

水圏における窒素固定に関する微生物学的研究 VI

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**Microbiological Studies on Nitrogen Fixation
in Aquatic Environments—VI.
On the *in situ* Nitrogen Fixation in Water Regions***

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In various water environments, the rate of nitrogen fixation was estimated by the use of the acetylene method which was modified by the present authors in the previous paper. The rate ranged from 0.3 to 4.46 mg N/hr/m³, depending on seasons, localities of the water regions, etc. Considerably high values were observed in the water of fish culture ponds and the rate estimated in the sea water of Maizuru Bay was similar to that in fresh water environments. Nitrogen fixation by microorganisms of size larger than 5 μ amounted to 30–50% of the total activity of the water in fresh water environments. In fresh water regions, nitrogen fixation proceeds actively in the bottom sediments rather than in the water.

In the previous paper¹, the authors reported the modified acetylene method for the purpose of estimating the rate of *in situ* nitrogen fixation. This paper concerns the *in situ* rate of nitrogen fixation measured by the method in various aquatic environments.

Methods

The measurements of the *in situ* rate of nitrogen fixation and the analysis of water were carried out according to the methods described in the previous papers^{1,2,3}. The investigation was carried out in Lake Biwa, Pond I, Pond II, Midoroga-ike Pond, Suyama Culture Pond, and Maizuru Bay. The location and the outline of these water regions were already mentioned in the previous papers^{2,3,4}.

Results and Discussion

***In situ* rate of nitrogen fixation in various aquatic environments.** The qualities of the water in Pond I, Pond II, Midoroga-ike Pond and Lake Biwa were shown in Tables 1 and 2.

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Table 1. Quality of the surface water in Pond I, Pond II and Midoroga-ike.

Pond (Depth)	Pond I (0.5 m)				Pond II (1.5 m)	Midoro- ga-ike (1 m)
	Date	6/17, 1969	7/11, 1969	9/3, 1969	3/16, 1970	11/13, 1969
Atm. temp. (°C)	21.2	23.5	31.8	5.9	15.1	17.0
Water temp. (°C)	20.7	20.8	27.0	8.9	13.0	14.0
NH ₄ ⁺ -N (μg atoms/L)	4.51	0.34	1.51	91.5	0.94	—
NO ₂ ⁻ -N (μg atoms/L)	0.08	1.47	0.24	5.30	1.74	0.13
NO ₃ ⁻ -N (μg atoms/L)	3.72	32.7	16.2	—	141	—
C. O. D. (O ₂ mg/L)	—	2.93	4.71	6.32	2.83	3.44
Sugars (μg/L)	1.88	0	4.80	—	3.65	—
PO ₄ ³⁻ -P (μg atoms/L)	—	13.4	38.7	226	0.79	4.74

Table 2. Quality of the water at Station Bj 1 in Lake Biwa (7/22, 1969).

Depth (m)	0	20	38
Water temp. (°C)	29.8	15.8	8.1
D. O. (O ₂ mg/L)	8.93	7.84	9.11
pH	8.4	7.1	6.9
NH ₄ ⁺ -N (μg atoms/L)	0.30	1.12	—
NO ₂ ⁻ -N (μg atoms/L)	0.28	0.41	—
NO ₃ ⁻ -N (μg atoms/L)	0.16	3.55	—
C. O. D. (O ₂ mg/L)	3.04	1.77	—
Sugars (μg/L)	5.09	3.77	—
PO ₄ ³⁻ -P (μg atoms/L)	ND	ND	—

ND; not detected, Depth of Station; 40 m, Atmospheric temperature; 30.2°C.

The contents of ammonia, nitrite and inorganic phosphate varied considerably with seasons in the Pond I. The extraordinary high levels of inorganic phosphate and ammonia in the water which were observed on March 16, 1970 seemed to be owing to an active mineralization of organic substances at that time. C. O. D. value of the water of the three ponds was always comparatively high, namely as high as 2–6 mg O₂/L. On the other hand, the nitrate concentration in the water of Pond II was extremely high on November 13, 1969. In Lake Biwa, C. O. D. value of the surface water was somewhat high at Station Bj 1 in Shiozu Bay. Both of ammonia and nitrite in the water were poor. The amount of nitrate was, however, very small in the surface water and increased with the depth. The stable stratification in summer was clearly observed at Station Bj 1.

Table 3 showed the rate of nitrogen fixation estimated from the acetylene reducing activity of the water in various water regions above mentioned. It was observed that the rate of nitrogen fixation of the surface water in Pond I varies from 0.30 to 2.15 mg N/hr/m³ during June, 1969—March, 1970. Although the water samples were incubated in the

Table 3. Rate of nitrogen fixation estimated from acetylene reducing activity of the water in different water regions.

Water region (Depth)	Date	Water temperature (°C)	C ₂ H ₂ reducing activity (μ moles C ₂ H ₄ /hr/m ³)	Rate of N ₂ fixation (mg N/hr/m ³)
Pond I	6/17, 1969	20.7	230	2.15
	7/11, 1969	20.8	32.3	0.30
	9/3, 1969	27.0	95.8	0.89
	3/16, 1970	8.9	108	1.02
Pond II	11/13, 1969	13.0 (*18)	478	4.46
Lake Biwa	7/28, 1969			
(0 m)		29.8 (*20)	66.3	0.62
Stn. Bj 1 (20 m)		15.8 (*20)	51.0	0.48
(38 m)		8.1 (*20)	0	0
Midoroga-ike	11/15, 1969	14.0 (*17)	251	2.35
Maizuru Bay				
Stn. E (5 m)	7/5, 1969	21.8	28.3	0.26
	7/18, 1969	23.8	135	1.62

* Temperature of incubation.

water bath at 18°C which was slightly higher than *in situ* temperature, the rate of nitrogen fixation was very high, i.e. about 4.46 mg N/hr/m³. The water sample in Midoroga-ike Pond was also incubated in the laboratory at 17°C, while *in situ* temperature was 14.0°C. The rate of nitrogen fixation of the water of Midoroga-ike Pond was also as high as about 2.35 mg N/hr/m³. On the other hand, the water samples from Lake Biwa were incubated at 20°C, and the rate of nitrogen fixation ranged from 0 to 0.62 mg N/hr/m³. The water samples of Maizuru Bay were collected from the depth of 5 m at Station E near the shore, and the *in situ* rate of nitrogen fixation was 0.26–1.62 mg N/hr/m³, which was similar to the rates observed in fresh water environments. In general, there was no clear relationship between the rate of nitrogen fixation and various environmental condition of the water region.

By the modified acetylene method, it has been demonstrated, in the present study, that the rate of nitrogen fixation ranges from 0.3 to 4.5 mg N/hr per 1 m³ of the water in fresh water as well as coastal sea water regions. DUGDALE *et al.*⁵⁾ and DUGDALE and DAGDALE⁶⁾ measured directly the rate of nitrogen fixation in Sanctuary Lake by the use of ¹⁵N. They found that the maximum daily rate of nitrogen fixation is as high as 130 μ g N per liter of the water in the epilimnion. The mean rate was 30 μ g N/L, which corresponds to 1% of the nitrogen content in the lake water. On the contrary, the daily rate ranged from 0 to 0.0047 μ g N/L in Alaskan lakes during July and August. STEWART *et al.*⁷⁾ observed the high nitrogen fixation rate ranged from 10 to 200 mg N/

hr/m³, in the surface water of Lake Mendota during heavy algal bloom in July, 1967. The rate was estimated from acetylene reducing activity. They also measured *in situ* rate of nitrogen fixation, 2–45 mg N/hr/m³, at various depths in Lake Mendota in July, 1967. The present authors' findings as to the rate of nitrogen fixation in various aquatic environments are in good agreement with their results, except in the water with heavy algal bloom.

Diurnal variation of *in situ* rate of nitrogen fixation. In order to find the relationship between the rate of nitrogen fixation and the light intensity in the water, diurnal fluctuation in the nitrogen fixing rate was investigated in the water of Suyama Culture Pond. The water samples were collected from the depths of 0 m and 2 m at 1:00 p.m., 9:00 p.m. and 4:00 a.m. on September 20–21, 1969. The quality of the pond water was shown in Table 4. D. O. value was 9–11 mg O₂/L and the pond water was well-oxygenated. C. O. D. value was somewhat low, i.e. 1–2 mg O₂/L. The concentration of ammonia, nitrate and inorganic phosphate was fairly abundant.

Table 4. Quality of the pond water in Suyama Culture Pond⁴⁾.

Depth (m)	0			2			
	9/20, 1969		9/21, 1969	9/20, 1969		9/21, 1969	
	Date	Time	Date	Date	Time	Date	
		13:00	21:00	04:00	13:00	21:00	04:00
Atm. temp. (°C)		27.9	19.4	17.5	27.9	19.4	17.5
Water temp. (°C)		24.5	22.0	21.5	22.8	22.2	22.0
D. O. (O ₂ mg/L)		11.4	11.0	10.6	9.1	9.4	9.5
pH		8.6	7.9	8.6	7.9	8.0	7.6
NH ₄ ⁺ -N (μg atoms/L)		69.5	59.4	64.7	92.2	75.9	70.0
NO ₂ ⁻ -N (μg atoms/L)		3.97	3.84	3.95	3.49	4.19	4.38
NO ₃ ⁻ -N (μg atoms/L)		84.3	59.1	90.7	32.8	87.4	51.3
C. O. D. (O ₂ mg/L)		1.63	1.77	1.85	1.82	2.55	1.61
Sugars (μg/L)		0.96	2.69	1.53	2.11	1.53	1.34
PO ₄ ³⁻ -P (μg atoms/L)		27.4	8.25	6.12	15.9	7.23	10.7

Transparency; 1.5 m (13:00).

Table 5. Rate of nitrogen fixation in the water of Suyama Culture Pond.

Depth (m)	Date	Time	Water temperature (°C)	C ₂ H ₂ reducing activity (μ moles C ₂ H ₄ /hr/m ³)	Rate of N ₂ fixation (mg N/hr/m ³)
0	9/20, 1969	13:00	24.5	355	3.31
		21:00	22.0	158	1.48
	9/21, 1969	04:00	21.5	247	2.30
2	9/20, 1969	13:00	22.8	403	3.76
		21:00	22.2	433	4.04
	9/21, 1969	04:00	22.0	345	3.22

As shown in Table 5, the rate of nitrogen fixation ranged from 1.48 to 4.04 mg N/hr/m³. In the surface water, the highest rate was observed during daytime and the lowest during night, which was in contrast with the almost equal rate in the water of 2 m depth. This may suggest that nitrogen fixation depends upon the light intensity in some extent, namely the photosynthetic microorganisms other than nitrogen fixing bacteria such as photosynthetic bacteria or blue green algae also may take part in the process in fresh water environments. The rate of nitrogen fixation in the water of Suyama Culture Pond was generally high as compared with other water regions.

Nitrogen fixing activities of the pond water fractionated by different pore-sized filters. In order to demonstrate the actual participation of nitrogen fixing bacteria in nitrogen fixation in fresh water environments, the pond water sample was fractionated by filtering through different pore-sized Millipore filters (0.22 μ , 0.45 μ , 0.8 μ and 5 μ), and was assayed the activities of nitrogen fixation of the each fraction. As shown in Table 6, the nitrogen fixation by the microorganisms attached on the suspending particles or microorganisms with the size of larger than 0.8 μ was about 78% of total activity of the water mass in Pond I. The activity of nitrogen fixation by the fraction with the size of larger than 5 μ was about 35% of the total activity in Pond II and about 46% in Midoroga-ike Pond. On the other hand, the nitrogen fixing activity of the fraction with the size of smaller than 0.45 μ was about 27% in the water of Pond II and about 35% in Midoroga-ike Pond. This fraction of the water may be consisted of nitrogen fixing bacteria which occur freely in the water.

Table 6. Distribution of nitrogen fixing activity in the water fractionated by different pore-sized filters.

Water region (Date)	Size of fraction (μ)	C ₂ H ₂ reducing activity (μ moles C ₂ H ₄ /hr/m ³)	N ₂ fixing activity (mgN/hr/m ³)	Percentage
Pond I (9/3, 1969)	>0.8	75.0	0.70	78.3
	0.8>	20.8	0.19	21.7
	Total	95.8	0.89	100
Pond II (11/13, 1969)	>5	168	1.57	35.2
	5—0.8	108	1.01	22.6
	0.8—0.45	70	0.65	14.6
	0.45—0.22	132	1.23	27.6
	0.22>	0	0	0
Total	478	4.46	100	
Midoroga-ike (11/15, 1969)	>5	116	1.08	46.2
	5—0.8	33.8	0.32	13.5
	0.8—0.45	11.7	0.11	4.7
	0.45>	89.5	0.84	35.6
Total	251	2.35	100	

In general, about 30–50% of total activity of nitrogen fixation in fresh water environments may be attributable to the microorganisms attached on the suspending particles or the microorganisms with the size of larger than 5μ . In addition to the diurnal changes in the activity in the surface pond water which has been observed in the above section, the facts that blue green algae are found in dense population contrary to somewhat small standing crop of nitrogen fixing bacteria and that the rate of nitrogen fixation is extremely high as compared with that in other water regions may introduce the assumption that the blue green algae play an important role on the nitrogen fixation during daytime in such culture ponds.

Vertical change in the rate of nitrogen fixation in Lake Biwa. In order to demonstrate the vertical change in the rate of nitrogen fixation in the water column of Lake Biwa, the water samples were collected from the different depths on July 28, 1969 from Station Bj 1 (Shiozu Bay) and on February 16, 1970 from Station Ie 1 (north lake) in Lake Biwa. The water samples were incubated in the water bath kept at 20°C .

The vertical distribution of nitrogen fixing activity was shown in Fig. 1. The results indicated that the activity of the water at Station Bj 1 is high in the surface water and decreased with the depth corresponding to the vertical change of the water temperature in July, 1969. On the contrary, the activity of the water at Station Ie 1 was low in the water from the surface to the bottom in February, the circulation period, as shown in Fig. 1. The amount of nitrogen fixed in the water column estimated from the vertical distribution of nitrogen fixing activity was 3.6 mg N/hr/m^2 at Station Ie 1 and 15.2 mg N/hr/m^2 , respectively. The fact that the biomass of nitrogen fixing bacteria as well as of blue green algae is huge at Station Bj 1 as compared with the other stations in Lake Biwa such as Station Ie 1, may certify the comparatively high nitrogen fixation in the water of Shiozu Bay.

Role of bottom sediments on nitrogen fixation in fresh water regions. The number of nitrogen fixing bacteria in the bottom sediments was as large as about $01^3 \text{ cells/g}^{2,4}$. In order to make clear the role of the bottom sediments on nitrogen fixation in fresh water environments, the nitrogen fixing rate in the bottom mud was compared with that in the water in Pond I.

The results in Table 7 indicated that the rate of nitrogen fixation in the bottom mud is 60–70 times higher than that in the water, while the standing crop of the responsible

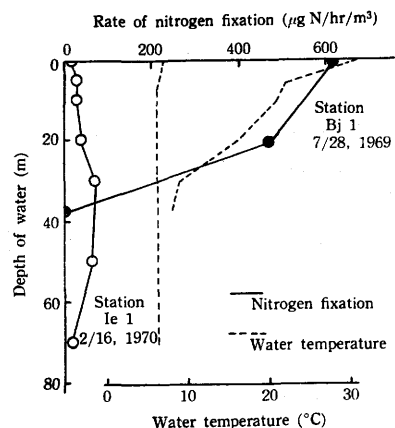


Fig. 1. Change in the rate of nitrogen fixation with water depth in Lake Biwa.

Table 7. Rate of nitrogen fixation in Pond I.

	Temperature (°C)	C ₂ H ₂ reducing activity (μ moles C ₂ H ₄ /hr/m ³ or ton)	Rate of N ₂ fixation (mg N/hr/m ³ or ton)
Water	20.8	32.3	0.302
Mud	20.8	2,020	18.9

bacteria in 1 g of the bottom mud was 10–100 times larger than that in 1 mL of the water. The rate of nitrogen fixation may not always depend upon the occurrence of nitrogen fixing bacteria, however, it seems likely that nitrogen fixing bacteria play a fairly important role on nitrogen fixation in the bottom mud. From the average depth of 0.5 m in Pond I, the hourly nitrogen fixation was estimated from these data as large as 0.151 mg N/m² in the water column, while 0.378 mg N/m² in the surface layer (0–2 cm) of the mud. Thus, the nitrogen fixation in the bottom sediments was about twice as active as that in the water mass in July, 1969 in Pond I.

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