Nitrogen Fixation by the Photosynthetic Sulfur Bacterium *Chlorobium phaeobacteroides* from Lake Kinneret

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N\textsubscript{2} fixation by *Chlorobium phaeobacteroides* from Lake Kinneret was dependent on ammonia concentration and light intensity. In the thermocline of Lake Kinneret, N\textsubscript{2} fixation and photosynthesis were low. It was concluded that the bacteria do not contribute significantly to the organic nitrogen load of the lake.

It is well known that photosynthetic bacteria, like blue-green algae (cyanobacteria), can fix nitrogen (4, 8, 15, 18, 21).

The nitrogen fixation activity of these bacteria has been shown to depend on light. A cell-free extract of the photosynthetic bacterium *Chromatium vinosum* strain D fixes nitrogen only in the light (22). Furthermore, it has been found that nitrogen fixation rises with increasing light intensity, with optimal activities for the photosynthetic bacterium *Rhodospirillum rubrum* and the blue-green alga *Mastigocladus laminosus* being at 135 × 10\textsuperscript{2} erg cm\textsuperscript{-2} sec\textsuperscript{-1} for white incandescent light bulbs and 1 × 10\textsuperscript{4} erg cm\textsuperscript{-2} sec\textsuperscript{-1} for Natur-Escent fluorescent light bulbs, respectively. (10, 13). Under higher light intensities, nitrogen fixation activity did not change. In Lake Mendota and Green Bay Lake (Wisconsin), nitrogen fixation by blue-green algae decreases with depth (14, 17, 19). A high correlation between nitrogen fixation, photosynthesis, and light intensity was found in Lake Mendota when the dominant phytoplankton was the alga *Aphanisomenon* sp. Nitrogen fixation and photosynthesis at the surface were maximal at a light intensity of 32,280 lx, and both decreased at midday, when light intensity was high (64,560 lx) (12).

Another factor that can influence nitrogen fixation is the external concentration of ammonia. In cultures of *Anabaena cylindrica*, rice roots, and the photosynthetic bacterium *Rhodopseudomonas palustris*, the presence of ammonia in the media inhibited nitrogen fixation (2, 3, 20). However, when a cell-free extract of *R. rubrum* was grown in chemostat culture with a low ammonia concentration as the sole nitrogen source, nitrogen fixation activity (after removal of the ammonia) was higher than when grown without ammonia (11).

Every summer between July and September, there is a bloom of the photosynthetic green sulfur bacterium *Chlorobium phaeobacteroides* in the thermocline of Lake Kinneret (1). The aim of this study was to find out under which conditions *C. phaeobacteroides* can fix nitrogen and whether there is any contribution to the organic nitrogen load in the lake.

The bacterium *C. phaeobacteroides*, isolated from Lake Kinneret, was grown as previously described (1) but without ammonia in the medium. Nitrogen fixation was determined by an adaptation of the acetylene ethylene reduction technique (16) as follows. In field and laboratory experiments, 100-ml bottles containing 50 ml of *C. phaeobacteroides* culture were flushed with a mixture of argon-acetylene (90:10) and incubated for various times at different depths in the lake, or under different light intensities in the laboratory. Gas samples were taken, and the ethylene content was determined by using gas chromatography with a flame ionization detector. Acetylene and ethylene were separated on a column (2 by 150 cm) of Porapak U at 70°C.

Photosynthetic activity was measured as described previously (1). Protein was determined by the method of Lowry et al. (9).

The bacteria were grown in medium containing different ammonia concentrations in the form of NH\textsubscript{4}Cl under an atmosphere of nitrogen and a light intensity of 25 μEin m\textsuperscript{-2} s\textsuperscript{-1}.

Ammonia concentrations of up to 5 mg of nitrogen per liter did not inhibit and even stimulated nitrogen fixation to some extent compared with media lacking ammonia (Fig. 1). This stimulation in nitrogen fixation may be explained by stimulation of cell growth and multiplication in the presence of ammonia, resulting in an increase in the total amount of nitrogen fixation. Concentrations higher than 5 mg of nitrogen per liter inhibited nitrogen fixation. It would appear that ammonia concentrations found in the thermocline of Lake Kinneret (0.3 to 0.5 mg of N per liter) (5-7) do not inhibit nitrogen fixation of *C. phaeobacteroides*.

Nitrogen fixation by *C. phaeobacteroides* de-
pended on light (Fig. 2). No nitrogen fixation took place in the dark, and increased light intensity raised activity.

The effect of light was also studied by incubating bottles containing a pure C. phaeobacteroides culture at different depths in the lake for 3 h. To minimize the effect of self-shading, the cultures were incubated in the lake at the beginning of the log phase (optical density at 715 nm, 0.4). A peak value of 5.5 μg of ethylene produced per μg of protein per h was obtained at a 3-m depth (Fig. 3). The activity decreased with depth, reaching 0.65 μg of ethylene produced per μg of protein per h at the C. phaeobacteroides bloom layer (20 m), where the low light intensity (0.3 to 1.0 μEin m⁻² s⁻¹) was the limiting factor. No acetylene reduction was obtained when cells from the bloom layer were tested. The exposure to air during sampling probably inactivated the nitrogenase, resulting in no acetylene reduction.

The photosynthetic activity pattern of a pure culture of C. phaeobacteroides incubated at different depths in the lake resembled that of nitrogen fixation. Maximal photosynthetic activity was also observed at a depth of 3 m and decreased to almost zero at the thermocline (20 m) (Fig. 4). The coincidence of limited nitrogen

![Figure 1](http://example.com/fig1.png)

**Fig. 1.** Effect of ammonia on nitrogen fixation by C. phaeobacteroides. Symbols: ○, no ammonia added; ×, 0.2 mg of ammonia-nitrogen per liter; △, 1 mg of ammonia-nitrogen per liter; □, 5 mg of ammonia-nitrogen per liter; ●, 10 mg of ammonia-nitrogen per liter.

![Figure 2](http://example.com/fig2.png)

**Fig. 2.** Nitrogen fixation by C. phaeobacteroides at different light intensities. Symbols: ×, 50 μEin m⁻² s⁻¹; ○, 20 μEin m⁻² s⁻¹; ●, 1 μEin m⁻² s⁻¹; △, no light.

![Figure 3](http://example.com/fig3.png)

**Fig. 3.** Nitrogen fixation by C. phaeobacteroides cultures at different depths in the lake.

![Figure 4](http://example.com/fig4.png)

**Fig. 4.** Uptake of [¹⁴C]bicarbonate by pure C. phaeobacteroides cultures in light (×) and darkness (●) at different depths in the lake.
fixation and of photosynthetic activity under low light intensities has also been found with algal studies (12). Because of this light limitation in the thermocline, there is probably no contribution to the organic nitrogen load in Lake Kinneret by the C. phaeobacteroides bloom.

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LITERATURE CITED


