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# Light inhibition of nitrification in sea-surface films

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## ABSTRACT

Nitrifying bacteria (ammonia- and nitrite-oxidizers) were isolated from sea-surface films collected in the Gulf of California. Laboratory studies indicate substrate oxidation by these isolates is inhibited by light. Ammonia-oxidizers showed lower nitrifying activity and a longer lag time when held in an 8-hr light:16-hr dark regime compared to those held completely in the dark, and nitrite-oxidizers showed nitrifying activity only when held in the dark. Tracer studies using <sup>15</sup>N-ammonia corroborate that little or no nitrification occurs in the surface films of subtropical seas.

## 1. Introduction

The sea-surface film is a microenvironment at the air-sea interface, and its study is thus important in understanding fluxes of heat, energy and materials between the ocean and the atmosphere. Dissolved organic carbon (DOC) and particulate organic carbon (POC) are enhanced in the surface film relative to their concentrations in subsurface water (Goering and Menzel, 1965; Williams, 1967; Nishizawa, 1971; Nishizawa and Nakajima, 1971; Sieburth *et al.*, 1976); surprisingly, dissolved inorganic nutrients such as nitrate and nitrite are also often reported to be enhanced (Table 1). In order to sustain an enhancement of nitrate in surface films,

Table 1. Literature values of average enhancement (concentration in surface film:concentration in control water) of nitrate in surface films.

Location	Average enhancement	(Range)	No. of samples	Reference
Off Peru*	4.1	(1.2-9.0)	6	Williams (1967)
Equatorial Pacific	1.2		1	Nishizawa (1971)
Central North Pacific*	3.0	(0.9-4.2)	4	IMR (1974)
Coastal U. K.	1.5	(0.5-5.3)	31	Chapman & Liss (1981)
Gulf of California*	1.6	(0.8-5.5)	11	This report
W. coast of Baja*	7.4	(0.2-24)	9	This report

\* These values have been corrected for the dilution of surface film water taken to be 150  $\mu$ m in depth, with the 10 cm subsurface water adhering to the screen frame.

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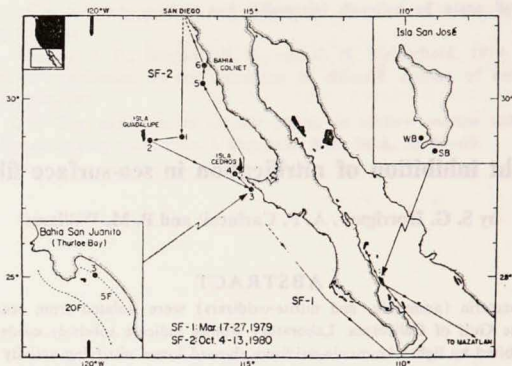


Figure 1. Cruise track for SF-1 and SF-2.

we assume that there must be either input of nitrate to the sea surface, production of nitrate in surface film water, or preferential utilization of nitrate just beneath the surface microlayer. Several mechanisms for input or production are possible: Williams (1967) suggested that the observed enhancement of nitrate in surface films was due to bacterial nitrification, but did not rule out the possibilities of fallout of gases, aerosols or precipitation high in nitrate, or the photochemical oxidation of organic nitrogen or ammonia.

In this study, we examined the possibility that bacterial nitrification is responsible for the observed enhancement of nitrate in the sea-surface film. We first looked for the presence of nitrifying bacteria in surface film water, and enrichments with nitrifying activity were obtained. We next asked if these organisms were active *in situ*. Nitrifying bacteria from soils and from below the euphotic zone in the ocean are inhibited by light (Müller-Neuglück and Engel, 1961; Ward and Olson, 1980; Olson, 1981), so we asked if light had an effect on the nitrifying activity of our enrichments from the sea-surface film. Finally, we examined the potential ammonia oxidation *in situ* using  $^{15}\text{N}$  as a tracer.

## 2. Materials and Methods

**Isolations.** Samples were collected from six stations on Cruise SF-1 (Fig. 1), using a modified screen technique described by Garrett (1965). The platform from which samples were collected was either a modified 18-foot "Hobie Cat" catamaran (Williams *et al.*, 1980, 1981), or a 12-foot rubber Zodiac. The screen used to collect samples was made of nylon monofilament mesh (Nitex, 1.8 mm mesh opening, 0.5 mm thread diameter, 61% open space) supported on a PVC frame.

The screen was lowered perpendicular to the surface film, and rotated so that it was lifted up through the surface film horizontally, thus entrapping the surface film layer plus extraneous water adhering to the PVC frame. The screen was then tilted so that collected water could be poured off into a container. Subsurface water was collected by holding a clean bottle  $\approx 10$  cm below the surface and allowing it to fill slowly.

The sample was processed using a modification of the Millipore filter technique of Carlucci and Strickland (1968). Aliquots of 500 ml of surface film and control (10 cm) water were filtered through sterile Millipore PH filters ( $0.3 \mu$  pore size) and placed into screw-capped test tubes containing 10 ml of media, either for ammonia-oxidizers or for nitrite-oxidizers (Carlucci and Strickland, 1968). Tubes were kept at room temperature in the dark. Nitrite production by ammonia-oxidizers or destruction by nitrite-oxidizers was measured using a qualitative modification of the colorimetric test for nitrite (Strickland and Parsons, 1972).

After demonstration of nitrifying activity, each enrichment was transferred to fresh medium. Enrichments were determined to be negative if no activity was seen after one year.

*Light inhibition studies.* Flasks with culture medium (Carlucci and Strickland, 1968) were incubated at  $20^{\circ}\text{C}$  under cool-white fluorescent light. Irradiance equalled approximately  $1 \times 10^{16}$  quanta  $\text{sec}^{-1} \text{cm}^{-2}$ . Light was adjusted by wrapping the flasks in aluminum foil when a dark period was desired, and removing the foil for the light period. Cultures were maintained under four conditions: (1) dark, (2) 8-hrs light : 16-hrs dark (8 : 16), (3) 16-hrs light : 8-hrs dark (16 : 8), and (4) light for 42 days. All cultures were then maintained in the dark for the remainder of the experiment. Activity was assayed quantitatively by production (ammonia-oxidizers) or disappearance (nitrite-oxidizers) of nitrite (Strickland and Parsons, 1972). Aliquots of the culture medium were diluted appropriately with filtered sea water, prior to nitrite determinations. Assays of activity were performed periodically (every 2-5 days) over the course of the experiment.

*Tracer ( $^{15}\text{N}$ ) studies.* Samples were collected at station 3 of Cruise SF-2 (Fig. 1). Because large volumes of film material were needed for the experiment, samples were collected with a "snowplow" (Williams *et al.*, 1981). The "snowplow" consists of two hinged sheets of Acrylite attached between the pontoons at the front of the catamaran, which traps films as the catamaran moves slowly forward under electric power. The film material was observed to be concentrated within the "snowplow." The sample was then dipped from between the pontoons with the screen sampler. Thus, we cannot quantitate what proportion of the sample was actually surface film material, but we can say from total organic carbon analysis that the sample was enriched in surface film material.

Four-liter aliquots of the sample were placed in each of four bottles: standard



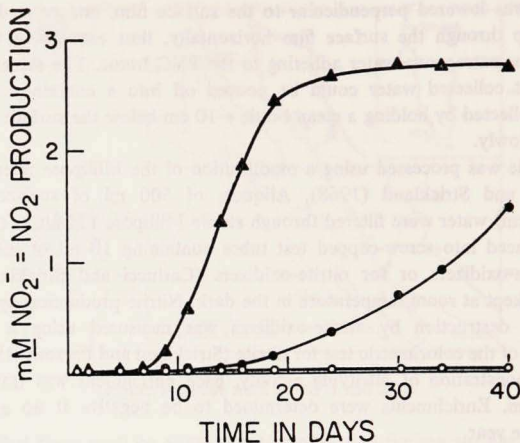


Figure 2. Activity of ammonia-oxidizers under various light-dark regimes: dark (▲), 8 hrs light:16 hrs dark (●), 16 hrs light:8 hrs dark (○). No activity was seen in the light incubation.

(with 10  $\mu\text{M}$   $\text{Na } ^{15}\text{NO}_2$  added), dark, light (under natural light conditions, total irradiance  $\approx 5.4 \times 10^{21}$  quanta  $\text{cm}^{-2}$ ), and killed control (with 75 ppm mercuric chloride). One  $\mu\text{M}$   $^{15}\text{NH}_4\text{Cl}$  with carrier  $\text{NaNO}_2$  was added to each bottle. Bottles were incubated in a flowing sea water bath for temperature control for 24 hrs. Samples were taken initially and finally for  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ , and  $\text{NO}_3^-$  analyses (Solòrzano, 1969; Strickland and Parsons, 1972). After 24 hrs, samples were filtered onto pre-combusted Reeve-Angel 984H filters, and the filtrate was frozen until analysis. The filtrate was extracted to recover the dissolved  $^{15}\text{NO}_2$  according to the methods of Olson (1981). Both particulate  $^{15}\text{N}$  on filters and dissolved  $^{15}\text{NO}_2$  were analyzed by mass spectrometry (Wada *et al.*, 1977).

### 3. Results

*Isolations.* From the six stations sampled on SF-1, two ammonia-oxidizing enrichments and four nitrite-oxidizing enrichments were obtained. Subsurface samples from the same stations yielded only one ammonia-oxidizing and one nitrite-oxidizing enrichment.

Due to their rapid activity, the ammonia-oxidizer from station 2 (surface film) and the nitrite-oxidizer from station 5 (surface film) were chosen for further study.

*Light inhibition studies.* Ammonia-oxidizers showed rapid nitrifying activity in the dark, as evidenced by nitrite production (Fig. 2). Activity on an 8:16 light-

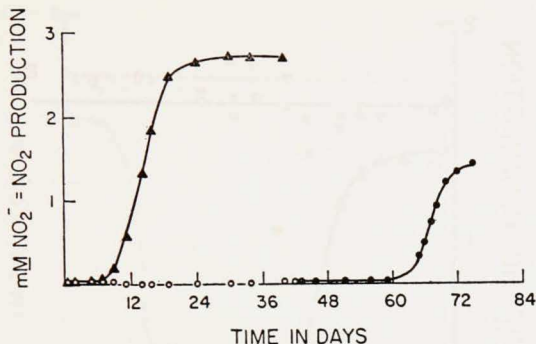


Figure 3. Recovery of ammonia-oxidizers, held under 16 hrs light:8 hrs dark, after being transferred to dark.  $\blacktriangle$  = culture held in dark;  $\circ$  = 16 hr light:8 hr dark,  $\bullet$  = same culture, switched to dark.

dark cycle was lower; both a longer lag time and slower production of nitrite was observed. No activity was seen in either the 16:8 light-dark cycle or the light cultures in 42 days. The lower production of nitrite in the 8:16 culture is probably due to inhibition of activity in the light.

The culture of ammonia-oxidizers held on a 16:8 light-dark cycle recovered to show nitrifying activity when it was placed in the dark (Fig. 3). After a lag, nitrifying activity proceeded at the same rate as the culture initially held in the dark.

Nitrite oxidizers also showed nitrifying activity in the dark, as evidenced by disappearance of nitrite (Fig. 4). No activity was observed in any of the other cultures (8:16, 16:8, or light) in 42 days. No recovery was seen in the 16:8 light-dark cycle culture when it was placed in the dark (Fig. 5).

*Tracer ( $^{15}\text{N}$ ) studies.* No evidence of ammonia oxidation was seen in the tracer studies (Table 2). If ammonia-oxidizing bacteria had been active, we would expect to see  $^{15}\text{N}$ -enrichment in the dissolved nitrite samples. Additionally, excretion of nitrite by phytoplankton in the light would contribute to the labelled dissolved pool (Carlucci *et al.*, 1970). Enrichment in the particulate samples is most likely

Table 2. Tracer studies with  $^{15}\text{N}$ . Surface film water sample was incubated with  $^{15}\text{NH}_4$  for 24 hrs, under natural light conditions (light) or in the dark (dark).

Initial (after addition)	Dissolved Nutrients ( $\mu\text{M}$ )			$^{15}\text{N}$ % Enrichment	
	$\text{NH}_4$	$\text{NO}_2^-$	$\text{NO}_3^-$	Particulate N	Dissolved $\text{NO}_2^-$
Light	1.12	1.06	.05	9.4%	0.2%
Dark	.31	.25	.01	5.7%	0%
Dark	1.64	.76	.06		

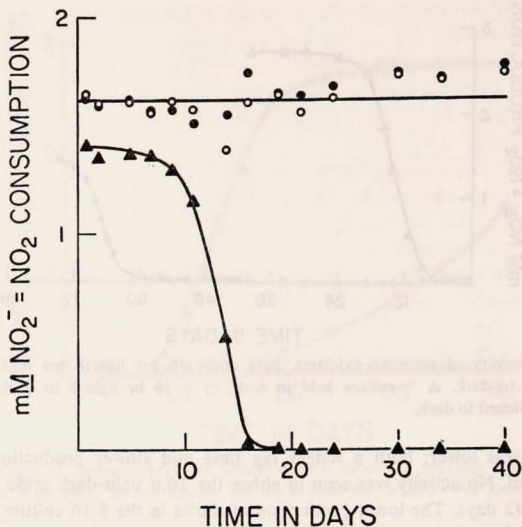


Figure 4. Activity of nitrite-oxidizers under various light-dark regimes. Symbols as for Figure 2.

due to uptake of  $^{15}\text{N}$ -ammonia by phototrophic organisms; enrichment in these samples is higher in the light bottle than in the dark. Such an hypothesis is substantiated by the changes in dissolved nutrients: the decrease in  $\text{NH}_4^+$  in the light bottle is probably due to uptake by phototrophs, whereas the increase in  $\text{NH}_4^+$  in the dark bottle is probably due to heterotrophic breakdown of organic matter (von Brand *et al.*, 1937).

#### 4. Discussion

Higher levels of nitrate in the sea-surface film compared to control water sampled from 10 cm or 1 m have often been observed (Goering and Menzel, 1965; Williams, 1967; Nishizawa, 1971; Chapman and Liss, 1981; Cruise SF-1 and SF-2, unpublished data). Possible mechanisms for this observed enhancement include: (1) differential utilization of nitrate, such that an initially constant level of nitrate throughout the euphotic zone becomes apparently concentrated in the surface film due to uptake of nitrate in the layers below the surface film, (2) input of nitrate to surface waters from deeper waters by bubble transport, (3) photo-oxidation of reduced forms of nitrogen, (4) introduction of nitrate from the atmosphere by precipitation, and (5) bacterial nitrification.

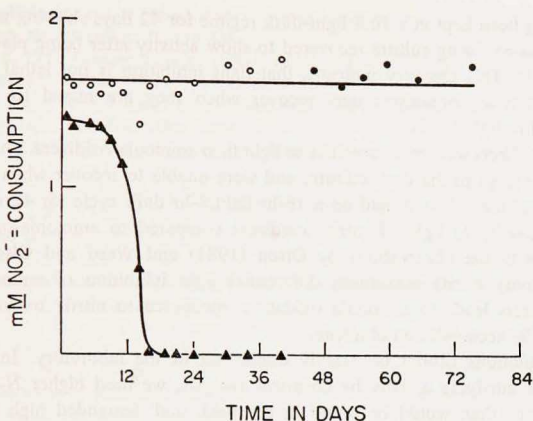


Figure 5. Recovery of nitrite-oxidizers. Symbols as for Figure 3.

In this study, we address the hypothesis that the enhancement of nitrate in sea surface films is due to production of nitrate in the surface film by the activities of nitrifying bacteria. To examine this hypothesis, we first asked whether nitrifying bacteria are present in the surface film, defined by our ability to obtain enrichments with nitrifying activity from surface film samples. (Note, however, that failure to obtain enrichments with nitrifying activity would not necessarily justify the conclusion that nitrifying bacteria are not present (Belser, 1979).) Indeed, we easily obtained enrichments with nitrifying activity.

Merely establishing the presence of the desired organisms is not sufficient to establish their role *in situ*, because organisms with the potential to perform certain reactions (nitrate production, in this case) may not be active *in situ*. Nitrifying bacteria are inhibited by light (Müller-Neuglück and Engel, 1961; Ward and Olson, 1980; Olson, 1981); therefore, we had to establish that our enrichments were not so inhibited before we could invoke nitrifying bacteria as the cause of nitrate enhancement in surface films.

We considered the possibility that surface film nitrifiers may only be active at night, because we found larger nitrate enhancements in the surface films at night stations compared to day stations (Cruise SF-1, unpublished data). Our laboratory studies were designed to test this possibility. Four different light regimes were used. Our results imply that activity of ammonia-oxidizers does indeed occur in the dark rather than in the light, but we could not detect activity of ammonia-oxidizers on a 16-hr light:8-hr dark cycle. Activity of a culture on an 8-hr light:16-hr dark cycle was slower and showed a longer lag time than that kept in the dark.



After having been kept in a 16:8 light-dark regime for 42 days showing no activity, the ammonia-oxidizing culture recovered to show activity after being placed in the dark (Fig. 3). This recovery indicates that light inhibition is not lethal and that, in field situations, organisms may recover when they are mixed to depths of sufficiently low light levels.

Nitrite-oxidizers were more sensitive to light than ammonia-oxidizers. They showed no activity except in the dark culture, and were unable to recover when placed in the dark after having been held on a 16-hr light:8-hr dark cycle for 42 days. This greater sensitivity to light of nitrite oxidizers compared to ammonia-oxidizers is consistent with the observations by Olson (1981) and Ward and Olson (1980) on the primary nitrite maximum: differential light inhibition of ammonia- and nitrite-oxidizers leads to ammonia oxidation uncoupled to nitrite oxidation, thus permitting the accumulation of nitrite.

Field conditions cannot be exactly duplicated in the laboratory. In order to demonstrate nitrifying activity by chemical analysis, we used higher N- substrate concentrations than would be found in the field, and demanded high detectable levels of nitrifying activity in our system. Furthermore, our original enrichments were kept in the dark in order to exclude photoautotrophs. Thus, we may have selected populations adapted to growth in the dark.

Although we found our light inhibition studies in the laboratory convincing, we complemented this study with a tracer ( $^{15}\text{N}$ ) experiment under simulated *in situ* conditions. No evidence of ammonia oxidation was observed.

It is concluded, then, that the observed enhancement of nitrate in surface film water is not due to bacterial nitrification.

*Acknowledgments.* We thank C. C. Price and K. J. Robertson for assistance in sampling, B. B. Ward and R. J. Olson for helpful discussions, H. Ducklow and O. C. Zafriou for reading the manuscript, and J. Stearns for typing. This work was supported by NSF GRANTS OCE77-26178 and OCE79-25942.

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