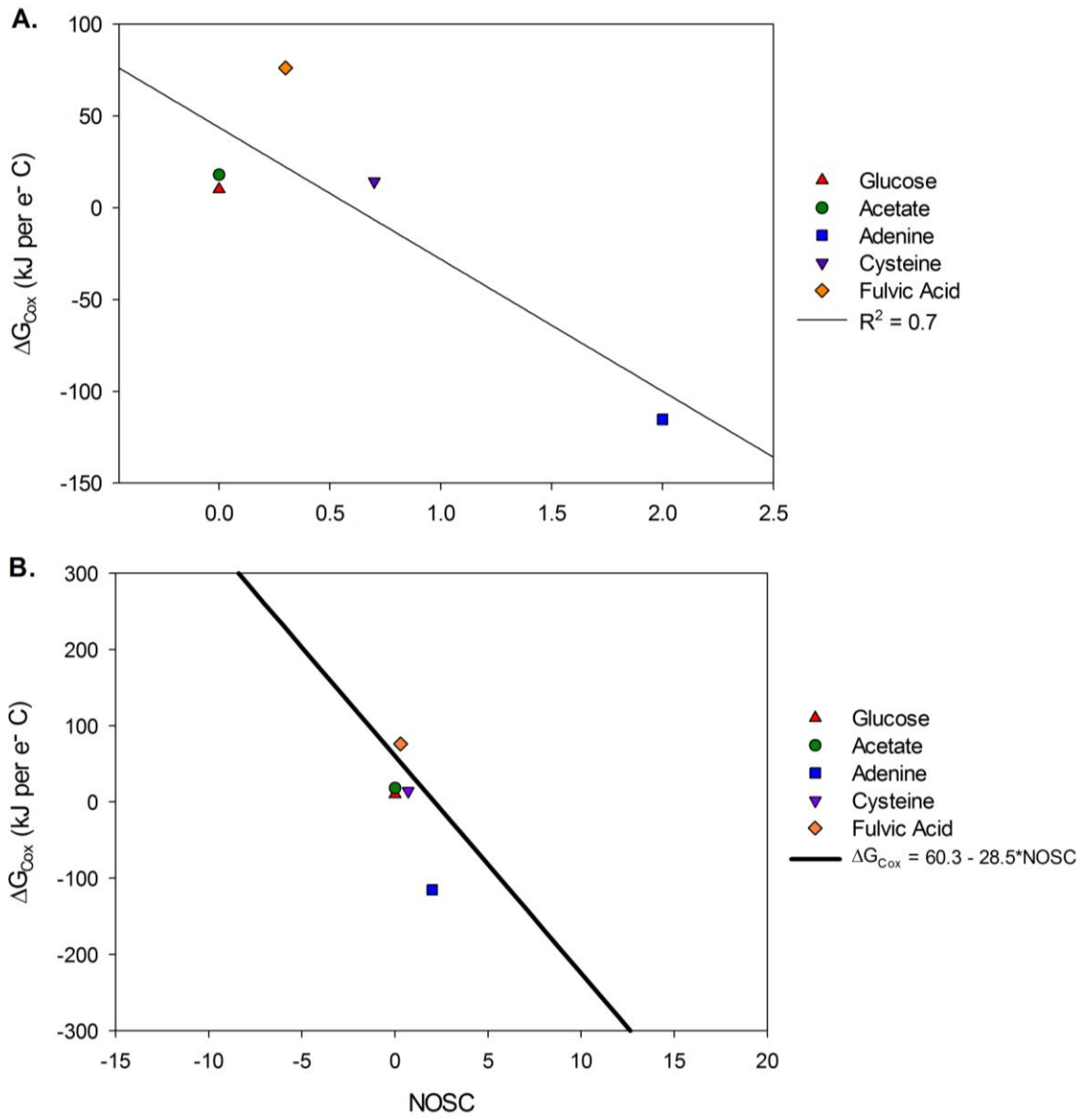


Figure 4.7: (A) A comparison between ΔG_{Cox} (kJ per e^- transferred from C) and NOSC showing a negative trend. (B) The comparison of the negative trend found in this experiment to the negative trend from LaRowe and Van Cappellen (2011).

No trend was found between NOSC to ΔG_R unless fulvic acid fed FTRs are excluded, then a strong positive trend is found (Figure 4.8). A positive trend proves the initial hypothesis is wrong, since it was predicted that a higher NOSC would mean a C-substrate would provide more energy, which would result in a negative trend. It is well known that different TEA's produce different amounts of energy and play an important role in C degradation. A C-substrate that

readily degrades with oxygen as a TEA, may not degrade so readily with NO_3^- or SO_4^{2-} as a TEA source and therefore a C-substrate that was considered labile will become more inert depending on the conditions presented (Appelo and Postma, 2005). The NOSC trends take into account ΔG_{Cox} and not ΔG_R, and so are unreliable since they do not incorporate the different energy yields in different redox environments. Attempting to predict reaction rates should be carried out on the basis of full reactions stoichiometry between TED and TEA, and not just based on the oxidation state of the C-substrates.

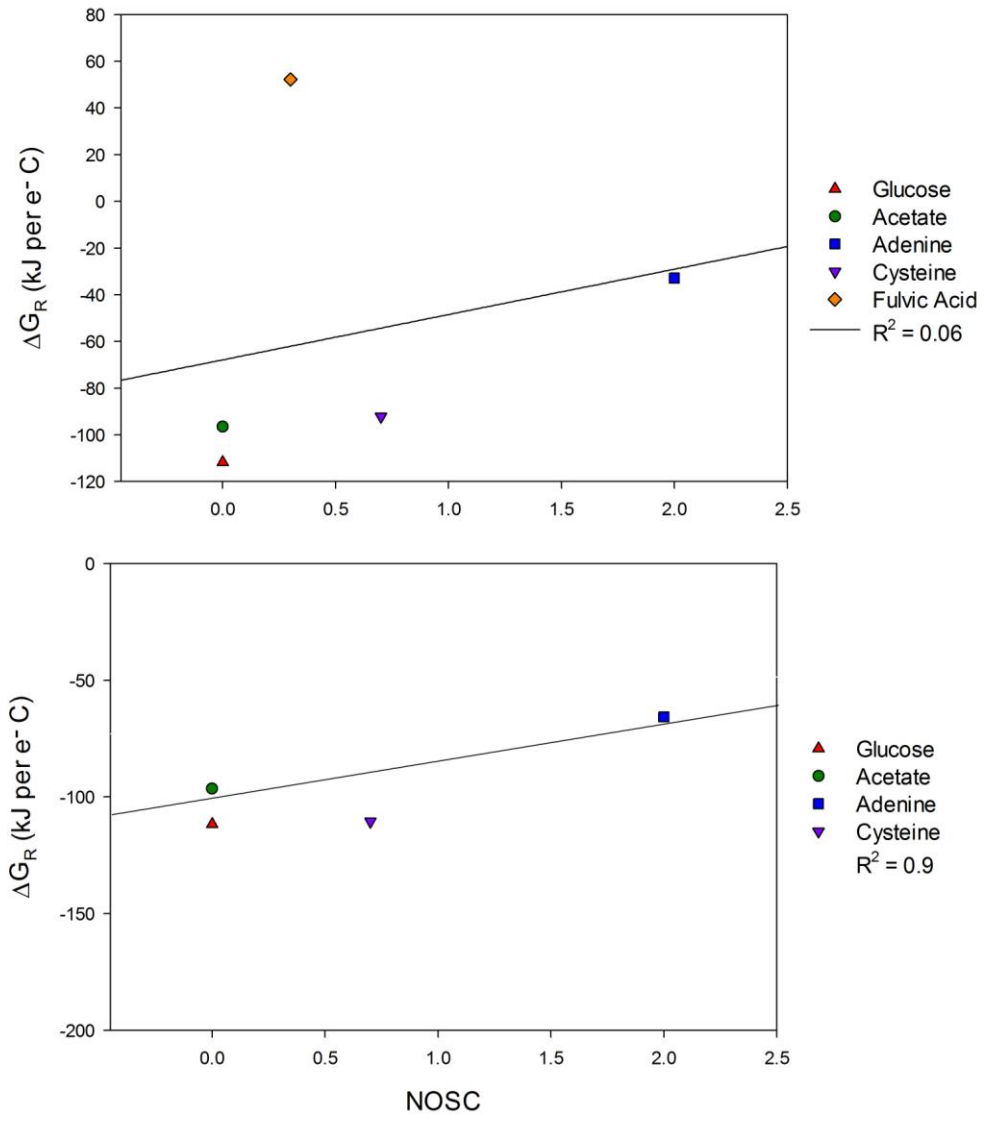


Figure 4.8: (A) A comparison of ΔG_R and NOSC including fulvic acid shows no trend and (B) A comparison of ΔG_R and NOSC excluding fulvic acid shows a positive trend.

The predicted order of preferred C-substrate using ΔG_R calculations is a close estimate but not an accurate one. In this experiment, utilization of C-substrates was predicted by ΔG_R to yield the order: glucose, cysteine, acetate, adenine and fulvic acid, from highest to lowest rates. Fulvic acid shows a positive ΔG_R and therefore the reaction with NO_3^- was predicted not to proceed. As shown on Figure 4.9, there is an inverse trend ($R^2 = 0.8$) between the rate and the ΔG_R. Although the order predicted by ΔG_R is not an exact match to the order of rates determined by the experiment, only two C substrates are out of order: glucose and acetate. Glucose supplied FTRs were predicted to have the fastest denitrification rate while, acetate supplied FTRs were predicted to have the 3rd fastest. The results show that these predictions should be switched, since acetate produced the fastest rate and glucose produced the 3rd fastest.

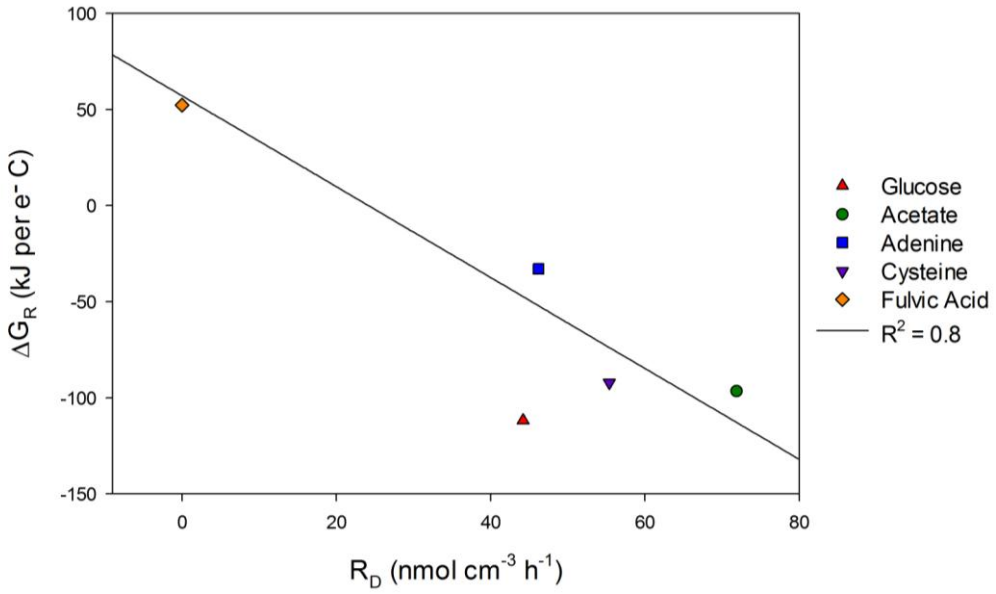


Figure 4.9: ΔG_R in kJ per e^- transferred from C-substrate compared to R_D (nmol cm^{-3} h^{-1}).

It is well known that denitrifying bacteria utilize acetate as a TED easily, however it has not clearly been determined why. Previous studies have been able to determine that microbes that degrade acetate use the citric acid cycle to produce energy from ATP (Thauer et al., 1989), as opposed to glycolysis, which is the breakdown of sugars such as glucose (Kaiser, 2009). Acetate, in many cases, is the lowest oxidation level of organic C that aerobic and anaerobic

microorganism can degrade C-substrates to (Thauer et al., 1989). That being said, and noting the small size of acetate, perhaps there is some kind of evolutionary pathway that led to the citric acid cycle and therefore acetate being easily degraded by denitrifying bacteria. Although there was no direct literature found proving microbial communities evolved to utilize acetate directly, it is implied by several articles in the literature that speculate on the evolutionary development of the citric acid cycle (Schnarrenberger and Maritn, 2002; Melendez-Hevia et al., 1996). Acetate, due to its small size, has a high diffusion rate and therefore can be up-taken by microbes more easily. Since many microorganisms can produce acetate from processes such as: fermentation, denitrifiers and other organisms, many have evolved to utilize the C-substrate that was most abundant in their surroundings, which in this case was acetate. Outliers like acetate suggests that ΔG_R cannot be used alone to predict denitrification rates.

4.2.2 Effects of Flow Rates and Microbial Communities

The denitrification rates were determined at 2 ml h^{-1} for each C-substrate. According to Pallud et al. (2006) R_D should be independent of flow, however, in this experiment rates increase as the flow rate increases (Table 4.9; Figure 4.10). The dependency on flow is potentially due to the selected concentrations of NO_3^- and C-substrates. It is likely that the C-substrate concentrations used in each case, excluding acetate, is a limiting factor, since NO_3^- was still present in the outflow of each. However, in the case of acetate, NO_3^- was the limiting factor since at flow rates of 1 ml h^{-1} and 2 ml h^{-1}, there was no detectable nitrate in the outflow. As shown on Figure 4.11, if the concentration of a substrate is too low, it will become a limiting factor, creating either a thermodynamically inhibited process or a thermodynamically limited process. At high concentrations, the reaction is far from equilibrium and the process becomes kinetically controlled assuming the reaction is thermodynamically favourable to the microbial community (LaRowe and Van Cappellen, 2011). This suggests that when working under ideal conditions with ideal concentrations, ΔG can be a useful tool in predicting denitrification rates. However, in natural environments, ideal conditions are highly unlikely and therefore flow rates or more specifically, substrate supply rates, will play a major role in degradation and denitrification rates.

Table 4.9: Potential denitrification rates (R_D ; in nmol cm^{-3} h^{-1}) for each C-substrate as a function of the imposed flow rates during the FTR experiments.

Flow rate (ml h^{-1})	Acetate	Glucose	Cysteine	Adenine	Fulvic Acid
1	36.0±13	31.0±11	31.0±10	30.4±10	0.9±0.3
2	71.9±26	44.2±16	55.4±17	46.2±15	0.0±0.5
4	114.5±41	67.3±25	N/A	N/A	N/A

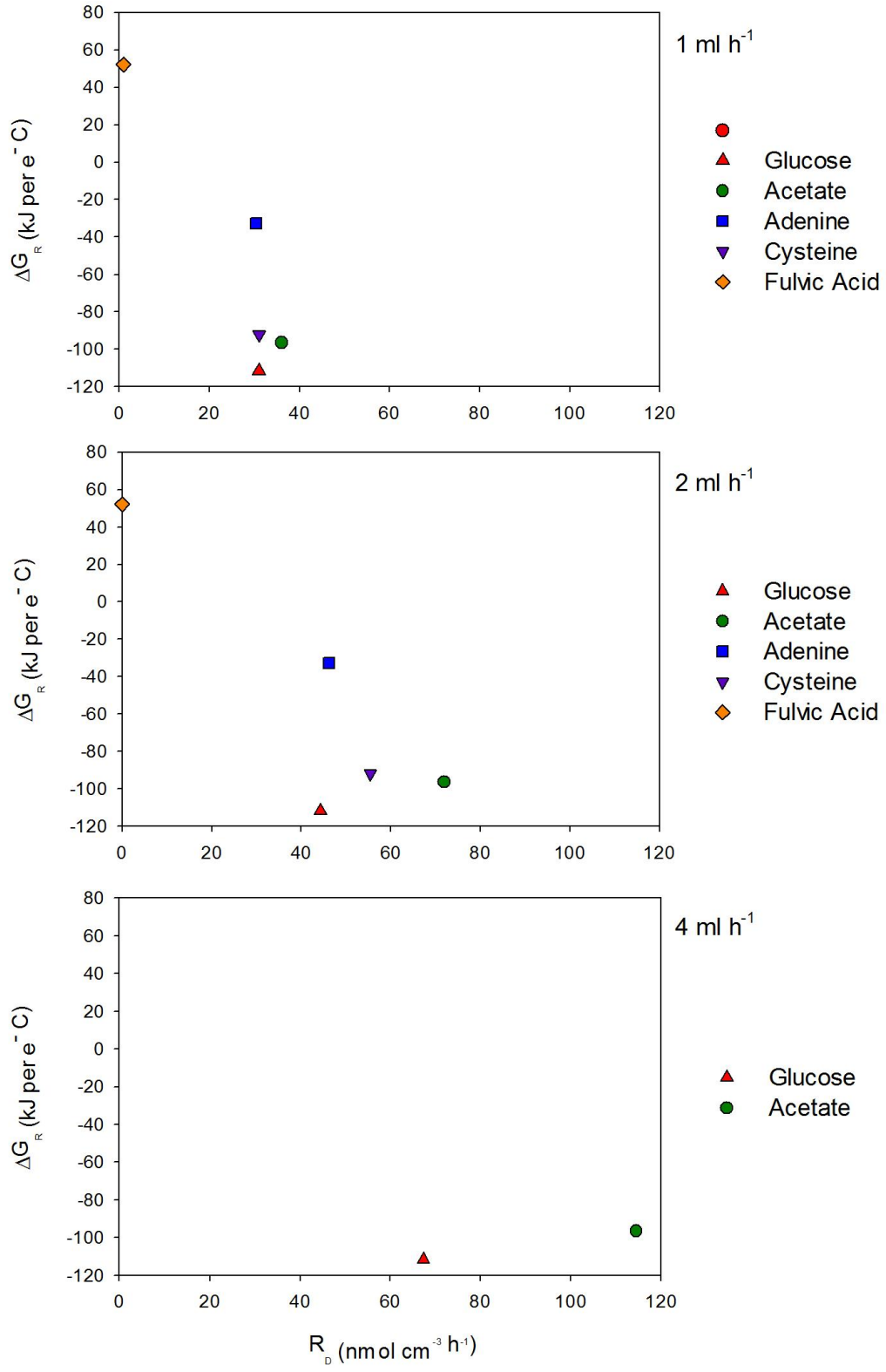


Figure 4.10: A comparison between the calculated denitrification rates at different flow rates. As the flow increases from 1 ml h^{-1} to 4 ml h^{-1} (top to bottom), denitrification rates increase in FTRs showing nitrate reduction.

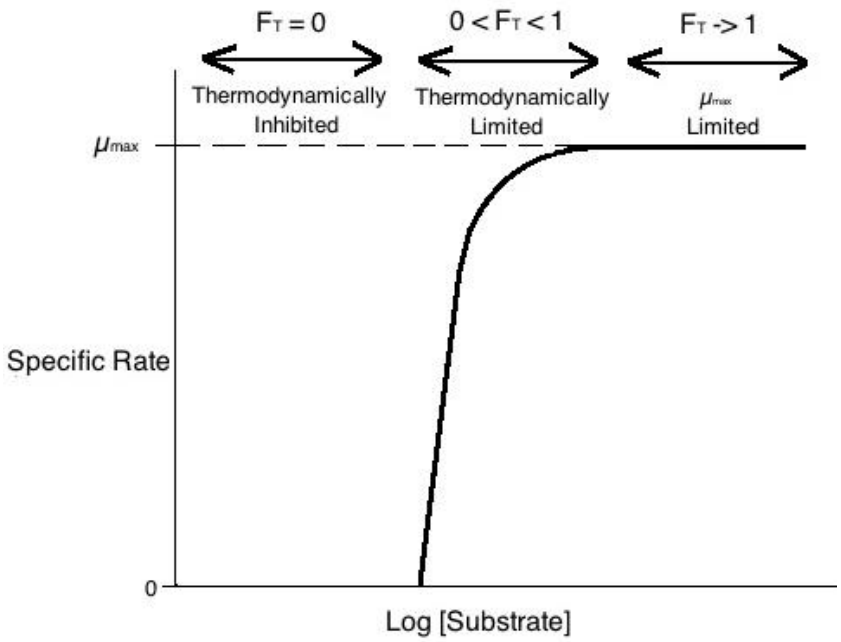


Figure 4.11: A representation of how rates, in general, are dependent on concentration in terms of thermodynamics (redrawn from LaRowe and Van Cappellen, 2011).

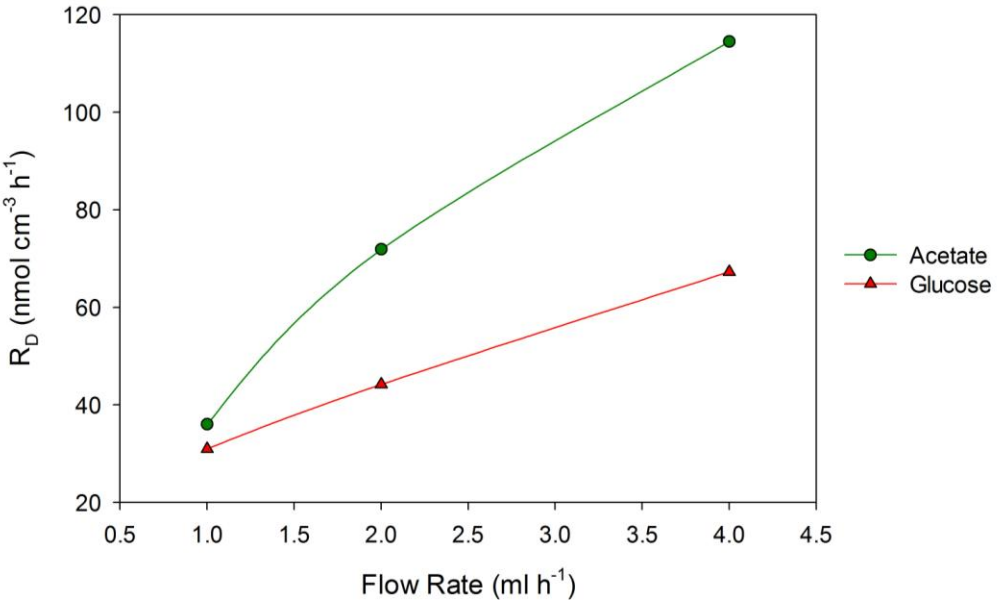


Figure 4.12: A comparison of how denitrification rates change with flow for FTRs supplied with acetate and glucose.

If Figure 4.11 and 4.12 are compared, it can be seen that either the concentration of N or of C is limiting for FTRs supplied with glucose and acetate. As the flow rate is increased the concentration inside the FTR is increased, which in turn increases the denitrification rate. Figure 4.12 appears to be following a similar trend to that shown in Figure 4.11. The denitrification rates in this experiment are not maximum rates and this is reemphasized by this analysis. Also, Figure 4.12 further demonstrates the significant differences in denitrification rates produced by supplying different C-substrates.

The microbial community present in the FTRs could also play a role in denitrification rates. It is assumed that in the FTRs the microbial populations started off more or less the same, since the sediment cores were all collected from the same m^2 area of Lake Belwood. It is likely that the conditions in the FTRs favored the growth of specific microbial communities due to the presence of certain C-substrates, thus changing the microbial communities from the initial populations. These speculations stem from the fact that cysteine supplied FTRs produced ammonium and sulphate in the outflow and that adenine supplied FTRs also produced ammonium but other C-substrates did not, which indicates the presence of different microbes. In order to produce sulfate, the present of sulfide oxidizing bacteria (SOB) is required. Their presence was shown in varying numbers through MPN indicating that cysteine fed FTRs had a much higher presence of SOB than FTRs supplied with adenine. Different microbial population evolution can likely play a large role on denitrification rates. Further research is currently being conducted to support or disprove this speculation. These speculations are also presented in works by Paul et al. (1989) when comparing different C-substrates as TEDs. They reason that when differing amounts of e^- are transferred per mole of C, that there is competition between different microbes trying to use the same C-substrate for different purposes. There is also varying complexity of different C-substrates, which require different enzymes in order to be broken down by different microbes. All of these factors very likely play a role in this experiment and further indicate the importance of microbial communities in natural systems. Although microbes such as NRB and SOB are ubiquitous in the environment, specific groups will thrive when the right geochemical conditions are present. It is highly unlikely, however, that the specific conditions provided by a simplistic input solution will be found in the environment and consideration of the effects of such an input solution should be taken when studying natural systems.

4.2.3 Denitrification studies containing different carbon substrates

There are a lot of denitrification studies in the literature focusing on various C-substrates such as: methanol, ethanol, glycerol, acetate and glucose (Calderer et al., 2010), but very little can be found on the effects of adenine, cysteine and fulvic acid (Table 4.10). Cysteine and adenine, although present in the environment since they are essential amino acids in all living organisms, are not considered in denitrification studies. So, information on the denitrification processes involving amino acids is hard to find. Amino acids are important to take into account should they be utilized by microbial populations as a TEDs in the environment, in terms of the byproducts amino acids can produce (i.e. SO_4^{2-}, HS^-, NH_4^+ etc.).

Table 4.10: Table summarizing conditions and rates (if available) from literature in alphabetical order and includes rates from this experiment at the end. K_m is the half saturation constant for NO_3^-, C:N is the carbon to nitrogen ratio used in the experiment and T is the temperature in degrees Celsius.

K_m µM	R_D nmol cm^{-3} h^{-1}	% NO_3^- Removed	Material	Method	C-substrate Added	C:N†	T °C	Location	Reference
N.A.	44.2	>99	Local Sediment	Batch/Column/Field	Acetate	1.25	20	New Mexico (USA)	Abdelouas et al., 1999
N.A.	793.8	99	Anaerobic sludge	Batch experiment	Glucose	5.4	30	Narbonne (FR)	Akunna et al., 1993
N.A.	259.6	99	Anaerobic sludge	Batch experiment	Acetate	4.8	30	Narbonne (FR)	Akunna et al., 1993
N.A.			Anaerobic sludge	Batch experiment	Glycerol	4.8	30	Narbonne (FR)	Akunna et al., 1993
N.A.			Anaerobic sludge	Batch experiment	Lactic Acid	5.0	30	Narbonne (FR)	Akunna et al., 1993
N.A.	n.d.		Anaerobic sludge	Batch experiment	Methanol	3.7	30	Narbonne (FR)	Akunna et al., 1993
4.2–6.3	110–130	82-108	Streamside soil	Slurries	Glucose		2-22	Copenhagen (DK)	Ambus (1993)
N.A.	3.8	52	Subsurface Soil	Batch experiment	Acetate	4	17	Argentona (SP)	Calderer et al., 2010
N.A.	4.5	63	Subsurface Soil	Batch experiment	Acetate + Glucose	4	17	Argentona (SP)	Calderer et al., 2010
N.A.	3.1	44	Subsurface Soil	Batch experiment	Glucose	2	17	Argentona (SP)	Calderer et al., 2010
N.A.	2.7	38	Subsurface Soil	Batch experiment	Glucose	4	17	Argentona (SP)	Calderer et al., 2010
N.A.	6.3	90	Subsurface Soil	Batch experiment	Glucose	7.9	17	Argentona (SP)	Calderer et al., 2010
N.A.	60.5	>96	Subsurface Soil	Batch experiment	Glucose	4	17	Argentona (SP)	Calderer et al., 2010
N.A.	89.4	N.A.	Lake Sediment	FTR	--	N.A.	N.A.	Haringvliet (NL)	Canavan et al. (2006)
N.A.	N.A.	34.8	Activated Sludge	Batch experiment	Acetate	4.5	N.A.	Chungli (ROC)	Chou et el., 2003
N.A.	N.A.	35.4	Activated Sludge	Batch experiment	Glucose	4.5	N.A.	Chungli (ROC)	Chou et el., 2003
N.A.	N.A.	38.5	Activated Sludge	Batch experiment	Methanol	4.5	N.A.	Chungli (ROC)	Chou et el., 2003
N.A.	23.8	>95	Sludge from WWTP	Batch experiment	Acetate	2.33	10	Auckland (NZ)	Elefsiniotis and Li, 2006
N.A.	23.8	<96	Sludge from WWTP	Batch experiment	Acetate	4.67	10	Auckland (NZ)	Elefsiniotis and Li, 2006
N.A.	29.6	<97	Sludge from WWTP	Batch experiment	Acetate	2.33	10	Auckland (NZ)	Elefsiniotis and Li, 2006

N.A.	29.6	<98	Sludge from WWTP	Batch experiment	Acetate	4.67	10	Auckland (NZ)	Elefsiniotis and Li, 2006
N.A.	42.5	<99	Sludge from WWTP	Batch experiment	Acetate	2.33	10	Auckland (NZ)	Elefsiniotis and Li, 2006
N.A.	42.5	98	Sludge from WWTP	Batch experiment	Acetate	4.67	10	Auckland (NZ)	Elefsiniotis and Li, 2006
13–640	0.4–119.4*	N.A.	River sediment	Intact cores	Fresh water medium	12.5-19.5	15	Swale-Ouse river (UK)	Garcia-Ruiz et al. (1998)
N.A.	N.A.	10-100	Anaerobic sludge	Batch experiment	Acetate	0-29	30	Tainan (ROC)	Her and Huang, 1995
N.A.	N.A.	10-100	Anaerobic sludge	Batch experiment	Glucose	0-29	30	Tainan (ROC)	Her and Huang, 1995
N.A.	N.A.	9.2-100	Anaerobic sludge	Batch experiment	Methanol	0-10.5	30	Tainan (ROC)	Her and Huang, 1995
N.A.	N.A.	69.7-92	Anaerobic sludge	Batch experiment	Benzoic Acid	2.1-3.4	30	Tainan (ROC)	Her and Huang, 1995
17–100	300–1500	N.A.	Lake sediment	Slurries	--	N.A.	8	Lake Vechten (NL)	Hordijk et al. (1987)
n.d.	1.8-5.4	20-60	Grazed grassland	Intact core, incubated	Glucose	1.9	15	Wexford (Ireland)	Jahangir et al. (2012)
n.d.	1.6-4.0	18-45	Grazed grassland	Intact core, incubated	DOC	1.9	15	Wexford (Ireland)	Jahangir et al. (2012)
2–170	1–8	N.A.	Subtidal Sediment	Intact cores	--	7.4	*in situ*	Tomales Bay (USA)	Joye et al. (1996)
270–800	274–933	80-100	Intertidal Sediment	FTR	Acetate	2-30	20-55	Appels (NL)	Laverman et al. (2006)
270–510	662–2400	80-100	Intertidal Sediment	Slurries	Acetate	2-12	4-30	Appels (NL)	Laverman et al. (2006)
250	100–325	80-100	Intertidal Sediment	FTR	Acetate	2-30	20	Waarde (NL)	Laverman et al. (2006)
220	98–155	80-100	Subtidal Sediment	FTR	Acetate	4-60	8-18	Haringvliet (NL)	Laverman et al. (2006)
1mM	179-233	N.A.	River Sediment	FTR	--	N.A.	21±2	Tresmes (FR)	Laverman et al. (2011)
N.A.	8.6-58.5	N.A.	Riparian buffer zone soil	Packed Flow Column	Citric Acid	0.8-3.2	N.A.	Goldsboro (USA)	Lin Wu MSc 2010
N.A.	2.8	N.A.	Riparian buffer zone soil	Packed Flow Column	Alginic Acid	0.8-3.2	N.A.	Goldsboro (USA)	Lin Wu MSc 2010
N.A.	n.d.	N.A.	Riparian buffer zone soil	Packed Flow Column	Suwannee River DOC	0.8-3.2	N.A.	Goldsboro (USA)	Lin Wu MSc 2010

218	89	9-23	Lake sediment	Slurries	--	N.A.	14-35	Lake Okeechobee (USA)	Messer & Brezonik (1983)
N.A.	30.3	43	Subsurface Soil	Batch experiment	Acetate	0.63	N.A.	Chungnam Province (SK)	Oa et al., 2006
N.A.	151.5	100	Mountain soils	Batch experiment	Fumerate	0.42		Chungnam Province (SK)	Oa et al., 2006
N.A.	53.0	100	Mountain soils	Batch experiment	Formate	2.5		Chungnam Province (SK)	Oa et al., 2006
N.A.	49.2	79	Mountain soils	Batch experiment	Lactate	0.42		Chungnam Province (SK)	Oa et al., 2006
N.A.	18.9	61	Mountain soils	Batch experiment	Propionate	0.36		Chungnam Province (SK)	Oa et al., 2006
N.A.	3.8	79	Mountain soils	Batch experiment	Ethanol	0.42		Chungnam Province (SK)	Oa et al., 2006
N.A.	37.9	48	Mountain soils	Batch experiment	Methanol	0.83		Chungnam Province (SK)	Oa et al., 2006
N.A.	56.8	53	Mountain soils	Batch experiment	Hydrogen	2		Chungnam Province (SK)	Oa et al., 2006
50	0.16-0.24*	8.5-17.6	Intertidal Sediment	Slurries	--	N.A.	20	San Francisco Bay (USA)	Oremland et al. (1984)
344	18	N.A.	Subtidal Sediment	Slurries	--	N.A.	12	Kysing fjord (DK)	Oren & Blackburn (1979)
n.d.	0.8	N.A.	Riparian zone sediment	insitu	--	N.A.	0.5-17.8	Wiilow Bush, Neiderneunforn (Switerland)	Peter et al. (2012)
n.d.	n.d.	N.A.	Desert soil	Slurries	Dextrose	1.1829545 45	30	Chihuahuan desert (USA)	Peterjohn (1991)
	7.9**	N.A.	River bed sediment	Batch experiment	Acetate	0.6-1.7	4-22	Colorado (USA)	Pfenning and McMahon (1996)
	5.4**	N.A.	River bed sediment	Batch experiment	Fulvic Acid (groundwater)	0.6	4-22	Colorado (USA)	Pfenning and McMahon (1996)
	6.0**	N.A.	River bed sediment	Batch experiment	Fulvic Acid (suface water)	0.6	4-22	Colorado (USA)	Pfenning and McMahon (1996)

200–1700	12–25	N.A.	Coastal marine	Cores and Slurries	Natural Sea Water	N.A.	22±2	Mediteranean coast (FR)	Raymond et al. (1992)
N.A.	892.5	>99	Methanogenic Culture	Batch experiment	Acetate	N.A.	35	Atlanta (USA)	Tugtas and Pavlostathis, 2007
N.A.	255	>99	Methanogenic Culture	Batch experiment	Glucose	N.A.	35	Atlanta (USA)	Tugtas and Pavlostathis, 2007
N.A.	0.2	99	Lake sediment	Microcosms	Glucose	5500	25	Lake Taihu (China)	Wang et al., 2007
N.A.	0.4	99	Lake sediment	Microcosms	Acetate	2032	25	Lake Taihu (China)	Wang et al., 2007
N.A.	114.5	63-96	Reservoir Sediment	FTR	Acetate	1.9	22±2	Lake Belwood (CA)	This Study
N.A.	67.3	47-86	Reservoir Sediment	FTR	Glucose	1.9	22±2	Lake Belwood (CA)	This Study
N.A.	55.4	76-85	Reservoir Sediment	FTR	Cysteine	2.3	22±2	Lake Belwood (CA)	This Study
N.A.	46.2	63-84	Reservoir Sediment	FTR	Adenine	3.8	22±2	Lake Belwood (CA)	This Study
N.A.	0	n.d.	Reservoir Sediment	FTR	Suwannee River Fulvic Acid	1.9	22±2	Lake Belwood (CA)	This Study

* nmol cm^{-2} h^{-1} **nmol g^{-1} h^{-1} †carbon to nitrogen ratio supplied in the experiment

The purpose of this literature comparison is to try and link a variety of studies together. There is a lot of variability in denitrification rates found in the literature as shown in Table 4.10. The rates differ by C-substrate, soil type, temperature, microbial population (natural versus lab culture) and C:N ratio. Redox conditions, pH the type of matrix being used to host the reaction (agar, broth, soil etc.) and NO_3^- concentration also play a role and vary in the presented studies. Most studies focus on the addition of acetate and glucose or *in situ* C in the sediment to estimate R_D, but use completely different sediment or experiment type. For example, studies looking at glucose as an added C source from Table 4.10 all operate under different parameters and produce very different results. Janhangir et al. (2012) used glucose as a TED and present R_D values ranging from 1.8 to 5.4 nmol cm^{-3} h^{-1}, using intact cores collected from grazed grasslands. Discordantly, Calderer et al. (2010) used subsurface soil in batch experiments and found the range of R_D to be 2.7 to 60.5 nmol cm^{-3} h^{-1}. Other studies that use glucose, such as those done by Akunna et al (1993) and Ambus (1993), find R_D value much higher 110 to 130 nmol cm^{-3} h^{-1} and 794 nmol cm^{-3} h^{-1} using anaerobic sludge in batch experiments and streamside soil in slurries, respectively. Due to these major differences in studies, it is hard to observe any consistent trends in denitrification patterns. Therefore, the following literature review and comparison is purely speculative based on available information.

There are conflicting studies that look at acetate and glucose as TEDs for denitrification. A study by Calderer et al. (2010) compared acetate and glucose, using a C:N ratio of 4, in subsurface soils. A C:N ratio is a good way to compare since you are using the same amount of C from each C-substrate and the same amount of N from NO_3^-. They determined that the combination of acetate and glucose produce the highest R_D and that glucose alone produced at the slowest rate compared to acetate. Although glucose showed the slowest rate, further experiments were continued with glucose due to the build up of NO_2^- found in the flasks when adding acetate or the combination of glucose and acetate. Another study by Chou et al. (2003) finds different results when comparing glucose and acetate in batch experiments; glucose gives a faster rate and has the highest NO_2^- build up. It should be noted however, that Chou et al. (2003) used activated sludge and not subsurface soil like the study conducted by Calderer et al. (2010). When looking at percentage of NO_3^- removal presented by Chou et al. (2003), there is little difference between glucose and acetate, 35.4% and 34.8% respectively. The experiment at hand agrees with parts from both Calderer et al. (2010) and Chou et al. (2003) in the sense that acetate fed FTRs were found to produce the highest rate, but glucose fed FTRs produced the most NO_2^- in the outflow.

According to Her and Huang (1995), a minimum C:N ratio of about 2 is required for both glucose and acetate in order to produce denitrification efficiencies over 97%. Their batch experiments consisted of acclimatized denitrifying sludge and were carried out at 30°C. Calderer et al. (2010) found that for glucose, a minimum C:N ratio of 7.9 was required to produce the efficient denitrification, however their batch experiments were performed with natural aquifer sediments at 17°C. The study carried out in this experiment used C:N values of approximately 1.9 for glucose and acetate and found NO_3^- removal to be 47-86% and 63-96% respectively, but was dependent on the flow rate since they were not batch experiments. Slower flow rate had lower denitrification rates and slightly lower NO_2^- accumulation, but higher percentage of NO_3^- removal.

Information on denitrification with C-substrates cysteine and adenine is lacking in the literature. The rates found in this experiment are presented here as first estimates for these C-substrates. Denitrification rates found with cysteine and adenine were 44.2 and 36.8 nmol cm^{-3} h^{-1} respectively, which yield nitrate removal efficiencies of 76% and 63% at a flow rate of 2ml h^{-1}. A large adaptation period was needed for the microbial communities when cysteine and adenine were supplied to the FTRs. Since byproducts, such as ammonium and sulphate were produced, it is assumed that ammonium and hydrogen sulfide had inhibiting effects on the present denitrifying communities. Ammonium and hydrogen sulfide can be toxic to certain bacteria (Shiskowski, 1993; Sprott and Patel, 1986; Reis et al., 1992) and therefore would negatively impact the overall system. Due to these potentially toxic byproducts, cysteine and adenine are not ideal for denitrification unless other microorganisms are present to also utilize these byproducts. Amino acids may not be the first choice for microbial populations to utilize as a TED, however, the knowledge that they can be used should be taken into account, since the R_D's found are within the range found by other studies for different C-substrates including some that use glucose and acetate as TEA in *in situ* studies (Table 4.10).

Fulvic acid is another C-substrate lacking in the literature for its effects on denitrification, likely because its exact structure is unknown and it is a complex mixture of C compounds. However, a few publications do exist. This study both agrees and conflicts with existing literature. In this study, fulvic acid was found not to induce denitrification. A very small R_D of 0.9 nmol cm^{-3} h^{-1} was found at a flow rate of 1 ml h^{-1}. However, denitrification is not found at a flow rate of 2 ml h^{-1}. With all other C-substrates used in this experiment, R_D increased as the flow rate increased, therefore the R_D calculated for fulvic acid at 1 ml h^{-1} is assumed to be in error. Fulvic

acid is assumed to not promote denitrification in this study. A study done by Wu (2010) also tested Suwannee River fulvic acid as a TED and found that it did not induce denitrification either. Conversely, a study carried out by Pfenning and McMahon (1996) found that surface water derived fulvic acid (SWFA) did induced denitrification, where ground water derived fulvic acid (GWFA) had a much smaller effect but still increased denitrification rates compared to natural conditions with no C-substrate added. Another study by Gundersen (2012), found that along the flow path from a recharge zone to discharge zone, denitrification decreased. After conducting a number of experiments to compare the different dissolved organic carbon (DOC) content available throughout the flow path of the recharge zone it was found that more labile C increased as humic acids converted to fulvic acids toward the discharge zone. Denitrification still decreased however, because although C became more liable along the flow path, there simply were not sufficient amounts of C present in the discharge zone. In all 4 studies, similar SWFA was used but only half showed that fulvic acid induced denitrification. It could be estimated that the cases, which did not show denitrification with fulvic acid, were not supplied with enough C to induce any significant denitrification. However, the study by Pfenning and McMahon, used fulvic acid on a µmol scale with a C:N ratio of about 0.6, whereas this experiment used concentrations on a mmol scale with a C:N ratio of about 1.9. Concentration of C is likely not the reason these studies differ. Differences are likely attributed to using different sediment types (i.e. river sediment versus reservoir sediment), the presence of different microbial communities and different types of experiments.

Overall, the literature review shows that there are still a number of gaps in past and present studies on denitrification. Present studies in the literature point to the conclusion that denitrification rates are highly dependent on the conditions present, whether they are experimental or environmental. It is clear that C-substrates play a large role on the rate of denitrification based on the varying R_D found in different experiments, it is also clear that different sediment types, microbial populations and types of experiments also have a significant influence on the results. This experiment has tried to provide more conclusive results by providing first estimates of denitrification rates with cysteine and adenine as TEDs and trying to achieve more realistic conditions in the FTRs. Future experiments need to focus more on different C-substrates in order to produce a clearer picture of how they affect denitrification.

4.2.4 Half Saturation Values

It is a risk that in this experiment, the concentrations of C-substrates supplied to the FTRs may not have been sufficient to reach max potential rates. As previously discussed, rates vary with flow rate even though they should remain constant as flow increases. This increase shows that there is a limiting factor present in the FTRs and is likely the concentration of C or N. The C:N ratios used in this experiment, according to some studies, seem to be within an acceptable range, however this number is debated in the literature (Calderer et al., 2010). Another option that could be used to determine if concentrations of N or C are high enough in the FTRs to not be limiting factors are half saturation values (K_m). K_m's are values predicted to be half of a reactant concentration required to achieve maximum reaction rates. If the concentrations used in this experiment are well above the required K_m values, then concentration is not a limiting factor. Not enough data was available to calculate specific K_m's for this experiment, so a literature search was conducted.

For the C-substrates used in this experiment, K_m values for denitrification are few in the literature. The K_m range was found for acetate in the literature of 0.4 to 1.2 mM (Ahring and Westerman , 1987; Cherchi et al., 2009). One K_m value for glucose was found for denitrification of 2.8 mM (Bowman and Focht, 1974). Another range of K_m values was found for glucose, not for denitrification, but for the entry of glucose into red blood cells, that was similar to the value found for denitrification: 1.28-2.36 mM (Nimmo, 1978). Cysteine and adenine K_m values for denitrification were not found. Values could be found for cysteine uptake by Bacillus subtilis ranging from 0.6-2.5µM (Burguière et al., 2004) and for transport through the blood-brain barrier in mice from 63-84µM (Hosoya et al., 2002). Available information on adenine uptake points to concentrations on the order of µM, as well for K_m values (Genchi et al., 1996; Puziss et al., 1983), but a majority of literature found is based on adenosine. No studies were found that provided a K_m value for fulvic acid. Assuming the literature values found can be applied to this experiment, it seems the concentration of C-substrates used in this experiment are sufficient to not be limiting factors with the exclusion of glucose.

Some of the K_m values found for acetate are below the concentration used in this study, but most were similar, suggesting acetate is a limiting factor in the FTRs supplied with it. However, FTRs ended up being NO_3^- limited, as all NO_3^- was consumed in the outflow. K_m values for NO_3^- range from 0.02 to 1.7 mM (Laverman et al., 2006; Oren and Blackburn 1987; Arango et al.,

2007; Evrard et al. 2012), with the majority of cases involving acetate having K_m's under 1 mM. Speeds of 1 and 2 ml h^{-1} were still too slow to produce any NO_3^- in the outflow to calculate an R_D, although, 1 mM originally was thought to be a high enough concentration to produce a maximum rate, while being low enough to be more environmentally relevant. NO_3^- was a limiting factor in the acetate supplied FTRs and a higher NO_3^- concentration is required for low flow experiments with acetate. It is likely at the highest flow rate for acetate fed FTRs, 4 ml h^{-1}, acetate then became the limiting factor since the concentration used is not more then double the k_m values found.

K_m values found in the literature for cysteine and adenine were on the order of µM, however, these numbers may or may not be comparable to this experiment based on the types of studies they were used in. If the values for glucose are compared from the human body versus microbial denitrification, they are very similar. It is hard to say based on 2 values that these show K_m's should be similar in all different types of experiments, however these similar values show it is possible to have similar K_m values. K_m values found for glucose show that FTRs supplied with glucose are C-limited since the concentration used in the input for this experiment is 0.3 mM (1.9 mM C) and the K_m value range is greater than 1 mM. K_m values may not be the same for every experiment and could change based on the soil type and microbial populations present. Most denitrification studies found do not look at K_m values unless they are modeling different input concentrations, and even then, most literature was found to report C:N, as discussed above, in order to compare their studies with others.

4.3 Reaction Stoichiometry and ΔG

Stoichiometric equations used to calculate the ΔG_R for each C-substrate is based on the assumption complete denitrification to N_2 occurring in each case. However, due to the presence of intermediate species such as NO_2^- and N_2O, complete denitrification is not occurring. NO_2^- and N_2O production change the total amount of electrons transferred from C-substrates to NO_3^- and therefore changes the potential energy produced overall. In order to see the effect of incomplete denitrification on potential energy, new stoichiometries and ΔG_R's were calculated (Table 4.11). Stoichiometries in Table 4.11 were calculated by combining the previously determined stoichiometric equations (Table 2.2, Chapter 2 and Table 3.2, Chapter 3) using the percent

composition of the outflow concentrations of N species. N_2O values were estimated to be at the detection limit (0.2 mM) since the data could not be quantified.

Table 4.11: Full reaction stoichiometries relative to 1mM NO_3^- and their revised ΔG_R (ΔG_{RV}) as well as the ΔG_R originally calculated.

C-Substrate	Full Stoichiometry	ΔG_R kJ per e^- to N	ΔG_{RV} kJ per e^- to N
Glucose	$0.12C_6H_{12}O_6 + NO_3^- \rightarrow 0.01N_2 + 0.58NO_2^- + 0.2N_2O + 0.7HCO_3^- + 0.22H_2O + 0.29H^+$	-110.5	-13.4
Acetate	$0.56CH_3COO^- + NO_3^- + 0.31H^+ \rightarrow 0.42N_2 + 0.16NO_2^- + 1.13HCO_3^- + 0.42H_2O$	-95.8	-82.7
Adenine	$0.44C_5H_5N_5 + NO_3^- + 4H_2O + 1.6H^+ \rightarrow 0.4N_2 + 0.2NO_2^- + 2.2HCO_3^- + 2.2NH_4^+$	-64.9	-59.5
Cysteine	$0.39C_3H_7NO_2S + NO_3^- + 0.4H_2O \rightarrow 0.18N_2 + 0.23NO_2^- + 0.2N_2O + 1.17HCO_3^- + 0.39NH_4^+ + 0.39HS^- + 0.4H^+$	-109.9	-91.3

As shown in Table 4.11, there is a significant difference in the ΔG depending on how it is calculated and if complete denitrification to N_2 or incomplete denitrification is considered. Table 4.12 highlights the differences in ΔG_R that occur if different units are used. For example, ΔG_R changes based on whether the unit is in kJ per e^- transferred to N or transferred from C. It is very important to consider the stoichiometry and methods being used to calculate ΔG when modeling or predicting. How the assumptions are made and how the calculations are carried out can affect the outcome when trying to use ΔG as a predictive tool. Different units or unsuspected reactions will yield different results (Figure 4.13).

Table 4.12: ΔG_R variation based on the units.

C-Substrate	kJ per mol	kJ per mol C	kJ per mol N	kJ per e^- from C	kJ per e^- to N
Glucose	-13258.1	-440.4	-552.4	-110.1	-110.5
Acetate	-3831.0	-383.1	-478.9	-95.8	-95.8
Adenine	-649.4	-129.9	-324.7	-32.5	-64.9
Cysteine	-1099.2	-366.4	-549.6	-91.6	-109.9
Fulvic Acid	18029.4	212.1	284.0	53.0	56.8

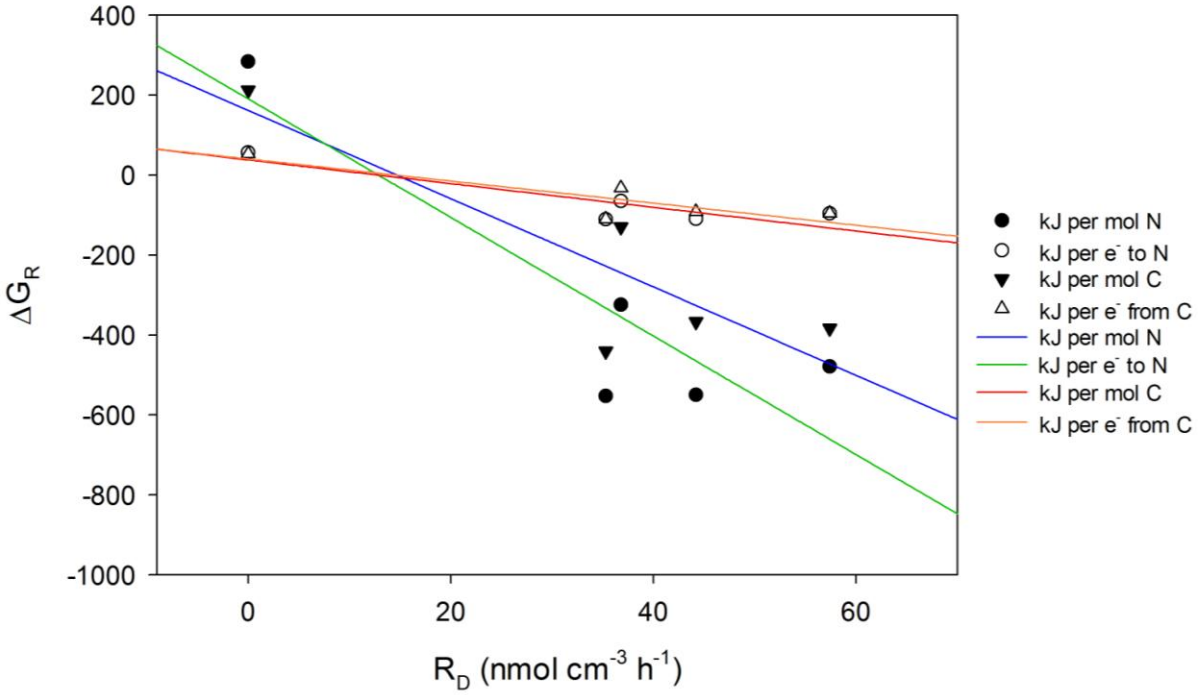


Figure 4.13: A comparison of the different trends found using different units for ΔG_R. The different units each yield different slopes and various y-intercepts.

4.4 Sources of Uncertainty

As with all lab experiments, some problems arose during the experiment. At the beginning of the experiment, outflow concentrations of NO_3^- were higher than the input concentration. FTRs were also run on the lab bench, and therefore outflow collection occurred in oxic conditions and FTRs were potentially exposed to oxygen although measures were taken to prevent it.

As mentioned in Section 3.2.1, NO_3^- concentrations measured for the outflow were corrected by Br^- measurements in order to correct for errors in sample injection volume within the instrument. However, at the beginning of the experiment, NO_3^- concentrations are still higher than input concentrations even with the Br correction. Measurements of the input solution show that the input concentration of NO_3^- is within error and cannot account for the NO_3^- increase. Calderer et al. (2010) also mention an increase in NO_3^- at the beginning of their experiments and attribute it to organic N in the soil being oxidized to NO_3^-. These authors also refer to other studies in which this phenomenon was noted.

FTRs are maintained as anoxic, closed systems. However, are prone to clogging or pressurization, causing leaks. A few leaks did occur throughout the experiment and were fixed as soon as possible. During leaks, it is possible that O_2 diffuses in the system, interfering with the reduced species present. For instance, nitrification might have occurred during leak incidents, which could explain the increase in NO_3^- in the outflow at the beginning of the experiment following this simple reaction:

$$NH_4^+ + 1.5O_2 \rightarrow NO_3^- + H_2O + 2H^+ \qquad [4.16]$$

Overall, despite measures to minimize O_2 contamination of the sediment and of the samples, it is important to stress that unless the experiment is entirely conducted in a anaerobic environment there will always be artifacts due to O_2 and to sample degassing.

Chapter 5
Conclusions

This study only focuses on one aspect of denitrification and contributes to efforts working towards a better understanding of denitrification and how to predict denitrification rates in the environment. Understanding denitrification with respect to anthropogenic disturbances of the global N-cycle is important to help remediation efforts. In this experiment it was shown that denitrification rates are influenced by the C-substrates available to natural denitrifying communities. Two theoretical predictors of denitrification rates coupled to specific C-substrates were compared to the experimental results to try and determine which C-substrate would produce faster denitrification rates: the nominal oxidation state of carbon (NOSC; LaRowe and Van Cappellen, 2011) and the ΔG_R based on thermodynamics. Our results suggest that ΔG_R is the best predictor of denitrification rates. The compounds with more negative ΔG_R were oxidized faster, with the notable exception of acetate, which yielded the fastest rate but did not have the lower ΔG_R. On the other hand, no correlations were found between NOSC and the observed denitrification rates.

Acetate stood out as the preferred substrate, likely because preferential pathways exist for its metabolism microbes. The fact that acetate was an outlier on a plot of predicted (using thermodynamics) and observed reaction rates highlight that factors other than ΔG_R must be considered in predicting reaction rates. Although the results found in this study cannot be conclusive on the factors causing acetate to be used more rapidly by microorganisms, they are consistent with what is expected if only simple steric encumbrance is considered as a factor. Indeed, in this experiment smaller compounds (e.g., acetate) are used faster than larger compounds (e.g., fulvic acid). The effect of C-substrates on microbial communities needs to be further investigated, and this study represents a step towards such systematic investigations.

A lot of questions still remain unanswered by this study. Cation interactions within the FTRs could not be fully explained, but undoubtedly play a large role in the environment. The study site chosen (Lake Belwood) is rich in carbonates, and its sediment contains low organic C. Denitrification rates could potentially change in different types of systems (i.e. non-carbonate systems) with different environmental factors influencing the microbial communities present. As well, sampling one small area in a reservoir cannot represent the whole system. Due to

heterogeneities in the subsurface (including sediment type, moisture content etc.), denitrification rates will vary along the flow path of the groundwater. Differences in environmental conditions could also change how microbial communities interact with the provided C-substrate. In a different part of Lake Belwood, or in a non-carbonate system, it cannot be ascertained that acetate would remain the most efficient compounds to promote denitrification.

The results overall show that predictions determined by thermodynamics alone were not completely successful in predicting higher versus lower denitrification rates. However, they do show that the addition of external C-substrates will promote denitrification and the rate at which denitrification occurs will depend on which C-substrate is added. From the C-substrates that were compared in this study, acetate appears to be the most suitable substrate to add since it produces the fastest denitrification rate and the least amount of potentially harmful byproducts (e.g. NO_2^-, NH_4^+ and SO_4^{2-}). Other factors still need to be accounted for in predicting degradation rates; thermodynamic calculations alone cannot be solely depended upon to make accurate predictions in realistic conditions.

5.1 Implications

This study strengthens the point that not all C-substrates are similarly favorable for microbial use, not only due to thermodynamic energy yields but also due to metabolic preferences that microbes have with respect to C compounds. Many studies have been found to generalize rates by either acknowledging only the maximum potential rate, only looking at denitrification and not considering competing reactions or only using 1 C-substrate in their experiment which, in the majority of cases, is acetate or glucose.

Although the rates determined in this study cannot be transferred to the study site, Lake Belwood has the potential to reduce incoming NO_3^-. When reservoir sediments are saturated, natural attenuation of groundwater NO_3^- has the potential to occur through denitrification. However, this is dependent on the natural microbial populations having access to sufficient, labile, C-substrates or other usable substrates. The denitrification rates found in this study are comparable with rates found in other studies. Again, the denitrification rates found were also dependent on the addition of external C. Without knowledge of the C-substrates present at the study site, it cannot be stated whether or not denitrification will occur sufficiently.

5.2 Future work and improvements

The findings of this experiment point towards the fact that more complex experiments are needed in order to predict denitrification rates in the environment. The effect of C-substrates is only one small part of a very complicated system. More realistic conditions were achieved in this experiment compared to others but were still very simple compared to natural conditions. Improvements to better this experiment would include the following:

1. Adding more then 1 C-substrate to the FTRs to see how multiple carbons sources of different type are utilized when together.
2. Using concentrations of NO_3^- representative of the environment the soil was collected from.
3. Analyzing for specific C-substrates in the outflow in order to determine exactly how much is being used instead of using DOC.
4. Using a more realistic input solution that includes phosphorus, magnesium, and other compounds can change the fate of how the C-substrates are utilized in denitrification.
5. Comparing saturated to unsaturated flowing soil columns
6. Oscillating the redox conditions to reflect the seasonal changes and track how denitrification changes from aerobic to anaerobic conditions
7. Use anthropogenic sources of C to determine how they are or aren't being utilized in the natural environment

Unfortunately, these experiments are not cheap and budgets are often limiting factors. In most cases, other than highly contaminated sites, nitrate and carbon concentrations in the water are low. If the experiments were to use these low natural concentrations, rates could likely not be determined, since the concentrations would be below the detection limit of our equipment. In FTRs, it is hard to achieve varying redox or unsaturated conditions since they are small, enclosed cores. However, should an experiment like this be devised to include these conditions, the results would be extremely useful in understanding impacted systems and how to remediate them.

Improvements and future work should also include analysis of the microbial populations themselves. By knowing the microbial populations present in the sediment and how they are influenced by different C-substrates, better remediation strategies can be planned to utilize the existing microbes. It is speculated in this experiment that changes to the initial microbial communities took place purely due to the change of conditions presented to them in the FTRs.

Verification of this speculation through RNA analyses are being conducted on the FTRs used in this experiment, however analyses are still in the works by another party.

Appendix A

Equipment Specifics

Table 1A: An overview of lab equipment and specifics where available.

Equipment	Species Analyzed	Practical Quantification Limit (respective to species, µM)	Run Time per Sample	Note/ Reference
Ion Chromatograph	NO_3^-, NO_2^-, SO_4^{2-}, Br^-, Cl^-, CH_3COO^-	1.3, 1.8, 0.7, 1.0, 1.8, unknown	20 min – Capillary system 45 min – Analytical System	
Inductively Coupled Plasma Optical Emission Spectrometer	Ca^{2+}, K^+, Mn^{2+}, Mg^{2+}, Na^+, Si^{4+}, total S, total P, total Fe, Al^{3+}	18.2, 10.0, 1.7, 190.4, 227.3, 10.7, 1.1, 1.5, 1.4, 4.3	2-4 min	
Ultraviolet-Visible Spectrophotometer	NH_4^+	4	3 sec	Bolleter et al., 1961
Total Organic Carbon Analyzer	DOC	16.7	Unknown	Method 5310B
Gas Chromatograph	CO_2, N_2O	250, 200	1.3 min	UW Biology Department
Bromide Probe	Br^-		1-2 min	
pH probe	pH		1-2 min	
CHNS Elemental Analyzer	C (organic and inorganic), N, S	0.01*, 0.05*, 0.02*	Unknown	

***** Measured in percent of dry weight of sediment

Appendix B
Calculations

i. Bromide Conversion (Breakthrough curve)

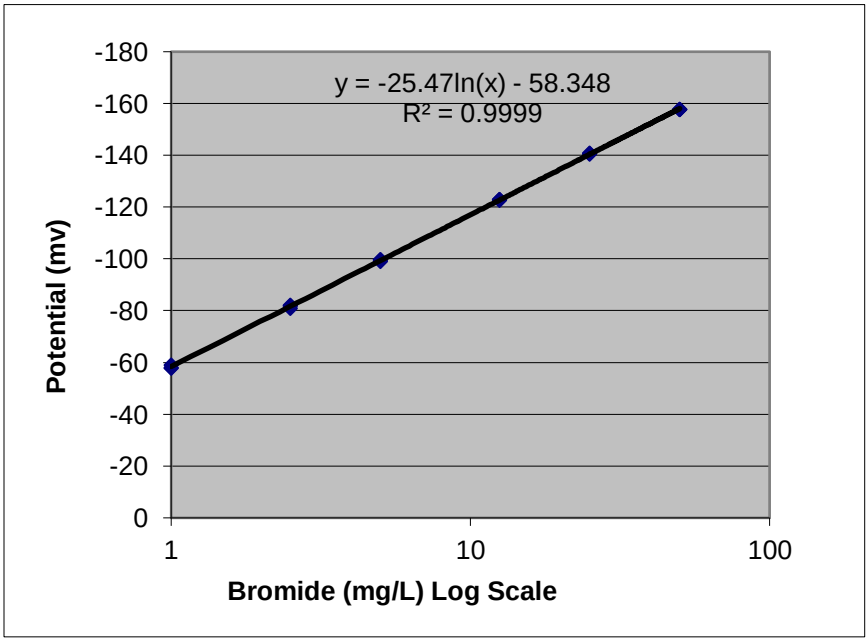


Figure 1: Bromide calibration curve.

Bromide data was calibrated using the equation of the best-fit line in Figure 1. Bromide data was then converted to mM by dividing by the molar mass of bromide, 79.9 mg $mmol^{-1}$.

ii. Mass Balance Figures

All mass balance figures were calculated for 1610 h due to limited DOC data. Integration was used to calculations were started from 1248 h when C-substrates were added:

$$X_{Tot} = \sum \frac{\Delta t \cdot \Delta x}{2} \cdot Q \qquad [B1]$$

Where X_{Tot} represents the total species being calculated, t is the amount of time in hours, x is the concentration measured in the outflow in mM and Q is the flow rate in m h^{-1}.

DOC data was used to help estimate the amount of N in the outflow from C-substrates adenine and cysteine as well as the amount of S from cysteine. The same calculation as equation B1 was used to calculate the total DOC in the outflow. DOC was assumed to be undegraded C-substrate and thus as estimate could be made by comparing the ration of N or S to C in the molecule. For example, adenine contains 5 N and 5 C therefore the concentration of DOC in the outflow should be equal to the amount of N. Raw DOC data and excel calculations for mass balance figures can be found in Table 1B below.

Table 1B: Average (Avg.) DOC data measured from the outflow of each FTR with the standard deviations (SD) as well as the total DOC calculated using integration (Int.) from the 1248 h to 2858 h.

C-Substrate	**Glucose**			**Fulvic Acid**			**Acetate**			**Adenine**			**Cysteine**			**No Carbon**		
Time (h)	Avg.	SD	Int.	Avg.	SD	Int.	Avg.	SD	Int.	Avg.	SD	Int.	Avg.	SD	Int.	Avg.	SD	Int.
548	3.45	0.48	0.86	3.20	0.26	0.78	3.27	0.12	0.87	3.27	0.40	0.79	3.07	0.12	0.80	2.90	0.00	0.77
788	3.68	0.15	0.55	3.27	0.40	0.73	3.97	1.16	0.59	3.30	0.17	0.50	3.60	0.62	0.53	3.50	0.28	0.55
932	3.95	0.24	0.11	6.83	4.65	0.14	4.20	0.26	0.12	3.67	0.32	0.91	3.80	0.26	1.03	4.15	0.07	0.93
1130	6.80	0.79	0.90	6.93	0.84	0.97	7.73	0.75	0.94	5.50	1.61	0.76	6.57	1.24	0.83	5.25	0.92	0.71
1298	3.88	0.31	0.79	4.60	1.82	0.14	3.50	0.35	0.89	3.53	0.40	3.05	3.37	0.15	1.76	3.20	0.42	0.59
1466	5.55	1.82	0.10	12.03	0.06	0.29	7.07	1.42	0.11	32.73	11.38	6.22	17.63	3.97	3.59	3.85	0.21	0.80
1682	4.03	2.13	0.57	14.47	0.57	0.22	3.20	0.30	0.55	24.83	11.29	3.65	15.57	3.27	2.48	3.60	0.99	0.55
1826	3.85	1.21	0.91	16.37	0.95	0.30	4.40	0.44	0.90	25.80	11.44	4.40	18.87	1.46	3.19	4.00	1.13	0.90
1994	6.98	1.85	0.10	19.60	0.61	0.29	6.37	0.67	0.10	26.53	7.96	3.79	19.17	2.20	2.88	6.70	0.71	0.92
2162	5.35	0.87	0.26	14.93	0.31	0.58	6.00	0.96	0.24	18.57	6.31	2.52	15.17	0.49	2.49	4.30	1.27	1.93
2354	8.33	6.28	0.26	15.33	3.15	0.51	6.50	N.A.	0.22	7.73	6.07	1.78	10.77	1.97	1.84	5.75	0.21	1.52
2522	7.20	2.60	0.61	15.20	2.03	0.55	6.87	1.91	0.71	13.40	5.05	6.75	11.10	3.08	4.13	3.30	0.14	1.10
2690	11.03	6.16	0.73	17.30	3.04	0.59	14.27	3.51	0.73	26.80	8.46	8.02	13.50	1.84	4.32	3.25	0.07	1.54
2858	10.63	6.23		17.70	1.80		7.57	2.11		20.93	3.84		12.23	0.67		5.95	3.32	

iii. Sulfur in sediment

Calculating the total amount of S in the outflow and subtracting it from total amount in the inflow determined the amount of S in the sediment. The difference in S between the outflow and inflow was estimated to have been converted into solid S in the sediment since S measured through CHNS analyses was below the detection limit of 0.02% by dry weight. Calculations are provided below and values are provided in Table 2B.

To calculate total S missing (S_s) in the outflow in mmol:

$$S_{s-aq} = S_{In-aq} - S_{Out-aq}$$

Convert S_{s-aq} to grams (S_s):

$$S_s = \frac{S_{S-aq} \cdot S_m}{1000}$$

Calculate the percentage of total dry mass of sediment:

$$S_{S\%} = \frac{S_S}{S_w} \cdot 100$$

Table 2B: Summary of values used to calculate S in the sediment.

S_{In-aq} mmol	**S_{Out-aq} mmol**	**S_{s-aq} mmol**	**S_m (molar mass) mg mmol^{-1}**	**Total S_S g**	**S_w (Average sediment weight) g**	**$S_{S\%}$ % dry weight**
1.42±0.21	0.92±0.47	0.50±0.68	32.065±0.005	0.016±0.03	28.01±4.9	0.0006%

Appendix C
Raw Data Tables

i. Cations

Table 1C: Average cation data from all the FTRs including calcium (Ca), iron (Fe), potassium (K), manganese (Mn), magnesium (Mg), sodium (Na), phosphorus (P), silicon (Si) and aluminum (Al) in mM.

Cation	C-Substrate	Glucose		Fulvic Acid		Acetate		Adenine		Cysteine		No Carbon	
	Time (h)	Avg.	SD	Avg.	SD	Avg.	SD	Avg.	SD	Avg.	SD	Avg.	SD
Ca	740	0.45000	0.11044	0.44998	0.21620	0.37262	0.07549	0.37199	0.14535	0.35099	0.11393	0.27977	0.08385
	956	0.37878	0.10134	0.25753	0.03568	0.22508	0.05036	0.30477	0.11888	0.28807	0.06908	0.24028	0.05557
	1154	0.31361	0.08254	0.23709	0.02129	0.21513	0.03576	0.24753	0.09593	0.26048	0.06292	0.22302	0.06350
	1442	0.21551	0.14339	0.22123	0.03297	0.17781	0.02602	0.21573	0.08896	0.38192	0.15589	0.19473	0.06487
	1610	0.34673	0.05548	0.21754	0.02016	0.13186	0.02264	0.19573	0.07633	0.51240	0.16559	0.17782	0.06663
	1778	0.30332	0.09045	0.21026	0.02428	0.09847	0.01953	0.12351	0.14533	0.52532	0.15279	0.15670	0.05792
	1946	0.34013	0.14979	0.23120	0.04444	0.11202	0.02564	0.19089	0.13615	0.52956	0.14015	0.15867	0.04661
	2114	0.40264	0.11317	0.23406	0.04168	0.10171	0.02123	0.19249	0.11253	0.50970	0.14010	0.14897	0.03950
	2378	0.34909	0.06984	0.23508	0.03861	0.10209	0.03389	0.19653	0.12358	0.50528	0.11492	0.15001	0.03102
	2546	0.31154	0.08293	0.22844	0.03310	0.09848	0.02709	0.14374	0.09391	0.47377	0.10765	0.14169	0.03681
	2666	0.24425	0.08795	0.14474	0.12748	0.09658	0.02735	0.11656	0.08289	0.44622	0.14514	0.13531	0.03522
Fe	740	0.00011	0.00006	0.00008	0.00004	0.00011	0.00001	0.00005	0.00004	0.00015	0.00014	0.00003	0.00013
	956	0.00009	0.00004	0.00014	0.00010	0.00012	0.00015	0.00018	0.00005	0.00014	0.00010	0.00019	0.00005
	1154	0.00008	0.00011	0.00007	0.00008	0.00004	0.00004	0.00006	0.00006	0.00004	0.00012	0.00006	0.00001
	1442	0.00012	0.00016	0.00018	0.00003	0.00012	0.00008	0.00018	0.00016	0.00045	0.00035	0.00023	0.00007
	1610	0.00010	0.00012	0.00011	0.00016	0.00009	0.00002	0.00035	0.00031	0.00079	0.00117	0.00011	0.00003

	1778	-0.00008	0.00004	0.00017	0.00004	0.00016	0.00006	0.00014	0.00017	0.00014	0.00022	0.00008	0.00002
	1946	0.00005	0.00004	0.00012	0.00008	0.00017	0.00006	0.00038	0.00059	0.00055	0.00066	0.00008	0.00009
	2114	0.00010	0.00007	0.00016	0.00005	0.00009	0.00007	0.00036	0.00036	0.00026	0.00016	0.00019	0.00003
	2378	0.00006	0.00012	0.00021	0.00008	0.00013	0.00010	0.00021	0.00025	0.00047	0.00039	0.00010	0.00014
	2546	0.00019	0.00009	0.00039	0.00029	0.00007	0.00007	0.00025	0.00023	0.00032	0.00016	0.00012	0.00004
	2666	0.00013	0.00006	0.00030	0.00027	0.00012	0.00009	0.00010	0.00012	0.00097	0.00068	0.00012	0.00009
K	740	0.53276	0.23644	0.68110	0.23483	0.77374	0.20389	0.54307	0.18616	0.59728	0.38557	0.90151	0.24663
	956	0.60865	0.24165	0.86876	0.07056	0.96518	0.07026	0.66843	0.14896	0.75093	0.23649	0.76532	0.21642
	1154	0.72238	0.20544	0.91688	0.03397	0.96992	0.04666	0.75782	0.11148	0.78961	0.17707	0.79448	0.17907
	1442	0.67535	0.45267	1.00487	0.04314	1.00506	0.03297	0.86899	0.13847	1.10240	0.21272	0.84417	0.19256
	1610	0.98442	0.15505	1.03290	0.01231	0.92268	0.00646	0.94904	0.15088	1.08327	0.15637	0.84882	0.15122
	1778	0.78615	0.15672	0.94938	0.00659	0.84148	0.02571	0.66671	0.57198	0.93271	0.02723	0.77208	0.15153
	1946	0.80882	0.27774	0.97326	0.05288	0.95175	0.01716	0.97580	0.04801	0.93104	0.02368	0.83994	0.10286
	2114	0.93717	0.09099	0.96497	0.00916	0.90522	0.01509	0.92726	0.06587	0.94002	0.01753	0.87446	0.07300
	2378	0.90099	0.03497	0.99834	0.01081	0.97742	0.01474	0.97735	0.05169	1.00611	0.01312	0.92002	0.09367
	2546	0.91721	0.03486	0.99221	0.00176	0.96923	0.00849	0.85075	0.06139	0.99743	0.01966	0.91709	0.05106
	2666	0.91856	0.02178	0.63308	0.54243	0.94082	0.01735	0.79384	0.09172	0.94410	0.03094	0.90607	0.08397
Mn	740	0.00003	0.00002	0.00006	0.00007	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
	956	0.00002	0.00000	0.00001	0.00000	0.00001	0.00000	0.00001	0.00000	0.00002	0.00002	0.00002	0.00001
	1154	0.00001	0.00000	0.00002	0.00003	0.00000	0.00001	0.00001	0.00001	0.00000	0.00000	0.00000	0.00000
	1442	0.00005	0.00004	0.00001	0.00002	0.00001	0.00002	0.00001	0.00000	0.00339	0.00293	0.00000	0.00000
	1610	0.00014	0.00022	0.00000	0.00001	0.00006	0.00010	0.00008	0.00007	0.00462	0.00323	0.00002	0.00001

	1778	0.00016	0.00026	0.00000	0.00001	0.00008	0.00006	0.00018	0.00016	0.00609	0.00248	-0.00001	0.00000
	1946	0.00032	0.00042	0.00001	0.00002	0.00007	0.00007	0.00026	0.00003	0.00949	0.00344	0.00000	0.00001
	2114	0.00070	0.00103	0.00002	0.00002	0.00007	0.00005	0.00022	0.00016	0.01273	0.00575	0.00002	0.00003
	2378	0.00075	0.00108	0.00009	0.00004	0.00006	0.00004	0.00025	0.00018	0.02475	0.01306	0.00000	0.00001
	2546	0.00049	0.00084	0.00006	0.00006	0.00006	0.00003	0.00026	0.00019	0.03291	0.02050	0.00000	0.00000
	2666	0.00033	0.00059	0.00003	0.00003	0.00009	0.00007	0.00019	0.00012	0.04190	0.03298	0.00000	0.00000
Mg	740	0.10811	0.04652	0.12414	0.06374	0.07277	0.00873	0.08609	0.03567	0.09456	0.01034	0.06275	0.00928
	956	0.08869	0.03912	0.06289	0.03178	0.05129	0.00451	0.07131	0.02692	0.06520	0.01781	0.07024	0.01975
	1154	0.07069	0.03095	0.05351	0.02689	0.04834	0.00730	0.05152	0.01777	0.05638	0.01400	0.06213	0.01622
	1442	0.04758	0.03807	0.04630	0.02273	0.03788	0.00698	0.04429	0.01800	0.08502	0.01166	0.05460	0.01010
	1610	0.07955	0.02650	0.04221	0.02188	0.02220	0.00506	0.03934	0.01917	0.12287	0.03283	0.04671	0.00683
	1778	0.07135	0.01189	0.04221	0.01977	0.01513	0.00392	0.01885	0.01908	0.16109	0.05995	0.04344	0.00596
	1946	0.08093	0.00661	0.04794	0.01774	0.01593	0.00432	0.02685	0.01109	0.18183	0.07341	0.04378	0.01024
	2114	0.09478	0.03678	0.05250	0.01919	0.01363	0.00455	0.03457	0.02303	0.17375	0.07123	0.04037	0.01150
	2378	0.08384	0.04618	0.06820	0.02342	0.01557	0.00664	0.03596	0.02482	0.17884	0.07450	0.04046	0.01529
	2546	0.07242	0.03547	0.06762	0.02101	0.01671	0.00812	0.02582	0.01707	0.16665	0.07236	0.03685	0.01334
	2666	0.04934	0.01761	0.04963	0.04772	0.01983	0.01061	0.02339	0.02026	0.14240	0.06927	0.03464	0.01252
Na	740	7.71395	0.04310	7.44667	0.03117	7.44287	0.06138	7.54565	0.09375	7.49022	0.06576	7.58650	0.17143
	956	7.49480	0.02844	7.28187	0.04404	7.35851	0.08739	7.44687	0.14756	7.38500	0.06230	7.32954	0.07228
	1154	7.34473	0.05968	7.34775	0.05821	7.34101	0.04509	7.33100	0.14731	7.31295	0.06633	7.26139	0.03535
	1442	5.45866	3.58987	7.30219	0.52900	7.54169	0.12227	6.95872	0.02074	7.17997	0.06061	6.94186	0.11138

	1610	6.82293	0.08634	6.75448	0.01133	7.22474	0.11632	5.81280	0.04730	6.65701	0.20222	6.54398	0.04374
	1778	5.65034	1.07084	6.46285	0.02203	6.62360	0.07088	4.13386	3.60202	6.38539	0.11371	6.37566	0.12818
	1946	5.36457	1.81810	6.43776	0.28377	7.28266	0.04244	6.37102	0.11754	6.44097	0.03412	6.43003	0.02664
	2114	7.82814	1.57431	6.29733	0.03217	6.80851	0.03251	6.50010	0.12541	6.32528	0.01942	6.37044	0.01055
	2378	6.99566	0.14297	7.09264	0.05329	7.31522	0.13551	7.05276	0.08725	6.89172	0.00477	6.69171	0.27869
	2546	6.81703	0.09553	7.19128	0.05209	7.43729	0.00726	6.11500	0.27957	6.84424	0.04850	6.71816	0.07526
	2666	6.74499	0.20885	4.51800	3.90907	7.21107	0.01250	3.58952	0.11785	6.72624	0.03342	6.75183	0.11191
P	740	0.00042	0.00004	0.00052	0.00012	0.00051	0.00005	0.00046	0.00009	0.00048	0.00007	0.00049	0.00011
	956	0.00038	0.00005	0.00045	0.00001	0.00044	0.00002	0.00045	0.00012	0.00053	0.00008	0.00040	0.00000
	1154	0.00039	0.00004	0.00042	0.00003	0.00040	0.00004	0.00041	0.00009	0.00038	0.00002	0.00037	0.00001
	1442	0.00010	0.00009	0.00020	0.00006	0.00014	0.00000	0.00024	0.00003	0.00014	0.00003	0.00029	0.00005
	1610	0.00019	0.00007	0.00021	0.00003	0.00029	0.00006	0.00027	0.00010	0.00015	0.00003	0.00031	0.00000
	1778	0.00008	0.00003	0.00015	0.00006	0.00027	0.00004	0.00018	0.00015	0.00014	0.00002	0.00025	0.00010
	1946	0.00012	0.00007	0.00012	0.00001	0.00035	0.00017	0.00025	0.00022	0.00008	0.00006	0.00021	0.00004
	2114	0.00009	0.00001	0.00012	0.00004	0.00025	0.00010	0.00011	0.00008	0.00004	0.00002	0.00024	0.00004
	2378	0.00010	0.00004	0.00016	0.00003	0.00016	0.00007	0.00015	0.00010	0.00007	0.00003	0.00017	0.00004
	2546	0.00004	0.00004	0.00013	0.00004	0.00012	0.00005	0.00019	0.00021	0.00006	0.00007	0.00012	0.00005
	2666	0.00007	0.00002	0.00007	0.00008	0.00014	0.00009	0.00017	0.00007	0.00004	0.00001	0.00021	0.00001
Si	740	0.11673	0.07527	0.06649	0.02462	0.05633	0.01020	0.12935	0.11000	0.06345	0.01823	0.93399	1.25040
	956	0.11619	0.07262	0.05730	0.00588	0.05442	0.00842	0.16625	0.17661	0.06449	0.01262	0.69625	0.87562
	1154	0.11480	0.06516	0.05704	0.00580	0.05424	0.00940	0.11723	0.10172	0.06102	0.01243	0.66913	0.84343
	1442	0.11300	0.08777	0.05782	0.00702	0.05731	0.01003	0.13950	0.14288	0.10231	0.04520	0.68829	0.82877

	1610	0.16180	0.11315	0.06459	0.00661	0.06866	0.01371	0.18504	0.21054	0.09317	0.02755	0.82563	1.03132
	1778	0.09238	0.01451	0.07306	0.00724	0.07564	0.01239	0.03920	0.03444	0.08785	0.01268	0.88581	1.13460
	1946	0.09750	0.01563	0.07552	0.01437	0.07428	0.01125	0.05984	0.01425	0.09205	0.00844	0.92010	1.19984
	2114	0.11908	0.06844	0.07763	0.00786	0.07019	0.01020	0.13292	0.12924	0.09790	0.01020	0.90782	1.17851
	2378	0.16013	0.18657	0.05554	0.01057	0.05668	0.01100	0.12451	0.11724	0.10100	0.01488	0.91508	1.20621
	2546	0.14412	0.16585	0.06468	0.00158	0.05955	0.00952	0.12017	0.10640	0.10354	0.01031	0.87165	1.14756
	2666	0.16952	0.24285	0.04381	0.03798	0.05316	0.00620	0.14789	0.17422	0.08695	0.00532	0.87731	1.14988
Al	740	0.00710	0.00249	0.00451	0.00445	0.00819	0.00297	0.00354	0.00271	0.00499	0.00148	0.01151	0.00583
	956	0.00801	0.00280	0.00832	0.00364	0.01130	0.00240	0.00330	0.00250	0.00907	0.00357	0.00810	0.00326
	1154	0.00876	0.00343	0.00926	0.00348	0.01144	0.00203	0.00442	0.00296	0.00948	0.00291	0.00878	0.00379
	1442	0.00341	0.00249	0.00914	0.00331	0.01097	0.00248	0.00403	0.00313	0.00403	0.00214	0.00688	0.00297
	1610	0.00398	0.00178	0.00875	0.00248	0.01633	0.00563	0.00412	0.00308	0.00235	0.00093	0.00730	0.00382
	1778	0.00333	0.00179	0.00877	0.00195	0.02081	0.00393	0.00347	0.00399	0.00265	0.00106	0.00848	0.00429
	1946	0.00297	0.00133	0.00806	0.00152	0.02253	0.00368	0.00670	0.00138	0.00260	0.00129	0.00999	0.00503
	2114	0.00474	0.00077	0.00740	0.00153	0.02394	0.00373	0.00647	0.00301	0.00233	0.00119	0.01106	0.00570
	2378	0.00418	0.00164	0.00766	0.00205	0.02738	0.00482	0.00686	0.00295	0.00219	0.00103	0.01356	0.00738
	2546	0.00449	0.00214	0.00865	0.00141	0.02865	0.00429	0.00846	0.00374	0.00186	0.00091	0.01486	0.00822
	2666	0.00637	0.00352	0.00542	0.00505	0.02632	0.00384	0.00785	0.00505	0.00174	0.00074	0.01664	0.00932

ii. pH

Table 2C: Average (Avg.) pH data from all FTRs and standard deviation (SD).

C-Substrate	Glucose		Fulvic		Acetate		Adenine		Cysteine		No Carbon	
Time (h)	Avg.	SD	Avg.	SD	Avg.	SD	Avg.	SD	Avg.	SD	Avg.	SD
380	8.00	0.29	8.01	0.12	7.84	0.18	7.94	0.79	7.95	0.33	7.37	0.57
428	7.60	0.11	7.45	0.03	7.46	0.03	7.16	0.49	7.20	0.18	7.24	0.13
524	7.88	0.09	7.80	0.17	7.92	0.06	7.65	0.46	7.84	0.07	7.75	0.14
596	8.05	0.18	7.99	0.05	8.00	0.01	7.66	0.76	7.92	0.33	7.65	0.32
692	8.19	0.22	8.10	0.08	8.08	0.02	7.68	0.91	7.98	0.27	7.88	0.14
908	7.95	0.06	7.96	0.05	7.99	0.01	7.63	0.39	7.86	0.07	7.80	0.06
980	7.87	0.05	7.94	0.02	7.95	0.02	7.59	0.35	7.80	0.10	7.75	0.09
1076	7.68	0.39	7.89	0.04	7.87	0.03	7.49	0.62	7.78	0.09	7.60	0.16
1250	7.82	0.09	7.84	0.00	7.83	0.03	7.37	0.46	7.70	0.13	7.68	0.05
1274	8.38	0.34	8.44	0.28	8.48	0.15	7.81	0.76	8.20	0.51	8.34	0.13
1322	8.64	0.28	8.51	0.30	8.77	0.11	7.93	0.89	8.71	0.33	8.47	0.04
1442	8.42	0.28	8.51	0.25	8.88	0.06	8.36	0.57	8.22	0.42	8.30	0.04
1514	8.38	0.33	8.35	0.16	8.98	0.05	8.49	0.51	8.31	0.22	8.19	0.16
1610	8.31	0.29	8.27	0.21	9.07	0.04	8.55	0.36	8.14	0.25	7.97	0.08
1682	8.32	0.19	8.38	0.25	9.17	0.04	8.48	0.41	8.10	0.31	8.01	0.21
1778	8.26	0.17	8.43	0.20	9.22	0.02	8.51	0.32	8.03	0.31	8.11	0.07
1850	8.20	0.07	8.36	0.34	9.22	0.08	8.60	0.31	8.03	0.31	8.18	0.10
1946	8.34	0.12	8.33	0.32	9.17	0.07	8.64	0.22	8.04	0.29	8.07	0.35
2018	8.26	0.08	8.39	0.12	9.25	0.04	8.67	0.21	7.98	0.31	8.46	0.14
2090	8.31	0.16	8.46	0.26	9.29	0.01	8.71	0.17	7.97	0.31	8.63	0.09
2186	8.23	0.10	8.45	0.24	9.34	0.07	8.71	0.15	7.95	0.24	8.40	0.01
2306	8.26	0.37	8.29	0.29	9.46	0.05	8.76	0.11	7.94	0.22	8.50	0.24
2378	8.22	0.34	8.36	0.28	9.47	0.07	8.69	0.12	7.87	0.23	8.50	0.10
2474	8.29	0.25	8.39	0.15	9.45	0.07	8.72	0.08	7.87	0.23	8.18	0.05
2546	8.10	0.06	8.20	0.12	8.80	0.64	8.84	0.12	7.84	0.25	8.65	0.15
2594	8.28	0.42	8.36	0.18	9.49	0.08	8.84	0.06	7.83	0.24	8.74	0.13
2666	8.36	0.42	8.06	0.18	9.38	0.11	8.83	0.11	7.74	0.26	8.23	0.04
2762	7.86	0.50	8.11	0.06	9.44	0.09	8.70	0.15	7.73	0.26	8.38	0.13
2834	8.22	0.29	8.31	0.17	9.33	0.18	8.79	0.11	7.75	0.24	8.71	0.10

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