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EVOLUTION OF THE PHYSICO-CHEMICAL AND BIOLOGICAL PARAMETERS IN A MODIFIED WINOGRADSKY COLUMN

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RESUM

S'estudia la dinàmica microbiana en una columna de Winogradsky modificada en relació als paràmetres biològics i físico-químics que s'esdevenen en la columna d'aigua.
Chlorobium limicola, C phaeobacteroides i *Chromatium minus* són bacteris fototròfics del sofre amb una dinàmica poblacional ben estudiada en condicions de camp, en els estanyols del Vilar i del Sisó (Banyoles, Girona). En aquest treball es compara la dinàmica d'aquestes poblacions entre el període de màxima estratificació en l'estanyol i en la columna de laboratori. Aquesta columna de laboratori permet controlar la temperatura de l'aigua i el cicle d'il.luminació.
En condicions naturals s'estudia la competència entre bacteris verds del sofre i bacteris porpres en base a raons fisiològiques i d'adaptació pigmentària a la llum disponible. En la columna de Winogradsky modificada s'observa una primera situació de competència entre dues espècies de bacteris verds del sofre; al final de l'estudi, la competència es produeix de forma similar a les observades en condicions de camp. Aquest fet seria provocat pel caràcter més oportunista dels bacteris fototròfics verds del sofre enfront les Cromatiàcies.

RESUMEN

Se estudia la dinámica microbiana entre poblaciones de bacterias fototróficas del azufre en una columna de Winogradsky modificada.
Chlorobium limicola, C phaeobacteroides y *Chromatium minus* son bacterias fototróficas del azufre con una dinámica poblacional bien estudiada en condiciones de campo. En este trabajo se compara la dinámica de estas poblaciones en el período de máxima estratificación en el campo y en la columna de Winogradsky modificada. Esta columna de laboratorio permite controlar el ciclo de iluminación y la temperatura del agua.
En estudios de campo, la competencia entre bacterias del azufre se establece entre poblaciones de bacterias verdes y bacterias púrpuras o Cromatiáceas. En la columna de laboratorio, la competencia se establece en un primer momento, entre especies de bacterias verdes del azufre y finaliza con la coexistencia entre poblaciones de bacterias verdes del azufre y Cromatiáceas. Las poblaciones de bacterias verdes del azufre se manifestan más oportunistas que las Cromatiáceas.

ABSTRACT

The microbial dynamics of phototrophic sulfur bacteria was studied in a modified Winogradsky column. *Chlorobium limicola, C phaeobacteroides* and *Chromatium minus* are phototrophic sulfur bacteria which population dynamics were well know on lake conditions in Sisó and Vilar lakes (Banyoles, Girona).
Evolution of these populations were studied in order to compare stratification both in lake and laboratory column. Laboratory water column was stratified in similar way to lake

column. Modified Winogradsky column has and electrical mechanism to stablish a thermal gradient and illumination cycle control.
In lake conditions, ecological competition were stablished between green and purple sulfur bacteria. In this work, we found previous competence between both *Chlorobium (C. limicola* and *C. phaeobacteroides)* and before the following competence were with *Chromatium minus* and *Chlorobium limicola*. These were due to mixing sediments to Vilar lake with *Chromatium minus* and *Chlorobium limicola* and Sisó lake with *Chromatium minus* and *Chlorobium phaeobacteroides* as phototrophic sulfur bacteria. The green sulfur bacteria was more fast to useful physico-chemical factors for grown than Chromatiaceae.

Keywords: Laboratory column experiments, Chlorobium limicola, Phototrophic Sulphur Bacteria, Chlorobium Phaeobacteroides, Chromatium minus competencia, Physico-chemical factors.

INTRODUCTION

Physico-chemical conditions of aquatic environments determine the presence of phototrophic sulfur bacteria populations (Abella & Garcia-Gil 1988). Laboratory columns for microbial studies of bacteria were developed by Sergei Winogradsky in 1880 (Winogradsky 1949).

The Winogradsky column consists of a glass cylinder tube (usually 50 cm x 15 cm). It can be prepared by filling one-third full with sediment rich in organic matter, preferably sulphide containing. The mud is covered with lake, pond or ditch water and placed in a north window so as to receive adequate sunlight. As a result of the production of sulphide, purple and green sulfur bacteria develop on the outer layer of the mud exposed to light. For isolation and enrichment of purple and green sulfur bacteria, the Winogradsky column has traditionally been used (Broock and Madigan 1988).

Recently a new large (1 m, 2 m , 20 m) laboratory columns have been developed for studying phytoplankton ecophysiology, Margalef (1963), Strickland et al. (1969), Booker et al. (1976), Booker & Walsby (1979, 1981), Heaney et al. (1989), Andersen and Nival (1989), how described the importance of a population enriched in a laboratory column to the limnological studies of aquatic systems in their natural environments.

In this work, we describe evolution of physico-chemicals factors as well as biological factors which occurred in a modified Winogradsky column. This column 130 cm in length enable us to control temperature and the illumination cycle.

MATERIAL AND METHODS

The column utilized was described previously by Pibernat et al (1991) with an illumination cycle about 12 hours light/dark. Thermical gradient occured at 50 cm and the intensity of gradient was selected about 14 °C/m.

Sediment used was formed by mixing half parts of compact and suspended sediment from Sisó and Vilar lakes respectively. The sediment was also mixed with 1% nutritive broth and 9% $CaSO_4$ as a source of sulphate (Schegel 1987). Water was taken out of "Riera Castellana" stream (Banyoles, Girona).

Water samples were collected at week periodicity with a peristaltic pump. Sampling rate was 0.4 - 0.6 l/h in order to keep water stratification.

Measures:

Temperature and conductivity were measured with a Crison thermometer T-637 and conductivimeter 523 respectively. Density was calculated by Bürher and Ambühl (1975) formula. Light intensities were measured by a photocell Megatron with lectures were on luxs. Sulphide was measured by a methyl blue method (Pachmayer 1960). The pH was measured by a pHmeter Crison 506, redox power was measured by a glass electrode with Pt Metrom 6.0401.100 and oxygen was measured by a Yellow Spring model 57 oxymeter.

Biological parameters as photosynthetic pigments samplings were studied folloowing the methods described by Montesinos (1982). Cells were observed through a Zeiss microscope.

RESULTS

Evolution of physico-chemical factors of water were studied in a modified Winogradsky column together with photosynthetic pigments of phototrophic bacteria.

Figures 1, 2 and 3 shows time-depth representation of temperature (°C), conductivity ($mS.cm^{-1}$) and density ($g.cm^{-3}$) respectively in a modified Winogradsky column. These parameters appeared as density discontinuities such thermocline with an intensity gradient of 14 °C. m^{-1} and chemocline with 2 $mS.cm^{-1}.m^{-1}$. Modified Winogradsky column was stratified in similar way as meromictic lake water column.

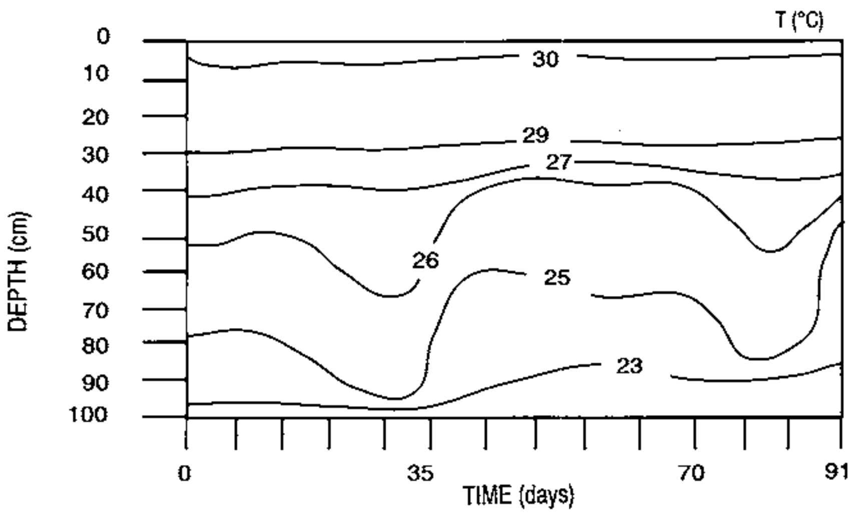

Figure 1. Time-depth representation of temperature (°C) in a modified Winogradsky column.

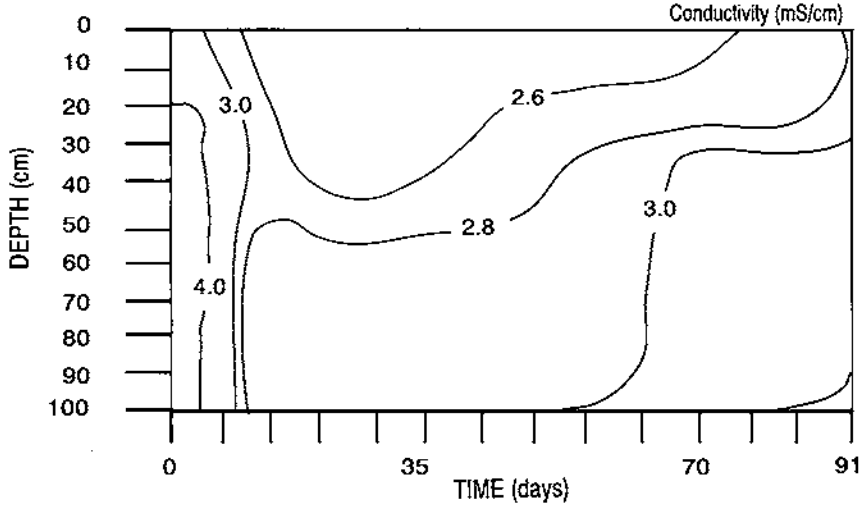

Figure 2. Time-depth representation of conductivity (mS/cm) in a modified Winogradsky column.

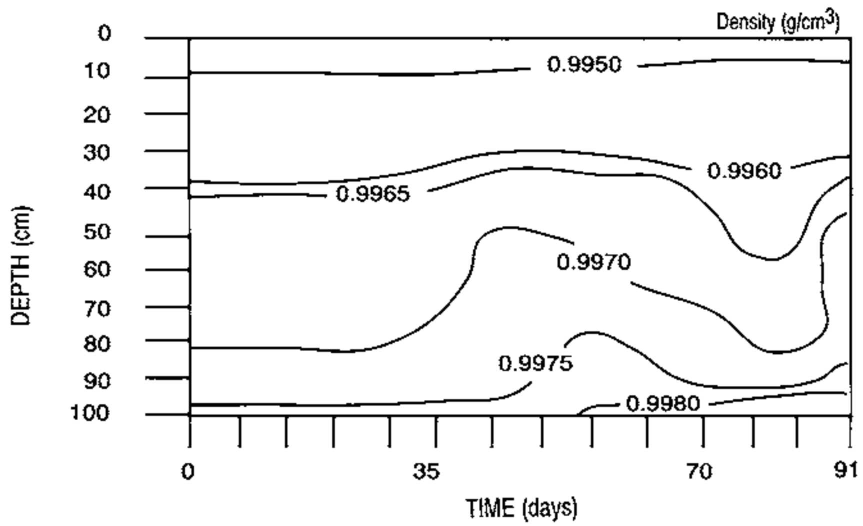

Figure 3. Time-depth representation of density (g/cm3) in a modified Winogradsky column.

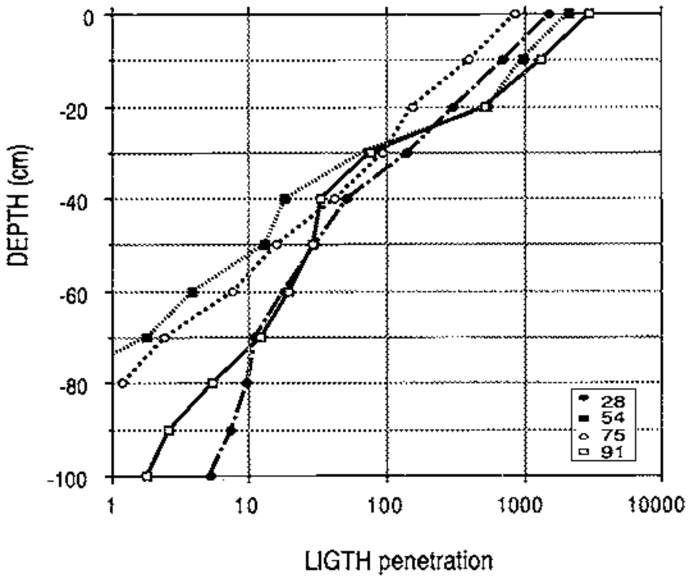

Figure 4. Representation of ligth penetration in water column on time.

Bacterial photosynthesis depends closely on light intensity and quality as well as H-donor, nutrients and temperature. In aquatic environments, light intensity decreases exponentially with depth, and the energy distribution of light also changes with depth. Figure 4 shows light penetration in a water laboratory column. The light profile in the column is also modified by photosynthetic pigments of phototrophic sulphur bacteria. Light extinction coefficient (2) shows the variation in the hypolimnetical zone. The 2 = 90 cm-1 at first time, between 20 and 30 cm-1 when populations of photosynthetic sulfur bacteria grew and 2 = 125 cm-1 at the end of this study.

Time-depth evolution of pH, redox potential and sulphide concentration were shown at figures 5,6 and 7 respectively. These factors describe main chemical conditions which explain the establishment of phototrophic sulfur bacteria and the following evolution.

pH:

Water original came from Banyoles lake area and has a shightly alcaly pH, 7.6. The pH we found in Winogradsky column 7.2 and 7.4 at the half water column is due to sulphide concentration. Sulphide is a weak acid and it's formed by sulphate reduction metabolism.

Near to sediment pH was 8.0 because was formed an accumulation area by concentration of sedimented particles and diffusion of substractes from sediment (Riera 1987).

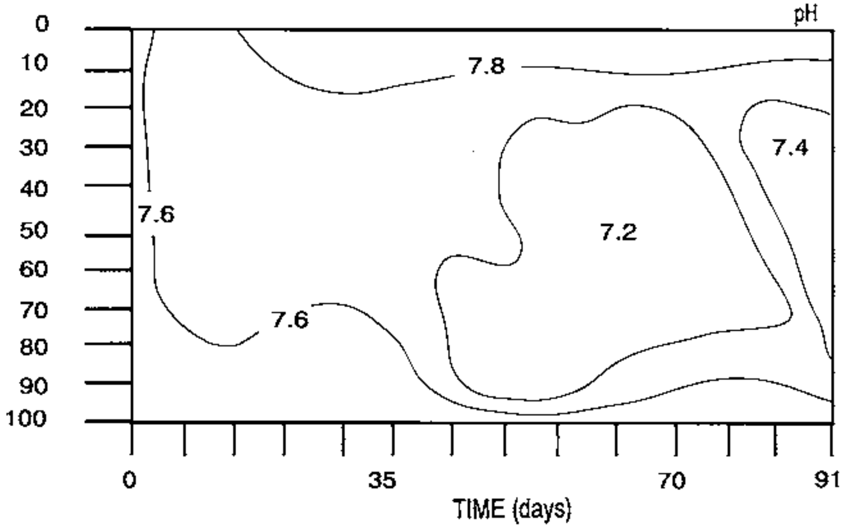

Figure 5. Time-depth representation of pH in a modified Winogradsky column.

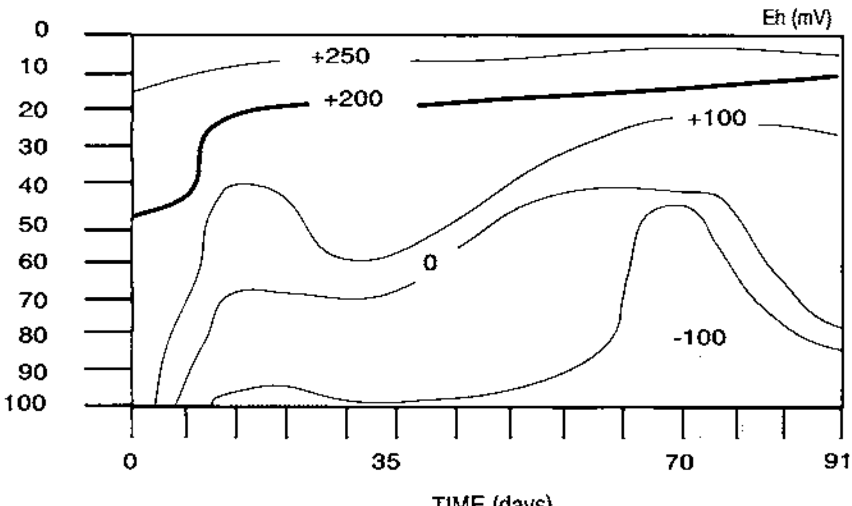

Figure 6. Time-depth representation of redox potential (mV) in a modified Winogradsky column.

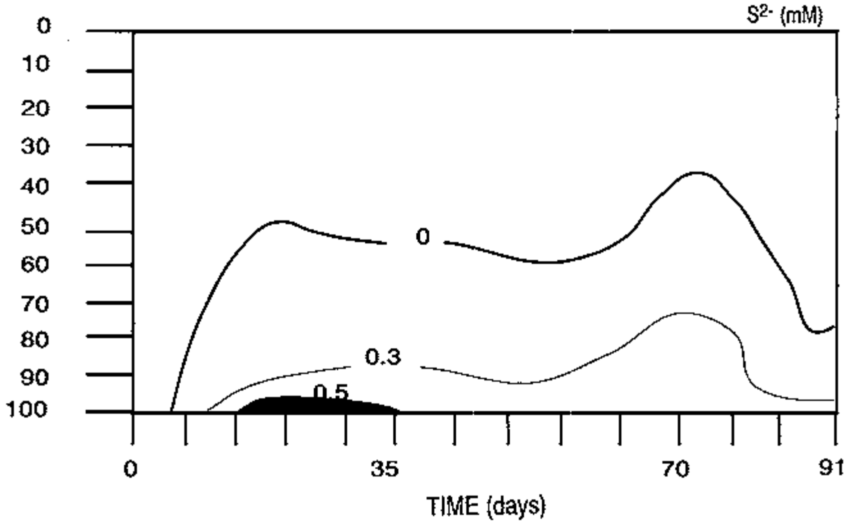

Figure 7. Time-depth representation of sulfide concentration (mM) in a modified Winogradsky column.

Redox:

The redox potential decreased in depth with time. Decreasing is due to bacterial metabolisms and presence of sulphide in the water column. The upper part of column rested aerobic but the bottom part remained anaerobic. The zone between aerobic and anaerobic life is formed by density gradient and indicated by line of + 200 mV redox potential.

Sulphide:

Sulphide appeared in the column after 7 days from the beginning or the study and suddenly high concentration were measured. When phototrophic sulfur bacteria developed, sulphide concentration decreased by consumption. Presence of sulphide in the water column occured during 91 days.

Figure 8 showed time-depth representation of photosynthetic pigments of *Chlorobium phaeobacteriodes* a brown phototrophic sulfur bacteria. Figure 9 showed photosynthetic pigments of *Chlorobium limicola,* a green sulfur bacteria; and figure 10 showed time-depth representation of Bclor-a and okenone concentration to *Chromatium minus,* purple sulfur bacteria.

DISCUSSION

– PHYSICO-CHEMICAL FACTORS:

In aquatic systems vertical heterogenity is created by density discontinuities such as thermocline and chemocline. These barriers result in a very effective differentiation of the water masses above and below the discontinuity. In stratified lakes, the

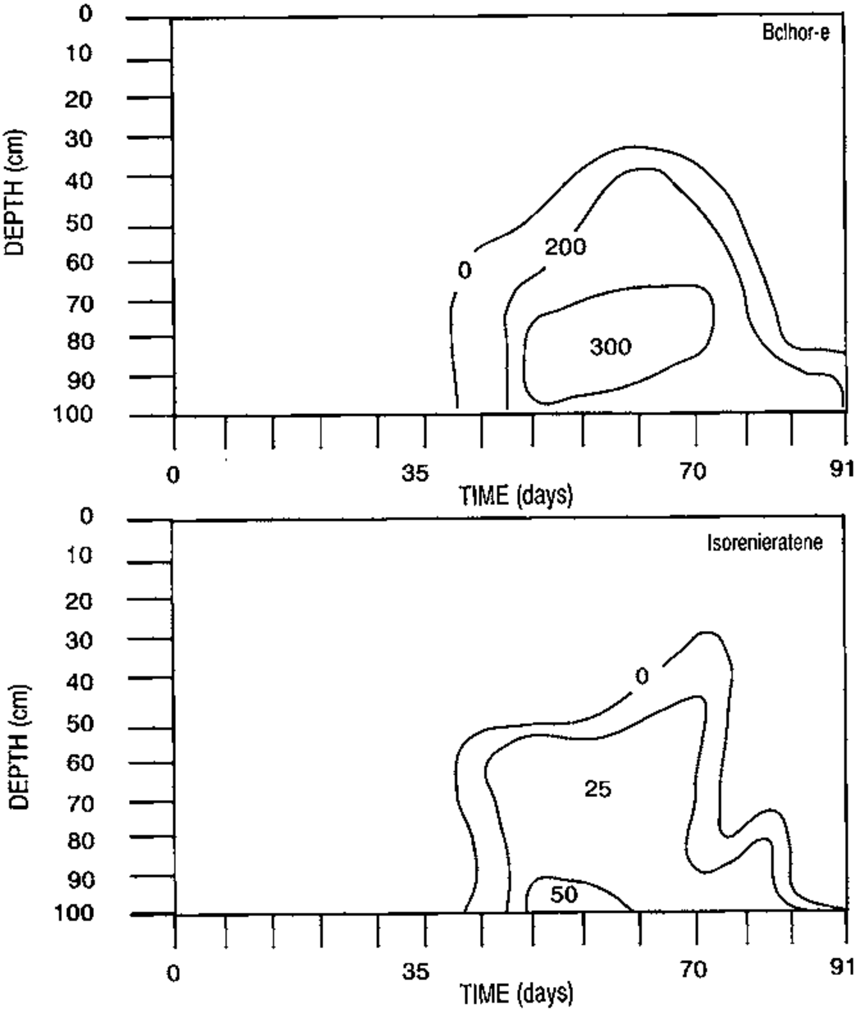

Figure 8. Time-depth representation of Bacteriochlorophyll-e (μg/l) and Isorenieratene concentration (U.A./l) in a modified Winogradsky column. Photosynthetic pigments of *Chlorobium phaeobacteroides*.

hypolimnion frequently becomes anaerobic. In the water laboratory column used, density discontinuities were created according to natural environments. Density gradient was stronger than natural ecosystem because the depth of the column was shorter (1.30 m in the laboratory column to 13 m in lake water column).

Chemical factors had been evolutioned in relation to prevail metabolism. According to the modified chemistry of the environment the population of microorganisms

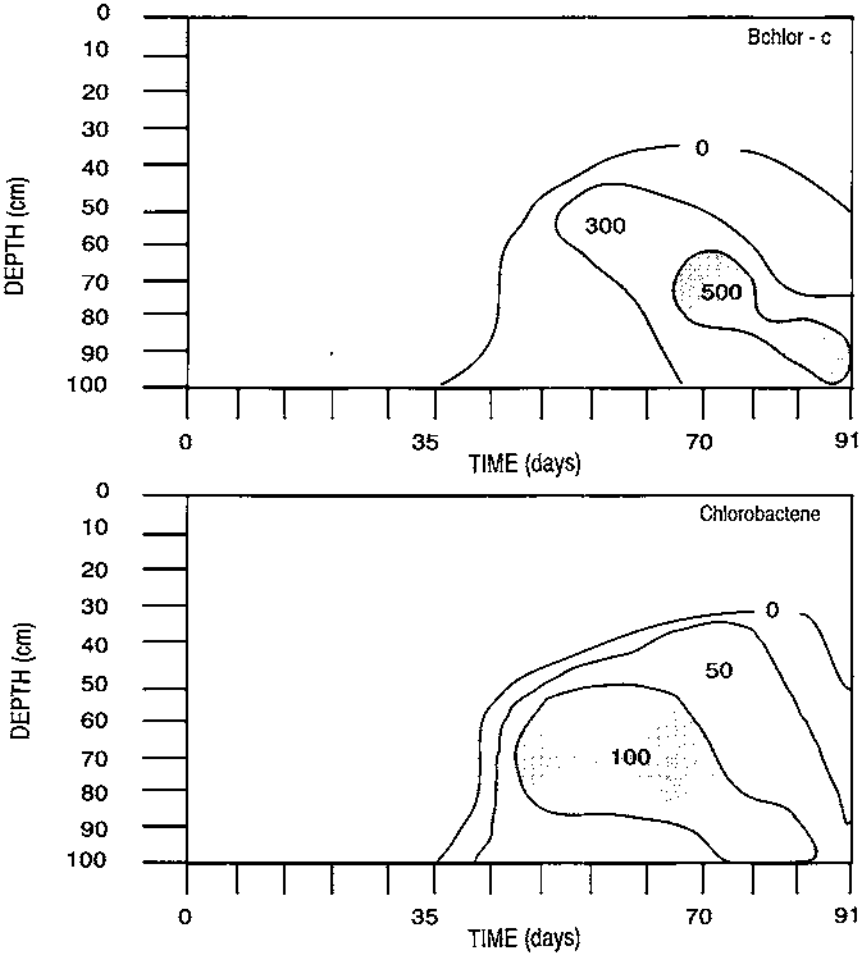

Figure 9. Time-depth representation of Bacteriochlorophyll-c (μg/l) and Chlorobactene concentration (U.A./l) in a modified Winogradsky column. Photosynthetic pigments of *Chlorobium limicola*.

was changed. When sulphide was present in the water column, the redox potential was negative, and the population of phototrophic sulfur bacteria began to develop.

- BIOLOGICAL FACTORS:

Populations of phototrophic sulfur bacteria developed were *Chlorobium limicola*, *C. phaeobacteroides* and *Chromatium minus* in the modified Winogradsky column.

Green phototrophic sulfur bacteria appeared 20 days before Chromatiaceae. In lake conditions, maxima values of Chromatiaceae and green sulfur bacteria were

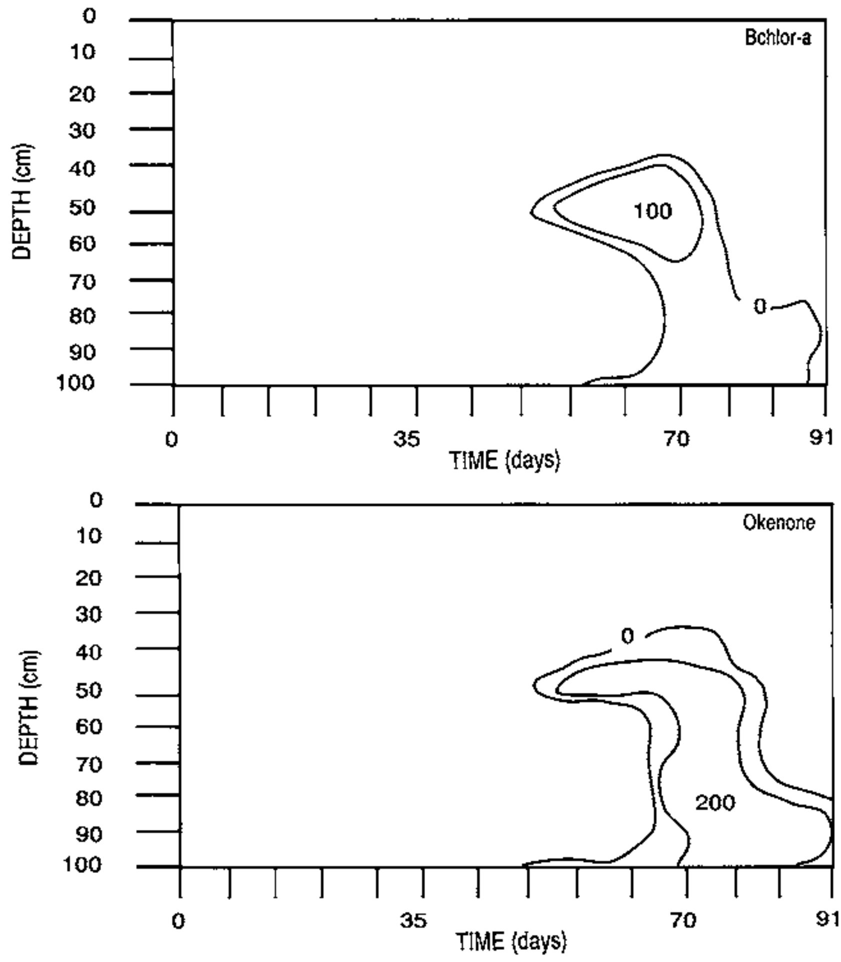

Figure 10. Time-depth representation of Bacteriochlorophyll-a (μg/l) and Okenone concentration (U.A./l) in a modified Winogradsky column. Photosynthetic pigments of *Chromatium minus*.

shift 15 - 30 days (Montesinos 1982). Growth of green phototrophic bacteria was fast 14 - 20 days on Vilar lake (Montesinos 1982) and 16 days in CIV of Banyoles lake (Garcia-Gil et al. 1987). Generation time of Chromatiaceae at lake conditions was 23 days on Vilar lake (Montesinos 1982).

Three bacterial populations developed in the modified Winogradsky column appeared in lake environment of which the sediment was used (Abella 1980).

The main difference between lake dynamics and laboratory column population dynamic was the simultaneous presence of *Chlorobium limicola* and *C. phaeobacte-*

roides. In Sisó lake, *Chromatium minus* grewd during summer whereas *Chlorobium phaeobacteroides* had the minimum population. In Vilar lake, the same population dynamics had been described (Montesinos 1982, Abella 1980, Guerrero et al 1980), but *Chlorobium limicola* appeared when the algal population of epilimnion were not abundant.

C. limicola and *Chromatium minus* coexist under laboratory conditions (van Gemerden and Beeftink 1981) because don't light competition for their photosynthesis.

In this work the population of green phototrophic bacteria, *C. phaeobacteroides* and *C. limicola* coexist in competition during approximately 20 days. These bacteria present a physiological competition for sulphide and for available light. *C. limicola* occuped the upper part of hypolimnion and *C. phaeobacteroides*, the bottom part, near to sediment where sulphide concentration was maxima. After a few days, *Chromatium minus* appeared at the upper part of hypolimnion and the brown sulfur bacteria disappeared for light competition and coexist green sulfur bacteria at the bottom part of column with Chromatiaceae at the upper part.

Purple sulfur bacteria (Chromatiaceae) and green sulfur bacteria thrive in the anaerobic parts of many ponds and lakes. Under suitable conditions, these bacteria develop profusely. Such blooms of phototrophic bacteria are often reported to be mixed population of green and purple sulfur bacteria (Takahashi and Ichimura 1970, Gorlenko et al. 1975, Guerrero and Abella 1978). For the composition of such mixed natural blooms a variety of parameters can be expected to be of importance. In this study we focused on the dynamics of population under physico-chemical conditions.

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