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EXPERIMENTAL GROWTH OF RHODOBACTER CAPSULATUS ATCC 11166 PURE CULTURES IN THE NATURAL LIGHT CONDITIONS OF THE FRESHWATER COLUMN OF ROTSEE, SWITZERLAND.

A proposal for a new biological method to detect light limitation levels for phototrophic bacteria.

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RESUM

Es proposa un nou mètode utilitzant indicadors biològics *(Rhodobacter capsulatus)* per detectar els nivells de limitació de llum en medis aquàtics naturals. S'incuben 100 mL de medi amb cultiu pur de *Rhodobacter* a fondaries escollides de la columna d'aigua a estudiar. Els paràmetres quantitatius del creixement de *Rhodobacter* s'utilitzen per la determinació de la limitació de la llum utilitzant bacteris fototròfics.

SUMMARY

A new method bassed on biological indicator to study light limitation in natural waters is propossed. Pure cultures of *Rhodobacter capsulatus* in 100 mL bottles are situated in a rope at selected depths of water column. Quantitative parameters of growth are used to determine the level of light limitation.

Key words: *Rhodobacter capsulatus*, light limitation, competition, light quality, phototrophic bacteria.

INTRODUCTION

The limiting factors for phototrophic growth in fresh-water bodies have been largely discussed (Parkin & Brock, 1980, Pedrós-Alio et al. 1984). Phototrophic sulfur bacteria need sulffur compounds, as main electron donor and light as energy source. The problem is how distinguish both kinds of possible limitation.

Phototrophic bacteria are kwown to form dense layers at the chemocline/thermocline level in lakes water column. There, both light and sulfur

reduced compounds could act as limiting factors, being the reduced sulfur compounds the major problem. Also some populations of phototrophic sulfur bacteria could thrive near the bottom where light is the major limiting factor due to water light extintion and shading by the biological filter effect.

In order to determine the specific role of light and reduced compounds as limiting factors in freshwater bodies, members of *Rhodospirillaceae* were used. *Rhodobacter capsulatus* doesn't need sulfide as electron donor, and hence, could be used to discriminate the influence of light independent of the reduced sulfur compounds limitation. *Rhodobacter* can use, for growth, organic acids as electron donor.

MATERIAL AND METHODS

Spectra of pigment extracts were obtained ffrom 350-800 nm in a Uvikon 610 CL spectrophotometer. Bacteriochlorophyll a quantification was obtained following Takahashi & Ichimura (1970).

Rhodobacter capsulatus, ATCC 11166, was grown in a medium described by Bielb & Pfennig (1981) which contains enough organic acids as electron donor (succinate and acetate) to support growth. Light limitation using these strain was studied directly in the lake, placing 125 mL bottles with 1 mL inoculated medium from an early stacionary phase culture. Then, bottles where situated in a rope at 1,3,5,7,9,10,11,13, and 14.5 m depth and left there for seven days. The control bottle was placed in the dark. These experiments were done in lake Rotsee (Switzerland) together with studies on sulfur cycle in the hypolimnion and sediments (Kholer et al. 1984).

After seven days A_{650}, total number, by epifluorescence counting method and bacteriochlorophyll *a* concentration where measured.

To discriminate between a possible diferencial temperature effects on the growth of *Rhodobacter capsulatus* in lakes, experiments were made at saturating light intensities, but under controlled temperature in thermostatized water baths. Chosen temperatures were similar to the prevalent in the water column (7, 11 and 15 °C).

Light irradiance was measured with a quantameter Lambda Instruments in $\mu E\ m^{-2}\ s^{-1}$. The sensor was placed in a spherical bulb in order to integrate light from any direction (Kirk, 1977). Global light extintion was measured with a selenium cell (Megatron, England). Also, different filters were used (BG 12, VG 6 and RG 660) combined with selenium cell to measure light extinction of different parts of light espectrum.

RESULTS

Light conditions in the water column of Rotsee. Light extintion measured as % from surface incident light is shown in figure 1. Three main slopes,

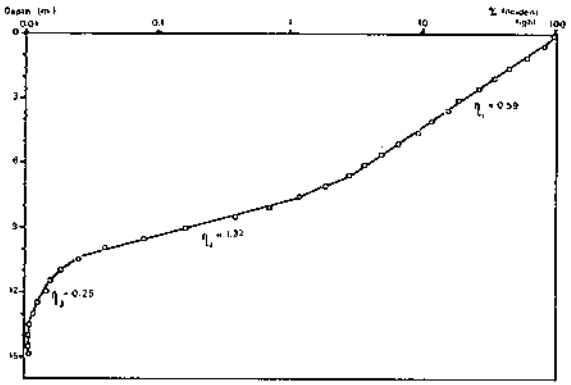

Figure 1. Global light extintion in the Rotsee water column (29.9.82). Three different extinction coeficients could be measured (η_1=0.59 from 0 to 7.5 m depth; η_2=1.32 from 7.5 to 10.5 m depth and η_3=0.25 from 10.5 to 13.5 m depth). The high extinction coefficient value from 7.5 to 10.5 m coincides with the presence of maximum concentration of pigments of phototrophic bacteria populations.

which correspond to different extinction coefficients could be seen. One from surface to 7.5 m depth, where purple phototrophic bacteria are first present (0.7 % of incident light, η=0.59). The second, from 7.5 m depth to 10.5 m (0.025 %, η=1.32) and the third slope between that depth and 13.5 m (0.012 %, η=0.25). From there to the bottom light values are out of detection by our selenium cell method.

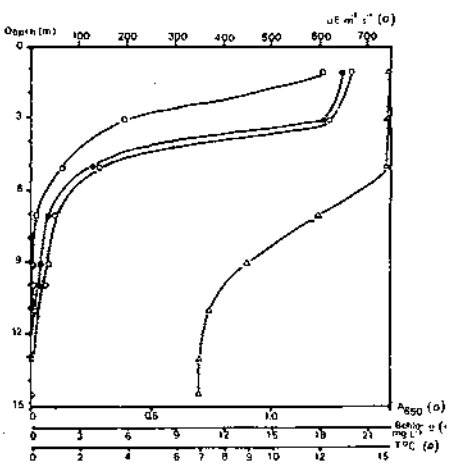

Figure 2. Light penetration as photosynthetic active radiation (PAR) (□). A_{650} (○) Bchlor *a* (mg L^{-1}) (●) and temperature distribution (°C) (△) in the water column of Rotsee (29.9.82).

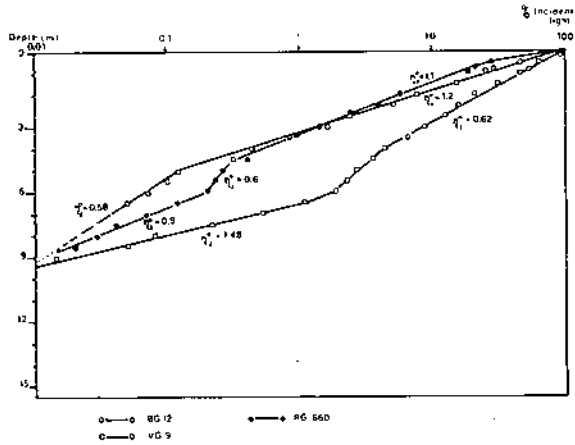

Figure 3. Extinction of filtered light at different depths in Rotsee water column. Extinction coefficient values are indicated.

In figure 2 light irradiance as photosynthetic active radiation (PAR) in $\mu E.m^{-2}.s^{-1}$ is shown. Quanta distribution show that light extinction follow a exponentially negative distribution. At 9.5-10 m depth, 0.8 to 1 $\mu E\ m^{-2}.s^{-1}$ are measured.

In figure 3 different filtered light extinction are studied. The extinction of light filtered by selected filters (see transmission spectra in figure 4), is shown. The light measured with VG-9 filter (yellow enriched light), has a higher penetration in Rotsee water column and, compared with total light extintion (figure 1) has only two slopes, one from surface to 7 m depth and the other till 10 m depth, where 520 nm light is lower than 0.01 %. Light filtered with RG 660 filter (blue enriched light) and light of BG-12 filter (red enriched light) crose their paths around 5 m depth. Between surface and these depth, blue enriched light has lower extintion coeficient. From 5

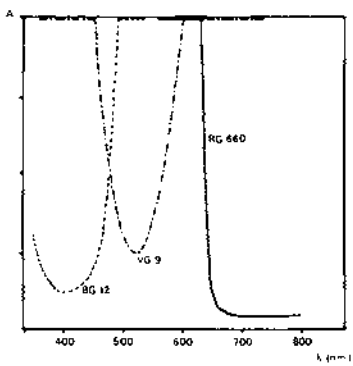

Figure 4. Visible light spectra with VG-9, RG 660 and BG 12 filters.

m, red enriched light has a lower penetration capacity. Yellow light at 10 m, red light at 9 m and blue light at 7 m, were also indetectable.

Light limitation using *Rhodobacter capsulatus* pure cultures

Also in figure 2 the results of A_{650}, bacteriochlorophyll *a* concentration together with light penetration and temperature of the water column is presented. Growth is maximum at 1 and 3 m with no light limitation, but becomes clearly limitant below 5 m depth. Scarce growth is also present at 10 m.

Since temperature also changes with depth, it can became also a limiting growth factor. To separate the effect of temperature differences, growth was quantified in the laboratory at saturated light with different temperature conditions. The results (figure 5) show that growth reaches similar yields at stacionary phase with different temperatures, indicating that temperature effects at the range of 7-15 °C are not significant in front of light saturating conditions.

DISCUSSION

The *Rhodobacter capsulatus* field experiment (figure 2), shows that light is the main limiting factor at the depths were phototrophic bacteria are found which is almost independent, in seven days incubation, of the temperature at values range of the water column of the lake (Aikin & Sojka, 1979).

The reduced sulfur compounds limitation could be important if its flow the sediment is not enough at the chemocline level.

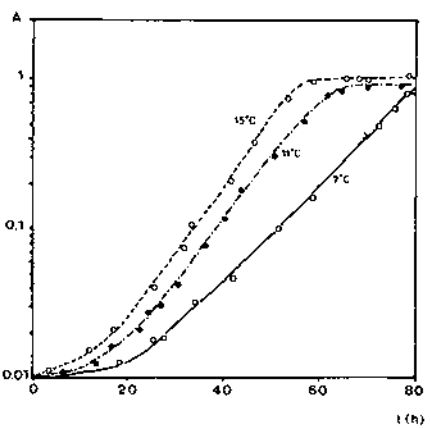

Figure 5. Temperature effect on *Rhodobacter capsulatus* at saturating light condictions. Growth was measured at 7, 11 and 15 °C, prevalent in Rotsee summer water column.

Montesinos (1982) has demonstrated that only the upper part of the phototrophic bacteria layer is active, showing the auto-shading effect of light absorbing cells.

Our results show that light limitation alone could be a limiting factor in natural conditions. Indeed, for sulfur bacteria, reduced sulfur compounds could act as limiting growth factor.

As a method, *Rhodospirillaceae* could be used for a prospective detection of light limitation conditions at different depths of lake water column. In table 1 general values of absorption, and per cent of light

Taula 1. Comparative light extinction values (in % from surface), between phototrophic populations found in Rotsee water column and *Rhodobacter capsulatus* at the depth with the minimum detectable growth (10 m).

	Algae	*Chromatiaceae (Thiopedia rosea)*	Brown *Chlorobiaceae*	*Rhodobacter capsulatus*
Light limitation at (% surface light)	1.0	0.70	0.04	0.04*
Depth (m)	7	8	10	10

* The corresponding experimental growth absorption (A_{665}) was 0.035 (a doubling time of seven days).

extintion are shown. They are related to the distribution of the different populations of photosynthetic organisms in Rotsee water column. The interest of using *Rhodobacter capsulatus* is demostrated by the fact that has light limiting levels similar to brown *Chlorobiaceae* kown by its very low light limitation level.

Abella et al. (1980), demonstrated that in lake Sisó (Spain), brown populations of *Chlorobium* could thrive under *Chromatiaceae* populations

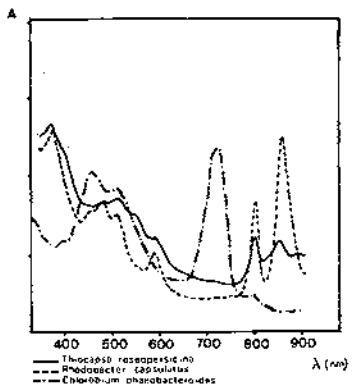

Figure 6. Absorption spectra of living cell suspensions of *Rhodobacter capsulatus, Thiopedia rosea* and *Chlorobium phaeobacteroides*.

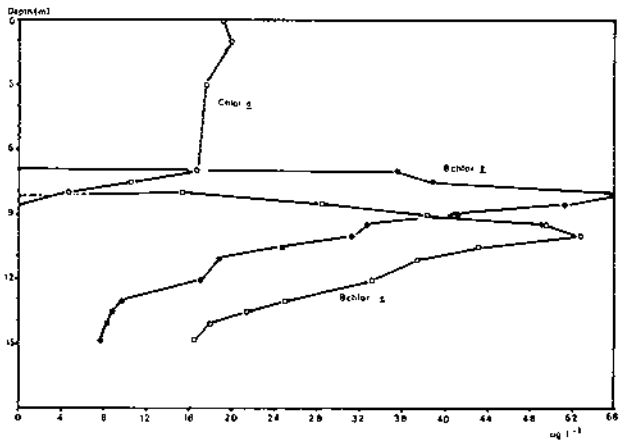

Figure 7. Pigment distribution in the water column of Rotsee (29.9.82). (○) chlorophyll *a*; (●) Bacteriochlorophyll *a* and (□) Bacteriochlorophyll *e*, corresponding to algal, *Thiopedia rosea* and «Pelochromatium roseum» populations.

even under competition for available light. In vivo absorption spectrum of *Chromatiaceae* upper layer has a shading effect on the 500-600 nm band that left only a short band of useful light for brown *Chlorobiaceae* (from 450-500 nm) (Fig. 6).

These light quality conditions are related to their carotenoid pigments, which absorb in the green part of the spectrum, okenone at 540 nm, for *Thiopedia rosea* and isorrenieratene at 458 nm for brown *Chlorobium* both found in Rotsee water column (see fig. 7). *Rhodobacter capsulatus* has spheroidenone (482 nm in vivo) and matches fairly the carotenoid band of brown chlorobia (Fig. 6).

These fact strengths the interest for using *Rhodobacter capsulatus* as a marquer strain for the «in situ» study of light limitation. The quality of light affects the strain in the same way as on brown populations found in the deepest part of lakes water column.

Acknowlegments

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