Microbiological Studies on the Nitrogen Cycle in Aquatic Environments—VI

Metabolic Rate of Ammonium Nitrogen in a Goldfish Culturing Pond^{*1}

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(Received January 23, 1979)

In order to make clear the mechanism of the nitrogen cycle in bodies of water, the metabolic rate of ammonium nitrogen and its incorporation rate into each trophic level of bio-communities, as well as into each nitrogenous compound in the water, were estimated using a ¹⁵N tracer technique in the model system of a goldfish culturing pond. The extremely high turnover rate of ammonium nitrogen in the water, 141%/h, was estimated. A large part of the ammonium nitrogen which metabolized was incorporated into "Bacteria" and "Phytoplankton" fractions; their incorporation rates were calculated to be 1.83 μ g atoms/l/h and 0.83 μ g atoms/l/h, respectively. In the "Zooplankton" fraction, the incorporation of ¹⁵N nitrogen which had been added as ammonium nitrogen in the water was detected after 30 min, and its incorporation rate was as low as 0.02 μ g atoms/l/h. Although no increase in ¹⁵N was found in "Nitrite and Nitrate" fraction, the distribution of ¹⁵N in each nitrogen fraction indicates that the process of nitrification as well as denitrification proceeded rapidly in the water body.

In the previous paper¹⁾, the authors have reported that ammonium nitrogen was incorporated rapidly into the organisms of primary trophic level and that only a small portion of the nitrogen thus incorporated was transferred into the higher trophic levels, zooplankton and fish, in the goldfish culturing pond. However, the metabolic rate of ammonium nitrogen as well as its incorporation rate into each trophic level of the bio-communities in the pond is still obscure. It seems to be necessary to estimate these metabolic rates in order to make clear the mechanism of nitrogen cycle in the water region.

From this viewpoint, the authors have attempted to estimate the metabolic rate of ammonium nitrogen in the goldfish culturing pond using the ¹⁵N tracer technique.

Materials and Methods

Fish Culturing Pond Used in the Study

The goldfish culturing pond A-70 in the previous study was employed again in the present work. The experiment was carried out on May 18th in 1974. The population density of the fish as well as its growth stage was almost same as that

was reported in the previous paper¹⁾. At that time, ca. 40,000 heads of the goldfish at the stage of 2 weeks after hatching were in the pond, and their bait was supplied only through the natural food chain.

Model System of the Goldfish Culturing Pond

In the present study, the experimental system was designed without bottom mud for the reason that the stirring of water at the tracer supplementation causes the up-roiling of bottom mud, which may increase the concentration of the nutrients in the water. Approximately 20 l of the water from the middle depth of the pond were poured gently into a transparent bag of polyethylene film, then 20 heads of the fish were put into the bag to give the same population density as it was in the pond, as shown in Fig. 1.

Bacteriological and Chemical Analyses

The enumeration of each group of the bacteria in the water and the bottom mud of the pond was carried out using the methods described in the previous paper¹⁾. The qualities of the water and the bottom mud were determined by the methods mentioned previously^{2,3)}.

^{*1} Supported in part by the Grant from the Ministry of Education, the Government of Japan.

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Fig. 1. Model system of the goldfish culturing pond used in the ^{15}N experiment.

^{15}N Tracer Experiment

After being supplemented a definite amount of ammonium sulfate with heavy nitrogen [$^{15}\text{NH}_4$] $_2\text{SO}_4$, 99.3 atom %, which was corresponded to about a quarter of the original amount of ammonium nitrogen, into the water, 1 l of the water containing 2 heads of the fish was taken out from the bag at the settled intervals over 6 h. To obtain the initial rates of ammonium nitrogen metabolism and of the incorporation into each nitrogen fraction, the first sampling was done at 2 min after the tracer supplementation, subsequently at every 10 min up to 30 min.

The fractionation of each nitrogen fraction in the water body was carried out by the methods shown in Figs. 2 and 3. The detailed description of the procedure is in the previous paper^{1,3,4}. The ^{15}N abundance ratio in each nitrogen fraction was analyzed by the mass spectrometry⁵. The rate of ammonia metabolism was calculated based on the change in ^{15}N content of each nitrogen fraction during the early stage of the experiment, in which the metabolic processes proceeded linearly with the elapse of time.

Fig. 2. Fractionation of the nitrogen fractions at each trophic level in the model system of the pond.

Results and Discussion

Qualities of Water and Bottom Mud in the Pond

Table 1 shows the qualities of the water and the bottom mud in the pond at 05:30 on May 18th, just before the supplementation of tracer ^{15}N ammonium salt. As it was in the early morning, dissolved oxygen in the water was extremely low in its concentration. The DO value increased with the elapse of time and rised up to 150% saturation at 11:30, due to the active photosynthesis by phytoplankton occurring in the water. The COD value of the water was quite high as compared with that in natural waters. The con-

Fig. 3. Fractionation of the dissolved nitrogen fractions in the model system of the pond.

Table 1. Quality of water and bottom mud in the gold fish culturing pond

	Water	Bottom mud
Temperature (°C)	23.0	23.0
DO (saturation %)	19.5	
Eh (mV)		-25
pH	7.35	7.30
Total N ($\mu\text{g atoms/l, g}$)*	353	114
NH ₄ -N ($\mu\text{g atoms/l, g}$)	3.5	9.6
NO ₂ -N ($\mu\text{g atoms/l, g}$)	trace	trace
NO ₃ -N ($\mu\text{g atoms/l, g}$)	1.26	0.15
PO ₄ -P ($\mu\text{g atoms/l, g}$)	25.8	58.5
SiO ₂ -Si ($\mu\text{g atoms/l, g}$)	192	233
COD (O ₂ mg/l, g)	71.7	12.5

* not included the nitrogen of "Goldfish" fraction.

centration of total nitrogen as well as the ratio of ammonium nitrogen to nitrate nitrogen, the redox balance of inorganic nitrogen, was lower than each value observed in the previous work in the same pond. The accumulation of organic matter in the bottom mud seemed to be somewhat less than in 1973.

Bacterial Flora in the Pond

The numbers of total heterotrophic bacteria, sulfate reducers and each group of the bacteria which have different biochemical activities of nitrogen metabolism are illustrated in Figs. 4 and 5. In the present study, the viable count of heterotrophic bacteria in the water was in the same order of magnitude as that in the previous survey in 1973¹¹. The ratio of nitrifiers to the other groups of the bacteria was, however, higher than that observed in 1973. In the bottom mud, the number of each group of the bacteria was also smaller than in 1973, with the exception of nitrifying bacteria.

Fig. 4. Bacterial Flora of the water in the goldfish culturing pond.

Rate of Nitrogen Metabolism in the Model System of the Pond

Fig. 6 shows the change in the concentration of

Fig. 5. Bacterial flora of the bottom mud in the goldfish culturing pond. **Fig. 6.** Change with the time elapsed in the concentration of ammonium nitrogen in the water of the model system of the pond.

ammonium nitrogen in the water of the model system of the pond. Ammonium nitrogen in the water was $3.5 \mu\text{g atoms/l}$ at the time just before the supplementation of ^{15}N ammonium nitrogen ($0.971 \mu\text{g atoms/l}$) as the tracer. Two minutes after the supplementation, the concentration was determined as $2.3 \mu\text{g atoms/l}$ which is considerably lower than the theoretical value of $4.471 \mu\text{g atoms/l}$ at 0 h. In response to this, about a half amount of ^{15}N nitrogen which had been supplemented into "Ammonia" fraction was recovered in the other nitrogen fractions at 2 min, as shown in Figs. 7, 8 and 9. This phenomenon is not attri-

Fig. 7. Change with the time elapsed in the ^{15}N content of the nitrogen fractions ("DON", "Ammonia" and "Nitrite and Nitrate").

Fig. 8. Change with the time elapsed in the ^{15}N content of the nitrogen fractions ("Phytoplankton" and "Bacteria").

Fig. 9. Change with the time elapsed in the ^{15}N content of the nitrogen fractions ("Zooplankton" and "Goldfish").

butable to biochemical processes, and can be explained by assuming that a part of ammonium nitrogen supplemented was adsorbed spontaneously on dissolved as well as particulated organic matter which was present abundantly in the water. Essentially linear increase or decrease of ^{15}N content in each nitrogen fraction was observed during the period from 2 min to 30 min, which might be due to the biochemical processes of nitrogenous compounds. From the decrease in the ^{15}N content of "Ammonia" fraction in the period, $0.622 \mu\text{g atoms/l/h}$, the metabolic rate of ammonium nitrogen was calculated as $3.53 \mu\text{g atoms/l/h}$.

The amount of ^{15}N in "Nitrite and Nitrate" fraction was $0.004 \mu\text{g atoms/l}$ at 2 min after the tracer was added. In the subsequent period of the experiment, no detectable amount of ^{15}N was found in the fraction, in spite of a large population of nitrifying bacteria in the water. It may be due to the rapid assimilation and reduction of the ^{15}N labeled nitrogen in the fraction by microorganisms.

The decrease in the ^{15}N content of "DON" fraction might be due to the low generation rate of ^{15}N labeled DON through its excretion and decomposition of ^{15}N labeled organisms. The rate

of decrease in the ^{15}N content of "DON" was $0.078 \mu\text{g atoms/l/h}$. From the value, the metabolic rate of "DON" was calculated as $59.1 \mu\text{g atoms/l/h}$.

The amount of ^{15}N in "Bacteria" and "Phytoplankton" fractions increased linearly from 2 min to 30 min after the ^{15}N tracer supplementation. The incorporation rate of the ^{15}N into these fractions were $0.322 \mu\text{g atoms/l/h}$ and $0.147 \mu\text{g atoms/l/h}$, respectively. The ^{15}N in these fractions may have originated not only from the uptake of ^{15}N labeled ammonium nitrogen but also from that of the labeled DON. However, most of the ^{15}N incorporated into the fractions might be derived from "Ammonia" fraction, because the ^{15}N atom percentage of "Ammonia" fraction was about 100 times higher than that of "DON" fraction. Assuming that all of the ^{15}N in these fractions originated from "Ammonia" fraction, the incorporation rate of ammonium nitrogen into "Bacteria" and "Phytoplankton" fractions can be calculated as $1.83 \mu\text{g atoms/l/h}$ and $0.83 \mu\text{g atoms/l/h}$, respectively.

The amount of ^{15}N in "Zooplankton" fraction began to increase after the lag period of about 30 min, while the incorporation of ^{15}N into "Bacteria" and "Phytoplankton" fractions began immediately after the ^{15}N supplementation. The results obtained here indicate that the incorporation of ^{15}N into zooplankton was done only through bacteria and phytoplankton. In the subsequent period of the experiment, the ^{15}N content of the "Zooplankton" fraction increased linearly. The incorporation rate of ^{15}N into the fraction was $0.004 \mu\text{g atoms/l/h}$. Then, incorporation rate of ammonium nitrogen was calculated as $0.02 \mu\text{g atoms/l/h}$ during the period of 0.5 h to 6 h.

Throughout the experiment, the increase in ^{15}N content of "Goldfish" fraction could not be detected. It may be caused by the low ^{15}N atom percentage of nitrogen in "zooplankton" which is the sole source of the fish bait.

Fig. 10 shows the change in the amount of ^{15}N present in the water body in the model system. The amount of ^{15}N nitrogen recovered at 2 min by making measurement in each fraction and summing them was nearly same as that of the supplementation. The ^{15}N content of the water body decreased almost linearly with the elapse of time in the period from 2 min to 30 min. The rate of denitrification was calculated as $0.85 \mu\text{g atoms/l/h}$ from the decrease rate of ^{15}N , $0.150 \mu\text{g atoms/l/h}$. Since the ^{15}N was scarcely found in "Nitrite and

Fig. 10. Change with the time elapsed in the ^{15}N content of the whole water body in the model system of the pond.

Nitrate" fraction, the rate of denitrification is regarded as the oxidation rate of ammonium nitrogen according to the reason mentioned in the previous paper^{1,4)}.

The results obtained in the ^{15}N tracer experiment

Table 2. Metabolic rate of ammonium nitrogen and DON in the goldfish culturing pond

Nitrogen	Concentration ($\mu\text{g atoms/l}$)	Metabolic rate ($\mu\text{g atoms/l/h}$)	Turnover rate (%/h)
Ammonia N	2.5	3.53	141
DON	178	59.1	33.2

Table 3. Incorporation rate of ammonium nitrogen in the goldfish culturing pond

Nitrogen fraction	Concentration ($\mu\text{g atoms/l}$)	Rate of incorporation ($\mu\text{g atoms/l/h}$)	Turnover rate (%/h)
Nitrite and nitrate	1.26		
Bacteria	78.8	1.83	2.3
Phytoplankton	90.0	0.83	0.9
Zooplankton	3.06	0.02*	0.7
Goldfish	39.5		
Gaseous N		0.85	
DON	178	0.02	0.01

* rate in the period from 30 min to 6 h.

were summarized in Tables 2 and 3. The turnover rate of ammonium nitrogen in the pond water was 141.2%/h (i.e. 42.5 min), which is extremely fast as compared with other aquatic regions⁴⁾. It may be due to the huge population of phytoplankton in the water. Approximately 75% of ammonium nitrogen metabolized was incorporated into "Bacteria" and "Phytoplankton" fractions, and about 24% was transformed into gaseous nitrogen. 0.5% might be incorporated into "DON" fraction, since the amount of ammonium nitrogen incorporated into "Zooplankton" and "Goldfish" fractions in the period of 30 min after ^{15}N supplementation was so small. The incorporation rate into "DON" fraction obtained here was extremely low as compared with that in the other water regions⁴⁾. Such the low rate might be ascribed to that the incorporation during the initial 30 min was slow because the incorporation of ammonium nitrogen into "DON" fraction might proceed only through organisms in the water of the pond. On the other side, the metabolic rate of DON was considerably high. A part of the DON might be utilized directly by microorganisms as nitrogen source for their growth, therefore, it seems probable that the turnover rate of "Bacteria" as well as "Phytoplankton" fraction obtained here might be underestimated.

References

- 1) A. KAWAI and M. SUGIYAMA: *Bull. Japan. Soc. Sci. Fish.*, **45**, 61-65 (1979).
- 2) A. KAWAI, M. SUGIYAMA, R. SHIOZAKI, and I. SUGAHARA: *Memoirs Res. Inst. Food Sci. Kyoto Univ.*, **32**, 7-15 (1971).
- 3) A. KAWAI and M. SUGIYAMA: *Bull. Res. Inst. Food Sci. Kyoto Univ.*, **40**, 1-6 (1977).
- 4) M. SUGIYAMA and A. KAWAI: *Bull. Japan. Soc. Sci. Fish.*, **44**, 351-355 (1978).
- 5) A. KAWAI and M. SUGIYAMA: *Bull. Res. Inst. Food Sci. Kyoto Univ.*, **39**, 1-7 (1976).
- 6) K. JONES and W. D. P. STEWART: *J. Mar. Biol. Assoc. (UK)*, **49**, 701-716 (1969).