PERFORMANCE AND MICROBIAL COMMUNITY STRUCTURE OF MIDWESTERN GROUNDWATER PRETREATMENT REACTORS

BY

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THESIS

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Abstract

Biological ammonia oxidation has received considerable industry research recently as water utilities seek to minimize chlorine demand and potential disinfection byproduct formation. Many plants are forced to chlorinate before primary filtration in order to meet disinfection requirements, so a pretreatment configuration is the one of the few feasible locations for biological activity.

Parallel fixed-bed column reactors were operated in order to investigate the applicability of biological pretreatment for Midwestern groundwater containing dissolved methane, ammonia, and iron. One column was loaded with high-porosity gravel, while the second contained sintered glass pellets. Both media materials were marketed as "biological support" for aquiculture applications, and both columns were operated at a 10 minute empty bed contact time (EBCT) with continuous aeration to maintain aerobic conditions.

Complete oxidation of influent ammonia was achieved within 50 days of startup without any nutrient amendment or microbial inoculation. Terminal restriction fragment length polymorphism revealed temporal and depth-related changes in microbial community structure throughout the ripening period in parallel with performance data. This study demonstrates that pretreatment systems could be a viable location for biological ammonia oxidation and a potential treatment choice for infrastructure-confined Midwestern utilities.

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1 Background

1.1 U.S. Drinking Water Sources

The northern glacial aquifer system of the United States is the largest aquifer system in the country, including portions of 26 states. It is the most-tapped aquifer in the U.S. and is also the most-used for drinking water production. The central and central-west regions, also known as the Midwest agricultural region, of the northern glacial aquifer system have the lowest dissolved oxygen of the system and an intermediate pH when compared to the east and west areas, corresponding to higher iron and manganese concentrations (Groschen et al., 2008). The Midwest agricultural region includes Illinois, which is the location of this study, and includes these interesting water characteristics.

According to U.S. Environmental Protection Agency (U.S. EPA) data, there were 52,873 community water systems (CWS) in the United States at the close of fiscal year 2010 serving over 300 million people. There are 48,656 of these systems (92%) classified as "small systems", serving 10,000 people or less, and 77% use groundwater as their source. These systems also represent 93% of all maximum contaminant level, maximum residual disinfectant level, or treatment technique violations (U.S. EPA, 2011). Clearly, improved methods for groundwater treatment would be beneficial to a significant portion of U.S. utilities. Several water constituents found in groundwater are of particular interest to this study, including ammonia, iron, and dissolved methane.

1.2 Midwestern Groundwater

1.2.1 Ammonia

Ammonia is a naturally occurring contaminant in groundwaters throughout the United States (U.S. Geological Survey, 2010), including source waters in the Midwest agricultural region. In the Midwest, ammonia is present due to leaching and microbial action on paleosols deposited during glaciation (Glessner and Roy, 2009). The well water used in this study originated in the

Mahomet aquifer in central Illinois and contained an average of 1.33 mg/L ammonia as nitrogen (Table 1). Ammonia present in source waters can present several treatment challenges.

Chlorination of water containing ammonia can foster the formation of nitrogenous disinfectant byproducts including nitriles and nitrosamines, while free ammonia in the drinking water distribution system provides a substrate for nitrification, that affects disinfectant demand and tap taste and odor (Schreiber and Mitch, 2007; Goodall, 1979; Wilczak et al., 1996). Nitrite and nitrate formed as the result of nitrification has also been linked to adverse developmental impacts in infants and, in extreme cases, death due to acute ingestion leading to methemoglobinemia (i.e. blue baby syndrome) (Fan and Steinberg, 1996). Because of these concerns, the U.S. EPA established a maximum contaminant level (MCL) for nitrite at 1 mg/L as N, and nitrate at 10 mg/L as N.

Ammonia oxidation (nitrification) can occur through biological processes. Aerobic, autotrophic, ammonia-oxidizing bacteria (AOB) and archaea (AOA) carry out biological nitrification in two steps following the reactions described in Equations 1 and 2.

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + H_2O + 2\ H^+ \quad (1)$$

$$NO_2^- + 0.5\ O_2 \rightarrow NO_3^- \quad (2)$$

Based on this stoichiometry, complete nitrification requires 4.6 mg/L oxygen for every 1 mg/L of ammonia as N consumed. This oxygen requirement is often the limiting reactant in natural waters, and unintentional air introduction during water treatment can stimulate growth of nitrifying populations within media filters. Several plants with low influent ammonia content have observed complete nitrification within the treatment plant without any engineered process support (Kurtz-Crooks et al., 1986; Lytle et al., 2007[b]).

1.2.2 Iron

Like ammonia, iron is present in many groundwaters. The Midwest agricultural region of the northern glacial aquifers of the United States has the highest concentrations of iron, manganese,

and other trace elements within the aquifer system (Groschen et al, 2008). While iron does not pose a direct health concern, the potential for staining and undesirable taste has resulted in a U.S. EPA secondary standard set at 0.3 mg/L.

Water from the Newmark well has a mean total iron concentration of 1.39 mg/L, 1.31 mg/L of which is present in the reduced ferrous form . In the presence of oxygen, ferrous iron is rapidly oxidized to insoluble ferric oxide according to Equation 3 (Stumm and Lee, 1961).

$$Fe^{2+} + ¼O_2 + 2OH^- + ½H_2O \rightarrow Fe(OH)_{3(s)} \quad (3)$$

If allowed to enter the distribution system, amorphous ferric oxide particles and colloids are a potential cause of red water complaints from consumers. Oxidized iron deposition within the treatment works is a potential source of fouling and head loss buildup.

1.2.3 Methane

Similar to ferrous iron, anoxic conditions in groundwater allow methane to remain stable in solution. Methane (CH_4) is commonly present in North American anoxic groundwaters as a result of biological degradation of organic matter and/or release from shallow deposits of fossil fuels (e.g. coal seams) (Conrad, 2007). Because methane is sparingly soluble at ambient pressure, air stripping and other related gas-transfer techniques are commonly used to liberate dissolved methane and other volatile organics to the atmosphere (Hand et al., 2011). Since methane, iron, and ammonia are all present in Newmark groundwater, they are of interest in this study.

1.3 Treatment for Potable Distribution

1.3.1 Current Practices in the U.S.

Currently, most groundwater-based community water systems provide disinfection with no additional treatment (U.S. EPA, 2009[a]). Twenty-nine percent of plants serving 501 people or

less (the largest subset of all plants) do not perform any treatment. When treatment is provided, the most common techniques are aeration, pressure filtration, sequestration, and ion exchange. Chemical addition and contact is also common for specific treatment needs, including potassium permanganate, corrosion control agents, and fluoride treatment (U.S. EPA, 2009[b]). In order to meet applicable disinfection requirements, small scale systems with naturally occurring ammonia typically use combined chlorine.

1.3.2 Combined Chlorine

Chloramination is becoming increasingly popular within the drinking water industry as regulations are promulgated on the byproducts of disinfection with free chorine. When source water contains ammonia, breakpoint chlorination can be used to chemically oxidize all available ammonia and create a free chlorine residual (Cohen and Friedman, 2006).

After the breakpoint is achieved, utilities may choose to add ammonia back into finished water to create a combined chlorine residual for distribution. Typical chlorine (as Cl_2) to ammonia (as N) ratios range from 3:1 to 5:1 by weight, with 4:1 being most common (U.S. EPA, 1999).

When both ammonia and hypochlorous acid (reactive chlorine) are present in solution, they will combine following one or more of the following reactions (Cohen and Friedman, 2006):

Monochloramine: $NH_3 + HOCl \rightarrow NH_2Cl + H_2O$ (4)

Dichloramine: $NH_2Cl + HOCl \rightarrow NHCl_2 + H_2O$ (5)

Nitrogen trichloride: $NHCl_2 + HOCl \rightarrow NCl_3 + H_2O$ (6)

Combined chlorine is often selected for residual disinfection because of its superior stability in a reactive distribution system (e.g. iron mains), but laboratory analysis using sample premise plumbing has shown that distribution system nitrification negates this advantage over free chlorine (Zhang and Edwards, 2009). Combined chlorine loss in a nitrifying distribution system is a partially self-fueling cycle. As monochloramine is destroyed in interactions with biomass and other material, free ammonia is released, which in turn fuels the growth of additional

nitrifying biomass. Therefore, the key to maintain a combined chlorine residual is to prevent nitrification from initially taking hold in the distribution system.

A 1996 phone survey of 67 utilities utilizing chloramination for residual disinfection revealed that 63% had some indication that nitrification was occurring in their distribution system (Wilczak et al., 1996). In a second component of the survey, utilities reported that maintenance of combined chlorine residual prevented the establishment of nitrification in the distribution system, but chloramine was unable to arrest nitrification once it had begun (Odell et al., 1996). Difficulty in suppressing existing nitrification is likely a result of microbial attachment to pipe walls and particulate matter. Similarly, researchers at the East Bay Municipal Utilities District in California found that chloramine stability was enhanced by minimizing the total organic carbon in finished water and providing contact time with free chlorine in order to satisfy a large portion of the initial disinfectant demand (Wilczak et al., 2003). A treatment train featuring biological treatment to remove dissolved ammonia and carbon would avoid these well-documented problems and allow utilities to choose whichever disinfectant they prefer.

1.4 Biological Drinking Water Treatment

1.4.1 Common Microbiological Processes

Biologically-active treatment techniques have only recently gained attention in North America, but the process has been employed in Europe and elsewhere for decades. Rittman and Snoeyink described several systems achieving biological ammonia removal in Germany, Britain, and France in 1984, and a few in-depth examples from literature are included below.

In the report of the 1955 International Water Supply Congress and Exhibition, Kooijmans states that:

> In France at the pumping station of Le Pecq (Ste Lyonnaise de Eaux) a contact bed filter has been constructed for the removal of about 7 ppm ammonia before filtration. Compressed air is first injected into the water that passes through the contact bed in an upward flow. Poudzzolane has been chosen as a contact medium, experiments having proved that this material is more suitable for fixing the bacterial conglomerations. In the course of time and abundant growth of nitrifying organisms has developed and the ammonia content is reduced to a very low level.

Even in the U.S., Richard wrote the following recommendation for the biological oxidation of ammonia in a U.S. EPA report on oxidation techniques (Richard, 1979):

> If the quantity of ammonia present in the untreated water is greater than 2.5 mg NH_4 per litre, the ammonia can be eliminated by means of a an immersed filter packed with pozzolana, into which air is blown to satisfy the oxygen demand required for nitrification. The most favourable air/water ratios lie between 0.2-1.2. The ammonia is eliminated at the pozzolana filter. The redox potential of the water increased considerably with aeration, and deferrization is effected by chemical means.

In a later chapter of the same EPA report, Sontheimer describes the effect that biological activity has on the run time of an activated carbon filter bed: "A prolongation of the running time by a factor of 3 – 5 is frequently observed…" (Sontheimer, 1979). He goes on to describe the total bed life between regenerations as "5 – 10 times as long as that in the case of pure adsorption" (Sontheimer, 1979).

Several studies were performed in central Illinois from 1960-1970 evaluating the effects of microbial action on the iron removal process. Ghosh operated rapid sand beds in Clinton, Illinois for the removal of iron following aeration. After an 8 to 10 week filter ripening period, reduction of ferric to ferrous iron was observed through the filter, along with a drop in ammonia and dissolved oxygen concentration. Control filers using antimicrobial agents did not produce similar results, and Ghosh concluded that microbial-mediated ammonia oxidation in the filter bed must results in a reducing environment (Ghosh, 1965).

Because iron removal was the process goal and ammonia was not considered a contaminant, Ghosh recommended instituting pre-chlorination to suppress biological activity and prevent the associated disruptions in iron removal efficiency. Shock chlorinating a "ripened" sand filter at 50 mg/L for 48 hours did not halt nitrification (Ghosh, 1965). In modern plant designs which include iron removal steps distinct from filtration; there is a renewed interest in biological oxidation of influent ammonia and dissolved carbon.

More recently, a few Midwestern U.S. utilities have adopted some form of biological treatment. In 2007 the American Water Works Association (AWWA) Opflow journal featured a utility in Hutchinson, MN biologically oxidizing ammonia, iron, and manganese in a side-stream to be

combined with permeate from their primary reverse osmosis process. Opflow also featured Dubuque, Iowa in 2011 reporting a 50% decrease in chlorine addition required to maintain a stable distribution system residual after re-ordering the treatment train to allow their main filters to biologically oxidize ammonia (Rodriguez, 2011).

Internationally, a new 0 mg/L trichloramine standard in Tokyo is causing the metropolitan government to convert most plants to biological filtration (Kasuga et al., 2010[a]). This practice is widely accepted because biologically active treatment systems have been used in Japan since the early 1990's (Kasuga et al., 2010[b]).

1.4.2 U.S. EPA Case Studies

Previous U.S. EPA research (data not published) with which the author assisted from 2007-2009 assessed the feasibility of biological ammonia oxidation in several Midwestern locations. These studies and others carried out by the EPA's Treatment Technology Evaluation Branch resulted in U.S. patent number US8,029,674 B2 (Lytle, 2011). Various support media were analyzed in several operational schemes in order to study the applicability of biological treatment. When used in pretreatment of Midwestern groundwaters containing iron and methane, granular media rapidly clogged with biomass and iron deposits. Beds of ¼ to ½ inch river gravel were tested to prevent clogging, but surface area limitations prevented full ammonia oxidation. It became clear that in order to pretreat water with iron, ammonia, and carbon present, large media size had to be combined with high surface area.

2 Experimental Design

2.1 Problem Statement

Due to potential nitrogenous disinfection byproduct regulations, high disinfectant cost, or a desire to maintain a more stable distribution system, the drinking water industry is adopting dissolved ammonia and carbon removal. Drinking water treatment plants which are constrained by regulatory preference for chlorinated filters or multiple barriers will need to employ ammonia and carbon removal processes early in the treatment train. There is a need to develop biological pretreatment systems to fill this niche. The overall goal for this research is the development of a robust biological pretreatment system for the reduction of dissolved nitrogen (i.e. ammonia) and carbon (i.e. methane) in Midwestern groundwater.

2.2 Specific Objectives

1) Determine whether Midwestern groundwater will support biological pretreatment
   a) Is chemical amendment (e.g. phosphorus, carbon source) beyond aeration required?
   b) Will the presence of methane inhibit the establishment of nitrification?
   c) Will ferrous iron oxidation and deposition inhibit nitrification or foul the apparatus?
2) Determine whether gravel or sintered glass material provides a better support material for biological growth
   a) Does the seeding/startup period differ?
   b) Will media type provide a competitive advantage to a particular group of organisms?

3 Materials and Methods

3.1 Approach

To study the removal of dissolved ammonia and methane through biological pretreatment, two pilot columns were constructed. The identical columns were operated in parallel, one packed with gravel (Matrix by the Seachem Corporation) and the second with sintered glass (Siporax Mini by the Sera Corporation). The columns were supplied with fresh un-amended groundwater containing 1.3 mg/L ammonia as N on average, and flow was controlled to maintain a ten minute empty bed contact time (EBCT).

The total heights of the columns were 60 inches, initially containing 24 inches of biological support media. Sampling ports were located at 22, 12, and 2 inches from the base of the column, so that water quality could be monitored at the wellhead, after 40 inches (20 minutes) of counter-current travel though freeboard, and after five and ten minutes of contact with the biological support media. A schematic and photograph are provided in Figure 1.

3.2 Water Source and Quality

Groundwater used in this study was supplied by a well underneath the Newmark Civil Engineering Laboratory on the campus of the University of Illinois at Urbana-Champaign. According to Baliga, the well was drilled by the Illinois State Water Survey (ISWS) in the spring of 1968 (Baliga, 1969). It is 152 feet deep in the Illinoisian formation above the Mahomet Aquifer. Table 1 provides a comparison of water quality data from the Illinois State Water survey immediately after drilling, from Baliga's study in 1969, and the current data. The water quality has remained remarkably consistent over the 44 year span. The warmer temperature noted in this study is likely because water was drawn from the fourth floor of the building after water has traveled through uninsulated pipes whereas previous studies were conducted in the basement next to the wellhead.

In this study, the dissolved oxygen concentration of the influent groundwater averaged 0.99 mg/L. This is likely a result of incidental oxygen addition during sampling and measurement.

Nearly all iron present in the samples is in the ferrous form, indicating that anoxic conditions are maintained in the bulk water prior to measurement. If oxygen were present in any concentration, the iron would rapidly be oxidized to the ferric form (Stumm and Lee, 1961).

The unintended introduction of air into the influent plumbing also prevented an accurate measurement of methane entrained in the groundwater. A peak indicative of methane was routinely detected in the gas released from raw water in a sealed collection bag (Hackley et al., 2010), but the peak was never more than 1% of the total gas volume analyzed, and parallel quality control samples using pure N_2 indicated that injecting samples into the gas chromatograph introduced 3% error. Since the gas concentration of interest was never more than the known method error, no quantitative conclusion can be made regarding methane in the groundwater. Future methane analysis with this water source will require water collection closer to the wellhead in order to minimize air intrusion and thus maximize the relative methane fraction for analysis. Qualitatively, evidence of methane is in agreement with the previous report by Hackley et al. (2010) and several personal correspondences with local water utilities drawing from the Mahomet aquifer. With iron, ammonia, and methane present in an anoxic environment, the Newmark well is an excellent example of Midwestern groundwater.

3.3 Reactor Design

Each column is made from a six-foot-long section of clear two inch inner diameter polyvinyl chloride (PVC) pipe obtained from the U.S. Plastics Corporation. Non-reactive plastic mesh was installed approximately two inches from the bottom of each column and a round stone course air diffuser from Fischer was installed to provide counter-current air flow. Air was supplied from the laboratory building's compressed air system, which was first run through a plug of cotton to prevent oil and other foreign material from entering the columns. An air:water ratio of 4:1 was maintained throughout the experiment so that saturated dissolved oxygen conditions would be maintained throughout the media bed and oxygen limitation would be removed as a research factor.

The columns were mounted in parallel on a Unistrut frame from McMaster-Carr which was fitted with an open section of PVC at the top to catch and discharge excess water to waste. As indicated in Figure 1, water from the Newmark well was added to the top of the column and flowed in a downward pattern against the added air. Water was added continuously and in excess, so that a constant head datum was maintained at the level of an overflow port. In this way, head loss could be measured through comparison of the drawn-down water level in a parallel piezometer to the water level in the actual research column.

All tubing used in the apparatus was clear flexible Tygon brand from the Masterflex corporation, and was replaced whenever visual inspection revealed mineral deposits or cracks. All materials used, including tubing, PVC, and fittings were NSF standard 61 (water treatment and potable distribution) and/or NSF-51 (food grade) compliant.

Peristaltic pumps metered water flow exiting the bottom of the columns, and they were calibrated bi-weekly using a graduated cylinder and timer. Once operation began, the apparatus was run continuously throughout the study period.

In order to prevent clogging, backwashing was performed once a week. Backwash dates are provided in Appendix A. First, the air flow to the column being backwashed was doubled to provide increased scour. Next, deionized water was supplied to the bottom of the column for ten minutes at a rate of two liters per minute, or approximately 17 times the water flow rate during standard operation. While this is a high flow rate compared to backwashes at regional water treatment plants, the relative infrequency of backwashing means that less than 2% of overall water treated would be wasted during a backwash procedure. The rate was similar to that used by Rittman et al. in 2002 for a biologically-active GAC bed. Deionized water was used to avoid introducing microorganisms or nutrients not present in the natural feed water.

3.4 Sampling and Analysis Schedule

Samples for wet chemistry analysis were collected from the influent and several sampling ports on each column. Influent samples were taken directly from the incoming tube feeding the

columns. The sampling ports are ½ inch PVC ball valves tapped directly into the column. Twenty five milliliters were wasted before each sample to flush out water which was stagnant in the port. Sampling was performed rapidly from the top-down to ensure that any draw-down effect (resulting in shortened EBCT) is not captured in subsequent samples. All samples for wet chemistry were collected twice weekly. Biomass samples were collected every third week throughout the study period.

Head loss was measured in a three-eighth inch tube which was attached to the apparatus outlet before the pump head and run parallel to the column. In this way, the differential water level can be read as a direct measurement of hydraulic head loss. The zero point was calibrated on the first day of operation with full air flow rate and no media present.

3.5 Analytical Methods

Wet chemistry was determined through a variety of methods. Dissolved oxygen concentration was measured using an Orion 4 Star model digital meter by Thermo Scientific. The probe was calibrated daily using tap water according to the manufacturer's procedure. Approximately 30 mL was collected in a well-rinsed container with care taken to minimize air bubble intrusion.

The pH was measured with an Accument AB15 pH meter with Accument probe #13-620-287A. Three-point calibration was performed daily using Fischer buffers at pH 4, 7, and 10. Approximately 30 mL was collected in a well-rinsed container for each sample.

Several chemical constituents were evaluated on a Hach DR/4000U spectrophotometer. Ammonia analysis was performed using the salicylate method (Hach product number 2668000) according to the standard procedure. The range is 0-0.5 mg-N/L, and detection limit is 0.02 mg-N/L. Combined nitrate and nitrite analysis was performed using the cadmium reduction method (product number 2106169) according to the standard procedure. The range is 0-5 mg-N/L, and detection limit is 0.2 mg-N/L. Nitrite analysis was performed using the diazation method (product number 2107169) according to the standard procedure. The range is 0-0.5 mg/L, and detection limit is 0.006 mg/L. Total iron analysis was performed using the FerroVer method

8008 (product number 2105769) according to the standard procedure. The range is 0-3 mg/L, and detection limit is 0.03 mg/L. Ferrous iron analysis was performed using the 1, 10 Phenanthroline method (product number 103769) according to the standard procedure. The range is 0-3 mg/L, and detection limit is 0.03 mg/L. Samples were diluted as required with water from a Barnstead NANOpure ultrapure water system.

Total organic carbon was analyzed on a Shimadzu TOC-V CPH Total Carbon Analyzer according to the standard combustion method using a 0-10 mg/L calibration curve. 20-30 mL of sample is filtered through a 0.22 µm syringe filter to remove particulate matter and acidified with 2µl of 4M H_2SO_4 per ml of sample. Glass sampling vials were baked in a 550^0C Barnstead furnace for a minimum of five hours before use to release any residual carbon contaminants.

Finally, alkalinity was determined through titration with 1M nitric acid to the bromcresol green-methyl red endpoint. Twenty five milliliters of sample was collected for each titration. Full analytical results are provided in Appendix B.

3.6 Biological Analysis

In addition to water quality analyses, biomass samples were collected to examine parallel changes in the microbial community. At three-week intervals, the influent water supply was briefly suspended and several pieces of media were removed through dedicated biomass sampling ports at the top, middle, and bottom of the media beds as seen in Figure 1. The media was placed in 15 mL centrifuge tubes, to which 5 mL of sterile room-temperature 1x TAE buffer was rapidly added. Vigorous hand shaking liberated iron and biomass from the media surface, which was then poured into a second centrifuge tube. After spinning down at 8500 RPM for 10 minutes, the supernatant was discarded. An additional 5 mL of 1x TAE buffer was then added, and the pellet was re-suspended through shaking and light vortexation before spinning down again at 8500 RPM for 10 minutes. After discarding the supernatant, 1 mL of 1x TAE was added, using the force of fluid exiting the pipette tip to gently re-suspend the pellet. The solution was then transferred to a 1.5 mL centrifuge tube to be spun down at 10,000 RPM

for 10 minutes. The supernatant was then discarded and the pellet stored at -80^{0}C for downstream use.

DNA was extracted using the FastDNA SPIN Kit for Soil (Q-Biogene/MPBiomedicals, Solon, OH, USA) standard protocol with centrifugation extended to 15 minutes after bead beating and incubation at 55^{0}C for 5 minutes before final elution. DNA concentration was measured using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and an aliquot was diluted to 10 ng/µL for polymerase chain reaction (PCR) amplification. PCR was performed with Bullseye Standard Taq DNA Polymerase 2.0x Master Mix Kit with 1.5 mM $MgCl_2$ (Midwest Scientific, St. Louis, MO, USA) according to the manufacturer's proportions using the 47 forward FAM-labeled and 927 reverse universal bacterial primers (Amann et al., 1995; Chen et al., 2004). Thermo cycling conditions were: initial denaturation at 95^{0}C for 3 minutes; 25 cycles of 30 second denaturation at 95^{0}C, 30 second annealing at 58^{0}C, and 90 second extension at 72^{0}C; and a final extension at 72^{0}C for 90 seconds. PCR products were examined by gel electrophoresis with 1.0% agarose gel in 1x TAE buffer at 100 Volts for 30 minutes. Single-stranded DNA was removed by digestion with 1% mung bean nuclease (New England Biolabs, Ipswich, MA, USA) for 1 hour (Egert & Friedrich, 2003), after which the products were purified with the Promega Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA) according to the manufacturer's protocol and the concentration was re-checked via NanoDrop (Hwang et al., 2012).

Purified DNA samples were digested with the tetrameric restriction enzyme *Msp*I (New England Biolabs, MA) at 37°C overnight with a final restriction enzyme activity of 2 units/µL, and incubated at 80°C for 10 min to inactivate the restrictive enzymes (Liu et al., 1997). Fragment analysis was performed on the ABI 373xl Analyzer (Applied Biosystems / Life Technologies, Carlsbad, CA, USA) and peak analysis was performed with GeneMapper 4.0 (Life Technologies).

Terminal restriction fragment (T-RF) peaks less than 50 base pairs in length or less than 0.5% of the total sample peak area were manually removed from the data set and the samples were compiled into a standardized matrix by T-RF length. In order to visualize differences in the microbial communities present in the samples, principal component analysis (PCA) was applied to the data set using the free Multibase add-in for Microsoft Excel

(http://www.numericaldynamics.com/). PCA is a statistical technique commonly used in microbial ecology to reduce complex population matrices into principal coordinates which define the maximum possible cumulative variation in the raw data (Ramette, 2007). In this application, the first principal coordinate expresses 88% of total sample variation and the second principal expresses 5%.

4 Results

Complete and consistent biological ammonia oxidation to nitrate was achieved without any chemical addition or initial seeding. In both the sintered glass and the gravel media bed, an initial ripening phase was followed by a rapid decrease in effluent ammonia concentration and subsequent increase in effluent nitrite concentration. After approximately ten days of subsequent operation, the effluent nitrite spike was replaced by increasing nitrate concentration until effluent levels of both ammonia and nitrite were near zero.

As illustrated in Figure 2, the ammonia-nitrogen concentration in the sintered glass column began to decrease after approximately 21 days of operation and the concentration after a 10 minute EBCT was consistently below 0.2 mg-N/L about 14 days later. In the gravel column, the initial decrease in effluent ammonia concentration did not take place until approximately 28 days had elapsed and the concentration at 10 minute EBCT was not below 0.2 mg-N/L until 28 days thereafter.

In both columns, samples taken after 5 minutes of EBCT contained higher ammonia concentrations than after 10 minutes. NH_4-N concentration in the sintered glass bed became steady around 0.2 mg-N/L after about 60 days, while the gravel column stabilized at about 75 days. Ammonia removal in the headboard water above the bed was between 0.2 and 0.4 mg-N/L in both columns. Nitrification in the headboard water was likely occurring in the thick layer of iron deposits and biofilm lining the column wall. Water takes approximately 20 minutes (in a simple plug flow model) to travel from the top of the column to the top of the media, so nitrifying organisms have ample time to begin oxidizing ammonia. A similar phenomenon was noted in Roxana, IL, where unintended but near-complete nitrification occurred without engineered process support in the iron floc blanket within an upflow clarifier (Crooks et al., 1986).

During the initial ammonia removal period in the sintered glass column, the nitrite concentration at 10 minutes of EBCT never exceeded 0.6 mg-N/L and was below 0.2 mg-N/L within 28 days after ammonia oxidation began (Figure 3). The 10 minute EBCT nitrite concentration in the gravel column remained below 0.8 mg-N/L and was also below 0.2 mg-N/L 28 days after nitrification was established. Neither column would have exceeded the EPA MCL

of 1 mg-N/L for nitrite since nitrite oxidation began soon after it started to be produced from ammonia oxidation.

PCA was used to visualize the changes in terminal restriction fragment abundance and distribution among the biomass samples taken from the top, middle (i.e. 5 minute EBCT), and bottom (i.e. 10 minute EBCT) of the media beds. Samples were taken from the top and middle on days 22, 43, 64, and 89 of the study period. To replace media removed during sampling, additional media was added on day 50, which was then sampled on days 64 and 89. The bottom portions of the columns were also sampled on days 64 and 89.

Influent samples cluster together separate from column samples in the PCA plot provided in Figure 4, confirming that selection occurred in the media beds. As the microbial communities in the columns mature, their T-RFLP profiles shift from the upper right to the lower left portion of the plot. On day 22, the communities in the top and middle of the gravel bed are close together, indicating similarity, while the community in the middle section of the sintered glass bed has shifted leftward away from the initial state. This more rapid shift in community in the sintered glass bed is consistent with the more rapid startup of nitrification in the column as well.

On day 43, the top and middle samples from the gravel column are similar to each other and both have shifted to group near the middle of the sintered glass bed three weeks prior. Again, this agrees with wet chemistry data indicating that nitrification was occurring in both columns at that time. In contrast, the middle of the sintered glass bed has continued to mature further down and to the left of the earlier samples.

By days 64 and 89, the microbial communities have mostly stabilized in both columns as revealed by the relatively small shifts in the PCA plot. Consistent with prior dates, deeper samples in each column plot further to the lower left of the PCA, and the sintered glass column always appears to be more mature than the gravel column. Complete and consistent ammonia oxidation during this time period is consistent with the stable microbial community.

Inversely related to the dissolved oxygen concentration, the ferrous iron concentration in each column rapidly decreased in the presence of oxygen. Ferrous iron was routinely below the method detection limit at 10 minute EBCT in both media beds from an initial concentration of 1.31 mg/L. The characteristic orange-red coloration of oxidized iron deposition was noticeable

on the media bed and walls of the columns within hours after initial startup. After only a few days, the columns appeared completely opaque from iron and biofilm deposition.

Significant pH decrease is often a concern among utilities considering biological treatment, but in this study the pH in each media bed was affected more by aeration and subsequent CO_2 stripping than by hydrogen ion liberation from nitrification or iron oxidation. The sintered glass and gravel media bed demonstrated nearly identical pH increase from 7.4 to approximately 8 above and throughout the media bed. This pH change was evident from the beginning of operation before nitrification began; further confirming that it was not the result of a biological process. Alkalinity remained consistent in both columns until nitrification was active and thereafter decreased from approximately 325 to 315 mg/L as $CaCO_3$ as EBCT increased. High alkalinity measurements at the beginning of the study are likely a result of well inactivity prior to the study. Steady alkalinity throughout the media beds is consistent with CO_2 stripping as the cause of pH change since the change in dissolved carbon dioxide would change pH without affecting alkalinity.

Head loss was never more than one inch in either column. Neither bed material experienced clogging during the study period. Aggressive weekly backwashing and larger media size likely contributed to the ease of operation since past pilot work using similar water from the same region did clog in the EPA studies. Head loss data is provided in Appendix A.

Over the course of the study period, both biologically active media beds achieved and maintained complete ammonia oxidation with no initial seeding or nutrient addition. Analyzing the water chemistry and microbial community of the columns in parallel provided insight into the operation of the system during ripening.

5 Discussion and Future Work

Fixed-bed biological pretreatment is a robust, user-friendly method to remove naturally occurring ammonia from Midwestern groundwater. The columns used in this study were not artificially seeded with microorganisms from an outside source, and microbial analysis using T-RFLP confirmed that the community in each column diverged over time from the influent water, which remained steady throughout the study period. Although they experienced identical conditions, the microbial community on the sintered glass bed shifted away from the initial (run day 22) structure more rapidly than the community in the gravel bed, which is consistent with observed shifts in nitrogen speciation. Ammonia oxidation began first in the sintered glass column after approximately twenty days of operation, and then began in the gravel column seven days later. In both columns, initial ammonia oxidation coincided with an increased nitrite concentration in the effluent water. After seven days of continued operation, the effluent nitrite in both columns fell to the detection limit and nitrate was the primary nitrogen compound leaving the column for the remainder of the study. Also in agreement with operational data, biomass samples taken on days 64 and 89 of operation reveal mostly stable microbial communities in both beds which are shifting much more slowly than during ripening.

The observed 20-30 day startup period before complete nitrification was shorter than several reported in the literature. Lytle et al. (2007[a]) observed near-complete ammonia oxidation after 60 days in an iron removal anthracite bed at 14^0C in southwest Ohio, and Anderssen et al. (2001) required 40 days to establish nitrification in activated carbon beds at 20^0C. Temperature significantly affects the growth of nitrifying bacteria, which follow the Arrhenius relationship, facilitating a two-fold increase in activity for every 10^0C increase in water temperature (Baribeau, 2006). However, rapid bed ripening at the Newmark well is reinforced by Baliga's 1969 thesis using the same water at 15.6^0C. His graphs clearly indicate unplanned and complete ammonia oxidation after only 20-30 days in every aerated sand bed operated for iron removal.

Because nitrification is carried out by aerobic autotrophic organisms, no supplements (e.g. carbon source) are required beyond continuous aeration of the naturally anoxic groundwater. Aeration also strips entrained carbon dioxide throughout the treatment process, offsetting the consumption of alkalinity during nitrification and causing an increase in pH from 7.4 to 8 in

both beds. This value is within the optimal pH range of 7.5-8 for ammonia and nitrate oxidizing organisms (Baribeau, 2006) and also within the range that utilities prefer for distribution system stability.

The gravity-fed configuration is simple to build and operate with minimal time required for routine maintenance. Air flow oxygenates the water and also prevents the media bed from becoming clogged with oxidized iron deposits and biomass. Since the media is large in diameter, iron loss in the bed occurs through deposition and adhesion onto media pieces rather than mechanical filtration. Solids do accumulate in the bed, but, as previously reported by Kooijmans (1955) and Richard (1979), an aggressive weekly preventative backwash schedule and large media size were sufficient to prevent head loss buildup. Longer-term studies are necessary to determine the effects of continued iron deposition in the bed and explore whether a pseudo-steady-state iron balance is achievable with only a weekly backwash.

Relatively few studies have used universal primers to measure diversity of the 16S rRNA gene in a nitrifying filter, opting instead to target only the *amoA* gene in the nitrification pathway. In the only other molecular study involving raw Midwestern groundwater, White et al. (2012) reported that a full-scale carbon bed oxidizing ammonia was dominated by nitrite oxidizing bacteria, followed in abundance by ammonia oxidizing bacteria and OTUs of the order *Rhodobacterales* performing an unknown function. In the future, pyrosequencing the biomass samples from the current study would allow a deeper analysis of the microbial community structure by providing insight into the potential function of the prokaryotes present and comparison to microbial communities previously reported under varying circumstances. Functional information would reveal the selection pressures inside the bed during ripening and maturation and also potentially expose interactions among organisms present.

Additionally, more research is needed regarding nitrogen fate in the process. The total aqueous nitrogen mass balance (i.e. the sum of ammonium, nitrate, and nitrite entering the columns subtracted from the total mass exiting) in both media beds is at steady state until nitrification rapidly begins to occur. While nitrification rapidly accelerates, more nitrogen species are exiting the columns than entering. Once steady ammonia oxidation has been achieved, the opposite imbalance occurs wherein aqueous nitrogen is lost in the media bed. Future research should examine the possibility of nitrogen fixation during the startup period and/or denitrification

occurrence inside anoxic pockets in the biofilm. The presence of simultaneous nitrification and denitrification on similar sintered glass media has been reported previously (Menoud et al., 1999), so this finding would be reasonable and could potentially facilitate full nitrogen mineralization to N_2.

In conclusion, this study demonstrated that complete ammonia oxidation is achievable in a short contact time with an aerated bed of large porous media. Several Midwestern utilities have documented significant improvements in disinfectant stability after adopting biological ammonia oxidation in their primary granular filters, including a 50% decrease in residual disinfectant required at an Iowa facility (Rodriguez, 2011).This study showed pretreatment can offer similar process benefits early in the treatment train, which would allow utilities to satisfy initial disinfectant demand with free chlorine as recommended by Wilczak et al. (2003), and also sidestep regulatory concerns about biological activity in the primary filters.

Biological treatment is an easily-achieved, natural process to remove excess ammonia in water treatment. Monitoring evolution of the microbial community in parallel with water quality analysis provided insight regarding population changes inside the beds and their relation to performance, providing a foundation for future process optimization through selection. As this and other studies demonstrate the ease and robust flexibility of biological treatment, barriers to implementation will fall and Midwestern groundwater utilities will begin producing more stable drinking water at lower cost.

6 Figures and Tables

Table 1: Water Quality Parameters.

	Raw Groundwater			Current Study Effluent (Final 20-day Average)	
Species	ISWS 1968	Baliga 1969	Current Research	Sintered Glass	Gravel
Alkalinity (mg/L as $CaCO_3$)	324	333.8	332	315	316
Ammonia (mg/L as N)	1.1	1.04	1.33	0.03	0.09
Dissolved Oxygen (mg/L)			0.99	8.58	8.80
Iron, Ferrous (mg/L)		1.28	1.31	0.01	0.02
Iron, Total (mg/L)	1.1	1.2	1.39	0.70	0.75
Nitrate+Nitrite (mg/L as N)	0		0.4	1.3	1.2
Nitrite (mg/L as N)			0.007	0.001	0.006
pH		7.17	7.4	8.0	8.0
Temperature (^{0}C)		15.6	22		
TOC (mg/L)			1.8	1.1	1.1

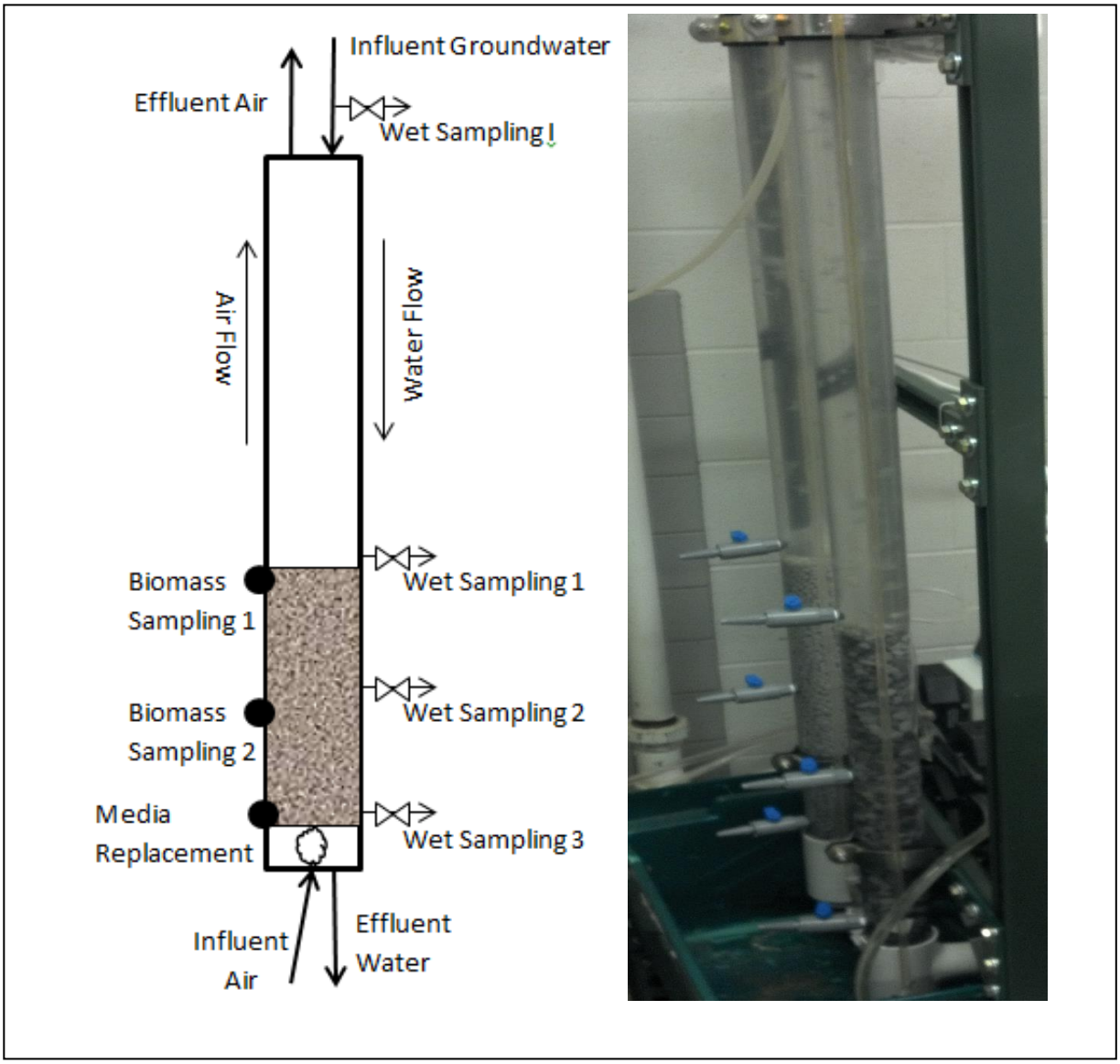

Figure 1: Column schematic (left) and photograph of operating apparatus (right).

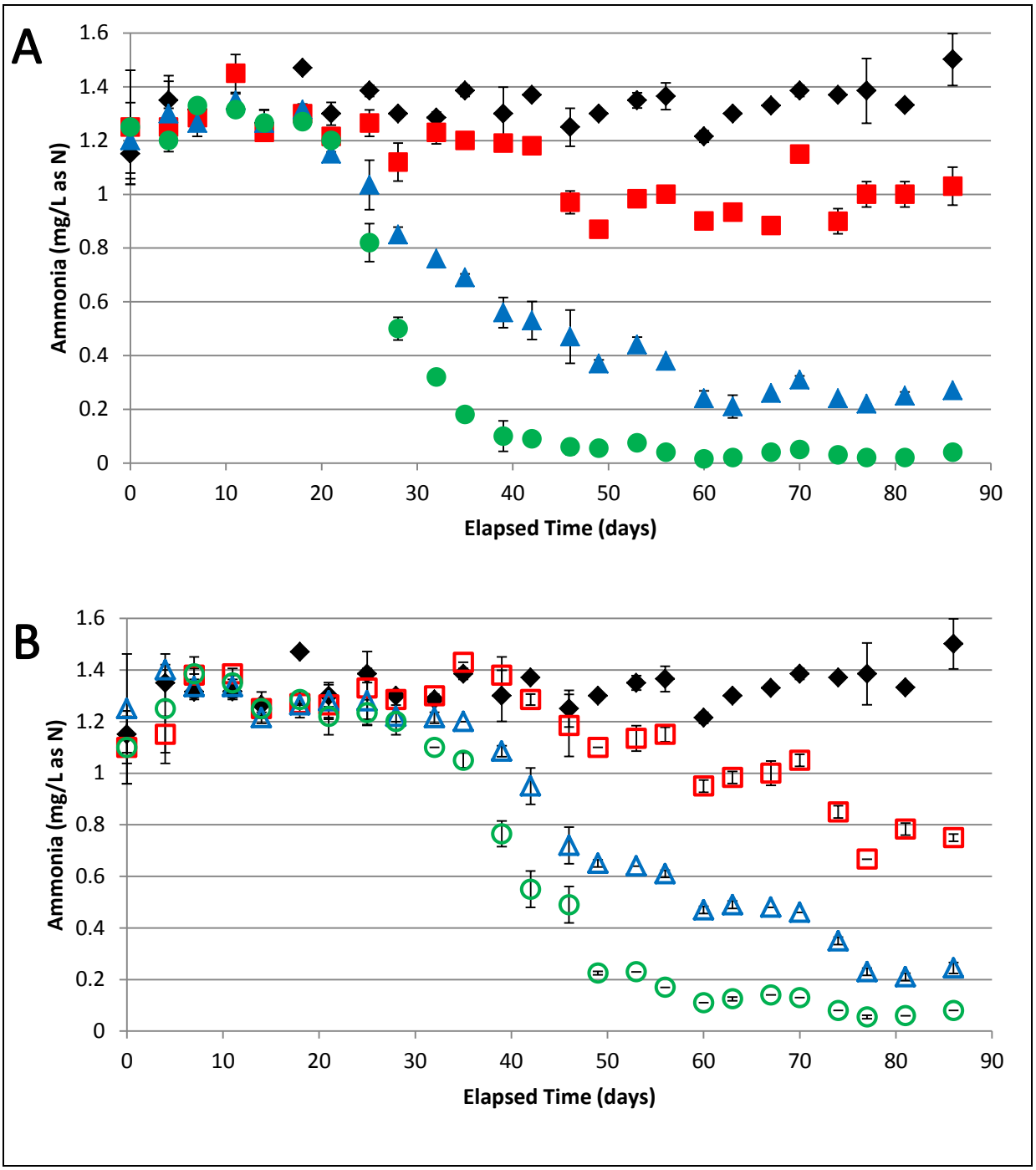

Figure 2: Ammonia concentration in the (A) sintered glass column and (B) gravel column. Symbols correspond to influent (◆), top of the media bed (■), 5 minute EBCT (▲), and 10 minute EBCT (●). Error bars represent standard deviations calculated from duplicate samples.

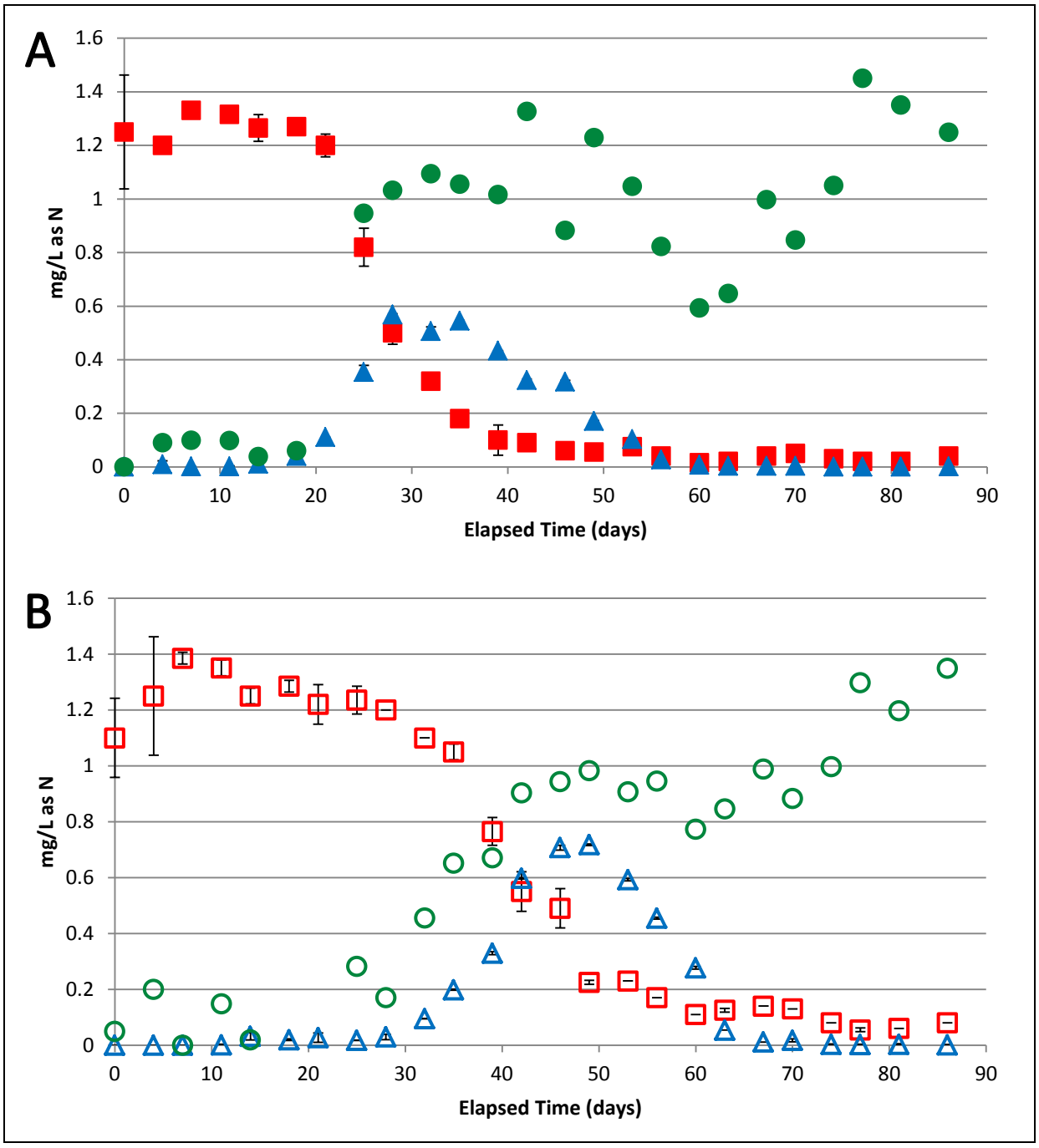

Figure 3: Effluent concentration of nitrogen species after 10 minutes EBCT in the (A) sintered glass column and (B) gravel column. Symbols correspond to effluent ammonia (■), effluent nitrite (▲), and effluent nitrate (●). Error bars represent standard deviations calculated from duplicate samples. The nitrate level shown is the arithmetic difference between averaged duplicate nitrite and combined nitrite + nitrate readings, and thus is not displayed with an error bar.

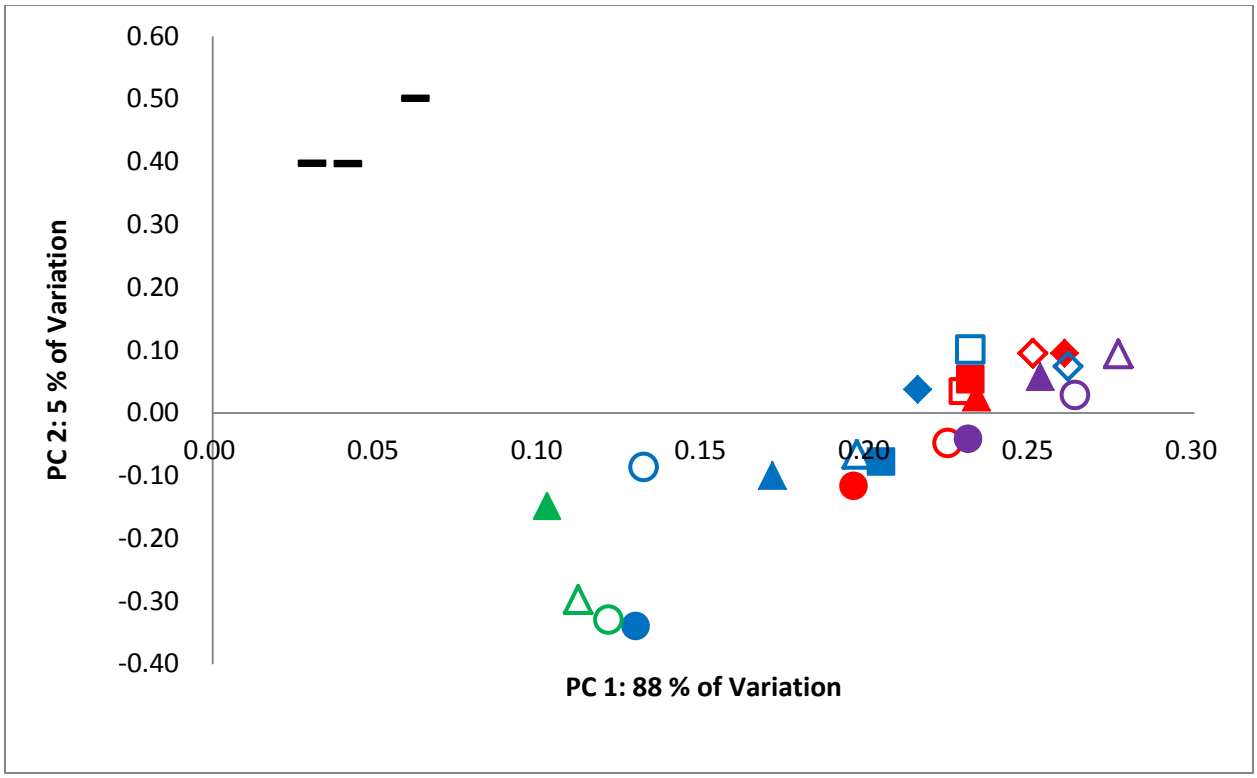

Figure 4: Principal component analysis (PCA) of community data obtained using T-RFLP. Symbols denote dates, fill denotes media type, and color denotes sampling location: day 22 (◆), day 43 (■), day 64 (▲), day 89 (●). Samples from the sintered glass column are filled while samples from the gravel column are hollow. The top of the bed is shown in red, 5 minute EBCT in blue, 10 minute EBCT in green, and media which was added to the top of the bed on day 50 in purple. Influent samples are represented with a black hash mark (-).

7 References

Amann RI, Ludwig W, Schleifer KH. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev. 1995 59(1):143-169.

Andersson, Anneli, Patrick Laurent, Anne Kihn, Michele Prevost, and Pierre Servais. 2001. "Impact of Temperature on Nitrification in Biological Activated Carbon (BAC) Filters used for Drinking Water Treatment." *Water Research* 35 (12): 2923-2934.

AWWA Opflow *Finished Water* profile, Blended Treatment Process Achieves Smooth Operation. American Water Works Association *Opflow*. Vol. 33, 11. Accessed 08/16/2011, 2007, http://www.ci.hutchinson.mn.us/pdf/opflowarticle.pdf.

Baliga, K.Y., Biologically Mediated Chemical Changes in the Filtration of Aerated Ground Waters, PhD Thesis, University of Illinois, 1969

Baribeau, Helene. 2006. "Growth and Inactivation of Nitrifying Bacteria." In *Fundamentals and Control of Nitrification in Chloraminated Drinking Water Distribution Systems*, edited by Mary Kay Kozyra. 1st ed., 6. Denver, CO: American Water Works Association.

Chen CL, Macarie H, Ramirez I, Olmos A, Ong SL, Monroy O, Liu WT. Microbial community structure in a thermophilic anaerobic hybrid reactor degrading terephthalate. Microbiology. 2004 150(Pt 10):3429-3440.

Cohen, Y. Koby and Melinda Friedman. 2006. "Introduction to Nitrification in Drinking Water and its Impact on Regulatory Compliance." In *Fundamentals and Control of Nitrification in Chloraminated Drinking Water Distribution Systems*, edited by Mary Kay Kozyra. 1st ed., 1. Denver, CO: American Water Works Association.

Conrad, Ralf. 2007. "Soil Microbial Communities and Global Climate Change - Methanotrophic and Methanogenic Communities as Paradigms." In *Modern Soil Microbiology*, edited by Jan Dirk Van Elsas, Janet K. Jansson and Jack T. Trevors. Second ed., 263. Boca Raton, FL: CRC Press.

Egert M, Friedrich MW. Formation of pseudo-terminal restriction fragments, a PCR-related bias affecting terminal restriction fragment length polymorphism analysis of microbial community structure. Appl Environ Microbiol. 2003 69(5):2555-2562.

Fan, Anna M. and Valerie E. Steinberg. 1996. "Health Implications of Nitrate and Nitrite in Drinking Water: An Update on Methemoglobinemia Occurrence and Reproductive and Developmental Toxicity." *Regulatory Toxicology and Pharmacology* 23 (1): 35-43.

Ghosh, M., Filtration as Related to the Removal of Iron from Ground Waters, PhD Thesis, University of Illinois, 1965

Glessner, Justin J. G. and William R. Roy. 2009. "Paleosols in Central Illinois as Potential Sources of Ammonium in Groundwater." *Ground Water Monitoring and Remediation* 29 (4): 56-64.

Goodall, J.B., Biological Removal of Ammonia. *Oxidation Techniques in Drinking Water Treatment* (H. Sontheimer and W. Kuhn, editors). EPA 57019-79-020, US EPA (1979)

Groschen, G.E., Arnold, T.L., Morrow, W.S., and Warner, K.L., 2008, Occurrence and distribution of iron, manganese, and selected trace elements in ground water in the glacial aquifer system of the Northern Unites States: U.S. Geological Survey Scientific Investigations Report 2009-5006, 89 p.

Hackley, Keith C., Samuel V. Panno, and Thomas F. Anderson. 2010. "Chemical and Isotopic Indicators of Groundwater Evolution in the Basal Sands of a Buried Bedrock Valley in the Midwestern United States: Implications for Recharge, Rock-Water Interactions, and Mixing." *Bulletin of the Geological Society of America* 122 (7-8): 1047-1066.

Hand, David W., David R. Hokanson, and John C. Crittensen. 2011. "Gas-Liquid Processes: Principles and Applications." In *American Water Works Association Water Quality and Treatment*, edited by James K. Edzwald. Sixth ed., 325. New York: McGraw Hill.

Hwang, C.C., Ling, F.Q., Andersen, G.L., LeChevallier, M.W., Liu, W.T., 2012. Evaluation of methods for the extraction of DNA from drinking water distribution system biofilms. Microbes and Environments 27 (1), 9e18.

[a]Kasuga, Ikuro, Hirotaka Nakagaki, Futoshi Kurisu, and Hiroaki Furumai. 2010. "Predominance of Ammonia-Oxidizing Archaea on Granular Activated Carbon used in a Full-Scale Advanced Drinking Water Treatment Plant." *Water Research* 44 (17): 5039-5049.

[b]Kasuga, Ikuro, Hirotaka Nakagaki, Futoshi Kurisu, and Hiroaki Furumai. 2010. "Abundance and Diversity of Ammonia-Oxidizing Archaea and Bacteria on Biological Activated Carbon in a Pilot-Scale Drinking Water Treatment Plant with Different Treatment Processes." *Water Science and Technology* 61 (12): 3070-3077.

Kooijmans, L. H. Louwe, ed. 1955. . Subject no. 4: Aeration and Deferrisation. [Report of the International Water Supply Congress and Exhibition]. London: International Water Supply Association.

Kurtz-Crooks, Jennifer, Vernon L. Snoeyink, Michael D. Curry, and Marty L. Reynolds. 1986. Technical note: Biological Removal of Ammonia at Roxana, Illinois. *Journal / American Water Works Association* 78 (5): 94-95

Liu, Wen-Tso, Terence L. Marsh, Hans Cheng, and Larry J. Forney. 1997. "Characterization of Microbial Diversity by Determining Terminal Restriction Fragment Length Polymorphisms of Genes Encoding 16S rRNA."*Applied and Environmental Microbiology* 63 (11): 4516-4522.

[a]Lytle, Darren A., Abraham S. Chen, Thomas J. Sorg, Sara Phillips, and Ken French. 2007. Microbial as(III) oxidation in water treatment plant filters. *Journal / American Water Works Association* 99 (12): 72,86+12.

[b]Lytle, Darren A., Thomas J. Sorg, Lili Wang, Christy Muhlen, Matthew Rahrig, and Ken French. 2007. Biological nitrification in a full-scale and pilot-scale iron removal drinking water treatment plant. *Journal of Water Supply: Research and Technology - AQUA* 56 (2): 125-36.

Lytle, Darren Alan U.S. 2011. *Biological Filter for Oxidizing Ammonia in Drinking Water Treatment*, edited by The United States of America as represented by the United States Environmental Protection Agency. Vol. 12457107. Patent number US8,029,674 B2 issued October 4,2011.

Menoud, P., C. H. Wong, H. A. Robinson, A. Farquhar, J. P. Barford, and G. W. Barton. 1999. "Simultaneous Nitrification and Denitrification using Siporax(TM) Packing."Elsevier Ltd, 1998.

Odell, Lee H., Gregory J. Kirmeyer, Andrzej Wilczak, Joseph G. Jacangelo, Joseph P. Marcinko, and Roy L. Wolfe. 1996. "Controlling Nitrification in Chloraminated Systems." *Journal / American Water Works Association* 88 (7): 13-13pp.

Qin, Ying-Ying, Xiao-Wen Zhang, Hong-Qiang Ren, Dao-Tang Li, and Hong Yang. 2008. "Population Dynamics of Ammonia-Oxidizing Bacteria in an Aerated Submerged Biofilm Reactor for Micropolluted Raw Water Pretreatment." *Applied Microbiology and Biotechnology* 79 (1): 135-145.

Ramette, Alban. 2007. "Multivariate Analyses in Microbial Ecology." *FEMS Microbiology Ecology* 62 (2): 142-160.

Richard, Y. Biological Methods for the Treatment of Groundwater. Oxidation Techniques in Drinking Water Treatment. (H. Sontheimer and W. Kuhn, editors). EPA 57019-79-020. U.S. EPA (1979).

Rittmann, Bruce E., Douglas Stilwell, Jason C. Garside, Gary L. Amy, Carl Spangenberg, Arseny Kalinsky, and Eric Akiyoshi. 2002. "Treatment of a Colored Groundwater by Ozone-Biofiltration: Pilot Studies and Modeling Interpretation." *Water Research* 36 (13): 3387-3397.

Rittmann, B. E. and V. L. Snoeyink. 1984. "Achieving Biologically Stable Drinking Water." *Journal / American Water Works Association* 76 (10): 106-114.

Rodriguez, Jacqueline. Sustainable Techniques Improve Water Quality, Lower Costs. American Water Works Association *Opflow*. Vol. 37, 8. Accessed 08/16/2011, 2011, http://www.awwa.org/publications/OpFlowArticle.cfm?itemnumber=57279.

Schreiber, I. M. and William A. Mitch. 2007. "Enhanced Nitrogenous Disinfection Byproduct Formation Near the Breakpoint: Implications for Nitrification Control." *Environmental Science and Technology* 41 (20): 7039-7046.

Sontheimer, H. Process Engineering Aspects in the Combination of Chemical and Biological Oxidation. Oxidation Techniques in Drinking Water Treatment. (H. Sontheimer and W. Kuhn, editors). EPA 57019-79-020. U.S. EPA (1979).

Stumm, W. & G.F. Lee (1961) "Oxygenation of Ferrous Iron", Industrial & Engineering Chemistry, 53(2)143-146.

[a] U.S. Environmental Protection Agency. *Community Water System Survey, 2006. Volume 1: Overview.* EPA/815/R-09/001. Washington, DC. Office of Water. http://water.epa.gov/infrastructure/drinkingwater/pws/cwssvr.cfm. 72p. 2009

[b] U.S. Environmental Protection Agency. *Community Water System Survey, 2006. Volume 2: Detailed Tables and Survey Methodology*. EPA/815/R-09/002. Washington, DC. Office of Water. http://water.epa.gov/infrastructure/drinkingwater/pws/cwssvr.cfm. 300p. 2009

U.S. Environmental Protection Agency. *Alternative Disinfectants and Oxidants Guidance Manual*. EPA 815-R-99-014. Washington DC. Office of Water. http://www.epa.gov/ogwdw/mdbp/alternative_disinfectants_guidance.pdf. 346p. 1999

U.S. Environmental Protcetion Agency**.** Fiscal Year 2010 Drinking Water and Ground Water Statistics, EPA 817K11001, 2011

U.S. Geological Survey. *The Quality of our Nation's Waters; Nutrients in the Nation's Streams and Groundwater, 1992-2004* 2010., edited by Neil M. Dubrovsky, Karen R. Burow, Gregory M. Clark, Jo Ann M. Gronberg, Pixie A. Hamilton, Kerie J. Hitt, David K. Mueller, et al U. S. Geological Survey, Reston, VA, United States.

White, Colin P., Ronald W. DeBry, and Darren A. Lytle. 2012. "Microbial Survey of a Full-Scale, Biologically Active Filter for Treatment of Drinking Water." *Applied and Environmental Microbiology* 78 (17): 6390-6394.

Wilczak, Andrzej, Joseph G. Jacangelo, Joseph P. Marcinko, Lee H. Odell, Gregory J. Kirmeyer, and Roy L. Wolfe. 1996. "Occurrence of Nitrification in Chloraminated Distribution Systems." *Journal / American Water Works Association* 88 (7): 12-12pp.

Wilczak, Andrzej, Linnea L. Hoover, and H. Hubert Lai. 2003. "Effects of Treatment Changes on Chloramine Demand and Decay." *Journal / American Water Works Association* 95 (7): 94-106+12.

Zhang, Yan and Marc Edwards. 2009. "Accelerated Chloramine Decay and Microbial Growth by Nitrification in Premise Plumbing." *Journal / American Water Works Association* 101 (11): 51-62.

Appendix A: Physical Parameters

Table 2: Head Loss Data.

Elapsed Time	Date	Head Loss (inches)	
		Sintered Glass	Gravel
0	1/12/2012	0	0
4	1/16/2012	0	0
7	1/19/2012	0	0
11	1/23/2012	0	0
14	1/26/2012	0.5	0.5
18	1/30/2012	0	0
21	2/2/2012	0	0
25	2/6/2012	0	0
28	2/9/2012	0	0
32	2/13/2012	0	0
35	2/16/2012	0	0
39	2/20/2012	0.5	0
42	2/23/2012	0.5	0.5
46	2/27/2012	0.5	0.5
49	3/1/2012	0.5	0.5
53	3/5/2012	0	0
56	3/8/2012	0.5	0.5
60	3/12/2012	0.5	0.5
63	3/15/2012	1	1
67	3/19/2012	0.5	0.5
70	3/22/2012	0.5	0.5
74	3/26/2012	0.5	0.5
77	3/29/2012	0.5	0.5
81	4/2/2012	0.5	0.5
86	4/7/2012	0.5	0.5
	Average:	0.3	0.3

Table 3: Backwash Schedule.

Date	Elapsed Time
1/12/2012	0
1/20/2012	8
1/27/2012	15
2/3/2012	22
2/11/2012	30
2/18/2012	37
2/25/2012	44
3/1/2012	49
3/10/2013	58
3/17/2013	65
3/30/2013	78

Appendix B: Analytical Data

Table 4: Ammonia Data.

ET	Inf-a	Inf-b	1S-a	1S-b	2S-a	2S-b	3S-a	3S-b	1G-a	1G-b	2G-a	2G-b	3G-a	3G-b
0	1.20	1.10	1.40	1.10	1.30	1.10	1.40	1.10	1.20	1.00	1.40	1.10	1.20	1.00
4	1.30	1.40	1.20	1.30	1.20	1.40	1.20	1.20	1.20	1.10	1.40	1.40	1.10	1.40
7	1.33	1.30	1.30	1.27	1.23	1.30	1.33	1.33	1.43	1.33	1.30	1.37	1.40	1.37
11	1.33	1.30	1.50	1.40	1.37	1.33	1.30	1.33	1.40	1.37	1.37	1.30	1.37	1.33
14	1.30	1.23	1.23	1.23	1.30	1.23	1.30	1.23	1.27	1.23	1.20	1.23	1.23	1.27
18	1.47	1.47	1.30	1.30	1.33	1.30	1.27	1.27	1.27	1.27	1.30	1.23	1.27	1.30
21	1.27	1.33	1.20	1.23	1.13	1.17	1.23	1.17	1.23	1.30	1.33	1.23	1.17	1.27
25	1.40	1.37	1.30	1.23	1.10	0.97	0.87	0.77	1.43	1.23	1.33	1.23	1.27	1.20
28	1.30	1.30	1.07	1.17	0.87	0.83	0.47	0.53	1.27	1.30	1.17	1.27	1.20	1.20
32	1.27	1.30	1.26	1.20	0.76	0.76	0.32	0.32	1.30	1.30	1.23	1.20	1.10	1.10
35	1.37	1.40	1.18	1.22	0.70	0.68	0.18	0.18	1.43	1.43	1.20	1.20	1.03	1.07
39	1.23	1.37	1.18	1.20	0.52	0.60	0.06	0.14	1.43	1.33	1.10	1.07	0.73	0.80
42	1.37	1.37	1.20	1.16	0.58	0.48	0.08	0.10	1.27	1.30	0.90	1.00	0.50	0.60
46	1.30	1.20	1.00	0.94	0.54	0.40	0.08	0.04	1.27	1.10	0.77	0.67	0.44	0.54
49	1.30	1.30	0.87	0.87	0.36	0.38	0.06	0.05	1.10	1.10	0.64	0.66	0.22	0.23
53	1.37	1.33	0.97	1.00	0.42	0.46	0.07	0.08	1.17	1.10	0.64	0.64	0.23	0.23
56	1.40	1.33	1.00	1.00	0.38	0.38	0.04	0.04	1.13	1.17	0.62	0.60	0.17	0.17
60	1.23	1.20	0.90	0.90	0.22	0.26	0.02	0.01	0.97	0.93	0.48	0.46	0.11	0.11
63	1.30	1.30	0.93	0.93	0.24	0.18	0.02	0.02	1.00	0.97	0.50	0.48	0.13	0.12
67	1.33	1.33	0.90	0.87	0.26	0.26	0.04	0.04	0.97	1.03	0.48	0.48	0.14	0.14
70	1.40	1.37	1.17	1.13	0.32	0.30	0.05	0.05	1.07	1.03	0.46	0.46	0.13	0.13
74	1.37	1.37	0.93	0.87	0.24	0.24	0.03	0.03	0.83	0.87	0.36	0.34	0.08	0.08
77	1.47	1.30	0.97	1.03	0.22	0.22	0.01	0.03	0.67	0.67	0.24	0.22	0.05	0.06
81	1.33	1.33	0.97	1.03	0.26	0.24	0.02	0.02	0.80	0.77	0.20	0.22	0.06	0.06
86	1.57	1.43	0.98	1.08	0.27	0.27	0.04	0.04	0.76	0.74	0.23	0.26	0.08	0.08

Table 5: Nitrite Data.

ET	Inf-a	Inf-b	1S-a	1S-b	2S-a	2S-b	3S-a	3S-b	1G-a	1G-b	2G-a	2G-b	3G-a	3G-b
0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
4	0.0000	0.0004	0.0000	0.0000	0.0000	0.0000	0.0000	0.0190	0.0012	0.0910	0.0000	0.0000	0.0000	0.0000
7	0.0019	0.0000	0.0000	0.0000	0.0000	0.0000	0.0019	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
11	0.0002	0.0017	0.0000	0.0000	0.0000	0.0000	0.0004	0.0033	0.0000	0.0000	0.0000	0.0000	0.0025	0.0000
14	0.0094	0.0012	0.0100	0.0084	0.0135	0.0063	0.0119	0.0106	0.0100	0.0317	0.0458	0.0335	0.0404	0.0222
18	0.0204	0.0222	0.0171	0.0242	0.0187	0.0208	0.0397	0.0407	0.0190	0.0336	0.0334	0.0189	0.0215	0.0173
21	0.0125	0.0157	0.0208	0.0106	0.0625	0.0600	0.1108	0.1108	0.0143	0.0290	0.0190	0.0150	0.0383	0.0146
25	0.0233	0.0161	0.0552	0.0548	0.2143	0.2028	0.3360	0.3719	0.0295	0.0212	0.0253	0.0193	0.0172	0.0180
28	0.0146	0.0165	0.0821	0.0815	0.2931	0.2935	0.5704	0.5652	0.0367	0.0124	0.0170	0.0581	0.0360	0.0227
32	0.0137	0.0139	0.0320	0.0364	0.2780	0.2828	0.4946	0.5180	0.0123	0.0128	0.0412	0.0433	0.0951	0.0946
35	0.0133	0.0136	0.0544	0.0492	0.2814	0.3112	0.5460	0.5436	0.0140	0.0138	0.0930	0.0953	0.1969	0.2002
39	0.0037	0.0013	0.0460	0.0477	0.3833	0.3863	0.4340	0.4330	0.0077	0.0113	0.1527	0.1410	0.3253	0.3330
42	0.0004	0.0000	0.0718	0.0656	0.3594	0.3478	0.3208	0.3270	0.0434	0.0434	0.2622	0.2576	0.5988	0.5954
46	0.0181	0.0122	0.1763	0.1713	0.2760	0.2870	0.3130	0.3215	0.0570	0.0618	0.4246	0.4242	0.7128	0.7010
49	0.0251	0.0266	0.2002	0.2088	0.1142	0.2376	0.1700	0.1730	0.0998	0.1056	0.4344	0.4416	0.7154	0.7194
53	0.0000	0.0000	0.1344	0.1278	0.1564	0.1558	0.0992	0.1072	0.0642	0.0644	0.3034	0.3158	0.5896	0.5954
56	0.0127	0.0102	0.1324	0.1336	0.0962	0.0994	0.0278	0.0278	0.1242	0.1262	0.3020	0.3072	0.4518	0.4566
60	0.0023	0.0000	0.0505	0.0525	0.0375	0.0393	0.0066	0.0072	0.1613	0.1593	0.2244	0.2334	0.2736	0.2804
63	0.0000	0.0036	0.0093	0.0093	0.0086	0.0080	0.0028	0.0031	0.1544	0.1577	0.1446	0.1510	0.0539	0.0538
67	0.0004	0.0026	0.0000	0.0000	0.0026	0.0027	0.0033	0.0033	0.1076	0.1057	0.0412	0.0398	0.0111	0.0113
70	0.0000	0.0020	0.0000	0.0013	0.0071	0.0103	0.0034	0.0033	0.0553	0.0557	0.0450	0.0502	0.0132	0.0206
74	0.0000	0.0014	0.0000	0.0000	0.0032	0.0054	0.0000	0.0000	0.0244	0.0241	0.0129	0.0135	0.0022	0.0042
77	0.0008	0.0000	0.0000	0.0008	0.0029	0.0004	0.0000	0.0000	0.0052	0.0088	0.0035	0.0034	0.0027	0.0015
81	0.0008	0.0036	0.0000	0.0000	0.0057	0.0030	0.0000	0.0000	0.0006	0.0029	0.0015	0.0018	0.0046	0.0014
86	0.0015	0.0000	0.0000	0.0000	0.0012	0.0000	0.0015	0.0010	0.0000	0.0014	0.0007	0.0007	0.0007	0.0034

Table 6: Combined Nitrite and Nitrate Data.

ET	Inf-a	Inf-b	1S-a	1S-b	2S-a	2S-b	3S-a	3S-b	1G-a	1G-b	2G-a	2G-b	3G-a	3G-b
0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.2	0.1	0.0
4	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.2	0.0	0.2	0.2	0.2	0.2	0.2
7	0.2	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
11	0.2	0.3	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.2
14	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.3	0.0	0.1
18	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25	0.3	0.3	0.4	0.5	0.9	0.9	1.3	1.3	0.0	0.2	0.2	0.2	0.3	0.3
28	0.4	0.2	0.7	0.7	0.8	0.9	1.4	1.8	0.3	0.3	0.5	0.4	0.1	0.3
32	0.3	0.3	0.5	0.3	1.1	1.1	1.7	1.5	0.5	0.4	0.3	0.4	0.6	0.5
35	0.1	0.4	0.3	0.3	1.2	1.2	1.6	1.6	0.3	0.1	0.5	0.3	0.8	0.9
39	0.2	0.1	0.4	0.4	1.3	1.4	1.4	1.5	0.1	0.2	0.4	0.4	1.0	1.0
42	0.3	0.2	0.4	0.5	1.1	1.0	1.8	1.5	0.3	0.2	0.9	0.8	1.4	1.6
46	0.4	0.3	0.8	0.8	1.0	1.0	1.2	1.2	0.5	0.4	1.1	1.0	1.6	1.7
49	0.4	0.5	0.8	0.9	1.1	1.0	1.4	1.4	0.5	0.5	1.2	1.2	1.8	1.6
53	0.4	0.3	0.5	0.5	1.0	1.0	1.2	1.1	0.3	0.3	1.1	0.9	1.5	1.5
56	0.4	0.4	0.7	0.7	0.8	0.9	0.9	0.8	0.7	0.6	1.0	1.1	1.4	1.4
60	0.4	0.3	0.5	0.5	0.6	0.7	0.5	0.7	0.7	0.7	0.9	0.9	1.1	1.0
63	0.4	0.5	0.4	0.5	0.6	0.7	0.7	0.6	0.8	0.8	0.9	0.9	0.9	0.9
67	0.6	0.6	0.7	0.5	1.0	0.9	0.9	1.1	0.9	0.9	1.2	0.9	1.1	0.9
70	0.5	0.4	0.7	0.5	0.9	0.9	0.9	0.8	0.7	0.7	0.9	0.9	1.0	0.8
74	0.8	0.9	0.7	0.7	1.4	1.0	1.1	1.0	0.9	0.8	1.1	0.9	1.1	0.9
77	0.9	0.8	0.8	0.9	1.1	1.1	1.5	1.4	1.2	1.1	1.3	1.2	1.4	1.2
81	0.9	0.8	1.0	0.9	1.3	1.3	1.4	1.3	1.1	0.9	1.4	1.3	1.3	1.1
86	0.7	0.8	0.9	0.8	1.3	1.1	1.3	1.2	1.0	0.9	1.2	1.1	1.3	1.4

Table 7: Ferrous Iron Data.

ET	Inf-a	Inf-b	1S-a	1S-b	2S-a	2S-b	3S-a	3S-b	1G-a	1G-b	2G-a	2G-b	3G-a	3G-b
0	1.950	1.226	0.449	0.065	0.163	0.041	0.123	0.000	0.643	0.378	0.128	0.039	0.034	0.018
4	1.372	1.360	0.294	0.379	0.034	0.049	0.026	0.011	0.396	0.460	0.101	0.121	0.022	0.042
7	1.330	1.262	0.434	0.437	0.031	0.058	0.000	0.000	0.351	0.472	0.105	0.151	0.003	0.004
11	1.181	1.218	0.078	0.080	0.000	0.008	0.000	0.000	0.433	0.444	0.094	0.112	0.000	0.000
14	1.307	1.255	0.109	0.090	0.018	0.063	0.000	0.000	0.216	0.187	0.017	0.045	0.000	0.000
18	1.166	1.382	0.270	0.271	0.051	0.057	0.043	0.013	0.282	0.263	0.092	0.090	0.048	0.036
21	1.157	1.232	0.038	0.055	0.000	0.000	0.000	0.000	0.182	0.166	0.023	0.011	0.003	0.000
25	1.362	1.417	0.131	0.091	0.068	0.066	0.000	0.010	0.437	0.386	0.066	0.028	0.047	0.067
28	1.346	1.364	0.166	0.147	0.062	0.061	0.016	0.029	0.363	0.354	0.076	0.071	0.020	0.053
32	1.402	1.325	0.427	0.422	0.127	0.151	0.000	0.000	0.401	0.347	0.085	0.065	0.033	0.013
35	1.304	1.338	0.343	0.355	0.100	0.101	0.000	0.000	0.386	0.381	0.082	0.104	0.054	0.018
39	1.278	1.268	0.300	0.295	0.044	0.015	0.016	0.017	0.380	0.382	0.080	0.069	0.008	0.037
42	1.245	1.327	0.312	0.287	0.054	0.061	0.000	0.000	0.488	0.441	0.162	0.152	0.016	0.021
46	1.255	1.248	0.295	0.300	0.084	0.070	0.040	0.030	0.525	0.493	0.073	0.063	0.051	0.020
49	1.375	1.240	0.180	0.164	0.104	0.106	0.000	0.025	0.240	0.220	0.102	0.055	0.040	0.044
53	1.211	1.264	0.515	0.523	0.170	0.117	0.039	0.029	0.604	0.634	0.251	0.279	0.047	0.045
56	1.382	1.361	0.408	0.412	0.047	0.046	0.000	0.039	0.384	0.368	0.110	0.084	0.021	0.021
60	1.414	1.495	0.391	0.366	0.085	0.050	0.008	0.051	0.424	0.405	0.135	0.131	0.010	0.039
63	1.039	1.273	0.253	0.212	0.000	0.000	0.000	0.000	0.275	0.266	0.053	0.072	0.039	0.001
67	1.429	1.346	0.372	0.373	0.050	0.017	0.000	0.000	0.333	0.347	0.068	0.052	0.017	0.014
70	1.177	1.042	0.654	0.658	0.084	0.097	0.009	0.009	0.432	0.429	0.065	0.084	0.000	0.000
74	1.450	1.436	0.372	0.346	0.068	0.085	0.000	0.000	0.219	0.213	0.031	0.030	0.040	0.010
77	1.355	1.327	0.517	0.455	0.085	0.073	0.015	0.014	0.195	0.176	0.029	0.062	0.018	0.026
81	1.207	1.267	0.486	0.445	0.140	0.104	0.022	0.060	0.221	0.242	0.035	0.038	0.006	0.047
86	1.263	1.264	0.537	0.561	0.157	0.182	0.013	0.000	0.263	0.296	0.117	0.155	0.004	0.037

Table 8: Total Iron Data.

ET	Inf-a	Inf-b	1S-a	1S-b	2S-a	2S-b	3S-a	3S-b	1G-a	1G-b	2G-a	2G-b	3G-a	3G-b
0	2.644	2.137	2.277	1.757	1.906	0.921	1.812	0.556	2.244	1.798	1.345	1.418	0.856	0.762
4	1.383	1.350	1.042	1.075	1.027	1.037	0.425	0.453	1.040	1.052	0.711	0.755	0.734	0.712
7	1.398	1.300	0.970	0.994	0.631	0.647	0.444	0.445	0.987	1.055	0.686	0.722	0.914	0.897
11	1.361	1.271	0.959	0.975	0.822	0.782	0.540	0.545	0.942	0.976	0.655	0.636	0.503	0.491
14	1.370	1.363	1.030	1.098	1.653	1.621	0.776	0.801	1.009	1.001	0.938	0.941	0.750	0.774
18	1.382	1.308	1.028	1.029	1.399	1.437	0.628	0.609	0.970	1.037	0.792	0.804	0.642	0.608
21	1.338	1.334	0.984	1.073	1.399	1.378	0.878	0.912	0.999	0.969	0.872	0.867	0.952	0.988
25	1.450	1.600	1.172	1.129	1.190	1.205	1.676	1.702	1.214	1.198	1.327	1.370	1.172	1.164
28	1.405	1.375	1.091	1.087	1.260	1.274	1.020	0.983	1.087	1.150	0.933	0.918	0.956	0.967
32	1.410	1.454	1.114	1.071	1.506	1.545	0.772	0.771	1.076	1.062	1.027	1.030	0.954	0.947
35	1.470	1.380	1.035	1.026	1.260	1.241	0.689	0.684	1.023	1.045	1.273	1.220	1.096	1.080
39	1.326	1.313	1.090	1.038	0.969	0.953	0.779	0.749	0.998	0.104	0.892	0.881	0.869	0.850
42	1.336	1.396	1.107	1.078	1.034	1.042	0.710	0.732	1.108	1.071	0.921	0.947	1.320	1.421
46	1.326	1.330	1.079	1.071	1.110	0.985	1.517	0.834	1.189	1.106	0.917	0.829	0.701	0.717
49	1.338	1.322	1.073	1.119	1.358	1.359	0.976	1.193	1.082	1.169	1.006	1.093	1.225	1.252
53	1.253	1.200	0.957	1.004	1.367	1.399	0.704	0.740	1.100	1.044	1.070	1.061	1.222	1.262
56	1.377	1.259	1.225	1.261	0.988	0.972	0.596	0.638	0.765	0.774	0.509	0.555	0.470	0.478
60	1.361	1.399	1.490	1.485	1.102	1.182	0.677	0.745	1.135	1.067	0.708	0.656	0.976	1.020
63	1.286	1.337	3.291	3.203	2.632	2.639	1.046	1.087	1.467	1.628	1.128	1.756	1.061	1.064
67	1.447	1.453	1.403	1.401	1.052	1.082	0.622	0.644	0.884	0.911	0.789	0.700	0.764	0.759
70	1.201	1.192	1.519	1.535	0.519	0.498	0.326	0.312	1.025	1.012	0.518	0.517	0.396	0.386
74	1.467	1.428	4.156	4.046	1.514	1.577	1.086	1.085	1.031	1.391	0.962	0.938	1.379	1.411
77	1.330	1.261	1.799	1.853	1.170	1.184	0.585	0.546	1.126	1.212	0.813	0.835	0.870	0.849
81	1.300	1.254	3.866	3.726	0.708	0.810	0.808	0.809	0.841	0.802	0.885	0.990	0.413	0.719
86	1.304	1.298	3.444	3.054	1.969	1.867	0.802	0.755	0.993	1.141	0.642	0.632	0.541	0.493

Table 9: pH Data.

ET	Inf-a	Inf-b	1S-a	1S-b	2S-a	2S-b	3S-a	3S-b	1G-a	1G-b	2G-a	2G-b	3G-a	3G-b
0	7.41	7.42	7.60	7.64	7.80	7.82	8.05	8.09	7.68	7.72	7.87	7.88	7.99	8.07
4	7.34	7.35	7.55	7.53	7.75	7.75	7.97	7.96	7.63	7.60	7.76	7.80	7.89	7.92
7	7.45	7.49	7.61	7.62	7.80	7.79	7.98	8.03	7.62	7.64	7.76	7.83	7.96	7.96
11	7.43	7.43	7.69	7.68	7.80	7.82	8.03	8.02	7.61	7.61	7.72	7.75	7.91	7.91
14	7.42	7.37	7.72	7.67	7.86	7.86	8.05	8.02	7.71	7.70	7.95	7.90	8.05	8.06
18	7.46	7.52	7.65	7.69	7.84	7.84	8.06	8.06	7.68	7.74	7.81	7.85	8.00	8.00
21	7.34	7.36	7.57	7.66	7.78	7.83	8.01	8.01	7.60	7.63	7.83	7.84	8.02	8.01
25	7.50	7.46	7.63	7.68	7.79	7.78	7.97	7.98	7.67	7.63	7.79	7.80	7.99	8.00
28	7.34	7.34	7.59	7.61	7.71	7.72	7.87	7.92	7.59	7.61	7.77	7.77	7.97	7.98
32	7.42	7.42	7.62	7.63	7.74	7.75	7.94	7.96	7.61	7.61	7.77	7.78	7.98	8.01
35	7.45	7.44	7.61	7.62	7.63	7.68	7.91	7.91	7.59	7.61	7.78	7.78	7.99	8.01
39	7.38	7.41	7.63	7.64	7.79	7.78	7.99	8.01	7.62	7.67	7.80	7.81	8.06	8.06
42	7.42	7.43	7.62	7.62	7.73	7.74	7.94	7.92	7.60	7.62	7.74	7.75	7.95	7.94
46	7.53	7.50	7.62	7.65	7.73	7.73	7.94	7.95	7.64	7.65	7.75	7.75	7.98	7.96
49	7.46	7.48	7.75	7.74	7.86	7.85	8.02	8.03	7.68	7.70	7.86	7.85	8.01	8.01
53	7.42	7.36	7.51	7.52	7.61	7.62	7.80	7.81	7.53	7.52	7.64	7.65	7.85	7.84
56	7.38	7.37	7.57	7.62	7.77	7.77	7.91	7.94	7.60	7.61	7.62	7.76	7.97	7.98
60	7.51	7.53	7.74	7.74	7.89	7.91	8.12	8.12	7.73	7.72	7.88	7.88	8.10	8.12
63	7.64	7.64	7.64	7.67	7.83	7.87	8.05	8.07	7.66	7.68	7.81	7.83	8.04	8.04
67	7.55	7.54	7.56	7.58	7.71	7.79	7.92	7.94	7.57	7.58	7.72	7.75	7.94	7.94
70	7.45	7.42	7.52	7.56	7.76	7.73	8.00	7.97	7.55	7.58	7.75	7.76	8.01	8.01
74	7.35	7.37	7.45	7.47	7.61	7.72	7.86	7.84	7.60	7.62	7.69	7.71	7.87	7.86
77	7.46	7.43	7.55	7.64	7.80	7.81	8.01	8.05	7.65	7.68	7.83	7.83	8.06	8.05
81	7.32	7.37	7.59	7.59	7.71	7.73	7.95	7.95	7.61	7.62	7.75	7.76	7.99	7.99
86	7.46	7.52	7.62	7.66	7.77	7.78	8.00	8.01	7.71	7.70	7.80	7.81	8.04	8.05

Table 10: Dissolved Oxygen Data.

ET	Inf-a	Inf-b	1S-a	1S-b	2S-a	2S-b	3S-a	3S-b	1G-a	1G-b	2G-a	2G-b	3G-a	3G-b
21	2.26	2.41	7.40	7.54	8.02	8.42	8.97	9.30	7.08	7.55	8.42	8.54	9.33	9.39
25	1.46	1.37	7.67	7.54	7.75	8.12	8.78	8.94	7.12	6.96	8.23	8.22	9.21	9.22
28	1.78	1.75	6.58	6.92	7.77	7.85	8.53	9.62	6.53	6.41	7.51	7.80	9.10	9.14
32	0.86	0.75	4.86	5.05	7.02	7.09	8.33	8.40	6.34	6.02	7.94	7.91	9.02	9.15
35	0.71	0.66	6.71	6.69	7.69	7.77	9.02	9.12	6.96	6.91	8.35	8.41	9.66	9.70
39	0.73	0.73	6.39	6.34	7.26	7.29	8.48	8.55	6.88	6.72	7.82	7.93	9.07	9.19
42	0.69	0.65	5.31	5.54	7.73	7.61	8.04	8.19	6.52	6.39	7.49	7.54	8.85	8.90
46	1.68	1.64	7.62	7.60	8.04	8.09	9.04	9.20	7.79	7.58	8.32	8.47	9.09	9.12
49			7.88	7.82	8.15	8.19	8.94	8.99	7.77	7.69	8.31	8.23	8.91	8.92
53			5.69	5.61	6.29	6.34	8.06	8.19	5.69	5.50	6.93	7.04	8.49	8.73
56	1.31	1.26	6.78	6.73	7.45	7.64	9.04	8.98	6.22	6.23	7.94	7.99	8.88	8.96
60	0.95	0.90	6.34	6.36	7.57	7.61	8.48	8.51	6.33	6.24	7.22	7.28	8.48	8.50
63	0.48	0.51	6.98	6.87	7.79	7.87	8.75	8.80	6.85	6.60	7.55	7.61	8.68	8.73
67	0.65	0.63	6.21	6.11	7.20	7.30	8.45	8.56	6.37	6.32	7.34	7.43	8.60	8.66
70	0.72	0.74	6.13	6.00	7.26	7.40	8.92	8.99	6.67	6.57	7.77	7.84	9.00	9.03
74	0.59	0.55	6.43	6.29	7.16	7.24	8.51	8.63	7.32	7.18	7.66	7.72	8.62	8.67
77	0.66	0.49	6.12	6.13	7.56	7.73	8.99	9.08	7.32	7.29	8.07	8.14	9.25	9.27
81	0.64	0.62	6.16	5.98	6.70	6.75	8.18	8.23	7.11	7.04	7.55	7.63	8.67	8.63
86			5.88	5.90	6.38	6.40	8.16	8.22	6.74	6.66	7.23	7.46	8.56	8.60

Table 11: Total Organic Carbon Data.

ET	I	1S	2S	3S	1G	2G	3G
0	2.55	2.004	1.915	1.969	1.959	1.972	2.017
4	2.188	1.923	1.871	1.951	1.983	2.136	1.895
7	1.802	2.131	2.129	1.944	1.68	1.904	2.048
11	2.257	1.875	1.993	2.029	1.964	2.336	2.259
14	2.287	1.972	1.918	2.022	1.955	1.997	2.204
18	1.859	1.794	1.71	1.756	1.79	1.835	1.813
21	1.817	1.702	1.96	1.611	1.932	1.672	1.809
25	1.926	2.029	1.733	1.825	2.011	2.374	1.833
28	1.931	1.75	1.755	1.7	1.826	1.807	1.803
32	1.973	1.877	2.209	1.948	1.675	2.288	1.926
35	1.597	1.55	1.494	1.574	1.537	1.507	1.527
39	1.507	1.532	1.589	1.667	1.617	1.552	1.579
42	1.62	1.697	1.886	1.721	1.936	1.689	1.815
46	1.73	1.612	1.764	1.664	1.606	1.667	1.656
49	1.651	1.8	1.83	1.89	1.771	1.886	1.917
53	2.17	1.704	1.919	1.699	2.003	1.743	0.772
56	1.657	1.713	1.741	1.68	1.744	1.793	1.756
60	1.785	1.905	1.829	1.881	1.828	1.881	1.94
77	1.935	2.289	1.617	0.7523	1.505	1.189	1.219
81	0.6748	0.6601	0.6042	0.7126	0.6553	0.6724	0.5987
86	1.71	1.607	1.606	1.694	1.704	1.76	1.571

Table 12: Groundwater Temperature Data.

ET	^{0}C
0	22
4	21
7	22
11	21
14	23
18	22
21	23
25	23.5
28	23.5
32	23.5
35	21
39	20
42	21
46	20
49	21.5
56	21
60	24
63	22
67	22
70	22
74	22
77	22
81	21
86	22

Table 13: Alkalinity Data.

ET	Inf-a	Inf-b	1S-a	1S-b	2S-a	2S-b	3S-a	3S-b	1G-a	1G-b	2G-a	2G-b	3G-a	3G-b
7	380	380	380	380	380	380	370	362	362	362	362	362	362	369
11	354	356	352	356	354	354	354	354	356	354	356	352	354	354
14	338	340	336	334	336	340	338	340	338	340	336	340	340	338
18	334	334	334	334	334	336	332	334	334	334	334	334	334	336
21	330	332	330	328	330	330	330	328	328	326	328	328	328	328
25	330	330	330	328	328	328	326	326	332	328	328	330	328	330
28	328	330	328	328	324	326	324	320	328	328	328	328	326	326
32	328	328	330	328	326	326	320	324	328	328	324	326	326	330
35	326	326	328	326	322	322	322	322	326	326	326	326	326	326
39	328	330	328	330	324	322	320	320	330	328	326	326	326	326
42	336	328	326	326	320	320	316	316	328	326	328	322	320	322
46	326	326	320	324	320	320	318	314	324	326	320	320	320	318
49	330	328	324	322	320	320	316	316	324	326	324	320	320	320
53	326	326	326	326	322	322	314	316	326	326	320	322	320	318
56	326	328	326	322	318	320	316	316	322	326	320	320	318	316
60	328	328	326	324	320	320	316	314	322	326	322	320	316	316
63	328	328	324	320	316	318	316	318	324	322	316	316	320	318
67	328	330	324	322	320	318	316	316	326	324	320	318	316	318
70	326	330	322	322	318	318	318	316	324	322	320	322	316	316
74	328	326	322	324	320	318	314	316	322	324	320	318	316	314
77	328	326	322	324	320	316	314	314	320	318	316	316	316	314
81	326	326	322	320	318	314	312	314	320	318	316	318	316	316
86	326	326	322	322	318	318	314	316	320	320	320	318	314	316