DENITRIFICATION IN GROUNDWATER AT URANIUM MILL TAILINGS SITES

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ABSTRACT

Nitrates are a major contaminant in groundwater at many Uranium Mill Tailings Remedial Action (UMTRA) sites. Microbial denitrification, the transformation of nitrate to nitrogen gas, may be occurring in groundwater at several UMTRA sites. Denitrification is a biologically mediated process whereby facultative anaerobes use nitrate for respiration under anaerobic conditions. Denitrifying bacteria are ubiquitous in soils, sediments, and water.

Denitrification requires nitrate, organic carbon, oxygen-limiting conditions, and trace nutrients, especially phosphorus. The lack of organic carbon is the most common limiting factor for denitrification. Denitrification occurs under a limited range of temperature and pH.

The uranium milling processes used at UMTRA sites provided a readily available source of carbon and nitrates for denitrifying bacteria. At the Maybell, Colorado, site, the denitrifying organisms *Pseudomonas*, *Flavobacterium*, and *Acinetobacter* were identified in core samples of materials from beneath the tailings. In addition, microcosm experiments simulating aquifer conditions beneath the tailings pile showed an average 40 percent decrease in nitrate concentrations over 13 days.

At the New Rifle, Colorado, site, aquifer conditions appear favorable for denitrification. Nitrate and organic carbon are readily available in the groundwater, and redox conditions beneath and downgradient of the tailings pile are relatively anoxic. Downgradient from the tailings, total nitrogen is being removed from the groundwater system at a greater rate than the geochemically conservative anion, chloride. This removal may be due to denitrification and adsorption of ammonium onto clay and silt particles.

INTRODUCTION

Nitrates are a major contaminant in groundwater at most Uranium Mill Tailings Remedial Action (UMTRA) sites. Nitrate concentrations exceed the proposed EPA groundwater standard (40 CFR 192) of 44 mg/L NO_3 at many of the 24 UMTRA sites. These elevated nitrate concentrations result from tailings seepage that is contaminated with residual nitrates from the uranium milling process.

However, there is evidence that microbially mediated denitrification (the transformation of nitrate to nitrogen gas) is occurring in groundwater at several UMTRA sites. Microbially mediated denitrification is beneficial because it removes nitrate from the groundwater.

This paper describes the conditions controlling microbially mediated nitrogen transformations in groundwater, and the evidence that these transformations are occurring at several UMTRA sites.

NITROGEN TRANSFORMATIONS IN GROUNDWATER

Nitrogen occurs as several species in groundwater, depending on the pH and redox potential of the hydrogeologic system. The relationships between the pH, redox potential, and the dominant species of nitrogen are shown in Fig. 1 (1). Nitrates are the most stable specie of nitrogen in oxidizing groundwater. They are geochemically conservative (i.e., nonreactive with geologic materials), and are extremely mobile.

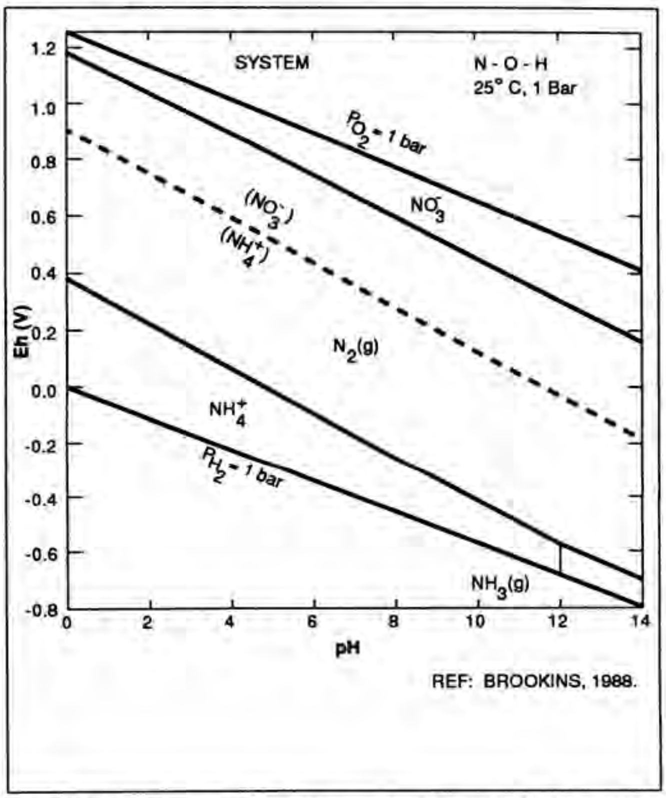

Fig. 1. Eh-pH diagram for nitrogen species at 1 ATM pressure and 25°C.

Figure 2 presents nitrogen inputs and transformations in groundwater (2). Nitrogen transformations in groundwater include ammonification, the conversion of organic nitrogen to ammonium; nitrification, the conversion of ammonium to nitrate; and denitrification, the conversion of nitrates to N_2O or N_2, depending on the temperature and redox conditions in the groundwater. These species, N_2O or N_2, are dissolved gases which are gradually removed from the groundwater by off-gassing (2). They are also biologically non-reactive, and therefore not considered groundwater contaminants.

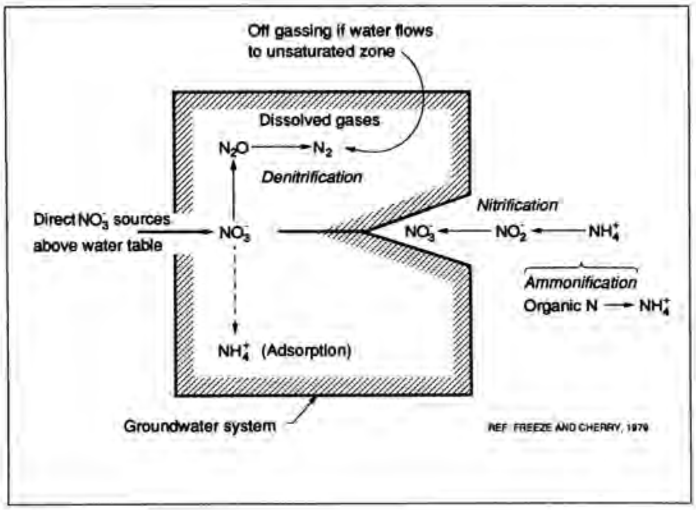

Fig. 2. Nitrogen inputs and transformations in the groundwater system.

Ammonium is also removed from groundwater by adsorption onto clays or silt particles. Due to biochemical reasons, relatively little nitrogen is transformed in this manner (2).

The Role of Microbes in Denitrification

Denitrification in groundwater is a biologically-mediated process. Denitrifying bacteria are facultative anaerobes which use nitrate rather than oxygen as a terminal electron acceptor for respiration under anaerobic conditions. This process requires organic carbon as the electron donor for microbial metabolism, with CO_2 as the byproduct (3).

Denitrification can be represented as follows:

$$\text{Organics} + NO_3^- + \text{trace nutrients} \rightarrow CO_2 + H_2O + N_2(g) + \text{bacteria} \qquad \text{(Eq.1)}$$

Bacteria capable of nitrate respiration are ubiquitous in soils, sediments, and water. They include the facultative autotroph *Micrococcus denitrificans*, and *Thiobacillus denitrificans*, an autotroph that oxidizes sulfur while reducing nitrate under anaerobic conditions. Heterotrophic denitrifiers include *Pseudomonas*, *Micrococcus*, *Achromobacter*, and *Bacillus* (4).

Environmental Factors Affecting Denitrification

Denitrification requires the following environmental conditions:

- Nitrate
- Organic carbon
- Oxygen-limiting (anoxic) conditions
- Trace nutrients (particularly phosphorus).

The most common limiting environmental factor for denitrifying bacteria in groundwater is the availability of a carbon source. This carbon source may be either soil organic carbon (5) or dissolved organic carbon in the form of simple sugars or other compounds such as phenols and aliphatic hydrocarbons. The rate of denitrification has been closely correlated to the availability of carbon in groundwater (6).

Denitrification has also been closely correlated to decreased oxygen content in groundwater. Before denitrification can occur, ammonia must first be oxidized through nitrification, and oxygen is required. Although oxygen in groundwater inhibits denitrification, limited oxygen is nonetheless required for denitrification to occur.

Temperature and pH also affect the rate of denitrification. Denitrification diminishes significantly above 45°C, and denitrification is virtually nonexistent below 5°C (7). The optimal pH range for denitrification is between 5.8 and 9.2 (8).

DENITRIFICATION AT UMTRA SITES

The uranium milling processes used at UMTRA sites often provided a readily available source of carbon and nitrates for denitrifying bacteria. In general, two primary methods of uranium recovery were used at UMTRA sites: the ion exchange method and the solvent extraction method. Both processes use nitrogen functional groups to remove uranium from solution. The solvent extraction method uses an organic solvent and an amine chelating agent. Although the spent solutions containing nitrogen were recycled in the milling processes, they were eventually discharged with the mill tailings, and the nitrogen species migrated to the underlying groundwater.

Ammonium is oxidized to nitrate under aerobic conditions, either in the vadose zone beneath the tailings pile, or in the groundwater. In oxidizing groundwater, nitrates are highly mobile, and nitrate contamination often extends downgradient of the tailings piles for hundreds of meters.

Kerosene, used in the solvent extraction process, often accompanies the nitrogen species. This source of organic carbon serves as an energy source for microbially mediated denitrification reactions.

Evidence indicates that microbially mediated denitrification may be occurring at some UMTRA sites. This evidence is discussed in detail below.

Denitrifying Bacteria at the Maybell Site

At the Maybell, Colorado, uranium processing site, denitrifying bacteria were identified in core samples collected from beneath the tailings (9). Two experiments were conducted to determine if conditions beneath the tailings pile could be suitable for microbial denitrification.

In the first experiment, using classical microbiological procedures, core samples from the tailings substrate materials were analyzed to determine the presence of denitrifying organisms. Five core samples were collected from an auger hole at depths ranging from 20.9 to 40.5 meters. Aliquots of the samples were diluted with sterile saline solution and plated on a variety of nutritional media. Colonies of six different microorganisms were isolated from these samples, and their distributions were quantified with respect to depth.

Microorganisms identified in the samples include *Pseudomonsa mendocina*, *Acinetobacter sp.*, *Micrococcus* *roseus*, *Pseudomonas sp.* or *Flavobacterium sp.*, and *Pseudomonas stutzeri*. These microorganisms occur in other soils (10) and in groundwater. Denitrification has been documented for members of the *Pseudomonas*, *Flavobacterium*, and *Acinetobacter* genera.

In the second experiment, anoxic microcosms were used to simulate conditions within the groundwater beneath the pile. Mixed culture inoculums of soil samples from the pile and a feed solution were added to the microcosms. The feed solution consisted of a phosphate buffer, a nitrate source, small concentrations of inorganic salts, a glucose carbon source, and a 1 mg/L of Resazurin (a redox indicator). The solution was incubated at 20°C.

Nitrate concentrations in the microcosms were monitored approximately every 2 days over a 2-week period using ion chromatography. Nitrate concentrations in each microcosm are shown in Fig. 3. The various curves are for samples from different depths.

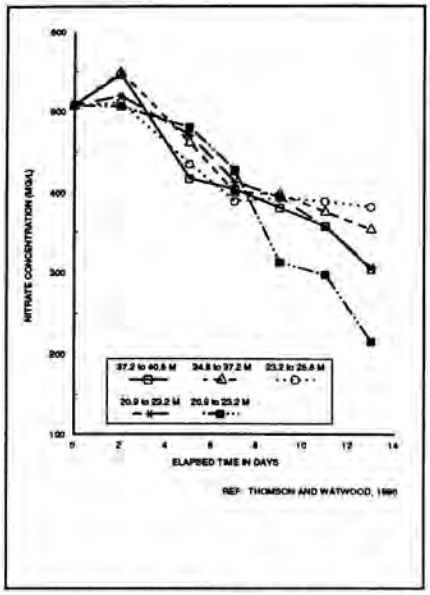

Fig. 3. Nitrate concentrations in the microcosms versus time.

Active denitrification was observed in all of the five samples. In each case, a several-day lag was noted in each microcosm during which there was no decrease in nitrate concentrations. Following this period, there was a consistent drop in nitrate levels with time. During the 13-day experiment, denitrification decreased nitrate concentrations by an average of 40 percent (Fig. 3). The sample collected from the shallowest depth in groundwater beneath the tailings pile yielded a nearly 60 percent nitrate reduction.

Evidence for Denitrification at the New Rifle Site

Denitrification may also be occurring in groundwater at the New Rifle, Colorado, UMTRA site. Environmental conditions appear to be well suited for denitrification because of the following:

- An ample supply of nitrate and organic carbon exists in the groundwater downgradient of the tailings (Figs. 4 and 5).
- Relatively high concentrations of Total Organic Carbon (TOC) (Fig 5) and dissolved iron (greater than 15 mg/L) are present in groundwater immediately downgradient of the tailings pile. This indicates that redox conditions are relatively anoxic downgradient of the tailings.
- Nitrogen is being removed from the groundwater downgradient of the tailings. Figure 6 shows the ratio of total nitrogen to chloride (a conservative anion) versus distance downgradient from the tailings. If nitrogen removal from the groundwater system is strictly due to dilution and dispersion, this ratio would remain relatively constant. However, the data indicates that, downgradient from the tailings pile, nitrogen is being removed at a greater rate than chloride. Probable mechanisms for this nitrogen removal include denitrification and adsorption of ammonium onto clay and silt particles.

Additional study is necessary to quantify redox conditions in groundwater and to verify microbial denitrification at the New Rifle site.

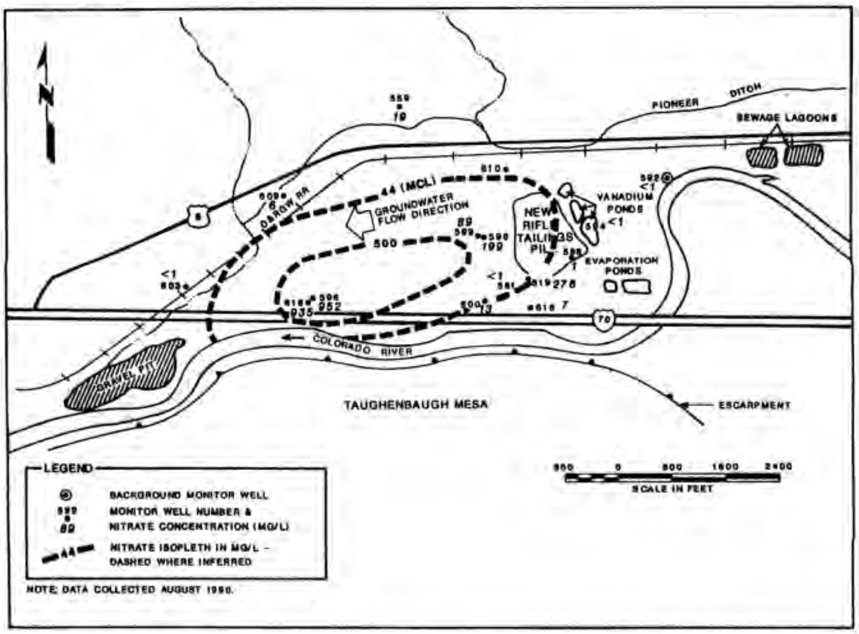

Fig. 4. Nitrate concentrations in alluvial groundwater, New Rifle Site.

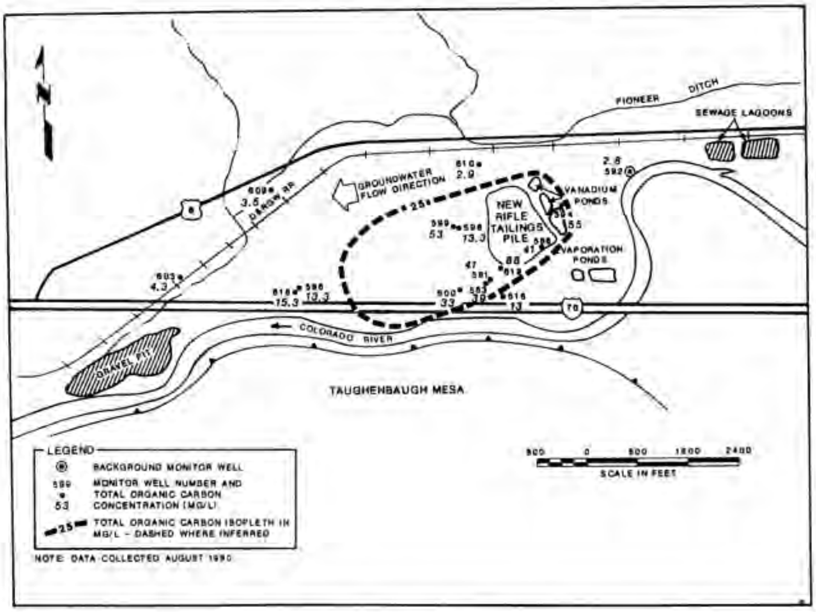

Fig. 5. Total organic carbon concentrations in alluvial groundwater, New Rifle Site.

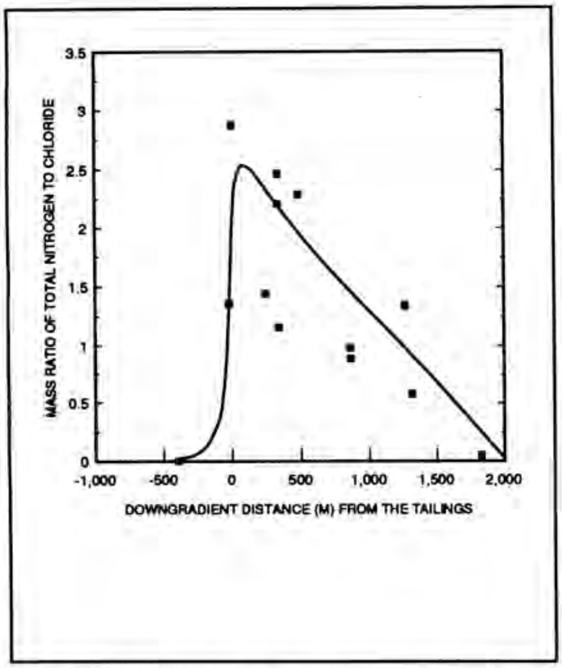

Fig. 6. Mass ratio of total nitrogen to chloride versus downgradient distance from the tailings.

CONCLUSIONS AND RECOMMENDATIONS

Denitrification will occur in groundwater if the right environmental conditions are present. These conditions include the presence of the following:

- A carbon and energy source
- Nitrate
- Trace nutrients including phosphorus
- An oxygen-limiting environment.

Microbially mediated denitrification may be occurring in groundwater at some UMTRA sites, including the Maybell, Colorado and New Rifle, Colorado sites. Denitrification is beneficial because it decreases nitrate concentrations in groundwater.

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