GROUNDWATER NITRATE REDUCTION IN A SIMULATED FREE WATER SURFACE WETLAND SYSTEM

A Thesis
Presented to
The Academic Faculty

by

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In Partial Fulfillment
of the Requirements for the Degree
Master of Science in Environmental Engineering in the
School of Civil and Environmental Engineering

Georgia Institute of Technology
December 2009

GROUNDWATER NITRATE REDUCTION IN A SIMULATED FREE WATER SURFACE WETLAND SYSTEM

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Date Approved: November 13, 2009

ACKNOWLEDGEMENTS

Foremost, I would like to thank my advisor, Dr. Spyros G. Pavlostathis, for his guidance and encouragement. Throughout this project, his suggestions and ideas have made a significant impact on the completion of my Master's research and will be invaluable to me as I continue to work on my Ph.D.

I am very thankful for my thesis committee members, Dr. Jim Spain and Dr. Ulas Tezel, for their time and valuable suggestions. I would also like to thank all the members of Dr. Pavlostathis' group who have offered endless help and support.

This research project was supported by the Columbus Water Works (CWW), Columbus, GA through Jordan, Jones and Goulding, Inc. (JJG), Norcross, GA.

Finally, I would like to acknowledge the financial support from Camp, Dresser and McKee, Inc. (CDM) in the form of a three-year fellowship during my graduate studies at the Georgia Institute of Technology.

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SUMMARY

A wastewater treatment facility located in Georgia, provides drinking water to a city and surrounding areas with a total population of approximately 230,000. The facility generates approximately 6000 dry tons per year of biosolids from the primary and secondary treatment processes. The solids consist of anaerobically digested primary and waste activated sludge. A portion of the solids are land applied as part of a biosolids application program and the nutrients are utilized by crops to enhance agriculture. Consequently, nitrate leaches into the soil and contaminates the groundwater. The wastewater treatment facility has proposed a constructed wetland system for treatment of the nitrate contaminated groundwater and if successful, all biosolids produced at the treatment facility can be land applied to fields surrounding the site. The purpose of this project is to investigate the feasibility of treating the nitrate contaminated groundwater with a constructed wetland.

The concentration of nitrate in the groundwater to be treated depends on the infiltration and pumping conditions at the site. For the duration of this project, the pumping rate was kept relatively constant and therefore, the nitrate concentration in the groundwater remained constant around 70 mg NO_3-N/L. The maximum contaminant level (MCL) for nitrate is currently 10 mg NO_3-N/L (USEPA, 2006). The treatment wetland must successfully reduce the nitrate concentration in the groundwater below the MCL before release into a nearby river.

The effectiveness of a constructed wetland on removing nitrate and other pollutants is dependent on many parameters, the most important being microbial activity in the sediment, biodegradable carbon availability, hydraulic retention time, and temperature. All the above factors were investigated through a series of laboratory experiments that were performed over a period of 12 months.

The results demonstrated that with sufficient available biodegradable carbon, the nitrate concentration of the groundwater contaminated by the biosolids application program can be reduced below the regulated 10 mg NO_3-N/L by use of a constructed wetland.

CHAPTER 1

INTRODUCTION

1.1 Project Overview

Wastewater created by commercial and residential activities is typically sent to wastewater treatment facilities. The sewage is screened for large debris before entering the plant where it is sent through primary, secondary and tertiary treatment stages. During these stages it undergoes physical, chemical and biological processes in order to achieve treated effluent to meet the final disposal requirements. The layout of a typical wastewater treatment facility is displayed in Figure 1-1.

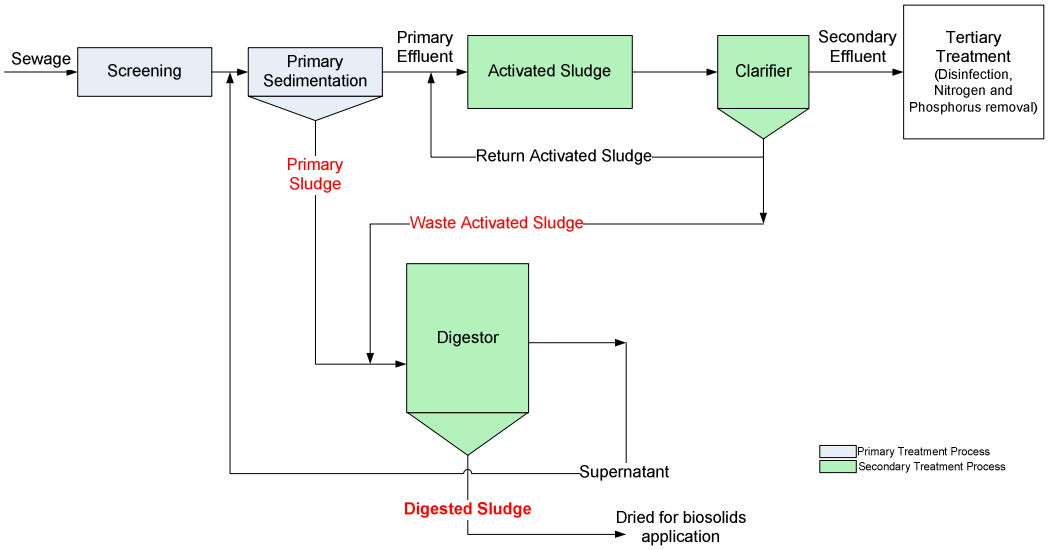

Figure 1-1. Schematic of typical sewage treatment facility.

Primary sludge and waste activated sludge consist of solids from the primary sedimentation tank and the clarifier, respectively. The primary and waste activated sludge are sent to the digester where they are digested and further processed, thereby creating biosolids. Biosolids, a nutrient-rich byproduct of domestic wastewater treatment, contain pathogens such as bacteria, viruses and parasites. As one of the final disposal methods, biosolids are applied to agricultural fields as a fertilizer to enhance plant growth and maintain productive soils. Before application, the biosolids must meet regulatory requirements and their pathogen level is indicated by two main classifications, Class A and Class B. Class A biosolids are essentially free of pathogens before being land applied, while Class B biosolids contain some pathogens which die off when applied to soil (USEPA, 2007).

1.2 Site Information

A wastewater treatment facility located in Georgia, services a city and surrounding areas with a total population of approximately 230,000. The facility generates approximately 6000 dry tons per year of biosolids. A portion of the solids are land applied and the remainder is sent to a landfill, a very expensive disposal method. Consequently with land application of biosolids, ammonia in the biosolids and/or produced as a result of biosolids degradation is released and oxidized to nitrate. As a result, nitrate leaches into the soil and reaches the groundwater in the upper aquifer. The nitrate levels in the groundwater at this site are in a range between 65 to 400 mg N/L, which are above the regulated limit of 10 mg NO_3-N/L, and therefore must be treated before being released into the nearby river.

The wastewater treatment facility has proposed a constructed overland-flow wetland system for treatment of the nitrate contaminated groundwater and if successful, all biosolids produced at the treatment facility can be land applied to fields surrounding the site. This approach would significantly reduce the operating cost for the facility and provide a nutrient rich fertilizer for local agriculture. Currently, there is a three-cell pilot scale wetland system set up at the site and a lab scale study was conducted in support of this project. The layout of the site is displayed in Figure 1-2.

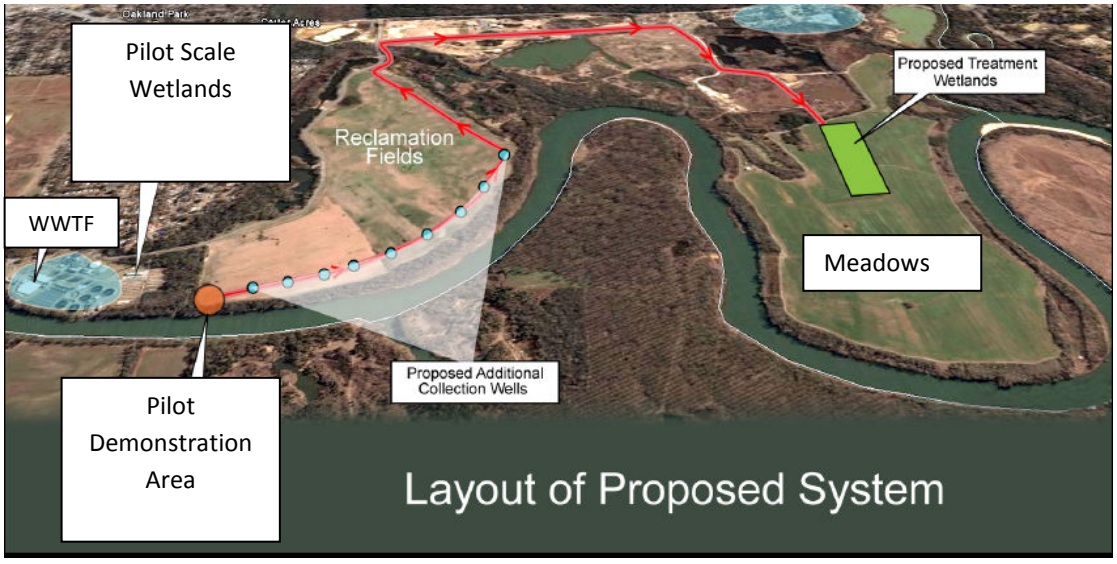

Figure 1-2. Schematic of wetland demonstration project.

The pilot scale wetland system is adjacent to the wastewater treatment facility (WWTF) highlighted in blue on the left side of the schematic. Contaminated water is pumped from the pilot demonstration area to the wetlands for treatment. If the full-scale wetland is constructed, the proposed collection wells will pump the nitrate contaminated water from the reclamation fields to the proposed wetland area, highlighted in green.

1.3 Research Objective

The effectiveness of a constructed wetland for removing nitrate and other pollutants is dependent on many parameters, the most important being microbial activity in the sediment, biodegradable carbon availability, hydraulic retention time, and temperature. All these factors were investigated through laboratory experiments that were performed over a period of 12 months.

The specific objectives of this study were to:

1. Verify that there is microbial activity in the soil and that nitrate reduction can be established.
2. Quantify the nitrate removal rates as a function of:
    a. Carbon sources
    b. Carbon loading
    c. Initial nitrate concentration
    d. Hydraulic retention time (HRT)
    e. Temperature
3. Determine the feasibility of treating the nitrate contaminated groundwater with a constructed wetland at the site of the wastewater treatment facility

CHAPTER 2

BACKGROUND

2.1 Nitrate Contamination

Nitrate (NO_3^-), which is one of the most common groundwater contaminants world-wide, is mainly introduced into the environment from sewage treatment processes and agricultural activities, mostly related to land application of nitrate-containing fertilizers (Burkart and Stoner, 2002; Rivett et al., 2008). In addition to inorganic fertilizers, primary and waste activated sludge from municipal wastewater treatment facilities, after stabilization, usually via anaerobic digestion, are land applied to serve as a nitrogen source and as an alternative to landfilling (Surampalli et al., 2008). The ammonia and nitrate in the fertilizers and biosolids enhance plant growth but also create a potential source for nitrate contamination of groundwater.

Nitrate, when introduced into natural aquatic systems through runoff, infiltration and wastewater discharge, leads to eutrophication, a process that increases the productivity of the ecosystem, such as algal blooms. However, the excessive activity decreases dissolved oxygen levels and reduces the overall quality of the water, creating conditions that are harmful to both plant and marine life (Maltais-Landry et al., 2009). A study on nitrate contamination of private wells in rural Alabama, USA found that nitrate concentrations in the groundwater were correlated with cropping activities in the region, such as irrigation and fertilizer use, but the distance of septic tanks and livestock was found to be insignificant (Liu et al., 2005). Similar studies in Florida and Alabama found that application of fertilizers, including a small portion of biosolids, contributed the

majority of annual nitrogen loading to the groundwater, with smaller contributions from septic tanks, livestock and atmospheric depositions (Katz et al., 2009; Murgulet and Tick, 2009). Algal blooms and fish death in the Canoochee River in Georgia, USA were reported downstream from a poultry processing facility and a wastewater land application site as a result of eutrophication and depletion of dissolved oxygen (Reichard and Brown, 2009).

Nitrate and nitrite are toxic and cause health problems in humans and animals. Nitrite, an intermediate of ammonia oxidation or nitrate reduction, oxidizes the iron atoms in the blood and inhibits the hemoglobin from carrying oxygen. It can affect both children and adults but is especially concerning for infants because they are more vulnerable to nitrate poisoning. Young children have more nitrate metabolizing bacteria than adults and these nitrate reducers convert the nitrate to nitrite, which oxidizes the hemoglobin to methemoglobin. When large quantities of nitrate are consumed by infants, their skin appears to have a bluish tint due to the lack of oxygen, a condition called methemoglobinemia or "blue baby syndrome" (USEPA, 1997). If not treated, high consumption of nitrate can lead to suffocation. Most cases of methemoglobinemia reported in infants were a result of water containing nitrate at a level higher than the maximum contaminant level for nitrate (Fan, 1996). Because nitrate is toxic to humans and animals, the minimum contaminant level (MCL) for nitrate in the United States is currently 10 mg nitrate-N/L (USEPA, 2006).

2.2 Constructed Wetlands

Wetlands are large water-saturated areas of land that are inhabited by a wide variety of plants and animals. They are one of the most biologically productive ecosystems on the planet due to the nutrient-rich sediments and abundance of water (Kadlec and Wallace, 2008). Wetlands occur naturally but can also be constructed for discharge and treatment of wastewater. Wetland-based treatment systems are mainly used for the biological removal of nitrogen, phosphorus, sulfur and heavy metals but are also used to filter out other pollutants. Typically, wastewater is pumped in and travels through the wetland with a certain retention time before it is discharged to bodies of surface water, such as rivers, lakes or streams. Thus, the wetland acts as a buffer between the pollution source and the natural aquatic ecosystem (Maltais-Landry et al., 2009; Paludan et al., 2002). Constructed wetlands are inexpensive to maintain, can handle fluctuating flowrates of water, and are aesthetically pleasing (USEPA, 2004).

Among all the nitrogen transformation processes taking place in wetlands, the one related to nitrate removal is denitrification, i.e., the reduction of nitrate all the way to nitrogen gas (N_2). Biodegradable organic matter required to drive denitrification (see Section 2.3 below) in constructed wetlands is made available by plant growth and decay within the wetland system. Relative to the nitrate removal capacity of constructed wetlands, based on performance data of 66 wetlands in the USA, Kadlec and Wallace (2008) reported that for influent nitrate up to about 50 mg N/L, mass loadings from 200 to 4,000 g N/m^2-year result in effluent nitrate-N of 10 mg/L or lower, demonstrating the high capacity of wetlands for nitrogen removal, especially for temperate climate conditions. A wetland in central Sweden, which receives effluent from a municipal

wastewater treatment plant with an average total-N and nitrate-N equal to 16.2 and 10.2 mg N/L, respectively, achieved an average nitrogen removal equal to 160 g N/m^2-year at an average hydraulic retention time of 7 days (Kjellin et al., 2007).

The three most common types of constructed wetlands are free water surface (FWS) wetlands, horizontal subsurface flow (HSSF) wetlands and vertical flow (VF) wetlands. Free water surface wetlands are open areas of water that contain floating vegetation and aquatic plants, similar to natural swamps. In FWS wetlands, water flows into the wetland at one end, passes over the soil/sediment surface and exits on the other end. Alternatively, horizontal subsurface flow wetlands are constructed so that water flows horizontally through a gravel bed of planted vegetation from inlet to outlet. In the third type, a vertical flow wetland, water is distributed throughout the surface of a bed of gravel and vegetation and it percolates downward through the root zone (Kadlec and Wallace, 2008).

2.3 Nitrogen Cycle

The nitrogen cycle involves nitrogen in many organic and inorganic forms and in seven valance states (-3 to +5). The most common forms of inorganic nitrogen are nitrate (NO_3^-), nitrite (NO_2^-), ammonia (NH_3), ammonium (NH_4^+), gaseous nitrogen (N_2), nitrous oxide (N_2O) and nitric oxide (NO) (Vymazal, 2007). The main processes involved in the N cycle are nitrification, denitrification (see Section 2.4.1), ammonification and anammox. All forms of nitrogen play an important role in the ecosystem of a wetland but some have a harmful effect on the atmosphere. Nitrous oxide and nitric oxide are known to be powerful greenhouse gases. Nitric oxide, NO, is relatively weak as a greenhouse

gas but nitrous oxide, N_2O, is 296 times as potent as carbon dioxide (IPCC, 2007). N_2O is a reactive intermediate of denitrification that is normally transformed to inert gaseous nitrogen. Partial denitrification can occur when carbon is limited, and/or the nitrous oxide reductase is inhibited, and N_2O could potentially be released to the atmosphere, magnifying the effects of global warming. The COD:N ratio plays an important role in controlling wetland greenhouse gas emissions. Studies have found that COD:N ratios below 3.5 can emit as much as 30% of the nitrogen as N_2O and that the optimal ratio is at or above 5:1 (Itokawa et al., 2001; Wu et al., 2009). The production of these toxic gases, especially N_2O, could ultimately affect the feasibility and environmental benefits of constructed wetland systems (Maltais-Landry et al., 2009).

2.4 Theoretical Considerations

2.4.1 Denitrification

Denitrification is a microbially facilitated process that reduces nitrate (NO_3^-) to nitrogen gas (N_2), through a series of intermediates. Denitrification is the main biological source of gaseous nitrogen, which in total represents 79% of the gases in the atmosphere (Madigan et al., 2009). The pathway, including intermediates, NO_2^-, NO and N_2O, is displayed in Reaction 2-1.

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_{2(g)} \qquad (2\text{-}1)$$

Nitrate is used as an electron acceptor by many facultative heterotrophs and autotrophs, which are capable of switching between oxygen and nitrogen respiration, though oxygen is favored based on bioenergetic grounds (Rittmann and McCarty, 2001). Denitrification is an anoxic process that mainly occurs in oxygen depleted environments,

which can be found in soil, sediments, groundwater, wetlands and many other poorly ventilated ecosystems (Vymazal, 2007). Denitrification is the most common pathway of nitrate reduction in both the environment and engineered systems. It is commonly used for the treatment of nitrate contaminated waters because the ultimate product, gaseous N_2, is released to the atmosphere, removing the nitrogen from the water.

2.4.2 Dissimilatory Nitrate Reduction to Ammonia (DNRA)

An alternative pathway of nitrate reduction is the dissimilatory nitrate reduction to ammonia (DNRA), which reduces nitrate to nitrite and then to ammonia, and thus does not lead to nitrogen removal. This pathway is less common than denitrification in most ecosystems, but it is favored under very low redox potential conditions, when sulfide is present or when there is a very high COD to nitrogen ratio (Tugtas and Pavlostathis, 2007). DNRA is the reduction of nitrate to nitrite to ammonia and is unfavorable for nitrate removal because it does not remove nitrogen from the system.

2.4.3 Stoichiometry

Denitrification reduces nitrate to nitrogen gas. The oxidation state of the nitrogen atom during denitrification is displayed in Reaction 2-2.

$$\underset{+5}{NO_3^-} \rightarrow \underset{+3}{NO_2^-} \rightarrow \underset{+2}{NO} \rightarrow \underset{+1}{N_2O} \rightarrow \underset{0}{N_{2(g)}} \qquad (2\text{-}2)$$

Thus, nitrate reduction to N_2 requires 5 electrons per atom N. Based on the fact that 8 g COD are required per electron equivalent (Rittmann and McCarty, 2001), and by

ignoring microbial growth, the theoretical COD requirement for denitrification is as follows:

$$\frac{5\text{eeq}}{\text{mol N}} \times \frac{8\text{ g COD}}{\text{eeq}} \times \frac{\text{mol N}}{14\text{ g N}} = 2.857 \frac{\text{g COD}}{\text{g N}}$$

Based on the above calculation, the COD requirement for denitrification, neglecting biomass growth, is 2.86 grams COD consumed per gram of NO_3-N reduced to N_2. Assuming that the above calculated COD requirement for nitrate reduction to N_2 represents about 50% of the total COD required for both nitrate reduction and microbial growth (i.e., $f_s = f_e = 0.5$; where, f_s and f_e is the fraction of the electron donor equivalents required for cell synthesis and energy, respectively), and neglecting microbial decay, the total COD requirement is about 5.7 g COD/g nitrate-N reduced to N_2.

2.4.4 Monod Kinetics

The rate of nitrate reduction can be described by a Monod kinetic equation containing two substrates, nitrate (N) and electron donor (D). The dual-substrate Monod equation is then as follows:

$$-\frac{dN}{dt} = \frac{kNX}{K_N+N}\frac{D}{K_D+D} \qquad (2\text{-}3)$$

where k is the nitrate reduction rate per unit biomass (mg nitrate-N/mg biomass VSS-day), N is the nitrate concentration (mg nitrate-N/L), X is the biomass concentration (mg biomass VSS/L), K_N is the half-saturation coefficient for nitrate reduction (mg nitrate-N/L), D is the electron donor (ed) concentration (mg ed/L) and K_D is the half saturation coefficient for electron donor utilization (mg ed/L). When the electron donor is at or in

excess compared to nitrate concentration based on stoichiometry (see above) and therefore $D >> K_D$), the electron donor effect can be neglected and equation 2-3 can be simplified to:

$$-\frac{dN}{dt} = \frac{kNX}{K_N + N} \quad (2\text{-}4)$$

Unless otherwise indicated, electron donor was supplied in excess in this study to avoid electron donor (i.e., carbon) limitations.

Based on the Monod model and taking into account microbial decay, the change in biomass concentration is as follows:

$$\frac{dX}{dt} = \frac{YkNX}{K_N + N} - bX \quad (2\text{-}5)$$

where Y is the true yield coefficient (mg biomass VSS produced/mg nitrate-N consumed) and b is the specific microbial decay rate (day^{-1}). The equations for both nitrate (equation 2-4) and biomass (equation 2-5) were implemented using the Matlab ode15 solver (MATLAB 7.0.1; The Mathworks, Natick, MA) to simulate batch systems used in this study. Given initial nitrate and biomass concentrations and estimated K_N and Y values, the model predicted the nitrate and biomass concentrations as a function of time. Because the soil active biomass at the beginning of each batch test could not be quantified, the specific substrate utilization rate (k, mg nitrate-N/mg biomass VSS-day) was estimated by adjusting the initial biomass concentration to obtain a good fit to the experimental nitrate concentration data.

CHAPTER 3

ANALYTICAL METHODS

3.1 pH

All pH measurements were performed using the potentiometric method with a ATI Orion Model 370 digital pH meter (Orion Research Inc., Boston, MA) and a gel-filled combination pH electrode (VWR International, West Chester, PA). The meter was calibrated weekly with pH 4.0, 7.0, and 10.0 standard buffer solutions (Fisher Scientific, Pittsburg, PA).

3.2 Dissolved Oxygen

Dissolved Oxygen in this study was measured using the polarographic method (APHA,2005) with a YSI Model 58 oxygen meter in conjunction with a YSI 5750 oxygen probe (Yellow Springs Instruments, Yellow Springs, OH). The instrument was calibrated to water-saturated air (at a given temperature) before each use and the probe electrolytic solution and membrane were changed periodically.

3.3 Total and Soluble Chemical Oxygen Demand (tCOD and sCOD)

COD was measured using the closed reflux, colorimetric method as described in *Standard Methods* (APHA,2005). An aliquot of 3 mL digestion solution composed of 4.9 g $K_2Cr_2O_7$, 6 g $HgSO_4$, 6 g Ag_2SO_4 and 500 mL H_2SO_4 was transferred to HACH COD digestion vials (HACH Company, Loveland, CO) and then 2 mL of sample was added to the vial. After tumbling the vial for 4-8 times, the content in the vials was digested at

150^{o}C for 2 hours and then cooled down to room temperature. The absorbance was measured at 620 nm with a Hewlett-Packard Model 8453 UV/Visible spectrophotometer (Hewlett-Packard Co., Palo Alto, CA) equipped with a diode array detector, deuterium and tungsten lamps and a 1 cm path length. Samples were centrifuged and filtered through a 0.2 μm nitrocellulose membrane filter if the sCOD was measured, otherwise well-mixed samples were used after appropriate dilution for tCOD measurements. All samples were prepared in triplicates and a calibration curve was prepared using standard solution of potassium hydrogen phthalate (KHP).

3.4 Dissolved Organic Carbon (DOC)

DOC measurements were performed using a Shimadzu TOC-5050A Total Organic Carbon Analyzer (Shimadzu Scientific Instruments Inc., Columbia, MD) equipped with a non-dispersive infrared detector for the analysis of total, organic and inorganic carbon of liquid samples. Liquid samples were filtered through 0.2 μm membrane filters (Fischer Scientific, Pittsburgh, PA), acidified below pH 2.0 using a 0.2 N HCl solution and purged with CO_2-free air for 2 minutes. Triplicate measurements were performed for each sample using 25 μL injection volume. Carbon analysis was based on catalytic combustion of the sample at 680^{o}C. A calibration curve was prepared using standard solution of KHP.

3.5 Ammonia

Ammonia was measured using the distillation method described in *Standard Methods* (APHA,2005). The samples were centrifuged at 12,000 rpm for 15 minutes and filtered through a 0.2 μm nitrocellulose membrane filter (Fisher Scientific, Pittsburgh, PA). The

ammonia distillation was performed using a Labconco distillation apparatus (Labconco Corp., Kansas City, MO). The distillate then was titrated with 0.2 N H_2SO_4 and the ammonia was quantified using the equation below.

$$[NH_3 - N] = \frac{14000N(V_{H2SO4} - V_{blank})}{V_{sample}}$$

In the above equation, [NH_3-N] is the concentration of ammonia-nitrogen in mg/L, N is the Normality of H_2SO_4 used for titration, V_{H2SO4} is the volume in mL of H_2SO_4 used for titration of the sample, and V_{blank} is the volume of H_2SO_4 in mL used for titration of the blank (DI water).

3.6 Total and Volatile Solids (TS and VS)

Total solids were determined according to procedures outlined in *Standard Methods* (APHA,2005). Samples were weighed in pre-ignited (550^{o}C) and cooled ceramic crucibles using an Ohaus AP250D Analytical Balance (precise to ±0.02 mg up to 52 g, and to ±0.1 mg between 52 and 210 g). The samples were then dried at 105^{o}C for 24 hours in a Fisher Isotemp Model 750G oven. After drying, the crucibles were transferred to a desiccator until cooled, and then the dry weight was measured. If VS were to be determined, the crucibles were transferred to a Fisher Isotemp Model 550-126 muffle furnace and ignited at 550^{o}C for 20 minutes. After ignition, the samples were cooled in a desiccator and the remaining solids weight was measured. TS and VS were then calculated using the equations below.

$$\text{TS (mg/L)} = \frac{\text{(Crucible weight after 105°C (mg))} - \text{(Crucible tare weight (mg))}}{\text{Sample volume (L)}}$$

$$\text{VS (mg/L)} = \frac{\text{(Crucible weight after 105°C (mg))} - \text{(Crucible weight after 550}^{o}\text{C (mg))}}{\text{Sample volume (L)}}$$

3.7 Total Gas Production

Total gas production in closed assay bottles and large volume reactors was measured by either the gas-water displacement method or with a VWR Pressure/Vacuum transducer (resolution –1 atm to 1.974 atm with an accuracy of 0.002 atm).

3.8 Gas Composition

The gas composition was determined by a gas chromatography (GC) unit (Agilent Technologies, Model 6890N; Agilent Technologies, Inc., Palo Alto, CA) equipped with two columns and two thermal conductivity detectors. Methane (CH_4) and dinitrogen (N_2) were separated with a 15 m HP-Molesieve fused silica, 0.53 mm i.d. column (Agilent Technologies, Inc.). Carbon dioxide (CO_2), nitric oxide (NO) and nitrous oxide (N_2O) were separated with a 25 m Chrompac PoraPLOT Q fused silica, 0.53 mm i.d. column (Varian, Inc., Palo Alto, CA). Helium was used as the carrier gas at a constant flow rate of 6 mL/min. The 10:1 split injector was maintained at 150°C, the oven was set at 40°C and the detector temperature was set at 150°C. All gas analyses were performed by injecting a 100 µL gas sample. The minimum detection limits for CH_4, CO_2, NO, N_2O and N_2 was 500, 800, 500, 7 and 50 ppmv, respectively.

3.9 Anions

Chloride (Cl^-), nitrite (NO_2^-), bromide (Br^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), and sulfate (SO_4^{2-}) anion concentrations were determined using a Dionex DX-100 ion chromatography unit (Dionex Corporation, Sunnyvale, CA) equipped with a suppressed conductivity detector, a Dionex IonPac AG14A (4x50mm) precolumn, and a Dionex IonPac AS14A (4x250 mm) analytical column. The unit was operated in autosupression mode with 1mM $NaHCO_3$/8mM Na_2CO_3 eluent and a flow rate of 1 mL/min. All samples were filtered through 0.2 μm membrane filters prior to injection. The minimum detection limit for each anion listed above was 0.03, 0.02, 0.03, 0.04, 0.02 and 0.05 mM, respectively.

CHAPTER 4

CARBON LOADING AND DENITRIFICATION KINETICS

4.1 Introduction

Nitrate contamination is a major problem worldwide. The severity of the contamination depends on many factors such as field and pumping conditions, source and exposure of contamination, and infiltration. Nitrate contamination is commonly treated by the biologically facilitated process, denitrification, which removes nitrogen from the water in the presence of an external carbon source (Rittmann and McCarty, 2001). Denitrifiers are heterotrophic bacteria, which are capable of using many different types of organic carbon. Many studies have found that denitrification kinetics are influenced by the type of available carbon and some carbon sources are more efficiently used than others (Christensson et al., 1994; Lorrain et al., 2004; Mohseni-Bandpi and Elliott, 1998).

The objectives of this study were to investigate the effect of two possible carbon sources used in this study (hay and MicroC G^{TM}) and initial nitrate concentration on the nitrate reduction capacity. Assays were conducted to assess the nitrate reduction capacity of bacteria in the surface soil collected at the study site using nitrate-bearing groundwater and three carbon sources (glucose, hay, and MicroC G^{TM}). MicroC G^{TM} is a plant-derived carbohydrate source that has been found to achieve similar nitrate reduction rates as methanol or acetate when used as a carbon source for denitrification (Cherchi et al., 2009).

In addition to carbon sources, the effect of nitrate concentration on nitrate reduction rates was investigated. In some cases, high levels of nitrate increased nitrite

accumulation and decreased nitrate reduction rates (Dhamole et al., 2007; Glass and Silverstein, 1999). At the study site, nitrate concentrations in the groundwater varied drastically from season to season depending on the pumping rate and precipitation and concentrations were reported in the range of 65 – 400 mg N/L. Thus, assays were conducted to investigate the denitrification kinetics (i.e., nitrate removal rate) at various initial nitrate concentrations.

4.2 Materials and Methods

4.3.1 Sample Collection and Characterization

Groundwater, surface soil, and hay were collected at the site and transported to the laboratory. The groundwater samples were stored in plastic containers under refrigeration ($4^{o}C$). The soil sample was passed through a US No. 10 sieve and then spread thin to air-dry for 24 hours at room temperature. The hay sample was spread and air dried for 24 hours before being coarsely chopped in a blender in small pieces (< 1 inch). Both the soil and hay samples were stored in covered plastic containers at room temperature. MicroC G^{TM}, a plant-derived complex carbohydrate mixture, was obtained from Environmental Operating Solutions Inc. (Bourne, MA). All samples were characterized by measuring pH, soluble and total chemical oxygen demand (sCOD and tCOD), dissolved organic carbon (DOC), moisture content, NH_4^+-N, NO_3^--N, NO_2^--N, and other ions following procedures outlined in *Standard Methods* (APHA,2005). Soluble COD was measured by the HACH colorimetric method and the total COD was measured by the Open Reflux Method. To measure pH, DOC, soluble COD, ammonia and ions, hay and soil filtrate solutions were prepared by adding 5 g of dry hay or dry soil to 300 mL of DI water and

mixing for 1 day at room temperature. The hay and soil solutions were then centrifuged at 10,000 rpm for 30 min and the supernatant was stored at 4°C. A MicroC G^{TM} solution was also prepared by 1000x dilution in DI water and stored in the dark at 4^{o}C. The concentrated MicroC G^{TM} was stored in the dark at room temperature.

The characteristics of the samples are given in Table 4-1. All samples were slightly acidic, with pH values ranging from 3.9 to 5.7. The nitrate concentration in the groundwater was in the range of 65 to 75 mg N/L. The soil sample was mostly inorganic matter (~95%) and did not contribute significant soluble COD or ions to the solution. On the other hand, the hay was 93% organic matter and contributed a significant amount of soluble COD as well ammonia, nitrate and nitrite, to the filtrate solution.

Table 4-1. Summary of sample analysis.

Parameter	Soil	Groundwater	MicroC G^{TM}	Hay
pH	4.4	5.7	3.9	4.2
Moisture content (%)	3.9±0.07[a]			9.1±0.8
Dry weight (%)	96.1±0.07			90.9±0.8
Organic matter (% of dry)	4.9±0.09			92.4±0.1
DOC (filtrate; mg C/L)	9.8±0.4	9.1±1.5	411±32[c]	1437±19
Soluble COD (filtrate; mg/L)	14.5±7	58.1±4.8	642±41[c]	2871±24
Total COD (mg/g dry weight)	68.3±5.2			1122±74
Ions (filtrate)				
Chloride (mg Cl/L)	ND[b]	14.4±0.75	ND	52.6
Nitrite (mg N/L)	ND	ND	ND	15.5
Nitrate (mg N/L)	0.3	69.3±1.3	ND	47.4
Sulfate (mg S/L)	0.2	28.4±0.1	ND	18.9
Phosphate (mg P/L)	1.3	ND	ND	43.2
Ammonia (filtrate; mg N/L)	ND	ND	ND	36.4±2.8

[a] Mean ± standard deviation (n = 3)
[b] ND, not detected
[c] 1000X diluted solution

Upon completion of the sample characterization, batch experiments were conducted. A summary of all batch experiments are given in Table 4-2. All batch experiments are described in detail in this chapter, excluding those for the effect of temperature which are discussed in Chapter 5.

Table 4-2. Summary of batch experiments.

Purpose	Reactor Type	Initial Nitrate (mg N/L)	Temperature (°C)	Carbon Source
Electron Donor Exhaustion	Closed Serum bottles	60	22 - 24	Glucose Hay MicroC G™
Electron Donor Exhaustion	Open Glass Reactor	60	22 - 24	Hay MicroC G™
Kinetics	Closed Serum bottles	35 - 400	22 - 24	MicroC G™
Kinetics	Open Open Plexiglas	70 - 400	22 - 24	MicroC G™
Temperature Effect (Chapter 5)	Open Open Plexiglas	150	5 - 22	MicroC G™
Seasonal Wetland In-situ Activity (Chapter 5)	Closed Serum bottles	40 - 150	10 , 22	MicroC G™

4.3.2 Carbon Exhaustion Assays

In order to investigate the biodegradability of the various carbon sources, batch assays were conducted under both open and closed conditions. The closed batch assay was conducted using 160-mL serum bottles closed to the atmosphere. The experimental matrix for the closed assay can be seen in Table 4-3.

Table 4-3. Details of the closed system carbon exhaustion assay.

Series	Description	Soil (g dry)	GW[b] (mL)	Nutrient Media (mL)	DI (ml)	Carbon Source
A	Control	5	100	0	20	None
B	Media	5	100	15	5	None
C	Glucose + Media	5	100	15	0	Glucose (5 mL)[a]
D	Glucose	5	100	0	15	Glucose (5 mL)[a]
E	MicroC G^{TM}/Media	5	100	15	0	MicroC G^{TM} (5 mL)[a]
F	MicroC G^{TM}	5	100	0	15	MicroC G^{TM} (5 mL)[a]
G	Hay + Media	5	100	15	5	Hay (0.283 g wet)
H	Hay	5	100	0	20	Hay (0.283 g wet)

[a] Stock solution of 14.4 mg COD/mL
[b]GW, Groundwater

The carbon sources used in the experiments included MicroC G^{TM} and hay. When this study was initiated the biological activity of the soil inocula was unknown. Glucose, which is a completely biodegradable carbon source known to be effectively used in heterotrophic denitrification, was chosen to test the biological activity of soil bacteria. In addition, a nutrients-rich medium was supplied to select series in order to determine any potential nutrient limitations.

The initial nitrate concentration in the groundwater was approximately 60 mg N/L. In an effort to estimate the extent of the carbon source biodegradability, upon exhaustion of the groundwater nitrate, a volume of nitrate ($NaNO_3$) solution were continuously added until the nitrate was no longer being removed, in which case, it was assumed that most bioavailable carbon was exhausted. Incubation was carried out at room temperature (22 to 24^{o}C).

In order to further assess the biodegradability of the two carbon sources (hay and MicroC G^{TM}) under open to the atmosphere conditions, similarly to the wetland conditions, three batch reactors were prepared using 2-L glass bottles. Each reactor was amended with 1000 g dry soil and 1.5 L of nitrate containing groundwater (65 mg NO_3-N/L). Initially, all three reactors were incubated for about a week without any carbon addition. Then, one reactor did not receive any auxiliary carbon source and served as the control, whereas the second and third reactor were amended with MicroC G^{TM} (432 mg COD) and hay (1.5 g wet weight), respectively. The reactors were stored and covered to reduce any direct light effect at room temperature (22 to 24^{o}C) for the duration of the experiment. During the incubation period the following measurements were periodically conducted: nitrate, nitrite, pH, D.O., soluble COD, and ammonia. The nitrate and nitrite concentrations were monitored until all were removed and then a volume of a nitrate stock solution was added to each reactor to bring the nitrate concentration to approximately 65 mg NO_3-N/L. In the control reactor, the pH was slightly acidic around 5. In the carbon amended reactors, the pH was near neutral in the range of 7 and 7.5 for the duration of the incubation period. D.O, was constant around 6 mg/L in the control reactor. After carbon addition in the hay and MicroC G^{TM} reactors, D.O. dropped from 6 mg/L to below 2 mg/L but increased throughout the incubation period until it reached the initial 6 mg/L.

4.3.3 Effect of Initial Nitrate Concentration on Denitrification Kinetics

Two batch assays were conducted to investigate the nitrate removal rate at different initial nitrate concentrations, under both closed and open to the atmosphere conditions.

The closed assay was conducted using duplicate 160-mL serum bottles sealed with rubber stoppers and aluminum crimps. Each serum bottle was amended with 5 g dry soil and 100 mL nitrate containing groundwater, except one series to which only 60 mL of groundwater was added (series B). A volume of K_2HPO_4 and $NaNO_3$ stock solutions was added to each serum bottle resulting in 1 mg phosphate-P/mg COD added and concentrations of NO_3-N ranging from 35 to 420 mg NO_3-N/L. The phosphate solution was added at a COD:P ratio of 100:1 in order to avoid possible P limitation at the relatively high initial nitrate-N levels tested. Excluding the control (series A), a volume of a Micro C G™ stock solution was added to each series resulting in a COD:N ratio of 6. The setup of this batch assay is summarized in Table 4-4. Incubation was carried out at room temperature (22 to 24°C). During the incubation period, the following parameters were measured: nitrate, nitrite, gas production and gas composition (CO_2 and N_2).

Table 4-4. Details of the closed bottle assay testing the effect of initial nitrate concentration on nitrate reduction.

Series	Soil (g dry)	GW[a] (mL)	Initial Nitrate (mg N/L)	MicroC G™ (mg COD/L)	Total COD (mg/bottle)	Total P (mg/bottle)
A	5	100	70	0	0	0
B	5	60	35	211	25	0.3
C	5	100	70	422	51	0.5
D	5	100	141	843	101	1
E	5	100	281	1687	202	2
F-1	5	100	422	2530	304	3
F-2	5	100	422	2530	304	0

[a] GW, groundwater

In order to quantify the kinetics of nitrate reduction in an open to the atmosphere system, a series of batch tests were conducted at different initial nitrate concentrations ranging from 70 to 400 mg NO_3-N/L. A 15-L cubic Plexiglas reactor was used which was prepared with 10.5 kg of soil and approximately 9 L of nitrate-bearing groundwater. For the tests conducted at higher than 70 mg NO_3-N/L, the groundwater was amended with a volume of a $NaNO_3$ stock solution. MicroC G^{TM} was used as the carbon and electron source in all tests at a COD:N ratio of 6. After each test was complete, the reactor was drained, flushed with de-ionized water three times and nitrate-bearing groundwater once before being refilled with groundwater for the next batch. This series of batch tests was conducted at room temperature (22 to 24^{o}C). Liquid samples were taken daily and the nitrate, nitrite, and COD concentrations were measured.

4.3 Results and Discussion

4.3.1 Carbon Exhaustion

In the closed batch assay, nitrate reduction was observed in all series without any lag, especially in those series amended with a carbon source. After the initial observations, the glucose series was abandoned and the assay focused on the other two carbon sources, consistent with the auxiliary carbon sources used in the wetland pilot study. Four of the eight series were amended with nutrients. No significant difference was observed between the series with and without nutrients, indicating that in soil-amended systems and for the duration of this assay, nutrients were sufficient to assist the microbial nitrogen reduction. The nitrate concentration in the control, MicroC G^{TM} and hay series

throughout this assay is shown in Figure 4-1. It is noteworthy that nitrite was not detected in any of the three series. At the end of the incubation period, there was no net ammonia production and the only nitrogen species derived from the groundwater nitrate was nitrogen gas (N_2). Therefore, under the conditions of this study, nitrate reduction followed the denitrification pathway and dissimilatory nitrate reduction to ammonia (DNRA) was not observed. A very low nitrate removal rate was observed in the control series (i.e., without any auxiliary carbon addition), which is attributed to the limited bioavailable, degradable organic matter in the surface soil.

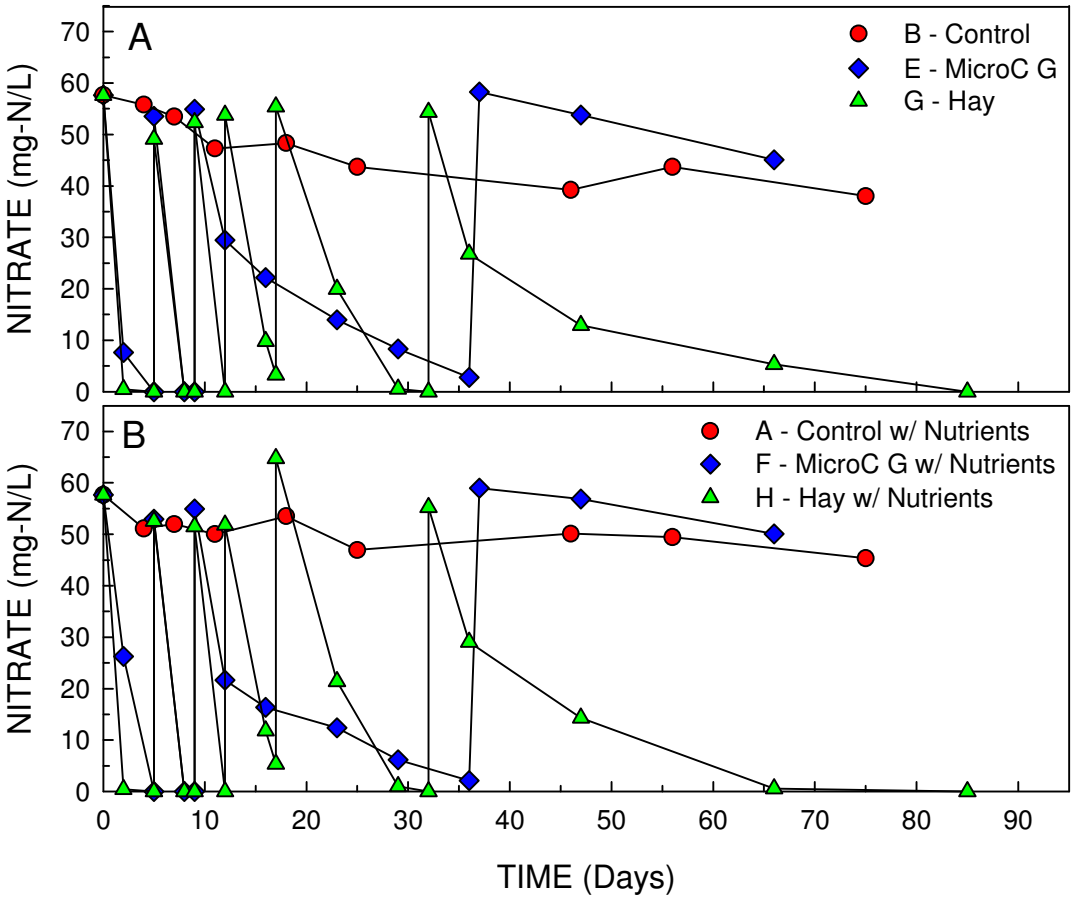

Figure 4-1. Nitrate profiles in series amended with (A) and without nutrients (B); closed system.

Each serum bottle in the MicroC G™ series was amended with 72 mg COD/bottle. Assuming that hay was less degradable than MicroC G™, about four times as much COD was added to the hay series (290 mg COD/bottle). This partly explains why each MicroC G™ series was only spiked four times with nitrate, while the hay series were spiked with nitrate six times. Ignoring microbial growth, the theoretical COD requirement for the reduction of NO_3-N to N_2 via denitrification is 2.85 mg COD/mg NO_3-N removed (see Chapter 2). Since all nitrate and nitrite were removed, this value was used to estimate the biodegradability of the carbon sources, ignoring biomass requirements due to the long-term incubation during which the denitrifying population decays, thus recycling electron equivalents to be used for nitrate reduction. Based on these assumptions, about 69 and 36% of the initially added COD was accounted for in terms of nitrate reduction in the MicroC G™ and hay series, respectively. Therefore, under closed conditions, both MicroC G™ and hay are effective carbon sources for denitrification, but MicroC G™ is significantly more biodegradable than hay.

Initially in the open assay, a slow rate of nitrate removal was observed in all three reactors, which was attributed to the low level of biodegradable organic matter in the surface soil before carbon addition. However, upon the addition of the external carbon sources in the two reactors, a very fast nitrate removal rate was observed in these reactors. As the carbon source was depleted, the nitrate removal rate decreased to a rate similar to that observed in the control (Figure 4-2). Nitrite and net ammonia production were not detected in these reactors.

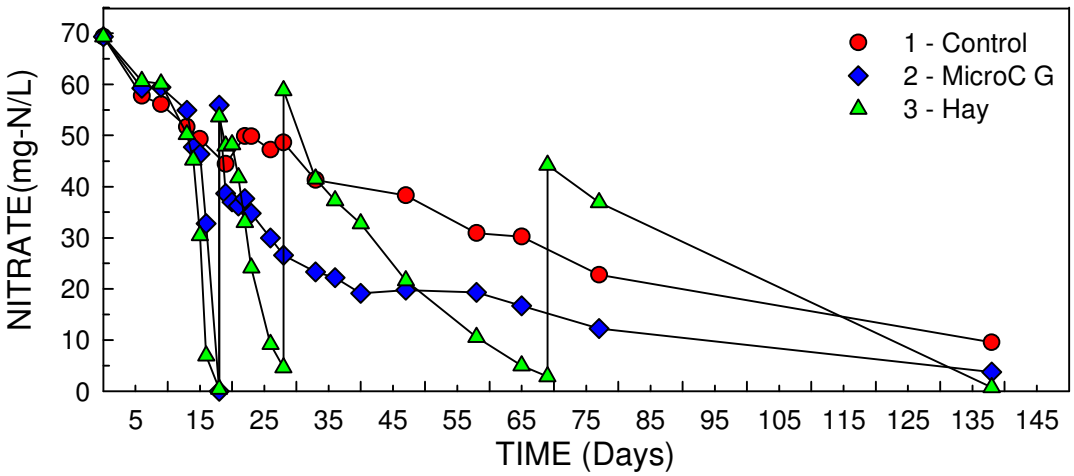

Figure 4-2. Nitrate profiles in the control, MicroC G^{TM}, and hay amended reactors; open system.

Based on the total amount of nitrate reduced and the initially added COD, about 53 and 39% of the initially added COD was accounted for in terms of nitrate reduction in the MicroC G^{TM} and hay amended reactor, respectively. This value is nearly the same for both the closed and open hay systems (36 and 39%, respectively). The fractions of COD utilized for nitrate reduction in each experiment are summarized in Table 4-5. The lower value obtained for the MicroC G^{TM} amended reactor compared to that obtained in the closed batch assay is due to COD consumption under the more thermodynamically favorable respiration conditions (i.e., using oxygen as the terminal electron acceptor as opposed to nitrate). Therefore, about 23% of the total MicroC G^{TM} COD consumed in the open to the atmosphere system was diverted away from nitrate reduction. The reactors in this assay were kept static (i.e., without any mixing). Under field conditions, the rate of reaeration (i.e., oxygenation) is expected to be higher as a result of wind and other factors. Overall, the conclusion is the same as for the closed system test, that both

MicroC G™ and hay are effective carbon sources for the denitrification of nitrate-bearing groundwater.

Table 4-5. Biodegradability of carbon sources under closed and open system conditions.

Parameter	**Closed System**		**Open System**	
	Hay	**MicroC G™**	**Hay**	**MicroC G™**
Total Nitrate Removed (mg)	36.4	17.4	237.6	80.4
COD Added (mg)	288.4	72.0	1739.1	432
Theoretical COD requirement (mg)	103.9	49.7	677.2	229.1
Biodegradable COD (%)	36.0	69.0	38.9	53.0

4.3.2 Effect of Initial Nitrate Concentration on Denitrification Kinetics

In the closed system experiment, nitrate reduction took place without any significant lag and the nitrate and nitrite profiles are shown in Figure 4-3. The relatively low nitrate removal rate observed in the first 20 hours of incubation is attributed to the low population size of active denitrifying bacteria in the soil. Significant nitrite levels were observed in series with an initial nitrate concentration of 125 mg N/L and above. Nevertheless, the nitrite reduction rate was fast and all series achieved complete denitrification in less than 6 days. As expected, nitrate removal in the control series (i.e., without any auxiliary carbon addition) was not observed due to the limited bioavailable, degradable organic matter in the surface soil.

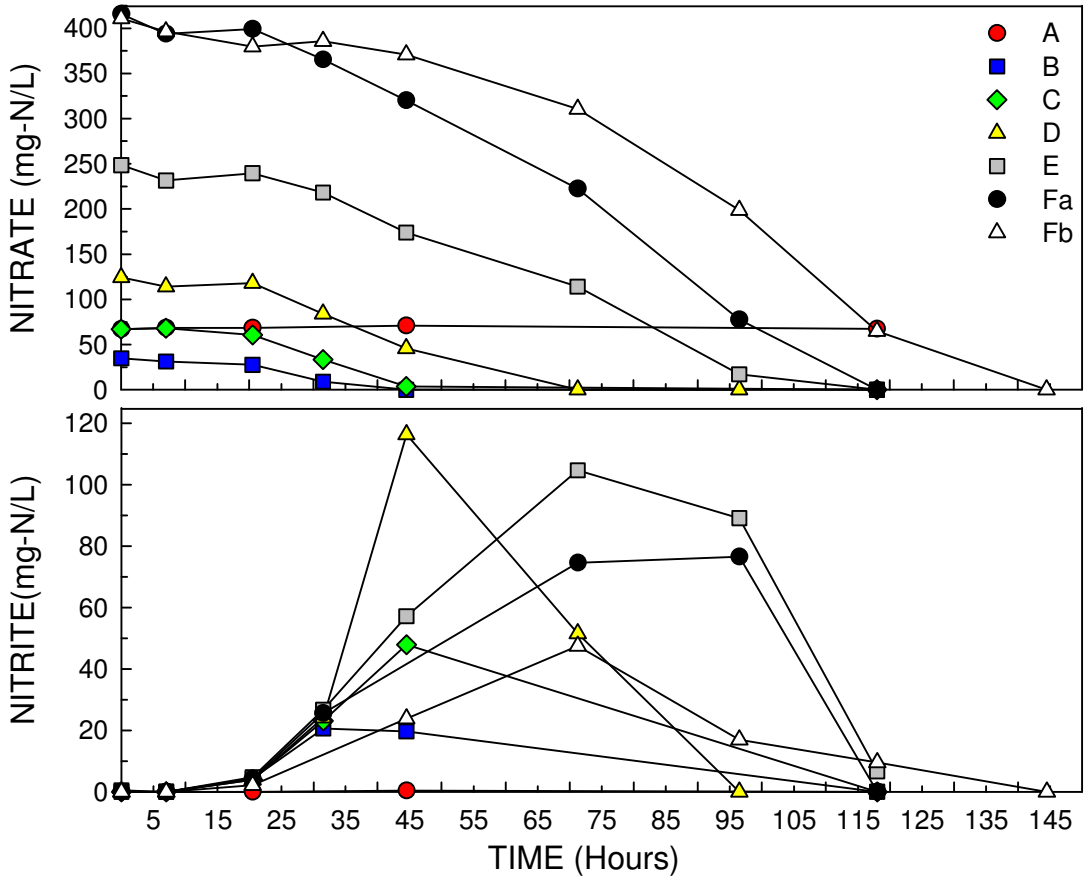

Figure 4-3. Nitrate (A) and nitrite (B) profiles in series amended with an initial nitrate concentration from 35 to 415 mg N/L and MicroC G^{TM}; closed system.

In order to estimate the nitrate reduction rate (k) using the Monod equation, other biokinetic parameters needed to be estimated for denitrification. As a result, based on reported literature values and experimental data, the values of the microbial yield coefficient (Y), half saturation constant (K_N), and microorganism decay rate constant (b), were estimated. As described in Chapter 2, the theoretical carbon requirement for denitrification is 2.86 g COD per g NO_3-N reduced to N_2. Rittmann and McCarty (2001) reported the fraction of electron donor (ed) used for synthesis, f_s, as equal to 0.55. For simplification of this model, the fractions of electron donor required for synthesis and

energy, f_s and f_e respectively, were assumed to be equal to 0.5. Therefore, the total electron donor requirement for energy and growth is equivalent to 5.7 g COD/g N. Rittmann and McCarty (2001) also estimated the true yield coefficient for denitrifiers using organic material as the electron donor to be approximately 0.26 g VSS/g ed COD. Since denitrifiers use NO_3^- as the N source for cell synthesis, the VSS_COD is 1.98 g VSS_COD/g VSS, rather than 1.42 g VSS_COD/g VSS when ammonia is used. Based on these reports, the yield coefficient used for all simulations was calculated as shown below:

$$Y = 0.26 \frac{\text{g VSS}}{\text{g ED\_COD}} \text{x } 1.98 \frac{\text{g VSS\_COD}}{\text{g VSS}} = 0.51 \frac{\text{g VSS\_COD}}{\text{g ED\_COD}} \text{ x } 5.7 \frac{\text{g ED\_COD}}{\text{g N}} = 2.86 \frac{\text{g VSS\_COD}}{\text{g N}}$$

The microorganism decay rate constant must also be estimated in order to model this system using Monod kinetics. The decay rate values for denitrifiers are generally in the range of 0.05 to 0.15 day^{-1} (Rittmann and McCarty, 2001; Tchobanoglous et al., 2003). A microorganism decay rate of 0.1 day^{-1} was chosen for all simulations; however, preliminary simulations using values of 0.05 and 0.15 day^{-1} were run and only small variations were observed in terms of nitrate concentration patterns.

After estimating the yield coefficient and the microorganism decay rate constant, the half-saturation coefficient, K_N, was estimated based on reported literature values for denitrification and experimental data. Typical K_N values for denitrification have been reported in the range of 4 to 153 mg N/L (Tugtas and Pavlostathis, 2007; Zumft, 1997). Based on preliminary simulations, the K_N value of 65 mg N/L was chosen. Given the fact that the initial, active denitrifiers concentration in the soil was not measurable, for each set of experimental data, an initial biomass concentration and a substrate utilization rate

were chosen to fit the nitrate experimental data from both closed and open batch systems. For all simulations, the values of Y = 2.86 mg VSS_COD/mg N, b = 0.1 day^{-1}, and K_N = 65 mg N/L were used.

Based on the data from the closed experiment and applying Monod kinetics with the biokinetic parameter values described above the specific substrate utilization rate for the closed system was determined to be 0.456 mg nitrate-N/mg biomass VSS-day
The simulated nitrate and biomass profiles and the experimental data are plotted for four different initial nitrate concentrations (70, 140, 300, and 400 mg N/L) and are shown in Figure 4-4. For each initial nitrate concentration, the initial biomass was assumed to be 150 mg/L.

For the open system assay, the nitrate and nitrite concentrations in each test over the incubation period are shown in Figure 4-5. Similarly to the closed system assay, a lag period of approximately 24 hours was observed in the first batch test performed with fresh soil and groundwater, which was attributed to the very low active denitrifying population size of the surface soil used in these tests. Nitrate reduction proceeded immediately in all subsequent tests, again pointing out the importance of a significant active denitrifying population size. Significant, transient nitrite concentrations were observed in all tests, but after the complete removal of nitrate, nitrite was also removed achieving complete denitrification in less than 4 days, except in the first test, in which nitrite reduction was slower.

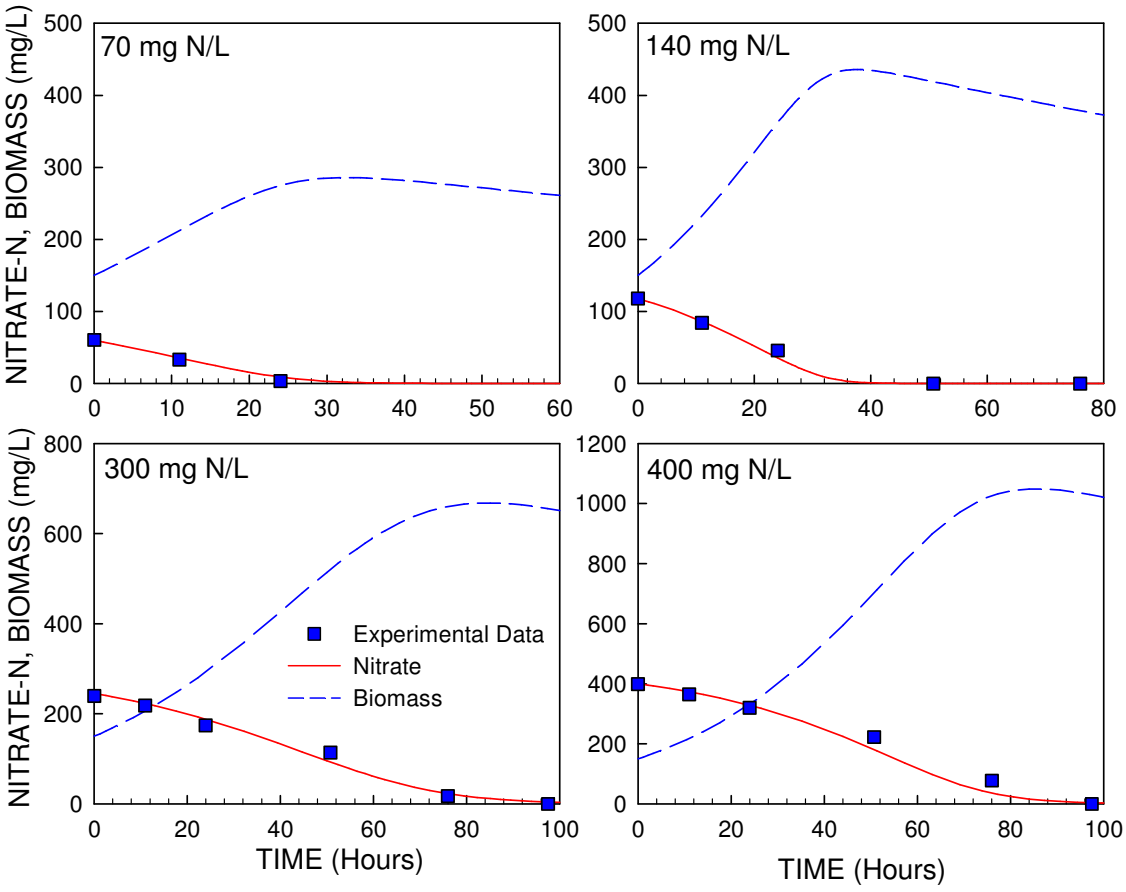

Figure 4-4. Nitrate and biomass profiles plotted with experimental nitrate data in batch tests conducted at different initial nitrate concentrations ranging from 70 to 400 mg N/L using MicroC G^{TM} as the carbon source (closed system).

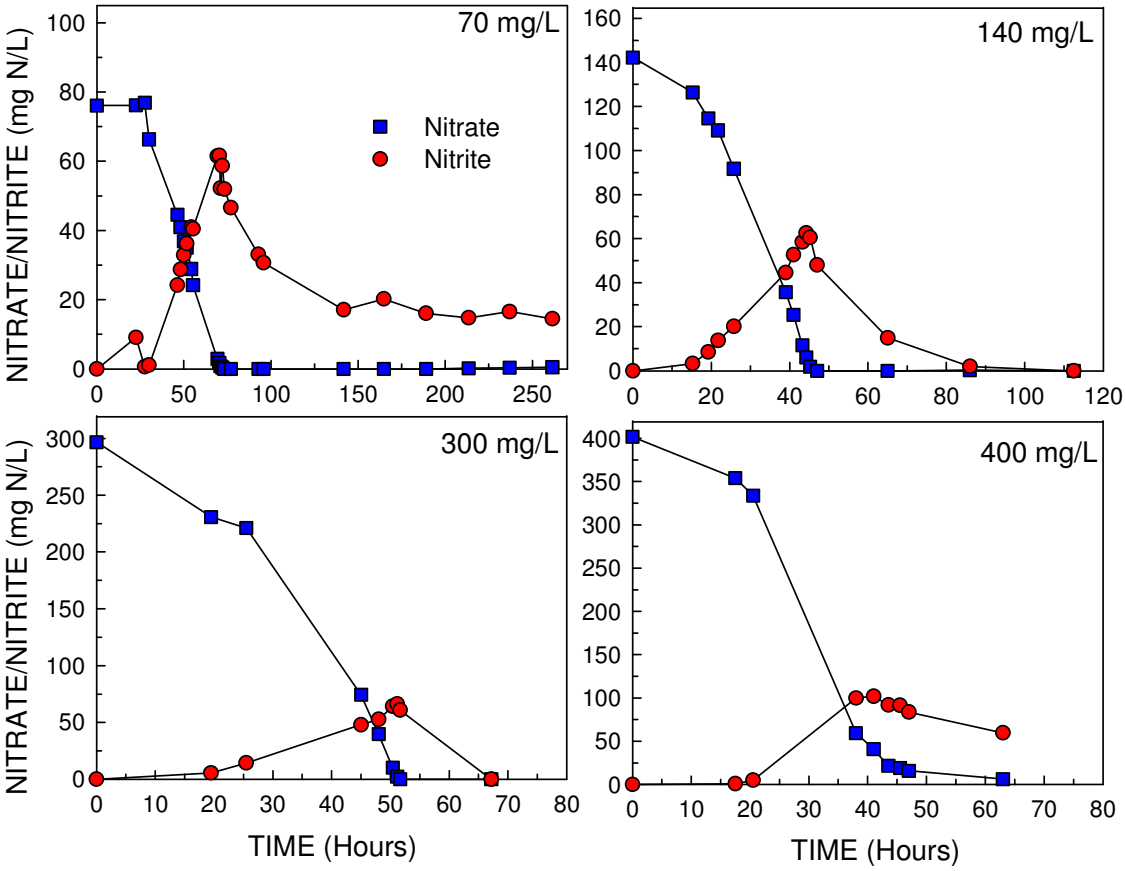

Figure 4-5. Nitrate and nitrite profiles in batch tests conducted at different initial nitrate concentrations ranging from 70 to 400 mg N/L using MicroC G^{TM} as the carbon source (open system).

Based on the data from the open experiment and applying Monod kinetics with the biokinetic parameter values described above the specific substrate utilization rate for the open system was determined to be 0.552 mg nitrate-N/mg biomass VSS-day. The simulated nitrate and biomass profiles and the experimental data are plotted for each initial nitrate concentration and are shown in Figure 4-6. For each initial nitrate concentration, the biomass was assumed to be 150 mg/L.

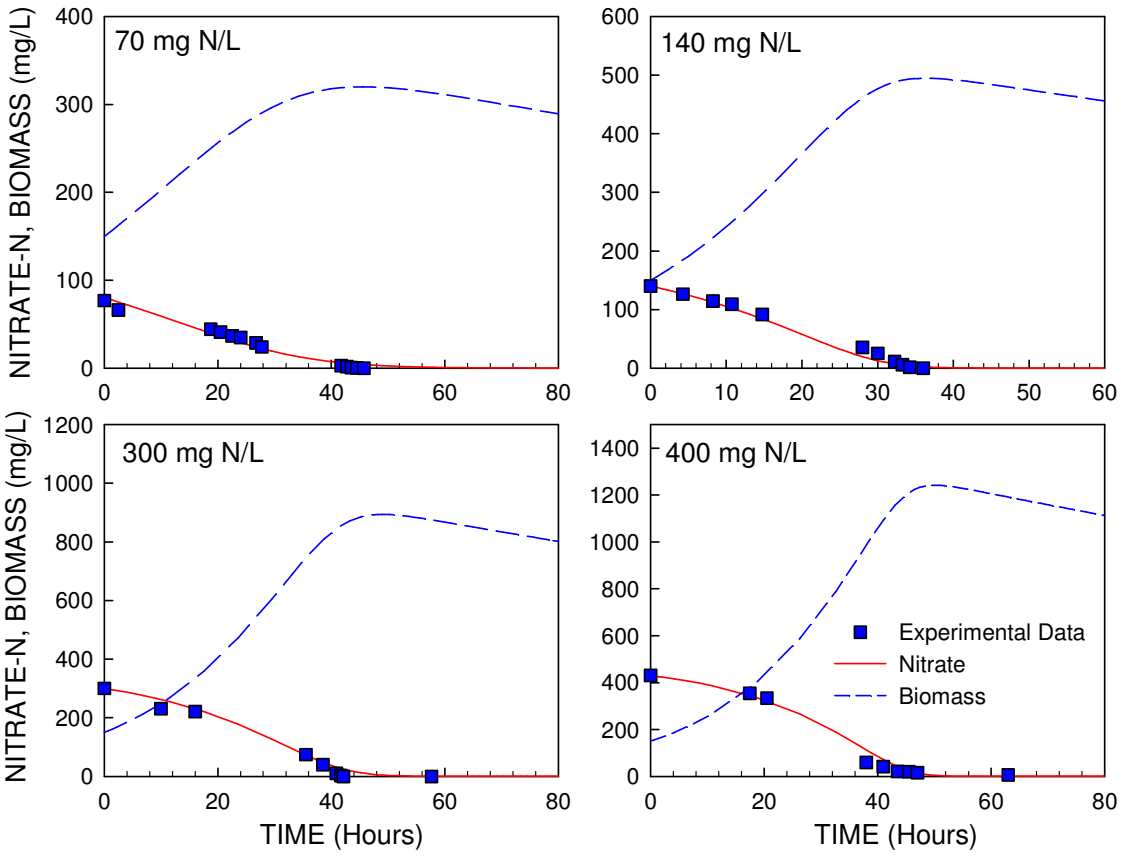

Figure 4-6. Nitrate and biomass profiles plotted with experimental nitrate data in batch tests conducted at different initial nitrate concentrations ranging from 70 to 400 mg N/L using MicroC G^{TM} as the carbon source (open system).

Compared to the nitrate reduction rate achieved in closed systems (0.456 mg nitrate-N/mg biomass VSS-day; the nitrate reduction rate achieved in open to the atmosphere systems was even higher. Therefore, as long as a bioavailable carbon source is supplied in excess of that required for the complete nitrate reduction, the nitrate reduction kinetics are not impacted by other alternative electron acceptors (e.g., oxygen for open systems) for similar systems (e.g., low mixing and reaeration). Similarly to the biodegradability assays, these laboratory reactors were static and mixing was avoided. However, under field conditions, a higher rate of aeration is expected (e.g., wind action), which may negatively impact the nitrate reduction rate as a result of a stronger competition for the carbon/electron source by oxygen.

4.4 Summary

Denitrification is a heterotrophic process that requires an external carbon and energy source to reduce nitrate to nitrogen gas. Many different carbon sources can be used, but some are more readily biodegradable than others. The two main sources investigated in this study, hay and MicroC G^{TM}, were found to have significantly different biodegradability, with slight variations under open and closed conditions. Both sources successfully drove denitrification to completion under both closed and open to the atmosphere conditions, but MicroC G^{TM} was more biodegradable than the hay.

Nitrate reduction kinetics were not affected by the initial nitrate concentrations tested (35 – 400 mg N/L). The nitrate reduction rates achieved under closed and open to the atmosphere conditions were comparable; however, the rate was slightly lower under closed conditions. Therefore, the nitrate reduction kinetics were not impacted by the

initial nitrate concentration or the presence of alternative electron acceptors (e.g., oxygen), as long as biodegradable carbon was supplied in excess.

CHAPTER 5

EFFECT OF TEMPERATURE ON DENITRIFICATION KINETICS

5.1 Introduction

Nitrate contaminated groundwater can often be treated in natural systems, depending on many environmental factors such as microbial activity, biodegradable carbon availability, and temperature. Treatment wetlands are often constructed in regions with moderate to cold climates that experience large seasonal variations in groundwater and sediment temperature. Biological and chemical processes controlling the efficiency of wetlands are enhanced by higher temperature (Kadlec and Reddy, 2001). The optimal temperature range for maximum nitrate reduction rates is between 20 and 25°C (Lee et al., 2009). In natural systems, denitrification commonly occurs in freshwater sediments; however, groundwater temperature values are usually around 10°C or lower (Rivett et al., 2008). These temperature values are well below the optimal range and therefore, it is important to understand the effect of temperature on the denitrification kinetics in order to assess the feasibility of a constructed wetland treatment option. The objective of this portion of the study was to investigate the temperature effect on denitrification kinetics in a temperature range of 5 – 22°C.

5.2 Materials and Methods

5.2.1 Temperature Test

In order to investigate the temperature effect on the nitrate reduction kinetics under open to the atmosphere conditions, four batch tests were performed at 22, 15, 10, and 5°C. The same 15-L cubic Plexiglas reactor used in previous experiments was also used in these tests. The reactor was housed in a controlled temperature room and its temperature was step-wise reduced from 22 to 5°C as shown in Figure 5-1. The rate of temperature change between the four target temperature values was 2°C/day. The initial groundwater nitrate concentration was adjusted to 150 mg N/L using a volume of a stock $NaNO_3$ solution. MicroC G™ was used as the carbon source at a COD:N ratio of 6:1. Nitrate and nitrite measurements were conducted throughout the incubation period.

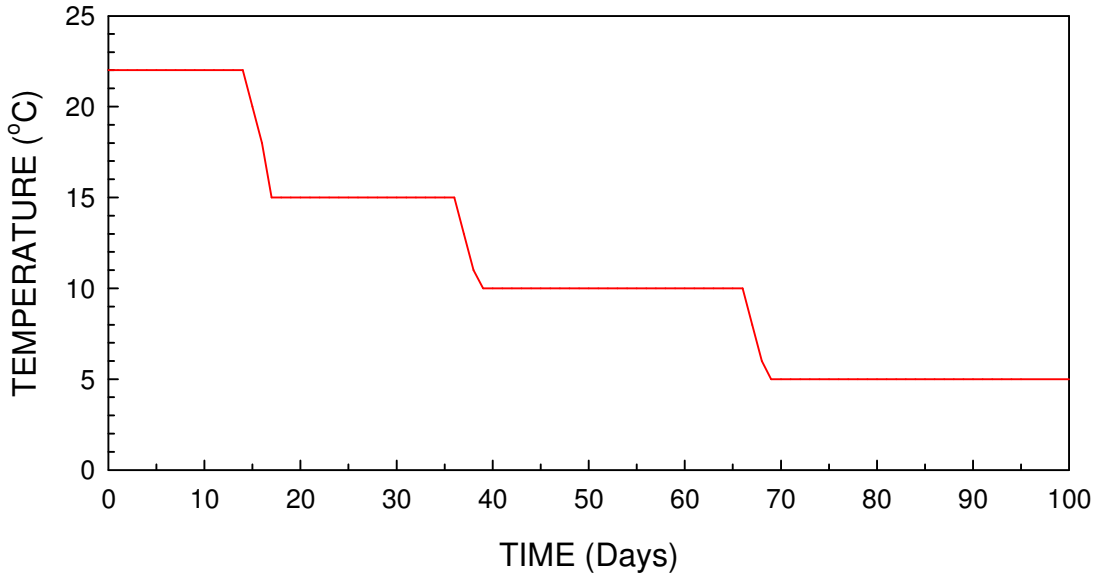

Figure 5-1. Temperature profile during the nitrate reduction tests conducted at progressively lower temperature (22 to 5°C).

5.2.2 Seasonal In-situ Wetland Activity

In order to investigate the seasonal variation of nitrate reduction at the wetland site under controlled laboratory conditions, a series of batch tests were performed with soil and water samples collected at the pilot-scale wetland system. Table 5-1 shows the sampling locations.

Table 5-1. Location of samples collected at the CWW pilot-scale wetland system.

Location	MicroC G™ Cell	Control Cell	Hay Cell
Head	x	x	x
Middle	x		x
Tail	x	x	x

Two sampling campaigns were completed. The first sampling took place on October 24, 2008, between 9 and 11am and the second on January 15, 2009 between 9 and 11am. Recorded temperature values are listed in Table 5-2.

Table 5-2. Temperature (°C) recorded during sampling campaigns.

	Campaign 1 October 24, 2008	Campaign 2 January 16, 2009
Ambient Air	15	-4
Groundwater Inlet	20	19
Cell Soil/Water	19	12.5
Tail Water	Not recorded	5.5

Batch tests were conducted by using duplicate 160-mL serum bottles sealed with rubber stoppers and aluminum crimps. Two types of batch tests were prepared, the first (serum bottles 1 – 8) without carbon limitations and the second (serum bottles 9 – 11) representing a core sample from each pilot wetland cell. The first batch assay was prepared using wetland soil from the site and fresh nitrate containing groundwater. The bottles in this series were amended with additional nitrate nitrogen to achieve an initial concentration of approximately 150 mg N/L and MicroC G^{TM} (6:1 COD:N ratio) in order to assess the in situ nitrate reduction rate achieved at the wetland system without any carbon limitations. The second batch consisted of a smaller number of bottles that were amended with wetland soil and water, both collected at the head of each pilot wetland cell, without any additional nitrate or carbon source. This batch assay was designed to represent core samples from each wetland cell in order to assess the nitrate reduction

rates in the wetland system at the time of sample collection. Both tests were conducted within five hours of field sample collection. Details of the batch assay setup are listed in Table 5-3. In order to simulate the field conditions at the time of sampling, incubation for the first campaign was conducted in the dark at 22°C. For the second campaign, incubation was carried out in the dark at 10°C, except one assay bottle (1-b) was incubated at 22°C. All serum bottles were incubated static.

Table 5-3. Details of seasonal wetland in-situ batch setup.

Bottle No	**Description**	**Soil (g wet)**	**Carbon Added**	**Fresh GW (mL)**	**Cell Water (mL)**	**Initial NO_3^- (mg N/L)**
1a,b	MicroC G™ Head	20	Yes	100		150
2	MicroC G™ Middle	20	Yes	100		150
3	MicroC G™ Tail	20	Yes	100		150
4	Control Head	20	Yes	100		150
5	Control Tail	20	Yes	100		150
6	Hay Head	20	Yes	100		150
7	Hay Middle	20	Yes	100		150
8	Hay Tail	20	Yes	100		150
9	MicroC G™ Head Core	62	No		80	65[a]
10	Control Head Core	62	No		80	65[a]
11	Hay Head Core	62	No		80	65[a]

[a] Water from each cell was used without any nitrate amendment; the initial nitrate concentrations varied from cell to cell

One serum bottle in each series was used for nitrate and nitrite measurements, which were conducted throughout the incubation period. Once all nitrate and nitrite was removed, gas production, gas composition and ammonia were measured in the duplicate serum bottle.

5.3 Results and Discussion

5.3.1 Temperature Test

The nitrate and nitrite concentrations over the incubation period for each test are shown in Figure 5-2. A lag of approximately 24 hours was observed, which is attributed to the low initial active denitrifying population as previously explained. The rates of both nitrate and nitrite reduction were very similar at 22 and 15^{o}C, with a maximum transient nitrite concentration slightly higher at 15^{o}C. At 10^{o}C, a significantly lower rate of nitrate and nitrite reduction was observed. At 5^{o}C, the time required for the complete removal of nitrate and nitrite was more than double of that under 10^{o}C. However, the effect of temperature was more pronounced at 5^{o}C where both the nitrate and nitrite reduction rates were much lower than at the other three temperature values, and the maximum transient nitrite concentration was the lowest.

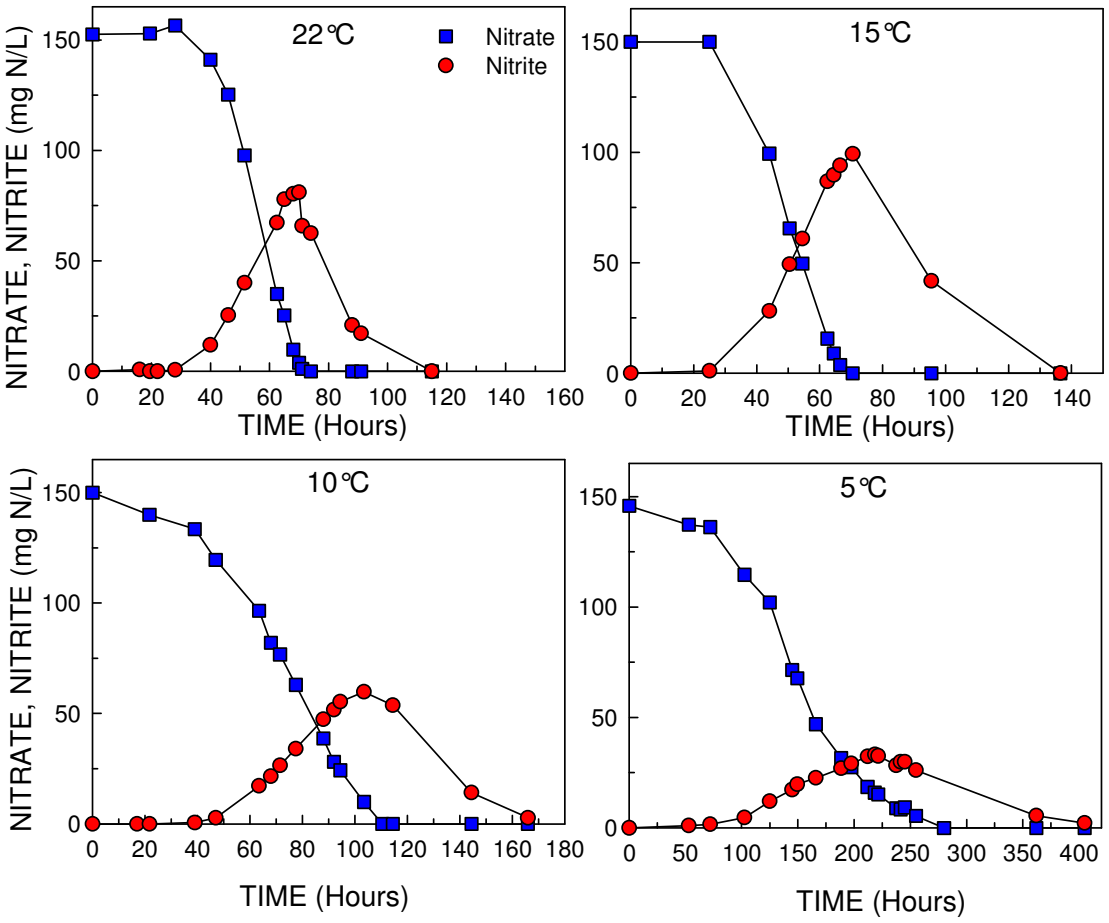

Figure 5-2. Nitrate and nitrite profiles during the batch assays of spiked groundwater at an initial nitrate concentration of 150 mg N/L at 22, 15, 10, and 5°C. The carbon source was MicroC G™ (open Plexiglas reactor).

The variation in the nitrate reduction rates at each temperature is more easily seen in Figure 5-3, which clearly illustrates that the reduction rates were not severely affected until the temperature dropped below 10°C, which agrees with the findings of other studies on denitrification at low temperatures (Burgoon, 2001; Darbi and Viraraghavan, 2004; Lee et al., 2009)

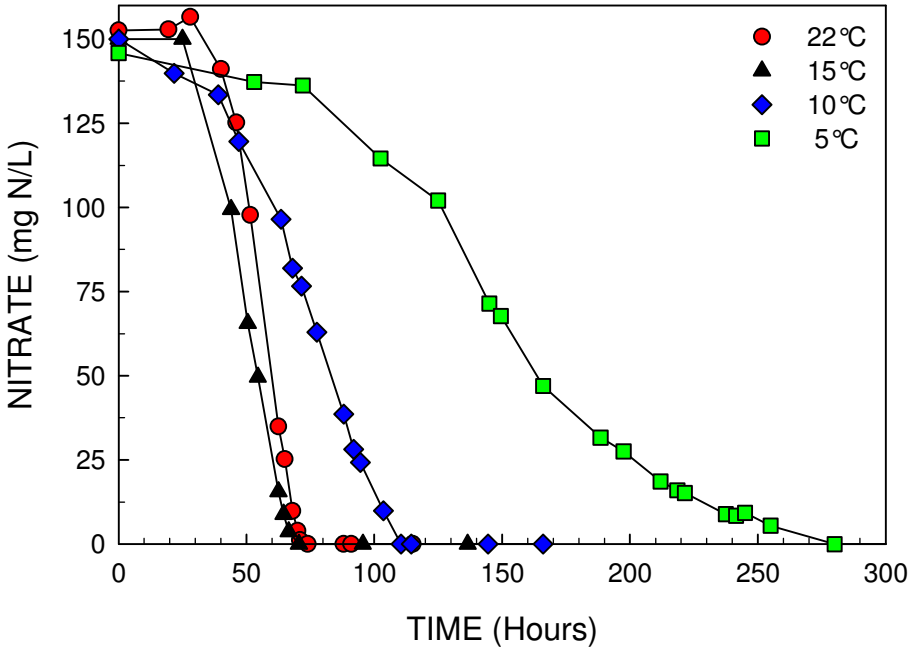

Figure 5-3. Comparison of nitrate reduction profiles at 22, 15, 10, and 5°C.

Once the tests were complete, the nitrate reduction rates were estimated at each temperature as described below. Since the soil biomass at the beginning of each test could not be quantified, the specific substrate utilization rate (*k*, mg nitrate-N/mg biomass

VSS-day) was estimated by adjusting the initial biomass to obtain a good fit to the experimental nitrate concentration data (Figure 5-4).

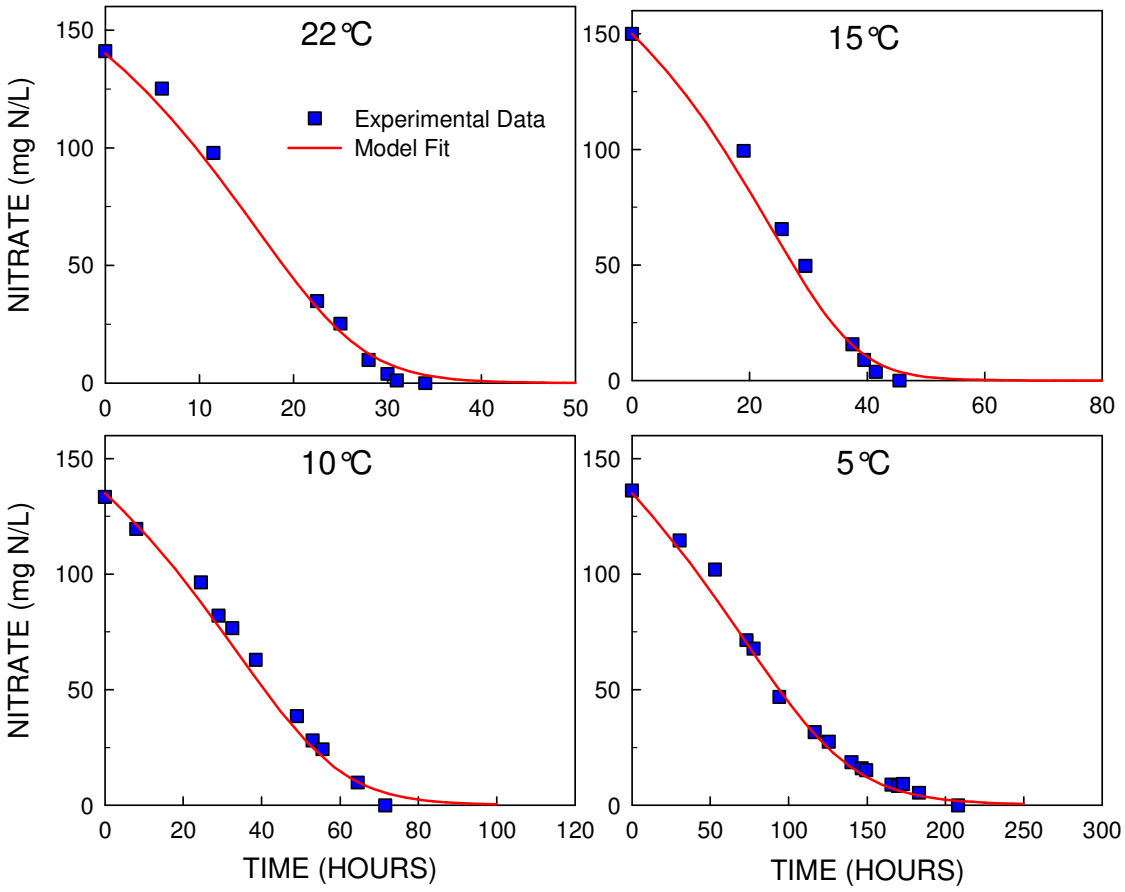

Figure 5-4. Nitrate profiles during the batch assays of spiked groundwater at an initial nitrate concentration of approximately 150 mg N/L at 22, 15, 10, and 5°C (Open Plexiglas reactor; lag phase not shown; lines are model simulations).

The nitrate and biomass concentration over the incubation period was simulated using the MatLab ode15s solver to numerically solve the system of the two ordinary differential equations (i.e., dN/dt and dX/dt), which were based on Monod kinetics (see Chapter 2).

The maximum specific nitrate removal rate value at each incubation temperature using the biokinetic parameters described in Chapter 4 are listed in Table 5-4. The removal rates were determined based on the nitrate experimental data and assumed initial biomass concentration. The data was best fit assuming an initial nitrate concentration of 150 mg/L at each temperature.

Table 5-4. Maximum specific nitrate removal rate (*k*) values as a function of incubation temperature.

Temperature (^{o}C)	Rate mg nitrate-N/mg VSS-day
22	0.816
15	0.540
10	0.360
5	0.168

A correlation of the maximum specific nitrate reduction rates and temperature was obtained based on the Arrhenius model:

$$k = A \exp\left(-\frac{E_a}{RT}\right) \quad (5\text{-}1)$$

where *k* is the maximum specific nitrate reduction rate (mg nitrate-N/mg biomass VSS-day), *A* is the frequency factor (mg nitrate-N/mg biomass VSS-day), E_a is the apparent activation energy (kcal/mol), *T* is the absolute temperature (K) and *R* is the gas constant

(= 1.987 x10^{-3} kcal/mol K). The rate data were plotted according to the linearized Arrhenius equation:

$$\ln k = \ln A - \frac{E_a}{RT} \quad (5\text{-}2)$$

The results of the rate data plotted according to the linearized Arrhenius equation are shown in Figure 5-5. The activation energy (E_a) estimated from the linear regression was 14.8 kcal/mol (61.9 kJ/mol). The temperature coefficient, Q_{10}, which is the ratio of the rates for a temperature difference of 10°C, was estimated to be approximately 2.46 using the following equation:

$$Q_{10} = \frac{k_{t+10}}{k_t} = \exp\frac{10E_a}{RT_1T_2} \quad (5\text{-}3)$$

Q_{10} values for denitrification based on various models ranged from 2 to 3 (Heinen, 2006). Pavlostathis and Zhuang (1991) reported an apparent activation energy of 14.5 kcal/mol and a Q_{10} of 2.6 over a temperature range of 5 to 20°C for denitrification by soil cultures developed with a contaminated subsurface soil. Thus, the E_a and Q_{10} values found in the present study agree well with those previously reported.

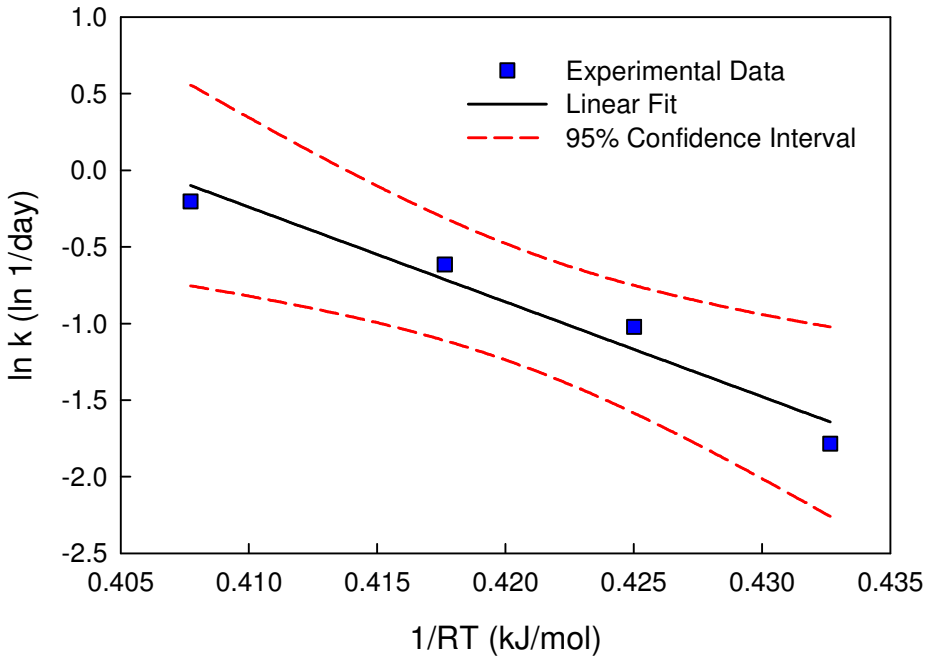

Figure 5-5. Linear regression according to the Arrhenius model.

An alternative expression is commonly used to express the effect of temperature on the biological rates by use of a dimensionless temperature coefficient (θ) taking 20°C as the basis:

$$k_T = k_{20}\theta^{(T-20)} \qquad (5\text{-}4)$$

Where k_{20} is the reduction rate at a base temperature of 20°C and k_T is the rate at any given temperature, T (Kadlec and Reddy, 2001). To derive an expression for temperature coefficient, θ, as a function of Q_{10}, Equation 5-4 can be rearranged by dividing by k_{20}.

$$\frac{k_T}{k_{20}} = \theta^{(T-20)} \qquad (5\text{-}5)$$

Equating equations (5-3) and (5-5) gives the resulting expression for Q_{10} as a function of temperature and temperature coefficient.

$$Q_{10} = \theta^{(T-20)} \quad (5\text{-}6)$$

Using the temperature value 10° higher than the base value of 20°C, the following relationship is formed:

$$Q_{10} = \theta^{10} \quad (5\text{-}7)$$

Using Equation 5-7 for any temperature coefficient, the Q_{10} value obtained indicates the effect of a 10°C increase in temperature on the substrate utilization rate. Therefore, a temperature coefficient greater than one indicates a positive relationship, in turn, an increase in substrate utilization rate with temperature. The opposite effect is observed with temperature coefficients less than one (Kadlec and Reddy, 2001).

By rearranging Equation 5-7, the following expression is obtained for the temperature coefficient as a function of Q_{10}:

$$\theta = (Q_{10})^{1/10} \quad (5\text{-}8)$$

For each Q_{10} value, a corresponding temperature coefficient can be estimated using Equation 5-8 to further quantify the effect of temperature on nitrate reduction rates.

5.3.2 Seasonal Wetland In-situ Activity

To compare the in situ activity at the pilot wetland site during two different seasons, nitrate and nitrite were monitored over the incubation period at 22 and 10°C, respectively. Figure 5-6 shows the nitrate and nitrite profiles corresponding to the two sampling campaigns for all series supplemented with additional initial nitrate and MicroC G™. Although some minor variation in the nitrate removal rate was observed between all 22°C series, nitrate reduction of 150 mg N/L was complete in less than 3 days. Thus, under the conditions of this test (i.e., with excess carbon), the nitrate reduction rate was relatively the same among all three wetland cells and at all locations in each cell.

In contrast, samples collected in January 2009 and incubated at 10°C achieved lower and different nitrate reduction rates under carbon saturation conditions. The highest rate was obtained by the series set up with soil collected at the head of the MicroC G™ cell (0.432 mg nitrate-N/mg VSS-day), whereas the lowest nitrate reduction rate was achieved by the series set up with soil collected at the head of the control cell (0.132 mg nitrate-N/mg VSS-day), in which case nitrate reduction of 150 mg N/L was complete in about 7.6 days.

Nitrate and nitrite profiles are not presented for the serum bottle (1-b) incubated at 22°C from the winter campaign because both species were removed in less than 24 hours from the start of the experiment. This rapid nitrate removal indicates that the fast temperature increase from approximately 10 to 22°C had a significant effect on the microbial activity. The Q_{10} for denitrification was estimated experimentally to be 2.46 (see Section 5.3.1), indicating that for a 10° increase in temperature, the substrate utilization rate, *k*, increases by a factor of 2.46. Therefore, when the temperature was

increased from 10 to 22°C, the nitrate removal rate should have increased by more than 2.46 times. This relationship explains the rapid removal of nitrate in the series incubated at 22°C. The increased nitrate reduction rate indicates that significant microbial activity is sustained under the colder winter conditions and when incubated at the higher, more optimal temperature of 22°C, the rates rapidly increase.

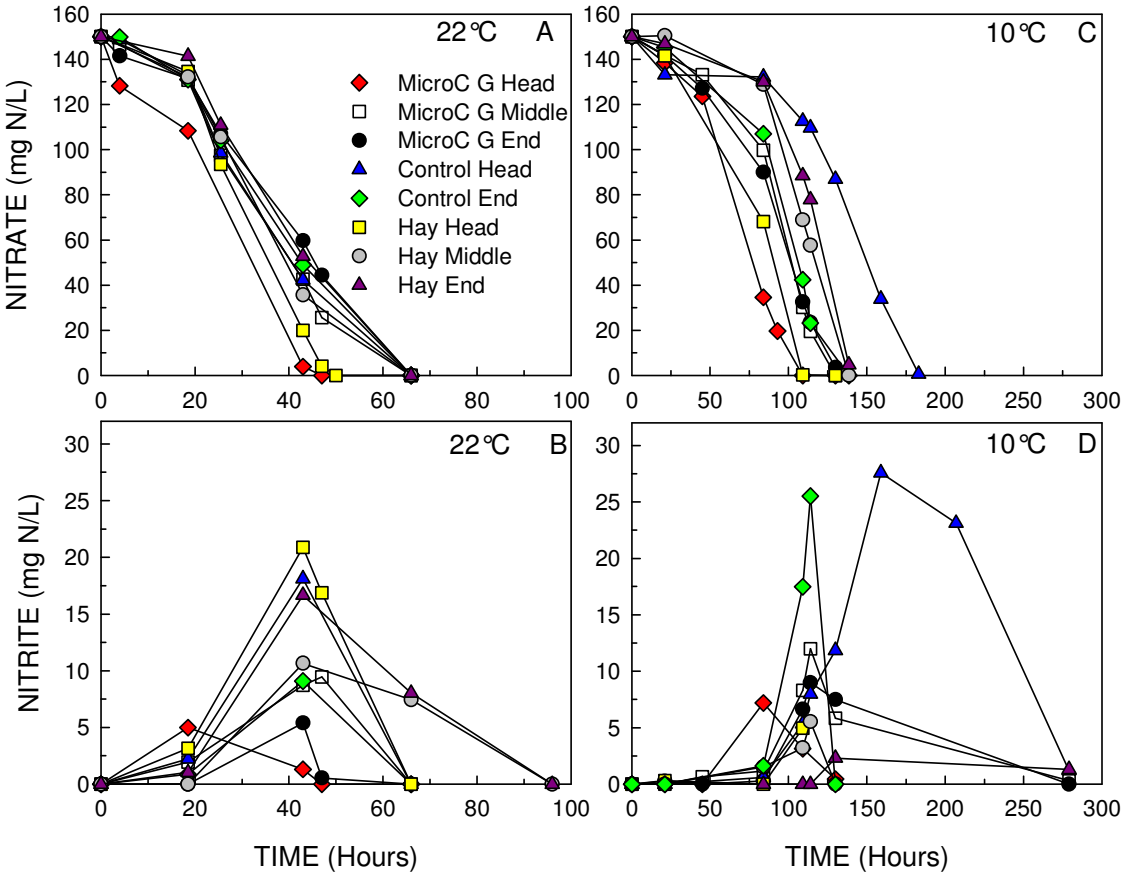

Figure 5-6. Nitrate (A and C) and nitrite (B and D) profiles in series set up with wetland soil and groundwater at an initial nitrate concentration of 150 mg N/L, amended with MicroC G™. Incubation was carried out at 22 (A and B) and 10°C (C and D) to simulate the field conditions in the Fall 2008 and Winter 2009, respectively.

Once the tests were complete, the specific substrate utilization rates (k, mg nitrate-N/mg biomass VSS-day) were estimated in each pilot-scale wetland cell during each incubation period as described above in Section 5.3.1 and the rates for each wetland cell are listed in Table 5-5.

Table 5-5. Nitrate removal specific substrate utilization rate (k) values (mg nitrate-N/mg VSS-day) ranges for each pilot-scale wetland cell for incubation temperature 10 and 22°C, respectively.

Wetland	10°C	22°C
MicroC G™	0.288 - 0.432	0.480 - 0.672
Control	0.132 - 0.168	0.480 - 0.504
Hay	0.144 - 0.240	0.480 - 0.552

Table 5-5 shows that for 22°C, the ranges are very similar for each wetland cell; however, at 10°C, the rates are much higher in the MicroC G™ cell than in the other two wetland cells. This indicates that the carbon source and feeding method did not significantly affect the rates at higher temperatures; however, at low temperatures, microbial activity is greater in the wetland cell with continuous organic carbon feed.

As mentioned in Chapter 2, in the context of biological nitrogen removal, a concern is related to the nitrate reduction to ammonia (i.e., DNRA) as opposed to dinitrogen gas. In previous batch tests conducted at room temperature, the production of dinitrogen gas was what was expected based on complete denitrification. To further test the possible occurrence of DNRA under relatively lower incubation temperature

conditions, a nitrogen balance was completed for the batch assay performed at 10°C and with excess carbon (MicroC G™; series 1 through 8 in Table 5-3) and the results are shown in Figure 5-7. Based on these results, an excellent nitrogen balance was achieved, and on average, about 16% of the initially added nitrate in these series was converted to ammonia, with the balance converted to dinitrogen. Other gaseous nitrogen oxides were not detected. The fraction of the nitrate converted to ammonia in this batch assay conducted at 10°C was more significant than the trace ammonia levels found in other assays performed at higher temperature values. Previous studies have reported nitrate conversion to ammonia through DNRA in the range of 1 to 34% (Bartlett et al., 1979; Cooke, 1994; Kadlec and Wallace, 2008).

The second batch assay representing wetland core samples was set up with soil and water from the head of each wetland cell without amendment of nitrate and carbon. This batch assay achieved much lower nitrate reduction rates compared with their counterparts, which were amended with both nitrate and carbon (Figure 5-8). The nitrate removal rate achieved by the MicroC G™ cell soil and water was relatively the same for both sampling campaigns, implying that significant microbial activity is retained in this cell even through the winter period as a result of the continuous addition of this carbon source in the field. In contrast, the bottle series set up with soil and water from the control and hay cells achieved similar nitrate reduction rates to that in the MicroC G™ cell during the Fall 2008 (incubated at 22°C), but significantly lower rates in Winter 2009 (incubated at 10°C). These results suggest that due to bioavailable carbon limitations, a lower microbial activity is retained in winter in these two cells as opposed to the MicroC G™ cell.

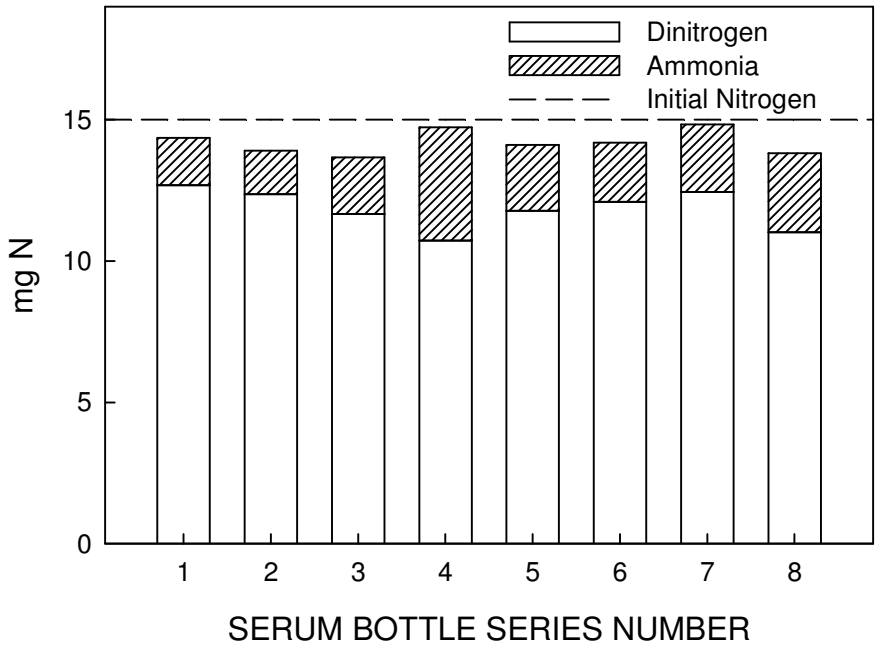

Figure 5-7. Nitrogen balance completed for the winter *in situ* batch assay incubated at 10°C.

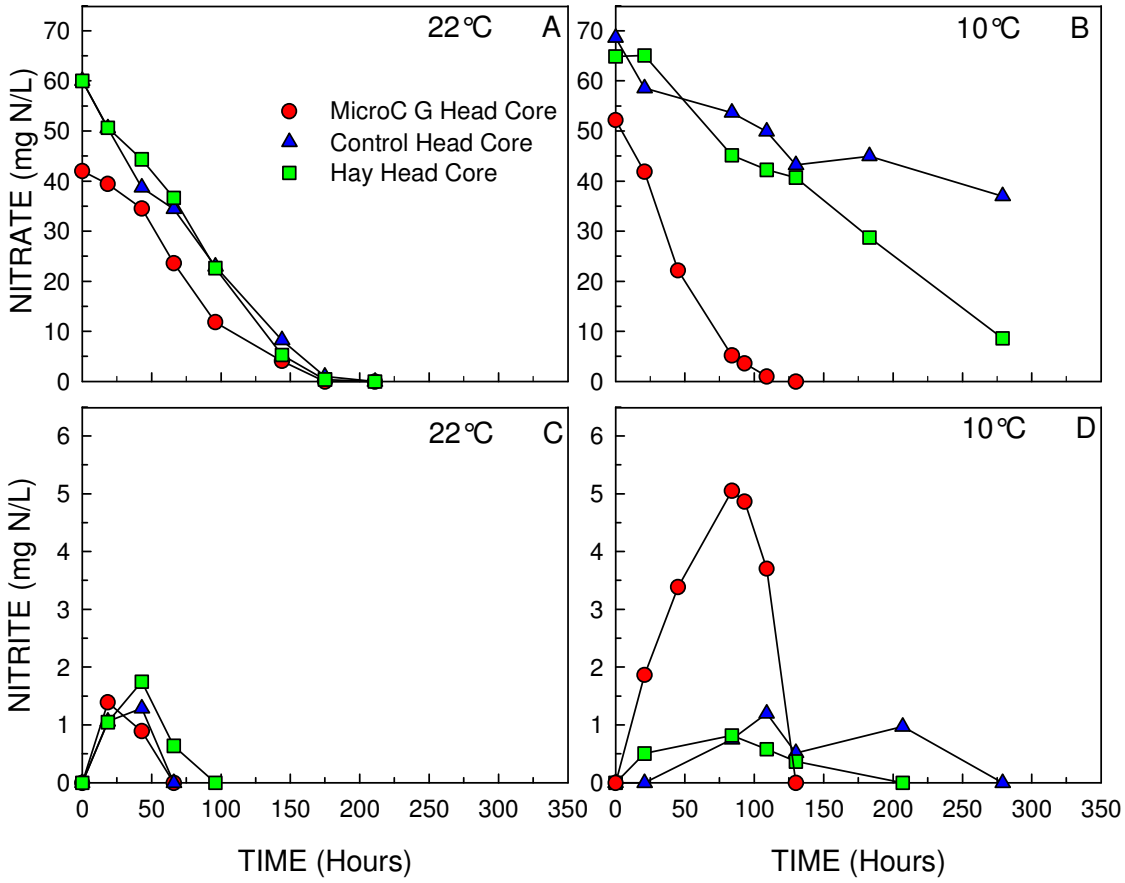

Figure 5-8. Nitrate (A and C) and nitrite (B and D) profiles in series set up with wetland soil and water without additional nitrate and carbon amendment. Incubation was carried out at 22 (A and B) and 10^{o}C (C and D) to simulate the field conditions in the Fall 2008 and Winter 2009, respectively.

5.4 Summary

Wetland removal efficiency is controlled by many biological and chemical processes that are strongly affected by variations in environmental and operational parameters. Microbial activity is negatively affected by decreasing sediment and groundwater temperatures; therefore, nitrate removal rates decrease with declining seasonal temperatures (Bachand and Horne, 1999). This study demonstrates that denitrification occurs in subsurface environments even at low temperature values. The decrease of the denitrification rate with decreasing temperature followed the Arrhenius model. For a temperature range from 22 to 5°C, the Q_{10} value of 2.46 was estimated. Although the denitrifying bacteria were still active at a temperature as low as 5°C, the significantly lower denitrification rate at this temperature could ultimately determine the feasibility of constructed wetlands under low temperature conditions imposed by either region and/or season).

CHAPTER 6

CONTINUOUS-FLOW SYSTEMS

6.1 Introduction

Free water surface (FWS) wetlands are a common type of constructed wetlands in which water enters at the inlet, flows through and over the surface of the soil/sediment and vegetation before it exits at the effluent side. FWS wetlands are areas of open water containing plants and floating vegetation, similar to natural swamps and marshes. They are commonly used for advanced treatment of effluent from secondary or tertiary treatment processes and are suitable in all climates (Kadlec and Wallace, 2008).

Lab-scale wetlands are often designed to investigate the effect of environmental and operational parameters on the nitrate reduction potential. In many cases, continuous-flow stirred tank reactors (CSTR) and plug flow reactors (PFR) are used to closely simulate the flow patterns in the FWS wetlands. The objective of this portion of the study was to investigate the effect of operational and environmental parameters, such as HRT, COD:N ratios and temperature, on denitrification in lab-scale, free-water surface flow reactors.

6.2 Materials and Methods

To test the effect of hydraulic retention time (HRT), carbon source COD:N ratio, and temperature, continuous-flow reactors experiments were conducted using 15-L cubic Plexiglas reactors. Each reactor was filled with 10.5 kg of soil and approximately 9 L of

nitrate-bearing groundwater and was kept static for 1 day in order to expel all air from the soil layer and uniformly wait the soil. A plastic reservoir filled with groundwater was attached to peristaltic pumps (Masterflex; Cole-Parmer) and the groundwater, with nitrate concentrations ranging from 65 to 150 mg N/L, was fed to the reactors continuously at a specific flow rate, depending on the desired retention time. When MicroC G^{TM} was used as the carbon source, a 200 g COD/L diluted solution was fed using a positive displacement pump (Fluid-Metering, Inc.). The MicroC G^{TM} was fed every 2 hours with the help of an electronic timer (ChronTrol) at a flow rate depending on the HRT and desired COD:N ratio. Each reactor was initially operated as a single compartment simulating CSTRs, verified by a tracer test. At a later time, in order to more closely simulate the flow regime at the pilot-scale wetland system, two baffles were inserted in each reactor, thus dividing the liquid volume to three, equal-volume compartments and a tracer test was conducted. Based on this test, the flow regime in the modified reactors was simulated by a system of 1.5 to 2 CSTRs in series (See Section 6.3.1). Overflow reactor effluent was periodically collected and analyzed for nitrate, nitrite, ammonia, DOC, and soluble COD.

Details of each continuous-flow run are summarized in Table 6-1. All runs were conducted open to the atmosphere to closely simulate the wetland pilot-scale system conditions and specific details for each continuous-flow assay are given in the Results and Discussion section, below.

Table 6-1. Summary of continuous-flow runs.

Run	Reactor Type	T (°C)	Mean Influent Nitrate (mg/L)	Carbon Source	Purpose
I-a	CSTR	22 - 24	67	None	Control
I-b	CSTR	22 - 24	67	MicroC G™	HRT Effect Carbon Exhaustion
I-c	CSTR	22 - 24	67	Hay	Carbon Exhaustion
II	1.5/2 Baffled CSTR	22 - 24	67	MicroC G™	Minimum COD:N ratio
III	1.5/2 Baffled CSTR	5 - 22	67	MicroC G™	Temperature Effect

6.3 Results and Discussion

6.3.1 Tracer Study

A common technique for analyzing the flow pattern in a reactor system is to introduce a tracer into the input of the reactor and monitor the tracer output signal in the effluent. An appropriate tracer is a soluble, inert substance that does not react or disrupt the flow pattern of the reactor, but that can be detected quantitatively (Grady et al., 1999).

Both a single- and a three-compartment (i.e., baffled) laboratory-scale continuous-flow reactors were used in this study. In order to proceed with modeling and simulation of the continuous-flow systems, tracer tests were conducted in which the groundwater nitrate was used as a tracer. In order to avoid loss of nitrate as a result of nitrate reduction, the soil in the Plexiglas reactors was rinsed several times with DI water over 24 hours to deplete all available organic carbon which could serve as electron donor for nitrate reduction. Then, nitrate-bearing groundwater was continuously fed to the inlet of

the reactor at a concentration of approximately 70 or 140 mg N/L for the single and three-compartment reactors, respectively. The effluent nitrate concentration was monitored frequently and its profiles were compared to reactor design equations for continuous tracer input in order to estimate the flow pattern of each reactor.

The effluent nitrate concentration data for the single-compartment reactor are shown in Figure 6-1. For an ideal continuous-flow, stirred tank reactor (CSTR), and assuming a reactive tracer with first-order depletion kinetics, the following equation holds:

$$\frac{C}{C_o} = \frac{1 - e^{-\left(t(\frac{1}{\theta}+k)\right)}}{1 + k\theta} \tag{6-1}$$

where C_o and C are the influent and effluent tracer (nitrate) concentration (mg N/L), t is time (days), θ is the hydraulic retention time (days), and k is the first-order rate constant (day^{-1}). For a conservative, i.e., non-reactive tracer, k = 0 and equation 6-2 becomes:

$$\frac{C}{C_o} = 1 - e^{-\left(\frac{t}{\theta}\right)} \tag{6-2}$$

The tracer profiles for an ideal CSTR according to equation 6-2 and a range of k values from 0 to 0.5 day^{-1} are shown in Figure 6-1. Based on a comparison of these profiles to the experimental data, it was concluded that the flow pattern of the single-compartment reactor closely resembled that of an ideal CSTR without reaction (i.e., k = 0 day^{-1}).

The effluent nitrate concentration data for the three-compartment reactor during the tracer study are shown in Figure 6-2. The flow pattern for a conservative, i.e., non-

reactive tracer and a multi-compartment reactor can be simulated as a series of ideal CSTRs according to the following equation (Grady et al., 1999):

$$\frac{C}{C_o} = 1 - \left[1 + \frac{t}{\theta} + \frac{\left(\frac{t}{\theta}\right)^2}{2!} + \frac{\left(\frac{t}{\theta}\right)^3}{3!} + ... + \frac{\left(\frac{t}{\theta}\right)^{N-1}}{(N-1)!} + \frac{\left(\frac{t}{\theta}\right)^N}{N!} \right] \exp\left(-\frac{t}{\theta}\right) \qquad (6\text{-}3)$$

where C_o and C are the influent and effluent tracer (nitrate) concentration (mg N/L), t is time (days), θ is the hydraulic retention time (days), and *N* is the number of CSTRs of equal volume *V* (i.e., $V = V_{Total}/N$).

The tracer profiles according to equation 6-3 for N = 1, 2, and 3 are shown in Figure 6-2. Based on a comparison of these profiles to the experimental data, it was concluded that the flow pattern of the three-compartment reactor was best simulated as a system of 1.5 to 2 CSTRs in series.

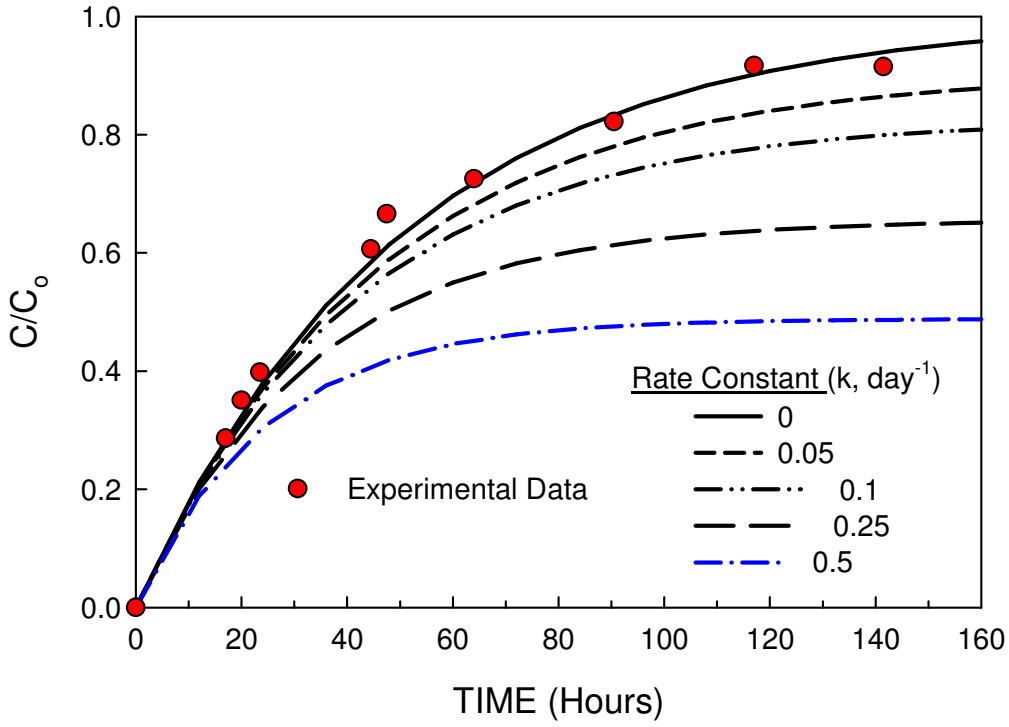

Figure 6-1. Experimental tracer data and simulation results for ideal CSTR systems with varying rate constants (0, 0.05, 0.1. 0.25, 0.5 day^{-1} (HRT, 2.1 days; Influent nitrate concentration, 70 mg N/L).

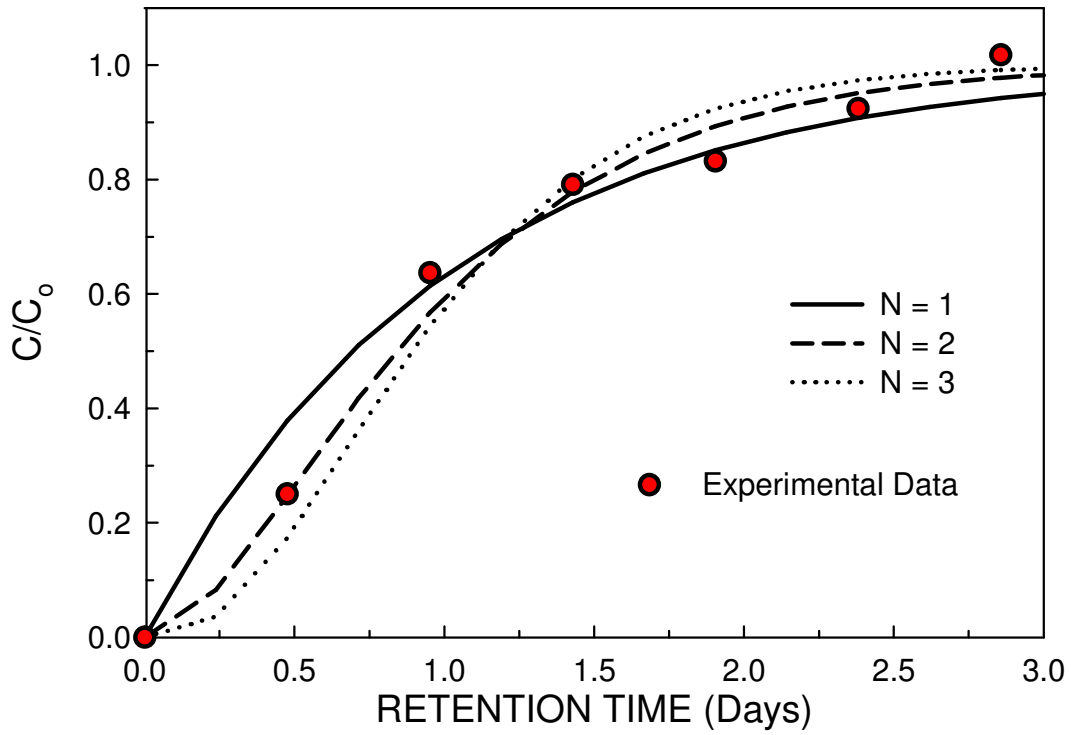

Figure 6-2. Experimental tracer data and multiple CSTRs in series simulation results (HRT, 2.1 days; Influent nitrate concentration, 140 mg N/L).

6.3.2 Continuous Flow Reactor Run I

The first continuous-flow run was conducted to investigate the effect of hydraulic retention time on the nitrate removal efficiency at an influent groundwater nitrate concentration between 67 and 70 mg N/L and compare the efficacy of two carbon sources in supporting nitrate reduction. Initially, for one week, and while groundwater was pumped, all three one-compartment reactors were operated without any external carbon addition. Then, one reactor (CFR1) did not receive any external carbon source and served as the control, the second reactor (CFR2) received MicroC G^{TM} at a constant COD:N ratio of 6:1, while the third reactor (CFR3) was amended only once with 27.5 grams of

hay, equivalent to the approximate hay loading at the pilot-scale wetland system (0.44 kg hay per square meter). Samples removed from the reactors' effluent port were frequently analyzed for nitrate, nitrite, COD, DOC and periodically for ammonia. COD and DOC over the incubation periods are shown in Figure 6-3. The effluent COD and DOC remained constant and low (DOC below 200 mg/L and COD below 75 mg/L) for the duration of the incubation period, excluding the initial peak after carbon addition on Day 6. No ammonia was detected in the three reactors from Continuous Flow Run I. D.O. and pH were frequently measured in the groundwater inside the reactors. For all continuous runs, the pH ranged from slightly acidic to neutral in between values of 5.5 and 7.5. D.O. in the control reactor CFR1 was in the range of 5.5 and 7 mg/L for the duration of the incubation period. D.O. remained below 2 mg/L in both reactors amended with carbon for the first 20 days after carbon addition; however, the D.O. gradually increased in the hay reactor, CFR3, throughout the incubation period, as carbon was only added once. D.O. remained consistently below 2 mg/L in CFR2 with continuous carbon loading.

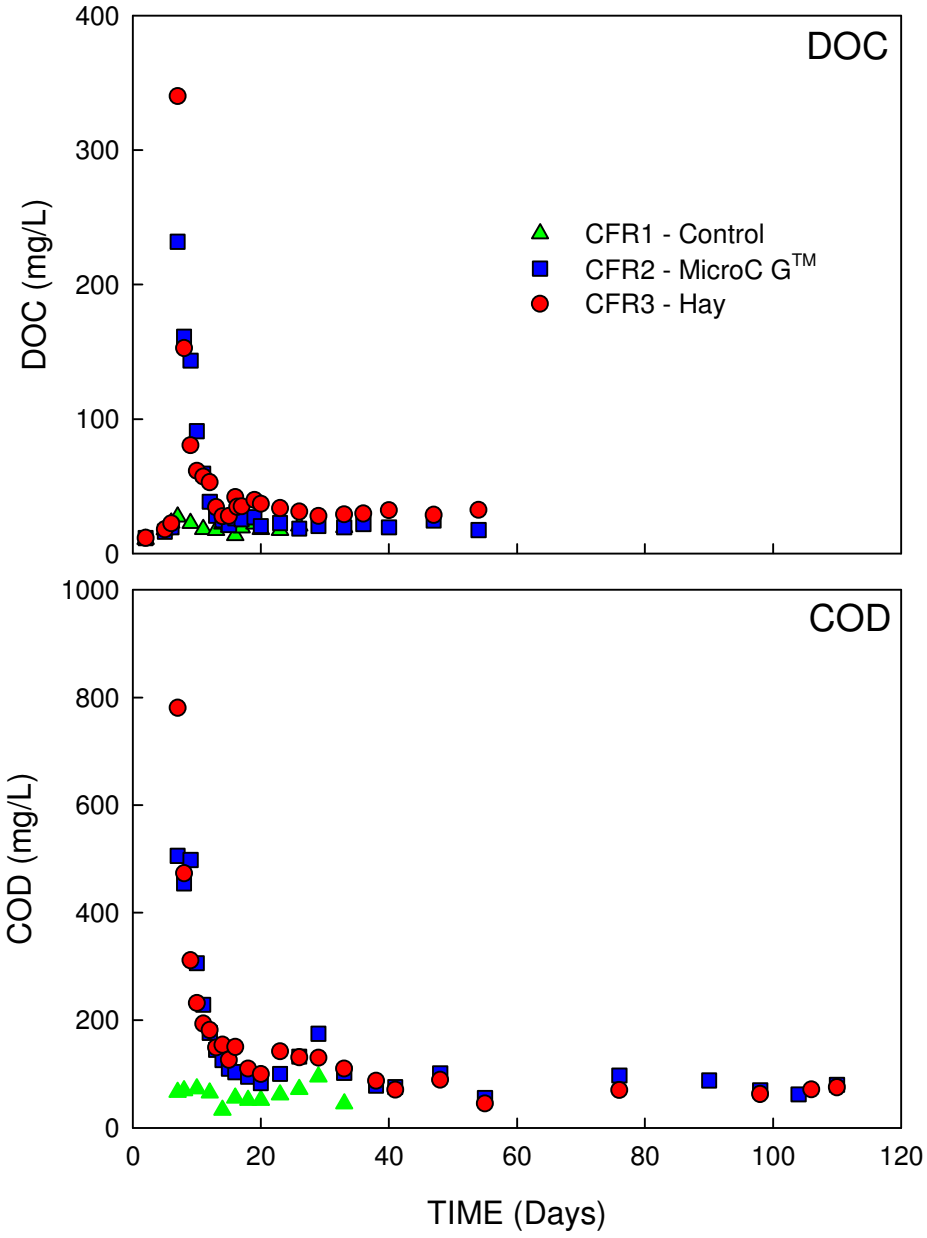

Figure 6-3. Effluent DOC and COD concentrations for CFR1, 2 and 3 over the incubation period.

Figure 6-4 shows the nitrate concentration in the control reactor (CFR1) over about one month. For the first 15 days this reactor was operated at an HRT of 5 days, which was then increased to 15 days. The effluent nitrate concentration had some slight variations and at times was even higher than that in the effluent, which averaged 67 mg N/L. The slightly elevated effluent nitrate concentration is attributed to water evaporation losses (about 175 mL/day) through the top of the open reactor operated at room temperature (22 to 24^{o}C). Based on this evaporation rate, the adjusted reactor nitrate concentration was estimated as equal to 76 mg N/L, which closely matches the observed nitrate concentrations. Overall, at the two HRT values that the control reactor was operated, the organic carbon in the soil at the bottom of the reactor was not able to support any sizable nitrate reduction, which in turn resulted practically in zero nitrate removal.

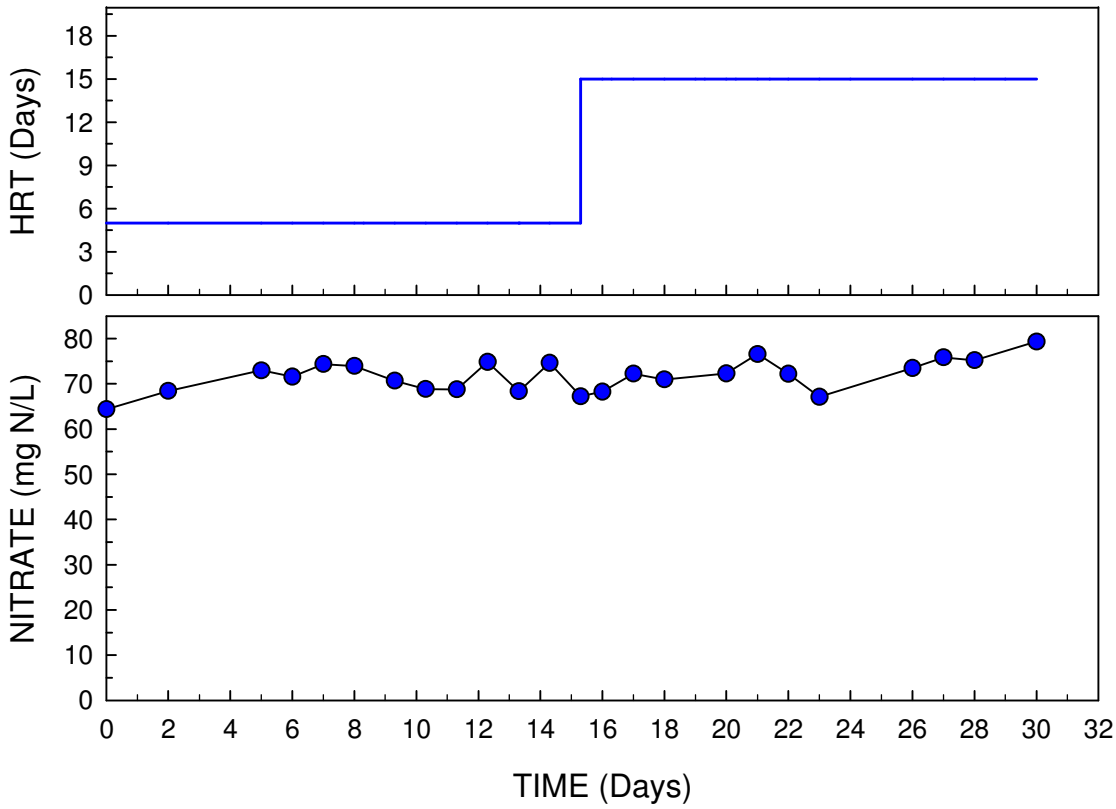

Figure 6-4. Effluent nitrate concentration in the continuous-flow control reactor (CFR1) operated without any external carbon addition at room temperature (22 to 24°C; mean influent groundwater nitrate, 67 mg N/L).

The second reactor (CFR2) was operated at three different retention times of 2.8, 3.5 and 5 days, while being continuously fed with groundwater and MicroC G™ at a COD:N of 6 after the first week during which external carbon was not added. The effluent nitrate concentration over the entire run period is shown in Figure 6-5. Upon addition of MicroC G™ directly to the reactor, the effluent nitrate concentration decreased and reached non-detectable levels within 3 days. For the remainder of this run, the effluent nitrate concentration did not exceed 20 mg NO_3-N/L at any of the three

retention times. At approximately 88 days, the concentration of the influent groundwater was increased to 130 mg NO_3-N/L to illustrate that the nitrate removal follows Monod kinetics, according to which the effluent nitrate concentration is not a function of influent nitrate concentration. As expected, the effluent concentrations slightly increased to approximately 7 mg N/L until steady state was achieved, after which the effluent concentration quickly returned to non-detectable levels.

In order to qualitatively evaluate the nitrate removal in this reactor and the effect of carbon source, at approximately 92 days, the MicroC G™ pump was turned off and only the groundwater at approximately 130 mg N/L was fed to the reactor, still at a HRT of 5 days. As shown in Figure 6-5, the effluent nitrate concentration increased and reached about 88 mg N/L within 10 days, further demonstrating the necessity of a continuous addition of a degradable carbon source. At that time, the MicroC G™ pump was turned on again supplying carbon at a COD:N ratio of 6 resulting in the gradual decrease of the nitrate concentration to about 20 mg N/L in 20 days. Thus, even at an elevated influent nitrate concentration and at a relatively low HRT, continuous addition of the biodegradable organic carbon in excess of stoichiometric levels achieved a high nitrate removal.

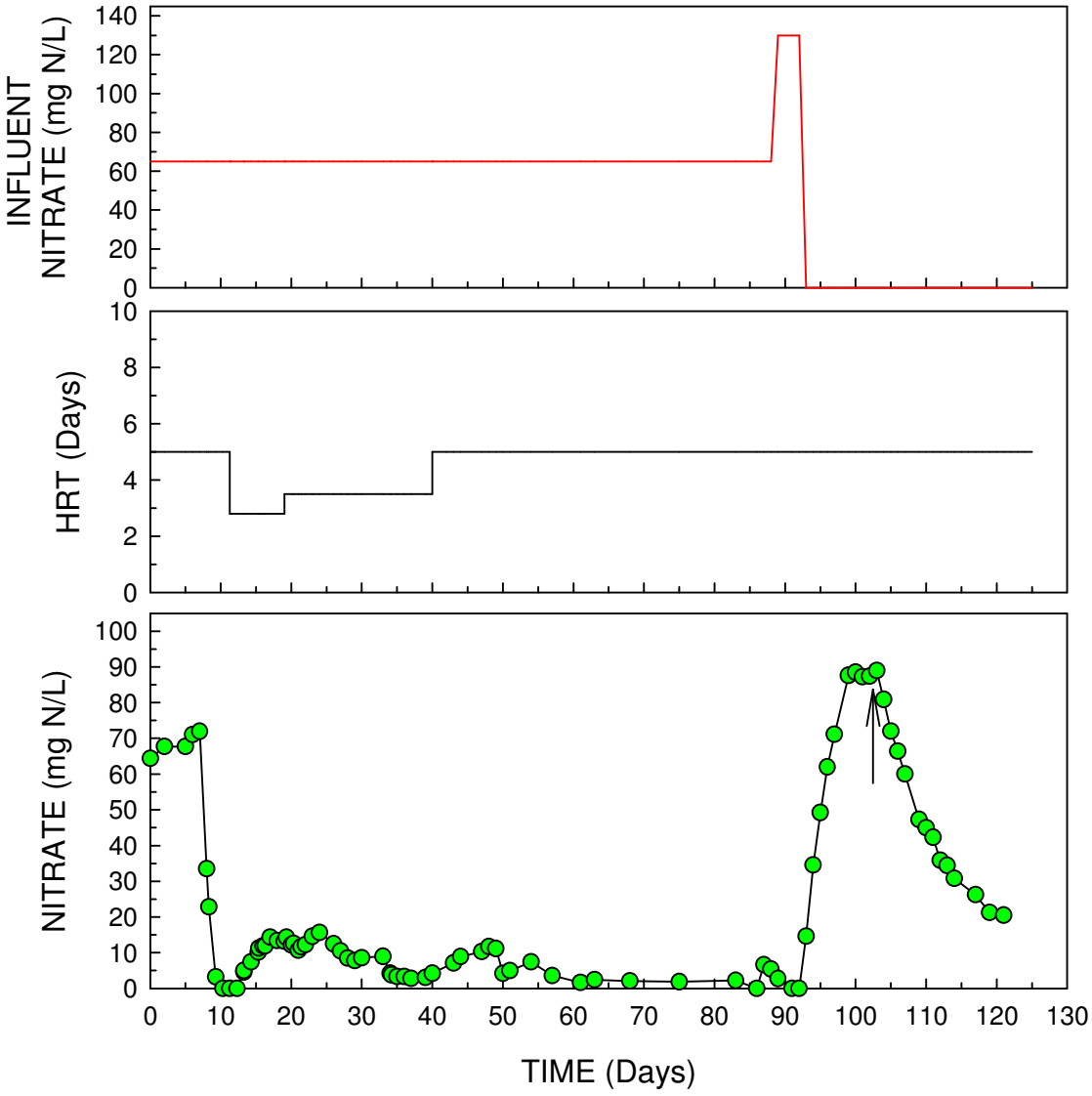

Figure 6-5. Effluent nitrate concentration in the continuous-flow reactor CFR2 operated with MicroC G^{TM} addition at room temperature (22 to 24^{o}C; mean influent groundwater nitrate, 67 mg N/L; arrow indicates MicroC G^{TM} pump on).

The third continuous-flow reactor (CFR3) was operated at two different retention times, 5 and 10 days. After one week of operation without any external carbon addition, hay was added once and in less than 2 days, the effluent nitrate concentration rapidly dropped to non-detectable levels (Figure 6-6). After a few days of achieving very low effluent nitrate concentrations, the effluent nitrate concentration began to gradually increase reaching 15 mg N/L at 20 days of operation. At this time the HRT was changed to 10 days to slow down the physical removal of the soluble hay carbon. The reactor was operated at an HRT of 10 days for over 80 days and during this time period the effluent nitrate concentration continued to gradually increase. At 105 days of operation, the groundwater pump was shut off, converting the CFR to a batch system. The reactor nitrate concentration was monitored for about 20 days, accounting for the water evaporation losses (175 mL/day). While under batch conditions, this reactor achieved a slow nitrate reduction rate, indicating that the residual hay could still provide some degradable carbon able to sustain a low rate of nitrate reduction.

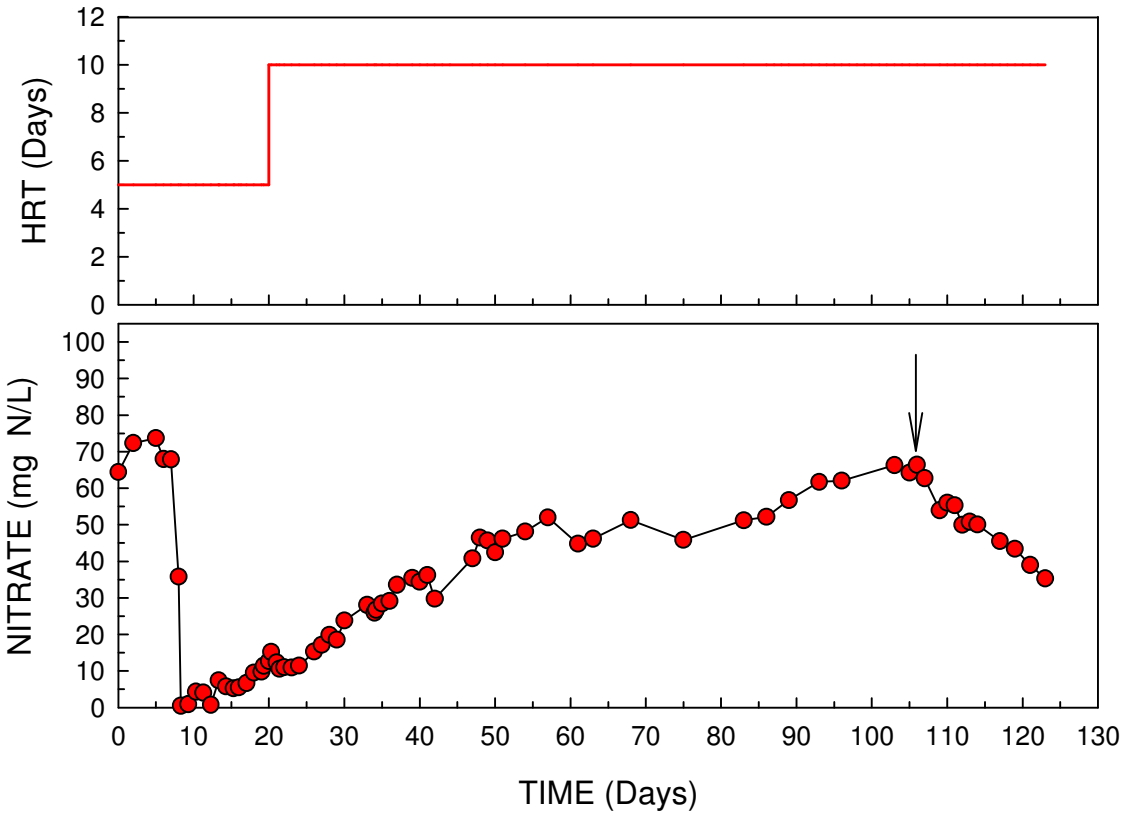

Figure 6-6. Effluent nitrate concentration in the continuous-flow reactor CFR3 operated with a single, initial hay addition at room temperature (22 to 24°C; mean influent groundwater nitrate, 67 mg N/L; arrow indicates groundwater pump off).

6.3.3 Continuous Flow Reactor Run II

All batch assays and the above-discussed continuous-flow run I were conducted with addition of MicroC G™ at a constant COD:N ratio of 6:1. In order to further assess the minimum level of carbon addition required for an efficient nitrate reduction, another continuous-flow run was conducted in which the influent groundwater nitrate concentration was kept constant at 70 mg N/L, the HRT was 2 and then 5 days, while the COD:N ratio was step-wise decreased to lower values. For this run, the reactor was retrofitted with two baffles dividing the reactor liquid volume in three, equal volume compartments, resulting in a flow regime that simulated 1.5 to 2 CSTRs in series. The reactor was operated at ambient room temperature (22 to 24°C).

Figure 6-7 shows the reactor effluent nitrate concentration along with other operational parameters. For the first 18 days, the reactor was operated at an HRT of 2 days, during which the effluent nitrate concentration decreased sharply to less than 10 mg N/L. The HRT was then increased to 5 days to achieve more stable operation and an effluent nitrate concentration of less than 10 mg N/L, similarly to the performance achieved by the CFR2 in run I. The COD:N ratio was step-wise decreased from an initial value of 6:1 to the lowest value of 0.5:1. On day 25, the COD:N ratio was decreased to 5:1, which initially did not have any impact on the nitrate reduction process. Between 35 and 47 days of operation the MicroC G™ pump was accidentally turned off, which resulted in a rapid increase of the nitrate concentration from less than 5 to about 45 mg N/L. When the MicroC G™ pump was turned on again, the effluent nitrate concentration gradually decreased to below 6 mg N/L. When on day 75, the COD:N ratio was further decreased to 4:1, the effluent nitrate concentration increased sharply to about 27 mg N/L.

A decrease of the COD:N ratio to 3:1 and then to 2:1 resulted in an effluent nitrate concentration ranging between 32 and 40 mg N/L. A further decrease of the COD:N ratio to 1 and then to 0.5:1 resulted in a gradual increase of the effluent nitrate concentration to 42 mg N/L. On day 232, the COD:N ratio was increased to 5:1, which resulted in a fast decrease of the effluent nitrate concentration to below 4 mg N/L. Based on these results, for an open to the atmosphere system at ambient temperature between 22 and 24^{o}C, and influent nitrate concentration of 67 mg N/L and an HRT value of 5 days, the minimum COD:N ratio is about 5:1 in order to achieve an effluent nitrate concentration of less than 10 mg N/L.

As previously discussed, the theoretical requirement for complete denitrification, ignoring microbial growth, is 2.85 mg COD/mg nitrate-N reduced to N_2. At relatively low COD:N values, incomplete denitrification is possible which could lead to the formation of nitric oxide (NO) and nitrous oxide (N_2O), both potent greenhouse gases. It has been reported that N_2O emissions in wetlands are highly dependent on the COD:N ratio, as well as the pH, dissolved oxygen, and temperature among other parameters (Wu et al., 2009; Inamori et al., 2008). Wu et al. (2009) found that significant amounts of N_2O were released from constructed wetlands at very high and very low COD:N ratios, with the minimum emissions at a ratio of 5:1. In order to investigate if nitrogen oxides were released in the laboratory reactors due to incomplete denitrification, on days 104, 143, 192 and 215 when the continuous-flow reactor was operated with a COD:N ratio of 3:1, 2:1, 1:1 and 0.5:1, respectively, gas bubbles and water were collected biweekly from the bottom of the reactor by using an inverted glass vial fully submerged in the water and sealed with a stopper while under water. Then, the vial was positioned upright and its

headspace was analyzed by gas chromatography (thermal conductivity detection). NO and N_2O were not detected at any of the COD:N ratios tested, confirming that complete denitrification, leading mainly to the production of nitrogen gas (N_2) was the main nitrate removal process in the laboratory reactors.

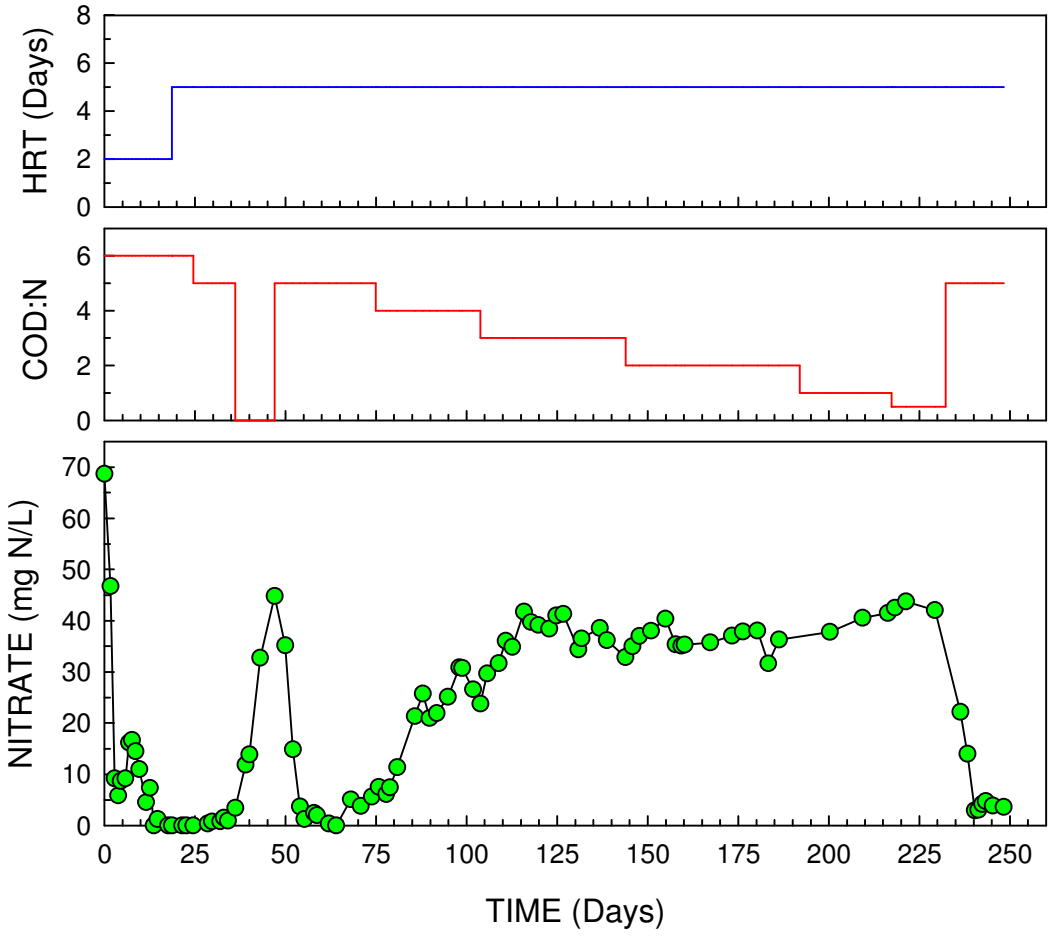

Figure 6-7. Effluent nitrate concentration in a continuous-flow reactor operated with MicroC G^{TM} addition at several COD:N ratios, at room temperature (22 to 24^{o}C; mean influent groundwater nitrate, 70 mg N/L;

6.3.4 Continuous Flow Reactor Run III

All continuous-flow runs discussed above were conducted at room temperature (22 to 24^{o}C). In order to evaluate the effect of lower temperature values on the nitrate reduction kinetics under continuous-flow conditions, a three-compartment, baffled reactor was operated at four different temperature values (22, 15, 10, and 5^{o}C). Both the groundwater reservoir and the reactor were housed in a temperature controlled room. The rate of temperature change between the four target temperature values was 2^{o}C/day (Figure 6-8). MicroC G^{TM} was used as the carbon source at a COD:N ratio of 6:1 throughout this run. The initial HRT was set at 2 days and then changed to 5 days.

Figure 6-8 shows the reactor effluent nitrate concentration along with other operational parameters. For the first 15 days, the reactor was operated at 22^{o}C with an HRT of 2 days, during which the effluent nitrate concentration decreased sharply to less than 13 mg N/L. The HRT was then increased to 5 days to achieve more stable operation and an effluent nitrate concentration of less than 10 mg N/L, similarly to the performance achieved by the CFR2 reactor in run I. On day 30 the room temperature was decreased and by day 32 reached 15^{o}C. While at 15^{o}C, the reactor performance did not change and the effluent nitrate concentration was kept at non-detectable levels. On day 51, the room temperature was decreased again and reached 10^{o}C by day 55. There was a slight increase in the effluent nitrate concentration at this time, but within 24 hours it returned to non-detectable levels.

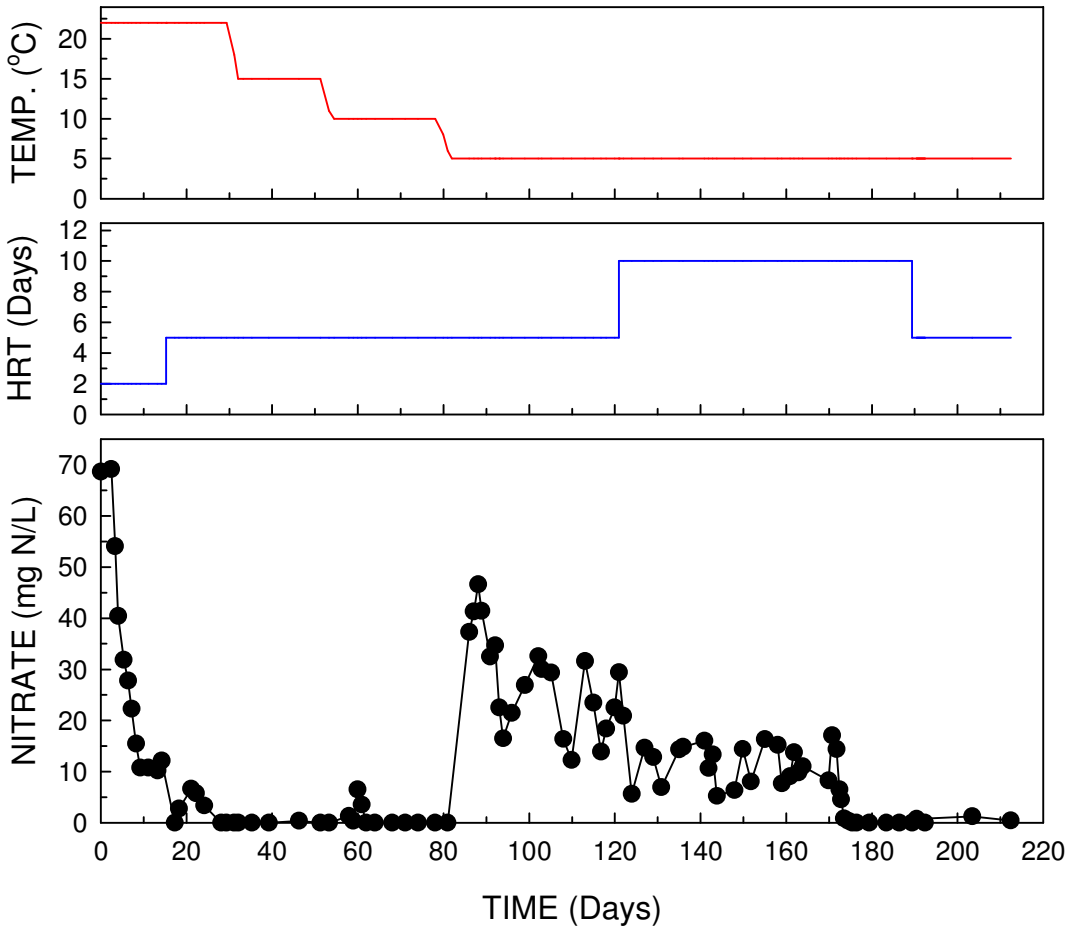

Figure 6-8. Effluent nitrate concentration in a continuous-flow reactor operated with MicroC G™ addition at a COD:N ratio of 6:1 and a range of temperature (22 to 5°C; mean influent groundwater nitrate, 67 mg N/L;

After the room temperature was reduced to 5°C by day 82, the reactor effluent concentration increased rapidly to a maximum 47 mg N/L, and then started to decrease. However, for over 35 days at 5°C, the reactor's performance was not stable and the effluent nitrate concentration fluctuated between 10 and 35 mg N/L, albeit with a downwards trend. In an attempt to achieve a stable effluent concentration, the HRT was increased to 10 days on day 120. Although the effluent nitrate concentration decreased significantly at an HRT of 10 days, it continued to fluctuate between 5 and 20 mg N/L. Upon further observation, it was realized that MicroC G™ was not well mixed with the groundwater in the reactor as it was delivered intermittently by a micro pump every 2 hours at the point where the groundwater was constantly pumped into the reactor (head of reactor). It appears that MicroC G™ has a limited solubility at 5°C. Therefore, the unstable and poor performance of the reactor was attributed to electron donor availability. To further test if mixing was the cause of the low and unstable reactor performance, on day 172 the reactor liquid was hand-mixed twice a day to help incorporate the MicroC G™ into the reactor groundwater. After mixing began, the effluent nitrate concentration decreased rapidly to non-detectable levels. To permanently remedy the situation, on day 176 a mixer was installed in the influent portion of the reactor and was turned on by a timer every 2 hours while the MicroC G™ was fed and for an additional 10 minutes after feeding was stopped. With intermittent mixing, even at 5°C MicroC G™ was well incorporated into the reactor groundwater, which resulted in a stable reactor performance with non-detectable effluent nitrate concentrations. On day 190, the HRT was returned to 5 days and the reactor was operated for approximately another 25 days, during which period the effluent nitrate concentration remained below 2 mg N/L (Figure 6-8). During

this period, biofilm formation on the reactor walls was more noticeable than during the operation at temperature values above 5°C. Based on the experimental results of the temperature study, an effluent nitrate concentration of 10 mg N/L or less can be achieved even at a water temperature as low as 5°C, as long as sufficient degradable carbon is provided and well incorporated into the groundwater. It is noteworthy that the conditions used in this test differ from what it was observed in the field demonstration site where the groundwater temperature did not change significantly throughout the year (ranged between 18 and 20°C). In addition, as mentioned above (see Section 5.2.2), during Winter with ambient air temperature between -4 and 15°C, the groundwater temperature just before the wetland cell inlets at the CWW site was 19°C and the soil and water in the wetland cells head and tail was 12-13°C and 5-6°C, respectively. Therefore, even during Winter, the impact of temperature on the wetland performance in the study site, where the lowest average monthly air temperature, usually in January, is about 8 to 9°C, is expected to be less drastic. Therefore, the results of the laboratory study at a water temperature as low as 5°C are conservative, but show the resilience and efficiency of the denitrification process at low temperature values.

6.4 Modeling and Simulations

6.4.1 Model Development

Based on Monod kinetics described in Section 2.4.4 and a reactor mass balance, the continuous flow systems were modeled using the following equations:

ACCUMULATION = INPUT – OUTPUT – BIOLOGICAL REMOVAL (6-4)

$$V\frac{dN}{dt} = Q(N_0 - N) - V\frac{kXN}{K_N + N} \quad (6\text{-}5)$$

where V = reactor volume (L), N_0 = initial substrate concentration (nitrate; mg N/L), N = effluent substrate concentration (nitrate; mg N/L), Q = CSTR flow rate (L/day), X = biomass concentration (mg VSS/L), k = substrate reduction rate (mg nitrate-N/mg VSS-day), K_N = half saturation constant (mg N/L).

To simplify, all terms were divided by V to obtain the expression below:

$$\frac{dN}{dt} = \frac{Q}{V}(N_0 - N) - \frac{kXN}{K_N + N} \quad (6\text{-}6)$$

Based on the relationship that:

$$\theta = \frac{V}{Q} \quad (6\text{-}7)$$

where θ is the hydraulic retention time (days), equation 6-6 can further be simplified to:

$$\frac{dN}{dt} = \frac{(N_0 - N)}{\theta} - \frac{kXN}{K_N + N} \quad (6\text{-}8)$$

The same can be done for the biomass mass balance as shown below:

$$V\frac{dX}{dt} = Q(X_0 - X) - V\frac{YkXN}{K_N + N} - bXV \quad (6\text{-}9)$$

$$\frac{dX}{dt} = \frac{Q}{V}(X_0 - X) - \frac{YkXN}{K_N + N} - bX \quad (6\text{-}10)$$

$$\frac{dX}{dt} = \frac{(X_0 - X)}{\theta} - \frac{YkXN}{K_N + N} - bX \quad (6\text{-}11)$$

where Xo is the initial biomass concentration (mg VSS/L, Y is the yield coefficient (mg COD/mg N), and b is the microorganism decay rate constant (day^{-1}).

The effluent nitrate and biomass concentrations were modeled in Matlab given biokinetic constants (k, Y, K_N, and b), initial biomass and nitrate concentrations and hydraulic retention time. The design equations for nitrate and biomass concentrations in a continuous flow system (Equation 6-8 and 6-11, respectively) are used to compare two different continuous reactors used in this study, CSTR and multiple CSTRs in series. To model the effluent nitrate and biomass in a true CSTR, Equations 6-8 and 6-11 were used exactly as displayed above. The multiple CSTRs in series used in this study were estimated to be equal to 1.5CSTRs (see Section 6.3.1) and were modeled using the equations below:

CSTR1: Volume = 1 x CSTR

$$\frac{dN_1}{dt} = \frac{(N_0 - N_1)}{\theta} - \frac{kXN_1}{K_N + N_1} \qquad (6\text{-}12)$$

CSTR2: Volume = 0.5 x CSTR

$$\frac{dN_2}{dt} = \frac{(N_1 - N_2)}{\theta} - \frac{kXN_2}{K_N + N_2} \qquad (6\text{-}13)$$

To simulate the multiple CSTR reactors, the total reactor volume was equal to 1.5 times the true CSTR ($V_{CSTR1} + V_{CSTR2} = 1.5$).

In addition to reactor volume, flowrate and initial concentrations, biomass retention is another variable parameter for each reactor. The amount of biomass retained in the reactor has a significant effect on effluent nitrate concentrations. To vary the

biomass retention in each continuous flow reactor, the biomass retention factor, β, was included in the CSTR equation:

$$\frac{dX}{dt} = \frac{(X_0 - \beta X)}{\theta} - \frac{YkXN}{K_N + N} - bX \qquad (6\text{-}14)$$

The biomass retention factor was varied between the values of β = 0 (100% biomass retention) and β = 1 (0% biomass retention) to estimate effluent nitrate and biomass concentrations in reactors with different biomass retentions.

6.4.2 Effect of Hydraulic Retention Time on Nitrate Removal

Using the biokinetic values calculated based on the open to the atmosphere batch assay (see Chapter 4) under carbon saturation conditions and the Monod model, the effluent nitrate concentration was simulated as a function of HRT for a true CSTR without biomass recycle (i.e., all biomass is in suspension, well mixed and subject to advection) and results are shown in Figure 6-9.

Based on the results of this simulation, an HRT value equal to or higher than 10 days is required to achieve an effluent nitrate concentration below the limit of 10 mg N/L. Note that according to the Monod model, the effluent nitrate concentration is not a function of influent nitrate concentration as long as sufficient carbon is provided in order to avoid carbon (i.e., electron donor) limitations.

The above-presented simulation relative to the minimum HRT does not agree with the results of the laboratory study, in which the CSTR reactor achieved effluent nitrate concentrations below 10 mg N/L even at an HRT value as low as 5 days. Thus, it

is most likely that this discrepancy is the result of the assumption that the biomass was all in suspension and there was no biomass retention in the reactor. However, a significant amount of biomass was retained in the laboratory reactor and very little biomass exited with the effluent. Therefore, to accurately predict the reactor performance, biomass retention must be considered in the simulation.

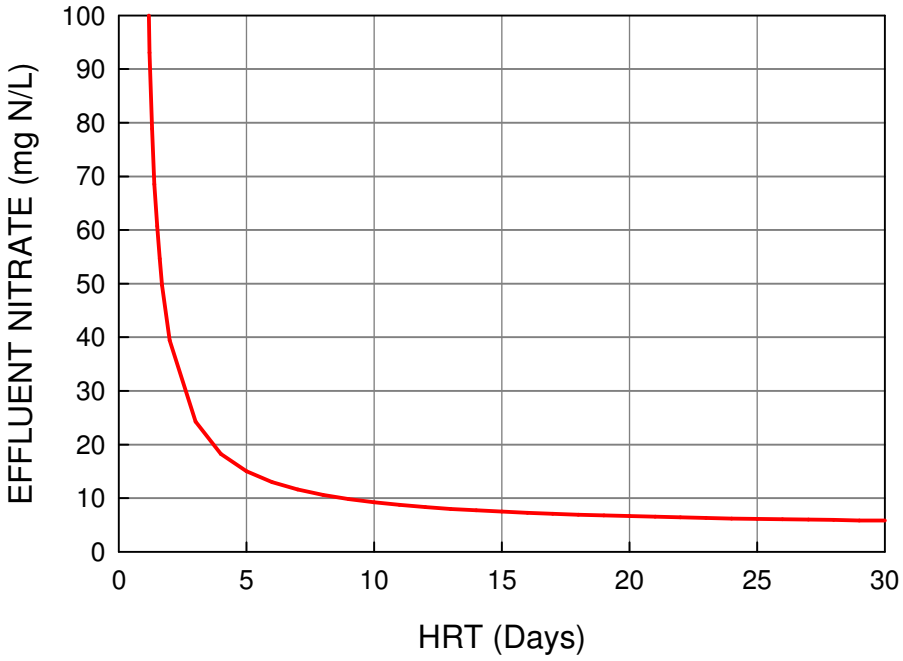

Figure 6-9. Effect of HRT on the effluent nitrate concentration (22^{o}C; without biomass retention).

Based on the above discussed observations, another model simulation was performed for an HRT value of 10 days and an influent nitrate concentration of 100 mg N/L, assuming that different degrees of biomass retention took place. To simulate different degrees of biomass retention, the biomass retention factor, β ($0 \leq \beta \leq 1$) was introduced into the CSTR biomass mass balance equation (see equation 6-14, above). The results of these simulations (Figure 6-10) show that at an HRT value of 10 days, without biomass retention, the minimum effluent nitrate concentration achievable is approximately 9 mg N/L and decreases to a value of 4 mg N/L as biomass retention increases to 100%.

Another simulation was performed to compare the effect of biomass retention on the two continuous-flow systems used in this study. Confirmed by the tracer tests (Section 6.3.1), the two different continuous-flow reactors used, without and with baffles, represented one CSTR and 1.5 to 2 CSTRs in series, respectively. The effect of biomass retention on the effluent nitrate concentration in these two reactor systems was modeled with $\beta = 0$ (100% biomass retention) and $\beta = 1$ (0% biomass retention) and the results are shown in Figure 6-10. As expected, the reactor representing multiple CSTRs in series achieved a lower steady-state effluent nitrate concentration with both 0 and 100% biomass retention. When no biomass was retained in the system, the effluent nitrate concentrations were higher compared to the system with 100% biomass retention. At 100% biomass retention, the multiple CSTRs in series and the single CSTR achieved effluent nitrate concentrations of approximately 0.7 and 4.2 mg N/L, respectively, compared to no biomass retention in which case the two reactors achieved effluent nitrate concentrations of 7.5 and 9.2 mg N/L, respectively.

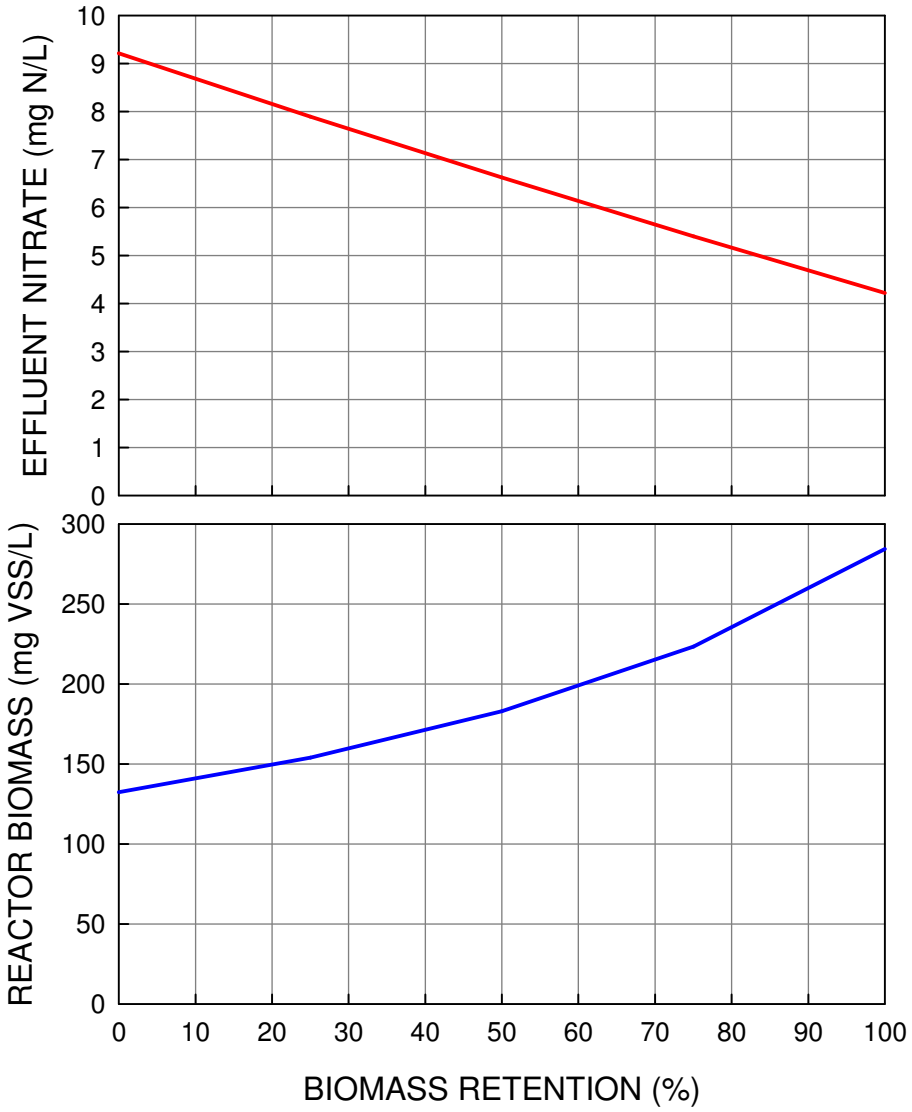

Figure 6-10. Effect of biomass retention on the effluent nitrate concentration and reactor biomass concentration (HRT, 10 days; influent nitrate concentration, 100 mg N/L; 22°C).

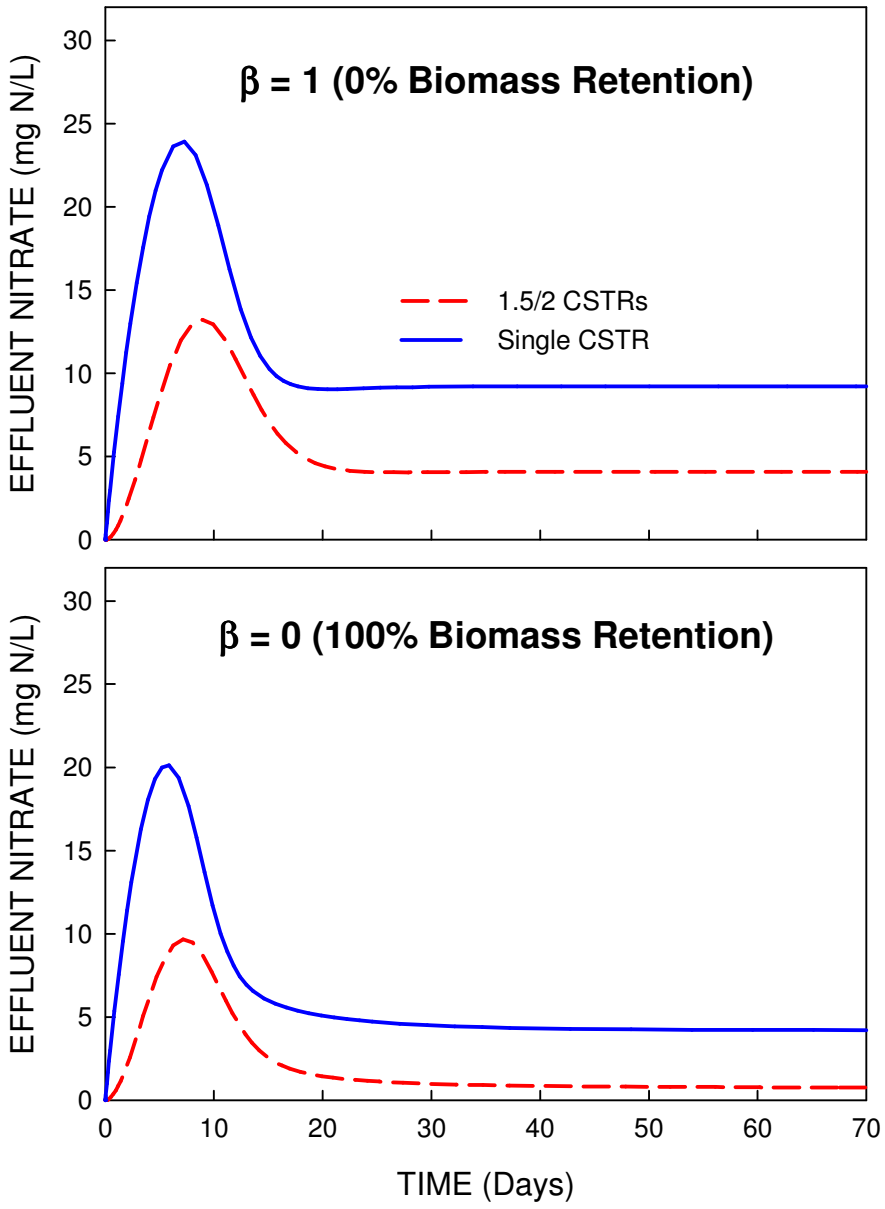

Figure 6-11. Single CSTR and multiple CSTR in series effluent concentrations at 0 and 100% biomass retention, respectively (HRT, 10 days; influent nitrate concentration, 100 mg N/L; 22°C).

Based on the observation that very little biomass escaped from the laboratory reactors, an additional simulation was performed to compare the model prediction with the laboratory data. The simulation was modeled after the laboratory reactor CFR2, which was operated at 22°C with an HRT of 5 days, open to the atmosphere and with carbon saturation. Figure 6-12 shows that under the above conditions, operating at an HRT of 5 days and assuming approximately 90% biomass retention, the effluent nitrate concentration is predicted to be approximately 5 mg N/L. The effluent nitrate concentration in the laboratory reactor at steady-state ranged from 0 to 4 mg N/L, which is slightly lower than the simulation results. Thus, the model closely predicts the performance of the laboratory continuous-flow system.

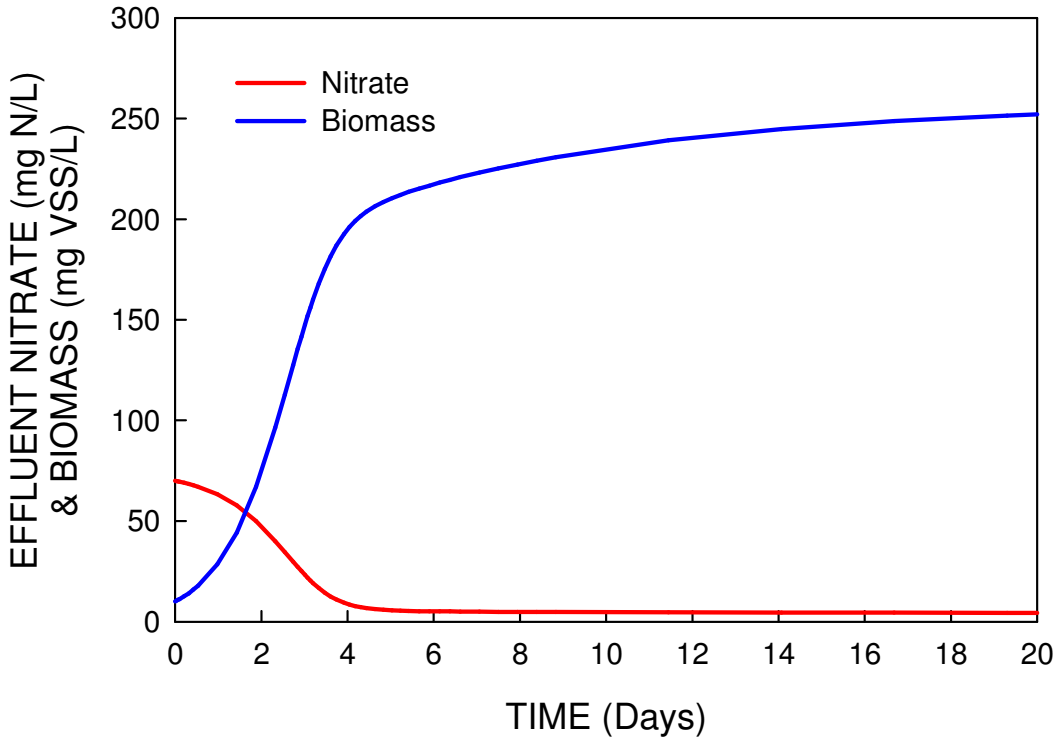

Figure 6-12. Model simulation of a continuous-flow reactor, CFR2 (HRT, 5 days; influent nitrate concentration, 70 mg N/L; 22°C; and 90% Biomass Retention).

6.4.3 Effect of Nitrate Reduction Rate on Nitrate Removal

Because the three biokinetic parameters (Y, K_N, and b) were kept constant and nitrate experimental data were used to estimate the maximum specific nitrate reduction rate (k) in two batch assays (see sections 4.2.2 and 4.2.4), another simulation was performed with a wide variation in the value of k and at a range of biomass retention in order to quantify the effect of possible variation of the value of k on the effluent nitrate concentration. The results of this simulation are shown in Figure 6-13. Based on the simulation results, decreasing the rate by 20% had a greater effect on the effluent nitrate concentration than increasing it by 20%, especially with low biomass retention. At 0% biomass retention, decreasing the rate by 20% affected the effluent nitrate concentration by approximately the same magnitude as increasing the rate by 50%. This simulation illustrates that large variations in the value of k do not drastically affect the effluent nitrate concentration, especially for systems with high biomass retention.

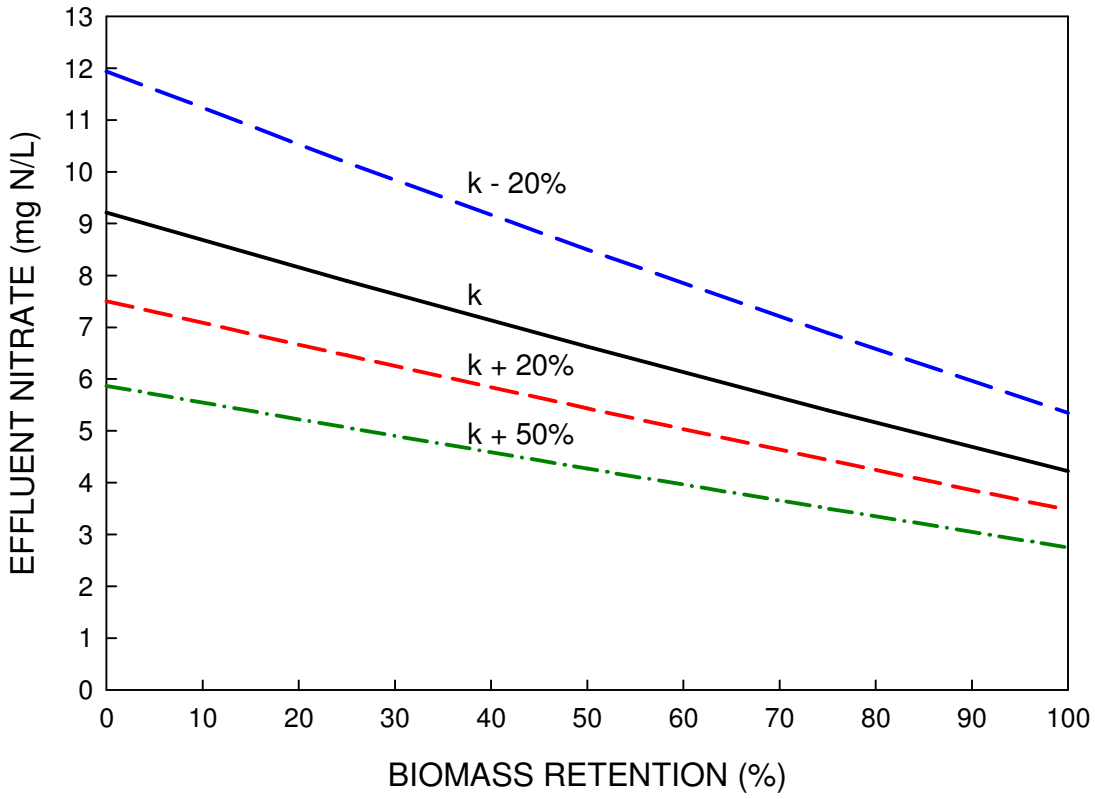

Figure 6-13. Effect of different nitrate reduction rate and biomass retention values on effluent nitrate concentration (HRT, 10 days; influent nitrate concentration, 100 mg N/L; 22°C).

6.4.4 Effect of Temperature on Nitrate Removal

The effect of temperature on the effluent nitrate concentration was simulated based on four literature Q_{10} values (1.6, 2.0, and 2.6) and one experimental value (2.46), where Q_{10} is a measure of the effect of a 10°C temperature change on the nitrate removal. Each Q_{10} value was used to estimate a temperature coefficient based on Equation 6-16:

$$\theta = (Q_{10})^{1/10} \qquad (6\text{-}16)$$

The literature Q_{10} values correspond to temperature coefficient (θ) values of 1.048, 1.072, and 1.100, respectively and the experimental Q_{10} value corresponds to a temperature

coefficient value of 1.094. The experimental Q_{10} value of 2.46 was estimated from biokinetic data obtained in the open to the atmosphere batch assay under carbon saturated conditions, and agrees with the literature values (Pavlostathis and Zhuang, 1991). The Q_{10} values and their corresponding temperature coefficients are summarized in Table 6-2.

Table 6-2. Q_{10} and corresponding temperature coefficient values.

Q_{10}	θ
1.6	1.048
2.0	1.071
2.46	1.094
2.6	1.100

Both the literature values and experimental value used in this study are within the temperature coefficient range of 1.04 and 1.16 reported by Kadlec and Reddy (2001) for removal of nitrate nitrogen. Kadlec and Wallace (2009) also reported a mean temperature coefficient of 1.11 for denitrification experiments conducted at temperatures ranging from 6 to 24°C. Based on these temperature coefficient values, the effluent nitrate concentration was simulated as a function of temperature at HRT of 10 and 15 days, and results are shown in Figure 6-14. As expected, for the same HRT value, the effluent nitrate concentration increases as the temperature decreases, especially below 10^{o}C. Both

figures show that the response to temperature changes is much greater below 15°C than it is at higher, more optimal, temperatures (>20°C). The strongest responses are seen at 5°C, where slight variations in Q_{10} results in drastic changes in effluent nitrate concentration. Increasing the HRT value from 10 to 15 days reduces the negative temperature effect to some degree.

Figure 6-14 also shows that the temperature effect is strongly correlated to the Q_{10} value. However, the experimental reactor maintained at 5^{o}C achieved effluent nitrate concentrations well below 5 mg N/L at an HRT of 10 and even at 5 days, which are well below the nitrate concentrations predicted based on the above-discussed Q_{10} values. Biomass retention, even the formation of a competent biofilm as discussed above, certainly plays a positive role in achieving low effluent nitrate concentrations at 5^{o}C.

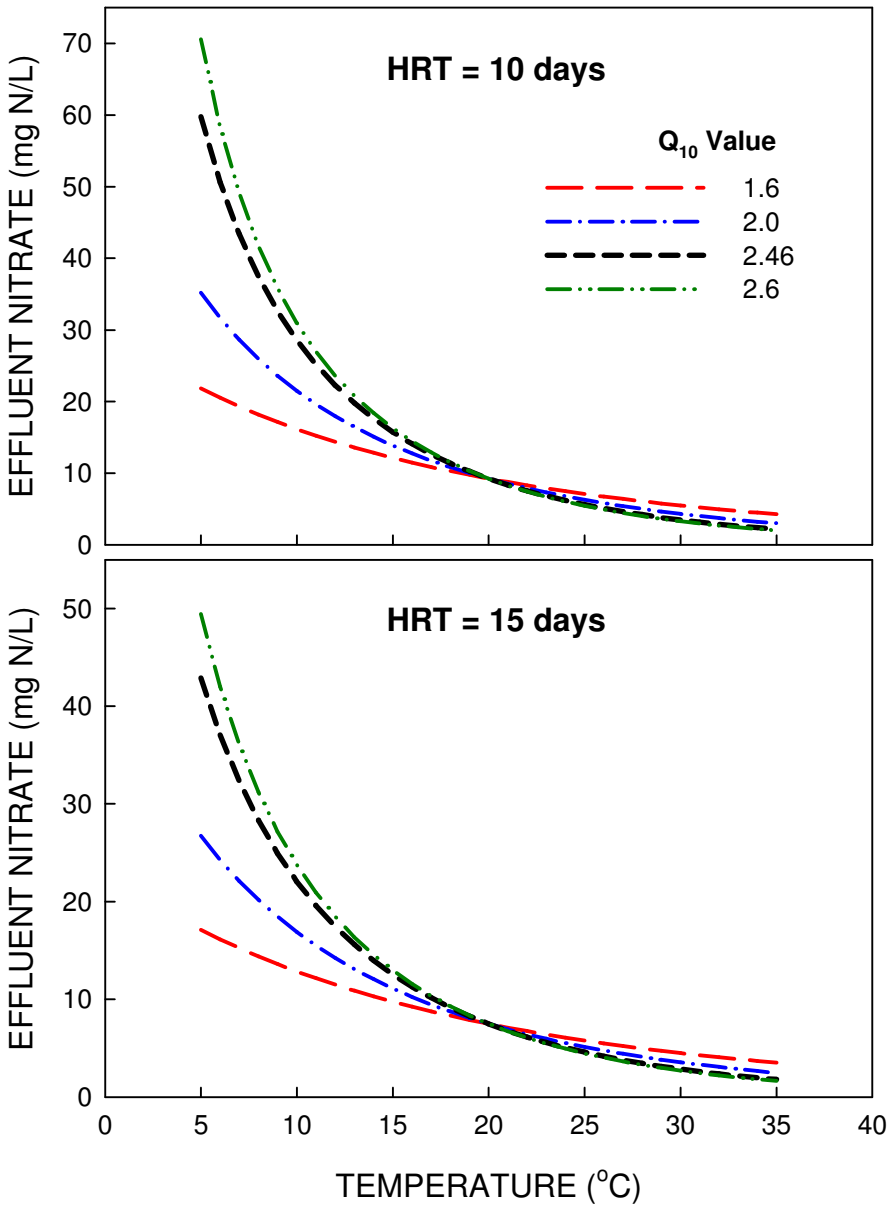

Figure 6-14. Effect of temperature on the effluent nitrate concentration at HRT values of 10 and 15 days.

6.5 Summary

Lab-scale continuous flow reactors were used to investigate the effects of different operational and environmental parameters on effluent nitrate concentrations. Based on the experimental data, the tracer study concluded that the flow pattern of the single compartment reactor (without baffles) simulated one single CSTR and the three-compartment reactor was best simulated as a system of 1.5 to 2 CSTRs in series.

Based on the experimental and simulation results, the optimal operating and environmental conditions for this wetland system to achieve an effluent nitrate concentration below 10 mg N/L are to operate at or above 5 days HRT, at a COD:N ratio of 5 or 6 and at temperatures above 10°C. If the temperature drops below 10°C, denitrification is still possible as long as there is sufficient degradable carbon incorporated into the nitrate-bearing groundwater and an increase of the HRT value to 10 days should result in a stable operation achieving effluent nitrate concentrations below the 10 mg N/L limit.

CHAPTER 7

CONCLUSIONS

This study investigated nitrate removal as a function of carbon sources, carbon loading, initial nitrate concentration, hydraulic retention time, and temperature in order to determine the feasibility of treating nitrate-contaminated groundwater with a constructed, overland flow wetland. Based on the results of the laboratory study, the following conclusions were reached:

1) Nitrate reducing bacteria are ubiquitous in soil and other environmental media. Thus, nitrate reduction can be established without a lag providing that a bioavailable carbon source is supplied.
2) Nitrate reduction is carbon-limited; that is, electron donor availability is a major condition for a successful denitrification process. For open to the atmosphere systems, an electron donor supply at a COD:N ratio of 5:1 is required for the complete denitrification of nitrate-bearing groundwater.
3) Fast nitrate reduction rates can be achieved even in open to the atmosphere systems. However, oxygen affects (increases) the carbon requirement for denitrification, but as long as the carbon source is not limiting (see conclusion 2, above), the kinetics of nitrate reduction are not affected.
4) At a temperature of 15^{o}C and above, model simulations assuming a continuous-flow CSTR system, showed that for an effluent nitrate concentration below 10 mg N/L a

HRT equal to or higher than 10 days is necessary. However, a laboratory-scale CSTR system fed with nitrate-bearing groundwater and a nitrate concentration range from 67 to 140 mg N/L at 22°C consistently achieved effluent nitrate levels below 3 mg N/L.

5) Biomass retention in a CSTR system further reduces the nitrate effluent concentration (e.g., from 9.2 to 4.8 mg N/L with 75% biomass retention at an HRT of 10 days). Experimental evidence supported a very high biomass retention level (above 95%).
6) Results of model simulation showed that the three-compartment, baffled reactor, simulated as 1.5 to 2 CSTRs in series, achieved a lower effluent nitrate concentration compared to the single-compartment, CSTR reactor (0.7 and 4.2 mg nitrate-N/L, respectively; HRT, 5 days; influent nitrate concentration, 70 mg N/L; 22°C; and 100% biomass retention).
7) The nitrate removal rate decreased as the reactor temperature decreased. The estimated temperature coefficient (θ) value was 1.094. However, in contrast to relatively high effluent nitrate concentrations predicted based on the temperature correlation derived in this study, a continuous-flow, baffled reactor operated with an influent nitrate of 67 mg N/L and an HRT of 5 days, fed MicroC G™ at a COD:N ratio of 6:1 achieved effluent nitrate concentrations below 3 mg N/L even at 5°C.
8) Incomplete denitrification is possible under low COD:N conditions, which could lead to the formation of nitrous oxide (N_2O), a potent greenhouse gas. However, when a continuous-flow, laboratory reactor operated with a COD:N ratio of 3:1, 2:1, 1:1 and 0.5:1 was monitored, N_2O was not detected at any of the COD:N ratios tested,

confirming that complete denitrification, leading mainly to the production of nitrogen gas (N_2) was the main nitrate removal process in the laboratory reactors.

Overall, the laboratory results validated the pilot treatment wetlands results and showed that further improvements in the field can be achieved by controlling the hydraulics of the system. Thus, constructed wetland technology is a technically feasible and attractive alternative for the treatment of the nitrate-bearing groundwater at the biosolids application site. It should be pointed out that this technology is "green", relying on carbon cycling via photosynthesis and is therefore environmentally sound, in addition to being an aesthetically attractive solution.

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