1998

A methodology for studying denitrification by recently formed sulfides

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A METHODOLOGY FOR STUDYING DENITRIFICATION BY RECENTLY FORMED SULFIDES

by

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Bachelor of Science, Bridgewater State College, 1995

A Thesis

Submitted to the Graduate Faculty

of the

University of North Dakota

in partial fulfillment of the requirements

for the degree of

Master of Science

Grand Forks, North Dakota
December
1998

This thesis, submitted by Bethany A. Bolles in partial fulfillment of the requirements for the Degree of Master of Science from the University of North Dakota, has been read by the Faculty Advisory Committee under whom the work has been done and is hereby approved.

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Department Geology and Geological Engineering

Degree Master of Science

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ACKNOWLEDGMENTS

I would like to express my sincere appreciation to several people who helped me throughout this project. First and foremost, I would like to thank my advisor, Scott Korom, for being an exceptional educator, advisor, and role model. Scott's determination and enthusiasm for education and research is unparalleled. I am also grateful to my other committee members, Phil Gerla and Ron Matheney, for contributing many valuable comments and suggestions.

I would also like to thank Charlene Crocker, at the Energy and Environmental Research Center (EERC), for the help in the Water Quality Lab. Allen Schlag also deserves sincere thanks for the many hours he spent building equipment for my project. I would like to express my appreciation to John Wagner for performing X-ray analyses of the site sediment. Geochron Laboratories, and Marshall Otter in particular, were very generous with their help and support involving the isotope analyses. I am very thankful to Gary Debuque for a great job constructing my column. Also, without the help and advice of Gloria Pederson, I would never have gotten things accomplished.

I am also very grateful for the support and encouragement of my best friend, Chad Tomforde, with whom I share many unforgettable experiences. Last, but not least, I would like to thank my mom and dad for shaping me into the person that I have become, and for teaching me how to keep an open mind through life's experiences.

ABSTRACT

Nitrate (NO_3^-) contamination due to anthropogenic sources is a serious and widespread health problem. In many groundwater systems, NO_3^- can be reduced to a harmless gas, N_2, through the bacterially mediated process of denitrification. During this process, bacteria gain energy by transferring electrons from an "electron donor" to nitrate, the "electron acceptor". It is typically believed that organic carbon is the most important electron donor in the denitrification process; however, increasing amounts of research suggest the importance of inorganic electron donors. The most extensive studies involving the reduction of NO_3^- by inorganic species have focused on pyrite (FeS_2) and Fe(II). Few studies have considered the possibility of denitrification by recently formed sulfide species, such as hydrogen sulfide (H_2S), iron monosulfide (FeS), mackinawite (FeS), and greigite (Fe_3S_4). The objective of this research was to design and begin implementing a methodology for investigating the effects of recently formed sulfides on denitrification.

In order to begin investigating the denitrification potential of recently formed sulfides, a 4-foot high, 6-inch diameter Plexiglas column was constructed, with tubing ports on the top and bottom. The column was filled with sediment and groundwater collected from a field site in Fertile, MN. Groundwater from the field site was spiked with organic carbon (glucose) and sulfate. The spiked water was injected into the column, until two pore-volumes of the original column water were flushed out. The

spiked water was left in the column for several months, during which the concentrations of several groundwater species were monitored, including: total organic carbon (TOC), total inorganic carbon (TIC), dissolved oxygen (DO), reduced iron (Fe^{2+}), reduced manganese (Mn^{2+}), nitrate (NO_3^-), sulfate (SO_4^{2-}), and pH.

The results of the laboratory study revealed that water in the column was reducing according to the thermodynamic model of reduction sequences. The dissolved oxygen concentrations decreased from 8.4 mg/L to 0.8 mg/L. Once concentrations of oxygen became limiting, dissolved manganese and then dissolved iron concentrations increased as the manganese and iron minerals in the sediment were reduced. Dissolved manganese concentrations rose from 2.07 mg/L to 4.40 mg/L, and dissolved iron concentrations increased from < 0.03 mg/L to 2.56 mg/L. Concentrations of total inorganic carbon increased concurrently with the oxidation of organic carbon and the reduction of oxygen, manganese, and iron. The next step in the sequence, sulfate reduction, was not observed during the course of this study. Once enough sulfate is reduced to form sufficient reserves of sulfides, the next step of the methodology is to add nitrate to the column. The expectation is that the sulfides will reduce the nitrate and be oxidized to sulfate.

CHAPTER I

INTRODUCTION

Purpose

Nitrate (NO_3^-) contamination due to anthropogenic sources is a serious and widespread problem. Infants are particularly susceptible to nitrate contaminated water, which, if ingested in high enough concentrations, results in the fatal condition methemoglobinemia or "blue baby" (Comly, 1945). In many aquifers, however, nitrate can be reduced to harmless nitrogen gas through the bacterially mediated process of denitrification. During this process, bacteria gain energy by transferring electrons from an "electron donor" to an "electron acceptor," in this case, nitrate. Denitrification is the only effective way for nitrate to be removed in-situ from aquifer systems (Postma *et al.*, 1991; Korom, 1992).

The types of electron donors used by bacteria fall into two categories: organic and inorganic. Most research has focused on organic carbon as the major electron donor in the denitrification process (Trudell *et al.*, 1986; Morris *et al.*, 1988; Smith and Duff, 1988; Starr and Gillham, 1989). However, several studies have shown that nitrate may be reduced by inorganic compounds such as sulfide and reduced iron (Fe^{2+}) (Korom, 1992 and references therein). Of those who have studied denitrification by sulfide minerals, none have determined whether the sulfides were formed recently (on the order

of 10^0 - 10^1 years) or over a long period of time (e.g. 10^2 years or more). This study presents a methodology for studying denitrification by recently formed sulfide species.

The first step of the methodology is to form reduced sulfide compounds in a sediment column through the process of sulfate reduction by organic carbon. The next step is to add nitrate to the sediment column once sulfate reduction has progressed long enough to form sufficient quantities of sulfide species. The goal of the methodology is to demonstrate that nitrate can be reduced by the oxidation of recently formed sulfides. A laboratory study was conducted to begin implementing the steps determined by the methodology. The continuation of the experiment should show whether denitrification by recently formed sulfides is an important process that needs to be investigated in greater detail.

Denitrification

Nitrate can be reduced by both organic and inorganic electron donors. Denitrification by organic carbon oxidation is the most commonly recognized way in which nitrate is reduced. During this process, bacteria use energy released from the transfer of electrons from organic carbon to nitrate, which serves as the electron acceptor. The reduction of nitrate follows a pathway of nitric oxide intermediates (Firestone, 1982; Payne, 1981). This sequence begins with nitrate and ends with nitrogen gas, as shown below:

$$NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$$

The overall reaction involving nitrate reduction by organic carbon with nitrogen gas as the end product can be expressed as:

$$CH_2O + 4/5\ NO_3^- + 4/5\ H^+ \rightarrow CO_2 + 2/5\ N_2 + 7/5\ H_2O \quad (1)$$

In this reaction, CH_2O represents a generic form of organic carbon. However, there are numerous organic compounds in nature (Thurman, 1985), and various oxidation states for carbon (Stumm and Morgan, 1981). For further information on denitrification by organic carbon, see the review by Korom (1992).

The reduction of nitrate is only one in a series of reactions that can take place during the oxidation of organic carbon in groundwater. For example, several inorganic species may serve as electron acceptors during organic carbon oxidation (Figure 1). The interaction of these inorganic species and organic carbon follows a distinct sequence (Stumm and Morgan, 1981) determined by the energy yield of the reaction. The first reaction in the sequence, and the one that releases the most energy to bacteria, is the transfer of electrons from organic carbon to dissolved oxygen (DO). Once the reserves of oxygen become limiting, the bacteria begin to use nitrate as an electron acceptor, then manganese(IV), then iron(III), and then sulfate. Depending on the availability and concentration of electron acceptors in the system, the result of organic carbon oxidation in groundwater is the formation of several reduced species. As long as supplies of organic carbon are non-limiting, NO_3^-, Mn(IV), Fe(III), and SO_4^{2-} present in the groundwater or aquifer sediment may be reduced to N_2, Mn(II), Fe(II), and HS^-, respectively (Figure 1).

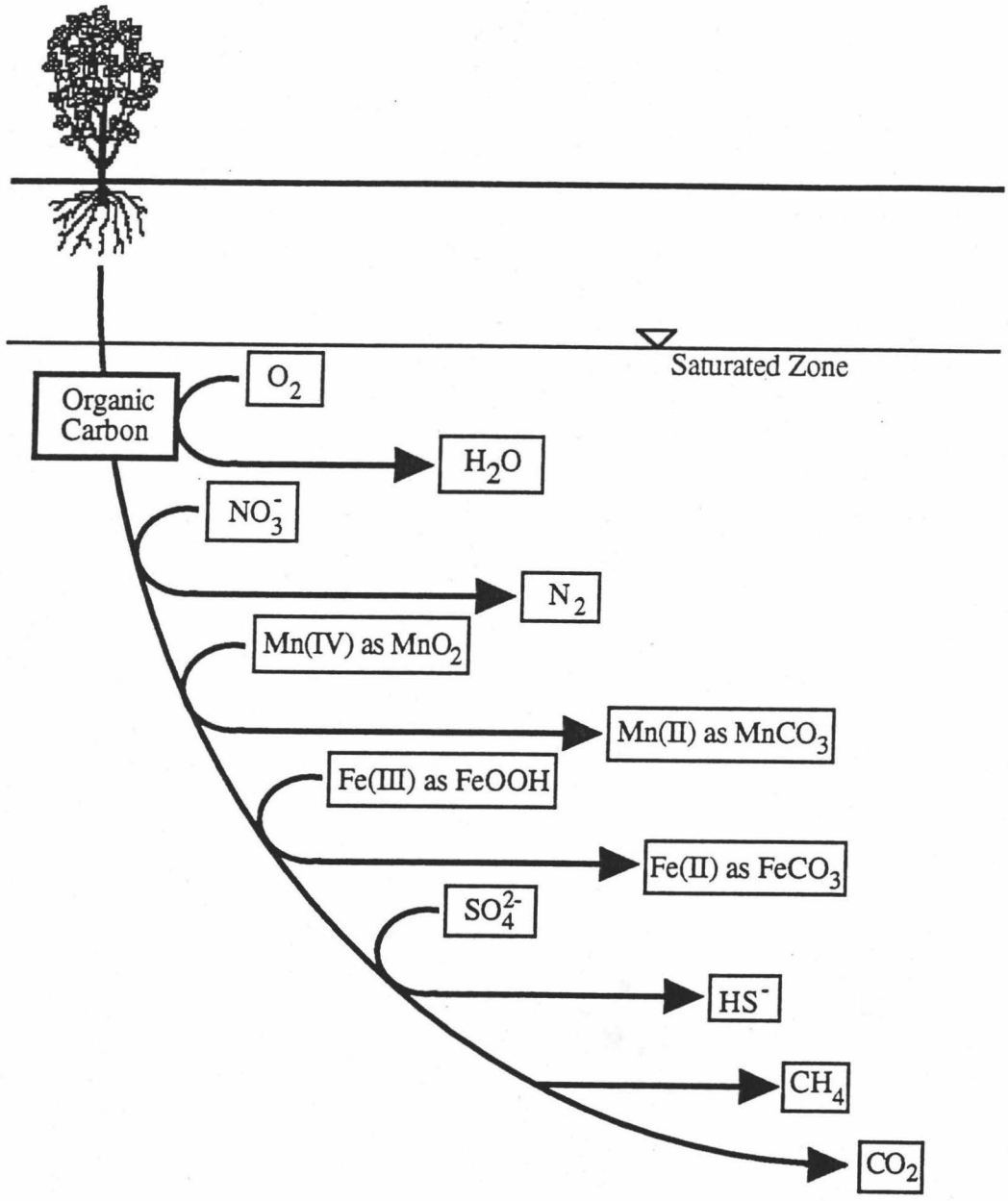


Figure 1. The oxidation of organic carbon in the saturated zone with the sequence of electron acceptors and the resulting reduced inorganic compounds (from Korom, 1992).

The reduced inorganic compounds present within groundwater and sediments store the electron potential originally present within the organic carbon. The lower the species in the reduction sequence (Figure 1), the greater the stored potential. Thus, any reduced inorganic compounds having a potential more negative than nitrate are capable of supplying electrons necessary for denitrification (Lind, 1977). In many cases, what is possible from a thermodynamic point of view is not always observed. However, in this case, several studies support the model discussed above (Buresh and Moraghan, 1976; Gouy *et al.*, 1984; Koelle *et al.*, 1985; Postma, 1985; Aller and Rude, 1988; Postma, 1990; Postma *et al.*, 1991; Van Cappellen and Wang, 1996 and references therein).

Of all the potential inorganic electron donors for denitrification, sulfides have been researched most extensively. By weight, sulfides are able to reduce more nitrate than any other common inorganic electron donor. For example, only 1.6 mg of pyrite-sulfide is needed to reduce 1 mg of nitrate-nitrogen according to:

$$5/14\ FeS_2 + NO_3^- + 2/7\ H^+ \rightarrow 5/7\ SO_4^{2-} + 5/14\ Fe^{2+} + 1/2N_2 + 1/7\ H_2O \quad (2)$$

By comparison, 9.8 mg of dissolved manganese (Mn^{2+}) is required to reduce 1 mg of nitrate-nitrogen (NO_3^--N), according to the following reaction:

$$5/2\ Mn^{2+} + NO_3^- + 2H_2O \rightarrow 1/2\ N_2 + 5/2\ MnO_2 + 4\ H^+ \quad (3)$$

The reduction of 1 mg of nitrate-nitrogen (NO_3^--N) requires 19.9 mg of dissolved iron (Fe^{2+}) according to:

$$5\ Fe^{2+} + NO_3^- + 12\ H_2O \rightarrow 5\ Fe(OH)_3 + 1/2\ N_2 + 9\ H^+ \quad (4)$$

The reduction of nitrate by dissolved manganese or dissolved iron does not appear to be of practical importance because of the large concentrations needed to reduce significant quantities of nitrate.

Reduced sulfide compounds may be formed by the reduction of sulfate by organic carbon oxidation. An example of such a reaction is:

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^- \quad (5)$$

Often, hydrogen sulfide (H_2S) reacts with dissolved iron in the groundwater or in the sediment to form iron sulfides. The first species to precipitate in this manner is non-crystalline iron monosulfide, FeS (Berner, 1984):

$$Fe^{2+} + H_2S + 2HCO_3^- \rightarrow FeS + 2CO_2 + 2H_2O \quad (6)$$

The FeS can further react with H_2S and/or elemental sulfur derived from H_2S to form pyrite (FeS_2) or iron sulfide intermediates, such as mackinawite (crystalline FeS) or greigite (Fe_3S_4) (Goldhaber and Kaplan, 1974; Berner, 1970).

Denitrification by pyrite has been investigated in three aquifers located in northern Europe: the Fuhrberger Feld aquifer in northern Germany (Koelle *et al.*, 1985, 1987; Frind *et al.*, 1990; Boettcher *et al.*, 1990); the Vierlingsbeek aquifer in the Netherlands (Van Beek *et al.*, 1989); and the Rabis aquifer in Denmark (Postma *et al.*, 1991). The aquifers were each composed of sandy sediments containing coal particles and iron sulfide minerals. Recharge to the aquifers occurred over forested and arable land. Despite large quantities of nitrate contamination due to fertilizer application and land tilling, concentrations of nitrate in the groundwater decreased to zero with depth below the water table. The absence of nitrate at depth was attributed to bacterially mediated

denitrification by pyrite (Koelle *et al.*, 1985, 1987; Frind *et al.*, 1990; Boettcher *et al.*, 1990). At the Vierlingsbeek aquifer, both organic carbon and pyrite apparently contributed to the reduction of nitrate (Van Beek *et al.*, 1989).

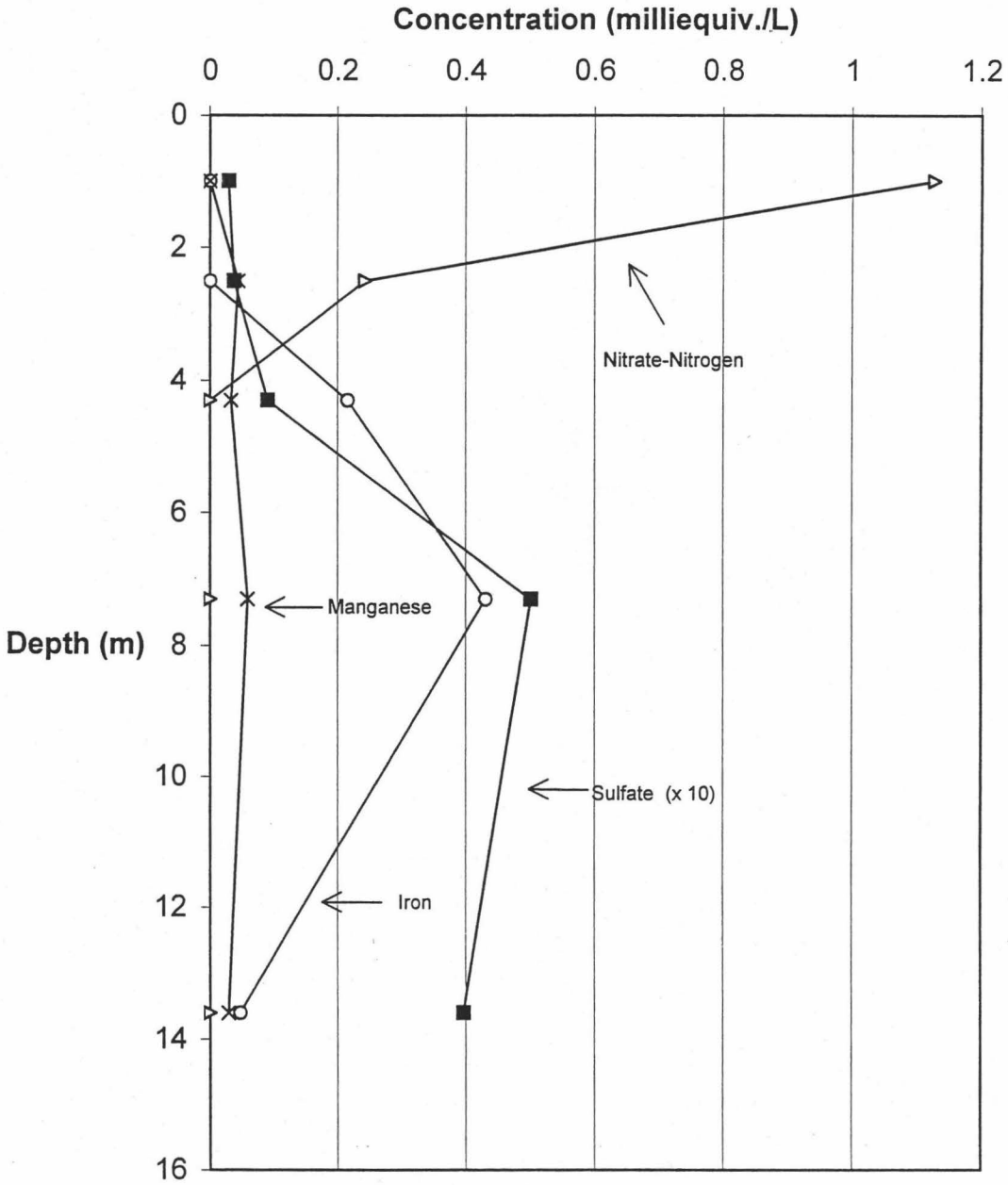
Denitrification by pyrite resulted in sharp increases of sulfate and ferrous iron in the zone of nitrate reduction. Figure 2 shows an example of the distribution of these species with depth in aquifers characterized by denitrification by pyrite. Once the nitrate was depleted, the sulfate was reduced by organic carbon contained within the aquifer sediments. Sulfate-S and sulfate-O isotope ratios at the Fuhrberger Feld supported the interpretation of Strebel *et al.* (1990) that the reduction of sulfate was a bacterially mediated process and indicated that pyrite, not organic carbon, was the preferred electron donor for denitrification in these aquifers.

At the Fuhrberger Feld aquifer, Frind *et al.* (1990) saw evidence of in-situ sulfide-mineral formation. They recognized that this process could play an important role in subsequent denitrification and advocated further research.

Through computer modeling, Postma *et al.* (1991) found that the largest influence on the movement of the nitrate front in the groundwater of the Rabis aquifer was pyrite in the aquifer sediments. Assuming that reaction rates were fast compared to the groundwater flow velocity, they found that aquifers containing small amounts of pyrite (<10 mmol/kg) were at a much higher risk of contamination by nitrate than aquifers containing large quantities of pyrite (>10 mmol/kg).

A situation similar to that of the northern European aquifers can be seen at the Elk Valley aquifer, located in northeastern North Dakota (Mayer, 1992). Geologically, there

Figure 2. Example of the distribution of nitrate, manganese, iron, and sulfate with depth in groundwater characterized by denitrification by pyrite. The concentrations of the different species were taken from water quality data reported by Mayer (1992). The concentrations are reported as milli-equivalents per liter, and depth is in meters below the water table. Sulfate concentrations were reduced by a factor of ten for display on the graph.



are many similarities between the Elk Valley Aquifer and the northern European aquifers. The sediments of the Elk Valley aquifer are composed of glacial outwash and deltaic sand and gravel deposits. The sediments in a section of the aquifer studied by Mayer (1992), contain 20 to 95 percent shale fragments at depths of 7.9 meters to 20.1 meters below the ground surface. The shale fragments were originally eroded from a shallow marine deposit. Coal fragments were also found within the sediments (Mayer, 1992).

Mayer (1992) conducted a study of denitrification within the Elk Valley Aquifer, focusing primarily on organic carbon as the major electron donor. He briefly mentioned the possibility of nitrate reduction by inorganic electron donors, and he suggested that more research be conducted concerning the influence of inorganic species on the system. Further analysis of his data supports denitrification by organic carbon, but it also provides evidence for the occurrence of nitrate reduction by pyrite. For example, in one nest of 5 wells screened at different depths, nitrate-nitrogen concentrations dropped from 15.8 mg/L 1 meter below the water table (about 5 meters depth) to below detection (< 1 mg/L) at a depth of 4.3 meters below the water table. Corresponding to the decrease of nitrate, sulfate levels increased from 14 mg/L near the water table to as much as 240 mg/L at a depth of 7.3 meters below the water table. Dissolved iron concentrations also increased from below detection (< 0.2 mg/L) in the upper portions of the aquifer to a maximum of 12 mg/L at 7.3 meters below the water table. Figure 2 depicts the distribution of the above species as a function of depth below the water table.

Robertson *et al.* (1996) investigated nitrate reduction by sulfide species at five sites in southern Ontario, each characterized by high inputs of anthropogenic nitrate. The

aquifer sediments all contained significant concentrations of pyrite and organic carbon. At each of the sites the nitrate-nitrogen concentrations in the upper 3 - 5 meters of the aquifer approached or exceeded the drinking water limit (10 mg/L). Nitrate levels quickly decreased with depth, mostly over a 1-meter interval. Also associated with the upper portion of the aquifer was a depletion of sulfide minerals. At the point at which dissolved oxygen sharply decreased and nitrate was depleted (the redoxcline), the concentrations of sulfide minerals in the sediment abruptly increased. The concentration of organic carbon in the upper sediment, decreased only slightly over the zone of nitrate reduction, and could not account for the amount of denitrification observed. The authors concluded that the forms of organic carbon in the sediment were less labile than the sulfide minerals present, and, therefore, sulfide oxidation was the dominant denitrification reaction. They also felt that, as the reserves of sulfide were depleted, the redoxcline and the zone of nitrate reduction would migrate to greater depth.

Aravena and Robertson (1998) conducted another study on denitrification by both organic carbon and sulfide. Their study focused on nitrate contamination by a septic system plume located at a campground on the north shore of Lake Erie. Nitrate concentrations decreased dramatically with depth, ranging from approximately 50 mg/L at the top of the water table to levels below 10 mg/L at depths of 3 meters below the water table. Sulfate concentrations ranged from 20 to 35 mg/L at the water table, and increased to as much as 100 mg/L through the zone of nitrate reduction.

Aravena and Robertson (1998) used nitrogen, oxygen, sulfur and carbon isotopes to document the processes of nitrate reduction by organic carbon and reduced sulfur

compounds. As denitrification occurred, enrichments of ^{15}N and ^{18}O (the heavier isotopes of nitrate) were observed in the residual nitrate, indicating microbial reduction of nitrate. In contrast, the zone of sulfate enrichment exhibited a trend towards reduced $\delta^{34}S$ values. The most depleted $\delta^{34}S$ values were found at the greatest measured depth (5 meters). This was attributed to the oxidation of biogenic pyrite, which is depleted in ^{34}S ($\delta^{34}S < 0$ per mil) compared to sulfates from other sources. Isotope data and mass balance calculations indicated that sulfides probably caused 25% of the denitrification, despite the presence of labile organic carbon supplies.

Significance of Recently Formed Sulfides

Most research involving denitrification by sulfides has focused on pyrite as the major electron donor. However, work conducted by Doyle (1968) and Berner (1967) indicated that the stable forms of sulfide found in recent sediments rich in hydrogen sulfide are mackinawite and greigite. Therefore, aquifer sediments containing recently formed sulfides (e.g., within wetlands, septic tank plumes, and riparian zones), are more likely to contain intermediate iron sulfide minerals, such as mackinawite and greigite, rather than pyrite. Furthermore, the kinetics of transformations involving various iron sulfides may be slow, resulting in their persistence in sediments (Goldhaber and Kaplan, 1974).

Apparently few studies have considered recently formed sulfides, such as hydrogen sulfide (HS^-; H_2S) and iron sulfide minerals (mackinawite, FeS; greigite, Fe_3S_4; pyrite, FeS_2), as electron donors for denitrification. As long as these species continue to

be overlooked, the denitrification potential of certain aquifers and environments could be underestimated or ignored. In order to gain a better understanding of the factors that may contribute to denitrification, the influence of recently formed sulfides on nitrate reduction needs to be addressed.

CHAPTER II

METHODS

To implement a study of denitrification by recently formed sulfides, a hollow Plexiglass column was filled with sediment and water from a field site in Fertile, Minnesota. The site was chosen as a location for duplicating the laboratory experiment where the sediment and groundwater contained no nitrate and little organic carbon and sulfides to interfere with the experiment. Organic carbon (in the form of glucose) and sulfate were added to the saturated sediment to induce sulfate reduction and the formation of sulfides. Throughout this experiment, the concentrations of total organic carbon (TOC), total inorganic carbon (TIC), dissolved oxygen (DO), pH, dissolved manganese (Mn^{2+}), dissolved iron (Fe^{2+}), and sulfate (SO_4^{2-}) were monitored to help document the processes occurring within the column.

Column Preparation

The column used for the laboratory study was constructed by sealing each end of a 4-foot high, 6-inch diameter Plexiglas pipe with plates bolted to flanges (Figure 3). The plates were two, ½-inch thick Plexiglas disks. An 8-inch O-ring formed the seal between each plate and flange. Both ends of the column contained sample ports. One end had an additional port, from which a 3/8-inch diameter, stainless steel sampling point extended

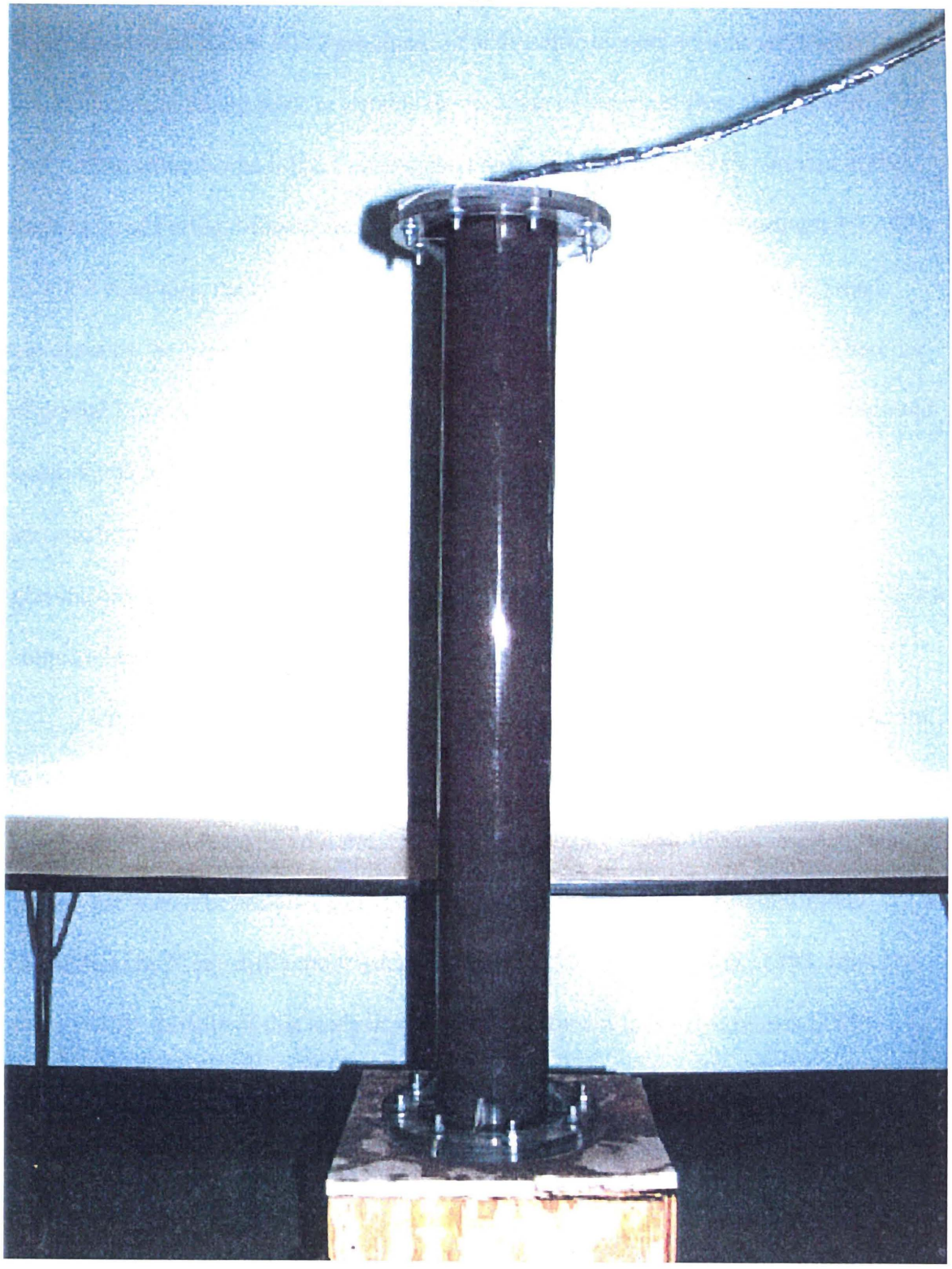


Figure 3. Picture of the laboratory column.

approximately 12 inches into the column. Small stainless-steel screens were placed on the inside of the tubing ports to prevent the removal of sediment from the column.

The column was filled with sediment and water from a field site located in the Sand Hills of Fertile, Minnesota (T.147N., R.44W., Section 32). Approximately 12,000 to 9,000 years ago, the sediment comprising the Sand Hills was carried from glacial moraines by the Sand Hill River, and was deposited in deltas along the eastern shoreline of glacial Lake Agassiz. Predominantly westerly winds reworked the sediment into sand dunes 9,000 to 4,000 years ago (The Nature Conservancy). At present, the dunes surrounding the field site are covered with a variety of vegetation including prairie grasses, oak savanna, and aspen woods. The field site itself is covered by prairie, and is a source of natural recharge.

Originally, the laboratory study was going to be duplicated in the field; therefore, the intent was to choose a field site where the laboratory experiments could be replicated using natural materials that did not contain large quantities of sulfide minerals, nitrate, or organic carbon. The mineralogical composition of the sediment (Appendix I) was determined by X-ray diffraction, using the Rietveld Method (Hill *et al.*, 1993; Bish and Post, 1993). Results of this analysis showed the sediment to be approximately 84% quartz, 14% plagioclase (albite), and 2% potassium feldspar by weight. No sulfide minerals were found, and any other minerals were present only in small amounts (<1%; Wagner, personal communication).

Water from the site was obtained from a two-inch diameter well screened at a depth of approximately 15 feet, which is about three to four feet below the water table.

An initial background analysis of this water was conducted before the water was used in the column (Appendix II). The results confirmed that the water had a low ionic strength and contained little organic carbon and no detectable nitrate-nitrogen (< 0.5 mg/L). Fifty liters of water were collected from the well and used to saturate the sediment within the column. To minimize entrapment of air bubbles, the sediment was slowly and evenly dropped into a standing pool of water maintained above the level of sediment in the column. The approximate volume of water needed to saturate the sediments was 7.8 liters. The total volume of the column was 20.9 liters; therefore, the porosity of the sediment was 0.37.

Analytical Techniques

All of the water analyses for this study were conducted in the Water Quality Laboratory of the Department of Geology and Geological Engineering at the University of North Dakota. Most cations and metals were analyzed on a Thermo-Jarrell Ash Inductively Coupled Plasma Emission Spectrometer (ICP). Anions were analyzed with an ion chromatograph (IC) assembled with an Alltech column heater and suppressor module and a Bacharach Tri-Detector. Total carbon (TC), total organic carbon (TOC), and total inorganic carbon (TIC) were determined using a Shimadzu Total Organic Carbon Analyzer. All samples and blanks analyzed on the IC, TOC Analyzer, or ICP were first filtered through 0.45-μm, hydrophilic polyethersulfone filter paper. Standards were not filtered because any contaminants introduced as a result of filtration would

appear in the blank samples. Analyses for pH, DO, and ammonia were determined using ion-selective electrodes.

To insure the accuracy of the measurements, procedures for sample storage and analysis from Standard Methods for the Examination of Water and Wastewater (American Public Health Association (APHA), 1992) were followed. When using calibration curves, at least one standard of higher and one of lower concentration than the sample were used. Blank samples were run for every analysis. If necessary, any excess chemical compounds found in the blank were subtracted to account for any background contamination in the distilled water and/or from the sampling technique. The average blank subtraction was 2.2 % of the sample concentration, excluding 2 extreme instances where the blank subtractions were 22 % (Table 17, Appendix IV) and 23% (Table 32, Appendix IV). In most cases, duplicate samples and standards were run. Sample spikes were measured with each ICP analysis to check for matrix effects. If the spike recovery was not within the range of 80 to 120 percent, as recommended by Standard Methods (APHA, 1992), the sample was analyzed again and a new sample spike was conducted. The results of the quality control procedures indicated that the data are reliable. The errors associated with measurement of each analyte were calculated with data obtained from multiple sample analyses (Table 1).

Samples for pH, DO, TOC/TIC, and NH_3 measurement were analyzed immediately after collection. Samples to be analyzed for anions were also measured within the recommended period. Samples for cation analysis were acidified to pH < 2 with concentrated nitric acid.

After the column was constructed and filled with saturated sediment, the major anions and cations in the water to be used in this study were determined. Three background analyses were conducted on different batches of site and column water (see Appendix II). Fifty liters of the site water used in the final background analysis were collected from the field site and stored at 4° C for use throughout the experiment. Also, several preliminary steps were taken before the primary experiment was started (Appendix II).

Table 1. The error associated with the analysis of each species.

Analyte	Standard Deviation (mg/L)
TOC	$\pm$ 0.2
TIC	$\pm$ 0.1
TC	$\pm$ 0.2
SO_4^{2-}	$\pm$ 0.2
NO_3^-	$\pm$ 0.3
Cl^-	$\pm$ 0.09
Br^-	$\pm$ 0.1
Fe^{2+}	$\pm$ 0.01
Mn^{2+}	$\pm$ 0.05
Si^{4+}	$\pm$ 0.2
Ca^{2+}	$\pm$ 0.5
Na^+	$\pm$ 0.2
Ba^{2+}	$\pm$ 0.003
Mg^{2+}	$\pm$ 0.2
K^+	$\pm$ 0.02

To begin the main part of the column experiment, 9 liters of site water were spiked with both sulfate and organic carbon. Approximately 100 mg/L of sulfate (as potassium sulfate) were added to the site water, which had an initial sulfate concentration of 11.5 mg/L. Enough organic carbon (as glucose) was added to reduce all the oxygen within the water (~ 10.6 mg/L) and about 85% of the sulfate. This water was injected into the column on 7/31/97 until one pore volume (7.8 L) of the column had been flushed out. The measured concentrations of sulfate and organic carbon added to the column were approximately 114.5 mg/L and 30.8 mg/L, respectively (see Appendix IV, Tables 19 and 20). This water remained in the column for almost 3 months (from 7/31/97 to 10/27/97), during which several samples were taken and analyzed for TOC, TIC, DO, pH, Mn^{2+}(as dissolved manganese), Fe^{2+}(as dissolved iron), and SO_4^{2-} (Table 2.).

CHAPTER III

RESULTS AND DISCUSSION

Table 2 and Figure 4 show several significant changes in the concentrations of the measured species between 7/31/97 and 10/27/97. After 89 days, the total organic carbon (TOC) decreased from 31.2 mg/L to 3.9 mg/L. This decrease was probably a result of attenuation onto the column sediments (Tipping, 1981; Davis, 1982; Davis, 1984), and reactions with DO and manganese. During the first 5 days, DO concentrations decreased from 10.6 mg/L to 1.3 mg/L. Although the DO meter detected minor concentrations of DO in the column, readings around 1.0 mg/L are probably close to the minimum detection limit of the meter due to atmospheric oxygen contamination of the sample and probe during sampling and measurement.

Concomitant with the decrease in TOC and DO, concentrations of total inorganic carbon (TIC) increased from 23.3 mg/L to 39.9 mg/L. Dissolved manganese (presumably Mn^{2+}) began increasing after 48 days, from a concentration of 0.95 mg/L to 4.08 mg/L. The pH of the water decreased slightly during the sampling period. Iron concentrations remained below detection and sulfate did not appear to change from the original concentration (see "sulfate check" in Table 21, Appendix IV).

By the end of the observation period, the reactions followed the expected reduction sequence; however, they proceeded at a much slower rate than expected. One explanation for this is that the bacteria within the column were nutrient-limited. The

Table 2. Concentrations of total organic carbon (TOC), total inorganic carbon (TIC), dissolved oxygen (DO), dissolved manganese (Mn), and pH in the column water.

Column Sample Date:	Time (days)	pH	TOC (mg/L)	TIC (mg/L)	DO (mg/L)	Mn (mg/L)
7/31/97	0	6.54	32.1	23.0	10.6	0.95
8/5/97	5	6.58			1.3	2.22
8/11/97	11					2.52
8/22/97	22	6.56	7.3	36.3	1.3	
8/28/97	28					3.19*
9/17/97	48					4.08
10/3/97	63	6.37			1.3	
10/27/97	88		3.9	39.9		

Missing data indicate that no measurement was taken. Column sample dates indicate the date the sample was taken, not necessarily the date of analysis. Data for sulfate and iron are not reported because no change in sulfate was observed, and no iron was present in any of the samples.

* This sample was measured on two separate occassions. The value from Table 23, Appendix IV was used here.

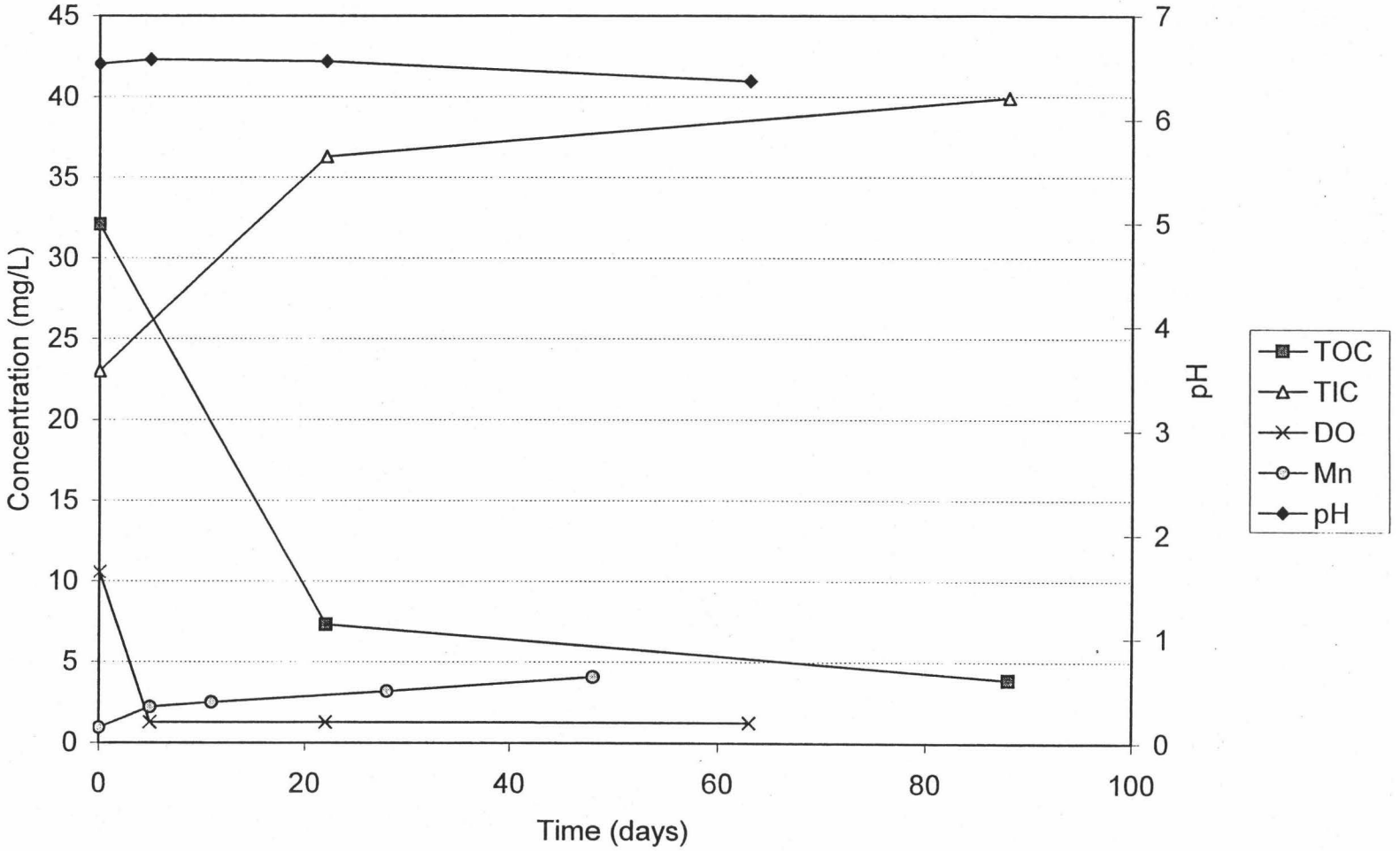


Figure 4. Changes in pH, TOC, TIC, DO, and dissolved Mn with time. Time = 0 days corresponds to 7/31/97 and Time = 88 days corresponds to 10/27/97.

nutrients essential to bacteria include carbon, nitrogen, phosphorus, potassium, sulfur, and smaller quantities of iron, calcium, magnesium, and chloride. If any of the nutrients essential to cell building is in short supply relative to the carbon present, competition for nutrients within the microbial communities may limit overall microbial growth (National Research Council, 1993) and slow reduction processes. Normally, only very small amounts of these nutrients are needed and sufficient amounts are often already present within the groundwater. However, no detectable concentrations of nitrogen and phosphorus were found in the groundwater and sediments used for this study. Thus, on 10/27/97, the column water was spiked with approximately 0.5 mg/L phosphorus and 2.1 mg/L nitrogen (Appendix V).

After spiking the column with nutrients, the water in the column was sampled periodically for 4 months (from 10/27/97 to 2/23/98). Table 3 and Figure 5 record the results of the water analyses conducted over this period. During the first two weeks, DO concentrations decreased from 8.4 mg/L to 0.8 mg/L. Manganese and TIC concentrations also began changing immediately. After 36 days, manganese concentrations increased from 2.07 mg/L to 4.92 mg/L, and then decreased to 4.40 mg/L towards the end of the sampling period. Concentrations of TIC rose from 23.6 mg/L to 39.2 mg/L over a period of 119 days. The reduction of the species within the column water was reflected by a change in the sediment color, from brown (10YR 4/3) just after the column was filled with sediment, to dark olive gray (5Y 3/2) on 2/23/98 as classified by a Munsell Soil Color Chart (Munsell, 1994).

Table 3. Concentrations of dissolved iron (Fe), dissolved manganese (Mn), silicon (Si), dissolved oxygen, total inorganic carbon (TIC), and total organic carbon (TOC) in the column water. These data represent changes is the column water composition after the addition of nutrients to the column.

Date	Time (days)	TOC (mg/L)	TIC (mg/L)	D.O. (mg/L)	Fe (mg/L)	Mn (mg/L)	Si (mg/L)
10/27/97	0	7.3	23.6	8.4		2.07*	
10/29/97	2	3.7	28.4	2.1			
10/31/97	4			1.5		2.32	15.0
11/3/97	7			1.1		2.51	12.6
11/6/97	10					2.87	15.8
11/10/97	14			0.8		2.96	15.0
11/17/97	21	3.5	31.3			3.24	14.5
11/24/97	28					3.42	
12/2/97	36				0.91	4.92	
12/4/97	38				0.91	4.57	
12/8/97	42	8.7	32.9				
12/11/97	45				1.51	4.31	
12/23/97	57				1.47	4.15	
1/3/98	68				1.70	4.23	
1/16/98	81				1.90	4.21	
2/12/98	108				2.56	4.40	
2/23/98	119	5.1	38.8				

Missing data indicate that no measurement was taken. Data for sulfate are not reported because no concentration changes were seen in this species.

* The manganese concentration from Table 30, Appendix IV was used.

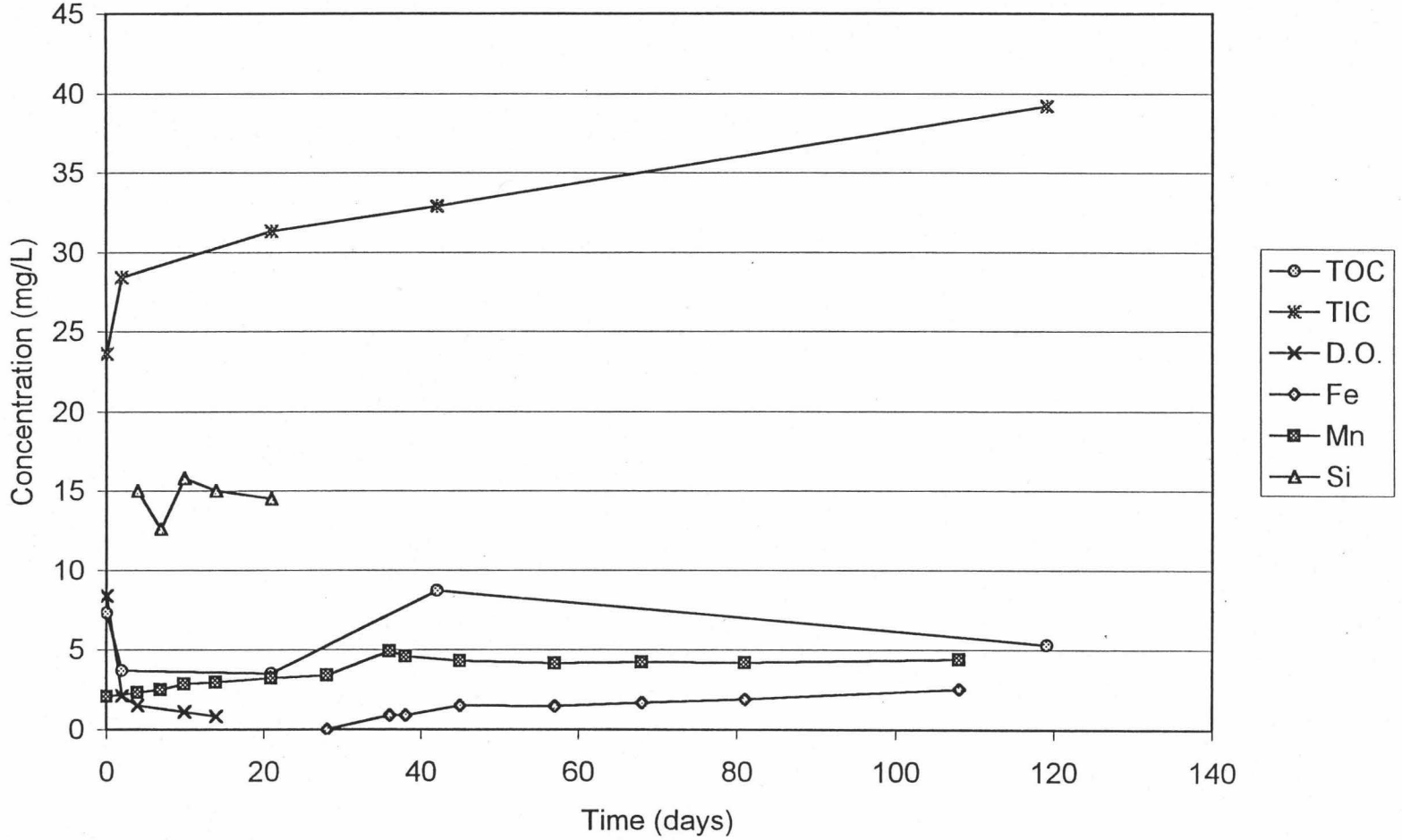


Figure 5. Changes in TOC, TIC, DO, dissolved Fe, dissolved Mn, and Si with time. Time = 0 days corresponds to 10/27/97 and time = 119 days corresponds to 2/23/98. The wastewater inoculation occurred at time = 30 days.

The microbes within the column water did not seem to be affected by the addition of nutrients to the column. A comparison of the increase in manganese before and after the addition of nutrients (Figure 6) shows no appreciable change in the rate of manganese reduction.

During the month after the addition of nutrients to the column, no changes in the sulfate concentration were seen, and, therefore, it was thought that perhaps sulfate-reducing bacteria were not present in the column. The column was inoculated on 11/26/97 with sulfate-reducing bacteria from a wastewater sample (see Appendix VI; Gullicks, personal communication). Six days later, manganese concentrations increased to 4.92 mg/L and then began to decrease, and the first detectable concentrations of iron (presumably Fe^{2+}) appeared. Iron concentrations continued to increase from a value of 0.91 mg/L to 2.56 mg/L during a 72-day period.

The concentration changes of the dissolved species within the column (Tables 2 and 3) can be explained in terms of reduction reactions. As previously mentioned, the processes involving reduction reactions in water and groundwater follow a set sequence of reactions (Figure 1) that are catalyzed by bacteria and governed by thermodynamics (Stumm and Morgan, 1981). In general, the oxidation of organic carbon, coupled with the reduction of oxygen, removes any available oxygen from the groundwater. Once the oxygen is used up, the organic carbon reduces the next electron acceptor, manganese, and then iron. Because the reduced forms of manganese (Mn^{2+}) and iron (Fe^{2+}) are soluble in water, concentrations of these species increase as reduction progresses. As the organic carbon fueling the reduction reactions is oxidized, it is converted into carbon dioxide,

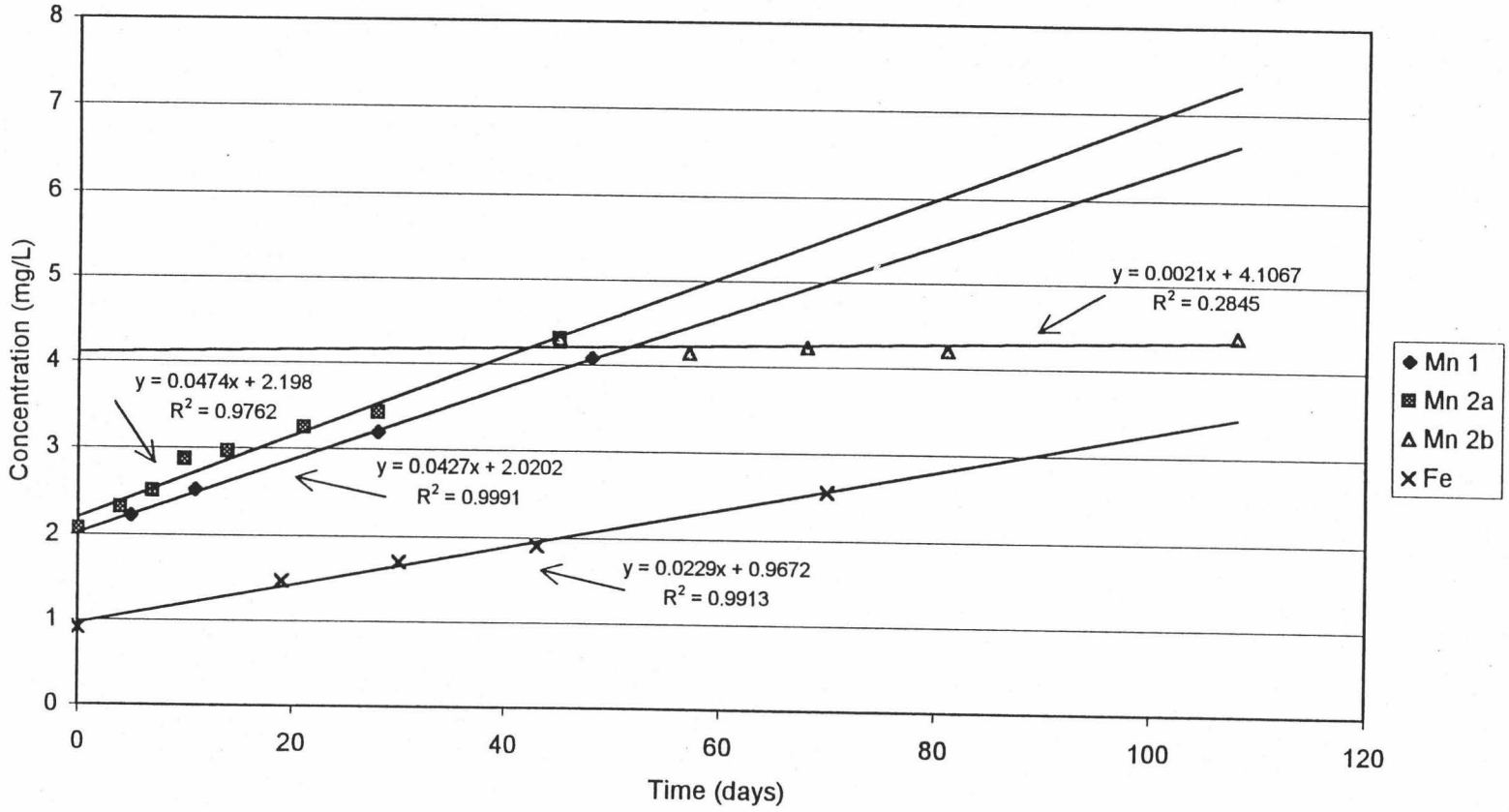


Figure 6. The increase of dissolved manganese before (Mn 1) and after (Mn 2a and Mn 2b) the addition of nutrients to the column water. The increase of dissolved iron in the column water is also shown (Fe). Each data set is adjusted so that Time = 0 days represents 7/31/97 for Mn 1, 10/27/97 for Mn 2a, and 12/4/97 for Fe. The first data point was removed for Mn 1 because it did not seem to represent the stabilized rate of manganese reduction. Two points were removed from the Mn 2a data set, and one point was removed from the Fe data set. These points seemed to represent abnormally high rates of microbial reduction due to inoculation of the column with the wastewater. Also, the manganese data were split into two sets, Mn 2a and Mn 2b, at the point where the rate of manganese increase seemed to stop.

thus increasing the concentration of inorganic carbon in the system. The reduction sequence discussed above and displayed in Figure 1 is clearly exhibited by the data (Tables 2 and 3 and Figures 4 and 5).

An unexplained result is the magnitude of TIC increase in the column water (Table 3 and Figure 5). The initial concentration of TIC was 23.6 mg/L just after addition of the nutrient-spiked site water to the column on 10/27/97. After 119 days, the TIC had increased to 39.2 mg/L. The mole for mole production of carbon dioxide from the oxidation of organic carbon related to these reduction reactions is seen in the following equations (Champ *et al.*, 1979), where CH_2O represents a generic form of organic carbon:

<u>Oxygen Reduction</u>: $CH_2O + O_2 \rightarrow CO_2 + H_2O$ (8)

<u>Manganese Reduction</u>: $CH_2O + 2MnO_2 + 4H^+ \rightarrow 2Mn^{2+} + 3H_2O + CO_2$ (9)

<u>Iron Reduction</u>: $CH_2O + 4Fe(OH)_3 + 8H^+ \rightarrow 4Fe^{2+} + 11H_2O + CO_2$ (10)

Based on these equations, an increase in TIC as H_2CO_3* (or HCO_3^-) was expected, but the actual increase is much greater than can be explained by the observed quantities of species reduced since the addition of nutrients to the column. The 8.4 mg/L reduction of DO in the site water accounts for a 3.2 mg/L increase in TIC. The 2.85 mg/L increase of reduced manganese and the 2.56 mg/L increase of dissolved iron corresponds to an additional 0.5 mg/L of TIC. Therefore, the total increase of TIC based on the observed changes in DO, manganese, and iron should have been about 3.7 mg/L. The

actual increase was 15.6 mg/L TIC, therefore, an additional 11.9 mg/L TIC is unaccounted for.

The dissolution of carbonate minerals contained within the sediment may be an explanation for the excess concentrations of TIC. Although calcium carbonate and dolomite were not detected by X-ray analysis, it would only take a small fraction of these minerals to significantly affect the chemistry of the column water. Therefore, the sediment was tested for carbonate using a simple titrimetric procedure from Methods of Soil Analysis (SSSA, 1996). The average concentration of calcium carbonate in the sediment was found to be 1.15×10^{-2} percent by weight (Appendix VII). If the entire percentage of calcium carbonate within the sediment dissolved into the column water, the concentration of TIC would increase by approximately 62.8 mg/L (Appendix VII). Calcium carbonate dissolution could definitely account for the increase of TIC not related to the formation of reduced species.

Another possible explanation for the high concentrations of TIC is that dissolved manganese and dissolved iron could be adsorbing onto sediment particles (Van Cappellen and Wang, 1996) or forming complexes with the attenuated organic carbon through processes like chelation (Stumm and Morgan, 1981; Tipping, 1981; Davis, 1982; Davis, 1984). If this were the case, the complexed metals would no longer be dissolved in the groundwater, and the concentrations of iron and manganese detected in the groundwater would be smaller than the quantities of iron and manganese actually reduced in the column. Therefore, the TIC concentrations in the water phase would be higher than expected when only considering the detected dissolved species from the oxidation-

reduction reactions. The detection of reduced metals attenuated unto sediment surfaces is beyond the scope of this study.

One explanation for the effects of the wastewater on the column water chemistry could be related to the difference in environments between the column and the reaction vessel, the source of the wastewater. The environment within the wastewater was conducive for bacterial activity, including sulfate reduction. Upon introduction to the column, the bacteria seemed to increase the rate of manganese reduction and begin the process of iron reduction. However, the drop in manganese concentrations and the decrease in the rate of iron reduction would indicate that the increased level of bacterial activity was unable to continue for an extended period within the column. This could have resulted because the conditions within the column were not as suitable as those within the wastewater reactor. Another possibility is that the sulfate reducing bacteria were unable to compete with the manganese and iron reducing bacteria, as long as the concentrations of manganese and iron in the column were sufficient to sustain the bacterial population.

CHAPTER IV

FUTURE WORK: USE OF STABLE ISOTOPES IN GROUNDWATER

The laboratory study is now at the point where the column needs to sit until sulfate reduction commences. Once the organic carbon is oxidized and sulfide minerals have been formed, the next step is to add enough nitrate to the column to oxidize the sulfide compounds. Sulfide oxidation should reduce the nitrate to nitrogen gas, and produce sulfate.

In addition to measuring concentrations of nitrate and sulfate, one method to help identify the processes of bacterially mediated sulfate and nitrate reduction is to monitor S and N isotopes (Mariotti *et al.*, 1981; Boettcher *et al.*, 1990; Strebel *et al*, 1990). Because bacteria preferentially select the lighter isotopes of nitrogen, carbon, and sulfur during a reaction (Thode *et al.*, 1961; Nakai and Jensen, 1964; Mariotti *et al.*, 1981; Boettcher *et al.*, 1990), the newly formed species will be enriched in the lighter isotopes of the elements involved, while the remainder of the original compound becomes enriched in the heavier isotopes. The isotopic enrichment or depletion of an isotope within a compound is often expressed with a delta notation (δ):

$$\delta_{(\text{heavy isotope})} = \{ [R_{(\text{sample})} - R_{(\text{standard})}] / R_{(\text{standard})} \} \times 1000 \, ^{o}/_{oo} \qquad (7)$$

where $R_{(\text{sample})}$ equals the ratio of concentrations of heavy to light isotope in the sample, $R_{(\text{standard})}$ equals the ratio of concentrations of heavy to light isotope in the standard and

$^{o}/_{oo}$ represents per mil, or parts per thousand. The isotope ratio standard commonly used with sulfur isotopes is that of the troilite within the Canyon Diablo meteorite ($^{32}S/^{34}S$ = 22.220 or $^{34}S/^{32}S$ = 0.0450045) (Nakai and Jensen, 1964).

The application of isotope ratio studies to this research is that the reduction of sulfate by organic carbon will yield sulfide compounds enriched in the light sulfur isotope, ^{32}S, while the remaining sulfate becomes increasingly enriched in the heavier isotope, ^{34}S. The initial sulfur isotope ratio of the column-water sulfate ($\delta^{34}S$ = + 0.9 $^{o}/_{oo}$) was determined by Geochron Laboratories. Once sulfate reduction begins and progresses, this value should become increasingly positive until sulfate reduction ceases. If nitrate is introduced into the system, the stored electron potential of the sulfide species will denitrify the nitrate and oxidize the sulfide species to sulfate. If all the sulfide is converted to sulfate, the final $\delta^{34}S$ of the sulfur in the sulfate will equal the initial $\delta^{34}S$ of the sulfate (+ 0.9 $^{o}/_{oo}$). In addition, as nitrate is reduced to nitrogen gas, the residual nitrate will become enriched in ^{15}N, the heavier nitrogen isotope. Monitoring the isotopic ratios of sulfur and nitrogen provides a tool to help confirm microbial reduction processes.

CHAPTER V

CONCLUSIONS

The goal of this research was to develop and begin implementing a methodology for examining the denitrification potential of recently formed sulfides. The reactions occurring within the column were kinetically slow and had not progressed to sulfate reduction by the end of the observation period. During the course of this study, two steps were taken to try to increase reaction rates. These steps included adding nutrients to create conditions more favorable for bacterial activity and inoculating the column with bacteria that may not have been present initially in the sediments and site water. Adding nutrients to the column did not seem to have any effects on the microbes over the observed time span.

The inoculation of the column with bacteria from wastewater (Appendix VI) apparently had an appreciable effect on the system as shown by the large increase in manganese and by the appearance of iron just after inoculation (Table 3 and Figure 5). These increases could not possibly be a result of iron and manganese contained within the wastewater, considering that only 5 mL of wastewater was diluted with 1.5 L of column water before being injected back into the column. In other words, the concentration of iron in the wastewater would have to be at least 270 mg/L to account for a 0.9 mg/L increase of iron. Soon after the rapid increases of manganese and iron, concentrations of

these species declined. Manganese concentrations continued to fluctuate slightly, while reduced iron concentrations increased.

By choosing a source of sediments with low concentrations of organic carbon, nitrogen, and sulfide, the column environment was not the best suited for bacterial activity and reproduction. This does not indicate that bacteria are not capable of living in the column environment; however, they are not as active as they would be in an optimal environment, such as wastewater sludge or a wetland (Mitsch and Gosselink, 1993). It seems likely that once a stable population of sulfate reducing bacteria is established, sulfate reduction will proceed more quickly.

Because of the slow reaction kinetics, the entire methodology was not tested. However, a solid base for ongoing research was developed. The reduction reactions that did occur followed the proposed sequence identically. An interesting aspect of the observed reduction processes are the seemingly linear rates of reduction exhibited by manganese and iron (Figure 6). Actually, the rates of manganese reduction before and after the nutrient spike are almost identical (before the manganese concentrations level off). Likewise, the iron reduction rate is not that different from the manganese reduction rate. If these linear rates continue for an appreciable period of time, they could be used as a tool in predicting the time needed to reduce certain quantities of iron and manganese in the column. For example, if the total iron concentration available for reduction was 8.0 mg/L, an estimate for the point at which iron reduction stops and sulfate reduction begins is approximately 307 days.

Further research needs to be conducted to broaden our knowledge of the different environments and conditions under which reduced species form. More specifically, to better understand the conditions that may affect the fate and transportation of nitrate in groundwater, attention needs to be given to the role of reduced inorganic species in denitrification.

Appendices

Appendix I

Sediment Mineralogy

Sediment Mineralogy

This appendix contains X-ray diffraction data from the analysis of a bulk sediment sample from the Sand Hills of Fertile, Minnesota. The sediment used was collected from the same depth as the monitoring well screen, about 3 feet below the water table (see discussion in main text, p. 15). Four random samples (A, B, C and D) of this sediment were used for analysis. John Wagner, from the Department of Physics at the University of North Dakota, ran the samples and analyzed the data using the Rietveld Method (Hill *et al.*, 1993; Bish and Post, 1993). This method involves finding a least-squares fit between the measured X-ray diffraction pattern and a profile calculated from the structural properties of several suspected mineral phases (Hill *et al.*, 1993). The scale of the calculated profile used to fit the measured diffraction pattern of each phase is used to determine the relative abundance of the phases present in the scan (Hill *et al.*, 1993).

Figure 7 is an example of the X-ray diffraction pattern of the sediment. Figures 8 and 9 depict the Rietveld refinement plots for the four samples. The plus signs represent the diffraction pattern of the sediment with the background subtracted. The solid lines represent a simulated diffraction pattern calculated directly from the structural properties of each component mineral phase. Tick marks depicted below the scan represent the Bragg peaks for each of the mineral phases examined. The lower scan depicts the difference between the observed and calculated scans at each point (or step). Table 4 shows the data obtained by using the Rietveld Method. The average mineralogical composition of the 4 samples by weight percent is: 84% quartz; 14% plagioclase (albite) and 2% orthoclase. The diffraction pattern of the sediment was also compared to the profiles of other minerals, including biotite, calcite, dolomite, pyrite, magnetite, pyroxene and amphibole. The calculated profiles of these mineral phases did not fit the diffraction pattern of the sediment. Quantities of any other minerals present in the sediment are less than 1% weight/volume (Wagner, personal communication).

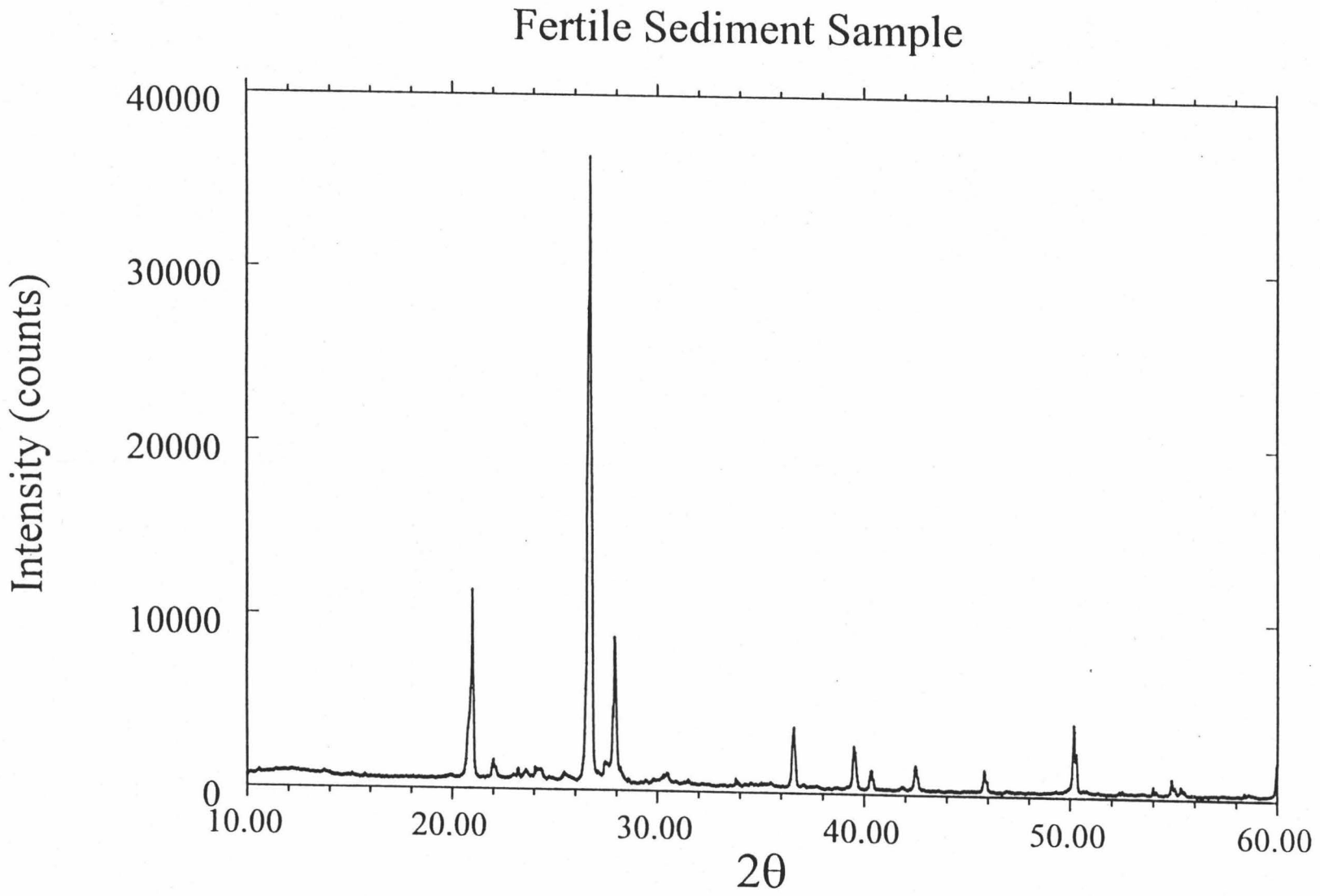


Figure 7. X-ray diffraction pattern of the sediment from the field site at Fertile, Minnesota.

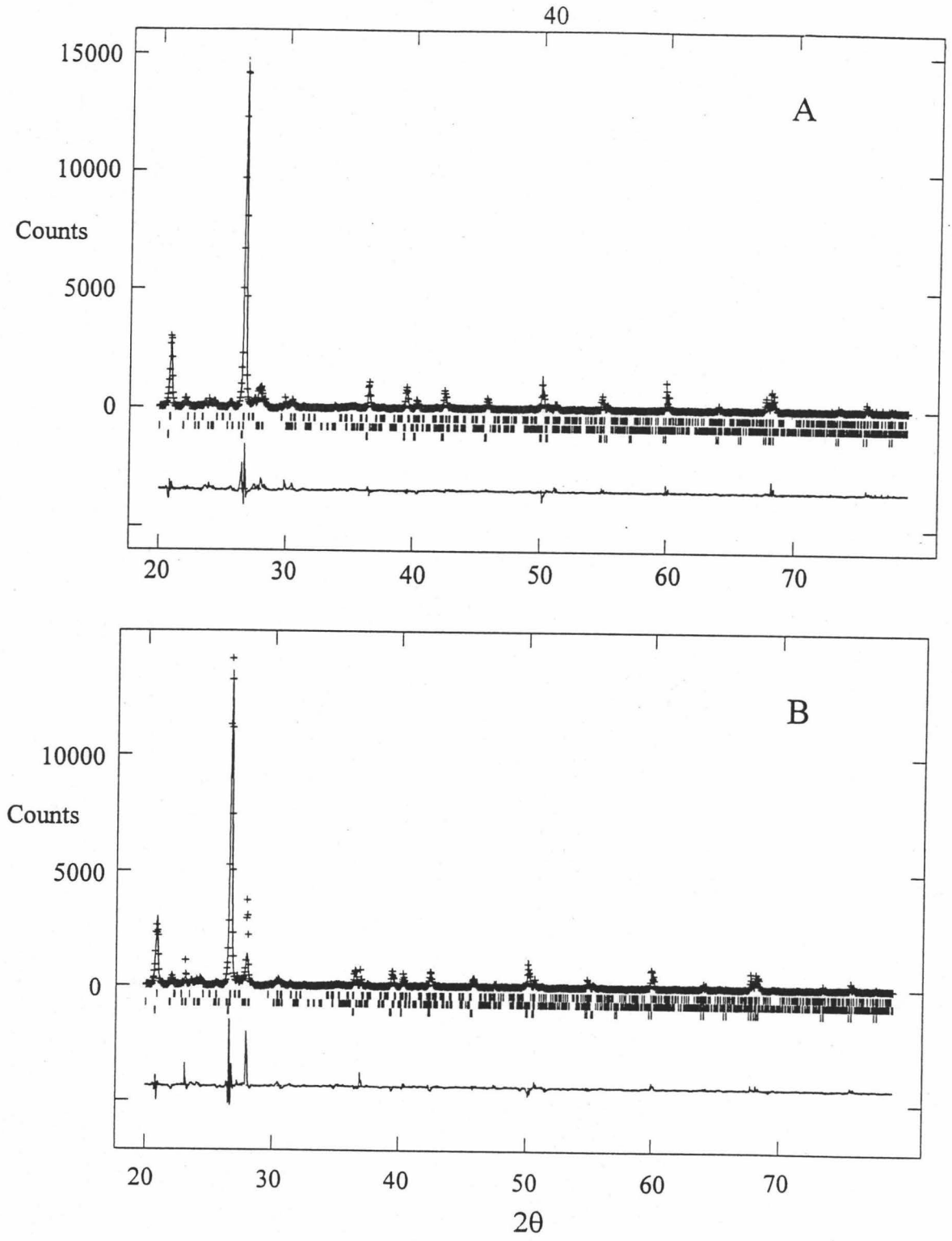


Figure 8. Rietveld refinement profiles of x-ray diffraction data for samples A and B. The plus signs (+) are the experimental data with the background subtracted. The solid line is the calculated multiphase profile. Tick marks below the profile indicate Bragg peaks for orthoclase, albite and quartz phases in descending order. The difference curve (observed minus calculated) is plotted at the bottom.

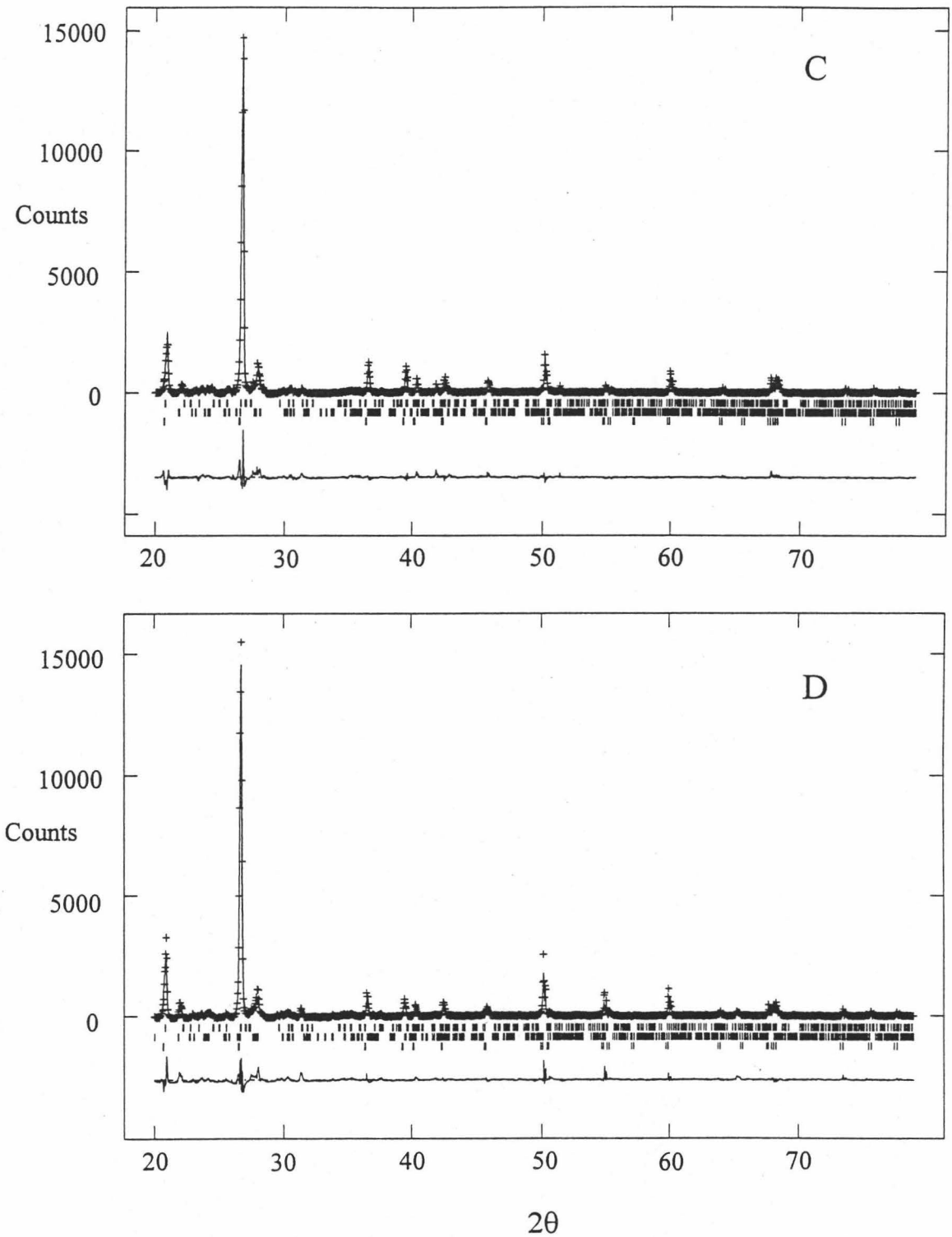


Figure 9. Rietveld refinement profiles of x-ray diffraction data for samples C and D. The plus signs (+) are the experimental data with the background subtracted. The solid line is the calculated multiphase profile. Tick marks below the profile indicate Bragg peaks for orthoclase, albite and quartz phases in descending order. The difference curve (observed minus calculated) is plotted at the bottom.

Table 4. Results of three-phase Rietveld refinements from four different sets of x-ray diffraction data from samples of Fertile sediment. Listed are the refined lattice constants, weight percents, and the angle between the a and c lattices (β). The refined uncertainties of each quantity are indicated in parentheses. For example, the number 5.4062(4) would have an uncertainty of ± 0.0004.
(From Profesor John Wagner, Department of Physics, UND)

	A	B	C	D	Statistical Average
Quartz - SiO_2:					
a = b	4.91460(27)	4.9132(4)	4.91574(25)	4.91388(31)	4.914(11)
c	5.4062(4)	5.4015(6)	5.4069(4)	5.4065(5)	5.4061(7)
Wt. %	85.6(6)	78.1(6)	85.3(6)	86.9(6)	84(4)
Albite - $NaAlSi_3O_8$:					
a	8.1668(26)	8.1672(19)	8.1741(23)	8.1786(33)	8.172(6)
b	12.786(4)	12.7953(34)	12.810(4)	12.798(5)	12.797(10)
c	7.1404(21)	7.1001(16)	7.1337(19)	7.1021(33)	7.12(2)
β	115.917(25)	115.686(21)	116.040(23)	115.62(4)	115.9(2)
Wt. %	12.0(4)	18.6(4)	12.0(4)	13.0(4)	14(3)
Orthoclase - $KAlSi_3O_8$:					
a	8.602(10)	8.589(9)	8.624(8)	8.589(9)	8.60(2)
b	12.967(11)	12.960(10)	12.966(9)	12.954(10)	12.962(6)
c	7.192(7)	7.196(6)	7.197(5)	7.191(6)	7.194(3)
β	115.52(8)	115.59(7)	115.75(7)	115.55(7)	115.6(1)
Wt. %	2.4(2)	3.3(2)	2.6(2)	0.1(2)	2(1)

Units: Lattice constants (a, b and c) in Angstroms; β in degrees.

Appendix II

Background Analyses

Background Analyses

This appendix contains the preliminary analyses of the site groundwater. An initial groundwater study was conducted on samples taken directly from the field site (Table 5). At the time, organic carbon, sulfate, and nitrate were the primary species of interest. Because sodium and potassium were not measured, an ion balance of the sample was not conducted.

After the initial site water study, three background analyses of the major cations and anions of the water were conducted (Tables 6, 7 and 8). The results of the first background study are presented in Table 6. The water for this study was taken directly from the column, and had been in contact with the column sediments for three days. The surprising result of this analysis was that the total organic carbon (TOC) concentration had increased from about 4.7 mg/L in the initial study of the site water (Table 5) to 78.2 mg/L once the site water had been in contact with the column sediments. Because there was such a difference in TOC concentrations, I conducted another analysis of the water for TOC. The water used for this analysis was in the column for 10 days more than the water used in the first analysis, and revealed a TOC concentration of 39.3 mg/L. Likewise, the total inorganic carbon (TIC) concentration had increased from 4.1 mg/L to 10.4 mg/L. Over this same period, the dissolved oxygen (DO) concentrations decreased from 5.7 mg/L to 4.7 mg/L.

Table 5. Initial site water analysis. Conducted on 11/15/96.

Dissolved Oxygen (DO) = 4.8 mg/L
Total Organic Carbon (TOC) = 4.7 (± 0.2) mg/L
Total Inorganic Carbon (TIC) = 18.6 (± 0.1) mg/L

Cations	Concentration (mg/L)	Concentration (milli eq./L)
Mn^{2+}	0.01 (± 0.05)	0.0004
Fe^{2+}	< 0.03	< 0.0005
Ca^{2+}	4.4 (± 0.5)	0.2196
Mg^{2+}	0.2 (± 0.2)	0.0140

Anions	Concentration (mg/L)	Concentration (milli eq./L)
NO_3^--N	< 0.5	< 0.0357
Br^-	< 0.5	< 0.0063
SO_4^{2-}	10.8 (± 0.2)	0.2249
Cl^-	2.20 (± 0.09)	0.0621

An electron balance was not completed for this analysis because sodium and potassium were not measured.

The determination of the error (in parentheses) associated with each analyte concentration is explained in Appendix III.

Table 6. Background Analysis #1. Column water analysis conducted on February 2, 1997

pH = 6.32
Dissolved Oxygen (DO) = 5.7 mg/L
Total Organic Carbon (TOC) = 78.2 (± 0.2) mg/L
Total Inorganic Carbon (TIC) = 4.1 (± 0.1) mg/L

Cations	Concentration (mg/L)	Concentration (milli eq./L)
Ba^{2+}	0.063 (± 0.003)	0.0009
Mn^{2+}	0.02 (± 0.05)	0.0007
Fe^{2+}	0.44 (± 0.01)	0.0159
Ca^{2+}	14.3 (± 0.5)	0.7146
Mg^{2+}	4.0 (± 0.2)	0.3308
Na^{+}	6.5 (± 0.2)	0.2819
K^{+}	23.00 (± 0.02)	0.5883

Anions	Concentration (mg/L)	Concentration (milli eq./L)
NO_3^--N	< 0.5	< 0.0357
Br^-	< 0.5	< 0.0063
SO_4^{2-}	11.9 (± 0.2)	0.2478
Cl^-	5.20 (± 0.09)	0.1467
HCO_3^- *	10.8 (± 0.5)	0.1720

Total Cations:	1.9330	milli eq./L
Total Anions:	0.5664	milli eq./L
Electroneutrality %:**	54.7	

* The pH of the water would put the inorganic carbon stability field between bicarbonate (HCO_3^-) and carbonic acid (H_2CO_3*). The following formula from Snoeyink and Jenkins (1980, p. 160) was used to calculate the concentration of HCO_3^-:

$$K_{a,1} = \frac{[H^+]\,[HCO_3^-]}{[H_2CO_3^*]} = 10^{-6.3} \quad \textbf{or} \quad [H_2CO_3^*] = \frac{10^{-(pH)}\,[HCO_3^-]}{10^{-6.3}}$$

Then, assuming $[TIC] = [HCO_3^-] + [H_2CO_3^*]$, and substituting the above equation for $[H_2CO_3^*]$ gives:

$$[HCO_3^-] = \frac{[TIC]}{(1 + 10^{-(pH)}/10^{-6.3})}$$

where all concentrations are calculated in moles/L.

** The electoneutrality percentage is calculated by the following equation:
E.N.% = [(Σ Cations - Σ Anions) / (Σ Cations + Σ Anions)] * 100

One explanation for the high concentrations of TOC in the column water is that the sediment used to fill the column was collected from just below the topsoil down to the water table depth. It is possible that the sediment just below the topsoil contained more organic carbon that the sediment at depth. When the site water, which was collected at depth, was used to saturate the column sediments, the organic carbon could have leached off the sediment. At any rate, 13 days after the first background study of the column water, the DO concentration in the column had decreased to 0.9 mg/L; therefore, it appeared the organic carbon was reacting with the oxygen in the system. Because the DO concentrations were becoming low, a new batch of site water was flushed into the column to further decrease the TOC concentration. The DO concentration in the site water was 10.6 mg/L; thus, plenty of oxygen would be available to react with the organic carbon. Two and a half pore volumes of site water were flushed into the column. The water was allowed to sit in the column for 24 hours before a sample was taken for TOC analysis. The concentration of TOC in the new column water was 22.1 mg/L, and the TIC concentration was 13.4 mg/L. The dissolved oxygen had decreased to 5.6 mg/L.

Because the results of the ion balance conducted in the first background analysis were poor, a second background analysis was conducted (Table 7). In addition to completing an accurate background analysis, it was decided that a precautionary step should be taken to verify that nitrate would not disappear in the column in the absence of organic carbon or sulfides. Therefore, 8 liters of site water were spiked with potassium nitrate and potassium bromide (with bromide being a conservative tracer) to an ionic strength equivalent to that which would be used in the study. The spiked site water was

Table 7. Background Analysis #2. Site water analysis conducted on March 4, 1997
The site water was spiked with bromide and nitrate for injection into the column.

Total Organic Carbon (TOC) = 3.6 (± 0.2) mg/L
Total Inorganic Carbon (TIC) = 16.5 (± 0.1) mg/L

Cations	Concentration (mg/L)	Concentration (milli eq./L)
Ba^{2+}	< 0.003	< 0.0000
Mn^{2+}	< 0.01	< 0.0004
Fe^{2+}	< 0.03	< 0.0011
Ca^{2+}	16.7 (± 0.5)	0.8333
Mg^{2+}	6.6 (± 0.2)	0.5431
Na^{+}	5.3 (± 0.2)	0.2305
K^{+}	79.10 (± 0.02)	2.0231

Anions	Concentration (mg/L)	Concentration (milli eq./L)
NO_3^--N	30.1 (± 0.3)	2.1482
Br^-	23.6 (± 0.1)	0.2954
SO_4^{2-}	9.3 (± 0.2)	0.1936
Cl^-	1.76 (± 0.09)	0.0496
HCO_3^- *	47.5 (± 0.5)	0.7785

Total Cations:	3.6301	milli eq./L
Total Anions:	3.4653	milli eq./L
Electroneutrality %:**	2.3	

* To view the calculations for the determination of HCO_3^- see the footnotes of Table 6. Also, because no pH reading was taken for this analysis, the pH measurement of the water from the first background study was used in the equation.

** The formula used for the determination of percent electroneutrality is shown in the footnotes of Table 6.

used to conduct the second background analysis before it was injected into the column. The original concentrations of nitrate-nitrogen and bromide in this water were 30.1 mg/L and 23.6 mg/L, respectively. After 18 days, the concentration of nitrate-nitrogen was 23.9 mg/L and the bromide concentration was 19.7 mg/L. The percent loss of nitrate (20.6) was close enough to the percent loss of bromide (16.5) to be attributed to dilution during addition to the column. After 3 weeks, this water was flushed from the column with more than 2 pore-volumes of fresh site water.

Before any organic carbon and sulfate were injected into the column, it was necessary to insure that the sulfate would not disappear by some other process in the absence of the added organic carbon. Twelve liters of site water were spiked with potassium sulfate (no organic carbon), and then injected into the column. The column water, which contained 90 mg/L sulfate, was allowed to sit for almost 2 months, during which samples were taken periodically. The sample peak heights (on the IC) were compared to the peak heights of a 100 mg/L standard. No significant changes in the sulfate concentration were observed in the column water during this period. (At the time, I did not realize that the kinetics of sulfate reduction in the column were going to be so slow. In actuality, this step only proved that sulfate reduction would not happen naturally over a 2-month period.) At this point, a new batch of site water was collected from the field site. The third background analysis was conducted on this water (Table 8), and it was decided that the ion balance results were satisfactory, considering the low ionic strength of the site water. A sample of this water was sent to the North Dakota State Health Department for comparison of analytical results with the third background

Table 8. Background Analysis #3. Site water analysis conducted between July 2, 1997 July 17, 1997.

pH = 6.44
Total Organic Carbon (TOC) = 0.7 (± 0.2) mg/L
Total Inorganic Carbon (TIC) = 27.5 (± 0.1) mg/L

Cations	Concentration (mg/L)	Concentration (milli eq./L)
Ba^{2+}	0.043 (± 0.003)	0.0006
Mn^{2+}	0.63 (± 0.05)	0.0218
Fe^{2+}	< 0.03	< 0.0011
Ca^{2+}	28.6 (± 0.5)	1.4261
Mg^{2+}	10.0 (± 0.2)	0.8229
Na^{+}	3.7 (± 0.2)	0.1614
K^{+}	0.74 (± 0.02)	0.0179

Anions	Concentration (mg/L)	Concentration (milli eq./L)
NO_3^--N	< 0.5	< 0.0357
Br^-	< 0.5	< 0.0063
SO_4^{2-}	11.5 (± 0.2)	0.2394
Cl^-	14.7 (± 0.09)	0.4146
HCO_3^- *	81.0 (± 0.5)	1.3270

Total Cations:	2.4508	milli eq./L
Total Anions:	1.9810	milli eq./L
Electroneutrality %:**	10.60	

* To view the calculations for the determination of HCO_3^- see the footnotes of Table 6.

** The formula used for the determination of percent electroneutrality is shown in the footnotes of Table 6.

analysis (Table 9). The water was stored in a 50-liter carboy at 4° C for use throughout the column study.

Overall, the state health department results compare well with the results of the third background analysis. The only major discrepancy occurred between the dissolved iron concentrations (< 0.03 vs. 3.5 mg/L). This value can be explained by the fact that the state health department acidified an unfiltered portion of the site water for cation analysis. Upon acidification, any ferric iron, Fe(III), in the site water would have been converted into dissolved iron, Fe(II), which would be detected during analysis. (Ferric iron was visible in the site sediments and in the carboy used to store the site water.) The range of bicarbonate values is quite large (81.0 mg/L for BG#3 and 120 mg/L for the Health Department), however, if the bicarbonate is converted into equivalent inorganic carbon concentration, the values are closer (27.5 and 23.6 mg/L, respectively). The difference between the bicarbonate concentrations is most likely a result of different methods used to calculate bicarbonate, in which case the different pH values (6.44 and 6.76) could significantly affect the results. If the inorganic carbon concentration obtained by the Health Department is converted into bicarbonate based on the formula used in each of the background analyses, the bicarbonate concentration becomes 89 mg/L. This value is much closer to the value obtained in the third background analysis.

To insure accurate sulfate analyses, one further precaution was taken. Sulfides and intermediate iron sulfide minerals (greigite, mackinawite) are quickly oxidized to sulfate when exposed to the atmosphere (Berner, 1967). Although colloids are defined as particles smaller than 1.0 μm (Fetter, 1993), there is no definition for how small they might be. There was a chance that sulfide colloids smaller than the filter size (0.45 μm)

Table 9. Comparison of the results of the third background analysis to the water analysis conducted by the North Dakota State Health Department.

Analyte	BG#3 Concentration (mg/L)	State Health Dept. Concentration (mg/L)
Ba^{2+}	0.043	0.046
Mn^{2+}	0.63	0.67
Fe^{2+}	< 0.03	3.5
Ca^{2+}	28.6	31.2
Mg^{2+}	10.0	10.4
Na^{+}	3.7	2.6
K^{+}	0.7	< 1
NO_3^--N	< 0.5	< 0.02
Br^-	< 0.5	No data
SO_4^{2-}	11.5	12
Cl^-	14.5	19.8
HCO_3^-	81.0	120
pH	6.44	6.76

would enter into the samples for sulfate analysis, and oxidize to sulfate before an analysis could be completed. If sulfides were to oxidize to sulfate in a sample, the measured sulfate concentration would be higher than the concentration of sulfate in the column. To eliminate the possibility of oxidizing any sulfide, samples were drawn directly from the column into a syringe containing one drop each of zinc acetate and sodium hydroxide per 20 to 30 ml of sample. The sodium hydroxide increases the pH and the sulfide precipitates out of solution as zinc sulfide (APHA, 1992). Samples then sat for 30 minutes before being filtered to remove the zinc sulfide precipitate.

To insure that the zinc acetate and sodium hydroxide did not affect the sulfate concentration in the water, a sample was taken and analyzed for sulfate. Zinc acetate and sodium hydroxide were added to a part of this same sample, and the sulfate concentration was re-measured (Table 20, Appendix IV). The percent difference in the sulfate concentrations of the two samples (1.2 %) was not considered significant.

Appendix III

Error Analysis

Error Analysis

The error associated with the analysis of each species is depicted in Table 10. Tables 11 - 14 show the data used to determine the error associated with the measurement of the following species: total organic carbon (TOC), total inorganic carbon (TIC), total carbon (TC), sulfate (SO_4^{2-}), nitrate (NO_3^-), chloride (Cl^-), bromide (Br^-), dissolved iron (Fe^{2+}), dissolved manganese (Mn^{2+}), silicon (Si^{4+}), calcium (Ca^{2+}), sodium (Na^+), barium (Ba^{2+}), magnesium (Mg^{2+}), and potassium (K^+). The data used in Tables 11 - 14 were chosen because they involved at least three measurements of the same sample. The standard deviations of each replicate were used as the error for that analyte. In cases where the same analyte was measured in several different sample runs, the standard deviations of each run were averaged together to represent the overall error for that analyte. The error determinations shown were used to represent the error in every analysis conducted, even if different samples and sample concentrations were measured. Technically, each time an analysis was conducted, that species or element could have its own standard deviation based solely on that one analysis. However, in most cases, a sample was only run twice, and, thus, the error determination for 2 samples was not as reliable as an error determination based on several runs. Errors were not determined for phosphorus and ammonia because too few analyses were conducted to determine a statistically significant error. All concentrations and standard deviations are expressed as mg/L.

Table 10. The error associated with the analysis of each species.

Analyte	Standard Deviation (mg/L)
TOC	$\pm$ 0.2
TIC	$\pm$ 0.1
TC	$\pm$ 0.2
SO_4^{2-}	$\pm$ 0.2
NO_3^--N	$\pm$ 0.3
Cl^-	$\pm$ 0.09
Br^-	$\pm$ 0.1
Fe^{2+}	$\pm$ 0.01
Mn^{2+}	$\pm$ 0.05
Si^{4+}	$\pm$ 0.2
Ca^{2+}	$\pm$ 0.5
Na^+	$\pm$ 0.2
Ba^{2+}	$\pm$ 0.003
Mg^{2+}	$\pm$ 0.2
K^+	$\pm$ 0.02

Table 11. Estimate of the error associated with measurement of total carbon (TC), total inorganic carbon (TIC), and total organic carbon (TOC) on the TOC analyzer.

Multiple Error Estimates for TC, TIC, and TOC:

	TC	TC	TC	TIC	TIC	TIC	TOC	TOC	TOC
Run #1	2.36	32.64	56.0	0.76	30.41	23.57	1.60	2.23	32.5
Run #2	2.11	32.81	55.6	0.70	30.56	23.44	1.41	2.25	32.2
Run #3	2.12	32.75	55.8	0.67	30.50	23.01	1.45	2.25	32.8
Run #4	2.07	33.08		0.70	30.47		1.38	2.61	
Run #5	2.05	32.93		0.64	30.50		1.41	2.43	
Mean:	2.14	32.84	55.80	0.69	30.49	23.34	1.45	2.35	32.50
Variance:	0.0151	0.0287	0.0400	0.0020	0.0030	0.0859	0.0078	0.0271	0.0900
Std. Dev:	**0.12**	**0.17**	**0.20**	**0.04**	**0.05**	**0.29**	**0.09**	**0.16**	**0.30**
% Dev. from mean:	5.74	0.52	0.36	6.38	0.18	1.26	6.09	6.99	0.92

Average Error Estimates for TC, TIC, and TOC:

	TC	TIC	TOC
Standard Deviation (mg/L):	**0.2**	**0.1**	**0.2**

Table 12. Estimate of the error associated with measurement of Cl^-, Br^-, NO_3^-, and SO_4^{2-} peak heights on the ion chromatograph. The data came from five consecutive measurements of a standard containing: 5 mg/L Cl^-, 16 mg/L Br^-, 10 mg/L NO_3^--N, and 20 mg/L SO_4^{2-}.

Error Estimates of Analyte Peak Heights:

		Cl^-	Br^-	NO_3^--N	SO_4^{2-}
Peak Height (cm)	Run #1	1.66	10.73	1.21	1.92
	Run #2	1.67	10.79	1.29	1.96
	Run #3	1.61	10.86	1.21	1.94
	Run #4	1.61	10.92	1.24	1.94
	Run #5	1.61	10.91	1.27	1.93
	Mean:	1.63	10.84	1.24	1.94
	Variance:	0.0009	0.0066	0.0013	0.0002
	Std. Dev:	**0.030**	**0.081**	**0.036**	**0.015**
	% Dev. from mean:	1.86	0.75	2.88	0.77

Concentration Measurement Errors:

	Cl^-	Br^-	NO^{3-}-N	SO_4^{2-}
% Dev. from mean peak height:	1.86	0.75	2.88	0.77
Analyte Concentration (mg/L):	5.0	16.0	10.0	20.0
Std. Dev. of Conc. (mg/L):*	**0.09**	**0.1**	**0.3**	**0.2**

* The standard deviation of the concentration of each analyte was determined by multiplying the analyte concentration by the percent deviation of the peak height from the mean peak height.

Table 13. Estimate of the error associated with measurement of Ba, Ca, Na, Mg, and K on the ICP. The data are from a site water analysis conducted on 7/3/97 and 7/10/97.

Multiple Error Estimates for Each Analyte:

	Ba	Ca	Ca	Na	Na	Na	Na	Mg	Mg	K	K
Run #1	0.041	28.99	29.02	4.52	4.61	4.63	4.98	10.04	10.25	0.72	0.75
Run #2	0.047	29.28	29.10	4.72	4.58	4.69	5.06	9.57	10.41	0.72	0.74
Run #3	0.042	28.16	28.65	4.59	4.50	4.46	4.96	10.08	10.46	0.70	0.78
Run #4	0.040	28.74	29.44	4.69	4.41	5.06	4.88	9.97	10.47	0.72	0.80
Run #5		27.69	28.56	4.91	4.72	5.12	5.05	10.02	10.15		
Run #6				4.74		4.87					
Run #7				4.70		4.53					
Run #8				4.23		4.52					
Run #9				4.25		4.47					
Mean:	0.042	28.57	28.95	4.59	4.57	4.71	4.99	9.94	10.34	0.71	0.77
Variance:	0.00001	0.413	0.127	0.052	0.013	0.064	0.005	0.043	0.020	0.0001	0.0009
Std. Dev:	**0.003**	**0.64**	**0.36**	**0.23**	**0.12**	**0.25**	**0.07**	**0.21**	**0.14**	**0.01**	**0.03**
% Dev:*	6.62	2.25	1.23	4.95	2.52	5.37	1.47	2.10	1.38	1.28	3.82

Average Error Estimates for Each Analyte:

	Ba	Ca	Na	Mg	K
Standard Deviation (mg/L):	**0.003**	**0.5**	**0.2**	**0.2**	**0.02**

* Percent standard deviation from the mean.

Table 14. Estimate of the error associated with measurement of Fe, Mn, and Si on the ICP. The data are from analyses conducted on 11/10/97, 11/17/97, and 2/12/98.

Multiple Error Estimates for Each Analyte:

	Fe^{2+}	Fe^{2+}	Mn^{2+}	Mn^{2+}	Mn^{2+}	Mn^{2+}	Mn^{2+}	Mn^{2+}	Si^{4+}	Si^{4+}
Sample Date:	2/12/98	2/12/98	2/12/98	11/17/97	7/2/97	2/12/98	2/12/98	11/17/97	11/17/97	11/10/97
Run #1	1.509	1.716	4.277	3.270	0.630	4.112	4.235	3.111	14.55	14.05
Run #2	1.505	1.698	4.408	3.219	0.642	4.256	4.207	3.207	14.69	13.62
Run #3	1.518	1.683	4.399	3.288	0.648	4.147	4.329	3.107	14.22	14.06
Run #4	1.515				0.610					14.10
Mean:	1.512	1.699	4.36	3.26	0.63	4.172	4.26	3.14	14.49	13.96
Variance:	0.00003	0.0003	0.0054	0.0013	0.0003	0.0056	0.0041	0.0032	0.0582	0.0509
Std.Dev:	**0.006**	**0.017**	**0.073**	**0.036**	**0.017**	**0.075**	**0.064**	**0.057**	**0.24**	**0.23**
% Dev. from mean:	0.39	0.97	1.68	1.10	2.65	1.80	1.50	1.80	1.67	1.62

Average Error Estimates for Each Analyte:

	Fe	Mn	Si
Standard Deviation (mg/L):	**0.01**	**0.05**	**0.2**

Appendix IV

Data from All Analyses

Data from All Analyses

The following tables (15 - 38) contain all the analyses conducted from July 2, 1997 to February 23, 1998. The analyses are listed by date, and include the data from the third background study and all analyses conducted thereafter. The complete data (i.e. quality control data) of the initial site water study and the second and third background studies are not included because those analyses were considered preliminary work and those batches of site water were not used for the main part of the experiment.

The determination of sample concentrations on all of the instruments requires use of a calibration curve. The calibration curve is a plot of the concentrations of known samples (or standards) versus the intensity of the instrument signal for each standard. The TOC Analyzer and ICP automatically create calibration curves. However, the IC or any of the ion-selective electrodes requires creation of calibration curves by the user. The calibration curves created for the analyses are not displayed in this appendix, but the R^2 values of each curve are reported. The R^2 values are a measure of how well the data points fit the calibration curve. Ideally, this value should equal one, indicating a perfect fit of the data to the curve. The calibration curves of the following analyses have R^2 values of at least 0.99, and in many instances 0.999.

Several quality control (QC) measures were used throughout the analyses. Sample blanks consisted of distilled, de-ionized water (DI water) that was processed exactly the same as a sample. For example, if a sample was filtered and stored in a sample bottle, the DI water was also passed through the same type of filter and stored in a bottle that had been handled in the same manner as the sample bottle. Sample blanks are indicators of contamination that may occur during the processing and handling of the sample before it is measured on the instrument.

Another method of quality control used was the measurement of standards and samples of known concentration on the instrument. These QC samples were analyzed on the instrument just like all the other samples. The concentration of the QC samples was compared to their known concentrations as a measure of how accurately the instrument was reading the sample concentrations. (This assumed the QC standards and samples were truly the reported concentration.)

The last method of quality control used was sample spikes. Often, the matrix in which an analyte is contained (water plus other species) may inhibit detection of the analyte. This phenomenon, known as a matrix effect, can be detected by measuring sample spikes. A sample spike involved measuring a sample and then increasing the concentration of analyte in that sample by 50 to 150 percent of the detected analyte concentration. Analysis of the spiked sample should detect the original analyte concentration plus the amount added during spiking. The comparison of the measured concentration of the spiked sample to the theoretical value of the spiked sample based on the amount of analyte added was referred to as the "spike recovery". The value of the spike recovery should be between 80 and 120 percent of the theoretical value to rule out any matrix effects.

The following list is an explanation of some of the terms used to report the data analyses:

QC: Refers to a quality control sample. The theoretical value of the analyte concentration in the sample follows this term.

Std: Indicates the measurement of a calibration standard as a sample for quality control purposes. The theoretical value of the analyte concentration in the standard is listed after this term.

Column sample: A sample taken from the sampling point within the column. The date on which the sample was taken is also displayed.

Bottom column sample: A sample taken from the tubing port at the very bottom of the column.

Top column sample: A sample taken from the tubing port at the top of the column.

Some of the data involving analyses conducted on the ICP contain terms like, "Mn260" or "Ca393". These numbers indicate the wavelength corresponding to one of the peaks that was measured for the analyte in the sample. The ICP has the capability of measuring several wavelengths of one element, and although some will display more accurate values than others, the readings from all the peaks should be close. The wavelength numbers were only listed when two different analyte peaks were used to analyze the same sample.

Table 15. Background Study #3. Analysis of TC, TIC, and TOC conducted on July 2, 1997. The samples were taken from a 50-liter carboy of site water refrigerated at 4°C.

Sample:	pH	Avg. pH
Site water #1:	6.44	6.43
Duplicate:	6.42	
Site water #2:	6.45	6.44
Duplicate:	6.43	

R^2 of Total Carbon calibration curve: 1.000
R^2 of Total Inorganic Carbon calibration curve: 1.000

Sample:	Total Carbon (mg/L)	Total IC (mg/L)	Total OC (mg/L)
Site water #1:	28.6	27.1	1.6
Site water #2:	29.4	28.8	0.6
Sample Blank:	0.9	1.5	< 0.1
WQ Lab Blank:	< 0.1	1.0	< 0.1
30 mg/L TC standard:	30.0	0.9	29.1

Concentrations with Blank Subtraction:

Sample:	Total Carbon (mg/L)	Total IC (mg/L)	Total OC (mg/L)
Site water #1:	27.7	26.6	1.2
Site water #2:	28.5	28.3	0.2
30 mg/L TC standard:	29.1	0.4	28.7

Average of Site Water #1 and #2:

Total Carbon: 28.1 (± 0.2) mg/L
Total IC: 27.5 (± 0.1) mg/L
Total OC (mg/L): 0.7 (± 0.2) mg/L

Table 16. Background Study #3. Analysis of chloride, bromide, nitrate, and sulfate conducted on July 16, 1997. The site water was collected from the carboy on July 2, 1997.

Sulfate Analysis:

R^2 value of calibration curve: 0.9914

Sample:	Sulfate Concentration (mg/L)
Site water:	11.7
Duplicate:	11.3
Sample Blank:	< 0.5

Average Sulfate Concentration = 11.5 (± 0.2) mg/ L

Chloride Analysis:

R^2 value of calibration curve: 0.9987

Sample:	Chloride Concentration (mg/L)
Site water:	14.53
Duplicate:	14.88
Sample Blank:	< 0.50

Average Chloride Concentration = 14.71 (± 0.09) mg/L

Bromide, nitrate-nitrogen, and ammonia concentrations were below the detection limits of 0.5 mg/L, 0.5 mg/L, and 0.1 mg/L, respectively.

Table 17. Background Study #3. Analysis of Ca, K, Mg, and Na conducted on July 3, 1997.
The samples were taken from a 50-liter carboy of site water refrigerated at 4°C.

R^2 for Ca393 calibration curve = 0.99776
R^2 for Ca396 calibration curve = 0.99985
R^2 for K calibration curve = 0.99934
R^2 for Mg calibration curve = 0.99850
R^2 for Na588 calibration curve = 0.99941
R^2 for Na589 calibration curve = 0.99935

Sample	Ca393 Conc. (mg/L)	Spike Recovery (%)	Ca396 Conc. (mg/L)	Spike Recovery (%)	K Conc. (mg/L)	Mg Conc. (mg/L)	Spike Recovery (%)	Na588 Conc. (mg/L)	Spike Recovery (%)	Na589 Conc. (mg/L)
Site water #1:	29.0		29.0		0.75	10.0		4.6		4.5
Duplicate:	29.3	89.1	29.1	92.5	0.74	9.6	97.0	4.7		4.7
Duplicate:	28.2		28.7			10.1		4.5	95.1	4.6
Duplicate:								5.1		4.7
Duplicate:								5.1		4.9
Site water #2:	28.7		29.4		0.78	10.0		4.9		4.7
Duplicate:	27.7		28.6		0.80	10.0		4.5		4.7
Duplicate:								4.5		4.2
Duplicate:								4.5		4.3
Sample Blank:	0.2		0.2		0.03	< 0.1		1.0		0.9
Duplicate:	0.2		0.2		0.03	< 0.1		1.0		0.9
QC: 2.0 mg/L	2.0		2.0		1.99	2.1		2.1		1.9
Duplicate:	2.0		2.0		2.03	2.1		2.0		1.9
Duplicate:	2.0		2.1		2.00	2.1		2.0		1.9

Average of Site Water #1 and #2, Including Blank Subtraction:

Sample:	Ca Conc. (mg/L)	K Conc. (mg/L)	Mg Conc. (mg/L)	Na Conc. (mg/L)
Site Water:	**28.6 (± 0.5)**	**0.74 (± 0.02)**	**10.0 (± 0.2)**	**3.7 (± 0.2)**

Table 18. Background Study #3. Analysis of Ba, Fe, and Mn conducted on July 3, 1997. The samples were taken from a 50-liter carboy of site water refrigerated at 4°C.

R^2 for Ba calibration curve = 0.99946
R^2 for Fe calibration curve = 0.99986
R^2 for K calibration curve = 0.99876
R^2 for Mn calibration curve = 0.99827

Sample	Ba Conc. (mg/L)	Spike Recovery (%)	Fe Conc. (mg/L)	Spike Recovery (%)	Mn Conc. (mg/L)	Spike Recovery (%)
Site water #1:	0.041	106.4	< 0.03	117.7	0.63	104.3
Duplicate:	0.047		< 0.03		0.64	
Site water #2:	0.042		< 0.03		0.65	
Duplicate:	0.040		< 0.03		0.61	
Sample Blank:	< 0.003		< 0.03		< 0.01	
QC: 0.5 mg/L Ba,Fe, Mn	0.500		0.49		0.51	
Duplicate:	0.509		0.50		0.51	

Average of Site Water #1 and #2, Including Blank Subtraction:

Sample:	Ba Conc. (mg/L)	Fe Conc. (mg/L)	Mn Conc. (mg/L)
Site Water:	**0.043**	**< 0.03**	**0.63**

Table 19. Analysis of pH, DO, TC, TIC, and TOC in a sample of site water spiked with sulfate and organic carbon. The analysis was conducted on July 31, 1997, just before and just after injection of the water into the column.

Spiked-site water:

	pH	DO (mg/L)
Reading #1:	6.55	10.6
Reading #2:	6.50	10.6
Reading #3:	6.56	10.5
Average:	6.54	10.6

R^2 of Total Carbon calibration curve: 1.000
R^2 of Total Inorganic Carbon calibration curve: 1.000

Sample:	Total Carbon (mg/L)	Total IC (mg/L)	Total OC (mg/L)
Spiked site water:	56.0	23.6	32.5
Duplicate:	55.6	23.4	32.2
Duplicate:	55.8	23.0	32.8
Sample Blank:	0.7	0.3	0.4
WQ Lab Blank:	0.7	< 0.1	0.7
Top Column:*	55.2	28.7	26.5
Bottom Column:*	54.7	23.8	31.0
Duplicate:	54.3	22.9	31.4
QC: 80 mg/L TC	80.77	< 0.1	80.8
QC: 60 mg/L TC	60.1	0.1	60.0

Average Concentrations with Blank Subtraction:

Sample:	Total Carbon (mg/L)	Total IC (mg/L)	Total OC (mg/L)
Spiked site water:	55.1 ($\pm$ 0.2)	23.0 ($\pm$ 0.1)	32.1 ($\pm$ 0.2)
Top Column:	54.5	28.4	26.1
Bottom Column:	53.8	23.0	30.8

* The top and bottom column samples are the spiked site water, just after injection into the column. The sample readings are a good indication of how much of the previous water was displaced from the column.

Table 20. Analysis of sulfate in a sample of site water spiked with sulfate and organic carbon. Conducted on July 31, 1997.

R^2 value of calibration curve: 0.9970

Sample	Sulfate Concentration (mg/L)
Spiked site water:	125.2
Duplicate:	125.4
Bottom Column:	114.5
Bottom Column w/ Zn Acetate:*	113.1
Top Column:	111.8
Sample Blank:	< 0.5

Average Sulfate Concentration:

Sample	Sulfate Concentration (mg/L)
Spiked site water:	125.3 (± 0.2)

* This sample was processed with sodium hydroxide and zinc acetate to insure that no significant changes in the sulfate concentration would occur by the addition of these compounds. For a more complete discussion of the zinc acetate method, see section titled "Analytical Techniques" in text.

Table 21. Analysis of sulfate, pH, and DO in a column sample taken on August 5, 1997. Conducted on August 5, 1997.

Sample	pH	DO
Column: 8/5/97	6.55	1.25
Duplicate:	6.55	1.25
Duplicate:	6.63	1.30
Average:	6.58	1.27

Sulfate check:* Sulfate concentration is greater than 100 mg/L.

* A sulfate calibration curve was not constructed. Instead, the sample intensity was compared to the intensity of the 100 mg/L standard. The original sulfate concentration of the column water was ~ 114.5 mg/L, thus, as long as the sulfate concentration in the sample was greater than 100 mg/L, a full calibration curve was not used. Also, several sulfate checks were conducted after this, but no sulfate reduction was seen, therefore, these were not reported.

Table 22. Analysis of pH, DO, TC, TIC, and TOC in a column sample taken and analyzed on August 22, 1997.

Sample:	pH	DO (mg/L)
Column: 8/22/97	6.56	1.27

R^2 of Total Carbon calibration curve: 1.000
R^2 of Total Inorganic Carbon calibration curve: 1.000

Sample:	Total Carbon (mg/L)	Total IC (mg/L)	Total OC (mg/L)
Column: 8/22/97	44.1	36.1	8.0
Duplicate:	44.0	36.5	7.5
Sample Blank:	0.5	< 0.1	0.5
QC: 60 mg/L TC	60.1	< 0.1	60.1
QC: 40 mg/L TIC	41.8	40.2	1.6
QC: 10 mg/L TC	10.4	< 0.1	10.4
QC: 10 mg/L TIC	11.2	10.1	1.1

Average Concentrations with Blank Subtraction:

Sample:	Total Carbon (mg/L)	Total IC (mg/L)	Total OC (mg/L)
Column: 8/22/97	43.6 ($\pm$ 0.2)	36.3 ($\pm$ 0.1)	7.3 ($\pm$ 0.2)

Table 23. Analysis of Fe and Mn conducted on August 28, 1997.

R^2 value for Mn calibration curve = 0.99959
All Fe concentrations were below detection, therefore, the data are not reported.

Sample:	Mn Concentration (mg/L)	% of spike recovery:
Tedlar Bag Sample:	4.53	87.0
Spiked site water from 7/31/97:*	0.66	94.5
Bottom Column: 7/31/97	0.95	94.0
Column: 8/5/97	2.22	85.2
Column: 8/11/97	2.52	80.9
Column: 8/19/97*	3.62	87.0
Column: 8/19/97*	3.61	
Column: 8/28/97	3.17	87.6
Duplicate:	3.20	
QC: 1.0 mg/L Mn	1.02	
Std: 1.0 mg/L Mn	1.00	

Average Sample Concentrations:

Sample:	Mn Concentration (mg/L)
Column: 8/28/97	3.19 (± 0.05)

* These samples were analyzed at the Energy and Environmental Research Center by myself and Charlene Crocker. Because these samples were not run on our instrument, they were not included in any charts or graphs in order to preserve the consistency of the measurements.

Table 24. Analysis of Fe, Mn, and Si conducted on September 17, 1997.

R^2 value for Mn257.610 calibration curve = 0.99456
R^2 value for Mn260.569 calibration curve = 0.99691
R^2 value for Si calibration curve = 0.99907
All Fe concentrations were below detection, therefore, the data are not reported.

Sample:	Mn257 Concentration (mg/L)	Mn260 Concentration (mg/L)	Si Concentration (mg/L)	% of spike recovery:
Column: 9/17/97	4.06	4.10	11.6	80.9, 82.1, 84.0
Column: 8/28/97	3.37	3.40	N.A.	80.3, 81.6, N.A.
Std: 5 mg/L Mn, Si	4.62	4.67	4.7	

Average Sample Concentrations:

Sample:	Mn Concentration (mg/L)	Si Concentration (mg/L)
Column: 9/17/97	4.08 ($\pm$ 0.05)	11.6 ($\pm$ 0.2)
Column: 8/28/97	3.39	N.A.

Table 25. Analysis of nutrient-spiked column water conducted on October 3 and 6, 1997. The sample was collected on October 2, 1997.

Ammonia-Nitrogen Analysis:

R^2 value of calibration curve: 0.9986

Sample:	Ammonia-Nitrogen Concentration (mg/L)
Nutrient-spiked column H_2O:	20.6
Duplicate:	21.6
Sample Blank:	0.2

Average Sample Concentration = 21.1 mg/ L

Average Nitrogen Concentration with Blank Subtraction = 20.9 mg/L

Phosphorus Analysis:

R^2 value of calibration curve: 0.9990

Sample:	Phosphorus Concentration (mg/L)
Nutrient-spiked column H_2O:	4.6
Sample Blank	< 0.5

Phosphorus Concentration = 4.6 mg/L

Table 26. Analysis of pH, DO, TC, TIC, and TOC of the site water, drained column water, and site water spiked with sulfate, organic carbon (OC), and nutrients. Samples taken and analyzed on October 27, 1997.

Sample:	pH	DO
Site water:	6.46	8.40
Duplicate:	6.45	8.35
Duplicate:	6.45	8.41
Average:	6.45	8.39

R^2 of Total Carbon calibration curve: 1.000
R^2 of Total Inorganic Carbon calibration curve: 1.000

Sample:	Total Carbon (mg/L)	Total IC (mg/L)	Total OC (mg/L)
Site water:	26.6	24.3	2.3
Duplicate:	26.6	24.4	2.3
Drained column w/ nutrients:	44.6	40.3	4.3
Duplicate:	44.6	40.4	4.1
Site water w/ sulfate and OC:	31.6	24.0	7.6
Sample Blank:	0.8	0.4	0.3
40 mg/L TC standard:	40.3	0.4	39.9

Average Concentrations with Blank Subtraction:

Sample:	Total Carbon (mg/L)	Total IC (mg/L)	Total OC (mg/L)
Site water:	25.9 ($\pm$ 0.2)	23.9 ($\pm$ 0.1)	2.0 ($\pm$ 0.2)
Drained column w/ nutrients:	43.8	39.9	3.9
Site water w/ sulfate and OC:	30.9	23.6	7.3

Table 27. Analysis of sulfate in the drained column water, the site water spiked with sulfate, organic carbon (OC) and nutrients, and the site water spiked with sulfate, organic carbon, and nutrients. Samples taken and analyzed on October 27, 1997.

R^2 value of sulfate calibration curve: 0.9961

Sample	Sulfate Concentration (mg/L)
Drained column with nutrients:	111.5
Duplicate:	113.7
Sulfate and OC-spiked site water:	113.7
Sulfate, OC and nutrient-spiked site water:	113.0
Sample Blank:	< 0.5

Table 28. Analysis of DO, TC, TIC, and TOC in a sample of column water. The sample was collected and analyzed on October 29, 1997.

	DO (mg/L)
Column Sample: 10/29/97	2.10

R^2 of Total Carbon calibration curve: 1.000
R^2 of Total Inorganic Carbon calibration curve: 1.000

Sample:	Total Carbon (mg/L)	Total IC (mg/L)	Total OC (mg/L)
Column: 10/29/97	32.1 (± 0.2)	28.4 (± 0.1)	3.7 (± 0.2)
Std: 50 mg/L TC	50.2	< 0.1	50.2

Table 29. Analysis of Fe, Mn, and Si conducted on October 29, 1997.

R^2 value for Mn calibration curve = 0.99779
R^2 value for Si calibration curve = 0.99990
All Fe concentrations were below detection (< 0.03), therefore, the data are not reported.

Sample:	Mn Concentration (mg/L)	Spike Recovery (%)	Si Concentration (mg/L)	Spike Recovery (%)
Tedlar Bag Sample: 10/27/97	2.37 (± 0.05)		14.3 (± 0.2)	
Spiked Site Water: 10/27/97	1.04	106.2	21.9	93.0
Bottom Column: 10/27/97	1.60		18.4	104.0
Top Column: 10/27/97	2.58		12.3	85.2
QC: 3 mg/L Mn,15 mg/L Si	3.06		16.3	
Std: 3 mg/L Mn,10 mg/L Si	2.79		9.6	
Std: 3 mg/L Mn,10 mg/L Si	2.86		9.3	
Std: 0.6 mg/L Mn, 2 mg/L Si	0.71		2.0	

Table 30. Analysis of pH, DO, Fe, Mn, and Si conducted on November 3, 1997.

	pH	DO (mg/L)
Column Sample: 11/3/97	6.48	1.10

R^2 value for Mn257.610 calibration curve = 0.99968
R^2 value for Mn260.569 calibration curve = 0.99979
R^2 value for Si calibration curve = 0.99998
All Fe concentrations were below detection, therefore, the data are not reported.

Sample:	Mn257 Concentration (mg/L)	Spike Recovery (%)	Mn260 Concentration (mg/L)	Spike Recovery (%)	Si Concentration (mg/L)	Spike Recovery (%)
Column: 10/27/97	2.09		2.05		N.A.	
Column: 10/31/97	2.34	95.7	2.28	95.5	14.9	81.8
Duplicate:	2.31		2.34		15.1	
Column: 11/3/97	2.40		2.65		N.A.	
Duplicate:	2.55		2.44		12.6	
QC: 3 mg/L Mn, 10 mg/L Si	3.11		3.08		9.7	
QC: 3 mg/L Mn, 10 mg/L Si	2.75		2.90		10.1	
Sample Blank:	< 0.01		< 0.01		< 0.04	

Average Sample Concentrations:

Sample:	Mn Concentration (mg/L)	Si Concentration (mg/L)
Column: 10/27/97	2.07 (± 0.05)	N.A.
Column: 10/31/97	2.32	15.0 (± 0.2)
Column: 11/3/97	2.51	12.6

Table 31. Analysis of pH, DO, Fe, Mn, and Si conducted on November 10, 1997.

	pH	DO (mg/L)
Column Sample: 11/10/97	6.58	0.83

R^2 value for Mn257.610 calibration curve = 0.99904
R^2 value for Mn260.569 calibration curve = 0.99920
R^2 value for Si calibration curve = 0.99998
All Fe concentrations were below detection, therefore, the data are not reported.

Sample:	Mn257 Concentration (mg/L)	Spike Recovery (%)	Mn260 Concentration (mg/L)	Spike Recovery (%)	Si Concentration (mg/L)	Spike Recovery (%)
Column: 11/10/97	3.02	96.0	2.89	92.5	14.9	107.0
Duplicate:	2.98		2.93		15.1	
Column: 11/6/97	2.82	103.2	2.79	104.2	16.0	109.2
Duplicate:	2.96		2.91		15.6	
QC: 4 mg/L Mn,15 mg/L Si	3.79		3.80		15.3	
Std: 3 mg/L Mn,10 mg/L Si	2.98		3.02		9.9	
Sample Blank:	< 0.01		< 0.01		< 0.04	

Average Sample Concentrations:

Sample:	Mn Concentration (mg/L)	Si Concentration (mg/L)
Column: 11/10/97	2.96 ($\pm$ 0.05)	15.0 ($\pm$ 0.2)
Column: 11/6/97	2.87	15.8

Table 32. Analysis of TC, TIC, and TOC in a sample of column water. The sample was collected and analyzed on November 17, 1997.

R^2 of Total Carbon calibration curve: 1.000
R^2 of Total Inorganic Carbon calibration curve: 1.000

Sample:	Total Carbon (mg/L)	Total IC (mg/L)	Total OC (mg/L)
Column: 11/17/97	34.8 (± 0.2)	31.3 (± 0.1)	3.5 (± 0.2)
Sample Blank:	0.8	< 0.1	0.8
QC: 50 mg/L TC	49.6	< 0.1	49.6
QC: 50 mg/L TIC	49.9	50.0	< 0.1

Table 33. Analysis of Fe, Mn, and Si conducted on November 17, 1997.

R^2 value for Mn257.610 calibration curve = 0.99891
R^2 value for Mn260.569 calibration curve = 0.99901
R^2 value for Si calibration curve = 0.99934
All Fe concentrations were below detection, therefore, the data are not reported.

Sample:	Mn257 Concentration (mg/L)	Spike Recovery (%)	Mn260 Concentration (mg/L)	Spike Recovery (%)	Si Concentration (mg/L)	Spike Recovery (%)
Column: 11/17/97	3.27	94.3	3.11	87.7	14.6	103.4
Duplicate:	3.22		3.21		14.7	
Duplicate:	N.A.		3.11	85.0	14.2	107.6
QC: 4 mg/L Mn, 15 mg/L Si	4.06		4.03		16.3	
Std: 3 ppm Mn,10 ppm Si	3.19		3.11		11.1	
Sample Blank:	< 0.01		< 0.01		< 0.04	

Average Sample Concentrations:

Sample:	Mn Concentration (mg/L)	Si Concentration (mg/L)
Column: 11/17/97	3.24 ($\pm$ 0.05)	14.49 ($\pm$ 0.2)

Table 34. Analysis of Fe and Mn conducted on November 24, 1997.

R^2 value for Mn257.610 calibration curve = 0.99992
R^2 value for Mn260.569 calibration curve = 0.99999
All Fe concentrations were below detection, therefore, the data are not reported.

Sample:	Mn257.610 Conc. (mg/L)	Spike Recovery (%)	Mn260.569 Conc. (mg/L)	Spike Recovery (%)
Column: 11/24/97	3.47		3.32	
Duplicate:	3.28	95.2	3.47	92.1
Duplicate:	3.63		3.37	
QC: 4 mg/L Mn	4.09		4.11	
Std: 1.5 mg/L Mn	1.60		1.55	
Sample Blank	< 0.01		< 0.01	

Average Sample Concentrations:

Sample:	Mn Concentration (mg/L)
Column: 11/24/97	3.42 ($\pm$ 0.05)

Table 35. Analysis of Fe and Mn conducted on December 4, 1997.

R^2 value for Fe calibration curve = 0.99924
R^2 value for Mn calibration curve = 0.99869

Sample:	Fe Concentration (mg/L)	Spike Recovery (%)	Mn Concentration (mg/L)	Spike Recovery (%)
Column: 12/2/97	0.88	96.4	4.86	90.0
Duplicate:	0.93		4.97	
Column: 12/4/97	0.91	92.8	4.57	95.7
Std: 3 mg/L Fe, Mn	3.09		3.05	
Std: 0.6 mg/L Fe, Mn	0.66		0.65	
Sample Blank:	< 0.03		< 0.01	

Average Sample Concentrations:

Sample:	Fe Concentration (mg/L)	Mn Concentration (mg/L)
Column: 12/2/97	0.91 ($\pm$ 0.01)	4.92 ($\pm$ 0.05)
Column: 12/4/97	0.91	4.57

Table 36. Analysis of TC, TIC, and TOC in a sample of column water. The sample was collected and analyzed on December 8, 1997.

R^2 of Total Carbon calibration curve: 1.000
R^2 of Total Inorganic Carbon calibration curve: 1.000

Sample:	Total Carbon (mg/L)	Total IC (mg/L)	Total OC (mg/L)
Column: 12/8/97	41.6 (± 0.2)	32.9 (± 0.1)	8.7 (± 0.2)
QC: 100 mg/L TC, 50 mg/L TIC	101.1	49.1	52.0
QC: 40 mg/L TC	40.5	< 0.1	40.5

Table 37. Analysis of Fe and Mn conducted on February 12, 1998.

R^2 for Fe calibration curve = 0.99983
R^2 for Mn257 calibration curve = 0.99984
R^2 for Mn260 calibration curve = 0.99972

Sample	Fe Concentration (mg/L)	Spike Recovery (%)	Mn257 Concentration (mg/L)	Spike Recovery (%)	Mn260 Concentration (mg/L)	Spike Recovery (%)
Column: 12/11/97	1.51		4.28		4.24	
Duplicate:	1.51	98.2	N.A.	96.5	4.21	98.6
Duplicate:	1.52		4.41		4.33	
Duplicate:	1.52		4.40		N.A.	
Column: 12/23/97	1.48		4.21		4.07	
Duplicate:	1.47		4.17		4.16	
Column: 1/3/98	1.72		N.A.		4.11	
Duplicate:	1.70	86.8	4.28	84.7	4.26	90.5
Duplicate:	1.68		4.28		4.15	
Column: 1/16/98	1.90		4.31		4.16	
Duplicate:	1.90		4.27		4.09	
Column: 2/12/98	2.59	95.8	4.46	88.6	4.37	88.6
Duplicate:	2.53		4.43		4.34	
QC: 3.5 mg/L Fe, Mn	3.64		3.49		3.42	
Std: 3.0 mg/L Fe, Mn	3.13		3.05		3.00	

Average Sample Concentrations:

Sample	Fe Concentration (mg/L)	Mn Concentration (mg/L)
Column: 12/11/97	1.512 (± 0.01)	4.31 (± 0.05)
Column: 12/23/97	1.47	4.15
Column: 1/3/98	1.70	4.23
Column: 1/16/98	1.90	4.21
Column: 2/12/98	2.56	4.40

Table 38. Analysis of TC, TIC, and TOC in a sample of column water. The sample was collected and analyzed on February 23, 1998.

R^2 of Total Carbon calibration curve: 1.000
R^2 of Total Inorganic Carbon calibration curve: 1.000

Sample:	Total Carbon (mg/L)	Total IC (mg/L)	Total OC (mg/L)
Column: 2/23/98	44.4	39.2	5.2
Duplicate:	44.4	39.1	5.4
Sample Blank:	0.6	0.4	0.2
QC: 50 mg/L TC, 50 mg/L TIC	50.7	49.6	1.1

Average Sample Concentration with Blank Subtraction:

Sample:	Total Carbon (mg/L)	Total IC (mg/L)	Total OC (mg/L)
Column: 2/23/98	43.8 ($\pm$ 0.2)	38.8 ($\pm$ 0.1)	5.1 ($\pm$ 0.2)

Appendix V

Nutrient Spike

Nutrient Spike

Originally, the nutrients were going to be added to the column water in a manner that disrupted the system as little as possible. The column water was drained into a vacuum-sealed Tedlar bag (similar to a plastic bag) to keep the water from coming in contact with the atmosphere. An 8-liter volume of site water was stripped of its oxygen through purging with argon gas, and used as a reservoir to replace the water as it drained from the column. The initial plan was to spike the drained column water with 2 mg/L nitrogen and 0.5 mg/L phosphorus, and re-inject the water into the column. However, I made a conversion error and added 10 times the desired concentration of nutrients. To compensate for this error, I decided to dilute a portion of the drained column water with refrigerated site water by a factor of 1 to 10. The pore volume of the column is about 8 liters; therefore, 0.9 liters of column water were diluted with 8 liters of site water, resulting in a dilution factor of 10 and a volume large enough to displace the water within the column. However, the site water needed to be adjusted so that the concentrations of organic carbon and sulfate were the same as those contained within the drained column water. Therefore, 8 liters of site water were spiked with organic carbon and sulfate concentrations equal to those within the drained column water. An additional amount of organic carbon was added to reduce the dissolved oxygen contained within the site water. After dilution, the concentration of nutrients in the mixture of site and column water was approximately 0.5 mg/L phosphorus and 2.1 mg/L nitrogen. The nutrient-spiked water was injected into the column on October 27, 1997 at a rate of 17 ml per minute.

Appendix VI

Column Inoculation

Column Inoculation

To insure the presence of sulfate-reducing bacteria, the column water was inoculated on November 26, 1997, with sample of wastewater from the final clarifier of a pilot biological wastewater treatment process (H. Gullicks, personal communication). To inject the wastewater into the column, approximately 1.5 liters of column water were drained into a Tedlar bag. The bag had been repeatedly flushed with argon gas to remove any oxygen. A 5-ml aliquot of wastewater was injected into the Tedlar bag through a septum valve. The bag was agitated to mix the wastewater with the column water before being pumped back into the column. Because of the 1 to 300 dilution factor, concentrations of any species contained within the wastewater would not have significantly affected the column water composition.

Appendix VII

Calcium Carbonate Analysis of the Column Sediment

Calcium Carbonate Analysis

The technique used to analyze the sediment for calcium carbonate involved a simple titrimetric procedure from Methods of Soil Analysis (SSSA). A sample of sediment was acidified with hydrochloric acid in a closed flask that contained a small vial of potassium hydroxide suspended above the sediment. Upon acidification, any carbonate in the sediment was converted to carbon dioxide (CO_2). The CO_2 reacted with the hydroxide ions (OH^-) in the potassium hydroxide to form CO_3^{2-}. After 24 hours, the vial containing the potassium hydroxide and CO_3^{2-} was titrated with hydrochloric acid to the phenolphthanlein endpoint (pH = 8.2). At this pH, the CO_3^{2-} was converted to HCO_3^-. The solution was then titrated with hydrochloric acid to the bromocresol green endpoint (pH = 4.5), at which point the HCO_3^- was converted to CO_2 (H_2CO_3*). The inorganic carbon content of the soil is proportional to the amount of hydrochloric acid consumed in the last titration.

The above procedure was conducted on four sediment samples. The weight percent of calcium carbonate for each of the four samples was: 2.14×10^{-3}, 1.63×10^{-2}, 4.88×10^{-3}, and 2.27×10^{-2}. Although these four values vary by an order of magnitude, this is within the margin of error of the experiment based on the accuracy of the scale used to weigh the sediment (accurate to four decimal places). It would only take one or two extra grains of calcium carbonate to account for the difference between the four

values. The average of these values is 1.15×10^{-2} weight percent calcium carbonate. To determine the amount of inorganic carbon this could produce if all of it dissolved into the column water, the following calculation was made:

Assume a sediment volume of 1 m^3, with a porosity of 0.37 and a density of 2.67 g/cm^3:

$(1\ m^3) \times (0.63) \times (2.67\ g/cm^3) \times [(100\ cm)^3/(1\ m)^3] = 1.68 \times 10^6$ g of sediment

Of this sediment, 1.15×10^{-2} % is calcium carbonate:

$(1.68 \times 10^6\ g) \times (1.15 \times 10^{-4}) = 193.44$ g of calcium carbonate

The porosity of the column sediment is 0.37, therefore, in 1 m^3 of sediment (or in 1000 L), there would be 370 L of water. That gives:

$(193.44\ g\ CaCO_3) / (370\ L\ H_2O) = 0.523\ g\ CaCO_3 / L = 523\ mg\ CaCO_3 / L$

In terms of inorganic carbon, this amount equals 62.8 mg / L of IC.

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