

Natural Denitrification in the Saturated Zone: A Review

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Denitrification is increasingly recognized for its ability to eliminate or reduce nitrate concentrations in groundwater. With this awareness comes a desire to predict the rate and extent of denitrification in aquifers. The limiting factor in making predictive models, however, is our limited knowledge of the physical characteristics of this process. This review synthesizes the published literature on natural aquifer denitrification. A background section discusses denitrification requirements and dissimilatory nitrate reduction to ammonium, which occurs in environments similar to those where denitrification occurs, and gives a historical perspective on denitrification. Other sections discuss denitrification with organic carbon serving as the electron donor (heterotrophic denitrification) and with reduced inorganic compounds serving as the electron donor (autotrophic denitrification). The section on heterotrophic denitrification is structured around two tables that summarize natural aquifer denitrification rates reported by laboratory studies and natural aquifer denitrification rates reported by field studies. The section on autotrophic denitrification discusses denitrification with reduced iron and reduced sulfur. Thus far, most studies only consider a single electron donor or donor type, whether heterotrophic or autotrophic. This review demonstrates, however, that multiple electron donors may be present in a given aquifer. Future research efforts are recommended to determine the factors affecting the availability of electron donors and their denitrification rates. Additional research is also suggested on how dissolved oxygen affects denitrification rates and on the factors influencing the partitioning of nitrate reduction products to nitrous oxide, a potential contributor to the destruction of the ozone layer, and to ammonium.

INTRODUCTION

Nitrate (NO_3^-) is the most common groundwater contaminant and, because of growing anthropogenic sources, NO_3^- pollution is increasing [Freeze and Cherry, 1979]. Nitrate is a stable nitrogen (N) species under certain natural conditions [Stumm and Morgan, 1981] and forms compounds that are highly soluble [Hook, 1983]. These characteristics allow NO_3^- to be transported in some groundwater systems to environments where it can be converted to N species that either promote surface water eutrophication or are hazardous to humans, livestock, and the environment.

Hallberg [1989] reported that agriculture is the most extensive anthropogenic source of NO_3^- to groundwater systems. Examples of such sources include the application of N fertilizers, mismanagement of irrigated crops, the disposal of livestock waste [Hallberg, 1989], and the cultivation of virgin land [e.g., Ronen *et al.*, 1983; Faillat, 1990].

With these concerns it is advantageous from a water quality perspective to exploit any process in the N cycle that functions as a sink for N in groundwater. Denitrification fulfills this criterion. In this process, bacteria in an anaerobic environment use NO_3^- as a terminal electron (e^-) acceptor in their metabolic processes. As shown later, the denitrification pathway terminates with molecular nitrogen (N_2). In this form the triple bond between the N atoms resists further chemical change. Additionally, once the concentration of N_2 in the groundwater exceeds saturation, it tends to migrate out of the saturated zone (the molar solubility of N_2 in water is approximately half the molar solubility of O_2 in water [Böckle *et al.*, 1984]).

Denitrification has been studied intensively with respect to surface water/sediment interfaces, septic tank systems, soil

environments, and water treatment processes. Denitrification in aquifers below the root zone, however, is a more recent addition to the literature. Only one comprehensive review has been written that focuses on this topic; Mariotti [1986] reviewed three methods for identifying groundwater denitrification.

A continued interest is likely in aquifer denitrification because it can naturally remove NO_3^- from groundwater and also for the following reasons:

1. Aquifers are being used as "bioreactors." An aquifer's natural denitrification ability can be increased by adding e^- donor amendments such as sucrose and methanol to encourage in situ denitrification.
2. Nitrate is added as an e^- acceptor amendment to anaerobic sites in aquifers contaminated with hazardous organic compounds to encourage in situ biological degradation of these wastes.
3. Denitrification releases N_2O to the atmosphere, which is believed to contribute to the "greenhouse effect" and to the destruction of the ozone layer.

With the current and expected interest in aquifer denitrification, an important goal is to attain predictive capabilities for it. Kinzelbach *et al.* [1990, p. 322], in a recent review on numerical groundwater quality models with a focus on nitrate transport in the saturated zone, concluded that present models' predictive capabilities are limited. The cause of this limitation is not in our modeling techniques, but by "limited knowledge of the parameters, data and boundary conditions." It is my hope that this review on natural groundwater denitrification in the saturated zone provides a synthesis of our current knowledge and a guide for our future efforts in understanding and modeling this process. Because of the paucity of groundwater data for some of the topics considered, data from other environments, such as water/sediment interfaces and soils, are considered. These instances are noted in the text.

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BACKGROUND

It is shown elsewhere [Korom, 1991a] that once NO_3^- is leached below the root zone there are four possible "fates" (other than continued leaching) that await it. These are soil retention, assimilatory reduction into microbial biomass, dissimilatory nitrate reduction to ammonium (DNRA), and denitrification. Only the latter may serve as a major N sink; the others only temporarily immobilize N. Of those that retard the groundwater transport of N, DNRA is a particularly important process for those studying denitrification because of its similarities to denitrification. Thus subsections on both denitrification and DNRA follow. These are followed by another subsection that gives a historical perspective on denitrification in aquifers.

Denitrification

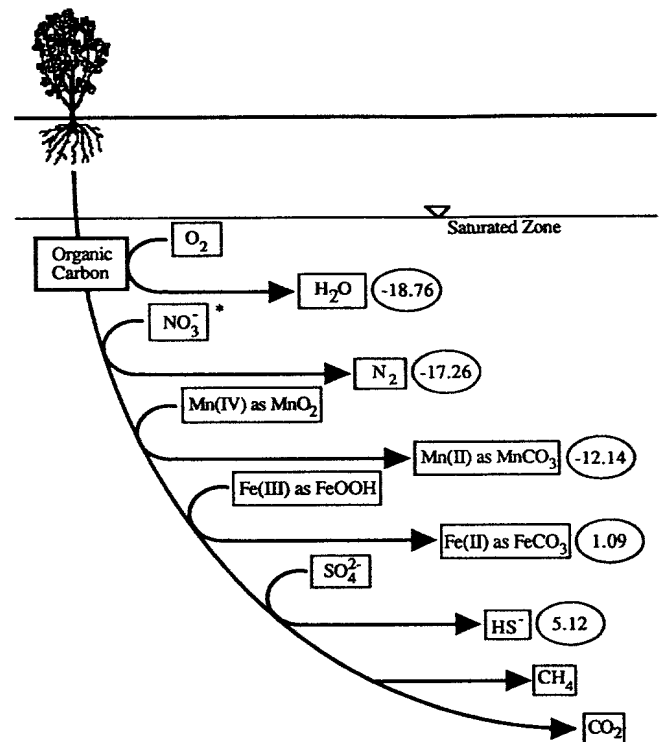
Denitrification refers to the microbially mediated process whereby NO_3^- is reduced to N_2O or N_2 . This process also goes by the less popular names of "biological denitrification" [Tiedje, 1982] and "enzymatic denitrification" [Paul and Clark, 1989]. "Chemodenitrification" refers to the abiotic process whereby nitrite (NO_2^-) is chemically reduced to N_2 and other gaseous nitrous oxides [Paul and Clark, 1989]. It is somewhat of a misnomer, however, as previous reviewers [Nelson, 1982; Chalk and Smith, 1983] indicated that the presence of NO_2^- in a soil typically results from biotic processes involving nitrifying or denitrifying bacteria. It is therefore debatable whether chemodenitrification removes NO_3^- from a system independent of bacteria. Hereinafter, any reference to biological denitrification will simply be termed denitrification.

The four general requirements for denitrification are [Firestone, 1982]: (1) N oxides (NO_3^- , NO_2^- , NO, and, N_2O) as terminal e^- acceptors, (2) the presence of bacteria possessing the metabolic capacity, (3) suitable e^- donors, and (4) anaerobic conditions or restricted O_2 availability. The first requirement is assumed in this review. The last three requirements are discussed below.

Denitrification requirements. The physiological property of denitrification belongs exclusively to bacteria; however, not all bacteria denitrify [Tiedje, 1982]. The ones that do are very diverse [Knowles, 1982]. As such, it is useful to define some terms for their classification.

Bacteria in aquifers obtain energy from the oxidation of organic or inorganic compounds (as opposed to gaining energy from the sun) and hence are chemotrophs. This designation is implied for all bacteria discussed in this review. If the e^- donor is organic, the organism is organotrophic. If the e^- donor is inorganic, the organism is lithotrophic. Inorganic e^- donors found in groundwater include reduced manganese (Mn^{2+}), ferrous iron (Fe^{2+}) and sulfides. An organotroph virtually always uses its organic energy source as a source for cellular carbon; thus it is also heterotrophic. [Brock and Schlegel, 1989]. Additionally, most lithotrophs obtain carbon from inorganic carbon dioxide (CO_2); thus they are also autotrophic [Brock and Schlegel, 1989]. Most authors seem to differentiate denitrifiers as either heterotrophic or autotrophic, implying that the bacteria either require organic carbon (OC) or do not require OC for growth and maintenance. This terminology is retained.

Payne [1981] catalogued known heterotrophic and autotrophic denitrifiers. Most of them are heterotrophic. Most



* See discussion in text concerning the reduction of nitrate by denitrification or dissimilatory nitrate reduction to ammonium.

Fig. 1. Oxidation of organic carbon in the saturated zone with the sequence of electron acceptors and the resulting reduced inorganic compounds (adapted from Jørgensen [1989]). Values in ellipses are the Gibbs free energies released (if <0) or consumed (if >0) by the electron-acceptor half reactions (in kilocalories/electron). Values for the Gibbs free energies were calculated from electron activities given by Stumm and Morgan [1981, Table 7.5]. This figure additionally depicts a generalized progression of reactions with depth below the water table.

are also facultative anaerobes [Firestone, 1982], that is, organisms that are capable of survival with or without O_2 .

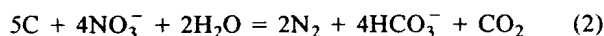
As stated above, all bacteria need an e^- donor as an energy source. To complete the oxidation-reduction (redox) reaction, an e^- acceptor is also required. Figure 1 (adapted from Jørgensen [1989] for a water/sediment interface) is a schematic representation showing the fate of organic matter, nature's most common e^- donor, in the presence of a variety of e^- acceptors in the saturated zone. Thermodynamically, OC tends to be oxidized preferentially by the e^- acceptor that yields the most energy to the bacteria [Stumm and Morgan, 1981]. In the saturated zone, therefore, aerobic bacteria use O_2 to oxidize OC until oxygen supplies become limiting. At this point, facultative anaerobes switch to using NO_3^- and O_2 as e^- acceptors. As O_2 levels decrease, obligate anaerobes begin to use nonoxygenous e^- acceptors. When O_2 levels increase, aerobic bacteria will return to O_2 respiration because of the increased energy economy. The cutoff point where O_2 concentrations are great enough for the cessation of denitrification varies among organisms. Robertson and Kuenen [1984a] isolated an organism (*Thiosphaera pantotropha*) from a wastewater treatment system that uses both O_2 and NO_3^- , albeit mostly O_2 , as terminal e^- acceptors at dissolved oxygen (DO) concentrations to 90% of air saturation [Robertson and Kuenen 1984b]. This corre-

given by Korom [1991a]. It revealed that denitrifying bacteria were typically found in both sample types. In some cases where they were not found, it may have been related to the enumeration method used [e.g., Trudell *et al.*, 1986] or because the aquifer consisted of material with physical characteristics unsuitable for bacteria (e.g., limestone with pore spaces too small for bacteria [Whitelaw and Rees, 1980]). Other studies on aquifer denitrification are reviewed in the remaining portion of this work. The next section reviews heterotrophic denitrification in aquifers.

HETEROTROPHIC DENITRIFICATION IN AQUIFERS

This section is structured around two tables that summarize findings on aquifer denitrification. The majority of the references given in these tables are for heterotrophic denitrification. Some autotrophic references are noted in the tables and in this section but are discussed more fully in the following section.

Below is an example of a heterotrophic denitrification reaction [Kölle *et al.*, 1983]:



Here, C represents an arbitrary organic compound with an oxidation state of zero. Naturally occurring organic compounds are numerous and diverse [Thurman, 1985] and may exist with their C atoms having a variety of different oxidation states [Stumm and Morgan, 1981]. This usually makes it unsatisfactory to model heterotrophic denitrification with a single organic compound serving as an e^- donor.

Table 1: Laboratory Denitrification Rates
From Aquifer Samples

Table 1 gives laboratory denitrification rates from aquifer samples. Some of the references listed on it report denitrification rates with samples amended with an OC source; however, only data that show the natural denitrification potential of aquifer sediments are included in this table. Additionally, denitrification rates from groundwater samples are not included in this table. Since most denitrifying bacteria seem to be attached to an aquifer's porous matrix [Korom, 1991a], denitrification rates in water samples are expected to be lower than rates with core samples and therefore are not representative of an aquifer's natural denitrification potential. There is evidence that this is, indeed, true [Smith and Duff, 1988].

Lind [1983] is the only study on Table 1 that included both in situ temperatures (10°C) and temperatures more typical of laboratory conditions (25°C). As expected, the cooler temperatures reduced microbial activity and led to slower denitrification rates. The rate increases with temperature varied considerably in Lind's three sites. However, the rates at in situ temperatures are all within an order of magnitude and compare well with the reported rates at similar temperatures given by Slater and Capone [1987], Bengtsson and Annadotter [1989], and Smith *et al.* [1991], that is, $\sim 10^{-1}$ mg N kg⁻¹ dry sediment per day. As discussed later, the denitrification reported by Lind may be autotrophic.

Francis *et al.* [1989] found that samples taken from depths down to 289 m had denitrification potential. They also found that samples with $\leq 50\%$ sand and $>30\%$ clay had no measurable denitrification at in situ concentrations of NO_3^- . The practical significance of this finding, however, is questionable

since the NO_3^- concentrations involved were too low to be of concern from a water quality perspective, that is, $\sim 10^{-1}$ mg NO_3^- -N L⁻¹. When NO_3^- was added, the denitrification rates were similar to the others reported in Table 1.

Bengtsson and Annadotter [1989] reported the only laboratory results that include aerobic incubations. They added small amounts of Na¹⁵NO₃ to microcosms of aquifer sediments. They measured the ratio of this N isotope to the predominant isotope of N in nature (¹⁵N/¹⁴N) in the resulting N compounds and compared this ratio to a standard value. In this way they traced how the NO_3^- in the microcosm was reduced. They determined for the aerobic microcosm that 89% of the ¹⁵NO₃⁻ was reduced to ¹⁵N₂, 25% of the ¹⁵NO₃⁻ was reduced to ¹⁵NH₄⁺ (DNRA), and $<0.01\%$ of the ¹⁵NO₃⁻ was assimilated into cell material. The DO concentrations of 9.9 mg L⁻¹ in the aerobic microcosm were reduced to 1.3 mg L⁻¹ in the effluent. They found in the anaerobic microcosm that 36% of the ¹⁵NO₃⁻ was reduced to ¹⁵N₂ and 55% of the ¹⁵NO₃⁻ was reduced to ¹⁵NH₄⁺ (DNRA). The DO concentrations remained at 0.6 mg L⁻¹ throughout the anaerobic experiment. No ¹⁵NO₃⁻ was detected in the effluent of either of these microcosms. The mean residence time in the microcosms was 5 days. A summation of the percentages of the reduced N species given above shows that they do not sum to 100%. The aerobic microcosm had a positive imbalance of 14%; the anaerobic microcosm had a negative imbalance of 9%. Despite these concerns, this study demonstrates that "aerobic" denitrification and the partitioning between denitrification and DNRA in aquifers are topics for further research.

Smith and Duff [1988] reported on a site where denitrification has reduced high influent NO_3^- concentrations. The site was a sewage-contaminated groundwater plume located on Cape Cod, Massachusetts. Near the contaminant source, NO_3^- -N concentrations up to 24 mg L⁻¹ were measured. The center of the plume 0.25 km downgradient had no detectable NO_3^- -N concentrations. This study is also one of the few that considered the possibility that NO_3^- can also undergo DNRA. They found no evidence of DNRA in the aquifer cores sampled. They also determined that this aquifer is carbon limited, that is, OC concentrations are limiting the denitrification process, not NO_3^- concentrations. The hypothesis of Tiedje *et al.* [1982] given earlier supports this observation; that is, NO_3^- reduction in carbon-limited aquifers favors denitrification over DNRA. Subsequent research at this site by Smith *et al.* [1991], using an isotopic fractionation method that is discussed later, did find evidence of DNRA but concluded that denitrification was predominantly responsible for the observed NO_3^- reduction.

The microcosms of Obenhuber and Lowrance [1991] from the Claiborne aquifer in south central Georgia also were carbon limited. Only those amended with glucose showed evidence of significant amounts of denitrification.

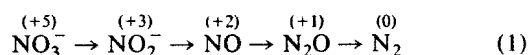
Unfortunately, the references given in Table 1 reveal little on the kinetics of denitrification at concentrations of concern for groundwater quality. Some references [Slater and Capone, 1987; Morris *et al.*, 1988; Smith and Duff, 1988] provide evidence that the kinetics of denitrification at concentrations >1 mg NO_3^- -N L⁻¹ are zero order, that is, independent of NO_3^- concentration. However, data given by Morris *et al.* [1988] and Smith and Duff [1988] can also be interpreted as showing a concentration dependence. There are not enough data in these studies to allow definite conclusions to be made.

sponds to a DO concentration of 6.9 mg L^{-1} (at sea level and a temperature of 37°C [American Public Health Association *et al.*, 1985]). Typically, however, most bacteria switch to using NO_3^- as an e^- acceptor at much lower DO concentrations [Robertson and Kuenen, 1984a].

Once DO is removed from the saturated zone it is replaced by diffusion from the unsaturated zone and by advection and/or diffusion from oxygenated regions of the aquifer. Typically, these processes are slow. Resulting oxygen deficits facilitate the use of other e^- acceptors in the oxidation of OC.

As shown in Figure 1, NO_3^- is the next e^- acceptor to oxidize OC. This is heterotrophic denitrification. It helps explain why NO_3^- is often found in much greater concentrations near the saturated surface than at depth in the aquifer. This phenomenon is referred to later in this section. After NO_3^- concentrations become limited, manganese (IV) and ferric iron (III) and then sulfate (SO_4^{2-}) are reduced. These latter reduced nonnitrogenous compounds are also important in aquifer denitrification. If NO_3^- is introduced into a manganese/iron or sulfate-reducing zone, even in the absence of OC, it is thermodynamically unstable. If the appropriate bacteria are present, they may denitrify using reduced manganese, reduced iron, and sulfides as e^- donors. This is autotrophic denitrification. The distinction between heterotrophic and autotrophic denitrification is very important. Most researchers consider only one or the other in their work. Nature, however, is not always so discriminating.

The pathway showing the e^- -accepting (reducing) steps beginning with NO_3^- is shown below with the oxidation state of the N atom (or atoms) in each molecule shown above it (or them) in parentheses [Firestone, 1982; Payne, 1981]:



The role of nitric oxide (NO) in this pathway is controversial [Tiedje, 1982]. Most denitrifiers can complete the entire pathway, but some can only mediate portions of the pathway [Tiedje, 1988].

Dissimilatory Nitrate Reduction to Ammonium

Both dissimilatory nitrate reduction to ammonium and denitrification are dissimilatory reduction processes in that they make energy available to the cell for growth and maintenance [Harris, 1982]. Both also occur in anaerobic environments. Dissimilatory reduction of NO_3^- to ammonium (NH_4^+) generally conserves a system's N; denitrification results in a N loss from the system. Tiedje *et al.* [1982] discussed the competition between DNRA and denitrification for available NO_3^- supplies and hypothesized that DNRA is favored when NO_3^- (e^- acceptor) supplies are limiting, and denitrification is favored when carbon (e^- donor) supplies are limiting. Data substantiating this conclusion in aquifers are scarce. This is unfortunate, as Tiedje *et al.* [1982] showed that in some natural systems DNRA can successfully compete with denitrification for available NO_3^- supplies. If NH_4^+ produced by DNRA later encounters aerobic conditions, such as from a receding water table, it may be converted back to NO_3^- via nitrification. High groundwater gradients, such as those often encountered in the spring, may flush the NO_3^- through the aquifer to a receiving body of water, well field, or other location where it is not wanted.

Historical Perspective

Payne [1981] reviewed the history of our observation of denitrification beginning with the introduction of the term "denitrification" in 1882 by U. Gayon and G. Dupetit. The history of our awareness of groundwater denitrification, however, can be traced to two more recent discoveries. The first is the discovery of bacteria in regions much deeper than the soil zone. This history is discussed in detail elsewhere [Dunlap and McNabb, 1973]. Suffice it to say that the notion held by many early microbiologists that the portions of the Earth's crust beneath the soil zone were devoid of life, or nearly so, is false. Geomicrobiology has been aided by recent developments of aseptic subsurface sampling techniques. By the 1980s a number of studies done under aseptic conditions provided convincing evidence that subsurface environments support an abundant microbial population of great diversity [e.g., Ghiorse and Balkwill, 1983; Hirsch and Rades-Rohkohl, 1983; Fredrickson *et al.*, 1989]. The latter [Fredrickson *et al.*, 1989] found a diverse bacterial community with a population that did not decrease with depth, even at 260 m below the land surface!

The second discovery that began to direct attention to the fate of NO_3^- in groundwater was that the ingestion of nitrate-contaminated drinking water supplies can be dangerous to infants. Reports of health problems associated with nitrate-contaminated groundwater appeared near the end of World War II when Comly [1945] reported that infants who had ingested well waters high in nitrates developed methemoglobinemia (also commonly known as cyanosis and "blue baby"), a potentially fatal condition. Before Comly's discovery the "presence or absence" of NO_3^- in groundwater was of "little consequence" except in only fairly complete groundwater studies [George and Hastings, 1951, p. 450].

The reports of methemoglobinemia also prompted the establishment of water quality standards by the World Health Organization (WHO) and the U.S. Public Health Service on nitrate levels in drinking water [U.S. Public Health Service, 1962]. Today, these levels are $11.3 \text{ mg NO}_3^- \text{-N L}^{-1}$ ($50 \text{ mg NO}_3^- \text{ L}^{-1}$) for the WHO and $10 \text{ mg NO}_3^- \text{-N L}^{-1}$ ($45 \text{ mg NO}_3^- \text{ L}^{-1}$) in the United States [Howard, 1985].

Studies of NO_3^- in groundwater in the 1950s and 1960s noted that NO_3^- concentrations tend to be stratified in an aquifer, with the higher concentrations near the water table surface. Others [Trudell *et al.*, 1986; Hallberg, 1989] listed references noting this phenomenon. George and Hastings [1951, p. 455] wrote

What becomes of the nitrate? One can hardly assume that the formation of nitrate is a recently acquired trick of nature or that not enough time has elapsed for water in the outcrop to move down to depths of several hundreds of feet. It must necessarily be assumed that something happens to the nitrate in transit.

During this era the disappearance of NO_3^- with depth was attributed to bacteria [e.g., George and Hastings, 1951; Ineson and Downing, 1963; Behnke and Haskell, 1968]; however, the presence of denitrifying bacteria in aquifers was largely speculation. Since the 1970s, however, studies specifically involving aquifer denitrification began and more substantial evidence of denitrifying bacteria and of denitrification was reported. A table listing numbers of denitrifying bacteria reported in groundwater and aquifer samples was

TABLE 2. Field Estimates of Denitrification Rates in Aquifers

Reference	Location	Contamination Source	Aquifer Material, m	Sample Depths	Temperature, °C	Initial [NO ₃ ⁻ -N], mg L ⁻¹	Denitrification Rates
<i>Vogel et al.</i> [1981]	Stampriet, south central Namibia	natural sources of unknown origin	sandstone	artesian wells from an aquifer 50 to 130 m deep	N.R.*	up to 21.2	up to 21.1 mg N L ⁻¹ in groundwater dated by ¹⁴ C to 13,990 years old
<i>Foster et al.</i> [1985]	Billingborough, Lincolnshire, England	agriculture	limestone	multiple depths to about 45 m (artesian conditions)	10	up to 7	1.5 mg N m ⁻² d ⁻¹
<i>Kölle et al.</i> [1985] and <i>Böttcher et al.</i> [1989]	Hannover, northern Germany	mainly agriculture	sand and gravelly sand	multiple depths to about 12 m (water table at 2 m)	8–10	up to about 40	first-order reaction with a half-life of 1.2 to 2.1 years†
<i>Trudell et al.</i> [1986]	Rodney, Ontario, Canada	typically agriculture but spiked for an in situ tracer experiment	sand	3 (water table at 1 m)	N.R.*	13.0	0.19 to 3.1 mg N L ⁻¹ d ⁻¹
<i>Van Beek and Van Puffelen</i> [1987]	dunes in western Netherlands	N.R.*	coarse sand with shells coarse sand with gravel	26 (HT26)	10	2.1 ± 0.2	0.12 to 0.20 mg N L ⁻¹ d ⁻¹ ‡
				44 (HT44)	10	2.1 ± 0.2	0.09 to 0.15 mg N L ⁻¹ d ⁻¹ ‡
<i>Starr and Gillham</i> [1989]	Rodney, Ontario, Canada	typically agriculture but spiked for an in situ reaction vessel experiment	sand	below water table which was 1 m below ground surface	N.R.*	about 6.4§	0.58 mg N L ⁻¹ d ⁻¹
	Alliston, Ontario, Canada	agriculture	N.R.*	below water table which was 4 m below ground surface	N.R.*	about 3.8§	<0.014 mg N L ⁻¹ d ⁻¹
<i>Fontes et al.</i> [1991]	Tombouctou, Mali	natural sources of unknown origin	various	30–100	27.0–31.9	up to 10.4+	up to 10.4 mg N/L ⁻¹ in groundwater dated by ¹⁴ C to 12,800 years old
<i>Korom</i> [1991a]	Heber, Utah	typically agriculture but spiked for an in situ tracer experiment	clay, silt, sand with cobbles	3.5 (water table at 1 m)	6–8	12.5 and 23.7	up to 0.73 mg N L ⁻¹ d ⁻¹ ‡

*N.R. not reported.

†Denitrification rates are claimed to be by autotrophic denitrification.

‡Denitrification rates are claimed to be by heterotrophic and autotrophic denitrification.

§Numerical values taken from graphs.

The only measurable property known that is specific for denitrifiers is the assay of the process itself, i.e., by measuring consumption of NO₃⁻ or NO₂⁻ and/or production of the gaseous products.

This assay is difficult to achieve in the field, however, and only a few of the references given in Table 2 have accomplished it.

As an alternative, *Hendry et al.* [1983] published results from a case study giving a number of geochemical criteria which demonstrate that denitrification best explains the nitrate distributions they observed at their research site.

These criteria include Eh, pH, DO, alkalinity, ion analysis, and isotope studies. This work was done convincingly without the measurement of any N gases. More discussion on some of the methods used in the references on Table 2, both geochemical and others, is given by *Mariotti* [1986].

Vogel et al. [1981] conducted a study at a lightly cultivated and sparsely populated location in the Kalahari Desert. They reported that N₂ and argon usually get into groundwater by the equilibration of infiltrating water with the atmosphere and by trapped air bubbles during infiltration. Measurements from water samples taken from wells downgradient showed

TABLE 1. Laboratory Denitrification Rates From Aquifer Samples

Reference	Location	Aquifer Sample Material	Sample Depths, m	Temperature, °C	Test Duration, days	Initial [NO ₃ ⁻ -N], mg L ⁻¹	Denitrification Rates ± Standard Deviation, mg N kg ⁻¹ dry sediment per day
<i>Lind</i> [1983]	Herlflagle, Denmark	clay with stones and clay loam with stones	8.0, 12.1, and 17.6	10	45	amended with 1000	0.44 ± 0.34
				25	45	amended with 1000	1.05 ± 0.17
	Bramminge, Denmark	coarse sand and sand	6.5, 11.6, and 15.9	10	45	amended with 1000	0.05 ± 0.005
			9.9 and 15.9	25	45	amended with 1000	1.16 ± 0.35
<i>Slater and Capone</i> [1987]	Long Island, New York	N.R.*	4	In situ temperatures	8.125	in situ = 3.8 ± 0.1	0.10 ± 0.001
						amended with 1000	0.17 ± 0.003
						amended to 5.2	0.24 ± 0.008
							0.15 ± 0.065
<i>Morris et al.</i> [1988]	Paris Island, South Carolina	sand and limestone	5, 9, 17, 32, 47, 88, 125, and 185	22	Up to 26	amended with 14	0.048 ± 0.050
<i>Smith and Duff</i> [1988]	Falmouth, Massachusetts	sand and gravel	2.1, 7.0, 14.4, and 21.3 below water table	22-25	2	in situ = 7.3 ± 0.7	0.082 ± 0.071†
			1.5, 6.1, 11.0, and 16.4 below water table	22-25	2	in situ = 15.7 ± 10.6	0.033 ± 0.015†
			2.1, 9.1, 14.9, and 21.0 below water table	22-25	2	in situ = 1.12 ± 2.24	0.015 ± 0.011†
			2.4, 10.4, 16.8, and 27.1 below water table	22-25	2	in situ = 0.49 ± 0.98	0.004 ± 0.002†
<i>Bengtsson and Annadotter</i> [1989]	Vomb, southern Sweden	N.R.*	7.1 m below water table	11	14	in situ = 2.5 plus 0.0875 mg L ⁻¹ labeled ¹⁵ N	0.52‡
							0.21§
<i>Francis et al.</i> [1989]	Savannah River Site near Aiken, South Carolina,	sands, clayey sands, and sandy clays	41 depths ranging from 31 to 289 m in 3 boreholes	24	3	in situ = <0.21 ± <0.36	0.015 ± 0.033
		≤50% sand and >30% clay	11 depths ranging from 75 to 278 m in 3 boreholes	24	3	in situ = 0.15 ± 0.23	0.73 ± 0.29
<i>Obenhuber and Lowrance</i> [1991]	South central Georgia,	coarse sand	6-12 m (in saturated zone)	20	302	in situ = 0.447	0
<i>Smith et al.</i> [1991]	Falmouth, Massachusetts	sand and gravel	4.7 below water table	corrected to 12°	1.67	in situ = 11+	0.60 ± 0.31
							trace
							0.036†

*Not reported.

†Denitrification rates for this reference were based on grams "wet" sediment. They were converted to grams dry sediment by assuming that the sandy sediments had a porosity of 0.40 and a mass density of 2.67⁻¹ g cm⁻³.

‡Aerobic samples had a positive mass imbalance of 14%.

§Anaerobic samples had a negative mass imbalance of 9%.

Table 2: Field Estimates of Denitrification Rates in Aquifers

Laboratory demonstrations of denitrification in aquifer samples indicate that denitrification is likely in an

aquifer; however, such observations are not conclusive evidence that denitrification is occurring in situ. With respect to identifying aquifer denitrification, *Tiedje* [1982, p. 1013] stated,

the top 6–15 cm of the water table. They [Ronen *et al.*, 1987] theorized that denitrification occurring in this zone explained the discrepancy between the high NO_3^- levels predicted for this aquifer (Coastal Plain aquifer, Israel) and the small rates of increase observed. They [Ronen *et al.*, 1987] attributed the high OC levels to the use of sewage effluents as irrigation water and suggested that the OC was not entirely decomposed by bacteria in the unsaturated zone due to the low moisture content there.

The paper by Foster *et al.* [1985] also presented evidence that denitrification is occurring in the Lincolnshire Limestone aquifer (near Billingham). The high concentrations of NO_3^- ($>10 \text{ mg NO}_3^- \text{-N L}^{-1}$) derived from agriculture have not penetrated as deeply into the aquifer as expected. They gave two possible explanations for this observation. Either NO_3^- is being physically retarded by diffusing from the mobile fissure water into the static pore water or it is being denitrified. The former is not reasonable, however, since thermonuclear tritium (^3H) at concentrations indicating that it is probably post-1960 was found in pore waters where only small amounts of NO_3^- ($<0.2 \text{ mg NO}_3^- \text{-N L}^{-1}$) were measured. Additionally, ^3H was found by 1969 in some locations where NO_3^- has never been detected. In both situations, had the concentrations of NO_3^- been conserved in the groundwater, NO_3^- should have been found with the ^3H . They theorized that denitrification explained the loss of NO_3^- and estimated that the aquifer had a denitrification rate of $1.5 \text{ mg N m}^{-2} \text{ d}^{-1}$. This was determined by assuming that NO_3^- was reduced at a rate of $10 \text{ mg NO}_3^- \text{-N L}^{-1}$ by flowing a distance of 2 km in 100 days through limestone with a volume/area ratio of 0.015 m. They concluded that at least some of the OC used as an e^- donor for the denitrification is derived from the limestone.

This observation brings up an important rule of thumb for heterotrophic denitrification. If the concentration of $\text{NO}_3^- \text{-N}$ in the groundwater exceeds the concentration of OC in the groundwater, the OC is not enough to denitrify the $\text{NO}_3^- \text{-N}$. This relationship can be verified by considering the organic compound that can donate the most electrons, that is, has the lowest oxidation state, and has the greatest proportion of its mass as C. The C atom in methane (CH_4) has an oxidation state of $-IV$ and accounts for 78% of the molecule's mass. Thus one mole of CH_4 going to CO_2 (oxidation state of C is $+IV$) can reduce 8/5 mole of $\text{NO}_3^- \text{-N}$ (oxidation state of N is $+V$) to N_2 (oxidation state of N is 0). Or in terms of mass, one unit mass of CH_4 can denitrify 1.4 times as much $\text{NO}_3^- \text{-N}$. CH_4 is produced under extremely reduced conditions; Figure 1 shows that it is formed after the sulfate-reduction sequence. The C in most organic compounds is not this reduced nor does it account for such a high fraction of the compound's mass; hence one unit mass of OC typically can reduce less than one unit mass of $\text{NO}_3^- \text{-N}$.

Thurman [1985] stated that the majority of all groundwaters have dissolved OC (DOC) $<2 \text{ mg L}^{-1}$. Thus in the absence of DOC, large amounts of denitrification are only possible in aquifers with large quantities of reduced inorganic compounds or solid labile OC in the porous matrix. Foster *et al.* [1985] reported that portions of the limestone in the aquifer of their study contain total OC in excess of 1000 mg kg^{-1} dry weight ($>0.1\%$ by dry weight). How much of this is able to be used by bacteria for denitrification is not known. They additionally noted that a few of the enrichment cultures using scraped limestone samples had positive

growth with thiosulfate (a reduced sulfur compound). Thus, in addition to heterotrophic denitrifiers, this aquifer had evidence of autotrophic denitrifying bacteria as well.

The work of Van Beek and Van Puffelen [1987] also provides evidence of autotrophic denitrification potential. They studied the chemical changes in water injected into an unconsolidated sandy aquifer. The recharge program lasted 7 years. Among the chemical changes noted was a reduction in NO_3^- . The e^- donors for this reaction were believed to be OC and reduced iron and sulfide (as FeS_2); thus heterotrophic and autotrophic denitrification may have accounted for the diminished NO_3^- concentrations. Autotrophic denitrification involving reduced iron and reduced sulfur is discussed in the next section.

If the supply of NO_3^- exceeds the supply of OC, denitrification will cease unless other e^- donors are found. There are two ways OC can be supplied to groundwater. One is that OC in such forms as sucrose and methanol is actually injected into the aquifer or mixed into water pumped from an aquifer and subsequently allowed to flow back into the groundwater system. Gayle *et al.* [1989] and Hiscock *et al.* [1991] reviewed this literature. Large quantities of OC may also be encountered when groundwater interacts with surface water or vegetation (roots). This could occur as groundwater exits an aquifer to streams, lakes, or oceans or interacts with wetlands or riparian vegetation. These topics are beyond the scope of this review.

AUTOTROPHIC DENITRIFICATION IN AQUIFERS

Figure 1 shows the various e^- acceptor sequences in the oxidation of OC. As mentioned previously, if NO_3^- is introduced to any reducing zone below a denitrifying zone, thermodynamic considerations show that the NO_3^- can serve as an e^- acceptor, and reduced inorganic species such as Mn^{2+} , Fe^{2+} , and HS^- can serve as e^- donors. Large quantities of these reduced inorganic species exist in some aquifers and are reported to participate in denitrification reactions. Two of the reduced inorganic species that have appeared in the literature on groundwater denitrification are discussed below. Specifically, the next two subsections concern autotrophic denitrification by reduced iron and by reduced sulfur.

Autotrophic Denitrification by Reduced Iron

It is commonly observed that groundwaters that contain Fe^{2+} have little or no NO_3^- [e.g., Lind, 1983; Böttcher *et al.*, 1985; Mariotti *et al.*, 1988]. The fate of NO_3^- in such an environment is discussed below.

Buresh and Moraghan [1976] demonstrated in a reaction vessel that Fe^{2+} chemically reduces NO_3^- to nitrogen gases (N_2O and N_2) and NH_4^+ in the presence of Cu^{2+} . NO_3^- reduction was negligible without the copper catalyst. They further reported that NH_4^+ formation was significant in all their experiments and was the dominant pathway at pH 9 and 10. Maximum nitrogen gas formation (N_2O and N_2) occurred at pH 8 and accounted for 61% of the original NO_3^- ; NH_4^+ formation accounted for 32% of the original NO_3^- .

Verdegem and Baert [1984, 1985], working in the root zone and below, reported chemical reduction of NO_3^- by Fe^{2+} . The possibility of reduction to NH_4^+ was apparently not considered. Verdegem and Baert [1985] found in the

that additional N_2 is getting into the water. This "excess nitrogen" increased as the groundwater's NO_3^- and DO concentrations decreased, which suggested that denitrification was responsible for these observations. By dating the groundwater using ^{14}C , denitrification rates in the aquifer were approximated. They estimated that 21.1 mg N L^{-1} were denitrified in the aquifer in the past 13,990 years. They attributed the slow denitrification rates to the minimal availability of OC to the groundwater. *Fontes et al.* [1991], working in the Azaouad Depression in the northern Mali, used a similar procedure and reported that up to 10.4 mg N L^{-1} were denitrified in groundwaters up to 12,800 years old, as determined by ^{14}C (uncorrected ages).

Additionally, *Vogel et al.* [1981] discussed the possibility that the isotopic fractionation of the denitrification they studied, as determined by the $^{15}N/^{14}N$ ratios in the groundwater, was analogous to Rayleigh distillation. In such a process the lighter N isotope (^{14}N) tends to be denitrified before the heavier isotope (^{15}N) such that the per mil enrichment of ^{15}N as a function of the logarithm of the unreacted residual NO_3^- fraction forms a straight line; that is, the enrichment factor is a constant. This technique is discussed in detail by *Mariotti* [1986].

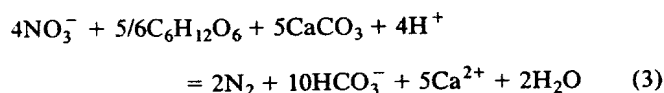
Three isotopic fractionation studies [*Mariotti et al.*, 1988; *Böttcher et al.*, 1990; *Smith et al.*, 1991] subsequent to *Mariotti* [1986] also reported that groundwater denitrification was a single-step unidirectional (Rayleigh) process with respect to the unreacted residual NO_3^- fraction. *Böttcher et al.* [1990] noted that the per mil enrichment of ^{18}O as a function of the logarithm of the unreacted residual NO_3^- fraction yielded a better linear regression than the ^{15}N counterpart in the Fuhrberger Feld aquifer in Germany. Thus they concluded that the $^{18}O/^{16}O$ ratio is better suited for identifying denitrification than the $^{15}N/^{14}N$ ratio.

One may think that since denitrification follows a Rayleigh process, the production of denitrification's end product, N_2 , does as well. However, isotopic fractionation studies by *Mariotti et al.* [1988] in the chalk aquifer of northern France and by *Wilson et al.* [1990] in the Lincolnshire Limestone aquifer in eastern England indicated that this notion may not be true.

An attempt to measure in situ denitrification rates was done by *Trudell et al.* [1986]. They placed a drive point into an unconfined sandy aquifer to a depth of 3 m, which was 2 m below the water table. They pumped 200 L of groundwater from the drive point, spiked it with NO_3^- (as KNO_3) and the conservative tracer bromide (as $NaBr$), and injected this slug back through the drive point. The dilution of this slug was monitored by the Br^- concentrations. Any loss of NO_3^- greater than that which could be accounted for by dilution was attributed to denitrification. They observed denitrification rates from 0.19 to $3.1 \text{ mg N L}^{-1} \text{ d}^{-1}$. An inverse linear relationship existed between the denitrification rate and NO_3^- concentration at higher NO_3^- concentrations. This observation should be viewed cautiously, as they make clear that when the slug was injected, DO concentrations at the drive point increased from 0 to 5.5 mg L^{-1} . This would allow O_2 to be used initially as an e^- acceptor in lieu of NO_3^- and thereby decrease the initial denitrification rate. An earlier study in the same geographic vicinity by *Gillham and Cherry* [1978] reported that 2.0 mg L^{-1} is the upper DO limit for denitrification to proceed. This represents an averaged esti-

mate, as some microsites within the porous matrix may have lower DO concentrations.

Reaction (2) makes it clear that the oxidation of organic compounds results in the production of carbon dioxide (CO_2). Bicarbonate (HCO_3^-) may result from other reactions involving this CO_2 or may be produced directly by heterotrophic denitrification. *Trudell et al.* [1986] and *Van Beek and Van Puffelen* [1987] demonstrated that CO_2 produced in a calcareous groundwater system results in the production of bicarbonate (HCO_3^-). *Trudell et al.* [1986] found that the production of HCO_3^- from denitrification agreed with their geochemical model that used glucose ($C_6H_{12}O_6$) as the organic e^- donor. This model was represented by



Reaction (3) states that each mole of NO_3^- denitrified produced 2.5 moles of HCO_3^- . This may be misleading as it implies that all denitrification was heterotrophic. This was not verified. Nor was it verified that the e^- donor at their site was glucose or that the average oxidation state of the organic e^- donors was the same as the C in glucose, that is, zero. Without such verification the possibility exists that autotrophic denitrification may have explained some of the observed decrease in NO_3^- concentrations.

Korom [1991a] performed a similar tracer test to that done by *Trudell et al.* [1986]. Not only did HCO_3^- increase with a decrease in NO_3^- but SO_4^{2-} did as well. The production of SO_4^{2-} with sulfide ($-I$ or $-II$) as an e^- donor, which is discussed in detail later, appeared to be responsible for about half of the decrease in NO_3^- concentrations observed in this field test.

Starr and Gillham [1989] also did an in situ denitrification experiment in the same aquifer as *Trudell et al.* [1986]. The water table at this site was also at a 1-m depth. They isolated a 2-L portion of the aquifer, pumped an aliquot of sample from this reaction vessel, spiked it with NO_3^- and acetylene, and reinjected it into the vessel. Acetylene inhibits the conversion of N_2O to N_2 [*Yoshinari et al.*, 1977] and is commonly used to measure denitrification rates. They found that the average denitrification rate for the 10-day experiment was $0.58 \text{ mg N L}^{-1} \text{ d}^{-1}$. They reported that $<0.1 \text{ mg NH}_4^+ \text{ N L}^{-1}$ was produced in the experiments, which indicated that the NO_3^- did not undergo significant DNRA.

Starr and Gillham [1989] performed the same experiment at a second agricultural site, only this one had a water table 4 m below the land surface. Here, they measured no denitrification ($<0.014 \text{ mg N L}^{-1} \text{ d}^{-1}$) despite the presence of denitrifying bacteria. The major difference cited between the two agricultural sites is that there was not enough labile OC at the second site to support denitrification. Their explanation was that deep water table conditions affected the amounts of OC transported to the saturated zone through the unsaturated zone. These results are supported by *Egboka* [1984], who worked in the same geographical vicinity as *Starr and Gillham*. The depth to water table, however, is not the only variable affecting the transport of OC through the unsaturated zone.

Ronen et al. [1987] give evidence of anoxic conditions caused by OC that was transported through a sandy unsaturated zone 30 m thick. Using a multilevel sampler [*Ronen et al.*, 1986], they showed that a eutrophic layer developed in

cally [Korom, 1991b] that apparently any type of OC used as an e^- donor in the sulfate-reducing zone would preferentially be used by bacteria for denitrification in a reaction similar to (2). This indicates that heterotrophic denitrification will also occur at the Fuhrberger Feld aquifer, at least after sulfide reserves are exhausted. Böttcher *et al.* [1991] concur with this statement but argue that the kinetics of such a reaction will not be significantly greater than the heterotrophic sulfate reduction observed and thus will be of little practical significance in reducing NO_3^- concentrations. Whether or not this is true is unknown. Tables 1 and 2 in this review give evidence that the kinetic rates for heterotrophic denitrification are generally faster than the heterotrophic desulfurification rates implied by the 76- to 100-year half-life given by Böttcher *et al.* [1989]. However, Böttcher *et al.* [1991] explained that they could find no evidence that heterotrophic denitrification was faster than heterotrophic sulfate reduction with the same organic e^- donor and thus assumed the rates were similar. This discussion by Korom [1991b] and Böttcher *et al.* [1991] illustrates the need for more data on natural groundwater denitrification in general and specifically with respect to reaction kinetics of various e^- donors.

Finally, Kölle *et al.* [1985] noted the following consequences associated with the autotrophic denitrification at the Fuhrberger Feld aquifer: (1) sulfate (SO_4^{2-}) concentrations in the groundwater increased (see reaction (6)) at a rate of up to $8.3 \text{ mg L}^{-1} \text{ yr}^{-1}$, causing drinking water standards (250 mg L^{-1}) to be exceeded in at least one well, (2) increases in SO_4^{2-} were accompanied by increases in water hardness and increases in the corrosion potential of the treated drinking water, (3) well performance was diminished by ferric iron precipitation, and (4) increased water treatment was required because of elevated concentrations of iron and manganese.

TOPICS FOR FUTURE RESEARCH

Contrary to statements by Howard [1985] and Hiscock *et al.* [1991], natural aquifer denitrification can, given the proper conditions, reduce NO_3^- contamination in modern recharge waters. This review illustrates, however, that our current capabilities to predict an aquifer's denitrification characteristics are site specific at best. The implication for future research is that more data covering a wide range of aquifer denitrification topics need to be collected and analyzed. Suggestions for future research are summarized as follows.

1. N_2O is a denitrification end product. This gas is a potential contributor to the destruction of the ozone layer [Crutzen, 1981] and to the "greenhouse effect" [Wang *et al.*, 1976]. Ronen *et al.* [1988] indicate that NO_3^- -contaminated aquifers are a forgotten component of the global N_2O budget. Research on the factors that influence the partitioning of denitrification end products between N_2O and N_2 and the contribution of N_2O by contaminated aquifers to the global budget needs to be done.

2. NO_3^- can be reduced to N gases or NH_4^+ . An area of future research is to determine the factors that influence this partitioning both via heterotrophic and autotrophic NO_3^- reduction. More verification is required on the hypothesis presented by Tiedje *et al.* [1982] concerning OC limitation and its impact on the partitioning of NO_3^- between heterotrophic denitrification and DNRA in aquifers.

3. Firestone [1982] gives three general denitrification requirements. They are (1) the presence of bacteria possessing the metabolic capacity, (2) anaerobic conditions or restricted molecular oxygen (O_2) availability, and (3) suitable e^- donors. Future research suggestions for each are given below.

First, the notion that aquifers have little or no microbial activity is invalid. Large and diverse populations of microorganisms can inhabit aquifers. We need a greater understanding of the microbial ecology of aquifers. Additionally, we need to identify the denitrifiers and document their behavior and microenvironments during denitrification. Currently, information on autotrophic denitrifiers lags that of heterotrophic denitrifiers.

Second, one of the conditions leading to denitrification is anaerobiosis or restricted DO availability. Suggested areas of future research are the determination of the DO concentrations that cause the onset of denitrification for specific denitrifiers and to what extent DO concentrations affect denitrification rates.

Third, perhaps the area of most practical concern for hydrogeologists assessing aquifer denitrification potential is the availability of e^- donors. OC is an important e^- donor. Some OC in pore water from a root zone is readily bioavailable; however, supplies are limited. In some locations, large reserves of OC are in an aquifer's solid phase. Thermodynamic considerations show, however, that over geologic time the OC remaining in a formation may not be very labile since the more labile forms of OC tend to be oxidized before less labile forms. It is important to ascertain what forms of OC are usable by bacteria for denitrification and how different types and sources of OC affect denitrification rates.

Likewise, availability of inorganic e^- donors and how different inorganic e^- donors affect denitrification rates need to be determined. Studies should incorporate a spectrum of potential e^- donors and not just be limited to a single e^- donor unless justified. Temporal and spatial variability in the supply of e^- donors within an aquifer also needs to be addressed.

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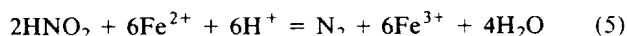
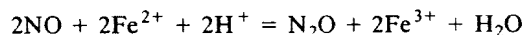
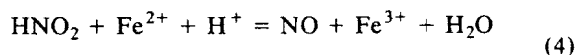
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three experimental agricultural fields studied that the stable ferrous iron minerals in the calcareous environment were FeCO_3 and Fe_3O_4 . The associated low Fe^{2+} activities are credited with chemically reducing the NO_3^- input in a matter of weeks or months, depending on the initial NO_3^- concentrations. They noted that the boundary of the permanently reduced zone gradually moved downward with the continued infiltration of the oxidants O_2 and NO_3^- . They additionally stated that this denitrification occurs at a site where the subsoil has a low OC (peat) content. The apparent, unverified implication is that heterotrophic denitrification did not explain their findings.

Lind [1983] reported that discussions with drinking water managers in Denmark indicated that groundwater having Fe^{2+} never contained NO_3^- . Lind's analysis of samples taken from three borings confirmed this finding. Samples taken below the oxidized zone typically had $[\text{Fe}^{2+}] > 20 \text{ mg L}^{-1}$ and $[\text{NO}_3^-\text{-N}] \ll 1 \text{ mg L}^{-1}$. The boundary between oxidized and reduced zones was delineated by visual inspection, that is, the oxidized profiles were reddish or brown and the reduced profiles were bluish or greyish.

The suspected denitrification was believed to occur entirely by abiotic means; however, attempts to demonstrate this in sterilized aquifer samples failed. Lind [1983] claimed that it is unlikely that bacteria could penetrate into the deeper clay layers (of the order of 10 m thick) at two sites, but subsequent findings at the Savannah River Site [Francis et al., 1989] showed that bacteria do exist below confining clay layers.

Whether Fe^{2+} abiotically reduces NO_3^- in an aquifer remains a moot point; however, there is evidence that at least one strain of bacteria can autotrophically denitrify in a reduced-iron environment. Gouy et al. [1984] reported that *Gallionella ferruginea* reduces NO_3^- to NO_2^- . This product can then be reduced abiotically in an OC-poor environment to gaseous N compounds by the oxidation of Fe^{2+} to Fe^{3+} by one of the following two reactions:



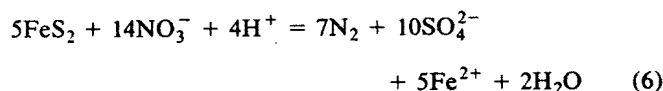
G. ferruginea are known to be indigenous to groundwater systems [Kucera and Wolfe, 1957]. They require some oxygen for growth [Kucera and Wolfe, 1957], although this requirement appears to be minimal, as Gouy et al. [1984] did an incubation with *G. ferruginea* under anaerobic conditions. Jørgensen [1989, Figure 7.6] also presented evidence that the DO requirement is very low ($<10 \mu\text{M}$ ($<0.32 \text{ mg L}^{-1}$)). A likely ecological niche for this bacterium is, then, at an oxic/anoxic interface where Fe^{2+} and DO meet in opposed diffusion gradients [Jørgensen, 1989].

The presence of *G. ferruginea* in an aquifer at least partially explains why groundwater poor in OC and in a reduced-iron environment has little or no NO_3^- present. A negative consequence of these bacteria is that they have such fast growth rates they clog wells and well screens [Hallbeck and Pedersen, 1986].

Autotrophic Denitrification by Reduced Sulfur

Portions of the Fuhrberger Feld aquifer in northern Germany that underlie arable land are subjected to the input of

large concentrations of NO_3^- . Strelbel and Böttcher [1989] reported that the arithmetic mean for the input of NO_3^- to the aquifer from arable land in this region is $30 \text{ mg NO}_3^- \text{-N L}^{-1}$. Yet the concentration of $\text{NO}_3^- \text{-N}$ in the water pumped from horizontal filter wells at a depth of about 25 m varied from less than 0.05 to a maximum of about $0.3 \text{ mg NO}_3^- \text{-N L}^{-1}$ [Kölle et al., 1985]. Evidence that NO_3^- concentrations were being denitrified was obtained by tritium dating of the groundwater [Böttcher et al., 1985], isotope fractionation of NO_3^- [Böttcher et al., 1990], and by the measurement of N_2 in the groundwater by a simple method (compared to gas chromatography) that incorporates the decreasing solubility of N_2 with increasing temperature [Böckle et al., 1984]. The stated conclusion is that the denitrification is based upon the oxidation of ferrous disulfide (or "pyrite") according to [e.g., Kölle et al., 1985; Böttcher et al., 1990; Frind et al., 1990]



This reaction is mediated by the bacteria known as *Thiobacillus denitrificans* [Kölle et al., 1983; Kölle et al., 1985]. In (6) the sulfide in FeS_2 (oxidation state of sulfur is $-I$) is oxidized to SO_4^{2-} (oxidation state of sulfur is $+VI$). In some regions of this aquifer a sulfate-reducing zone lies below the denitrification zone [Böttcher et al., 1989; Frind et al., 1990] (an observation predicted by Figure 1). Any SO_4^{2-} produced by the above reaction that is transported to the sulfate-reducing zone could be reduced back to sulfide with OC serving as the e^- donor [Böttcher et al., 1989; Frind et al., 1990]. Extensive field studies of the recharge area near the Fuhrberg waterworks' well 1 revealed that reaction (6) has a first-order kinetic rate constant that yields a half-life of 1.2 to 2.1 years and that the sulfate-reducing reaction has a half-life of 76 to 100 years [Böttcher et al., 1989; Frind et al., 1990].

Böttcher et al. [1989] and Frind et al. [1990] modeled denitrification at the Fuhrberger Feld aquifer assuming that it is based solely on the aquifer's sulfide reserves (reaction (6)). These models demonstrated the importance of these reserves in maintaining low concentrations of NO_3^- in the aquifer. They indicated that quantitative predictions will require more data on the sulfide reserves. There is evidence, however, that denitrification in this aquifer does not only depend on this aquifer's sulfide reserves.

There is evidence that Fe^{2+} was also a participant in the denitrification observed at the Fuhrberger Feld aquifer. Reaction (6) shows that for denitrification with ferrous disulfide, both SO_4^{2-} and Fe^{2+} are products. At some wells (S3 and N10) Böttcher et al. [1985] reported that increased concentrations of SO_4^{2-} do appear in the denitrification zone; however, Fe^{2+} does not appear in solution until all detectable NO_3^- is denitrified. This follows the scenario given in the previous subsection and is evidence that the Fe^{2+} participates in autotrophic denitrification via a reaction similar to reaction (4) or (5). The practical significance of this is limited, however, as Fe^{2+} is a less prolific e^- donor than $\text{S}(-I)$. Fe^{2+} only donates one e^- going to Fe^{3+} ; $\text{S}(-I)$ donates seven electrons going to SO_4^{2-} .

Furthermore, there is evidence that organic e^- donors are also available in this aquifer. As stated above, the sulfate-reducing zone uses OC as an e^- donor [Böttcher et al., 1989; Frind et al., 1990]. Elsewhere, it is shown thermodynamically

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