INTERNAL REPORT 143

NITROGEN TRANSFORMATION

James T. Staley and Frieda Taub
University of Washington

ABSTRACT

Nitrogen fixation rates were determined periodically by the acetylene reduction technique at three depths in each of the lakes. Fixation began in June in Lakes Washington and Sammamish and peaked during July and August. Chester Morse and Findley Lakes did not exhibit acetylene reduction activity until August and the rates encountered were much lower than in the other two lakes. Nitrogen fixation in the lakes correlates closely with the concentrations of blue-greens.

OBJECTIVES

The primary objective of this past year's study has been to assess the rates of nitrogen fixation in the water column of each lake. Secondary objectives, to be pursued if there was sufficient time, were to determine nitrogen fixation rates in the sediments of the lakes, to initiate studies on denitrification in the sediments, and to initiate studies to determine the relative significance of nitrate and ammonia as sources of nitrogen for the phytoplankton. This report is restricted to the results we have obtained to date on nitrogen fixation in the water column. Some preliminary data are already available on sediment fixation, and procedures are being tested now for assessing denitrification and nitrate and ammonia uptake. Inasmuch as these latter studies have been undertaken just recently the data are incomplete and are therefore not incorporated in this year's report.

MATERIALS AND METHODS

Nitrogen-fixation rates were determined with only slight modifications of the acetylene reduction procedure described by Mague and Burris (1973). Fifteen-liter water samples were collected periodically in a Scott-Richard's water bottle from three depths in each lake at the primary sampling site of that lake (Station No. 1). These samples were filtered onto a 48-µm plankton net to concentrate the phytoplankton. The phytoplankton were gently rinsed off the net into a graduate cylinder and equal portions were transferred to 14.5-ml serum-stoppered bottles which were then sealed. Part of the sample was also fixed with Lugol's iodine solution for direct counting of phytoplankton. To initiate the reaction acetylene was injected into the serum-stoppered bottle with a syringe to give a final concentration of about 0.2 atm. The bottles were then shaken to mix the gas and equilibrated to atmospheric pressure by removal of a volume of the gas equal to that of the added acetylene. The bottles were incubated on deck in a bucket of lake water from the appropriate depth and the reaction was stopped at 0, 30 and 60 min by the addition of 1.0 ml of $5\underline{N}$ H_2SO_4. A silicone sealant was used to seal the bottles for storage prior to analysis. The reaction bottles were refrigerated upon return to the lab until they could be analyzed.

A Hewlett-Packard 5700A gas chromatograph with a hydrogen flame ionization detector was used for the analysis of acetylene and ethylene. Nitrogen was

used as a carrier gas with a flow rate of 40 cc/min. The 6-ft, 1/8 inch diameter stainless steel column was packed with Porapak R (80-100 mesh) and operated at 40 C with 22.5 psi hydrogen, 24 psi air, and 80 psi nitrogen. Plastic tuberculin syringes were used to remove the 1.0-cc sample from the serum-stoppered bottle (1.0 cc of distilled water was injected with the syringe prior to sampling). The amount of ethylene produced was determined by measuring the areas under the peaks (they were assumed to be isosceles triangles) and comparing these values to a standard curve prepared from a standard, diluted ethylene-air mixture (1 part ethylene to 10^6 parts air). Concentrations of blue-green algae were determined in unconcentrated as well as filtered samples from each depth at which the nitrogen fixation rates were measured. Cells were enumerated in settling chambers with a Zeiss inverted phase microscope according to the procedure of Utermohl (1931) and Lund et al. (1958). Each chamber contained a known volume of sample (ca. 5.0 ml). After filling the chambers, they were incubated at room temperature in a cabinet free from appreciable temperature gradients for at least 24 hr. In excess of 400 microorganisms were counted in randomly selected fields of each sample using a 40X phase contrast objective coupled with a 1.25 Optovar and 12.5X oculars.

A portion of the water sample collected at each depth was stored in a sterile bottle and iced until return to the laboratory. Viable numbers of aerobic, heterotrophic, nitrogen fixing bacteria (azotobacters) were determined by spread-plating 0.2-ml portions in triplicate on Ashby's medium containing 0.1% glucose as carbon source. Plates were counted after 3-4 weeks' incubation at room temperature.

RESULTS

The first analyses of nitrogen fixation were conducted in Lake Washington in mid-June (Table 1). Even at this early time there were substantial concentrations of blue-greens in the water column and significant rates of nitrogen fixation. By the end of July both the concentration of blue-greens and the rate of nitrogen fixation had increased markedly and apparently remained high until the latter part of August. At the time of the diel study on 9-25-73 the concentration of blue-greens had dropped appreciably and the levels of nitrogen fixation were undetectably low.

The results for Lake Sammamish parallel those for Lake Washington. Nitrogen fixation occurred at significant rates in late June and the rates increased in July after which they remained high until the last sampling period in September. The concentrations of blue-greens remained at a high level throughout the summer whereas nitrogen-fixing bacteria were in relatively low concentrations except for the lower depths on September 7.

In contrast to Lakes Washington and Sammamish there was no detectable nitrogen fixation in Findley Lake and Lake Chester Morse until late summer. In Chester Morse appreciable rates of nitrogen fixation were measured on August 28 (Table 3). In Findley the rates of nitrogen fixation were still relatively low, albeit they were detectable in the surface waters on August 30 (Table 4). The numbers of blue-greens in Findley remained low throughout the summer months.

DISCUSSION

There is a good correlation between nitrogen-fixation rates and the concentrations of blue-greens in the lakes. This evidence coupled with their high cell densities relative to the heterotrophic nitrogen-fixing bacteria strongly suggests that they are primarily responsible for the fixation of nitrogen in the lakes. However, since the nitrogen-fixation analysis was conducted on the material retained by the 48-μm filter, we cannot conclusively eliminate the possible involvement of the bacteria in this process in the lakes. Nonetheless, it would appear that the bacteria in the aerobic, photic zones of these lakes are insignificant in nitrogen fixation because of their low cell densities.

These data conform well with previously published studies which indicate that nitrogen fixation occurs at more rapid rates in lakes of increasing nutrient enrichment (cf. Mague and Burris 1973). Consequently those lakes with low nitrogen concentrations do not have the capability of nitrogen fixation whereas they have the greatest need for it. This is explained by the fact that the blue-greens which are responsible for the fixation never attain high densities in oligotrophic lakes.

Surface water samples from Lake Erie and Green Bay, Lake Michigan, during midsummer fixed nitrogen at rates ranging from 0.033 to 35.0 nm per liter per hour (Mague and Burris 1973), well within the range for the lakes in this study. Higher values of fixation are recorded by Rusness and Burris (1970) for eutrophic Little Arbor Vitae Lake on 28 August 1968. They found rates in excess of 200 nm ethylene per liter per hour in the surface waters. As in our studies they noted that the rate of fixation generally decreased with depth in the Wisconsin lakes they studied.

It should also be noted that the rates are given in terms of the amount of ethylene formed by reduction of acetylene. If the nitrogenase enzyme complex in the communities would utilize the acetylene (even in the presence of nitrogen) as it would normally use the nitrogen as a substrate for the fixation reaction, then one could assume that the rate of ethylene formation in nanometers corresponds exactly with rate of nitrogen fixation in nanometers. Studies that have assessed this conclude that, although acetylene-reduction rates are not identical with nitrogen-fixation rates, they closely approximate them (Stewart et al. 1971).

ACKNOWLEDGMENTS

We thank David Tison, Rae Cooper, and Fred Palmer for their excellent technical assistance. David Tison was supported in part on an NSF/URP award.

REFERENCES

LUND, J. W. G., C. KIPLING, and E. D. LE CREU. 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting Hydrobiologia 11:143.

MAGUE, T. H., and R. H. BURRIS. 1973. Biological nitrogen fixation in the Great Lakes. Bioscience 23:236.

RUSNESS, D., and R. H. BURRIS. 1970. Acetylene reduction (nitrogen fixation) in Wisconsin lakes. Limnol. Oceanogr. 15:808.

STEWART, W. D. P., T. MAGUE, G. P. FITZGERALD, and R. H. BURRIS. 1971. Nitrogenase activity in Wisconsin lakes of differing degrees of eutrophication. New Phytol. 70:497.

UTERMOHL, H. 1931. Neue Wege in der quantitativen Efassung des Planktons. Proc. Int. Assoc. Theor. Appl. Limnol. 5:567.

Table 1. Nitrogen fixation in Lake Washington.

Date	Depth (m)	Fixation rate (nmoles ethylene $\ell^{-1}hr^{-1}$)	No. blue-greens (cells/ml)	No. azotobacters (cells/ml)
6-15-73	0-1	4.6	127,900	na
	5-6	na[a]	174,800	na
	15-16	3.7	128,700	na
7-31-73	0-1	17.5	261,700	358
	5-6	7.2	na	na
	15-16	5.1	15,200	2,320
8-23-73	0-1	15	165,600	66
	3-4	14	222,100	>1,000
	7-8	9	136,700	>1,000
9-25-73 (1100 hr)	0	<0.1	100,900	1,540
	4	<0.1	142,200	1,330
	12	<0.1	81,000	1,230
	30	<0.1	7,300 (0700 hr)	1,210

1[a] Data not available.

Table 2. Nitrogen fixation in Lake Sammamish

Date	Depth (m)	Fixation Rate (nmoles ethylene ℓ^{-1} lake water hr^{-1})	No. blue-greens (cells/ml)	No. azotobacters (cells/ml)
6-25-73	0-1	na[a]	216,700	na
	5-6	3.4	270,500	na
	15-16	3.4	150,290	na
7-13-73	0-1	12.1	123,200	201
	3-4	9.0	93,700	151
	10-11	8.2	107,700	574
8-2-73	0-1	12.6	153,800	81
	3-4	na	237,700	74
	10-11	12.4	270,100	80
8-10-73	0-1	13.2	91,500	169
	3-4	18.5	105,900	166
	5-6	7.3	218,100	161
9-7-73	0-1	15.6	60,700	66
	3-4	14.8	49,800	>1000
	6-7	na	51,900	>1000

[a] Data not available.

Table 3. Nitrogen fixation in Lake Chester Morse.

Date	Depth (m)	Fixation Rate (nmoles ethylene ℓ^{-1} lake water hr^{-1})	No. blue-greens (cells/ml)	No. azotobacters (cells/ml)
7-10-73	0-1	not detectable[a]	15,000	152
	10-11	not detectable[a]	5,000	178
	24-25	not detectable[a]	5,600	277
8-28-73	0-1	7.4	na	171
	3-4	na[b]	3,500	na
	6-7	5.8	44,000	na

[a] Rates are less than 0.1 nm per ℓ per hr.

[b] Data not available.

Table 4. Nitrogen fixation in Findley Lake.

Date	Depth (m)	Fixation Rate (nmoles ℓ^{-1} lake water hr^{-1})	No. blue-greens (cells/ml)	No. azotobacters (cells/ml)
7-5-73	0-1	not detectable[a]	900	<20/ml
	5-6	not detectable[a]	3,500	<20/ml
	15-16	not detectable[a]	1,300	<20/ml
8-30-73	0-1	m[b]	2,400	40
	3-4	4.5	2,500	<20
	5-6	not detectable[a]	1,650	na[c]

[a] Rates are less than 0.1 nm per ℓ per hr.

[b] Measurable, but below recorded values in nonlinear region of standard curve (actual value is >0.1 nm per ℓ per hr).

[c] Data not available.