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ENUMERATION AND CHARACTERIZATION OF MICROORGANISMS ASSOCIATED WITH THE URANIUM ORE DEPOSIT AT CIGAR LAKE, CANADA

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ENUMERATION AND CHARACTERIZATION OF MICROORGANISMS ASSOCIATED WITH THE URANIUM ORE DEPOSIT AT CIGAR LAKE, CANADA

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ABSTRACT

The high-grade uranium deposit at Cigar Lake, Canada, is being investigated as a natural analog for the disposal of nuclear fuel waste. Geochemical aspects of the site have been studied in detail, but the microbial ecology has not been fully investigated. Microbial populations in an ore sample and in groundwater samples from the vicinity of the ore zone were examined to determine their effect on uranium mobility. Counts of the total number of bacteria and of respiring bacteria were obtained by direct microscopy, and the viable aerobic and anaerobic bacteria were assessed as colony forming units (CFUs) by the dilution plating technique. In addition, the population distribution of denitrifiers, fermenters, iron- and sulfur-oxidizers, iron- and sulfate-reducers, and methanogens was determined by the most probable number (MPN) technique.

Direct microscopic counts of total and respiring bacteria in the water samples ranged from 10^2 to 10^4 cells ml^{-1}, and zero to 10^4 cells ml^{-1}, respectively. Total viable plate counts of aerobic bacteria were 10^4 to 10^7 CFU ml^{-1}. Direct microscopic counts of the cells in the water samples yielded values one to three orders of magnitude lower than the plate counts due to the presence of particulate material in the samples which interfered with the direct counts. We could not make direct microscopic counts from the ore sample

because of the interference by particulates and the radiation hazard. However, plate counts showed 10^5 CFU g^{-1} of aerobic bacteria. Anaerobes were not detected in the water nor in the ore samples by the dilution plating technique.

Denitrifiers were detected in the ore and in all the water samples, except the water from well 220 which is in the ore zone. Fermenters and sulfate reducers were present in the water samples but their numbers were much lower (2 to 4 orders of magnitude lower) than those of the denitrifiers. Small numbers of methanogens were detected in the water samples from wells 67 (altered sandstone zone), 75 (upper sandstone zone), and 81 (near the clay-sandstone interface). Iron oxidizers, sulfur oxidizers, and iron reducers were not detected in the ore or water samples in MPN analyses. However, iron reducers from the ore sample and water samples #67, #81, and #211, and sulfur oxidizers from samples #67, #79, and #219 were isolated from enrichment cultures.

Denitrifiers were the predominant group of microorganisms in all the water samples tested. The rates of denitrification in the water samples were determined by the acetylene blockage technique, with and without the addition of carbon and/or nitrate. Nitrous oxide was detected in nitrate and nitrate-plus-carbon amended samples, but not in the carbon amended or unamended samples. In nitrate-amended samples, all added nitrate was converted to N_2O in groundwater samples #67, #75, and #81 at the rate of 4.06, 2.50, and 2.74 nmol h^{-1} ml^{-1}, respectively. These results suggest that an adequate supply of metabolizable carbon is present in the samples, but denitrification is limited by the availability of nitrate.

INTRODUCTION

The deposit of high-grade Cigar Lake uranium ore in Northern Saskatchewan, is one of the richest ones in the world (Bruneton, 1986, 1993). It is located in a sandstone formation, where the uranium is surrounded by a clay-rich halo just above the unconformity in contact with the underlying Archean basement complex (Figure 1). The 1.3 Ga old deposit is overlain by about 400 m of sandstone and about 30 m of sandy and bouldery glacial sediments derived from the bedrock source.

Despite the presence of major aquifers in the permeable sandstone host rocks, the clay-hosted uranium ore has been well preserved for 1 Ga. Uranium solubility is predominantly influenced by redox processes. Uranium in the oxidized state (U^{6+}) is fairly soluble, while the reduced form (U^{4+}) is quite insoluble and tends to precipitate out of solution. In addition to geochemical reactions, microbial activity also can alter the redox conditions. Although geochemical aspects of the Cigar Lake deposit have been studied in detail (Cramer and Smellie, 1994), the microbial ecology of the site has not been fully investigated.

The objectives of this study were (i) to determine the numbers and types of microorganisms in the ore and the surrounding groundwater samples, and (ii) to determine the effects of microbial activity on the dissolution and precipitation of uranium. In this report, we discuss the results of our first objective, the population distribution of microorganisms in the ore and water samples collected in September 1992.

MATERIALS AND METHODS

Water Sample

Water samples were collected from piezometers located throughout the deposit down to 450 m below the surface (Figure 2). Samples were collected by J. Cramer, AECL-Research, Pinawa, Canada, using a down-hole sampling rig (Cramer and Smellie, 1991). Of the six water samples, two were from piezometers in the ore zone (#79 and #220), two from the sandstone (#67 and #75), and two from near the clay-sandstone interface (#81 and #211). A string of stainless-steel pressure vessels (55-110 ml), with one-way check-valves at each end of the string were attached to a pressure hose and lowered down a piezometer to approximately 15 m above its bottom. The vessels and the pressure hose were overpressured with a N_2-gas up to the check-valve between the bottom of the lowermost vessel and the intake port. When the sampling string had been lowered into place, the N_2-pressure was bled off at the surface, and the bottom check-valve was opened, allowing water to rise up through the string of vessels and inside the pressure hose. When the water levels inside and outside the hose had equilibrated, the check-valves automatically shut, thereby trapping the water sample under ambient pressure at that depth. A N_2-gas over-pressure then was applied to the top of the pressure hose and the whole assembly was raised to the surface.

The microbiology sampling vessel (55 ml), including its two valves was sterilized at 550°C for 4-8 hours in a muffle furnace and was placed at the bottom of the string of vessels directly above the intake port. This arrangement ensured that the vessel was filled last after it had been thoroughly rinsed with all of the formation water filling the other vessels.

The length of the pressure hose used was between 150 m and 400 m, depending on the sampling depth.

The samples were brought to surface under the ambient pressure at the sampling depth. Once the microbiology vessel was isolated from the string, the contents were transferred to a sterile glass serum bottle, sealed with a sterile septum, and filled with N_2 gas. A sterile stainless-steel connector and needle were attached to one end of the vessel, and a He-gas over-pressure was used to transfer the water sample through the septum into the bottle; a second sterile needle bled the gas over-pressure from the bottle during the transfer. The sample bottles were labelled, stored in the dark at 4°C, and then shipped to the laboratory on ice in coolers.

Ore Sample

The Cigar Lake ore sample used in this study was provided by J. Cramer, AECL. As part of their test drilling into the ore deposit, the Cigar Lake Mining Corporation (CLMC) drilled several HQ3 diamond drill holes (60 mm diameter) to install freeze pipes. The freeze holes were drilled upwards through the basement rock underneath the ore zone from the 480 m level excavation located beneath the ore body; they generally extended another 5 m into the overlying clay-rich halo (Cramer and Smellie, 1991). Ore samples (2 to 4 cm long) were obtained by freezing the ore zone and drilling the ore. The frozen ore was collected immediately, transferred to a sterile plastic bag, and stored frozen under N_2 for microbiological studies. This procedure minimized the radiological hazard, use of drilling fluid, and contamination of the ore samples.

About 120 g of uranium ore sample (CS 456) was delivered to Brookhaven National Laboratory (BNL) on 8/12/92, and surveyed for the amount and type of radiation by the Health Physics (HP) and Safety and Environmental Protection (S&EP) Division. The ore was stored and handled in consultation with HP and S&EP Divisions. The ore was stored inside a beta-shielding box, and the box was secured in a locked refrigerator. It was handled inside a sterile glove bag filled with UHP nitrogen gas, sterilized by passing through a 0.22 μm Millipore © Millex filter, in a fume hood under negative pressure.

The surface of the ore was dark gray. The outer portion of the sample (4 g) was pared away with a sterile razor blade and was designated as Cigar Lake Ore Surface sample (CLOS). It then was ground to a finer powder in a sterile mortar and pestle, and stored in a sterile, glass screw-cap tube.

The inner pared core material (20 g) was transferred to another sterile glove bag and designated as Cigar Lake Ore Inner sample (CLOI). The inner core was light gray, and was readily ground into fine particles using a sterile mortar and pestle; it was stored in a sterile, screw-cap tube.

I. MICROBIOLOGICAL ANALYSES

The microbial populations in the water and ore samples were estimated by (i) direct microscopy using the DAPI staining method for total bacteria and the CTC method for respiring cells, (ii) the dilution-plating technique for viable aerobic and anaerobic microorganisms as colony forming units (CFUs), and (iii) the most probable number (MPN) technique for denitrifiers, iron- and sulfur-oxidizers, fermenters, iron- and sulfate-reducers, and methanogens. To

determine the viable microbial populations, serial dilutions of the water and ore samples were made in sterile phosphate buffered saline (PBS) solution (Wollum, 1982). PBS was pre-reduced by boiling and purging with UHP N_2 gas, and then it was sealed in serum bottles fitted with butyl rubber stoppers in a nitrogen atmosphere. The serum bottles were sterilized by autoclaving. Ten grams of the CLOI sample were weighed and suspended in 90 ml of sterile, pre-reduced deionized water containing 1% $Na_4P_2O_7$, in a serum bottle inside the anaerobic chamber. For the CLOS sample, we used 1 g in 9 ml water. The samples were placed on a wrist-action shaker and mixed for 30 minutes at room temperature (Balkwill and Ghiorse, 1985). Sterile needles and syringes were used to transfer aliquots of the water or ore samples to make serial dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}. The ore samples were manipulated under N_2 gas to maintain anoxic conditions. Their radioactivity was monitored with a Geiger counter at contact with the outside of the dilution bottles.

Direct Counts

Bacteria in the water samples were counted directly by epifluorescent microscopy using a double-strand, DNA specific stain 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) (Dann et al., 1971). For cell counts, one ml aliquots of each water sample (as received) were removed with a sterile needle and syringe, placed in a snap-cap vial, and preserved by adding 5% glutaraldehyde (v/v). The water samples contained a considerable amount of debris which interfered with the conventional DAPI staining (Schweizer, 1976); hence, a modified procedure was used (Huber et al., 1985) that involved adding low concentrations of the surfactant, sodium dodecyl sulfate (SDS), with the DAPI, and incubating the samples for 7 minutes in the dark, to make the cells

permeable to the dye. Samples were filtered through a 0.22 μm pre-stained black polycarbonate membrane filter (Poretics, Inc.) and then placed on a slide and examined under the microscope. Triplicate slides were prepared for each sample, and the fluorescent cells were counted directly using a calibrated grid eyepiece under ultraviolet light. DAPI staining facilitated differentiation of cells and detritus: DNA-containing material was blue, and the unstained detrital material was yellow.

DAPI stains DNA in live, dormant, and dead cells. To detect actively respiring cells, we used a redox dye, 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) (Rodriguez et al., 1992). In actively respiring cells, the enzymes of the electron transport chain reduce the dye to a bright red, fluorescent form that is insoluble and precipitates intracellularly. Samples were incubated with 5 mM CTC in sterile test tubes for 4 h at 28°C, and filtered through 0.2-2 μm pre-stained black polycarbonate membrane filters (Poretics, Inc.). The filters were air dried and observed under an oil immersion lens (x100) with a blue excitation filter (420 nm). Respiring bacteria were counted by epifluorescent microscopy.

Viable Counts

The number of viable aerobic bacteria were determined by the pour-plate technique (Wollum, 1982) using a dilute peptone-tryptone-yeast-glucose (PTYG) agar (Balkwill and Ghiorse, 1985). The medium consisted of the following (g/L distilled water): glucose, 0.1; yeast extract, 0.1; peptone, 0.05; tryptone, 0.05; $MgSO_4.7H_2O$, 0.6; $CaCl_2.2H_2O$, 0.07; agar, 15.0, at pH, 7.0. The dilute medium allowed organisms present in the oligotrophic environment to grow

(Balkwill and Ghiorse, 1985). Anaerobic bacteria were determined by plating in dilute pre-reduced PTYG medium, supplemented with L-cysteine HCl (0.2 g/L) and thioglycollic acid (0.2 g/L) to provide a low Eh in the medium. The ore samples (CLOS and CLOI), as well as the water samples, were plated in triplicate and incubated at 22 ± 2°C. Anaerobes were incubated in a GasPak jar (BBL) containing a CO_2/H_2 generator and palladium catalyst to maintain an anaerobic environment. Colonies were counted weekly.

Most Probable Number (MPN) Analyses

Media for the growth of iron-reducers, fermenters, sulfate-reducers, and methanogens were pre-reduced and 5 ml of each was dispensed into 10 ml serum bottles fitted with butyl rubber stoppers. Five milliliters of the iron-oxidizer, sulfur-oxidizer, and denitrifier media were dispensed in 13 x 125 mm screw-cap tubes. One milliliter of a series of dilutions of the ore (CLOI) and water samples were added to each of the media and incubated at 22 ± 2°C in the dark. The MPN was analyzed as described elsewhere (Alexander, 1982).

Denitrifiers

The denitrifier medium contained: nutrient broth (BBL), 8.0 g; KNO_3, 0.5 g; deionized water, 1 L, at pH, 7.0 (Focht and Joseph, 1973). Each tube with 5 ml of medium contained 25 μmoles of NO_3^-. A small glass tube (Durham tube) was inverted in the culture tube to detect gas production. The tubes were visually checked for turbidity and gas generation. The presence of nitrate and nitrite in the medium was determined by the diphenylamine reagent (Tiedje, 1982).

Iron Oxidizers

To determine the presence of iron oxidizers, the following medium was used: $(NH_4)_2SO_4$, 3.0 g; KCl, 0.1 g; K_2HPO_4, 0.5 g; $MgSO_4.7H_2O$, 0.5 g; $Ca(NO_3)_2$, 0.01 g; 10 N H_2SO_4, 1.0 ml, were dissolved in 700 ml of deionized water, and autoclaved. After cooling, 300 ml of filter-sterilized ferrous sulfate ($FeSO_4.7H_2O$, 15g/300 ml) was added, and the pH of the medium adjusted to 3.0 (Silverman and Lundgren, 1959). Five milliliters of the medium were dispensed into sterile 13 x 125 mm screw-cap tubes. The medium was observed weekly for the formation of a yellow-orange precipitate, indicating iron oxidation, and the pH was determined at the end of incubation.

Sulfur Oxidizers

The sulfur-oxidizer medium consisted of (per liter deionized water): $(NH_4)_2SO_4$, 0.2 g; $MgSO_4.7H_2O$, 0.5 g; $CaCl_2$, 0.25 g; KH_2PO_4, 3.0 g; and $FeSO_4$, 0.005 g (Frederickson et al., 1989). The medium (5 ml) was dispensed in 13 x 125 mm screw-cap tubes and autoclaved. Elemental sulfur (S^0) was steam-sterilized in aluminum foil for four hours over a boiling water bath. The sulfur was added to the sterile medium to a final concentration of 1% w/v (0.05g S^0). The pH of the medium was 4.4. Sulfur oxidizers were assayed after one month by measuring the change in the pH medium due to the formation of H_2SO_4.

Iron Reducers

Two types of media were used for iron reducers, one with acetate, and the other with lactate as the carbon source to isolate those organisms capable of using lactate or acetate as the electron donor (Lovley and Phillips, 1986; Nealson and Myers, 1992).

The acetate medium consisted of (g/L deionized water): $NaHCO_3$, 2.5; $CaCl_2.2H_2O$, 0.1; KCl, 0.1; NH_4Cl, 1.5; NaH_2PO_4, 0.6; NaCl, 0.1; $MgCl_2.6H_2O$, 0.1; $MgSO_4.7H_2O$, 0.1; $MnCl_2.4H2O$, 0.005; $NaMoO_4.2H_2O$, 0.001; Na-acetate, 2.7; yeast extract, 0.05; pH adjusted to 7.3. Amorphic iron was added to yield a final concentration of 100 mM. The acetate concentration in this medium was 30 mM (Lovley and Phillips, 1986).

The lactate medium consisted of (g/L deionized water): $NaHCO_3$, 2.5; $CaCl_2.2H_2O$, 0.1; KCl, 0.1; NH_4Cl, 1.5; NaH_2PO_4, 0.6; NaCl, 0.1; $MgCl_2.6H_2O$, 0.1; $MgSO_4.7H_2O$, 0.1; $MnCl_2.4H_2O$, 0.005; $NaMoO_4.2H_2O$, 0.001; Na-lactate, 0.27; yeast extract, 0.05; pH adjusted to 7.3. Amorphic iron was added to a final concentration of 10 mM. The concentration of lactate in this medium was 3 mM (Nealson and Myers, 1992).

Both media were checked weekly for turbidity, and assayed for Fe(II) in solution, by the ortho-phenanthroline method (American Public Health Association, 1975).

Fermenters

The fermenter medium consisted of (g/L deionized water): glucose, 5.0; NH_4Cl, 0.5; glycerophosphoric acid, 0.3; $MgSO_4.7H_2O$, 0.2; $FeSO_4.7H_2O$, 0.0025; $CaCl_2.2H_2O$, 0.5; peptone (Difco), 0.1; yeast extract (Difco), 0.1, at pH, 7.0 (Francis and Dodge, 1988). One month after inoculation, the medium was assayed for turbidity and a change in pH using 0.002% w/v Bromcresol purple to confirm organic acid production due to fermentation.

Sulfate Reducers

The sulfate reducers were determined using Postgate's medium B containing (g/L deionized water): KH_2PO_4, 0.5; NH_4Cl, 1.0; $CaSO_4$, 1.0; $MgSO_4.7H_2O$, 2.0; $FeSO_4.7H_2O$, 0.5; yeast extract (Difco), 1.0; thioglycollic acid, 0.1; sodium lactate, 3.5. The pH was 7.1 after autoclaving, and contained a precipitate, as described by Postgate, to aid in the growth of tactophilic strains (Postgate, 1984). The samples were checked for a black precipitate after one month, indicating the formation of iron sulfide due to sulfate reduction.

Methanogens

Methanogenic bacteria were determined using a complex mineral salts/trace element medium containing acetate, with carbon dioxide and hydrogen in the headspace, to promote the growth of both acetate, and CO_2 and H_2 metabolizing methanogens.

The trace elements solution contained (g/L deionized water): Nitrilotriacetic acid, 4.50; $FeCl_2.4H_2O$, 0.40; $MnCl_2.4H_2O$, 0.10; $CoCl_2.6H_2O$, 0.17; $ZnCl_2$, 0.10; $CaCl_2$, 0.02; H_3BO_3, 0.019; $NaMoO_4$, 0.01. The basal medium contained (g/900 ml deionized water): KH_2PO_4, 0.75; K_2HPO_4, 1.45; NH_4Cl, 0.90; $MgCl_2.6H_2O$, 0.20; Na_2CO_3, 2.00; $Na_2S \cdot 9H_2O$, 0.50; L-cysteine HCl, 0.50; peptone, 2.00; yeast extract, 2.00; sodium formate, 2.00; sodium acetate, 2.00, at pH, 7.5.

One milliliter of a 0.1% solution of resazurin and 9 mL of the trace mineral solution were added to the basal medium to obtain a final volume of one liter. The medium was boiled for five minutes and purged with nitrogen

gas before adding the reducing agents (L-cysteine-HCl and sodium sulfite) to prevent the formation of toxic compounds. Carbon dioxide was bubbled through the medium until it became colorless. The medium was cooled on ice and placed in the anaerobic glove box. L-cysteine HCl (0.05% w/v final concentration) and sodium sulfide (0.05% w/v final concentration) were added, and the pH adjusted to 7.0 with KOH. Nitrogen was then bubbled through the solution. The medium was transferred to serum bottles with butyl rubber stoppers. The bottles were autoclaved, cooled, and five milliliters of a sterile 50% CO_2/H_2 gas mixture was injected into the headspace before inoculation. Samples were observed for turbidity and assayed for methane production by gas chromatography using a flame ionization detector (Zeikus, 1977).

II. DENITRIFICATION IN WATER AND ORE SAMPLES

The rate and extent of denitrification in water samples was determined by the acetylene blockage technique (Yoshinare and Knowles, 1976). The effects of the addition of carbon, nitrate, and carbon-plus-nitrate on denitrification rates also were examined (Francis et al., 1989).

One milliliter of sterile pre-reduced deionized water containing the appropriate amendments was added to 10 ml serum bottles placed inside an anaerobic glove box filled with N_2. Treatments consisted of (i) no additions; (ii) 300 nmol nitrate; (iii) 3 mmol succinate; (iv) 300 nmol nitrate plus 3 mmol succinate; and (v) 300 nmol nitrate and 3 mmol succinate, plus 10% formalin (control). The bottles were sealed with butyl rubber stoppers, and autoclaved. One milliliter of the water sample (undiluted) or the ore suspension (10^{-2} dilution) was added to the medium. Two milliliters of acetylene

gas were injected into the headspace. Denitrification was determined by measuring the accumulation of N_2O by gas chromatography. The concentrations of N_2O were corrected for the solubility of N_2O in water. At the start, and periodically afterwards, 0.5 ml of the headspace gas was removed and analyzed for N_2O.

Quality Control

All chemicals and biologicals used were reagent grade or high purity (Difco). Gas chromatographic analysis for methane and N_2O was performed in conjunction with a range of analyzed standards (Scott Specialty Gases, South Plainfield, NJ) using a three point calibration. The diluent used for direct microscopic counts was checked with DAPI stain for any contaminating cells. Uninoculated samples of the pour plates and MPN bottles were incubated and assayed as appropriate to check for contamination. Specific MPN samples also were inoculated with sterile water to check for contamination.

RESULTS

Radiological Characteristics of Samples

The water and ore samples were surveyed for radioactivity with a Geiger counter (Table 1). Counts from water samples were obtained at a point of contact outside the sample bottle, and the ore samples--CLOS and CLOI, outside the plastic bag. Both samples showed significant levels of beta-radiation, and, therefore, were stored in a beta-box and handled with extra caution. The water samples were relatively low in radioactivity, but nevertheless, were handled cautiously.

Chemical Characteristics of Water Samples

Table 2 shows selected chemical characteristics of the groundwater samples (Cramer, personal communication, 1993) used in this study. The pH of all the samples ranged from 6.2 to 7.2, except for sample #220 which was alkaline (pH 8.9). Water samples #67, #79, and #81 were oxidizing (Eh 67-139 mV), whereas samples #75, #211, and #220 were reducing. The nitrate content in the water samples was low (0.01 to 0.32 mg/L). Total organic carbon was detected only in samples #81 and #220. Samples #67, #81, and #220 showed the highest uranium concentration in solution.

I. MICROBIOLOGICAL ANALYSES

Direct Counts

Table 3 shows the numbers of total and respiring bacteria in the water samples; the former ranged from 10^2 to 10^4 cells ml^{-1}, whereas respiring bacteria ranged from zero to 10^4 cells ml^{-1} and were detected in all water samples except sample #220. The bacteria in the ore samples (CLOI and CLOS) could not be counted directly because of the interference by the particulates, also, the high radioactivity of the ore sample rendered the counting under a microscope hazardous.

Viable Counts

Viable counts of bacteria determined by the pour-plate technique showed the presence of aerobic bacteria, but not anaerobes, in both the ore sample and the water samples. The aerobic populations in the water samples ranged from 10^4 to 10^7 colony forming units (CFUs) ml^{-1}. The ore sample contained 10^5 CFUs g^{-1}. On aerobic plates, various types of bacterial colonies were seen.

Some colonies were fast growing and were visible after three-four days, whereas others were slow growing and were seen only after two weeks. The types of colonies observed include transparent with serrate margins, mucoid, and pigmented ones. The control plates had no bacterial colonies. There were no differences in bacterial numbers between the outer and inner samples (CLOS and CLOI).

MPN Analysis

Table 4 gives the results of the MPN analyses for denitrifiers, fermenters, sulfate reducers, and methanogens in water samples and in the ore sample (CLOI).

Denitrifiers

Denitrifiers were the predominant group of microorganisms in the water and ore samples. Their numbers in the groundwater samples ranged from 10^4 to 10^7 organisms ml^{-1}, except the water sample #220 where none were detected. The ore sample contained 2.7×10^5 denitrifiers g^{-1}.

Fermenters

Fermenters were found in water samples #67, #75, #79, #81, and #211, ranging from < 1 to 2200 organisms ml^{-1}. Sample #211 had the highest number, while sample #79 had the least. No fermenters were detected in sample #220, nor in the ore sample.

Sulfate Reducers

Few sulfate-reducing bacteria were detected in all the water samples;

their numbers ranged from 1 to 110 organisms ml^{-1}. Reduction of sulfate caused blackening of the medium due to the formation of sulfide. Sulfate reducers were not detected in the ore sample.

Methanogens

Methanogens were detected in water samples #67, #75, and #81, but none in samples #79, #211 and #220, nor in the ore sample.

Iron and Sulfur Bacteria

No iron-oxidizing, iron-reducing bacteria, or sulfur-oxidizing bacteria were detected in the ore or the water samples by MPN analysis. However, sulfur-oxidizing bacteria were isolated from samples #67, #79, and #219, and iron reducers from samples #67, #81, and #211, and the ore sample in enrichment cultures.

Microbiological Analyses--Summary

Tables 1 and 2 of the Appendix summarizes the microbiological analyses of the water and ore samples.

II. DENITRIFICATION IN WATER AND ORE SAMPLES

Figures 3-8 show the rate and extent of denitrification and the effect of adding carbon, nitrate, and carbon plus nitrate. The sample from well 220 was not analyzed since no denitrifiers were detected during MPN analysis. Water samples amended with nitrate alone showed that the production of nitrous oxide, and denitrification of the added nitrate was almost complete. Carbon plus nitrate amended samples showed only partial denitrification of the added

nitrate. None of the unamended samples nor the carbon-amended samples showed production of nitrous oxide, even after two weeks. Formalin-treated samples with the carbon plus nitrate amendment showed no denitrification.

Denitrification activity was detected in the ore sample amended with nitrate, but not with carbon nor carbon plus nitrate. The unamended, as well as the formalin treated carbon plus nitrate amended ore samples, showed no dentrification.

The rates of denitrification were determined for samples #67, #79, and #81 (Figures 9-11). All three showed denitrification after a 20-hour lag. The rates of denitrification (as nmol of N_2O produced per ml of water) in water samples #79 and #81 were similar, 2.5 nmol h^{-1} and 2.74 nmol h^{-1}, respectively, and slightly higher in #67, 4.06 nmol h^{-1}. Samples #67 and #81 were from the unaltered sandstone, but #81 was close to the interface with the clay lens. Sample #79 was from the ore zone.

None of the unamended nor carbon-amended samples showed any denitrification. Nitrate-amended samples showed denitrification, indicating that metabolizable carbon is present in the water samples, which the denitrifying bacteria can use.

DISCUSSION

Direct microscopy, viable counts, and MPN analyses confirmed the presence of bacteria in groundwater samples of the Cigar Lake deposit, including aerobic and anaerobic bacteria (denitrifiers, sulfate reducers, fermenters,

and methanogens). Aerobic bacteria and denitrifiers were present in the ore sample. Whether these bacteria are indigenous or are introduced through the mining operations is not known.

Usually, direct microscopic counts give higher numbers than viable counts, as both live and dead cells are counted. In this case, direct microscopic counts were lower than the plate counts because the particulate material present in the water samples interfered with direct counting.

Of particular interest is the low number of the microbial populations observed in sample #220, which is from the ore zone; this may be due to the radiation effect as well as the high pH. Further studies are warranted to determine the presence and activity of microorganisms in this zone.

Denitrifiers constituted a majority of the microbial population in the ore and water samples. An assesment of the denitrification potential in the samples revealed the following: (i) nitrate-amended samples showed complete denitrification of the added nitrate; (ii) carbon plus nitrate-amended samples showed only partial denitrification; and (iii) there was no denitrification in carbon-amended samples. Therefore, adequate amounts of metabolizable carbon are present in the water sample, but it is limited in nitrate. The reason for the reduced denitrification observed in the carbon plus nitrate-amended samples is not known. Further studies on the bacterial metabolism of the carbon sources are needed, including those unidentified compounds present in the water samples. The presence of denitrifiers in the groundwater samples and the ore sample implies that under anaerobic conditions, they may generate

nitrogen gas when nitrate is present. For example, nitrogen gas was detected in all the water samples tested, with #79 showing the highest amount (Cramer and Smellie, 1991). Bacterial denitrification is a possible source of the N_2 gas, although the source of nitrate is not known. Humic and fulvic acids were identified in the water samples (Allard and Petterrson, 1991). Alpha-radiolysis of nitrogen-containing organic matter may be one potential source of nitrate. Jorgensen and Tiedje (1993) reported that denitrifiers survive over long periods in nitrate-free, anaerobic environments. In survival experiments under starvation conditions, these bacteria showed a low-level of fermentation under anaerobic conditions sufficient to maintain viability, but not enough for growth.

The presence of a variety of organisms in the water samples suggests that the microbes could alter the mobility of uranium by direct or indirect actions. Dissolution or precipitation of uranium can be brought about directly due to enzymatic reduction, or indirectly due to production of metabolites (Francis et al., 1994). In the absence of nitrate, Fe(III) or U(VI) may serve as alternate electron acceptors since an adequate supply of metabolizable carbon is available in the subsurface. Additional studies are underway to determine the extent of dissolution or precipitation of uranium by selected microbes isolated from the ore and water samples.

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Table 1. Radioactivity of Samples (at contact outside the container)

Sample	Counts per Minute
Cigar Lake Ore	
Dilution 10^{0}	25,000
CLOI	
10^{-1}	2,000
10^{-2}	200
10^{-3}	150
10^{-4}	100
$>10^{-5}$	< 100
10^{-1}	2,000
10^{-2}	200
10^{-3}	150
10^{-4}	100
$>10^{-5}$	< 100
Cigar Lake Water	
10^{0}	100
10^{-1}	< 100

CLOI = Cigar Lake Ore Inner sample
CLOS = Cigar Lake Ore Surface sample

Table 2. Chemical Characteristics of the Cigar Lake Water Samples*

Well	Depth (m)	Environment	pH	Eh (mV)	T (°C)	TOC (mg/L)	NO_3^- (mg/L)	N_2 gas (cm^3/L)	U (µg/L)
67	345-348	Altered sandstone	7.0	67	10.6	NA	0.01	38.32	8.07
75	NA	Altered sandstone	6.3	-104	14.1	NA	0.13	36.15	0.31
81	440-444	Altered sandstone clay interface	6.5	84	8.3	1.09	0.03	64.99	2.1
211	NA	Altered sandstone clay interface	7.2	- 44	2.2	NA	<0.065	98.91	0.76
79	430-433	Ore Zone	6.2	139	11.1	NA	0.32	203.06	0.28
220	432-439	Ore Zone	8.9	-156	11.5	2.53	0.2	50	3.73

* Data provided by J. J. Cramer, AECL.
NA: Not analyzed.

Table 3. Bacterial Populations in Cigar Lake Samples

Sample	Direct Cell Counts		Colony Forming Units[3] (Aerobes)
	DAPI[1]	CTC[2]	
Water: (ml^{-1})			
Well 67	4.2×10^4	1.3×10^4	$2.4 \pm 0.5 \times 10^7$
Well 75	1.4×10^4	5.6×10^3	$2.6 \pm 0.75 \times 10^5$
Well 79	1.1×10^4	2.1×10^3	$6.7 \pm 1.0 \times 10^5$
Well 81	2.1×10^4	1.3×10^4	$3.6 \pm 0.5 \times 10^7$
Well 211	4.4×10^4	6.2×10^3	$4.5 \pm 0.6 \times 10^5$
Well 220	4.7×10^2	ND[4]	$1.8 \pm 0.2 \times 10^4$
Ore: (g^{-1})			
CLOI (interior)	NA[5]	NA[5]	$1.4 \pm 0.9 \times 10^5$
(surface)	NA[5]	NA[5]	$1.6 \pm 0.7 \times 10^5$

1: 4',6-diamidino-2-phenylindole dihydrochloride.
2: 5-cyano-2,3-ditolyl tetrazolium chloride.
3: No colonies were observed in anaerobic plates.
4: Not detected.
5: Not analyzed.

Table 4. Analysis of Microbial Populations in Cigar Lake Samples

Sample	Denitrifiers	Fermenters	Sulfate Reducers	Methanogens
Water (MPN/ml):				
Well 67	1.6×10^7	7.9×10^2	1.1×10^2	1.1×10^3
Well 75	4.9×10^4	4.4×10^1	1.1×10^2	1.2×10^2
Well 79	9.2×10^6	1	1	ND
Well 81	1.0×10^7	2.1×10^2	1.1×10^1	4.9×10^1
Well 211	1.0×10^7	2.2×10^3	1.1×10^2	ND
Well 220	ND	ND	1.1×10^2	ND
Ore (MPN/g):				
CLOI	2.7×10^5	ND	ND	ND
	NA	NA	NA	NA

ND: None detected.
NA: Not analyzed.

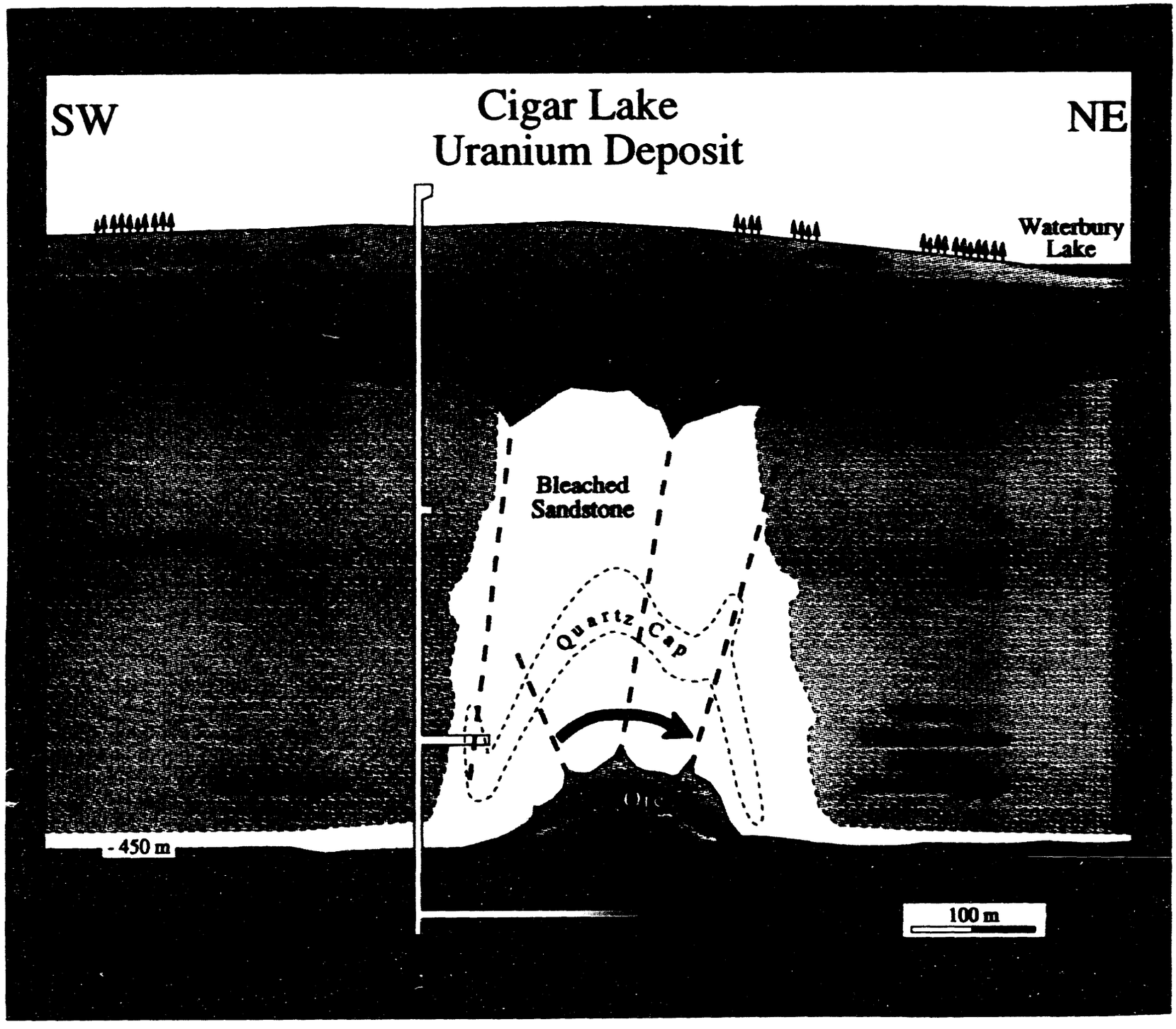

Figure 1. Schematic Cross-section of the Location of the Ore in the Cigar Lake Uranium Deposit.

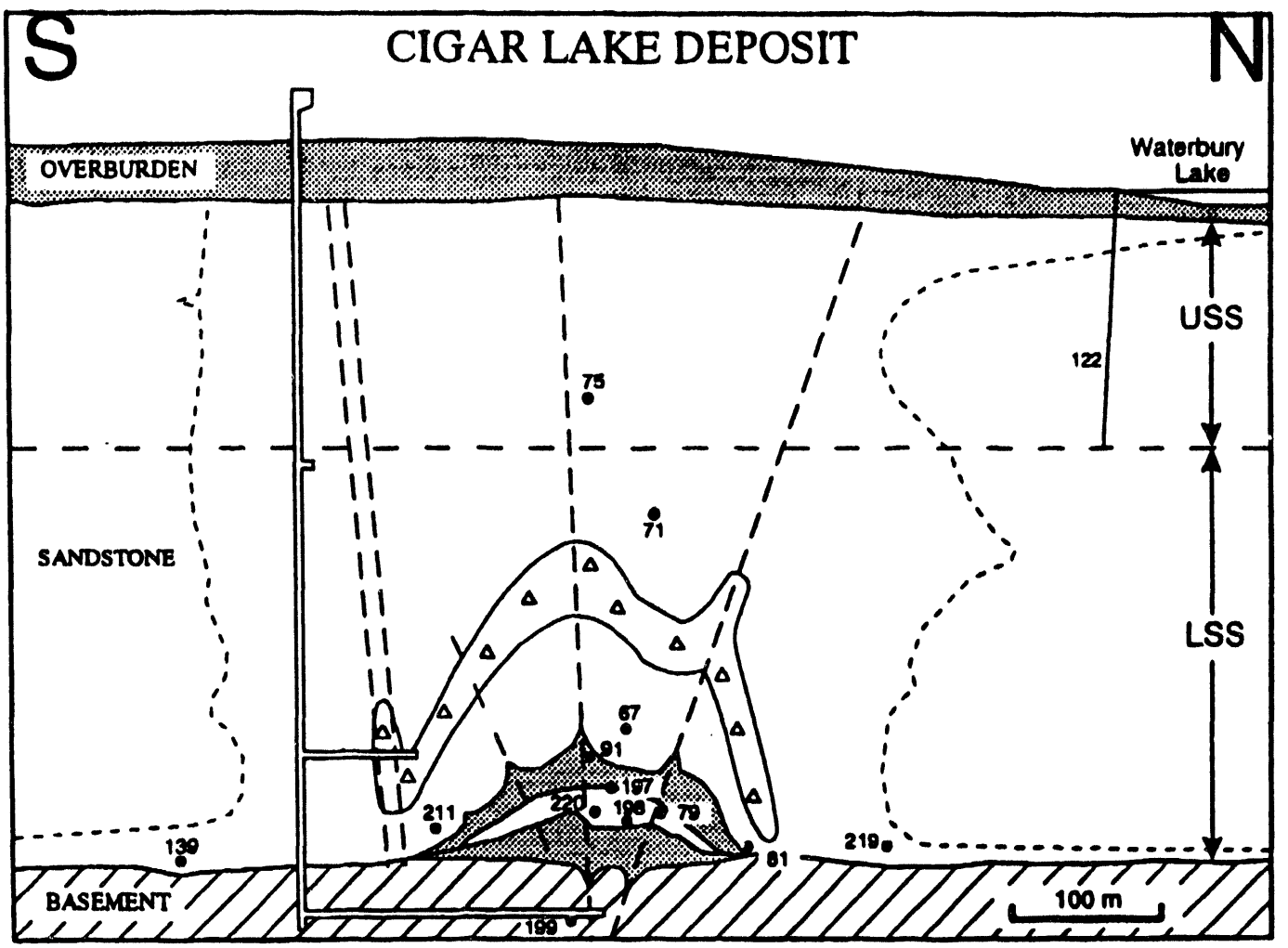

Figure 2. Schematic Cross-section of the Cigar Lake Uranium Deposit Showing Locations of Sampling Wells.

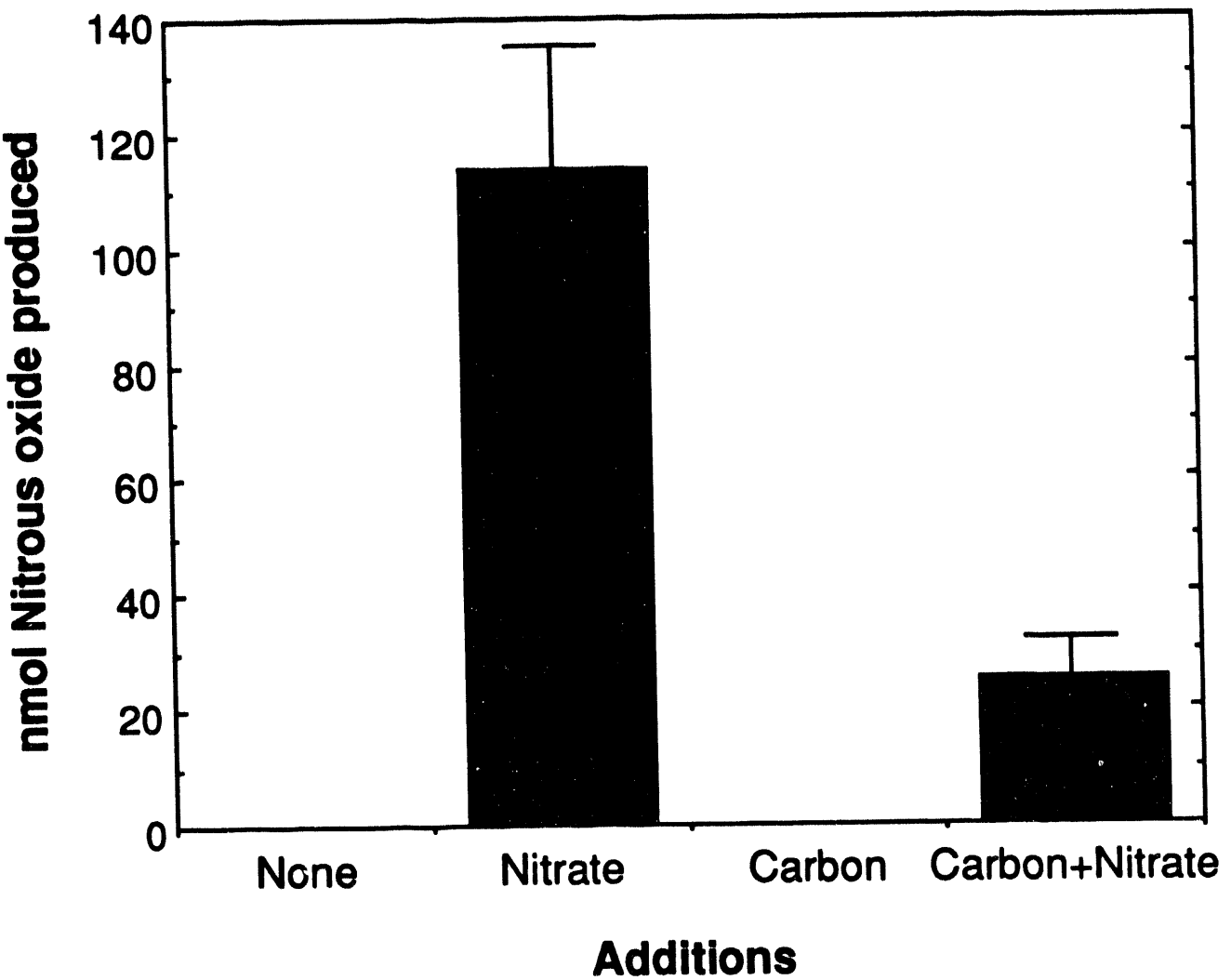

Figure 3. Denitrification in Groundwater Sample 67.

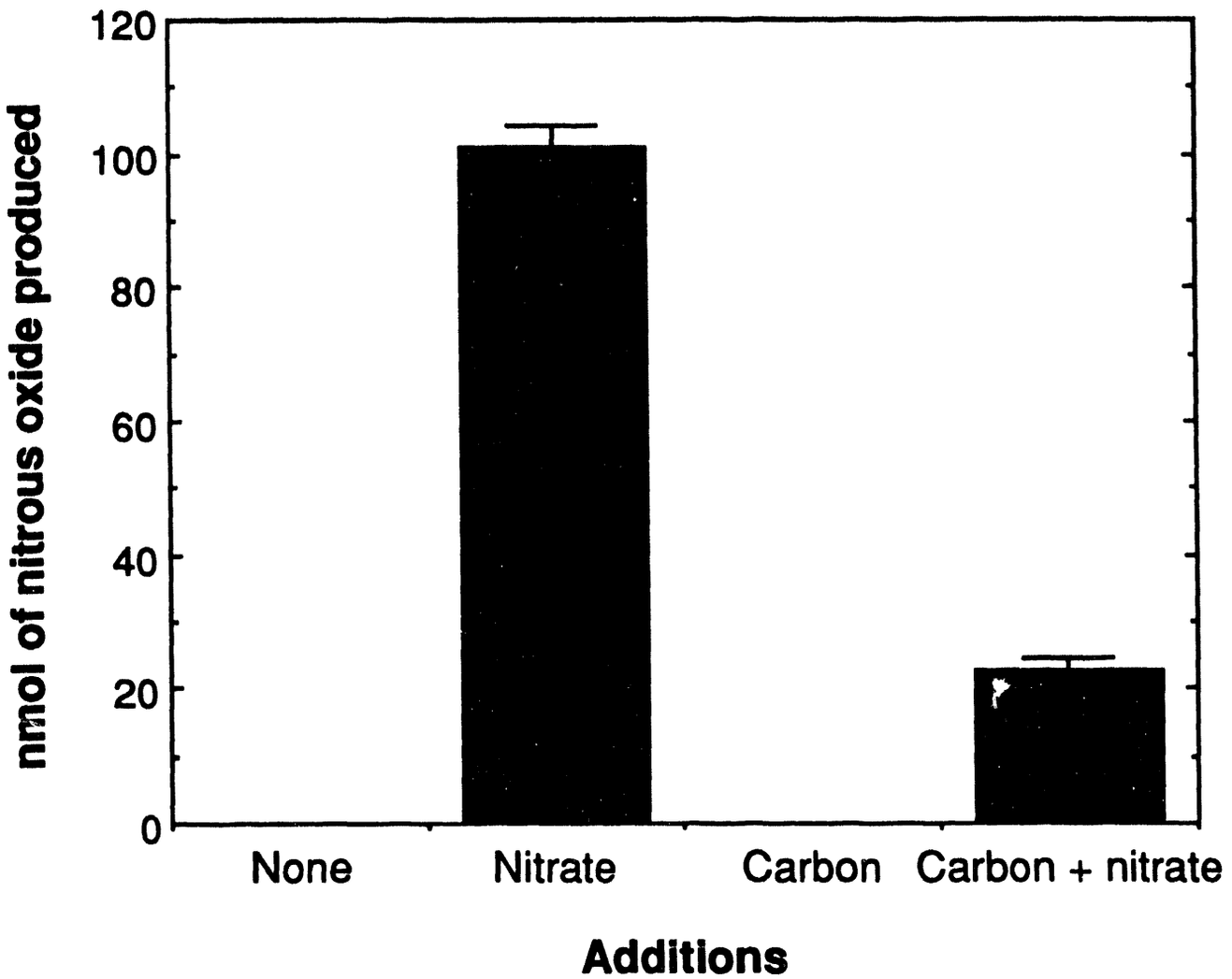

Figure 4. Denitrification in Groundwater Sample 75.

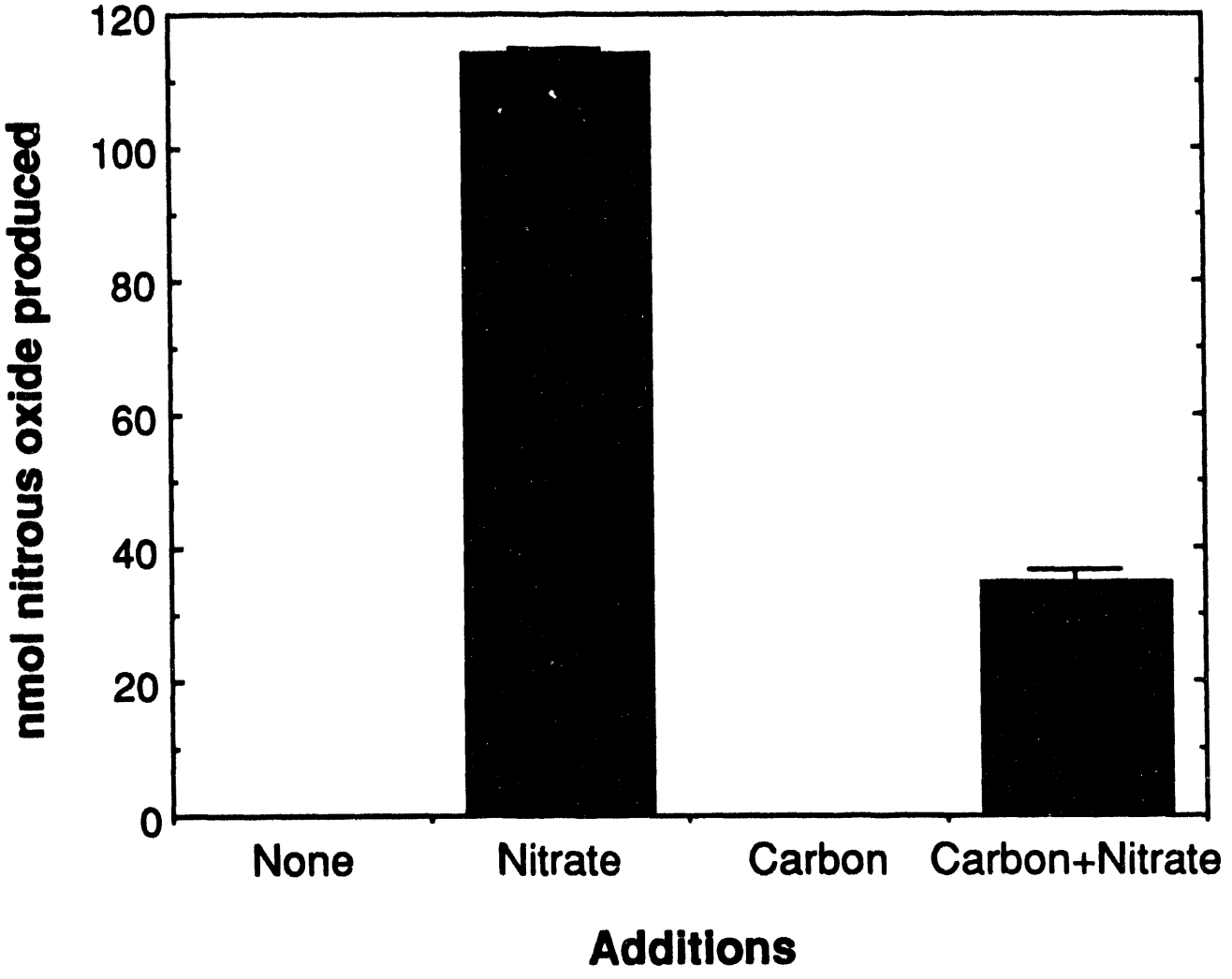

Figure 5. Denitrification in Groundwater Sample 79.

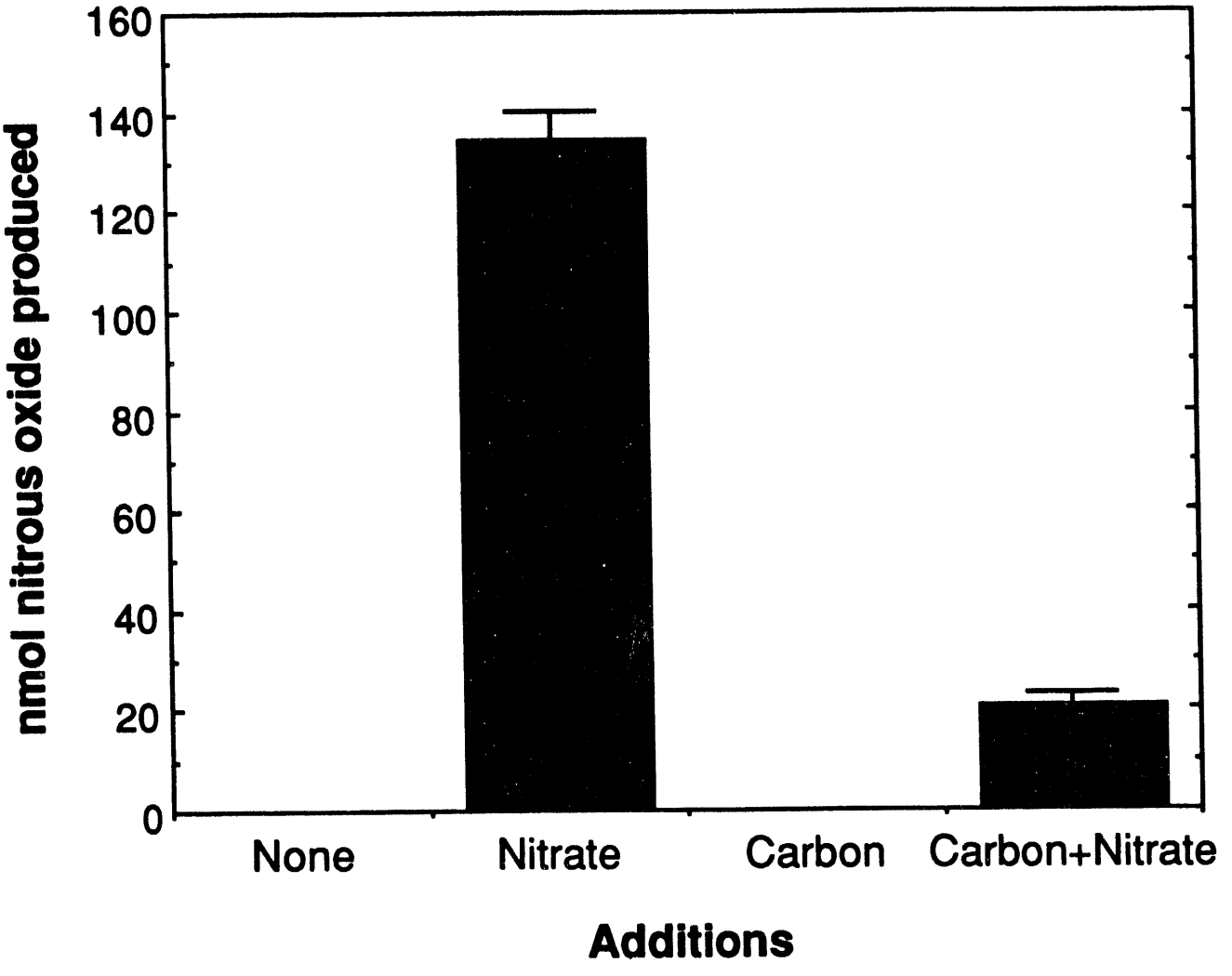

Figure 6. Denitrification in Groundwater Sample 81.

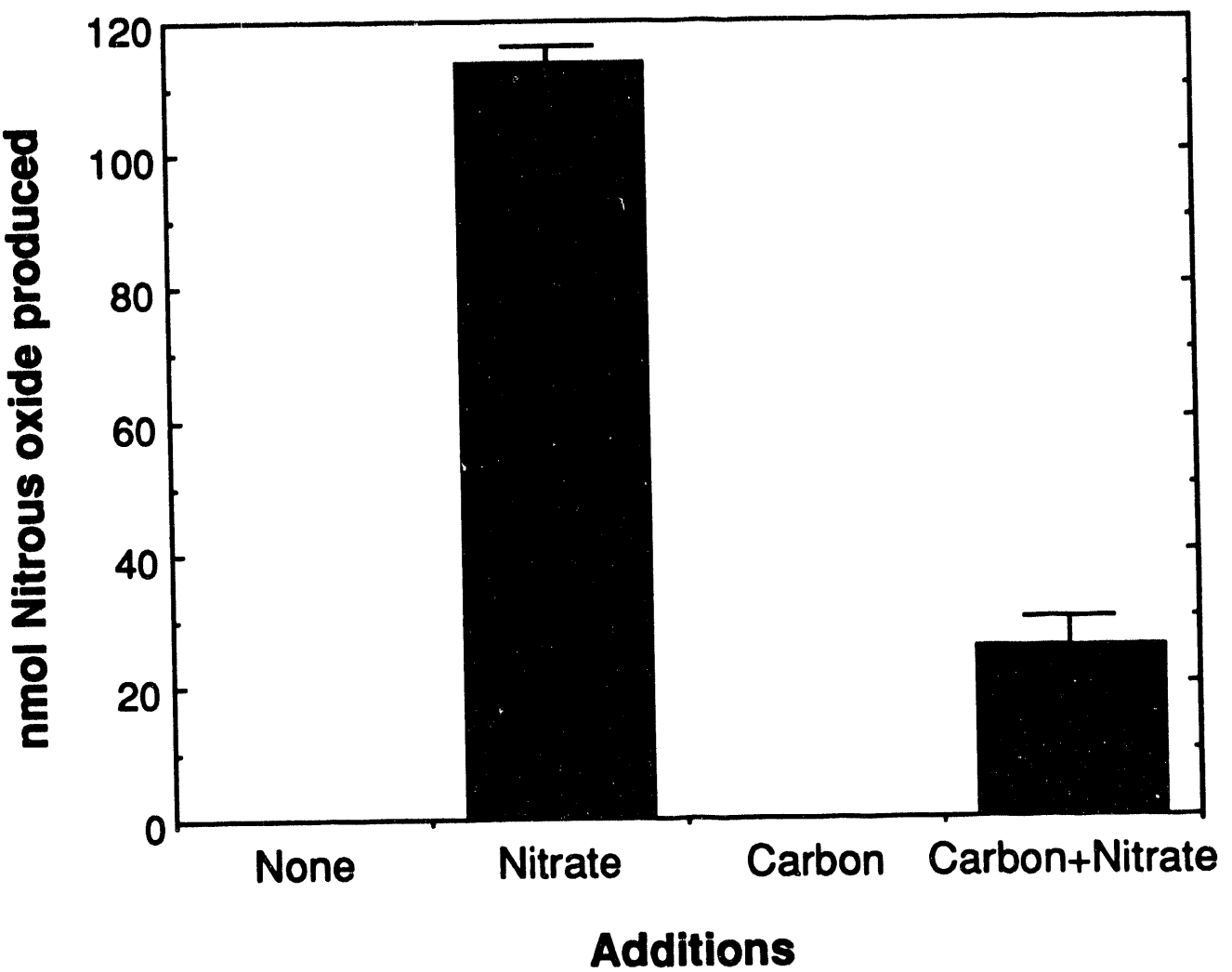

Figure 7. Denitrification in Groundwater Sample 211.

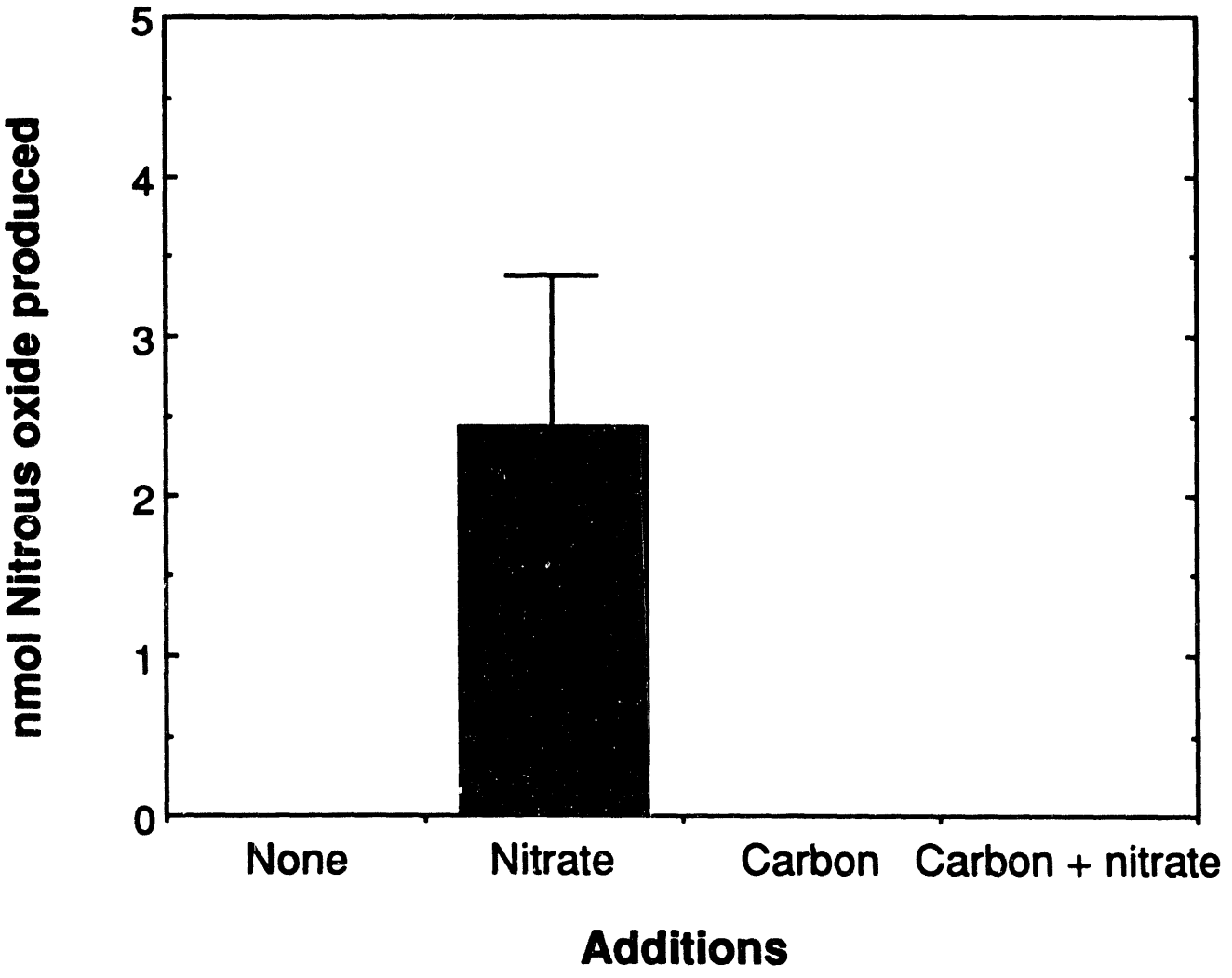

Figure 8. Denitrification in the Ore Sample.

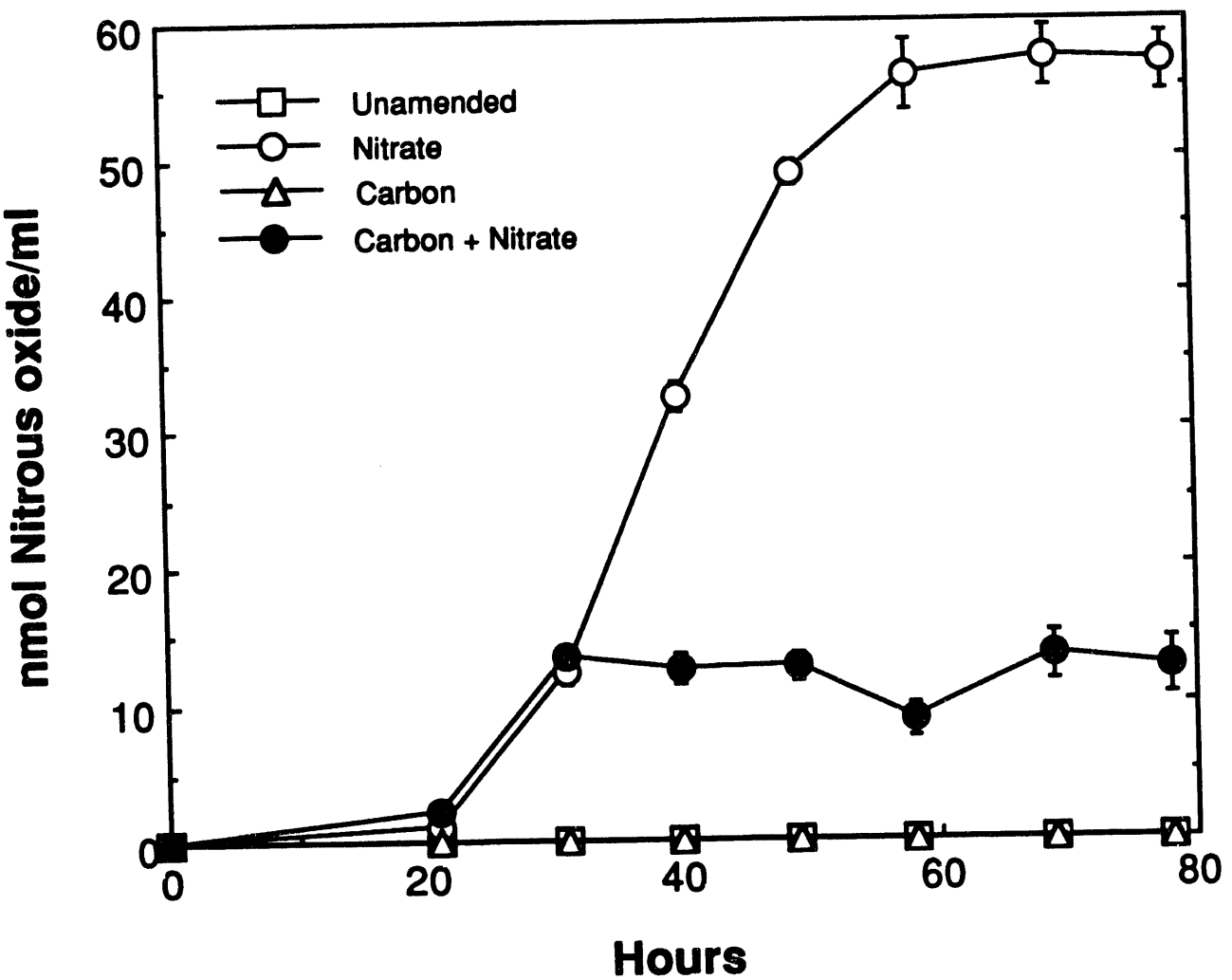

Figure 9. Denitrification in Groundwater Sample 67.

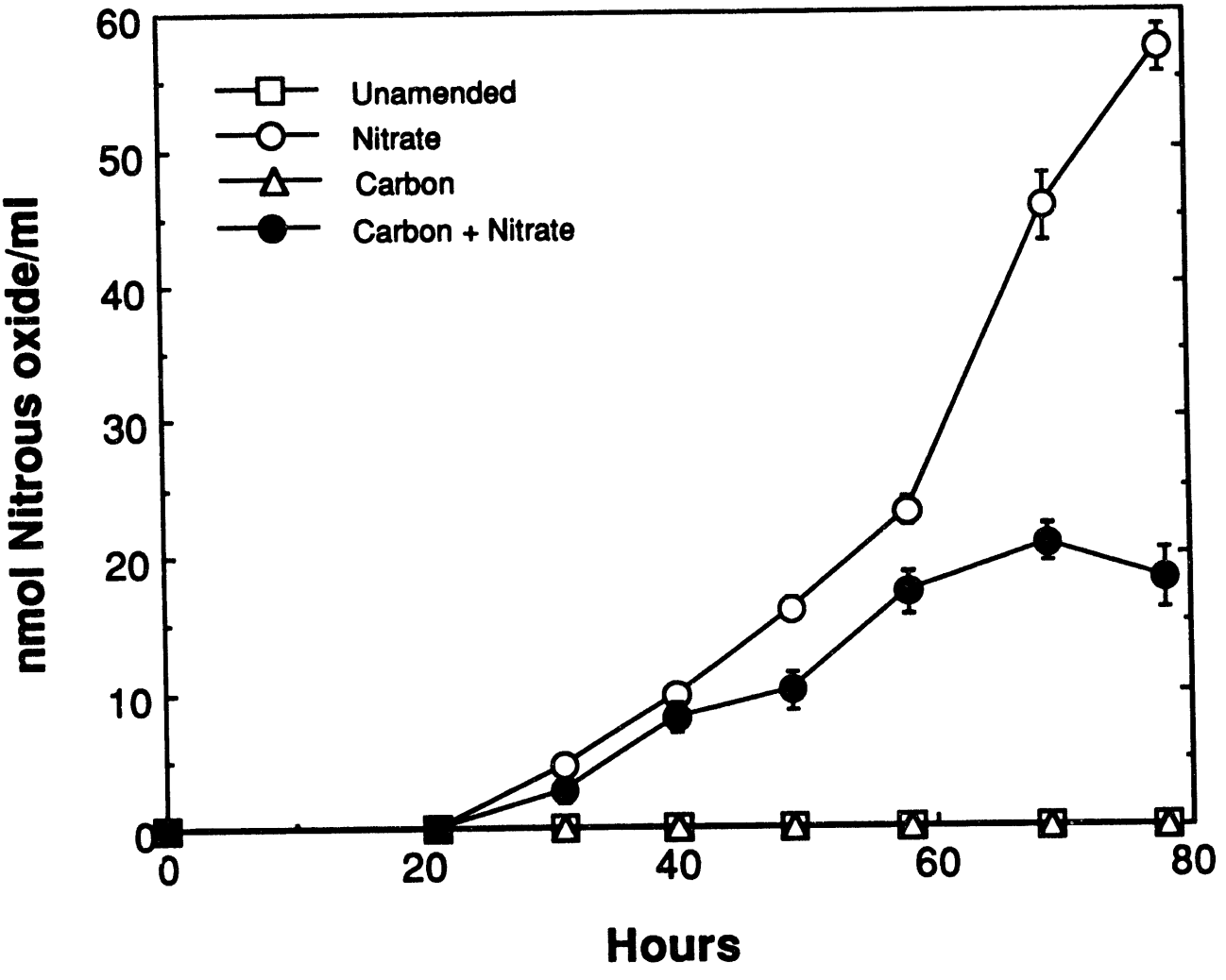

Figure 10. Denitrification in Groundwater Sample 79.

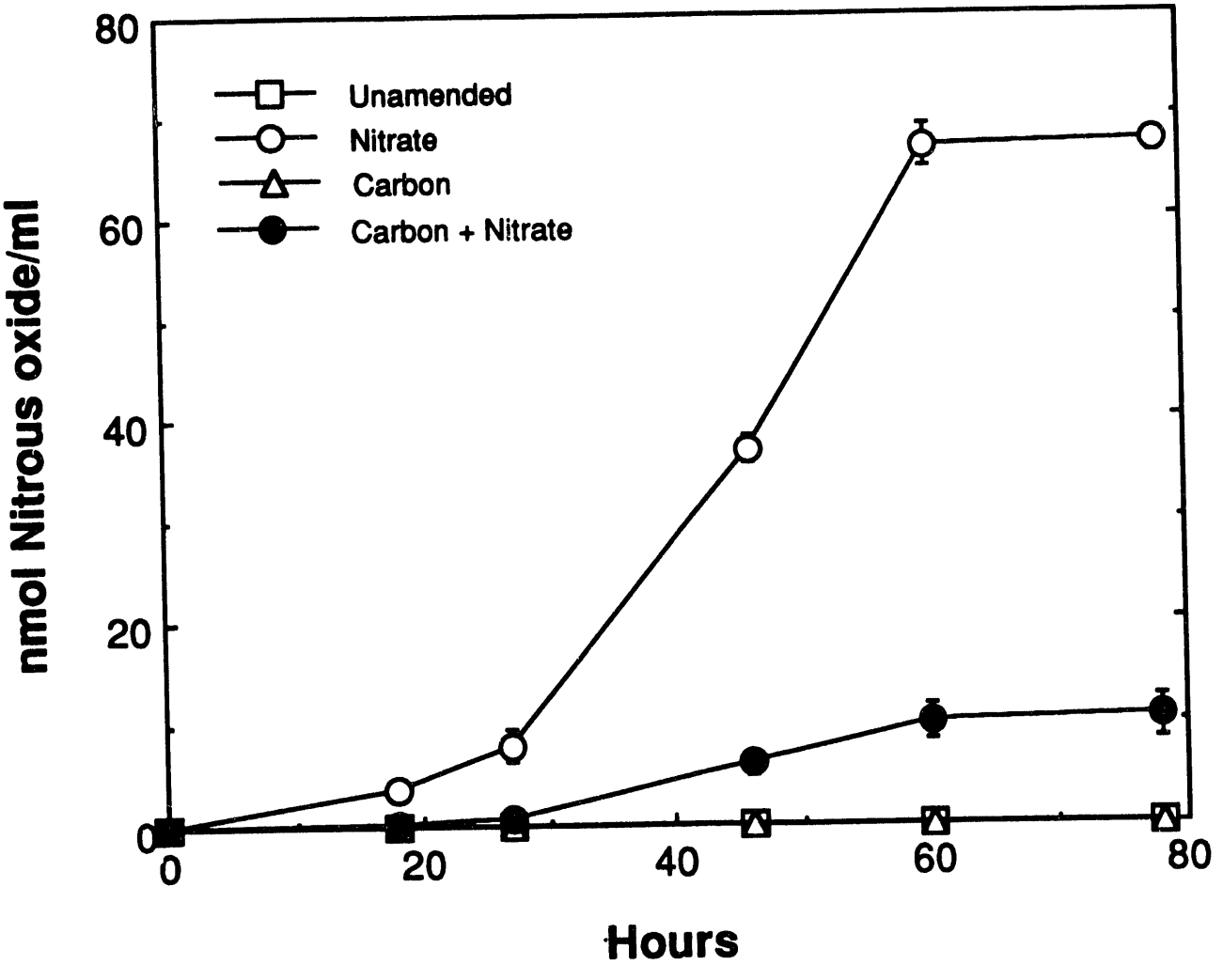

Figure 11. Denitrification in Groundwater Sample 81.

APPENDIX

APPENDIX

Table 1. Microbiological Analyses of Cigar Lake Samples

	Direct Counts[a]					Plate Counts[b]					
	DAPI			CTC		Aerobes			Anaerobes		
Sample	9/91	9/92	9/93	9/92	9/93	9/91	9/92	9/93	9/91	9/92	9/93
Ore (g^{-1})	NA	NA	NA	NA	NA	NA	1.6×10^5	NA	NA	0	NA
Water (ml^{-1})											
67	4.2×10^3	4.1×10^4	1.6×10^3	1.3×10^4	0	9.5×10^3	2.4×10^7	3.8×10^5	2.0×10^4	0	0
75	NA	1.4×10^4	NA	5.6×10^3	NA	NA	2.7×10^5	NA	NA	0	NA
79	2.3×10^3	1.1×10^4	7.8×10^3	2.1×10^3	0	9.1×10^4	6.7×10^5	2.1×10^5	1.0×10^5	0	0
81	7.3×10^3	2.1×10^4	NA	1.3×10^4	NA	2.0×10^4	3.6×10^7	NA	4.9×10^4	0	NA
139	2.9×10^3	NA	NA	NA	NA	2.7×10^5	NA	NA	2.7×10^5	NA	NA
197	6.5×10^3	NA	NA	NA	NA	4.0×10^5	NA	NA	3.2×10^5	NA	NA
211	2.4×10^4	4.4×10^4	NA	6.2×10^3	NA	1.4×10^5	4.6×10^5	NA	2.1×10^5	0	NA
219	2.9×10^3	NA	1.5×10^3	NA	0.8×10^3	2.3×10^5	NA	5.8×10^4	1.6×10^5	NA	0
220	5.1×10^2	4.7×10^2	NA	NA	NA	3.2×10^1	1.9×10^4	NA	2.0×10^2	0	NA

[a] = Cells ml^{-1}.
[b] = Colony forming units ml^{-1}.
NA = Not analyzed.
0 = Below the detection limit.

APPENDIX (cont.)

Table 2. Microbiological Analyses of Cigar Lake Samples

	Denitrifiers			Fermenters			Sulfate-reducers			Methanogens		
Sample	9/91	9/92	9/93	9/91	9/92	9/93	9/91	9/92	9/93	9/91	9/92	9/93
Ore (MPN g^{-1})	NA	$2{\cdot}7x10^{5}$	NA	NA	0	NA	NA	0	NA	NA	0	NA
Water (MPN ml^{-1})												
67	$>10^{2}$	$1{\cdot}6x10^{7}$	$3x10^{4}$	$>10^{2}$	$7{\cdot}9x10^{2}$	$4x10^{1}$	0	$1{\cdot}1x10^{2}$	<1	0	$1{\cdot}1x10^{3}$	$1{\cdot}0x10^{0}$
75	NA	$4{\cdot}9x10^{4}$	NA	NA	$4{\cdot}4x10^{1}$	NA	NA	$1{\cdot}1x10^{2}$	NA	0	$1{\cdot}2x10^{2}$	NA
79	$>10^{2}$	$9{\cdot}2x10^{6}$	$1{\cdot}1x10^{4}$	0	<1	<1	$1{\cdot}0x10^{0}$	$1{\cdot}0x10^{0}$	0	0	0	0
81	$>10^{2}$	$1{\cdot}0x10^{7}$	NA	$3{\cdot}0x10^{2}$	$2{\cdot}1x10^{2}$	NA	NA	$1{\cdot}1x10^{1}$	NA	NA	$4{\cdot}9x10^{1}$	NA
139	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
197	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
211	$>10^{2}$	$1{\cdot}0x10^{7}$	NA	$1{\cdot}0x10^{0}$	$2{\cdot}2x10^{3}$	NA	NA	NA	NA	NA	ND	NA
219	NA	NA	$7x10^{3}$	NA	NA	$7x10^{0}$	NA	$1{\cdot}1x10^{2}$	$7x10^{0}$ND	NA	NA	$1{\cdot}0x10^{0}$
220	0	0	NA	0	0	NA	$2{\cdot}0x10^{0}$	$1{\cdot}1x10^{2}$	NA	0	0	NA

MPN = Most probable number.
NA = Not analyzed.
0 = Below the detection limit.

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