PHOTOINHIBITION AND RECOVERY OF NH_4^+ - OXIDIZING BACTERIA AND NO_2^- - OXIDIZING BACTERIA

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The effects of illumination on nitrifying bacteria were investigated with and without substrate. NH_4^+ -oxidizing bacteria (strain H-1, probably *Nitrosospira* sp.) and NO_2^- -oxidizing bacteria (strain O-1, probably *Nitrobacter* sp.), isolated from Lake Kizaki, were inhibited by light (about $75 \mu\text{Einsteins/m}^2/\text{sec}$, 12-hr light: 12-hr dark). Photoinhibited H-1 and O-1 cells recovered after several to tens of days under dark conditions. However, H-1 cells, illuminated for 7 to 10 days in the absence of NH_4^+ , did not produce NO_2^- significantly for 120 to 350 days in a medium containing $50 \mu\text{M}$ NH_4^+ . The substrate (NH_4^+) was found to protect H-1 from photoinhibition, while such an effect of NO_2^- on O-1 was not so clear. O-1 was more sensitive to light than H-1 in the short illumination period. However, the inhibitory effect of prolonged illumination was greater on H-1 than on O-1. It was indicated from growth kinetic analysis that the photoinhibitory effects on both NH_4^+ -oxidizing bacteria and NO_2^- -oxidizing bacteria were not only lethal but also bacteriostatic.

Nitrification, which is an important microbiological process in the nitrogen cycle, is affected by substrate (NH_4^+ and NO_2^-) concentration, temperature, oxygen content, pH, and Eh. Light has an inhibitory effect on nitrifying bacteria (1, 2). OLSON (3, 4) and WARD et al. (5) suggested that the formation of the primary nitrite maximum in the ocean was due to the differential photoinhibition of nitrifying bacteria. HERRIGAN et al. (6) found that nitrifying bacteria isolated from sea-surface film were inhibited by light and that no nitrification occurred in the surface films of subtropical seas. From the point of view of the recovery from photoinhibition of nitrifying bacteria, HOOPER and TERRY (7) observed that *Nitrosomonas europaea* cells 90%-inactivated by illumination recovered their full activity after 10 hr. HERRIGAN et al. (6) cited the fact that light inhibition was not lethal and that nitrifiers might recover when they mixed to depths of sufficiently low light levels in the sea. In Lake Kizaki, the nitrifying activity of lake water is very low in

the spring overturn period and rapidly increases in the early summer stratification period (8).

The present authors consider that nitrifying bacteria distributed in the whole water column of Lake Kizaki may suffer from photoinhibition in the spring overturn period and recover in the dark in the hypolimnion. However, knowledge of the recovery from photoinhibition of nitrifying bacteria, and the nature of photoinhibition (lethal or bacteriostatic) is still limited. And there has been no growth kinetic analysis of the photoinhibition of nitrifying bacteria.

In the present work, the effects of illumination on the growth of nitrifying bacteria were studied by growth kinetic considerations. The effects of substrate concentration on the recovery from photoinhibition were also studied.

MATERIALS AND METHODS

Organisms. NH_4^+ -Oxidizing bacteria (strain H-1) and NO_2^- -oxidizing bacteria (strain O-1), isolated from the bottom mud of Lake Kizaki (9), were used in this study. The cells of H-1 were non-motile and lobular or stumpy and rod-like when the cells were examined with a phase-contrast microscope (9). However, when the cells were examined with an electron microscope (Jeol., 100-CX, 80 kV), the cells were spiral shaped, having 3 to 6 turns (Fig. 1). The size of the cells was

Fig. 1. Electron micrograph of H-1 showing the overall of the cell.

0.5–0.7 by 0.7–1.2 μm and the width of the spiral filament was about 0.3 μm . From these morphological characters, it seems that H-1 probably belongs to *Nitrosospira*. O-1 seems to belong to *Nitrobacter*, judging from the cell shape as described in the previous report (9). The compositions of pre-culture media were described in the previous study (9).

Illumination of cells. H-1: Two hundred milliliters of the pre-culture of H-1, which had reached the late exponential growth phase, was aseptically filtered through 0.2 μm Nuclepore filters. In order to eliminate NH_4^+ and NO_2^- , the filters were washed three times with a small amount of buffer solution on the filtration apparatus and then put into 20 ml of the same solution. The buffer solution contained: KH_2PO_4 , 0.5 g; K_2HPO_4 , 0.5 g; NaCl , 0.1 g; and distilled water, 1,000 ml. The pH of this solution was adjusted to 7.6 to 7.8 with 5% Na_2CO_3 solution. The buffer solution was autoclaved (121° , 15 min). Each 5 ml of the cell suspension was inoculated into two Roux Bottles with 200 ml of buffer solution. The final density of bacterial cells was about 1.1×10^7 cells/ml. One bottle admitted light and the other was a dark bottle wrapped with aluminum foil. These two bottles were incubated at $20 \pm 3^\circ$ under cool-white fluorescent light (Toshiba, FLR 40 S·W). The light intensity measured with a Quantum meter (LI-COR, Inc., Model LI-185 B) was about 75 $\mu\text{Einsteins}/\text{m}^2/\text{sec}$ at the surface of the bottle (about 5% of full sunlight). The illumination cycle was 12-hr light: 12-hr dark.

Incubation started at the initiation of the dark period. On the 1st, 2nd, 3rd, 5th, 7th and 10th days, samples were aseptically removed from each bottle, and each ml of subsample was inoculated into a 200 ml Erlenmeyer flask with 100 ml of growth media, which contained 50 μM or 1 mM NH_4^+ . Bacteria were counted at inoculation time using the acridine orange direct counting method (9) and the most probable number (MPN) method (8). These flasks were incubated at 20° in the dark. At appropriate intervals, the NO_2^- concentration was measured. In order to estimate the effect of illumination on the growth of H-1 in the growth media, H-1 was inoculated into media whose NH_4^+ concentrations were 50 μM and 1 mM. Initial cell density was about 1.1×10^5 cells/ml. The cultures were maintained under 12-hr light: 12-hr dark conditions, and increases in NO_2^- concentration were measured. The cultures for the dark control were wrapped with aluminum foil.

O-1: Each 5 ml of the pre-culture of O-1, which had consumed 0.1 g of NaNO_2 per 100 ml, was inoculated into two Roux Bottles with 200 ml of buffer solution. The final density of bacterial cells was about 1.1×10^6 cells/ml. Other procedures were the same as those for H-1, except for the substrate concentrations (NO_2^- : 10 μM and 100 μM). Direct count and MPN count during the illumination experiments were not carried out.

All experiments were replicated in triplicate or duplicate.

NO_2^- assay. NO_2^- concentration was assayed by the method of BENDSCHNEIDER and ROBINSON (10).

RESULTS

Effect of illumination in the absence of substrate

NH_4^+ -Oxidizing bacteria (H-1). Figure 2 shows the time courses of NO_2^- production in the flasks inoculated with illuminated and non-illuminated cells of H-1 in the absence of NH_4^+ . So long as the illumination period in the buffer solution was less than 2 days, there was hardly any difference in the NO_2^- production by the illuminated and the non-illuminated cells in both $50 \mu\text{M}$ and 1 mM NH_4^+ (Fig. 2-a and b). The production of NO_2^- by the cells illuminated for 3 days was delayed in comparison with that by the non-illuminated cells (Fig. 2-c). It appeared that the longer the illumination period, the larger the delay time (Fig. 2-c to f). Table 1 shows the delay times (or the differences between the time courses of NO_2^- production by the illuminated and the non-illuminated cells) estimated

Fig. 2. Effect of illumination on H-1 in the absence of NH_4^+ .

Time courses of NO_2^- production in the flasks inoculated with the illuminated (dashed line) and the non-illuminated (solid line) cells. Concentrations of NH_4^+ in the flasks were $50 \mu\text{M}$ ($\blacktriangle$ and $\triangle$) and 1 mM ($\bullet$ and $\circ$). Illumination periods: a, 1 day; b, 2 days; c, 3 days; d, 5 days; e, 7 days; f, 10 days.

from Fig. 2. In 1 mM NH_4^+ , the delay times caused by the illumination for 3, 5, 7 and 10 days were about 0.3, 1.2, 7.5 and 42 days, respectively. In $50 \mu\text{M}$ NH_4^+ , the delay times caused by the illumination for 3 and 5 days were 2 and 26 days, respectively. When the cells were illuminated for 7 and 10 days, significant NO_2^- production was not observed for over 120 and 350 days, respectively.

Table 2 shows the bacterial numbers determined by epifluorescent microscopy. The direct counts of bacterial cells in both the illuminated and the non-illuminated bottles decreased by less than 10% on the 20th day. The viable counts (MPN)

Table 1. Effect of illumination on H-1: the delay time in the absence of NH_4^+ .

Concentration of NH_4^+	Illumination period (days)					
	1	2	3	5	7	10
50 μM	0.3	0	2.0	26	>120	>350
1 mM	0	0	0.3	1.2	7.5	42

Units are days.

Table 2. The change in direct counts of H-1 cells during illumination in the absence of NH_4^+ .

Illumination period (days)	Light bottle	Dark bottle
0	1.1×10^7	— ^a
1	1.1×10^7	1.0×10^7
2	1.1×10^7	1.1×10^7
3	1.1×10^7	1.0×10^7
5	1.1×10^7	1.0×10^7
7	9.7×10^6	8.5×10^6
10	9.7×10^6	1.0×10^7
20	9.4×10^6	9.2×10^6

^a Not determined.
Units are cells/ml.

Table 3. The change in MPN values of H-1 during illumination in the absence of NH_4^+ .

Illumination period (days)	Light bottle	Dark bottle
0	3.6×10^5	— ^a
1	1.4×10^5	1.7×10^5
2	1.7×10^5	3.6×10^5
3	— ^b	2.1×10^5
5	— ^b	4.7×10^5
7	1.5×10^5	1.5×10^5
10	$<9.0 \times 10^3$	2.2×10^5
20	$<9.0 \times 10^3$	3.7×10^5

^a Not determined.

^b MPN values were indeterminate, since the growth in MPN tubes was irregular.
Units are cells/ml.

were very low in comparison with the direct counts (about 1 to 30% of the direct counts, Table 3). Although the variation in MPNs was very large and the trend in MPNs was not consistent, the MPNs after 10 and 20 days illumination were remarkably low, less than 9.0×10^3 cells/ml (the lower limit of the MPN method

Fig. 3. a-d.

adopted in this study). Therefore, it seems that 10 to 20 days illumination ($75 \mu\text{Einsteins}/\text{m}^2/\text{sec}$) in the absence of NH_4^+ , at least, had a lethal effect on H-1.

NO_2^- -Oxidizing bacteria (O-1). Figure 3 shows the time courses of NO_2^- consumption in the flasks which were inoculated with illuminated and nonilluminated O-1 cells in the absence of NO_2^- . Although delays were observed in the case of the illuminated cells, NO_2^- consumption rates of the illuminated cells were approximately the same as those of the non-illuminated cells. The delay times are shown in Table 4. In O-1, different results were observed in comparison with H-1. The inhibitory effect of light on the growth of O-1 was already exerted after one day's illumination (Fig. 3-a). However, the prolongation of the delay time associated with the illumination period was not so large in comparison with H-1 (Tables 1 and 4). When O-1 cells, which were illuminated for one day in the

Fig. 3. Effect of illumination on O-1 in the absence of NO_2^- .

Time courses of NO_2^- consumption in the flasks inoculated with the illuminated (dashed line) and the non-illuminated (solid line) cells. Concentrations of NO_2^- in the flasks were $10 \mu\text{M}$ ($\blacktriangle$ and $\triangle$) and $100 \mu\text{M}$ ($\bullet$ and $\circ$). Illumination periods: a, 1 day; b, 2 days; c, 3 days; d, 5 days; e, 7 days; f, 10 days.

Table 4. Effect of illumination on O-1: the delay time in the absence of NO_2^- .

Concentration of NO_2^-	Illumination period (days)					
	1	2	3	5	7	10
$10 \mu\text{M}$	5.2	5.9	6.5	8.3	9.6	22
$100 \mu\text{M}$	3.1	3.7	3.5	4.2	5.9	15

Units are days.

absence of NO_2^- , were inoculated into media containing $10 \mu\text{M}$ and $100 \mu\text{M}$ NO_2^- , NO_2^- consumption were delayed for 5.2 and 3.1 days, respectively. However, even with 7 days illumination, the delay times were 9.6 and 5.9 days, respectively.

Effect of illumination in the presence of substrate

H-1. Figure 4 shows the time courses of NO_2^- production by the growth of H-1 in the light and the dark flasks containing $50 \mu\text{M}$ and 1 mM NH_4^+ . In the case of 1 mM NH_4^+ , NO_2^- increased even in the light flask during the first 14 days, although the rate of NO_2^- production was considerably lower than that in the dark flask. In this period, the direct counts of bacterial cells increased slightly (Table 5). Thereafter, NO_2^- increased hardly at all in the light flask up to 31st day. Three light flasks (1 mM NH_4^+) were placed under dark conditions, one each on the 8th, 14th and 31st days. Delays occurred in NO_2^- production and they increased with

Fig. 4. Effect of illumination on H-1 in the presence of NH_4^+ .

Time courses of NO_2^- production by the growth of H-1 in the light (dashed line) and the dark (solid line) flasks. Arrows indicate the times at which each light flask was switched to dark. a: NH_4^+ concentration was 1 mM. Each one of three light flasks was switched to dark at the 8th (■—), 14th (■—) and 31st (■—) day, respectively. b: NH_4^+ concentration was 50 μM . One of the light flasks was switched to dark at the 8th day (□—). Another one was switched to dark at 31st day (□—).

the illumination period (Fig. 4-a). However, it appeared that the production rates of NO_2^- in these flasks finally reached the same level as those in the dark flasks. In the case of 50 μM NH_4^+ , no significant production of NO_2^- was found in the light flask up to the 123rd day (Fig. 4-b). One of the light flasks was switched to dark on the 8th day and another one on the 31st day. In the flask switched to dark on the 8th day, NO_2^- was gradually produced after the 47th day. In the flask switched to dark on the 31st day, NO_2^- production began after the 110th day.

Table 5. The change in direct counts of H-1 cells during illumination in the presence of NH_4^+ .

Illumination period (days)	NH_4^+	Light flask		Dark flask	
		50 μM	1 mM	50 μM	1 mM
2		1.1×10^5	1.2×10^5	1.2×10^5	1.2×10^5
6		1.2×10^5	1.1×10^5	1.6×10^5	5.5×10^5
10		1.2×10^5	1.6×10^5	2.1×10^5	5.1×10^5
14		1.4×10^5	2.1×10^5	3.0×10^5	5.8×10^5

Units are cells/ml.

Table 6. Effect of illumination on H-1: the delaytime in the presence of NH_4^+ .

Concentration of NH_4^+	Illumination period (days)		
	8	14	31
50 μM	65	— ^a	103
1 mM	1	4	19

^a Not determined.
Units are days.

Fig. 5. Effect of illumination on O-1 in the presence of NO_2^- .

Time courses of NO_2^- consumption by the growth of O-1 in the light (dashed line) and the dark (solid line) flasks. Arrows indicate the times at which each light flask was switched to dark (second day, —; 4th day, ———). NO_2^- concentrations; a, 100 μM ; b, 10 μM . One of the dark flasks was switched to light at the second day ($\square$ and $\blacktriangledown$).

The delay times estimated from the figures are shown in Table 6. Compared with the data in Table 1, the delay times in the presence of NH_4^+ were considerably

Table 7. Effect of illumination on O-1: the delay time in the presence of NO_2^- .

Concentration of NO_2^-	Illumination period (days)	
	2	4
10 μM	5	7
100 μM	3	6

Units are days.

shorter than those in the absence of NH_4^+ .

O-1. Figure 5 shows the time courses of NO_2^- consumption by the growth of O-1 in the light and the dark flasks containing 10 μM and 100 μM NO_2^- . In both NO_2^- concentrations of media, O-1 consumed hardly any NO_2^- during the time they were illuminated. Moreover, when the dark flasks were switched to light during incubation, O-1 cells ceased to consume NO_2^- in a few days. The delay times are shown in Table 7. Although NO_2^- consumption rates after switching to dark were slightly lower as shown in Fig. 5, these delay times were consistent with those in the absence of NO_2^- .

DISCUSSION

It is known that nitrifying bacteria (NH_4^+ -oxidizing bacteria and NO_2^- -oxidizing bacteria) experience photoinhibition. MÜLLER-NEUGLÜCK and ENGEL (1) reported that *Nitrobacter winogradsky* was reversibly inactivated by a relatively low light intensity (4,900 lx) and that illumination for 28 days had not a lethal but a static effect on the growth of *N. winogradsky*. SCHÖN and ENGEL (2) suggested that *Nitrosomonas europaea* cells were killed outright when they were illuminated for a long period with a sufficient intensity of light. In this study, also, it was found that NH_4^+ -oxidizing bacteria (H-1) and NO_2^- -oxidizing bacteria (O-1), isolated from Lake Kizaki, were inhibited by illumination with cool-white fluorescent light (about 6,000 lx). HOOPER and TERRY (7) found that *Nitrosomonas europaea* was protected from photoinhibition in the presence of NH_4^+ and that the protection was more effective at higher concentration of NH_4^+ . In the present work, also, H-1 were protected from photoinhibition by the presence of NH_4^+ . Moreover, it appeared that a higher concentration of NH_4^+ (1 mM) was more effective in the recovery from photoinhibition (Tables 1 and 6). However, in the experiments involving illumination in the presence of NH_4^+ , chelated iron, a component of growth medium, was also present. Since the chelated iron has a protective effect (7), a part of the protection from photoinhibition observed in the presence of NH_4^+ may be due to the chelated iron. In O-1, the protection from photoinhibition by NO_2^- and the effectiveness of NO_2^- in the recovery from photoinhibition were not so clear.

Fig. 6. The relationship between the delay time and the illumination period. H-1: ○, 1 mM NH₄⁺; ●, 50 μM NH₄⁺. O-1: □, 100 μM NO₂⁻; ■, 10 μM NO₂⁻.

In order to compare the inhibitory effect of illumination in the absence of substrate on H-1 and O-1, the relationship between the illumination period and the delay time is shown in Fig. 6. ULKEN (11) found that the *Nitrobacter* cell was more sensitive to light than the *Nitrosomonas* cell. However, in this study, although NO₂⁻-oxidizing bacteria were more sensitive to light than NH₄⁺-oxidizing bacteria at least in the short illumination period, the inhibitory effect of illumination for a long time was larger on NH₄⁺-oxidizing bacteria than on NO₂⁻-oxidizing bacteria (Fig. 6). This may be due to the decrease of viable H-1 cells. In fact, 10 to 20 days illumination in the absence of NH₄⁺ had a lethal effect on H-1, as described in the RESULTS section. It was examined whether the photoinhibition of nitrifying bacteria (H-1 and O-1) was due to the lethal or the bacteriostatic effect of light. Assuming that light has only a lethal effect on nitrifying bacteria, the delay time (shown in Tables 1, 4, 6 and 7) may be the result of a decrease in bacterial cell numbers in the inocula due to the death of bacteria. The following equation is derived from this assumption,

$$N_i = N_0 e^{\mu \times T}, \text{ or } N_0 = N_i e^{-\mu \times T},$$

where N_i is the apparent inoculum size, N_0 is the inoculum size effective enough to cause the delay time T , and μ is the specific growth rate. In the experiments on the presence of substrate, N_i is the bacterial density at the time the flask was switched to dark.

In the cases of 1 mM NH₄⁺ with H-1, N_i and N_0 are shown in Table 8. μ , calculated from the equation $\mu = 0.58 \times S / (0.2 + S)$ as shown in the previous paper (9), is 0.48 day⁻¹. In both experiments in the absence and presence of NH₄⁺, N_0 decreased as the illumination period increased. When the cells of H-1 were illuminated for 10 days without NH₄⁺, the effective inoculum size was very low. The specific growth rate of the cells illuminated for 10 days was lower than

Table 8. Effect of illumination on H-1: apparent inoculum size (N_i), effective inoculum size (N_o) and delay time in 1 mM NH_4^+ .

Illumination period (days)	N_i (cells/ml)	N_o (cells/ml)	Delay time (days)
In the absence of NH_4^+			
3	1.1×10^5	8.3×10^4	0.3
5	1.1×10^5	5.6×10^4	1.2
7	9.7×10^4	2.6×10^3	7.4
10	9.7×10^4	1.7×10^{-4} 4.9×10^{-3a}	42
In the presence of NH_4^+			
8	1.3×10^5b	8.1×10^4	1
14	2.1×10^5	2.9×10^4	4
31	2.3×10^5b	2.8×10^4	19

^a When $\mu=0.40$ was used for calculation.^b Estimated values from the data shown in Fig. 4 and Table 5.Table 9. Effect of illumination on H-1: observed and calculated delay time in 50 μM NH_4^+ .

Illumination period (days)	Delay time (days)	
	Observed	Calculated
In the absence of NH_4^+		
3	2.0	2
5	26	5
7	>120	30
10	>350	170
		140 ^a
In the presence of NH_4^+		
8	65	4
31	103	75

^a See Table 8.

that of the cells illuminated for 1 to 7 days. The μ of the cells illuminated for 10 days was estimated from the change in NO_2^- concentration to be about 0.4 day^{-1} . Even if this is so, the number of cells inoculated into the flask with 100 ml medium on the 10th day was less than one.

The delay times in the case of 50 μM NH_4^+ are calculated by the following equation,

$$t = \frac{\mu}{\mu'} \times T,$$

where $\mu' (=0.12 \text{ day}^{-1})$ is the specific growth rate of H-1 in 50 μM NH_4^+ and t is the calculated delay time in the case of 50 μM NH_4^+ . The observed and calculated delay times are shown in Table 9. The observed delay times were considerably

Table 10. Effect of illumination on O-1: apparent inoculum size (N_i), effective inoculum size (N_o) and delay time in $100 \mu\text{M NO}_2^-$.

Illumination period (days)	N_i (cells/ml)	N_o (cells/ml)	Delay time (days)
In the absence of NO_2^-			
1	1.1×10^{4a}	1.3×10^3	3.1
2	1.1×10^{4a}	8.3×10^2	3.7
3	1.1×10^{4a}	9.5×10^2	3.5
5	1.1×10^{4a}	5.8×10^2	4.2
7	1.1×10^{4a}	1.8×10^2	5.9
10	1.1×10^{4a}	3.0×10^{-1}	15
In the presence of NO_2^-			
2	1.1×10^{4b}	1.3×10^3	3
4	1.1×10^{4b}	1.6×10^2	6

^a Direct count at the initiation of the experiment was used.^b N_i was assumed to be equal to the initial value, since hardly any NO_2^- was consumed during illumination.Table 11. Effect of illumination on O-1: observed and calculated delay time in $10 \mu\text{M NO}_2^-$.

Illumination period (days)	Delay time (days)	
	Observed	Calculated
In the absence of NO_2^-		
1	5.2	7
2	5.9	8
3	6.5	8
5	8.3	9
7	9.6	13
10	22	33
In the presence of NO_2^-		
2	5	7
4	7	13

longer than the calculated ones. As shown in Fig. 4, the NO_2^- production rates by the cells illuminated for over 14 days with 1 mM NH_4^+ were negligible. If only the lethal effect of light was considered, there would be no viable cells in these flasks after the 14th day. However, the NO_2^- concentration in those flasks which had been placed in the dark increased at almost the same rates as that in the dark flasks, although delays were observed. The above observations suggest that light may have not only a lethal effect but also a bacteriostatic effect on H-1.

In $100 \mu\text{M NO}_2^-$ and O-1, N_i and N_o are shown in Table 10. μ is 0.70 day^{-1} . In the absence of NO_2^- , N_o for 1 to 3 days illumination was about one-tenth of N_i . Then N_o decreased with the illumination period and reached a low level ($3.0 \times 10^{-1} \text{ cells/ml}$). From the delay times in $100 \mu\text{M NO}_2^-$, those in $10 \mu\text{M NO}_2^-$

($\mu' = 0.32 \text{ day}^{-1}$) were calculated (Table 11), as described for H-1. In contrast with H-1, the observed delay times were nearly equal or slightly shorter than the calculated ones. This suggests that light might have a mainly lethal effect on O-1. However, by the lethal effect, it is not possible to explain why the prolongation in the delay time with increased illumination period was not so large, despite the considerable inhibition after only one day of illumination. The photoinhibition of O-1 cells may be reversible and the recovery from photoinhibition may require the dark condition for three to four days, as long as the illumination period is short. Thereafter, the inhibition may become irreversible, or lethal. However, in the case of 10 days illumination in the absence of NO_2^- , the observed delay time was only two-thirds of the calculated one. This indicates that the effect of light on O-1 is bacteriostatic rather than lethal.

In natural aquatic environments, the light condition may affect the nitrification process. OLSON (4) suggested that the differential photoinhibition of marine nitrifying bacteria contributed to the formation of the primary nitrite maximum in the ocean. In Lake Kizaki, active nitrification occurred in early summer (8). In the course of our study on the trigger for this phenomenon in Lake Kizaki, it was suggested that nitrifying bacteria attached to rapidly sinking particles were inactive (submitted to Arch. Hydrobiol.). This inactivation may be due to the exposure to sunlight in the euphotic zone of the lake. HERRIGAN et al. (6) suggested that photoinhibited nitrifying bacteria may recover when they are dispersed to depths with sufficiently low light levels. In Lake Kizaki during June to August, NH_4^+ was oxidized to NO_3^- without the accumulation of NO_2^- and the NO_2^- oxidation rates were greater than the NH_4^+ oxidation rates (8). This suggests that the inactive NO_2^- -oxidizing bacteria during the circulation period (March to early April) recovers earlier than the NH_4^+ -oxidizing bacteria. In this study, it was suggested that if the nitrifying bacteria were illuminated by sufficient light, the NO_2^- -oxidizing bacteria recovered earlier than the NH_4^+ -oxidizing bacteria. Therefore, a possible mechanism for the active nitrification in the hypolimnion of Lake Kizaki in early summer may involve the recovery of nitrifying bacteria from the photoinhibition suffered during the circulation period of lake. The studies on the role of light in nitrification in Lake Kizaki are ongoing.

In this study, it was indicated that NH_4^+ -oxidizing bacteria H-1 (probably *Nitrosospira* sp.) and NO_2^- -oxidizing bacteria O-1 (probably *Nitrobacter* sp.) isolated from Lake Kizaki were inhibited by light (about 5% of full sunlight) and that the photoinhibitory effect was not only lethal but also bacteriostatic. Photoinhibited H-1 and O-1 cells recovered after several to tens of days in the dark.

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