# Use of $^{13}NO_3^-$ to Determine the Reduction of Nitrate to Ammonia in Anaerobic Environments

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Very little is known about the ecology of the bacterial reduction of nitrate to ammonia. Earlier work in our laboratory[1] indicated that this generally assimilatory process can be used as a means of electron disposal in the energy metabolism of an organism (Clostridium) prevalent in nature. The present report defines the quantitative importance of microbial nitrate-reduction to ammonia in a variety of long-term anaerobic, methanogenic environments.

Earlier studies on the fate of nitrate in freshwater and marine sediments have been done with $^{15}N$[2,3]. However, incubation times of several hours were required because of the sensitivity of the $^{15}N$-analysis. Thus, it was essentially impossible to determine whether nitrate was reduced directly to free ammonia or whether the reduced nitrogen was incorporated into biomass and subsequently mineralized to free ammonia. The use of $^{13}NO_3^-$ and an HPLC analytical system[4] provided a powerful tool for short-term incubations not only because of the specificity of the detection but also because of its high sensitivity.

A. Digested sludge

As demonstrated in Table 1, digested sludge in 30 min completely reduced both $^{13}NO_3^-$ and $^{13}NO_2^-$ at initial $NO_3^-$-concentrations up to 0.3 mM. An autoclaved control showed that this reaction was biologically mediated. About 60% of the label reduced further than $^{13}NO_2^-$ was recovered following membrane-filtration in the extracellular $NH_4^+$-pool. The remaining 40% of label was found in the end products of denitrification, $N_2$ and $N_2O$. Very little radioactivity remained on the filters, suggesting that N-immobilization was insignificant. Pasteurized samples (30 min at 70° C) showed a quick recovery of both the nitrate-reducing and the ammonia-forming activity, indicating that spore-forming organisms were at least in part responsible for both reactions. However, a smaller fraction of the reduced label was found in the extracellular $NH_4^+$-pool as well as in the products of denitrification, $N_2$ and $N_2O$. Thus some immobilization may have occurred in pasteurized samples.

B. Lake sediment

As demonstrated by Table 2, sediment of a hypereutrophic lake had considerably less $NO_3^-$-reducing capacity than the digested sludge. Moreover, only about 10% of the label reduced further than $^{13}NO_2^-$ could be recovered in the extracellular $NH_4^+$-pool. This means that $NO_3^-$ reduction to $NH_4^+$ is of less quantitative importance in this lake sediment than in the digested sludge. Pasteurization did not significantly affect both $NO_3^-$-reducing and $NH_4^+$-forming activities. SInce after 50 min incubation only very little radioactivity was found in the solid fraction of the sediment, immobilization of $^{13}NO_3^-$ and subsequent release of $^{13}NH_4^+$ through mineralization can be ruled out as a major pathway of $NO_3^-$-reduction.

Table 1. Reduction of $^{13}NO_3^-$ and $^{13}NO_2^-$ and formation of $^{13}NH_4^+$ by samples of digested sludge.

| Treatment | Initial $NO_3^-$ conc. (moles/l) | Label reduced further than $^{13}NO_2^-$ (%) | Reduced label found as $^{13}NH_4^+$ (%) | HPLC-label recovery (%) |
|---|---|---|---|---|
| Fresh | $3.10^{-4}$ | 95 | 60 | 58 |
| Fresh | $3.10^{-5}$ | 100 | 70 | 70 |
| Fresh | $3.10^{-6}$ | 100 | 67 | 67 |
| Pasteurized | | | | |
| 1 day before exp. | $3.10^{-6}$ | 99 | 50 | 51 |
| 3 days before exp. | $3.10^{-6}$ | 91 | 37 | 43 |
| 6 days before exp. | $3.10^{-6}$ | 100 | 45 | 45 |
| Autoclaved, 6 days before exp. | $3.10^{-6}$ | 0 | -- | 112 |

Table 2. Reduction of $^{13}NO_3^-$ and $^{13}NO_2^-$ and formation of $^{13}NH_4^+$ by samples of a lake sediment.

| Treatment | Initial $NO_3^-$ conc. (moles/l) | Label reduced further than $^{13}NO_2^-$ (%) | Reduced label found as $^{13}NH_4^+$ (%) | HPLC label recovery (%) |
|---|---|---|---|---|
| Fresh | $3.10^{-5}$ | 13 | 0 | 87 |
| Fresh | $3.10^{-6}$ | 77 | 7 | 28 |
| Fresh | $3.10^{-7}$ | 25 | 4 | 77 |
| Pasteurized | | | | |
| 1 day before exp. | $3.10^{-6}$ | 18 | 5 | 83 |
| 3 days before exp. | $3.10^{-6}$ | -- | 2 | 104 |
| 6 days before exp. | $3.10^{-6}$ | 32 | 13 | 72 |
| Autoclaved, 6 days before exp. | $3.10^{-6}$ | 0 | -- | 75 |


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# Inhibition by Sulfide of Nitric and Nitrous Oxide Reduction by Denitrifying Pseudomonas fluorescens

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In a search of environmental factors that influence the overall activity and the differential release of gas products during denitrification, reference is most often given to parameters such as $O_2$, available carbon, pH and temperature. Much less attention has been paid to the parameters characteristic of the reduced environment, e.g., iron and sulfur compounds. In some environments, in particular the marine sediments, denitrification takes place in close proximity to zones of active transformation of iron and sulfur. In an earlier study in costal marine sediments significant accumulation of the denitrification intermediates, NO and $N_2O$, was noted in the redox transition zone near the sulfide-rich deeper layers; it was suggested that these accumulations were caused by either the low redox potential or the presence of sulfide in this zone[1]. The present study was undertaken to establish if a low redox potential or the presence of sulfide caused accumulation of NO and/or $N_2O$.

The denitrifying bacterium, Pseudomonas fluorescens, was incubated anaerobically in sealed vials with $^{13}NO_3^-$ or $^{13}NO_2^-$, in the presence of no reductant (Eh, + 300 mv), 15 mM Ti(III)-citrate (Eh, - 200 mv), or 0.3 mM $H_2S$ (Eh, - 200 mv) as the three treatments. After short incubations headspace gas was sampled and the quantity of $^{13}N$ gases determined by gas chromatography - proportional counting.[2]

The pattern of $^{13}N$ gas production from $^{13}NO_3^-$ is shown in Fig. 1. All three gases ($^{13}NO$, $^{13}N\text{-}N_2O$, $^{13}N\text{-}N_2$) were produced in all three treatments but the production pattern was influenced by treatment. The upper part of Fig. 1 shows the accumulation of $^{13}NO$ in the three treatments. The most pronounced accumulation of $^{13}NO$ was observed in the sulfide-containing series, which showed a 3-fold increase in NO.

Sulfide, however, exerted a stronger inhibitory effect on the reduction of $^{13}N\text{-}N_2O$ since $^{13}N\text{-}N_2O$ accumulated at the expense of $^{13}N\text{-}N_2$ in the $H_2S$-containing treatment. This was opposite to the result from the two other treatments where the production of $^{13}N\text{-}N_2O$ was small compared to that of $^{13}N\text{-}N_2$. The total gas production was similar in the three treatments which indicates that there was no major effect by $H_2S$ at the level of $^{13}NO_3^-$ and $^{13}NO_2^-$ reduction. A most important result was the similar gas composition in the presence of Ti(III) reductant and in the absence of any reductant. This suggested that the inhibitions were not caused by the low redox potential but rather induced by some specific action of the sulfide compound.

The use of strong reducing agents, such as Ti(III) and $H_2S$, involves a risk of chemical reactions and general toxic effects to the bacterium but the inclusion of sterile controls and observations for significantly altered rates of the total gas production should provide a control for any chemical reactions caused by these compounds. Since nitrite (as opposed to nitrate) is more prone to chemical reactions, a source of pure $^{13}NO_2^-$ was used in the sterile control experiment. This experiment is directly comparable to the previous one in terms of treatments and $^{13}NO_2^-$ substrate. Table 1 shows the activities of $^{13}NO$, $^{13}N\text{-}N_2O$ and $^{13}N\text{-}N_2$ in the treatment without reductant and in two treatments with Ti(III) and $H_2S$. Only traces of $^{13}NO$ and no $^{13}N\text{-}N_2O$ and $^{13}N\text{-}N_2$ were detected in the treatments without reducing agent and with $H_2S$. The production of $^{13}NO$ in the sterile $H_2S$-containing control was much lower than in the viable experiments. Though $^{13}NO$ was detectable in the sterile controls, it was apparent that most $^{13}NO$ production in the viable experiments was biological.

The Ti(III)-containing sterile treatment showed a significant accumulation of $^{13}NO$ prior to a further reduction to $^{13}N\text{-}N_2O$ and $^{13}N\text{-}N_2$. The accumulations of the latter were insignificant as compared to the $^{13}N\text{-}N_2O$ and $^{13}N\text{-}N_2$ production in the viable Ti(III)-containing treatment, but the result illustrates the strength of Ti(III) as a reducing agent. Any concurrent chemical reduction of $^{13}NO_2^-$ to $^{13}NO$ in the viable, Ti(III)-containing series was most likely inferior to the biological, since the total gas production (mostly as $^{13}N\text{-}N_2$) was much higher than the rate of $^{13}NO_2^-$ reduction to $^{13}NO$ in the chemical controls.

In conclusion, the experiments showed that sulfide rather than the associated low redox potential was responsible for the increased accumulations of $^{13}NO$ and $^{13}N\text{-}N_2O$ during denitrification by P. flourescens. A partial inhibition of $^{13}NO$ reduction and a strong inhibition of $^{13}N\text{-}N_2O$ reduction is suggested. This finding seems to be generally true for all denitrifiers since we noted this same response to $H_2S$ for three other denitrifiers, Alcaligenes faecalis (strain 191), Flavobacterium (strain 175) and P. aeruginosa (strain 156). Our results suggest that sulfide may influence the production of $N_2O$ and NO in natural environments. Thus habitats high in sulfide, e.g. coastal marine environments, might be expected to be a more important source of global $N_2O$.

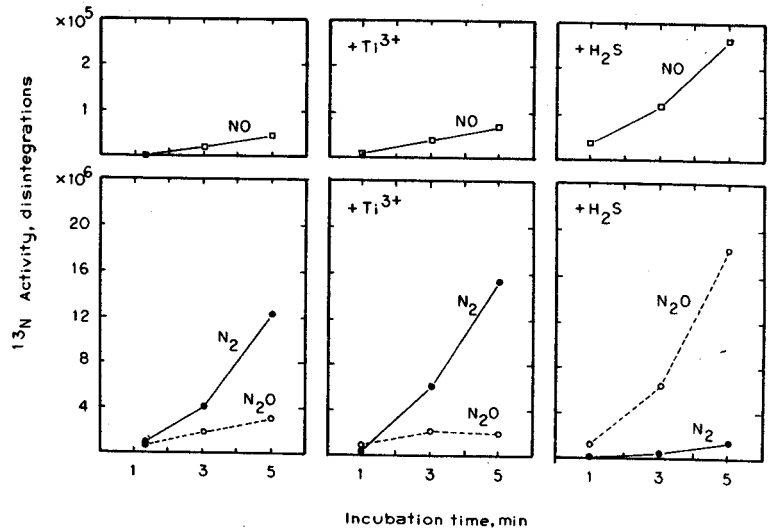


Fig. 1. $^{13}NO$, $^{13}N\text{-}N_2O$ and $^{13}N\text{-}N_2$ production by P. fluorescens from $^{13}NO_3^-$ substrate without reducing agent (left column), with 15 mM Ti(III) (middle column) and with 0.3 mM $H_2S$ (right column).

Table 1. $^{13}N$ gas production from $^{13}NO_2^-$ under sterile conditions (autoclaved cells) compared to live cells as influenced by reductant.

| Reductant | Cells | Time (min) | $^{13}N$ Gases Detected ($10^4$ disintegrations) $^{13}NO$ | $^{13}N\text{-}N_2O$ | $^{13}N\text{-}N_2$ |
|---|---|---|---|---|---|
| None | Dead | 1 | 0.01 | nd[a] | nd |
| | Dead | 3 | nd | nd | nd |
| | Dead | 5 | nd | nd | nd |
| $N_2S$ | Dead | 1 | 0.04 | nd | nd |
| | Dead | 3 | 0.03 | nd | nd |
| | Dead | 5 | 0.01 | nd | nd |
| | Live | 5 | 1.53 ( 100)[b] | 28.0 ( 1000) | 0.01 |
| Ti(III) | Dead | 1 | 0.90 | 0.05 | 0.01 |
| | Dead | 3 | 1.34 | 0.10 | 0.01 |
| | Dead | 5 | 0.96 | 0.12 | 0.03 |
| | Live | 5 | 0.36 | 10.9 | 27.2 |

a) nd = none detected

b) Fold increase in $^{13}N$ gases in vial of live cells over that found in analogous sterile control.


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# Short Term Studies of Nitrogen and Carbon Assimilation in Nodulated Leguminous and Non-Leguminous Plants Using $^{13}N$ and $^{11}C$ Tracers

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Bergersen and Turner[1] demonstrated that the first step of biological nitrogen fixation is the reduction of $N_2$ to form $NH_4$+. In leguminous plants the $NH_4$+ produced is assimilated and exported to the plant in the form of amino acids, amides, and ureides. Our objective has been to elucidate the mechanism by which $NH_4$+ is assimilated and determine how the various export products are synthesized. Our investigations have focused on leguminous and non-leguminous plants capable of symbiotic $N_2$ fixation including soybeans (Glycine max), cowpeas (Vigna unguiculata), and alder (Alnus species).

In early studies in which nodules from leguminous or non-leguminous plants were exposed to $^{15}N_2$, glutamate seemed to be labeled most rapidly with lesser amounts of label appearing in other amino acids and the amides (2-4). These findings supported the prevalent dogma that $NH_4$+ was assimilated via the glutamate dehydrogenase (GDH) pathway and according to many researchers probably occurred in the bacterial component of the nodule.

Using $^{13}N$ Wolk and co-workers[5] demonstrated that in the cyanobacteria $NH_4$+ derived from $N_2$ was assimilated via the glutamine synthetase (GS)/glutamate synthase (GOGAT) pathway. Similar findings were obtained when soybean root nodules were exposed to [$^{13}N$] $N_2$.[6] These results suggest that the GS/GOGAT pathway is the principal mechanism for the primary assimilation of $NH_4$+ formed from the reduction of $N_2$. Presumably the glutamine and glutamate formed serve as amide and amino donors for the synthesis of asparagine and other nitrogenous compounds found in the xylem exudate. Although labeling of aspartate, alanine, and an unknown compound migrating close to aspartate and glutamate has been observed in both intact nodulated roots as well as detached nodules exposed to [$^{13}N$] $N_2$, very little label is detected in the region of asparagine.

In order to determine whether asparagine, which is the major amino acid found in soybean root exudate, is formed after longer incubations, detached nodules or nodulated roots of intact plants were exposed to [$^{13}N$] $NH_4$+. Treatment with $NH_4$+ instead of $N_2$ increased the amount of radioactivity incorporated and decreased the time necessary to prepare the source. Ammonia was prepared by reduction of [$^{13}N$] $NO_3$- formed from bombardment of 0.8 ml $H_2O$ targets via the $^{16}O(p,\alpha)^{13}N$ reaction. The $NO_3$- is reduced using Davardi's alloy and the $NH_4$+ trapped after steam distillation.

When intact nodulated roots of soybeans were exposed to [$^{13}N$] $NH_4$+, the labeling pattern was similar to that observed after exposure to [$^{13}N$] $N_2$. Similar results were obtained after treatment of detached soybean nodules or intact nodulated cowpea plants with $^{13}N$ $NH_4$+. These results suggest that [$^{13}N$] $NH_4$+ can be used in place of [$^{13}N$] $N_2$ to study nitrogen assimilation in $N_2$- fixing plants. Attempts to identify the unknown compound observed previously have not been successful. The compound may be a ureide or a precursor of the ureides. Likewise, significant radioactivity has never been observed in the form of asparagine, nor have significant amounts of radioactivity in any form been detected in the stem of the plants. There are a number of possible explanations for the failure to observe movement of radioactivity from the nodules to the stem including water stress produced during treatment. Alternative methods are being tested to circumvent these difficulties.

The assimilation of $NH_4$+ in nodules appears to take place in the nodule cytosol and not in the bacteriods. [$^{13}N$] $NH_4$+ was rapidly incorporated by the cytosol fraction of an extract of soybean nodules whereas isolated bacteriods did not assimilate measurable quantities of [$^{13}N$] $NH_4$+. These observations support the idea that bacteriods excrete $NH_4$+ formed from $N_2$ and do not actively assimilate $NH_4$+.

The procedures above were used, however, to study nitrogen assimilation in the nodulated $N_2$-fixing non-legume, Alnus (alder). Two species were examined, A. glutinosa (European black alder) and A. crispa (speckled alder). Plants were collected from the Kellogg Forest and surrounding area or from the MSU campus. Detached nodules were exposed to [$^{13}N$] $NH_4$+ for 30, 120, 300, 600, and 900 seconds. Glutamine was the major compound labeled after 30 seconds. Glutamate was labeled subsequently but glutamine still predominated as the major labeled compound. Even after 15 minutes the majority of the radioactivity was in glutamine and glutamate.

Similar results were obtained after exposure of roots of intact plants to [$^{13}N$] $NH_4$+ although label was detected in the region of aspartate and alanine after 11 minutes of treatment. Preincubation of nodules with 1 mM azaserine, an inhibitor of the transfer of the amide-group from glutamine to oxoglutarate, for 10 minutes prior to incubation with [$^{13}N$] $NH_4$+ for 10 minutes produced an apparent decrease in the amount of radioactivity in the form of glutamate. On the other hand, preincubation for 10 to 20 minutes with 1 or 10 mM methionine sulfoximine (MSX), an inhibitor of glutamine synthetase, had no detectable effect. This may reflect a failure of MSX to be taken up by nodule tissue.

In order to determine whether labeled nitrogen first appeared in the amide or amino nitrogens of glutamine, nodules were exposed to [$^{13}$N] $NH_4$+ and extracted with 80% MeOH. The extract was dried and redissolved three times to remove [$^{13}$N] $NH_4$+. A portion of the extract was then incubated with glutaminase for 10 to 20 minutes to hydrolyze the amide bond. The products were then separated by ion exchange chromatography and radioactivity in the amino and amide fractions measured. The majority of the label appeared in the amide group at the earliest time point. Subsequently label increased in the amino-nitrogen. These results support the idea that nitrogen is assimilated in Alnus via the GS/GOGAT pathway. Further studies are essential to confirm these results and characterize subsequent steps in the assimilation of nitrogen in Alnus.

In addition to $N_2$ and $NH_4$+, roots and nodules of soybean and cowpea plants grown on a N-free medium (low concentration of $NO_3$- may be present in tap water) actively assimilate nitrogen in the form of $NO_3$-. Nodules or roots exposed to [$^{13}$N] $NO_3$- incorporated label producing primarily glutamine, glutamate, aspartate, and alanine. The labeling pattern was similar to that observed after exposure to labeled $N_2$ or $NH_4$+. These results suggest that both roots and nodules contain active $NO_3$- and $NO_2$- reductases which may function to assimilate soil nitrate. The $NH_4$+ formed from the reduction of $NO_3$- is assimilated via the GS/GOGAT pathway and used in the biosynthesis of other amino acids.

The biosynthesis of amino acids, amides, and ureides requires a supply of carbon skeletons. To avoid depleting the cell of TCA cycle intermediates, alternative mechanisms for producing carbon skeletons may be required such as the glyoxylate cycle or phosphoenol pyruvate (PEP) carboxylase. Although the glyoxolate cycle is present in plants, there is no evidence that this pathway is functional in nodules of leguminous plants. There is evidence, however, that PEP carboxylase which catalyzes the formation of oxalacetate from PEP and $CO_2$ does exist in roots. Christeller et al.[7] recently reported evidence to suggest that the major role of this enzyme in lupin nodules is to produce oxalacetate to use in the assimilation of $NH_4$+ forming asparagine in the process.

Soybean roots and nodules also have an active PEP carboxylase. Nodulated roots of intact soybean plants were exposed to [$^{11}$C] $CO_2$. Malate which is formed rapidly from oxalacetate was the major product labeled after short exposures. Label was subsequently detected in aspartate and glutamate. Alnine and several other unknown compounds were labeled to lesser extents. Significant quantities of labeled asparagine were not observed. In experiments in which soybean plants were exposed to both labeled $NH_4$+ and $CO_2$, however, the unknown compound observed previously after exposure to [$^{13}$N] $N_2$ or [$^{13}$N] $NH_4$+ also contained labeled carbon. This may be significant in terms of the mechanism of synthesis of the unknown.

In contrast to the story with lupins, oxalacetate formed from PEP and $CO_2$ does not appear to be utilized solely for the production of asparagine under the conditions used, although aspartate is highly labeled. The oxalacetate formed is used for the production of glutamate, alanine, and other compounds and thus may be significant in terms of $NH_4$+ assimilation and amino acid biosynthesis. Results of pulse labeling experiments or double labeling experiments in which nodulated roots were exposed to [$^{11}$C] $CO_2$, then [$^{14}$C] $CO_2$, suggest that part of the newly assimilated carbon is used for production of aspartate. The remainder is metabolized presumably via the TCA cycle to produce carbon skeletons for glutamate/glutamine and alanine synthesis or is respired to form $CO_2$. Factors which alter the process of $N_2$ fixation such as $C_2H_2$ seem to alter the distribution of label between various products. Similar labeling patterns were observed in cowpea and alder nodules. Thus, PEP carboxylase may function as a general mechanism for replenishing the TCA cycle intermediates used to assimilate $NH_4$+ in $N_2$-fixing plants.

The following schematic diagram summarizes our model of the processes of $N_2$ fixation, $NH_4$+ assimilation, and carbon metabolism in nodules of $N_2$-fixing leguminous plants. Through the use of short lived isotopes of nitrogen and carbon in combination with other studies, we hope to be able to substantiate this model. Furthermore, we hope to apply the same techniqeus to define these processes in non-leguminous $N_2$-fixing plants.

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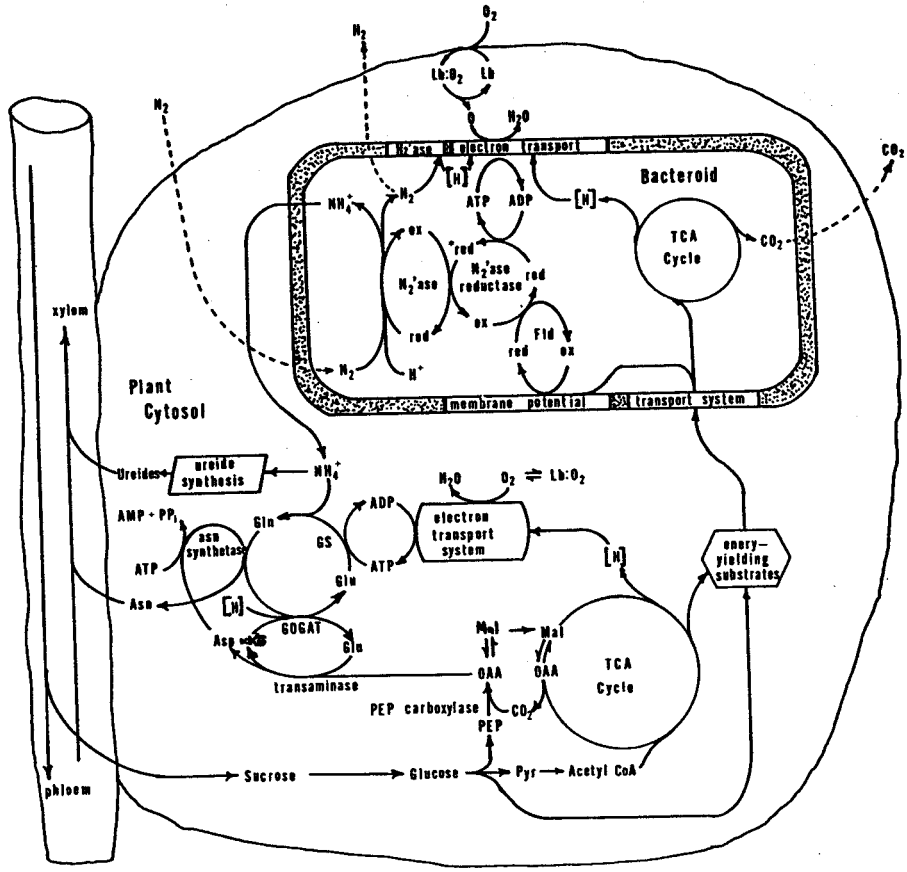


Fig. 1. Schematic diagram of the processes of $N_2$ Reduction and $NH_4$+ assimilation in nodulated roots of leguminous plants.

# Factors Effecting Denitrification in an Anaerobic Lake Sediment

A.J. Sexstone, J.M. Tiedje. R.B. Firestone

Denitrification processes in lake sediments have not been extensively studied. Rapid reduction of nitrate entering the hypolimnia and anoxic sediments of eutrophic lakes, coupled with characteristically low (ppb) or non measurable nitrate pool size make $^{13}NO_3^-$ the ideal tracer for studying denitrification in these systems. The present study examined the effect of environmental variables such as temperature, concentration of $NO_3^-$ or $NO_2^-$ as available electron acceptors, and concentrations of $Fe^{+3}$ as a possible alternate electron acceptor on the production of gaseous denitrification products in anaerobic sediments of a hypereutrophic lake. All reported experiments were performed using the continuous flow gas stripping system which has been previously described (2).

Initial study showed that $^{13}N$ gases could be detected within a minute after addition $^{13}NO_3^-$ to the sediment. The majority of the $^{13}N$ gas production occurred during the first 10-30 minutes in the presence of low $^{14}NO_3^-$ or $^{14}NO_2^-$ concentrations ($10^{-5}$ to $10^{-9}$M). A maximum of 70% of the added label could be recovered as N-gases in this time.

The percentage of $^{13}N$-gas occuring as $^{13}N_2O$ from $^{13}NO_3^-$ or $^{13}NO_2^-$ in the presence of $\mu M$ $^{14}NO_3^-$ or $^{14}NO_2^-$ respectively is shown in Table 1. The ratio of $N_2O$ produced to total gases ($^{13}N_2O/^{13}N_2O + {}^{13}N_2$) was generally greater at 10 min. than 30 min. At 25°C $N_2$ was generally the predominant gaseous product of denitrification. The percentage of N-gas occurring as $N_2O$ increased with decreasing temperature. At 1°C 30% of the gas produced from $NO_3^-$ occured as $N_2O$ after 30 minutes. High temperature (45°C) perturbations also increased the percentage of $N_2O$ produced.

The temperature effect on $N_2O$ production was enhanced by increasing concentrations of $^{14}NO_3^-$ or $^{14}NO_2^-$. At 1°C in the presence of mM $^{14}NO_3^-$ (not shown) 65% of the total gases produced occurred as $N_2O$.

No significant difference was observed in the percentage of $N_2O$ formed from $^{13}NO_2^-$ when compared with that formed from $^{13}NO_3^-$. This contrasts with recent similar studies performed in soil which showed that in that system $NO_2^-$ has more potent effects on increasing $N_2O$ production than did $NO_3^-$(1).

It has been suggested that $Fe^{+3}$might serve as an alternate electron accepter in conjunction with nitrate reductase (3,4). The effects of added $Fe^{+3}$ on the rates of denitrification from $\mu M$ $^{14}NO_3^-$ + $^{13}NO_3^-$ in sediments is shown in Fig. 1. Decreasing rates of N-gas production in the presence of increasing concentrations of $Fe^{+3}$ suggests that $Fe^{+3}$ might compete with $NO_3^-$ as an electron acceptor. Further work would be needed to confirm this interesting preliminary finding.


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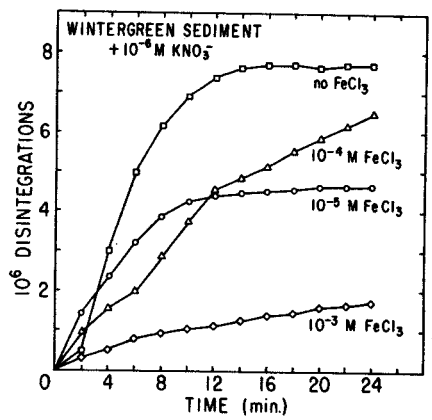


Fig. 1. Effect of $Fe^{+3}$ addition on rates of denitrification from sediments in the presence of $\mu M$ $^{14}NO_3^-$ + $^{13}NO_3^-$.

Table 1. Percentage of N-gases accumulating as $N_2O$ from sediment at various temperatures in the presence of either $\mu M$ $^{14}NO_3^-$ + $^{13}NO_3^-$ or $^{14}NO_2^-$ + $^{13}NO_2^-$

| Treatment | Temp. (°C) | Percentage of $N_2O$ produced | |
|---|---|---|---|
| | | 10 min | 30 min |
| $\mu M$ $KNO_3^-$ + $^{13}NO_3^-$* | 1 | 59 | 33 |
| | 5 | 24 | 14 |
| | 15 | 9 | 5 |
| | 25 | 4 | 4 |
| | 45 | 21 | 16 |
| $\mu M$ $KNO_2^-$ + $^{13}NO_2^-$** | | | |
| | 5 | 28 | 23 |
| | 15 | 10 | 6 |
| | 25 | 4 | 4 |

* Added as [90% $^{13}NO_3^-$ + 10% $^{13}NO_2^-$]

** Added as 100% $^{13}NO_2^-$

# Production of $^{13}N$, $^{11}C$, $^{15}O$, and $^{18}F$ at 25 MeV per Nucleon

R.B. Firestone, M.K. Firestone, and J.M. Tiedje

For several years we have been developing an extensive program for the production of short-lived radioactive tracers to be used in biological experiments. This work has been done at low energies with proton beams from the 50 MeV MSU Cyclotron. Proton bombardment energies were chosen to eliminate the production of unwanted impurities, and very pure sources of $^{13}N$ or $^{11}C$ labeled activities were produced. With the advent of new heavy-ion accelerators at MSU, we have performed a series of experiments to determine the feasibility of producing these tracers with heavy ions. This prospect is attractive because such experiments could operate parasitically to nuclear physics experiments by acting essentially as their beam dump.

In order to simulate conditions of heavy ions at 25 MeV per nucleon we bombarded targets of $H_2O$ with at 75-MeV $^3He$ beam. This energy is well above the threshold for the production of $^{11}C$, $^{13}N$, $^{15}O$ and $^{18}F$, the primary expected activities. The beam was completely stopped in the $H_2O$. After a 10 minute bombardment, a raw sample of activated $H_2O$ was multiscaled in a 2x2 NaI well Counter at 15 sec intervals for a total period of 80 min.

A second sample was first made alkaline and reduced to dryness, and then made acid and reduced to dryness. The residue was reconstituted with $H_2O$, and similarly counted in a well counter. This alkaline treatment was previously established to be effective in removing $^{13}NH_3$ plus $^{13}N$ gases. The acid treatment should have removed $^{11}C$ carbonate forms. Finally, both samples were analyzed by a high pressure liquid chromatograph (HPLC) with a coincidence NaI detector to separate the various ionic species in the source.

An analysis of the raw sample well counter data is hown in Table I where it is compared with previous data obtained with the usual 15 MeV proton beam. The sample was found to contain large quantities of $^{13}N$, $^{15}O$ and $^{18}F$ but no detectable $^{11}C$. Calculations were subsequently performed with the ALICE particle evaporation code of M. Blann and F. Placil to compare with these results. These calculations are also shown in Table I where we find that the $^{13}N$ and $^{18}F$ yields are predicted well for both proton and $^3He$ beams. The predicted large yields of $^{11}C$ and $^{15}O$ are not observed. This may be explained by the fact that the bombardment rabbit was not gas tight and we expect most of the $^{11}C$ to be produced as $CO_2$ or $CH_4$ which could escape before counting. The $^{15}O$ products would be $O_2$, $H_2O$ and $OH^-$ so some activity should remain after bombardment. $^{13}N$ and $^{18}F$ are produced substantially in ionic form explaining their greater recovery. Finally, in figure 1 we show a comparison of the raw and treated source HPLC scans. In the raw source we see several peaks including $NO_3^-$, $NO_2^-$, $NH_4^+$ and possibly $OH^-$. After treatment only the $NO_3^-$ peak remains. The treated sample was observed to decay with the $^{13}N$ half-life although a small $^{18}F$ impurity remained.

With this work we have shown the feasibility of producing short-lived tracers with heavy ions. Simple established chemical separation techniques allow the recovery of pure sources with high yield. A calculation predicting production with 300 MeV $^{12}C$ is included in Table I. The yield in this reaction is predicted to be quite comparable with the current results, especially if we include the production of tracers from reactions on the beam projectile which were not calculated here.

Calculated isotope production for various beams on $H_2O$ targets.

| | Yield (mCi/uA per 10 min)[1] | | | | |
|---|---|---|---|---|---|
| | 15 MeV - p | | 76 Mev - $^3He$ | | 300 Mev - $^{12}C$ |
| Isotope | Calculated[2] | Experiment | Calculated[2] | Experiment | Calculated[2] |
| $^{13}N$ | 32 | 26 | 81 | 55 | 26 |
| $^{11}C$ | 0 | 0 | 124 | 0 | 51 |
| $^{15}O$ | 0 | 0 | 385 | 45 | 109 |
| $^{18}F$ | 0.6 | 0.2 | 5.8 | 6.5 | 4.1 |

[1] Assuming beam stops in the target.
[2] Calculated using the ALICE compound nucleus particle evaporation code.

Table 1.

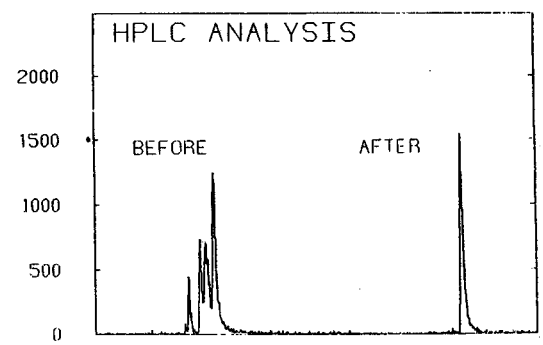


FIG. 1. HPLC analysis of activity produced by 76 MeV $^3He$ before and after purification.