

INCIDENCE OF THE IRON CYCLE ON THE DEVELOPMENT OF SULPHUR PHOTOTROPHIC BACTERIA IN LAKE VILAR

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RESUM

En el treball present s'estudia la desaparició d'una població de *Chlorobium phaeobacteroides* i l'aparició simultània d'una de *Chromatium minus* a l'estanyol del Vilar a l'estiu de 1987. La presència de Chlorobiaceae estava lligada a condicions de baixa concentració de sulfhídric (0.054 mM), mentre que la població de Chromatiaceae comença a desenvolupar-se quan la concentració de sulfhídric va augmentar (0.160 mM). Això va produir una limitació en la qualitat i en la quantitat de la llum que rebien les Chlorobiaceae, implicant-ne el creixement. Alhora vam detectar un increment en el contingut específic de ferro particular (SCPI), a mesura que l'efecte d'ombrejament de les Chromatiaceae es feia cada vegada més important.

RESUMEN

En este trabajo se estudia la desaparición de una población de *Chlorobium phaeobacteroides* y la aparición simultánea de una de *Chromatium minus* en la laguna del Vilar en el verano de 1987. La presencia de Chlorobiaceae estaba ligada a condiciones de baja concentración de sulfhídrico (0.054 mM), mientras que la población de Chromatiaceae empezó a desarrollarse cuando la concentración de sulfhídrico aumentó (0.160 mM). Este hecho produjo una limitación en la calidad y en la cantidad de luz que recibían las Chlorobiaceae, lo que impidió su crecimiento. Al mismo tiempo, se detectó un incremento en el contenido específico de hierro particulado (SCPI), a medida que el efecto de sombreado producido por las Chromatiaceae se hacía cada vez más importante.

ABSTRACT

The present paper deals with the disappearance of a population of *Chlorobium phaeobacteroides* and the simultaneous appearance of a population of *Chromatium minus* in the lake Vilar during the summer of 1987. The presence of Chlorobiaceae was related to conditions of low sulphide concentration (0.054 mM); Chromatiaceae started to develop when the sulphide concentration increased (0.160 mM). This produced a limitation in the quality and quantity of the light received by the Chlorobiaceae, and therefore, they could not keep on growing. At the same time, an increase in the specific content of particulated iron (SCPI) in Chlorobiaceae was detected, while the shadowing effect due to Chromatiaceae was becoming more important.

Key words: FeS, light competition, phototrophic bacteria, poblational succession.

INTRODUCTION

Recent papers have dealt with the relation between the iron cycle and phototrophic bacteria in Banyoles lake, a quite new aspect of the eco-physiology of these microorganisms (Garcia-Gil & Abella 1988, Garcia-Gil et al., 1988). Ferrous iron is released mainly from both the compact sediment, by leaching processes, and the suspended sediment of the bottom springs, by the incoming water. This water contains dissolved ferrous iron, which diffuses through the hypolimnion or monimolimnion (depending on the kind of lake, holomictic or meromictic, respectively). When iron reaches the oxygenated layers is then oxidized to ferric iron which forms insoluble yellowish hydroxides. If there is a high flux of incoming water, the supply of dissolved oxygen (1 mg.L^{-1}) is high enough to oxidize all the ferrous iron; this happens in the bottom springs 1 and 7 of the Banyoles lake, in which ferrous iron is almost inexistent (unpublished data).

In the same way, in lake Vilar it is not possible to find free ferrous iron in the monimolimnion, due to the precipitation with the sulphide produced by the sulphate-reducing activity of the sediment. Sulphide concentration, 3.8 mM (Montesinos et al., 1983), is much higher than iron concentration, $7.8 \text{ }\mu\text{M}$. Therefore, sulphide combines with ferrous iron to produce amorphous insoluble iron sulphide particles.

It has been reported by several authors (Van Gernerden 1984, Brugada 1986) that green sulphur bacteria (Chlorobiaceae) of the genus *Chlorobium* tolerate the highest sulphide concentration ($4\text{--}8 \text{ mM}$), in front of the purple sulphur bacteria (Chromatiaceae) of the genera *Chromatium*, *Thiocystis* and *Thiocapsa* ($0.8\text{--}4 \text{ mM}$) and the purple non-sulphur bacteria (Rhodospirillaceae, $0.4\text{--}4 \text{ mM}$) (Pfennig, 1975). Moreover, it is well known that species of the genus *Chlorobium* have high affinities for sulphide (around $70 \text{ h}^{-1}\text{mmol}^{-1}$), specially *Chlorobium phaeobacteroides*, which reaches values of $120 \text{ h}^{-1}\text{mmol}^{-1}$ (Van Gernerden, 1984).

The dominant species in the monimolimnion of the lake Vilar is the brown freshwater Chlorobiaceae *Chlorobium phaeobacteroides*, reflecting the light limitation produced by the algae living in the mixolimnion. These algae act as a natural filter and have a strong absorption in the wavelengths between 400 and 500 nm ; this kind of light is absorbed by brown Chlorobiaceae (maximum peaks at 470 and 520 nm), but not by green Chlorobiaceae (maximum at $440\text{--}450 \text{ nm}$) which cannot develop under these conditions (Montesinos et al., 1983). Therefore, the bacterial population and its dynamics in a certain moment are controlled mainly by the dynamics of algae, because of its effect on the light transmission properties through the water column (Montesinos & Esteve, 1984). Other sulphur phototrophic bacteria which can be found in this lake after enrichment cultures are *Chlorobium limicola* and *Chromatium minus* (Montesinos et al., 1983).

The aim of this work is to study the establishment of a dense population of *Chlorobium phaeobacteroides* in lake Vilar during the late spring and the early summer, with conditions of extremely low sulphide concentrations in the monimolimnion (0.054 mM) as a consequence of both a total mixing period occurred last winter, and the presence of soluble iron, which combined with the low sulphide produced in the sediment (Garcia-Gil et al., 1988). Another important subject studied in this paper are the reasons for the progressive disappearance of this population and its replacement by a population of *Chromatium minus* along the summer. It took place parallelly with the fact that the concentration of sulphide in the water was increasing, exceeding the soluble iron concentration.

MATERIALS AND METHODS

Lake Vilar is located in the Banyoles karstic area in the NE of Spain (42° 08'N, 2° 45'E), near the town of Girona. It is composed by two basins each with one bottom spring feeding the lake (see figure 1).

Samples were taken along the summer in the maximal depth point of the basin one. Temperature, conductivity, oxygen and light extinction were measured *in situ*. For temperature measurements a Crison T-637 thermistor was used. Conductivity was recorded with an YSI model 33 and corrected for temperature. Oxygen concentration was measured with an YSI oxymeter. Light extinction was measured with a selenium photoelectric cell. These measurements were performed in order to determine the location of the redoxcline and the bacterial layers before starting the sampling.

Water samples from different depths were taken with a pumping system and were stored in hermetically closed bottles. All analysis were carried out in the laboratory within 24 hours after sampling. Redox potential was determined with a platinum Metrohm 6.041.100 (JB) electrode. pH was measured with an Orion glass electrode (ORION 81-85). Total iron was determined according to the thiocyanate method (Vogel, 1978). Iron sulphide was measured in the following way: water samples were filtered, and the filter was kept for one hour in acidified water (950 mL of distilled water + 50 mL of sulphuric acid). Because of this treatment, iron solubilized in the form of iron (II), which was analysed by the o-phenantroline method (9.5 mL of treated sample + 0.5 mL o-phenantroline, measuring absorption at a 510 nm wavelength) (Golterman et al., 1978).

Samples for sulphide analysis were stored in screw capped tubes and preserved from oxidation by addition of a known volume of SAOB II (sulphide antioxidant buffer). Analysis were performed by potentiometric methods with an Orion 94-16 sulphide selective electrode and an Orion 90-92 double junction reference electrode.

Pigments were extracted from cells retained in membrane filters with a layer of MgCO₃ with 90 % acetone according to the method of Montesinos

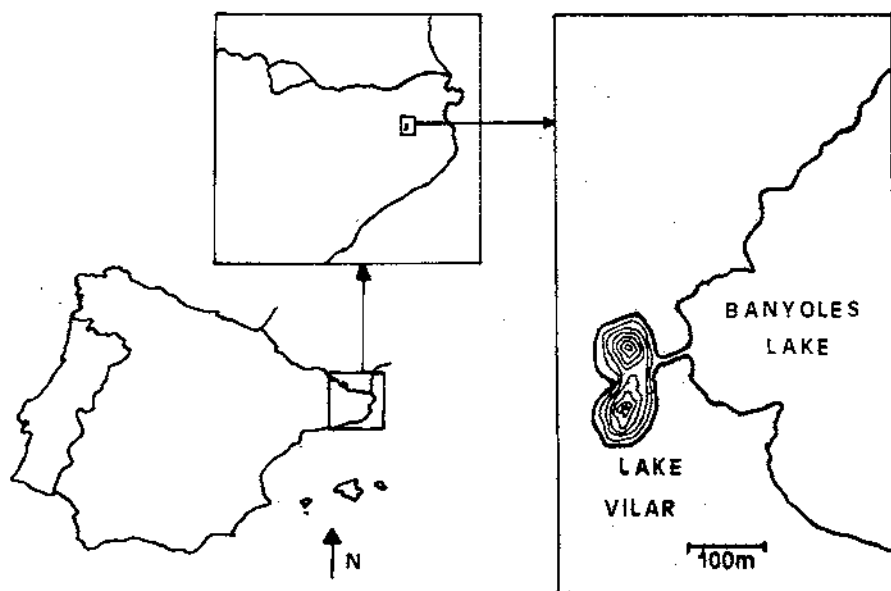


Figure 1. Location of lake Vilar.

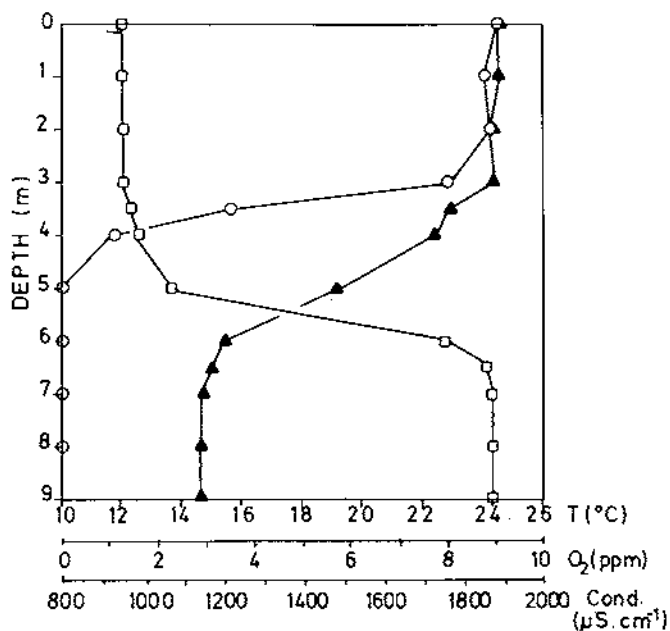


Figure 2. Physicochemical data of lake Vilar measured in August 10th, 1987.

(1982) also within 24 hours. Pigment concentration was calculated in $\mu\text{g.L}^{-1}$ using $\text{Bchlor } e = 10.2 \times (\text{A}_{650}-\text{A}_{830}) \times f$ and $\text{Bchlor } a = 25.2 \times (\text{A}_{775}-\text{A}_{830}) \times f$, where $f = v/V \cdot d$ and v is the extract volume in milliliters, V is the sample volume (filtered) in liters, and d is the pathlength of the used cuvette (Smith & Benitez 1955, Takahashi & Ichimura 1970, Gloe et al. 1975).

RESULTS

The physico-chemical data (Fig. 2) show a thermocline between 4 and 6 m depth, and a sharp redoxcline between 5 and 6 m depth. Oxygen is completely depleted below 5 m, where sulphide is formed. Light penetration is less than 0.8 % at 5 m depth, as a consequence of the strong algal absorption.

When FeS and bacteriochlorophyll «e» (Bchlor «e») values corresponding to the early summer are plotted together, a close relationship can be observed (figure 3a). Profiles present similar shapes, with both peaks almost coinciding. This relation disappears with depth due to the light limitation, and also with time, because of the disappearance of the *Chlorobium phaeobacteroides* population along the summer. Chromatium cells, which become dominant, do not show the same feature (figure 3b). Therefore, at the end of the summer it is possible to distinguish clearly the bchlor *a* peak from the FeS peak, which is found below. Tridimensional plots of FeS, bchlor *e* and bchlor *a* were performed in order to visualize their spatial-temporal evolution (figures 4,5 and 6). In these graphics the coincidence between the appearance of Chromatiaceae and the decreasing of Chlorobiaceae populations is observable.

Sulphide concentration reaches its maximum value (0.16 mM) (see table 1) at the end of July at 5.75 m depth, and moves downwards to 6 m depth, due to the activity of the dense *Chromatium* population, which established between 4.75 and 5.25 m depth. A portion of the iron-combined sulphide has to be included in these data of sulphide concentration, because of the response of the selective electrode. This feature will be discussed below.

DISCUSSION

Relation between iron and phototrophic bacteria

Our data agree with those in which iron and bchlor *e* (or bchlor *c*) peaks coincide (see figure 7). In these cases iron acts with valence 2+, independently of its chemical form, which can be soluble free cation (ferrous iron) or FeS.

Population dynamics

Tridimensional plots of pigments versus both time and depth show clearly the establishment of the population of *Chromatium minus* and the disappearance of the population of *Chlorobium phaeobacteroides*. It is due mainly to the clo-

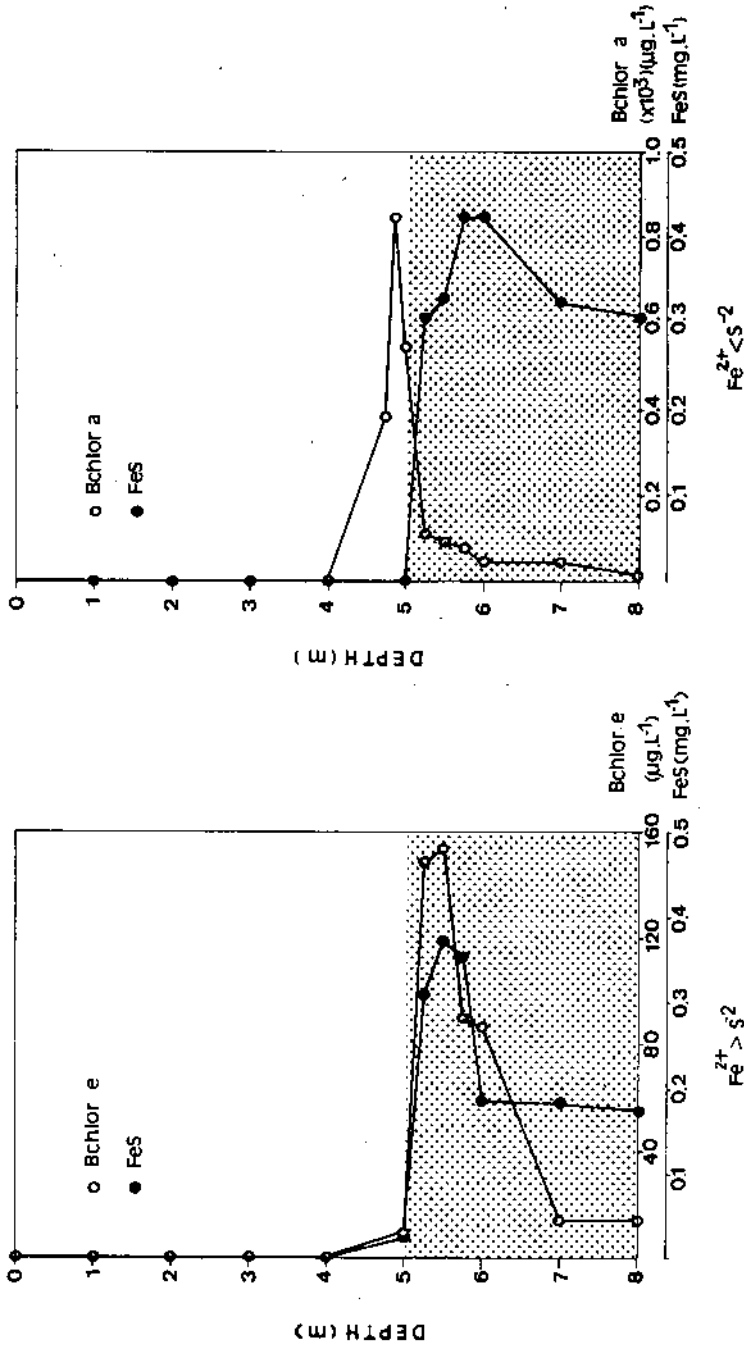


Figure 3. Vertical profiles of pigments (bchlors e and a) and FeS. Notice the correlation between bchlors e and FeS profiles (a). That could indicate the existence of some kind of physical relation between green phototrophic bacterial cells and ferrous iron. On the other hand Chromatiaceae seems to distribute independently of iron (b), since there was not a parallel peak of particulated iron in the chemocline.

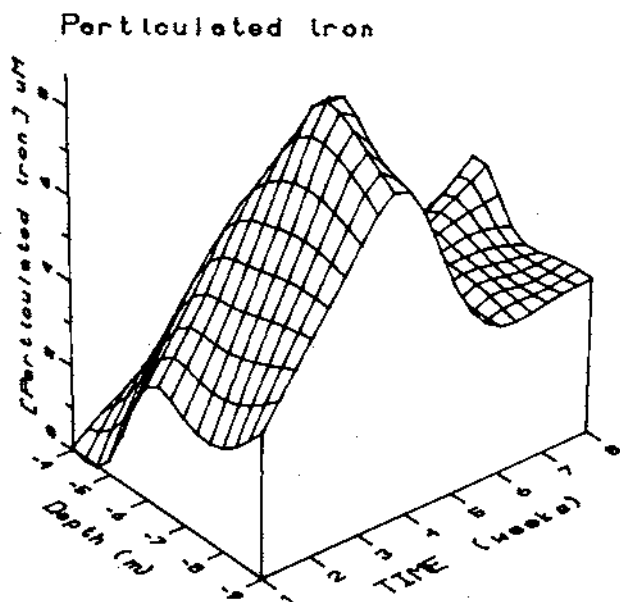


Figure 4. Spatial-temporal distribution of particulate iron (mainly FeS) from July 6th, 1987 (week number 1) till August 24 th, 1987 (week number 8).

Bacteriochlorophyll "e"

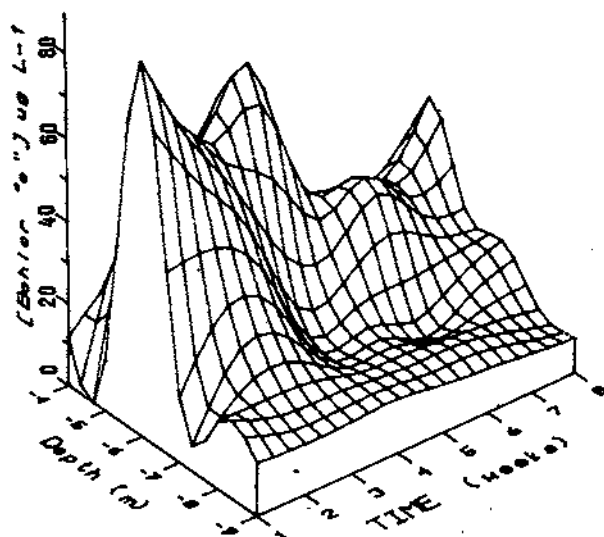


Figure 5. Spatial-temporal distribution of bacteriochlorophyll e. The decrease of this parameter in the top of the monimolimnion coincides with the start of the development of Chromatiaceae.

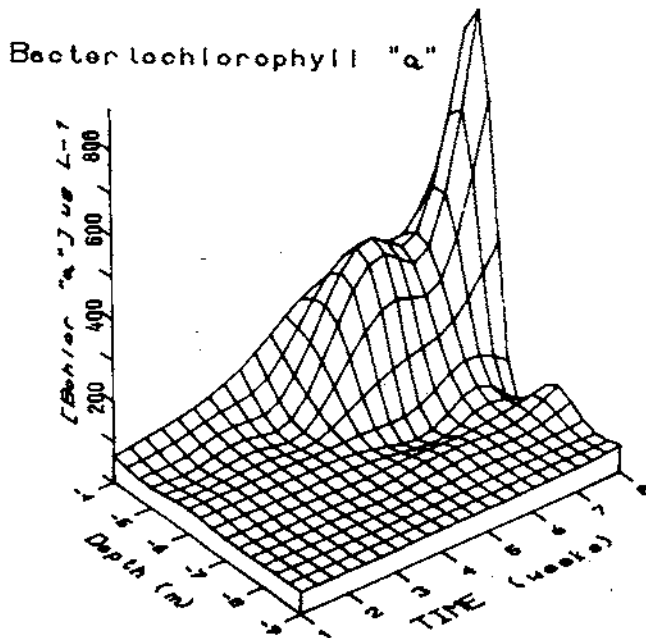


Figure 6. Spatial-temporal distribution of bacteriochlorophyll *a*. The high concentration of Chromatiaceae produces a strong shading effect, affecting to the development of Chlorobiaceae.

Table 1. Free sulphide concentration in lake Vilar at different moments

Time	FeS peak	[S ²⁻] (free)	Dominant species
1983	+?	3.800 mM	Chlorobiaceae
Winter 1987	Total mixing period		
June 1987	+	0.054 mM	Chlorobiaceae
August 1987	-	0.160 mM	Chromatiaceae

sing of the 540 nm wavelength window, which was still opened and was used by *Chlorobium* cells. There is no possibility for the establishment of a population of green Chlorobiaceae as it uses to happen many times (Montesinos et al. 1983), because of the dense algal population living in the mixolimnion.

FeS profiles

The shape of the first FeS profile, corresponding to the early summer, (figure 8, solid line) allows us to suppose that it is the result of the addition of two

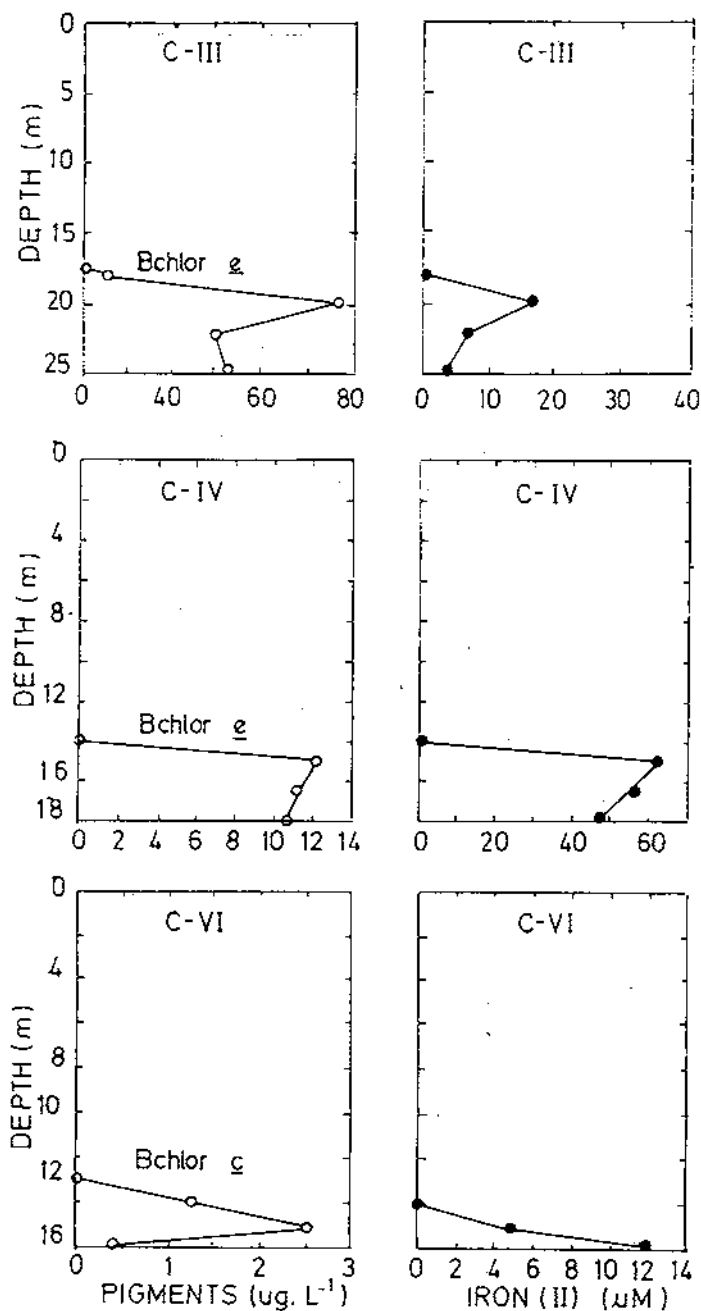


Figure 7. Vertical distribution of iron II and pigments of phototrophic bacteria in C-III, C-IV and C-VI of Banyoles lake. Bchlors e corresponds to *Chlorobium phaeobacteroides* and Bchlors c represents the population of *Chlorobium limicola*.

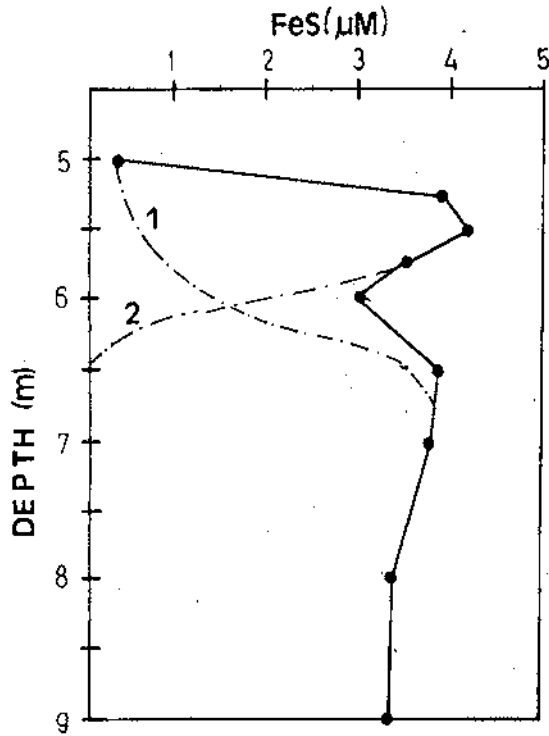


Figure 8. Typical iron sulphide profile (solid line) at date August 4th, 1987. At this moment the population of *Chlorobium phaeobacteroides* was completely developed. This profile corresponds to the measured data. FeS sedimentation profile (discontinuous line 1), and BFeS profile (discontinuous line 2) are both supposed.

independent profiles: the first is the typical profile of the FeS sedimentation (discontinuous line 1), and the second is the profile of the «bacterial» ferrous sulphide BFeS, (discontinuous line 2), this is, the FeS which accumulates in the *Chlorobium* plate. In each case we can calculate this BFeS as the measured FeS value minus an estimated value which would correspond to the one of the sedimentation profile.

This BFeS reduces as the population of *Chromatium minus* increases, just the same as bchlor *e*. We used a coefficient (SCPI, specific content of particulated iron) to give a numeric value to this feature. SCPI was calculated dividing the integrated values (along the plate) of BFeS (μM) by those of bchlor «*e*» ($\mu\text{g. L}^{-1}$), which gave the following units: $\mu\text{mol BFeS } \mu\text{g bchlor } e^{-1}$. The reduction rate did not appear to be the same for BFeS that for bchlor *e*; in fact, bchlor *e* reduced faster than BFeS. For this reason SCPI increased the summer while the light was becoming the most important limiting factor (Fig. 9). The shading effect of the Chromatiaceae cells seems to collapse Chlorobiaceae cells with ferrous sulphide (BFeS) in their surroundings.

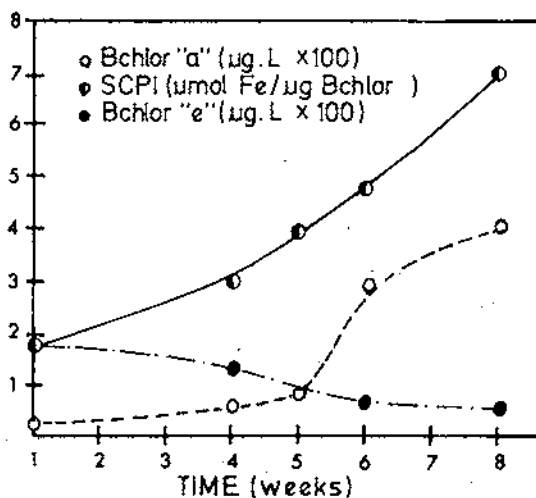


Figure 9. Population and SCPI dynamics along the summer of '87. These data are the integrated values in the bacterial plate. Notice the direct relation between SCPI and bacteriochlorophyll *a* (shading effect). Plotted values for the SCPI are one hundred times higher.

Role of the BFeS in the metabolism of Chlorobium

Recent data obtained in our laboratory show the ability of *Chlorobium phaeobacteroides* to use iron sulphide as electron donor for photosynthesis (Garcia-Gil & Abella, 1988). Light is required by *Chlorobium* cells for the release of sulphide from FeS, before its usual well-known uptake. When there is no light available, BFeS cannot be removed by the cell, and remains in its external part. This hypothesis could explain our field data presented in figures 3 and 9.

Sulphide measurements

Although the absolute concentration values are between 10 and 15 times higher for free sulphide, the sulphide and FeS profiles appear to take similar shapes. We must consider that the sulphide concentration measured does not correspond to free S^{2-} concentration only, because it includes part of the FeS. It has also been tested in our laboratory that the selective electrode responses proportionally to FeS (unpublished data). This electrode contains silver, which has a higher affinity for sulphide than iron, and this is the reason why a portion of the sulphide is removed from the iron and measured as free sulphide. So, we conclude that the real free sulphide concentration values must be lower than the measured. Nevertheless, the shape of the sulphide profile is not modified.

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