INTERNAL REPORT 86

NITROGEN TRANSFORMATIONS

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ABSTRACT

The feasibility and enumeration studies have led to the following conclusions: The nitrogen fixation studies should be done in 1973, using acetylene reduction technique with some verification by N^{15}. The denitrification studies should be done with N^{15} technique. The decision to continue more enumeration sampling of nitrogen transforming bacteria should be based on comparisons of the 1972 enumerations with the chemical budget data of 1972.

The pertinent literature is listed in the appendix of the report; the reprints are housed in Dr. Taub's office, 212 Fisheries Center, University of Washington. The enumeration data are presented in the report.

OBJECTIVES

The prime objective of the 1972 year was to evaluate the feasibility of measuring the magnitude of various nitrogen transformations. The abundance of nitrogen transforming bacteria was estimated periodically in the four IBP lakes (Washington, Sammamish, Chester Morse, and Findley).

METHODS

A collection of pertinent reprints was collected. A visit to Dr. W. J. Payne's laboratory at the University of Georgia was made to discuss chromatographic methods. (The trip was in conjunction with other business and was no cost to IBP.)

Bacterial enumeration samples of water were collected aseptically from each of the four IBP lakes from February through November 1972. The methods used to enumerate nitrate reducing bacteria were based on the recommendations of Skerman.[1] A nitrate peptone medium consisting of Bacto-peptone, 1%; sodium chloride, 0.5%; and sodium nitrate, 0.2%, was dispensed into screw capped Durham tubes. Samples were serially diluted, inoculated in triplicate and incubated at 12°C for at least four weeks. The tubes were observed at intervals. Turbidity was used to indicate numbers of aerobic heterotrophs and gas was used as an indication of denitrification. Tests were made for nitrite. Where gas and nitrite were not detected in positive tubes, analyses were made for nitrate. If the nitrate values found were significantly less than in the original medium the culture was recorded as representing reduction below nitrite and it was assumed that ammonium had been produced. The detection of bacteria able to oxidize ammonium or nitrite was attempted by inoculating a mineral salts medium containing the relevant substrate with 20 ml of sample and incubating for at least six

[1] Skerman, V.B.D. 1959. A Guide to the identification of the genera of bacteria. Williams & Wilkens Co.

weeks at 12°C. The medium used was medium 63[a] prepared with either 0.4 meq/l NH_4^+ or NO_2^- in place of nitrate. Analyses were made for nitrite and nitrate.

RESULTS

Literature Review

A collection of pertinent reprints has been assembled in Dr. Taub's office, 212 Fisheries Center (listed in appendix). The best nitrogen cycle presentation, Figure 1, has been provided by Dr. W. J. Payne.

Nitrogen Budget

The nitrogen inputs (rain and river and surface runoff), exports (river output), deposition to sediment, and changes between nitrate, nitrite, ammonia, and particulate organic portions are expected from the chemical budget studies of Dr. Demetrios Spyridakis, Civil Engineering.

Nitrogen Fixation ($N_2 \rightarrow NH_3$)

The literature indicates that significant nitrogen fixation may be expected to occur year round by bacteria in anoxic waters and sediments and seasonally in the photic zone by heterocystous blue-green algae (Granhall and Lundgren 1971, Howard et al. 1970, Brezonik and Harper 1969). In Lake Erken (Sweden) a moderately eutrophic and unpolluted lake, the pelagic nitrogen fixation increased the annual loading of combined nitrogen by 40% (Granhall and Lundgren 1971). Significant blue-green algal blooms do occur in Lakes Washington and Sammamish.

No measurements of nitrogen fixation were made on our lakes in 1972 because an appropriate gas-liquid chromatograph for the acetylene reduction technique (Klucas 1969, Howard et al. 1970) was not available to either Dr. Taub or Dr. Spyridakis. Dr. James Staley of the Microbiology Department has agreed to supply the required equipment and to undertake the study in 1973. It is felt that some 15N measurements should be made to verify the conversion factor for estimating the weight of nitrogen fixed per mole of acetylene reduced. The importance of verifying the conversion factor will be estimated after a few measurements indicate the relative importance of fixation in the total nitrogen budget.

Denitrification ($NO_3^- \rightarrow N_2$)

This process is known to occur in anoxic sediments (Kenney, Chen and Graetz 1971, Chen et al. 1972) and is presumed to be of major importance in lake nitrogen budgets (Hutchinson 1957). The demonstration of its relative importance is under study in Lake Mendota (Chen et al. 1972) and in the Tundra (Alexander 1970). The methods involve the use of 15N labeled NO_3 and the subsequent production of 15N labeled N_2. Direct estimations of this process from NO_3 disappearance cannot be used because NO_3 is also assimilated

[a]Taub, F.B. and A.M. Dollar, 1968. The Nutritional Inadequacy of *Chlorella* and *Chlamydomonas* as food for *Daphnia pulex*. Limnol. and Oceanogr. 4:607-617.

by organisms and reduced to NO_2^- which may or may not be further reduced. The appearance of N_2 gas cannot be used to estimate this process because N_2 is also stripped out of the dissolved state by the formation of other gases such as H_2S and CH_4 which are produced in the same area.

A highly sophisticated gas chromatography method for the study of the physiological properties and products of denitrification has been developed by W. J. Payne and colleagues at the University of Georgia. They are currently applying these methods to the study of denitrification in tidal marine muds. Their technique involves placing a funnel over the sediment and flushing the water with helium containing a tracer of neon. The sediments are enriched with nitrate and the overlying water in the funnel is analyzed for N_2 gas. The neon tracer acts as an internal standard to insure that the N_2 measured is not from leakage. The major limitation of their method is the inability to measure *in situ* $NO_3^- \rightarrow NO_2^- \rightarrow N_2$ rates since there is no detectable NO_3^- in the sediments being measured. The NO_3^- enrichment is necessary to provide a substrate for the bacteria. Apparently, the bacteria process the incoming NO_3^- at the rate it is received, but this rate is unknown. Another major limitation lies in the complex and delicate manipulation of the sample. Although they hope to be able to use it in the field, they are currently only working on sediments which have been removed to the lab. Given these limitations and the specialized equipment necessary, the older method of 15N studies might be more feasible here. Drs. Staley and Spyridakis each wish to explore this area further.

It had been erroneously thought that the occurrence of denitrification would be indicated by the absence of NO_3^-, since it had been reported that NO_3^- inhibits the reduction of NO_2^- to N_2 (Payne and Riley 1969). The inhibition is, however, only partial (Payne, personal communication). The time and efforts of Dr. Payne and his colleagues, Dr. J. H. Nuver and Dr. Claude Crawford are appreciated.

The presence of bacterial populations capable of denitrification are shown in Table 1, as "Gas Producers" and NO_3 reduced below NO_2^-." These activities were shown in Lakes Washington, Sammamish and Chester Morse, and occurred in the surface waters where, presumably, they would not be active.

Nitrate Reduction ($NO_3^- \rightarrow NO_2^-$ and $NO_2^- \rightarrow NH_3$)

The reduction of nitrate to ammonia occurs in algae as preparatory to protein synthesis as well as in bacteria which may assimilate or release NH_3. The numbers of bacterial nitrate reducers were estimated, Table 1, and appear to be a significant proportion of the total number of heterotrophic bacteria which would grow aerobically.

Nitrification ($NH_3 \rightarrow NO_2^-$ and $NO_2^- \rightarrow NO_3^-$)

Nitrification by bacteria is probably a major bacterial nitrogen transformation. Its presence is demonstrated by the reappearance of NO_3^- during the winter, presumably as a result of the decay of organic material resulting in the release of NH_3. During the summer, the released NH_3 would be taken up by the algae in the photic zone in competition with nitrification. Although the algae would be presumed to remove NH_3 preferentially

to NO_3^-, both will be removed by algae. For these reasons, the appearance of nitrate usually underestimates nitrification. The occurrence of bacteria capable of these reactions are shown in Table 1.

Other Comments

It appears there are only a few statements which can be made from the data. Generally, the nitrate reducers are nearly as numerous as the total bacteria. The ability to reduce nitrate is found in many species.

Late summer counts appear to be greater than fall counts.

Denitrification occurred most frequently in cultures from Lake Sammamish, also occurred in Chester Morse and Lake Washington samples and did not occur in Findley Lake samples.

Nitrifiers are a specialized group and in general may be considered to be obligatorily so. They were found in samples from all of the lakes. Their occurrence was seemingly random. This may be an artifact resulting from failure of nitrifiers to grow unless the medium is conditioned by NH_3 oxidizers.

It was not unusual for denitrifiers, which are favored by low O_2 tensions and nitrifiers which are aerobic, but are also favored by low O_2 tensions, to be found together.

Some of the Lake Washington data of Dr. Staley were examined. His total numbers were higher and he found many more denitrifiers. His workers tested several media including two that we used and settled on Dr. Ordal's Cytophaga medium with nitrate added. We made comparisons of the Cytophaga with Nitrate-peptone water suggested by Serman and found little or no difference. My impression is that Dr. Staley's data was similar on that point.

It seemed that the differences in total numbers and denitrifiers between Dr. Staley's data and ours might be the result of toxicity in our sampler or to the incubation temperature. We incubate at 12°C and Dr. Staley incubates at room temperature. We compared total numbers using a pipet with total numbers using the sampler and found little or no difference. However, incubation at room temperature resulted in numbers roughly ten times as high as at 12°C. I suspect that the effect on numbers of denitrifiers found would be even greater.

CONCLUSIONS

The nitrogen fixation studies should be done in 1973, using acetylene reduction technique with some verification by 15N.

The denitrification studies should be done with 15N techniques.

The decision to continue more enumeration sampling of nitrogen transforming bacteria should be based on comparisons of the 1972 enumerations with the chemical budget data of 1972.

Lake	Date (1972)		Total numbers/ml	Nitrate reducers/ml	Gas*	NO_3^- reduced below NO_2^-	Nitrification $NH_3 \rightarrow NO_2$	Nitrification $NO_2 \rightarrow NO_3$
Sammamish	2 Feb	Surface	460	43	+	4.3		
		Mid-depth	1100	1100	+	2		
		Bottom	1100	1100	+	3.9		
Washington	23 May	Surface	93	43	+	0.4	+	-
		Mid-depth	93	43	-	0.4	-	+
		Bottom	43	43	-	0.4	+	-
Findley	7 Jul	Surface	2.3	2.3	-	2.3	-	-
		Mid-depth	24	1.5	-	0.4	-	-
		Bottom	46	46	-	0.9	-	-
Findley	20 Jul	Surface	24	9.3	-	2.1	+	+
		Bottom	460	460	-	3.0	-	-
Chester Morse	25 Oct	Surface	24	4.3	-	-	+	+
		Mid-depth	43	24	-	-	-	-
		Bottom	43	43	-	-	-	-
Lake Washington	15 Nov	Surface	110	46	+	1.1	-	-
		Mid-depth	46	46	+	0.7	-	-
		Bottom	110	110	-	0	-	-
Lake Sammamish	29 Nov	Surface	1100	460	$\pm$	2.3	-	-
		Mid-depth	240	46	+	0.9	-	+
		Bottom	24	24	+	0.4	-	+

*Gas is presumed to be N_2 or N_2O

LITERATURE LIST OF *NITROGEN* FILE

(Housed in Dr. Taub's Office, 212 Fisheries Center)

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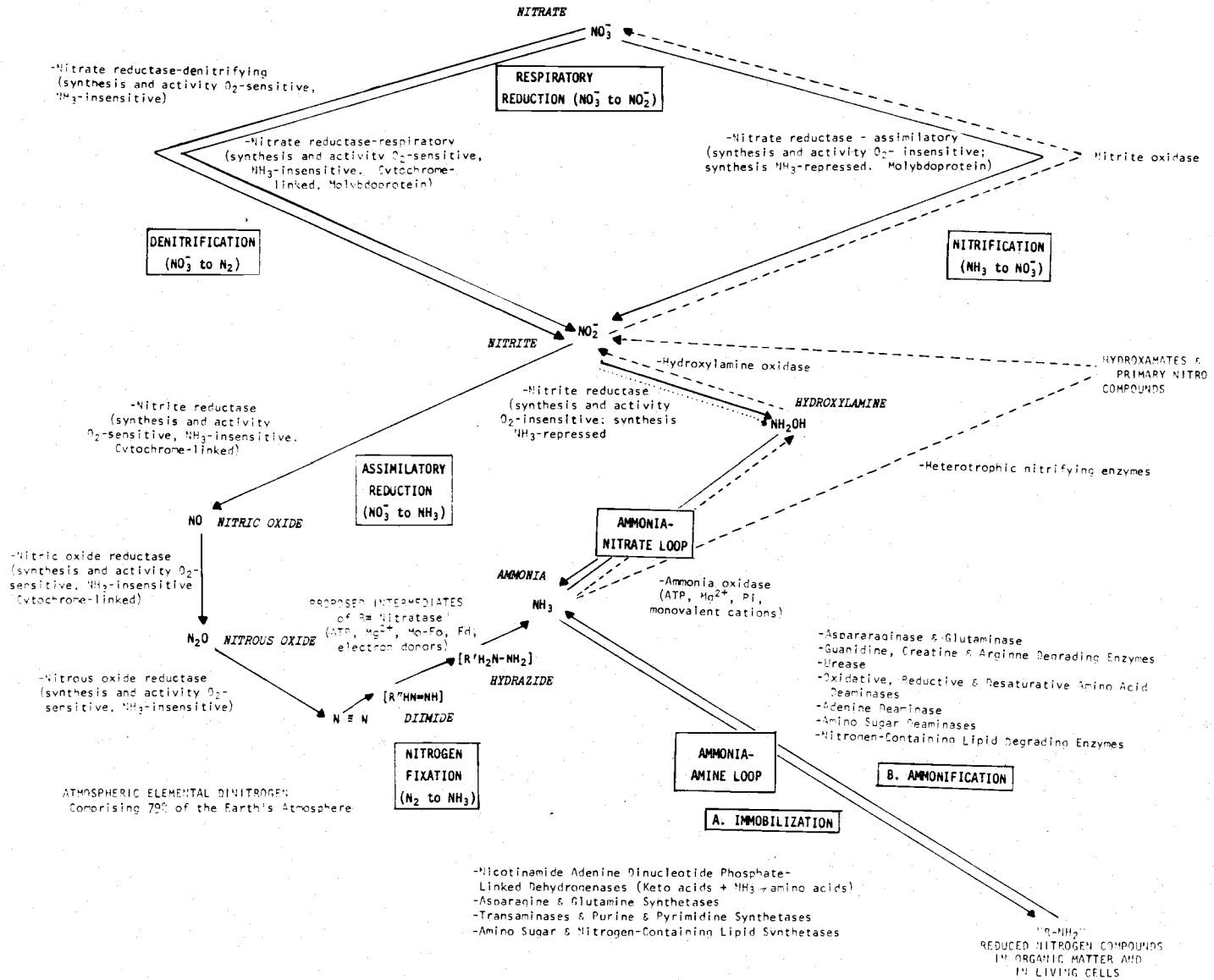

Figure 1. Summary of the biological nitrogen transformations (supplied by Dr. W. J. Payne).