Differential photoinhibition of marine nitrifying bacteria: a possible mechanism for the formation of the primary nitrite maximum[1]

by Robert J. Olson[2, 3]

ABSTRACT

^{15}N-tracer experiments using natural populations of marine nitrifying bacteria revealed photoinhibition of nitrite- and ammonia-oxidizing activity at light intensities less than 1% of sunlight. Nitrite oxidation was more sensitive to light than was ammonia oxidation. Calculations of biologically effective dose rates suggest that the depths at which ammonia oxidation exceeds nitrite oxidation are consistent with the depths of observed primary nitrite maxima in subtropical waters.

1. Introduction

The primary nitrite maximum found near the bottom of the euphotic zone is a prominent feature over much of the world's oceans. Several sources for this nitrite have been suggested, including ammonia oxidation to nitrite by autotrophic nitrifying bacteria (Brandhorst, 1959), bacterial nitrate reduction (Wada and Hattori, 1971), and excretion of nitrite during nitrate assimilation by phytoplankton (Vaccaro and Ryther, 1960). Recent work using ^{15}N tracers indicated that ammonia oxidation by *Nitrosomonas*-like organisms was the major source of nitrite in Southern California coastal waters and in the central North Pacific gyre, and it was shown that the rate of ammonia oxidation was reduced by exposure to light (Olson, 1981). In addition, ammonia oxidation was shown to occur at substantial rates below as well as within the nitrite maximum layer, while nitrite oxidation (presumably by *Nitrobacter*-like organisms) took place only below the nitrite maximum. It is the purpose of this paper to suggest that differential photoinhibition is a possible mechanism for this displacement of the two steps of nitrification which allows the accumulation of nitrite in a narrow band at depth.

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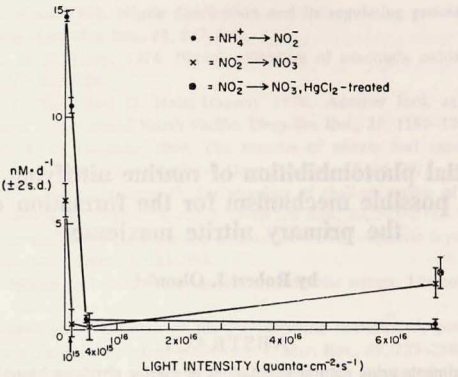

Figure 1. Effect of light intensity on nitrite oxidation and ammonia oxidation. Laboratory incubation of a sample from the SIO pier (4 m below the surface in a 7-m water column). Nitrite oxidation in a killed control (75 ppm $HgCl_2$) was also measured at the highest light intensity. Lines connecting points are for identification only.

Photoinhibition of pure cultures of *Nitrobacter winogradskyi* and *Nitrosomonas europaea* was reported by Müller-Neuglück and Engel (1961) and Schön and Engel (1962), respectively. They found that the inhibition was caused by blue light (wavelengths below 480 nm) and that light intensities as low as 200-300 Lux (for *Nitrobacter*) and 1000 Lux (for *Nitrosomonas*) were effective (full sunlight is approximately 100,000 Lux). Bock (1965) found that light caused oxidation of cytochrome *c* in the presence of oxygen in both nitrifiers, and confirmed that *Nitrobacter* was more sensitive than *Nitrosomonas* to light. He suggested that this greater sensitivity was associated with the relatively low cytochrome *c* content of *Nitrobacter* compared to *Nitrosomonas*.

It was suggested by Ulken (1963) that the higher sensitivity of the nitrite oxidizers to light might be of ecological significance. She found higher nitrite accumulation in water samples from the Elbe River incubated in light than in darkness after a period of two weeks and suggested that this effect could contribute to the high nitrite concentrations found in the Elbe.

Reported here are the results of measurements of photoinhibition of ammonia- and nitrite-oxidizing activity in natural seawater samples from Southern California coastal waters. Also presented are calculations of the effect of natural light regimes on the production and consumption of nitrite by nitrifying bacteria in two representative water columns.

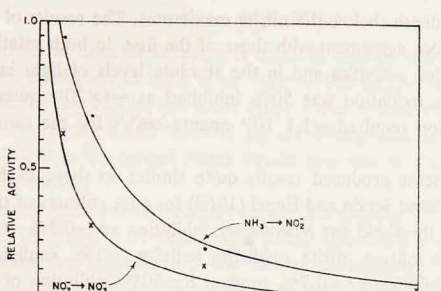

Figure 2. Effect of light intensity on nitrite oxidation and ammonia oxidation. Laboratory incubation of a sample from three miles off SIO, at 75 m depth (below the nitrite maximum). The smooth curves are hyperbolas fit to the data (see text).

2. Materials and methods

Incubation conditions and ^{15}N tracer techniques were as described by Olson (1981), except that light conditions were constant for 24 hours.

3. Results and discussion

a. Laboratory experiments. Available evidence for photoinhibition of marine nitrification is of three types: 1) *in situ* or simulated *in situ* experiments in which dark bottles show higher activity than light bottles; 2) *in situ* or simulated *in situ* profiles of activity *vs* depth in which activity decreases near the surface; and 3) laboratory experiments in which a natural seawater sample is divided and incubated under varying light intensities. Evidence of types 1 and 2 were presented in a companion paper (Olson, 1981); the results of laboratory experiments are presented in Figures 1 and 2. Figure 1 shows the results of an experiment in which seawater from the SIO pier (drawn from 4 m below the surface in a 7-m water column) was incubated at light intensities ranging from 0 to 7.1×10^{16} quanta/cm^2/s (full sunlight is about 1.5×10^{17} quanta/cm^2/s). Nitrite oxidation activity was very low in all except the dark bottle, while ammonia oxidation activity was about 70% of the dark rate at 1×10^{15} quanta/cm^2/s. (It should be noted that a killed control (75 ppm $HgCl_2$) gave a result nearly identical to the live sample at the highest light intensity, providing direct evidence for chemical photooxidation of nitrite as suggested by Zafiriou and True (1979).

In order to investigate this difference more closely, another experiment was done over the lower range of light intensities, using seawater from three miles offshore

at SIO, at 75 m depth (below the nitrite maximum). The results of this experiment (Fig. 2) are in close agreement with those of the first, in both relative responses of the two nitrification activities and in the absolute levels of light intensity effecting inhibition. Nitrite oxidation was 50% inhibited at $\sim 4 \times 10^{14}$ quanta/cm^2/s, while ammonia oxidation required $\sim 1.1\ 10^{15}$ quanta/cm^2/s for the same degree of inhibition.

These experiments produced results quite similar to those of Müller-Neuglück and Engel (1961) and Schön and Engel (1962) for pure cultures of the soil nitrifiers: ~0.2% sunlight threshold for *Nitrobacter* inhibition *vs* ~0.3% sunlight for 50% inhibition of the marine nitrite oxidizing activity; ~1% sunlight threshold for *Nitrosomonas* inhibition *vs* ~0.7% sunlight for 50% inhibition of the marine ammonia oxidizing activity. This finding encouraged me to use the published action spectra for photoinhibition of *Nitrobacter winogradskyi* (Bock, 1970) and *Nitrosomonas europaea* (Hooper and Terry, 1974) to make a calculation of the biologically effective light doses received by the organisms in the sea. The procedure, similar to that used by Smith *et al.* (1980) who examined photoinhibition of photosynthesis, was as follows.

b. Calculation of spectral irradiance at depth. Since no data are yet published for full-day integrated spectral irradiance measurements over both the UV and visible ranges of the spectrum, it was necessary to combine calculated and measured spectral irradiances to obtain coverage of the region of interest. The model of Green *et al.* (1974) with the marine air parameters of Baker *et al.* (1981) was used to calculate surface irradiance for wavelengths between 280 and 340 nm as a function of sun angle. Measurements by the Visibility Laboratory of SIO supplied data from 380 to 480 nm as a function of sun angle. The interpolation between these ranges to complete the coverage is a reasonable one because of the relatively flat spectral irradiance distribution in this part of the spectrum.

Spectral irradiance was integrated over time as follows:

(a) a graph of sun angle versus time (from Visibility Laboratory measurements, K. Baker, pers. comm.) was used to divide the daylight hours into eight intervals with sun angle weighted according to duration;

(b) for each angular interval, sky light and sunlight penetration through the sea surface were calculated separately (using the Fresnel equations at 10 nm intervals for the sunlight and a value of 0.94 for diffuse light penetration through the air-sea interface). The total (multiplied by the 10-nm interval width) gives the full day integrated irradiance for each wavelength, just below the surface.

(c) This value was divided by two to account for 12 hours of darkness during the night.

Propagation of this light field through the water column was calculated at 10 nm intervals according to

$$E_{(\lambda,z)} = E_{(\lambda,0-)}\exp(-k(\lambda)z)$$

where E is irradiance in units of W/m^2, z is depth in meters, and k is the spectral diffuse attenuation coefficient in units of $m^{-1}nm^{-1}$. The diffuse attenuation coefficients of Smith and Baker (1979) for water types 1 and 3 were used. These correspond to waters containing 0.025 and 0.5 μg chl *a*/liter, and are roughly representative of conditions in the central North Pacific gyre and off the coast of Southern California, respectively.

It should be mentioned that the method chosen for treating the daily light dose (integration of the light over a 24-hour period rather than using a step or sine wave function) ignores the day/night cycle of the natural environment. This implies that the nitrifiers do not recover from light inhibition during the night period and so respond to integrated and not instantaneous light doses. The available experimental evidence indicates that this is the case. Schön and Engel (1962) showed that pure cultures of nitrifiers required time on the order of days to recover from light inhibition, depending on the intensity given; Hooper and Terry (1974) found that the photoinactivation effect in *Nitrosomonas* depended on the total number of quanta given; and marine ammonia oxidizers exposed to light required several days of darkness before activity resumed (S. Horrigan, pers. comm.). Finally, *in situ* 24-hour ^{15}N-tracer experiments revealed that surface samples gave very low rates of nitrification (much lower than would result from bacteria that were inhibited by light but active at night) (Olson, 1981). (That nitrifiers are *present* at the surface as well as deeper in the water column has been shown by growth on enriched medium (A. F. Carlucci, S. Horrigan, pers. comm.) and by immunofluorescent assay (B. Ward, pers. comm.). It is probable that at a low enough light level, the dark period would become important, and this point awaits clarification.

c. Calculation of biologically effective dose. The spectral irradiance multiplied by the spectral response (action spectrum) for the photoinhibition effect gives the biologically effective dose. The action spectra for *Nitrobacter* and *Nitrosomonas* are shown in Figure 3. The *Nitrosomonas* action spectrum was reported in terms of the rate constant for inhibition; this measurement was converted to the equivalent fraction of the dark activity by assuming essentially complete inhibition at $\lambda = 250$ nm (where the rate constant was maximum) and then using the relationship between rate constant (r) and activity (A), $A_t = A_o\exp(-rt)$ where $t = 30$ minutes, the standard exposure time in the other work. For the *Nitrobacter* action spectrum, it was assumed that there was no inhibition at wavelengths greater than 470 nm (Müller-Neuglück and Engel, 1961) and that the inhibition continued to increase into the UV region not tested (in any case, light intensity at these wavelengths is so low at the depths of interest that this assumption is not important here).

Figure 4 shows the results of the calculation of (spectral irradiance) (spectral response) as a function of depth for the two water types. It can be seen that the bio-

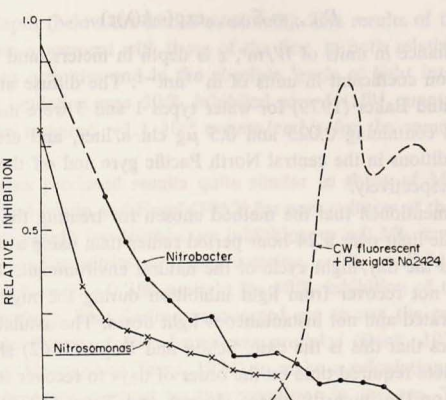

Figure 3. Action spectra for photoinhibition of *Nitrobacter winogradskyi* (Bock, 1970) and *Nitrosomonas europaea* (Hooper and Terry, 1974). Also shown is the relative spectral distribution of the combination of Cool-White fluorescent light and No. 2424 Plexiglas used in the laboratory experiments.

logically effective dose for *Nitrobacter* is greater than that for *Nitrosomonas* at any given depth.

The results of the laboratory experiments shown in Figures 1 and 2 provide a check on the validity of using action spectra for pure cultures of nonmarine nitrifiers to describe effects of light on natural populations in the sea. The biologically effective doses for the two organisms were calculated and compared to the measured inhibition levels, as follows.

The relative spectral irradiance of the Cool-White fluorescent bulbs as given by Duro-Test Corp. (1977; N. Bergen, New Jersey) was multiplied by the absorption spectrum of the Plexiglas used (No. 2424), to give the spectral distribution incident on the samples (See Fig. 1). Total light intensity from 400 to 700 nm, as measured with a quantum scalar irradiance meter (Booth, 1976), was then weighted by this spectral distribution to give absolute spectral irradiance. The spectral irradiance values multiplied by the relative response curves for the two organisms give the biologically effective doses corresponding to the measured levels of inhibition. Because the *Nitrosomonas* relative response curve is biased toward the UV compared to the *Nitrobacter* curve, the total calculated *Nitrosomonas* dose was only 1/7 that of the total *Nitrobacter* dose. The measured inhibition of "*Nitrosomonas*" activity

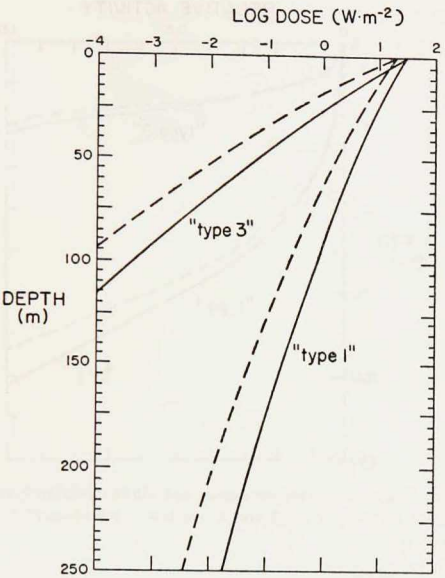

Figure 4. Biologically effective dose *vs* depth for water types 1 and 3, corresponding to 0.025 and 0.5 μg chl *a*/liter, respectively. *"Nitrobacter"* = ————; *"Nitrosomonas"* = – – – – –.

was about 1/2 that of the "*Nitrobacter*" activity; thus the calculated doses agree within a factor of about 3 with the measured inhibition.

d. Prediction of light-limited activity in the sea. The effect of light in the sea on the two stages of nitrification has been estimated by calculating the respective biologically effective doses from the spectral irradiance data and the published action spectra, and then weighting the doses according to the experimentally-determined dose-response relationships shown in Figure 2. The dose-response data have been described by hyperbolas analogous to those used to describe enzyme kinetics, with "dose" substituted for "substrate concentration" and "inhibition" for "velocity" such that

$$\text{inhibition} = \frac{(\text{dose})\ (\text{maximum inhibition})}{\text{dose} + \text{dose}_{0.5}}$$

The "$\text{dose}_{0.5}$" term is analogous to the K_m of enzyme kinetics, and is the dose at which inhibition is half-saturated. Because the dose-response curves apparently

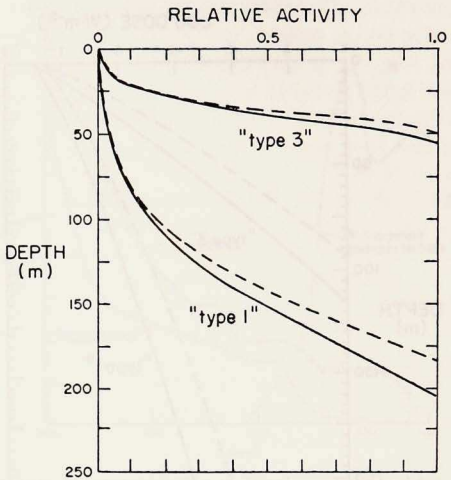

Figure 5. Relative activity profiles for ammonia- and nitrite-oxidizing bacteria as determined by light penetration for water types 1 and 3 (see text). *"Nitrobacter"* = ————; *"Nitrosomonas"* = – – – – –.

reach the level of minimum inhibition at nonzero dose levels, threshold values for each process were estimated and subtracted from the dose values. The following values have been used for the activity calculations: a) ammonia oxidation; $dose_{0.5} = 0.036\ W/m^2$, threshold = $0.0095\ W/m^2$; b) nitrite oxidation; $dose_{0.5} = 0.074\ W/m^2$, threshold = $0.0364\ W/m^2$. The relative activities (relative activity = 1 − inhibition) for ammonia and nitrite oxidation, as determined by photoinhibition, are plotted against depth in Figure 5. The data reveal that the activities increase dramatically near depths corresponding to the 1% light level (115 m for water type 1; 45 m for water type 3), and that the ammonia oxidizing activity is higher than the nitrite oxidizing activity at a given depth.

It is possible in principle to construct a depth profile of ammonia and nitrite oxidation rates, given information on the kinetic characteristics and distributions of natural populations of nitrifiers. Although these data are as yet far from complete, it is interesting to see the results of such calculations using the estimates obtained in field work off the coast of Southern California (Olson, 1981). The estimates and assumptions used in the following calculations are:

1) Both types of nitrifiers are distributed uniformly throughout the upper water column. (This assumption has been shown to be correct within an order of magni-

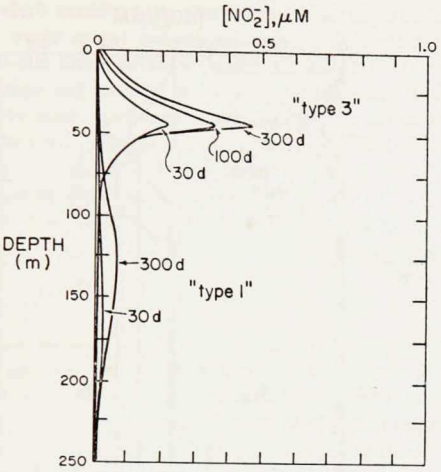

Figure 6. Calculated nitrite profiles in water types 1 and 3 after varying amounts of time (see text).

tude for at least two species of nitrifiers in Southern California coastal waters (B. Ward, pers. comm.);

2) Maximum rates for ammonia and nitrite oxidation are equal at 5 nM/d for water type 1 and 40 nM/d for water type 3;

3) Half-saturation constants for ammonia and nitrite oxidation are equal at 0.07 μM; and

4) Ammonia concentrations are constant with depth in the euphotic zone (0.05 μM for water type 1; 0.2 μM for water type 3) and decrease logarithmically to 0.01 μM at twice the depth of the euphotic zone. (The effects of varying the conditions set here are discussed below.)

The depth profile of nitrite concentration can then be determined from the depth profiles of the nitrite-producing and nitrite-consuming activities and the initial nitrite profile; for each depth the nitrite accumulation after each day will be

$$NO_2 = NO_{2(o)} + \frac{A_{NH_3} \cdot NH_3 \cdot Rate_{max}}{0.07\ \mu M + NH_3} - \frac{A_{NO_2} \cdot NO_2 \cdot Rate_{max}}{0.07\ \mu M + NO_2}$$

where A = relative activity allowed by light. Figure 6 shows calculated depth profiles of nitrite concentration after varying numbers of iterations, assuming no nitrite was initially present. The nitrite levels approach typical nitrite maximum values after about 100 days for "coastal" water and greater than 300 days for "gyre"

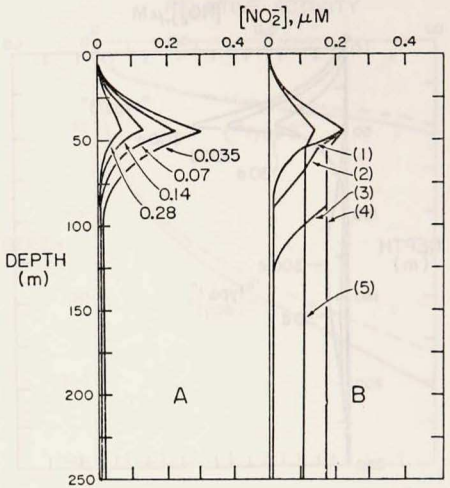

Figure 7a. Calculated nitrite profiles in water type 3 ("coastal") using the indicated values for $K_{s(NH_3)}$. $K_{s(NO_2)} = 0.07$ μM, time = 30 days.

Figure 7b. Calculated nitrite profiles in water type 3 using different NH_3 profiles. Time = 30 days.

1) $NH_3 = 0.2$ μM to 45 m, decreasing logarithmically to 0.01 μM by 90 m.
2) $NH_3 = 0.2$ μM to 45 m, decreasing linearly to 0.01 μM by 90 m.
3) $NH_3 = 0.2$ μM to 90 m, decreasing logarithmically to 0.01 μM by 135 m.
4) $NH_3 = 0.2$ μM uniformly.
5) $NH_3 = 0.1$ μM uniformly.

water. These are about an order of magnitude greater than the measured turnover times for nitrite in these waters, but it must be remembered that these curves represent net accumulation rather than turnover of nitrite.

Figure 7a shows the effects of changing the K_s value for ammonia oxidation over an 8-fold range, while keeping the K_s for nitrite constant. The position of the nitrite maximum is not affected, although the intensity of the peak increases by a factor of four as the affinity for ammonia is increased relative to that for nitrite.

Figure 7b shows the effects of changing the amount or distribution of ammonia in the water column. It can be seen that the lower slope of the nitrite maximum is dependent in large part on the rate of decrease of ammonia with depth. At depths where neither activity is inhibited by light, the nitrite concentration approaches the ambient ammonia concentration, given equal K_s values.

The nitrite profiles shown do not take into consideration the uptake of nitrite by

phytoplankton (which would tend to sharpen the upper slope of the maximum) or diffusion (which would spread out the maximum and decrease its intensity). In addition, the variables discussed above, which are known only approximately, can affect both the shape and intensity of the maximum formed. As formulated here, the structure of the nitrite maximum is affected most by the structure of the ammonia profile, which has been found to be both variable and difficult to determine precisely. However, the depth of the maximum nitrite concentration, which is the result least affected by the values chosen for the kinetic parameters, is consistent with observations of nitrite maxima in both the "coastal" and "open ocean" water types.

4. Conclusions

Results of the calculations carried out here, while encouraging in their similarity to field observations, serve to emphasize that very few aspects of marine nitrification are yet understood in other than semi-quantitative terms. Almost no quantitative information is available on the distribution of nitrifying organisms in the sea; data on the influence of environmental parameters such as substrate concentration and light on nitrifying activity are extremely limited. In the case of photoinhibition, *in situ* dose-response experiments and the determination of action spectra using natural populations or marine isolates are necessary next steps toward understanding.

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