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# Photoinhibition of marine nitrifying bacteria. II. Dark recovery after monochromatic or polychromatic irradiation

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ABSTRACT: Nitrifying bacteria ($NH_4^+$ and $NO_2^-$ oxidizers) are capable of recovery from photoinhibition in the dark. After short-term (2 to 4 h) irradiations, significant differences were found between the 2 groups. $NH_4^+$ oxidizers subjected to longer wavelengths (>400 nm; 25 W $m^{-2}$) or polychromatic light (15 W $m^{-2}$) regained activity faster (0.5 to 1 h) than if exposed to shorter wavelengths (<400 nm; 25 W $m^{-2}$) or sunlight (360 to 400 W $m^{-2}$). In contrast, $NO_2^-$ oxidizers only failed to recuperate activity after sunlight and near-UV (300 to 375 nm) treatment. Artificial light (5 to 25 W $m^{-2}$) did not affect nitrite oxidation. Thus, recovery of $NH_4^+$ and $NO_2^-$ oxidizing activities exhibited both dose and wavelength dependencies. These distinct recovery responses imply that nitrogen turnover in aquatic ecosystems depends on a number of factors among which light transmission properties of different water types (i.e. from lakes, rivers, estuaries, coastal marine and oceans) and physiological differences between nitrifying bacteria play significant roles.

KEY WORDS: Recovery · Nitrification · Nitrifying bacteria

## INTRODUCTION

Light has been recognized as a controlling factor for nitrification ($NH_4^+$ and $NO_2^-$ oxidation) for some time (Müller-Neuglück & Engel 1961, Schön & Engel 1962). However, the importance of this in natural ecosystems was not appreciated until recently when its ecological relevance was demonstrated (Olson 1981, Yoshioka & Saijo 1984, Diab & Shilo 1988, Vanzella et al. 1989). In general, there are at least 3 aspects to photoinhibition: intensity, duration, and spectral composition of the incident radiation. Photoinhibition becomes especially important in aquatic environments where light intensity and/or quality can vary depending on the characteristics of the water (dissolved and particulate organic matter, etc.), depth, time of year, latitude, cloud cover, etc. (Jerlov 1968, Craig 1973, Gieskes et al. 1989). In addition, natural waters transmit individual wavelengths only as a percentage of their values at the water surface (Jeffrey 1984). The likelihood of a bacteriostatic rather than a bactericidal light effect on nitrifiers becomes apparent from the ability to detect net nitrification in marine samples subjected to 24 h incubations (natural light cycle) (Ward et al. 1984). Evidence from studies which used natural populations or pure cultures also supports the prospect of dark recovery from photoinhibition (Carlucci et al. 1970, Horrigan et al. 1981, Yoshioka & Saijo 1984, Vanzella et al. 1990). Nevertheless, the various recovery times reported are based on the premise that uncoupling due to light occurs at the second step of nitrification: $NO_2^-$ to $NO_3^-$. Previous work in our laboratory has demonstrated that the first step, $NH_4^+$ to $NO_2^-$, is affected by light to a greater extent (Vanzella et al. 1989). The contrasting results prompted a reevaluation of photosensitivity among nitrifying bacteria (Guerrero & Jones 1996 in this issue) along with the present investigation on recovery in the context of the initial photoinactivation.

This study analyzed recovery times for $NH_4^+$ and $NO_2^-$ oxidation following broadband (artificial and sunlight) and narrowband (monochromatic) light exposure, in an attempt to assess its ecological consequences.

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## MATERIALS AND METHODS

**Microorganisms and growth conditions.** The marine ammonium oxidizing bacteria *Nitrosomonas cryotolerans* (Jones et al. 1988) and *Nitrosococcus oceanus* (ATCC 19707) along with the marine nitrite oxidizing bacteria *Nitrococcus mobilis* (ATCC 25380) and *Nitrobacter* sp. (Nb 297, provided by S. W. Watson) were grown and harvested as described in a companion paper (Guerrero & Jones 1996).

**Light exposure of cells.** Recovery experiments followed irradiations with either monochromatic (1 kW xenon lamp) or polychromatic (cool-white fluorescent, sunlight) light sources. A standard cell suspension inoculated in a continuous flow system was used for monochromatic or sunlight inhibition studies. Continuous cultures (chemostats) were only used in the case of cool-white fluorescent irradiations. The details of each procedure are described by Guerrero & Jones (1996).

**Recovery after monochromatic or polychromatic exposure.** After monochromatic or polychromatic (sunlight) irradiations, using the continuous flow system, 20 ml from the cell suspension was collected from cuvettes (experimental and control), centrifuged (10000 × *g*, 15 min, 5°C), and resuspended in 1 ml of filtered sea water (FSW). From this cell suspension, 0.2 ml was inoculated into duplicate sets of control and experimental 60 ml serum bottles that contained 10 ml of FSW with the substrate of interest (final concentrations: $NH_4^+$, 100 µM; or $NO_2^-$, 2 µM). Final cell densities for $NH_4^+$ and $NO_2^-$ oxidizers were approximately $1 \times 10^6$ cells $ml^{-1}$. Aliquots were collected every hour during the first 6 h and at the end of the experiment (20 to 24 h). The serum bottles were all incubated in the dark on a rotatory shaker at 150 rpm at 25°C.

In the case of nitrite oxidizers, recovery experiments were done only after near-UV and sunlight irradiation, since fluorescent light had no effect (Vanzella et al. 1989, Guerrero & Jones 1996).

**Analytical methods.** Nitrifying activity was evidenced by $NO_2^-$ determinations using the spectrophotometric method of Bendschneider & Robinson (1952). Nitrite production was used as an index of ammonium oxidation and nitrite disappearance as an indication of nitrite oxidation. Activities reported represent a percentage of dark controls. Rates were expressed at a density of $1 \times 10^6$ cells $ml^{-1}$.

The data are the result of 2 sets of experiments. Replicate samples were run for each experiment. Samples were collected in duplicate, and the data points correspond to the mean of the duplicates. Correlation analyses (r) and Student's *t*-tests were used to establish relationships between variables and statistical significance, respectively.

## RESULTS

### Recovery from monochromatic irradiation

Recovery of $NH_4^+$ oxidizing activity after monochromatic illumination proceeded in dose- and wavelength-dependent manners (Figs. 1 & 2). As expected, the longest recovery times were observed after irradiation with shorter wavelengths (i.e. 300 to 375 nm). Cells took as long as 20 h to regain 5 to 10% of their $NH_4^+$ oxidizing activity when previously illuminated with 300 nm light, as opposed to 1 h to restore up to 80% when exposed to 450 nm light. Dose also played a key role in this aspect, since even the shorter wave-

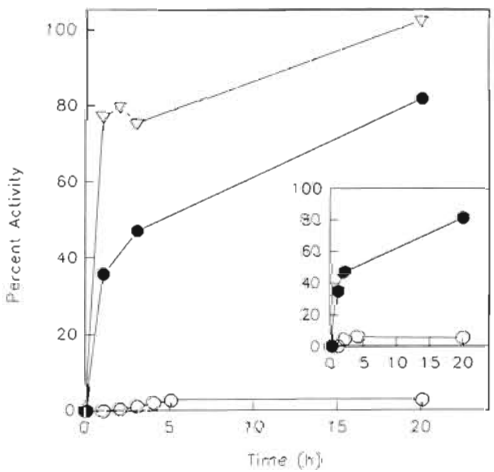


Fig. 1 Recovery as percent activity of *Nitrosomonas cryotolerans* after 2 h of monochromatic irradiation. Inset: Percent activity after 1 h of photoexposure. (○) 300 nm; (●) 400 nm; (▽) 450 nm

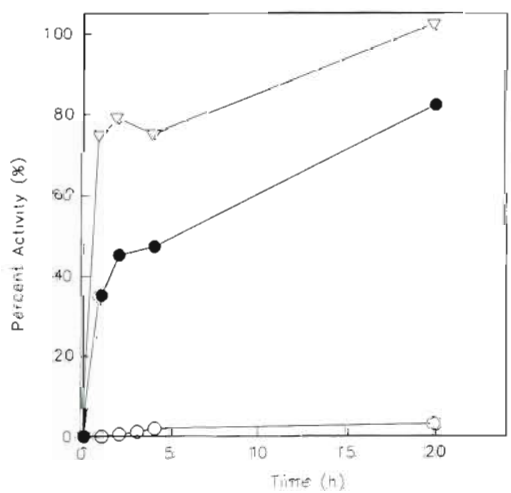


Fig. 2. Recovery as percent activity of *Nitrosococcus oceanus* after 2 h of monochromatic irradiation. (○) 350 nm; (●) 400 nm; (▽) 450 nm

lengths were not as inhibitory if the irradiation periods were decreased (i.e. from 2 to 1 h).

Another factor in looking at recovery times is the initial activity rates in the bacterial suspensions. What appears to be a more rapid recovery of *Nitrosomonas cryotolerans* when compared to *Nitrosococcus oceanus* may be due to the faster rate of ammonium oxidation displayed by *N. cryotolerans* (12 vs 0.82 μM $h^{-1}$; r = 0.93).

Previous work has shown that with monochromatic light, the only effective wavelengths for nitrite oxidizers were in the mid- and near-UV ranges (300 to 375 nm) (Guerrero & Jones 1996); consequently these were the only ones tested. It is interesting to note that as a result of a higher dose (4 h light exposure; Fig. 3), there was an increase in the final nitrite concentration (no recovery), whereas lowering the dose or using longer wavelengths allowed nitrite oxidation to proceed (recover) within 4 h (Figs. 3 & 4). However, when there was recovery, *Nitrococcus mobilis* was favored by its faster rate of nitrite oxidation as compared to *Nitrobacter* sp. (0.35 vs 0.02 μM $h^{-1}$; r = 0.98).

### Recovery from sunlight

Typically, ammonium oxidizers were able to recuperate from natural sunlight exposure very slowly. Even though high concentrations of $NH_4^+$ (100 μM)

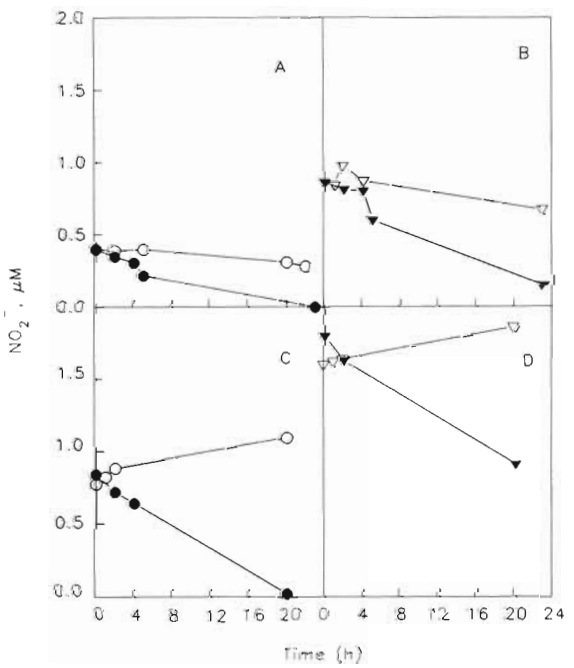


Fig. 3. *Nitrobacter* sp. (25W30N) Recovery as nitrite consumed after monochromatic irradiation. (A, B) After 2 h; (C, D) after 4 h. (○, ●) 300 nm; (▽, ▼) 325 nm. Solid symbols represent controls

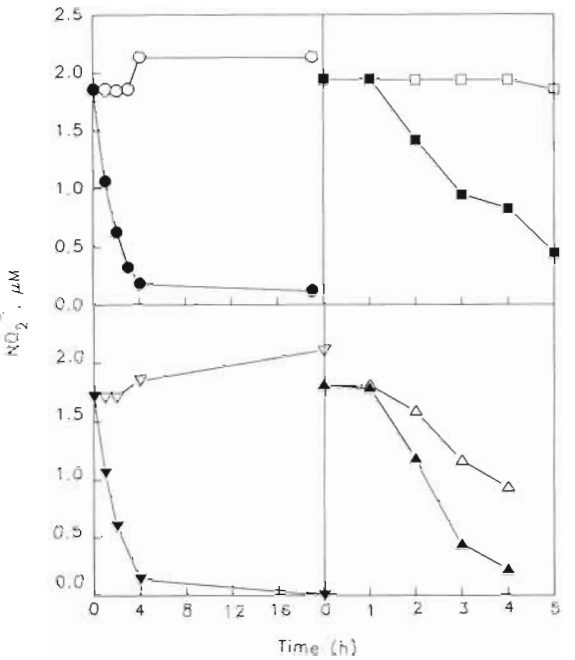


Fig. 4. *Nitrococcus mobilis*. Recovery as nitrite consumed after 2 h of monochromatic irradiation. (○, ●) 300 nm; (▽, ▼) 325 nm; (□, ■) 350 nm; (▽, ▼) 375 nm. Solid symbols represent controls

were included in the recovery flasks, the correlation between higher activity rates and faster recovery was not meaningful. It took 24 h for both organisms to recover 3% of their original ammonium oxidizing activity (Fig. 5). On the other hand, nitrite oxidizers were never able to recover their activity after the same sunlight treatment (Fig. 6), although both nitrite oxidizers had shown an apparent sunlight tolerance as detected by nitrite dissapearance (Guerrero & Jones 1996). Not only were nitrite oxidizers unable to resume their activity, but at the same time nitrite concentrations continued to increase instead of decreasing. In fact, the species that exhibited the higher nitrite oxidizing activity (*Nitrococcus mobilis*, 0.35 μM $h^{-1}$) was also capable of accumulating more nitrite as a result of the light treatment when compared to the one with lower nitrite oxidizing rates (*Nitrobacter* sp., 0.02 μM $h^{-1}$).

### Recovery from artificial light

Only $NH_4^+$ oxidizers were tested since $NO_2^-$ oxidizers were insensitive to this type of light. The results in Fig. 7 present the pattern for both $NH_4^+$ oxidizers. Ordinarily, recovery times depended on the light treatment (i.e. dose) imposed, such that they were faster for 8:16 h light:dark cycles than for 12:12 h light:dark cycles. Therefore, $NH_4^+$ oxidizers were able to recover

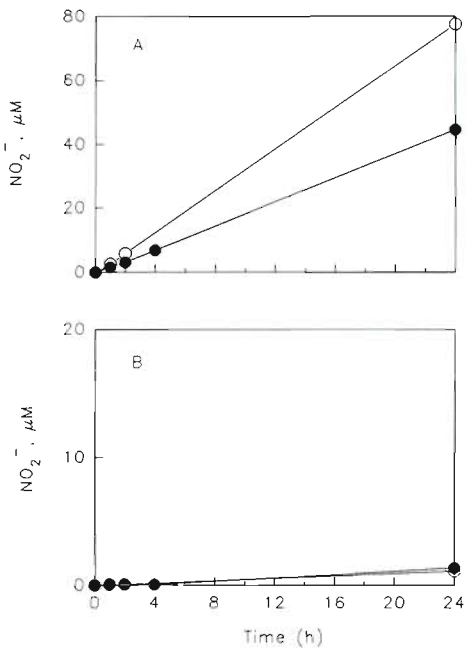


Fig. 5. Dark recovery as nitrite accumulation after 2 h of sunlight irradiation of ammonium oxidizers. Light intensity was 360 to 400 W $m^{-2}$ (A) Control; (B) sunlight-treated. (○) *Nitrosomonas cryotolerans*; (●) *Nitrosococcus oceanus*

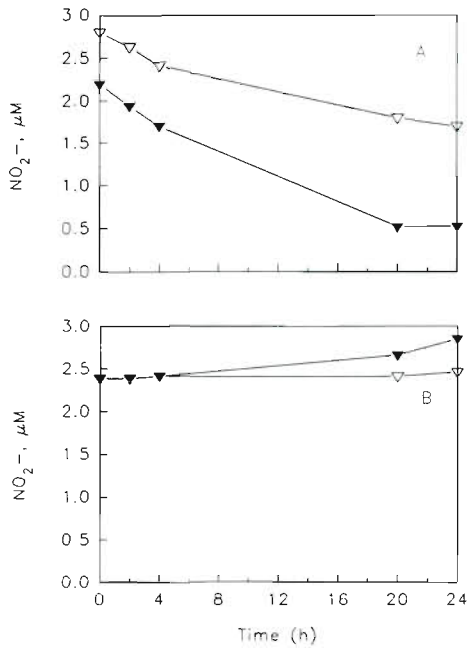


Fig. 6. Dark recovery as nitrite disappearance after 2 h of sunlight irradiation of nitrite oxidizers. Light intensity was 360 to 400 W $m^{-2}$ (A) Control; (B) sunlight-treated. (▽) *Nitrobacter* sp.; (▼) *Nitrococcus mobilis*

regardless of the light treatment administered. That recovery occurred faster for the shorter light cycle is an indication of dose responsiveness. Ultimately both variables, pH and nitrite concentration, returned to initial ($T_0$) values within the first 3 and 4.5 h of dark treatment, respectively.

## DISCUSSION

An important factor to consider in natural environments is the ability of nitrifying bacteria to recover from photoinhibition during dark periods. As expected, the recuperation of $NH_4^+$ and $NO_2^-$ oxidizing activities depended on the dose and the type of light to which they had been exposed previously.

In the case of ammonium oxidizers subjected to artificial (cool-white fluorescent) light, the speed of recovery depended only on the dose (i.e. time of exposure). If the light cycle was changed to 12 h instead of 8 h (Fig. 7A, B), the recovery time increased from 3 to 4.5 h, but it still occurred. Longer recovery times just indicate that cell activity was proportionately more affected by the longer light treatment. With monochromatic irradiations both factors, dose and quality of light regulated recovery. However, at the shortest wavelengths (300 nm) recovery was always longer, independent of the dose. Sunlight (2 h; approx. 400 W $m^{-2}$) caused the greatest drop in activity, followed by the

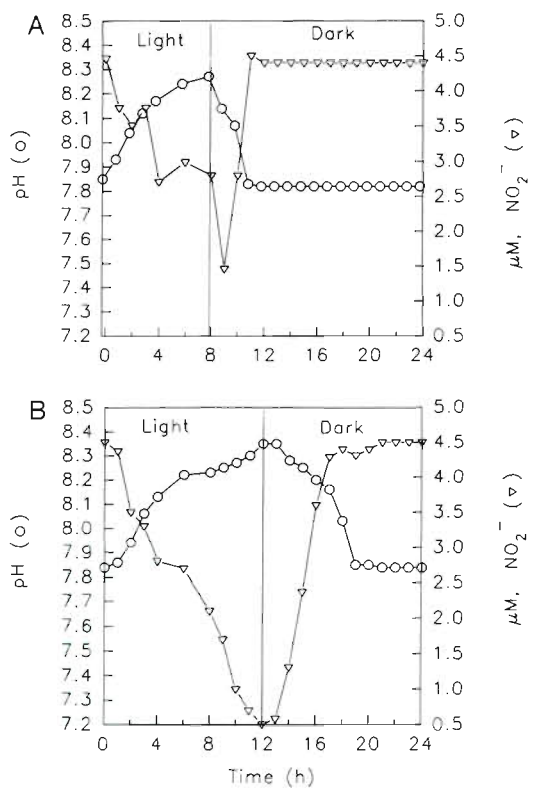


Fig. 7. *Nitrosomonas cryotolerans*. Effect of periodic illumination on chemostat culture. (A) 8:16 h light:dark; (B) 12:12 h light:dark

longest time (24 h) to restore 2 to 3% of the original activities. This might have been due to the fact that for natural sunlight experiments, sunlight was not screened. But in general, broadband results (artificial and sunlight) are consistent with narrowband results, since, for instance, natural sunlight, which is richer than artificial light in near-UV, brings about the longest recovery times just as monochromatic near-UV exposures did. Recovery of $NH_4^+$ oxidizing activities from artificial light (blue light enriched) was comparatively fast and agreed with the monochromatic blue light results.

Nitrite oxidation was not affected by artificial light as was ammonium oxidation. Post-irradiation behavior after near-UV (300 to 350 nm) exposures resulted in an increase in the final concentration of nitrite as opposed to the expected decrease of substrate. Nitrite oxidation was resumed when wavelengths >350 nm were used. Photochemical formation of nitrite and hydrogen peroxide was investigated under the abovementioned experimental conditions and was found to be negligible. Therefore, these were discounted as tentative causes for the decrease of nitrite oxidation/increase of nitrite concentrations. One alternative explanation for this unexpected increase is that near-UV light may have caused changes in the permeability of the cell membrane, as has been shown in other Gram-negative bacteria (Kelland et al. 1983), and transformed them into leaky cells capable of releasing endogenous nitrite. The other possibility would involve the nitrite oxidoreductase of $NO_2^-$ oxidizers. This enzyme has both nitrite dehydrogenase (oxidation of nitrite), as well as nitrate reductase activity (Bock et al. 1988, Freitag & Bock 1990). Perhaps near-UV light affected $NO_2^-$ oxidizers by prompting this enzyme to the reverse reaction (reduction of nitrate). Until now, the reversal of the reaction has only been observed under $O_2$ limited conditions (Tanaka et al. 1983, Sundermeyer-Klinger et al. 1984), yet the fact that increased levels of nitrite appear as a result of near-UV irradiation, particularly with *Nitrococcus mobilis* (faster $NO_2^-$ oxidation rate), suggested nitrate reduction as an alternative possibility. As expected, sunlight yielded the same type of nitrite accumulations, which was taken as a confirmation of the near-UV post-irradiation behavior The impact of near-UV in aquatic environments would be limited to the initial few centimeters (surface microlayer) because of the poor water penetration properties of these wavelengths. Field measurements of nitrification rates in the oceans exhibit subsurface maxima (Olson 1981, Ward et al. 1982) which is consistent with the aforementioned near-UV sensitivity of nitrifiers. On the other hand, depending on the water's properties/composition, blue light can be transmitted deeper through natural bodies of water (Jerlov 1968, Craig 1973, Wolken 1986).

Bactericidal or bacteriostatic action of the different types of light was not determined, yet the recovery data obtained after near-UV (Figs. 3 & 4) and sunlight (Fig. 6) exposure suggested that this type of light most probably has lethal effects on $NO_2^-$ oxidizers but not on $NH_4^+$ oxidizers. *Nitrosomonas cryotolerans* and *Nitrosococcus oceanus* were capable of a slow recovery after exposure to the same type of light (Figs. 1, 2 & 5). A theoretical model for the formation of a primary nitrite maximum in the water column is proposed based on the assumption that 'laboratory nitrifying bacteria' are physiologically similar to the 'naturally occurring nitrifying bacteria'. According to this model both types of nitrifying bacteria would be inactive at the surface which is where most of the UV radiation strikes. Yet, water circulation (mixing) will permit $NH_4^+$ oxidizers to recover by allowing them to go into a 'dark recovery zone'. The same does not apply to $NO_2^-$ oxidizers, which are irremediably injured while at the surface. We suggest that this differential recovery among nitrifying bacteria leads to an incomplete nitrification and in turn contributes to the formation of a primary nitrite maximum in at least the euphotic zone of aquatic habitats.

This study has followed the process optimization guidelines set by other investigators (Horrigan et al. 1981, Yoshioka & Saijo 1984, Vanzella et al. 1990) and demonstrated recovery of nitrifying bacteria from photoinhibition caused by visible light. But in reality, the nature of some habitats like open oceans (low nutrient availability, low temperature) may not facilitate the recovery process. The question that arises is not whether bacteria can recuperate given unlimited substrate concentrations, but whether they can at *in situ* substrate concentrations, typically <1 µM. Other factors such as substrate availability, temperature, $pO_2$ and attachment to particles need to be considered when attempting to extrapolate laboratory data to the environment. In the meantime, it is proposed that the differential recovery among nitrifying bacteria could subsequently be a factor in the formation of the primary nitrite maximum.

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