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Effect of CO and light on ammonium and nitrite oxidation by chemolithotrophic bacteria

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ABSTRACT: The effects of CO and light on the ammonium oxidizing bacterium *Nitrosomonas cryotolerans* and the nitrite oxidizing bacterium *Nitrobacter* sp. Nb297 were investigated. Ammonium oxidation was inhibited by CO concentrations ranging from 2 n*M* to 11.4 μ*M*. CO inhibition was cell density dependent and increased if the cells had been either previously starved or deprived of iron. Ammonium oxidation was inhibited by 63 % at intensities as low as 5 W m^{-2} of artificial light. *Nitrobacter* sp. Nb297, on the other hand, was able to tolerate CO concentrations as high as 400 μ*M*. Artificial light (25 W m^{-2}) slightly reduced nitrite oxidation while sunlight (628 W m^{-2}) decreased activity to ca 20 % of its original value. In addition, CO and CH_4 oxidation by *N. cryotolerans* and natural assemblages of bacteria were inhibited by light. It is apparent from these studies that the mechanisms of light and CO inhibition are different for the NH_4^+ and NO_2^- oxidizers examined. Characterization of light and CO responses by marine nitrifying bacteria is critical in any attempt to explain the formation of the subsurface primary nitrite maximum.

INTRODUCTION

In marine environments, the nitrogen cycle is largely controlled by microorganisms and can be regulated by different environmental factors (Yoshioka & Saijo 1984, Ward 1986). One of these is light, which has been known for some time to inhibit the activity of chemolithotrophic ammonium oxidizing and nitrite oxidizing bacteria (Müller-Neuglück & Engel 1961, Hooper & Terry 1974, Horrigan et al. 1981, Olson 1981a, b, Yoshioka & Saijo 1984, 1985, Shears & Wood 1985, Ward 1985). Furthermore, it has been suggested that a differential light inhibition between the ammonium and nitrite oxidizers is associated with the formation of the primary nitrite maximum (Olson 1981b). Another factor affecting nitrifying bacteria is CO (Hooper & Terry 1973, Suzuki et al. 1976, Jones & Morita 1984a, b). But, since CO can be photochemically formed (Conrad & Seiler 1980, Conrad et al. 1982, Conrad & Seiler 1985), it has been difficult to establish the exact mechanism for light/CO inhibition in these microorganisms.

This study investigated CO and light effects on NH_4^+ and NO_2^- oxidation by 2 chemolithotrophic bacteria, using different substrate concentrations. Starved cells and iron-deprived cells of *Nitrosomonas cryotolerans* were also included to test the possible role of cytochrome c_{554} in CO detoxification by ammonium oxidizers (Tsang & Suzuki 1982). In addition, the extent to which CO and light affect CO and CH_4 oxidation by *N. cryotolerans* was examined.

MATERIALS AND METHODS

Cultures. The marine ammonium oxidizing bacterium *Nitrosomonas cryotolerans* (Jones et al. 1988), and the nitrite oxidizing bacterium *Nitrobacter* sp. Nb297 (provided by S. W. Watson) were grown in the dark, in 4 l chemostat culture units (0.14 d^{-1} dilution rate) equipped with automatic pH control that maintained a pH of 7.8 ± 0.05 by addition of 5 % K_2CO_3. Ammonium oxidizer medium contained 0.011 *M* of NH_4^+ as $(NH_4)_2SO_4$ and salinity was adjusted to 30 ‰ using Instant Ocean Synthetic Sea Salts (Aquarium Systems, Inc.; Jones & Hood 1980). Nitrite oxidizers were cultured in the same manner in the medium of Watson & Waterbury (1971). The concentration of NO_2^- as $NaNO_2$ was 0.029 *M*. Cultures were vigorously aerated, agitated by a magnetic stirring bar, and kept at a constant temperature of 25 °C.

Preparation of standard inoculum. Cells were har-

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vested from 250 ml of chemostat culture by centrifugation (6000 × *g*; 15 min; 5 °C), washed twice with filtered (Whatman GF/C, 1.2 µm) Sargasso Sea water (SSW), containing 0.02 *M* N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES) pH 7.8, and then resuspended in 10 ml of SSW. Cell density was determined using a Petroff-Hauser counting chamber. From this suspension, 0.1 ml was inoculated into a 60 ml sterile serum bottle containing 25 ml of SSW. Final cell density was ca 5×10^5 cells ml^{-1} for the ammonium oxidizers and 1.1×10^6 cells ml^{-1} for the nitrite oxidizers. The SSW used for the preparation of the cell suspension had been previously purged for 15 min with CH_4^- and CO-free air in order to remove dissolved CO and CH_4 (Jones & Morita 1985). The SSW was low enough in nutrients that it did not interfere with the results (< 20 n*M* NH_4^+; < 10 n*M* NO_2^-; < 20 n*M* NO_3^-). Neither ammonium nor nitrite oxidation activity was detected in SSW.

All experiments described below were replicated and samples within each experiment were performed in triplicate. All data points reported are mean values of the replicates in individual experiments and fell within a 95 % confidence interval.

Effects of CO on NH_4^+ and NO_2^- oxidation. After inoculation, $(NH_4)_2SO_4$ was added to a final concentration of 10 µ*M* NH_4^+, bottles were sealed with serum stoppers and CO was injected into the headspace to give final CO concentrations ranging from 0 to 11.4 µ*M* CO (Schmidt 1979). These bottles were incubated in the dark at 25 °C on a rotatory shaker at 150 rpm. Incubation was stopped when ca 10 % of the added NH_4^+ was consumed (2 to 3 h). The bottles were then assayed for nitrite (Bendschneider & Robinson 1952). Nitrite production was used as an index of ammonium oxidation.

To test the effects of CO on NO_2^- oxidation, bottles were prepared as above, using 2 µ*M* of NO_2^- as $NaNO_2$ instead of NH_4^+. Eight additional CO concentrations from 22.8 to 457 µ*M* were used, since no inhibition was observed with 11.4 µ*M*. Nitrite oxidation was assayed by measuring its disappearance assuming that NO_2^- was converted to NO_3^-.

The effect of CO on *Nitrosomonas cryotolerans* was examined using cell densities from 4×10^4 to 1×10^6 ml^{-1} and NH_4^+ concentrations from 1 to 100 µ*M*.

To test the effect of CO on substrate-starved *Nitrosomonas cryotolerans*, the washed cells (standard inoculum) were starved in filter-sterilized (0.2 µm) suspension medium (SSW). Ammonium oxidation measurements were taken at different intervals for a period of 21 d. The effect of CO was also examined on cells grown under iron deprivation. Cells were grown in medium without added iron and containing 10^{-5} *M* filter-sterilized (0.2 µm) human serum transferrin (TF).

Effects of light on NH_4^+ and NO_2^- oxidation. Standard inocula of *Nitrosomonas cryotolerans* and *Nitrobacter* sp. were inoculated into screw-cap test tubes (25 × 117 mm) containing 25 ml of SSW. Substrate concentrations were 1, 10 or 100 µ*M* of NH_4^+ for the ammonium oxidizer and 2 µ*M* of NO_2^- for the nitrite oxidizer. Tubes were incubated in a water bath at 25 °C under a daylight fluorescent light (Philips F30T12/DS/R). The temperature-controlled water bath consisted of a flat-back painted box with circulating distilled water Light intensity was measured with a LI-COR (model 1800) calibrated spectroradiometer. Irradiance was ca 25 W m^{-2} at the surface. Tubes lay horizontally 1 cm under the water surface in a rack placed in the water bath. Neutral density screens of different mesh sizes (8 to 70 % transmission) were placed over the bottles during incubation, in order to obtain different light intensities. Control tubes were wrapped in aluminum foil. In order to compare the effects of sunlight on NH_4^+ or NO_2-oxidation, the water bath was placed under full sunlight. The sunlight irradiance was ca 628 W m^{-2}.

Measurement of photochemically formed CO. Tubes prepared as described above but without added NH_4^+ or NO_2^- were exposed to artificial light and sunlight for 4 and 2 h, respectively. After incubation the sample was passed through a gas stripper and CO was measured by gas chromatography (Butler et al. 1987).

Effects of light on CO and CH_4 oxidation. Seawater samples were collected from Biscayne Bay, Miami, Florida, USA (10 cm below the surface) in 4 l polypropylene bottles. Sampling was conducted at night, 2 h after sunset. The water was purged overnight with CO- and CH_4-free air to remove dissolved CO and CH_4; 25 ml were dispensed into each screw-cap test tube (25 × 125 mm), tubes were sealed with teflon/silicon septa, capped and 0.5 ml of ^{14}CO diluted in nitrogen (0.5 µCi ml^{-1}; specific activity, 56 mCi $mmole^{-1}$; Amersham Corp.) or 1 ml of $^{14}CH_4$ diluted in nitrogen (1 µCi ml^{-1}; specific activity, 59 mCi $mmole^{-1}$; Amersham Corp.) was injected into the headspace (Jones & Morita 1983b). Final ^{14}CO and $^{14}CH_4$ concentrations in solution were ca 3 n*M* and 14 n*M*, respectively. Tubes were then incubated for 3 h with ^{14}CO or for 24 h with $^{14}CH_4$ in a water bath at 25 °C under different light intensities. Dark controls were run with each experiment. The light sources and light intensities used were identical to those described above. After incubation, the reaction was terminated by the addition of 0.5 ml of 5.0 *N* NaOH with a syringe through the septa. The tubes were then shaken for 1 h at room temperature to allow the $^{14}CO_2$ to be absorbed into solution. Subsequently, the caps and septa were removed and each sample was transferred to 60 ml serum bottles. The bottles were shaken an additional 30 min in an exhaust hood to remove the remaining labeled gases. $^{14}CO_2$ was released by adding 1 ml of 10

N H_2SO_4 to the sample, and trapped on Whatman chromatography paper saturated with β-phenylethylamine. Radioactivity was measured in a Beckman scintillation counter (LS 3801) using omnifluor as scintillation cocktail (Griffiths et al. 1982).

To test the effects of light on CO and CH_4 oxidation by pure cultures of an ammonium oxidizer, 0.1 ml of the standard inoculum of *Nitrosomonas cryotolerans* was inoculated into screw-cap test tubes containing 25 ml of SSW and then incubated and analyzed as described in the previous section. Since nitrite oxidizers do not oxidize CO or CH_4 (Jones & Morita 1983b), these organisms were not tested.

RESULTS

Effect of CO on NH_4^+ an NO_2^- oxidation

Ammonium oxidation by *Nitrosomonas cryotolerans* was inhibited by CO, but the degree of CO inhibition could be reversed by increasing the concentration of ammonium in the medium (Fig. 1). For all CO concentrations tested, inhibition of ammonium oxidation was greatest at the lowest NH_4^+ concentration (1 μ*M*). Cell density influenced the CO inhibition of ammonium oxidation independent of the substrate concentration present (10 or 100 μM NH_4^+). At lower cell densities a greater inhibitory effect of CO was exhibited (Fig. 2).

Ammonium oxidizers that were starved for 3 wk showed greater CO inhibition than those starved for shorter periods (Fig. 3). In fact, CO concentrations above 2.3 μ*M* completely inhibited ammonium oxidation in cells starved for 3 wk (Fig. 3); while the cells starved for shorter intervals were still (5 to 15 %) active at this concentration.

Nitrosomonas cryotolerans grown under iron-deficient conditions was less active (25 % of iron-containing culture) and more inhibited by CO than cells grown in iron-containing medium (Fig. 4). There was a significant difference ($p \leq 0.025$, using the t-test) between the ammonium oxidation rates at all CO concentrations.

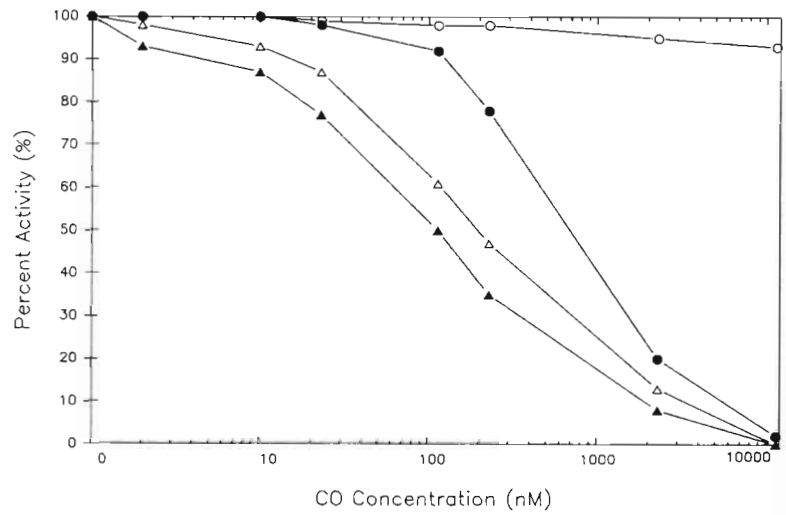

Fig. 1. *Nitrosomonas cryotolerans*. CO effects on NH_4^+ oxidation. (○) 1000 μ*M* NH_4^+; (●) 100 μ*M* NH_4^+; (△) 10 μ*M* NH_4^+; (▲) 1 μ*M* NH_4^+ NO_2^- production rates by controls (100 % activity) were 21.7 μmol l^{-1} h^{-1}, 8.9 μmol l^{-1} h^{-1}, 0.2 μmol l^{-1} h^{-1}, and 0.04 μmol l^{-1} h^{-1}, respectively. Activities are expressed at a density of 1×10^6 cells ml^{-1}

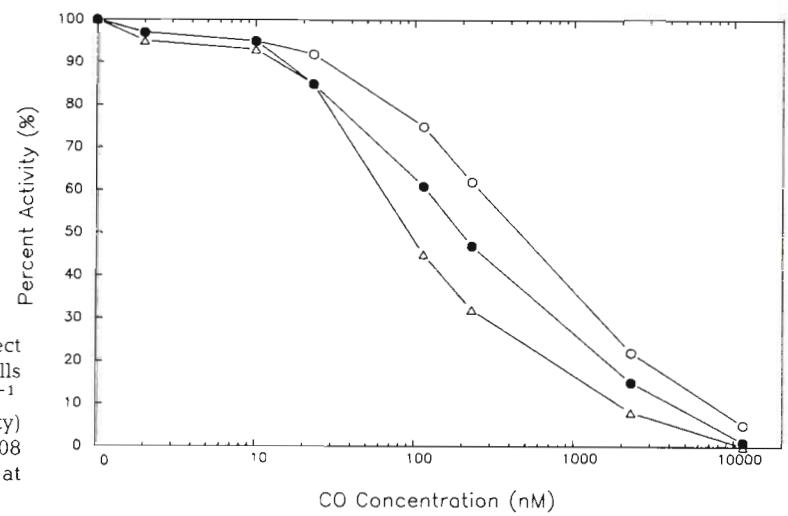

Fig. 2. *Nitrosomonas cryotolerans*. Cell density effect on CO inhibition with 10 μ*M* NH_4^+. (○) 1×10^6 cells ml^{-1}; (●) 5×10^5 cells ml^{-1}; (△) 4×10^4 cells ml^{-1} NO_2^- production rates by controls (100 % activity) were 0.17 μmol l^{-1} h^{-1}, 0.15 μmol l^{-1} h^{-1} and 0.08 μmol l^{-1} h^{-1}, respectively. Activities are expressed at a density of 1×10^6 cells ml^{-1}

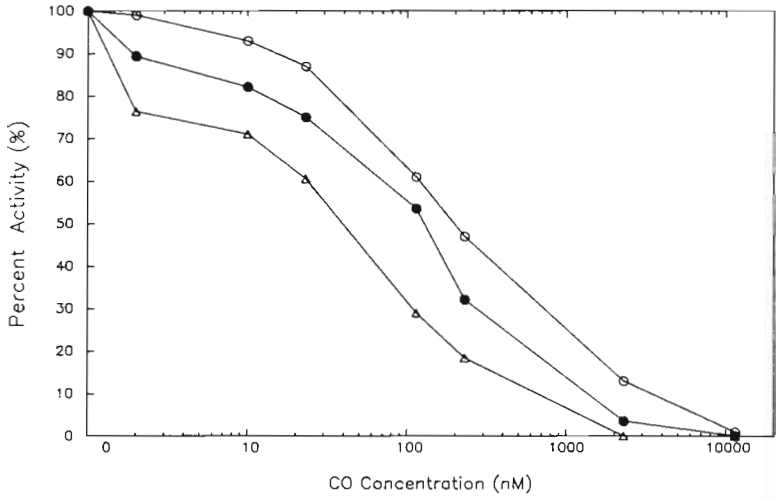

Fig. 3. *Nitrosomonas cryotolerans*. CO effects on NH_4^+ oxidation by substrate starved cells with 10 μM NH_4^+. (○) After 0 d; (●) after 1 wk; (△) after 3 wk of starvation. NO_2^- production rates by controls (100 % activity) were 0.2 μmol l^{-1} h^{-1}, 0.14 μmol l^{-1} h^{-1}, and 0.09 μmol l^{-1} h^{-1}, respectively. Activities are expressed at a density of 1×10^6 cells ml^{-1}

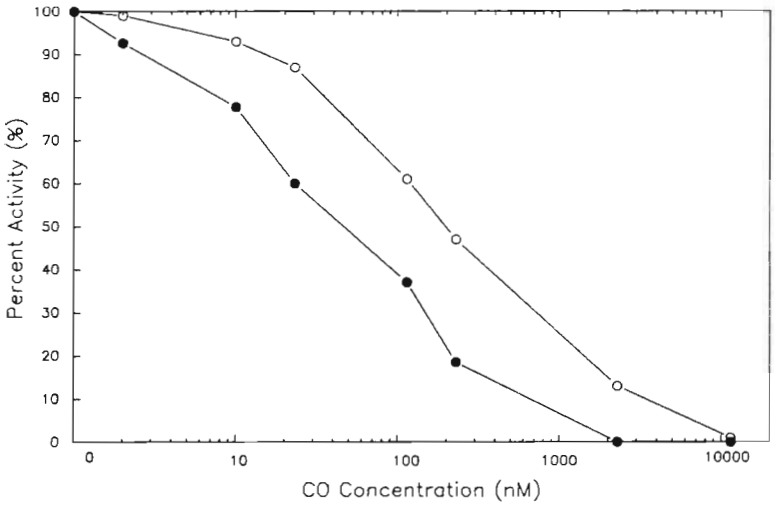

Fig. 4. *Nitrosomonas cryotolerans*. CO effect on NH_4^+ oxidation by cells grown in iron-free (TF) medium with 10 μM NH_4^+ (○) Cells grown in iron-containing medium; (●) cells grown in iron-free medium (TF). NO_2^- production rates by controls (100 % activity) were 0.20 μmol l^{-1} h^{-1} and 0.05 μmol l^{-1} h^{-1}, respectively. Activities are expressed at a density of 1×10^6 cells ml^{-1}

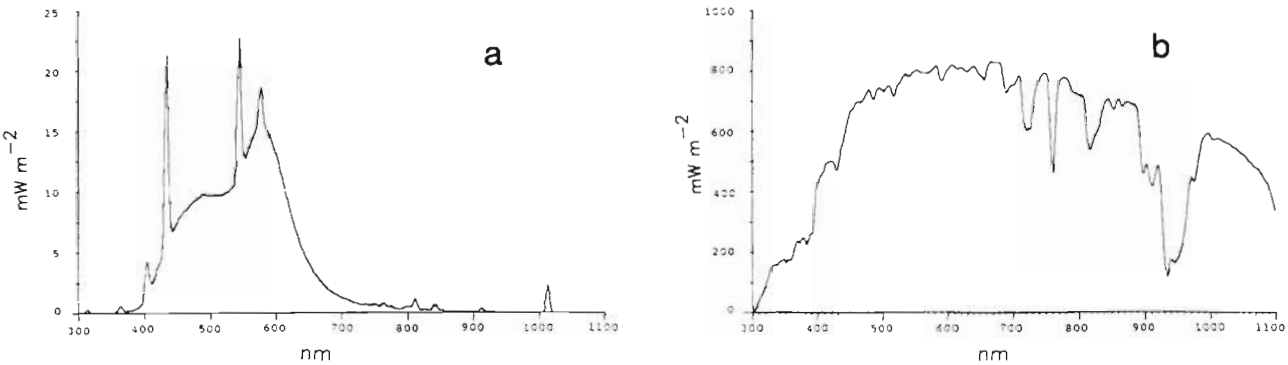

Fig. 5. Light spectra. (a) Artificial light (Philips F30T12/DS/R). (b) Sunlight

Nitrite oxidation by *Nitrobacter* sp. Nb297 was clearly insensitive to most of the CO concentrations examined: only 10 % inhibition was observed at 400 μM CO (results not shown).

Effects of light on NH_4^+ and NO_2^- oxidation

The intensity and spectral quality of sunlight (628 W m^{-2}) and artificial light (25 W m^{-2}) used were signifi-

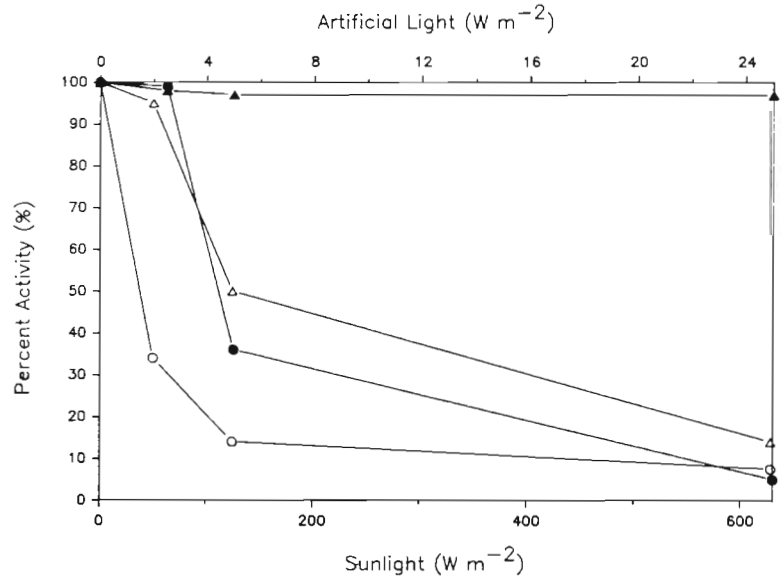

Fig. 6. *Nitrosomonas cryotolerans* and *Nitrobacter* sp. Effect of light on NH_4^+ and NO_2^- oxidation. *Nitrosomonas:* sunlight (○), artificial light (●); *Nitrobacter:* sunlight (△), artificial light (▲). NO_2^- production rates by controls (100 % activity) was 0.40 μmol l^{-1} h^{-1} NO_2^- oxidation rate by controls (100 % activity) was 0.11 μmol l^{-1} h^{-1} Substrate concentrations were 10 μ*M* NH_4^+ and 2 μ*M* NO_2^-. Activities of both are expressed at a density of 1 × 10^6 cells ml^{-1}

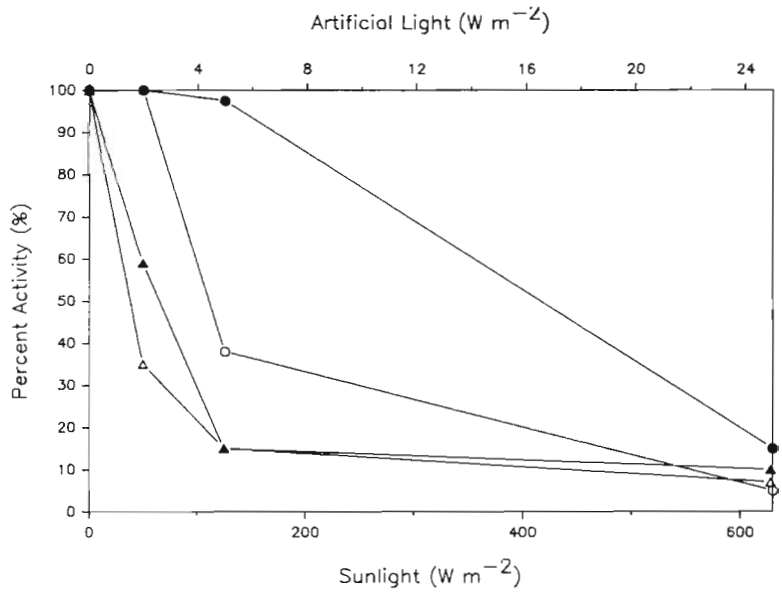

Fig. 7. *Nitrosomonas cryotolerans*. Effects of NH_4^+ concentrations on photoinhibition. 100 μ*M* NH_4^+: sunlight (○), artificial light (●); 10 μ*M* NH_4^+: sunlight (△), artificial light (▲). NO_2^- production rates by controls (100 % activity) were 14.42 μmol l^{-1} h^{-1} and 0.34 μmol l^{-1} h^{-1}, respectively. Activities are expressed at a density of 1 × 10^6 cells ml^{-1}

cantly different (Fig. 5a, b). Despite these differences it appears that sunlight had a greater inhibitory effect on both ammonium and nitrite oxidation (Fig. 6).

Overall, *Nitrobacter* sp. Nb297 showed more resistance to both types of light; artificial light reduced NO_2^- oxidation only slightly. Sunlight at low intensities (50 W $m^{-2)}$ produced only 5 % inhibition whereas 125 W m^{-2} showed a sharp decrease to 49 % activity. However, nitrite oxidation activity, even under full sunlight (626 W m^{-2}) did not drop below 20 % of the control; but since these are not rate measurements, this activity may not be sustained. With *Nitrosomonas cryotolerans* inhibition was observed with both types of light at most light levels examined (Fig. 6).

Ammonium concentration affected the photoinhibition caused by different light levels (Fig. 7). With 10 μ*M* NH_4^+ at a light intensity of 5 W m^{-2}, the activity sharply declined to 37 %. With 100 μ*M* of NH_4^+ at the same light intensity, ammonium oxidation activity decreased only slightly (3 %). Using 25 W m^{-2} of artificial light and 10 μ*M* NH_4^+ only 5 % of the original activity remained; whereas with 100 μ*M* NH_4^+, the activity only dropped to 53 %. With sunlight, ammonium oxidation rates at all light levels above 50 W m^{-2} and with either 10 or 100 μ*M* NH_4^+ were considerably lower.

Nitrosomonas cryotolerans grown under iron-deficient conditions was slightly more sensitive (10 to 15 %) to the inhibitory effects of artificial light, when compared to cells grown in the iron-containing medium. However, the ammonium oxidation rates were significantly different ($p \leq 0.025$, t-test).

Table 1. Inhibition by artificial light of CO and CH_4 oxidation by bacteria in Biscayne Bay water samples

Light intensity ($W\ m^{-2}$)	nmol $l^{-1}\ h^{-1}$ oxidized to $^{14}CO_2$ CO	(% Activity)	CH_4	(% Activity)
0	250	(100)	0.370	(100)
2	230	(89)	0.295	(79)
5	210	(84)	0.285	(77)
7	190	(77)	0.280	(76)

Measurement of photochemically formed CO

In full artificial light (25 W m^{-2}) and sunlight (628 W m^{-2}) the amount of CO formed in SSW without added cells was 7.0 n*M* after 4 h and 11.5 n*M* after 2 h respectively.

Effects of light on CO and CH_4 oxidation

CO and CH_4 oxidation rates by natural populations of bacteria from Biscayne Bay water decreased slightly as the level of artificial light increased (Table 1). At the highest artificial light intensity examined (7 W m^{-2}) the oxidation rate for both CO and CH_4 decreased to 77 %. With pure cultures of *Nitrosomonas cryotolerans* the effect of light on both CH_4 and CO oxidation was more pronounced (Table 2). Methane oxidation was more sensitive than CO oxidation to the inhibitory effects of both sun and artificial light.

DISCUSSION

Our results confirm previous observations in which CO inhibited ammonium oxidation (Hooper & Terry 1973, Suzuki et al. 1976, Jones & Morita 1984a, b). Further, this study shows the inhibitory effect of CO at concentrations (2 to 100 n*M*) that closely resemble those found in marine ecosystems (Conrad et al. 1982).

Increasing the concentration of NH_4^+ decreased the CO inhibition on ammonium oxidation. Consequently, in the open ocean where NH_4^+ levels are often very low ($\ll 0.5\ \mu M$) (Sharp 1983), in situ CO concentrations could affect ammonium oxidation to a greater extent. In fact, when inhibition experiments were conducted with cells starved for 3 wk (Fig. 3), the inhibitory effect of CO was enhanced.

The inhibitory effect of light on ammonium oxidation has been repeatedly demonstrated (Schön & Engel 1962, Hooper & Terry 1974, Horrigan et al. 1981, Olson 1981b, Yoshioka & Saijo 1984, 1985, Ward 1985). However, in this study, the experiments used light intensities (< 25 W m^{-2}) and exposure lengths different from those used by the other researchers. Interestingly, the strongest inhibition was obtained with 5 W m^{-2} of artificial light or with 50 W m^{-2} of sunlight (Fig. 6). This implies that inhibition is a function of specific wavelengths and their relative energies. But, whether the photoinhibition is due to these specific wavelengths and/or the quantity of light is unknown.

The exact mechanism of CO/light inhibition on NH_4^+ oxidation is not yet understood. It has been suggested that the capability of CO oxidation by *Nitrosomonas* (Jones & Morita 1983b, Jones et al. 1984) and the inhibition caused by CO and light on ammonium oxidation could interact to form the nitrite maximum. This hypothesis is supported by: (1) the photochemical formation of CO in the photic zone of open oceans (Wilson et al. 1970, Conrad et al. 1982, Conrad & Seiler 1985; Table 1), and (2) the inability of very low levels of NH_4^+ in this environment to prevent CO inhibition of ammonium oxidation. In addition, ammonium monooxygenase (AMO) has a low Km for CO, viz. 97 to 222 n*M* (Jones & Morita 1983b), hence CO can still be utilized by AMO at these low (n*M*) concentrations. Under such circumstances, CO can definitely inhibit NH_4^+ oxidation in the marine environment. Below the photic zone, CO levels can drop to 0.05–0.6 n*M* (Swinnwerton et al. 1976, Conrad & Seiler 1980); at these concentrations NH_4^+ oxidation is probably no longer affected (Fig. 1) and NO_2^- production can occur producing the nitrite maximum. Thus, light would indirectly

Table 2. *Nitrosomonas cryotolerans*. Light inhibition of CO and CH_4 oxidation. Oxidation rates expressed as $^{14}CH_4$ or ^{14}CO nmol $l^{-1}\ h^{-1}$ oxidized to $^{14}CO_2$ by *N. cryotolerans* at a density of 1×10^6 cells ml^{-1}. Numbers in parentheses represent percent activities calculated using the controls as 100 % activity

Artificial Light			Sunlight		
Intensity ($W\ m^{-2}$)	Oxidation Rate CO (% Act.)	CH_4 (% Act.)	Intensity ($W\ m^{-2}$)	Oxidation Rate CO (% Act.)	CH_4 (% Act.)
0	430 (100)	5.15 (100)	0	510 (100)	3.3 (100)
2	280 (65)	2.75 (53)	50	360 (70)	2.1 (64)
5	200 (47)	1.20 (23)	126	90 (18)	1.2 (36)
7	150 (35)	0.95 (18)	170	80 (16)	0.7 (21)

photoinhibit NH_4^+ oxidation in the photic zone through the photochemical production of CO.

The CO intensitivity observed in the NO_2^- oxidizer *Nitrobacter* Nb297 is consistent with the response of other aerobic bacteria that do not have CO oxidizing capabilities (Cypionka & Meyers 1982). Our experiments show that the NO_2^- oxidizer was more resistant to light inhibition than the NH_4^+ oxidizer. These results do not agree with previous findings of Olson (1981b) and Horrigan et al. (1981), where nitrite oxidation by natural populations only took place in the dark. The contrasting results may be due to the fact that the aforementioned studies used natural populations and diverse light treatments whereas these experiments used pure cultures and light from a daylight fluorescent bulb. Additional studies with other representative species of ammonium and nitrite oxidizers under standardized conditions are necessary in order to establish comparisons.

When the cells were exposed to different levels of artificial light or sunlight, by the use of neutral density screens, it was assumed that the quantity but not the quality of light was changed. Radiation reached the samples indiscriminately and not at particular wavelengths, as is the case at different depths in marine environments. Another problem when the cells were exposed to direct sunlight was UV radiation (< 400 nm, high energy). UV light is not normally a problem for nitrifiers in their natural environments, and exposure to it can be detrimental to almost any type of cell (Bridges 1976).

These experiments demonstrate that lower cell densities exhibit greater inhibition by CO (Fig. 2). Since CO is known to act as a competitive substrate for AMO (Jones & Morita 1983b), it follows that if fewer cells are present to oxidize CO to CO_2, the inhibition caused by CO will be greater.

If on the other hand, CO binds to the cytochrome system of these cells, it could be anticipated to result in greater inhibition at lower cell densities. Cytochromes are fundamental in the oxidation of NH_4^+ in that reduced cytochromes are the electron donors required for the hydroxylation of NH_4^+ (Suzuki et al. 1974, Tsang & Suzuki 1982) and cytochrome c_{554} from cell-free extracts of *Nitrosomonas* sp. is readily oxidized by CO (Tsang & Suzuki 1982). As expected, *N. cryotolerans* grown in an iron-deficient medium was more susceptible to CO inhibition (Fig. 4). Cytochrome content of this organism needs to be determined, in order to verify that TF effectively decreased cytochrome synthesis as it does in *Vibrio harveyi* (Makemson & Hastings 1986).

Jones & Morita (1983a) have shown that *Nitrosomonas* sp. are capable of oxidizing CO and CH_4. The results reported here corroborate their findings, using natural populations (Table 1). Photoinhibition observed with pure cultures of *N. cryotolerans* was greater than with natural populations. The presence of a mixed population and/or other unknown factors existent in the Biscayne Bay water samples may account for these results. The fact that under artificial light greater inhibition was observed for CH_4 oxidation than for CO oxidation is not clearly understood. A tentative explanation could be that as CO is continuously formed during incubation, the cells will tend to oxidize the photochemically formed CO rather than the $^{14}CH_4$ present since the affinity of AMO for CO is greater than for CH_4 (Jones & Morita 1983a, b).

Under full sunlight cell damage due to UV radiation might also be an important factor. This could be supported by the fact that the levels of CO detected for the SSW controls and samples after their exposure to sunlight were almost identical: 11.5 n*M* and 11.4 n*M* respectively. With artificial light, the CO levels were clearly different between the controls and samples: 7.0 n*M* and 2.8 n*M*, respectively. It is likely that under full sunlight the cells lost their CO oxidizing ability. However, interpretation of these results is limited since ^{12}CO is being formed in greater amounts than the ^{14}CO added (3 n*M*).

Nevertheless, these experiments indicate that the mechanisms of light and CO inhibition are different for the ammonium and nitrite oxidizers examined. Hence, if these bacteria are considered as representative of the marine nitrifiers, the occurrence of nitrite maxima in the oceans cannot be explained simply by a differential light and/or CO inhibition between these 2 groups of bacteria. It is suggested, however, that light has an indirect effect on ammonium oxidizers and that the photochemical formation of CO, rather than light itself, inhibits surface water ammonium oxidation. It is also possible that both light and CO have inhibitory effects on the ammonium oxidizers but with different effective sites.

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