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DISSIMILATORY SULFATE-REDUCING ACTIVITY IN LAKES OF BANYOLES KARSTIC AREA (GIRONA)

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RESUM

Hem estudiat l'origen del sulfhídric en els estanys de la zona càrstica de Banyoles. L'activitat reductora de sulfats s'ha mesurat mitjançant la transformació de sulfat radiactiu fins a sulfhídric radiactiu. La màxima activitat de reducció de sulfats es detectà en els primers 10-20 cm de sediment. Els valors de reducció integrats oscil·laven entre 40 i 1245 nmol SO_4^{2-},m^{-2}.dia^{-1}. En les zones anaeròbies de la columna d'aigua l'activitat fou generalment més baixa, entre 10 i 150 mmol SO_4^{2-}.m^{-2}.dia^{-1}. Una part substancial del sulfhídric produit per reducció de sulfat era retinguda en el sediment en forma de FeS (37-98%) mentre que la resta difon a la columna d'aigua. S'ha observat una relació hiperbòlica directa entre l'activitat sulfat-reductora i el pes sec del sediment.

RESUMEN

Se ha estudiado el origen del sulfhídrico en los lagos de la zona càrstica de Banyoles. La actividad máxima de reducción de sulfatos se detectó en los primeros 10-20 cm del sedimento. Los valores de reducción integrados oscilaban entre 40 y 1245 mmol $SO\text{-}_4^{2-2}$.m^{-2}.dia^{-1}. En las zonas anaerobias de la columna de agua la actividad generalmente fue más baja, entre 10- y 150 mmol.SO_4^{2-},m^{-2}.dia^{-1}. Una parte sustancial del sulfhídrico producido por reducción de sulfatos se retenía en el sedimento en forma de sulfuros metálicos (37-98%) mientras que el resto difunde hacia la columna de agua. Se ha observado una relación hiperbólica directa entre la actividad sulfato-reductora y el peso seco del sedimento.

ABSTRACT

The origin of sulfide in lakes of Banyoles karstic area has been studied. The activity of sulfate reduction has been measured by means of transformation of radiolabelled sulfate $^{35}SO_4^{2-}$ into $^{35}S^{2-}$. The maximum activity of sulfate reduction was located in the first 10-20 cm of sediment. Integrated values accounted for 40 to 1245 mmol SO_4^{2-} m^{-2}.day^{-1}. In the anaerobic zones of the water column the activity was generally lower accounting for 10-150 mmol SO_4^{2-},m^{-2}.day^{-1}. A substantial amount of the sulfide produced by sulfate reduction was retained into the sediment as FeS (37-98%) whereas the remaining diffused to the water column. A direct hyperbolic relationship has been observed between the activity of sulfate reduction and the dry weight of the mediment.

Key words: Sulfate-reduction, karstic lakes, sediment, *Desulfovibrio*, activity.

INTRODUCTION

In lakes located in the Banyoles karstic area the most abundant anion is sulfate, because underground waters dissolve gypsum strata. Some of the lakes are crenogenic meromictic (Vilar and Banyoles III). others are holomictic (Sisó and Nou). A few kilometers distance from lake Banyoles there are two small eutrophic lakes (Negre 1 and Coromines) in which carbonate is the dominant anion whereas the concentration of sulfate is 20 to 40 times lower.

All of these lakes have in common the presence of (1) anaerobic layers of water, specially during summer stratification, which exhibit high concentration of sulfide (up to 10mM), and (2) anaerobic «black sediments» very rich in decomposing organic matter. In some cases sulfide diffuses to the very surface of the lake. Till now nobody has studied the origin of sulfide in such lakes. It seems plausible at first sight that due to the abundance of sulfate, sulfate-reducing activity may substantially contribute to the production of sulfide.

The present work tries to ascertain the contribution of sulfate-reducing bacteria to the production of sulfide and to know factors controlling this activity in lakes of the Banyoles karstic area.

MATERIAL AND METHODS

Limnological characteristics and physico-chemical analysis were assessed following the standard methods described in Strickland & Parsons (1968) and Goltermann *et al.* (1978). Primary productivity in the water column was measured *in situ* by the ^{14}C method of Steeman-Nielsen (Vollenweider 1974).

Sulfate-reduction activity was estimated by the method of Jorgensen (1978a) using $^{35}SO_4^{2-}$. Samples of anaerobic sediments have been collected with a home-made core sediment sampler. The cores were transported to the laboratory and at small depth intervals, injected with 50 μL of a solution of $^{35}SO_4^{2-}$ (40 μCi.mL^{-1}). Incubation was performed in the dark at 20 °C during 24 hours. Afterwards the cores were freezed, cutted into small pieces of 1 cm thick, suspended in oxygen-free water and anaerobically purged with nitrogen gas. The resulting $^{35}S^{2-}$ was trapped into a solution of 1 M zinc acetate. Radioactivity in the precipitate was measured by liquid scintillation counting.

Sulfate- reducing bateria were cultivated in Postgate medium in environmental chambers (Postgate, 1979).

Table 1. Physicochemical characteristics of Banyoles karstic lakes (holomictic group) in summer 1979.

		pH	Cond. (mS/cm)	Eh (mV)	SO_4^{2-} (mM)	HCO_3^- (mM)	PO_4^{3-} (mM)	NO_3^- (mM)	NH_4^+ (mM)	H_2S (mM)
Sisó	Epilimnion	7.0	2.0	+130	10.4	5.0	3.0	0.4	120	0.0
	Hipolimnion	6.8	2.1	−310	7.3	7.5	0.0	0.0	250	7.4
Nou	Epilimnion	8.8	1.8	+ 10	11.8	5.5	0.0	1.0	—	0.0
	Hipolimnion	6.8	2.1	−225	12.5	10.0	1.0	0.0	—	1.2
Coromines	Epilimnion	9.0	0.6	− 80	0.7	5.4	4.2	0.0	—	0.0
	Hipolimnion	7.8	0.8	−380	0.3	7.3	21.3	0.0	—	1.5
Negre 1	Epilimnion	8.5	0.6	−100	0.4	4.9	3.5	0.0	—	0.0
	Hipolimnion	8.0	0.8	−275	0.4	6.7	15.3	0.0	—	0.2

Table 2. Pchysicohemical characteristics of Banyoles karstic lakes (meromictic group) during summer 1979.

Banyoles	Mixolim.	8.0	1.2	+-275	5.2	3.5	0.6	2.0	5.0	0.0
III	Monimolim.	7.4	2.2	−110	9.5	5.0	0.0	0.5	90.0	0.8
Vilar	Mixolim.	8.0	1.1	+130	5,3	2.9	0.2	4.4	—	0.0
	Monimolim.	6.8	1.5	−175	11.6	7.8	1.2	0.1	—	5.9

RESULTS AND DISCUSSION

1. Physicochemical and biologial characteristics, and sulfate-reduction activity in the water column

Tables 1 and 2 show the main physicochemical characteristics of water from the six studied lakes. All the lakes have in common the presence of an hypolimnion or monimolimnion containing anoxic water with a red-ox potential between —110 and —380 mV, rich in reduced inorganic substances such as ammonium (up to 250 μM) and sulfide (up to 7.4 mM). In the lakes Banyoles III, Vilar, Sisó and Nou, sulfate and bicarbonate are the dominant anions (5 to 12.5 and 5-10 mM respectively), whereas in the lakes Coromines and Negre the concentration of sulfate was considerably lower ranging from 0.3-0.7 mM and being bicarbonate the most abundant (5.4 to 7.3 mM).

Table 3 shows the integrated concentration of photosynthetic pigments, biomass of phototrophic sulfur bacteria and primary productivity for summer 1979. The highest biomass of phototrophic bacteria has been recorded for Lake Sisó (98.3 g fresh weight.m^{-2} and 1900 mg Bchl.m^{-2}) whereas the lowest in Banyoles III (4. 2 g fresh weight.m^{-2} and 150 mg Bchl.m^{-2}). The highest productivity was observed in the lakes Nou and Vilar (1535 and

Table 3. Biological parameters of Banyoles karstic lakes in summer 1979.

		Integrated photo-synthetic pigments (Chl or Bchlor in $mg.m^{-2}$)	Biomass (fresh weight of phototrophic bact. ($g.m^{-2}$)	Integrated primary production ($mg.m^{-2}$ day^{-1})
Banyoles	Mixolimnion	39.0	—	367
	Monimolimnion	150.0	4.2	53
Vilar	Mixolimnion	45.0	—	923
	Monimolimnion	203.0	17.4	57
Sisó	Epilimnion	10.0	—	406
	Hipolimnion	1900.0	98.3	458
Nou	Epilimnion	250.0	—	448
	Hipolimnion	580.0	13.2	1087
Coromines	Epilimnion	185.0	—	279
	Hipolimnion	450.0	27.5	59
Negre 1	Epilimnion	138.0	—	683
	Hipolimnion	410.0	14.2	167

Table 4. Integrated activity of sulfate-reduction (mmol $SO_4^{2-}.m^{-2}.day^{-1}$) in the anaerobic water and sediments of several lakes of Banyoles karstic area.

Lake	Total sulfate reduction activity	Anaerobic water	Anaerobic sediments.
Sisó	190	150 (79)[a]	40 (21)
Nou	119	60 (50)	59 (50)
Vilar	1365	120 (9)	1245 (91)
Banyoles III	850	65 (8)	785 (92)
Coromines	376	15 (4)	361 (96)
Negre 1	264	10 (4)	254 (96)

[a] in brackets, percentage related to the total sulfate reduction values in the lake.

980 mg $C.m^{-2}.day^{-1}$ respectively) whereas the lowest were in the Banyoles III and Coromines (420 and 338 mg $C.m^{-2}.day^{-1}$ respectively).

The activity of sulfate reduction in the anaerobic waters is presented in table 4. The values ranged from 10 to 150 mmol SO_4^{-2},$m^{-2}.day^{-1}$ suggesting an important contribution of this process in the water column to the total sulfate-reducing activity in certain lakes (Sisó and Nou).

2. Physicochemical characteristics and activity of sulfate reduction in anaerobic sediments

Table 5 shows the main physicochemical characteristics of pore water (Eh and pH) and dry weight of anaerobic sediments as vell as the integrated sulfate reduction activity. In all cases redox potentials are negative, ranging from -110 to -400 mV and the pH from 6.5 to 7.2. Dry weight increased from the top to the bottom parts of the cores. The highest activity of sulfate reduction was found for lakes Vilar and Banyoles III (1245 and 785 mmol $SO_4^{2-}.m^{-2}.day^{-1}$) whereas the lowest were observed in lake Sisó (40.3 mmol $SO_4^{2-}.m^{-2}.day^{-1}$).

Table 5. Sediment characteristics of Banyoles karstic lakes during summer 1979.

Lake sediment	Depth (cm)	Eh (mV)	pH	Dry weight ($g.cm^{-3}$)	Integrated SO_4^{2-} reduction activity (mmol $SO_4^{2-}.m^{-2}.day^{-1}$)
Banyoles III	0	—310	7.0	0.30	785.0
	10	—350	6.7	0.40	
	20	—400	6.5	0.60	
Vilar	0	—225	7.2	0.27	1245.00
	10	—275	7.1	0.41	
	20	—275	7.1	0.55	
Sisó	0	—220	7.0	1.03	40.36
	10	—280	7.0	1.03	
	20	—150	7.0	1.55	
Nou	0	—300	6.9	0.78	59.10
	10	—250	6.9	0.90	0.90
	20	—180	7.0	0.98	
Coromines	0	—150	6.6	0.31	361.36
	10	—110	6.6	0.50	
	20	—110	6.7	0.60	
Negre 1	0	—180	7.3	0.40	254.04
	10	—300	7.0	0.55	
	20	—280	6.9	0.70	

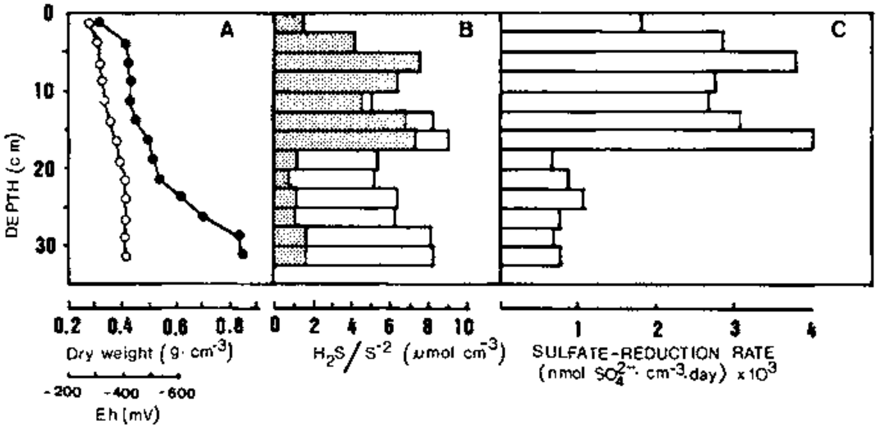

Figure 1. Vertical distribution of red-ox potential of pore water (o), dry weight (•) (panel A); concentration of free sulfide (shaded area), acid volatile sulfide (empty area) (panel B)) and the activity of sulfate reduction in anaerobic sediments of Banyoles basin III on june 29, 1979.

Figure 1 shows the vertical distribution of red-ox potential and dry weight in relation to the concentration of sulfide and the activity of sulfate reduction in a core from Banyoles III taken on June 29, 1979. The profiles and patterns are essentially the same for the other lakes and fit well with that obtained by Jorgensen (1978b). Water content decreased with depth being about 75% in the very top, to 15% at 30 cm depth. The concentration of total sulfur is relatively constant in the first 30 cm. In the first 20 cm most of the sulfur is in the form of gaseous sulfide able to diffuse to the water column, whereas below 20 cm most of the sulfur is in the form of FeS. Depending on the lake the amount of sulfide retained into the sediment as FeS ranged from 37 to 98% of the total produced. The lowest values were found for lakes Vilar and Banyoles III. In Coromines and Negre 1 more than 90% of sulfide produced was retained into the sediment. Most of the activity of sulfate-reduction was detected in all cases in the first 20 cm of sediment.

An hyperbolic relationship has been observed between the integrated rate of sulfate-reduction and dry weight of the sediment (Fig. 2). These results are consistent with the hypothesis that sediment density, and hence porosity, is an important environmental factor controlling rates of dissimilatory sulfate reduction. Sediment porosity determines not only the diffusion of sulfate (electron acceptor) and organic matter (carbon and energy source) from the water column, but also the free space that can be colonized by the microbial community. Thus the highest activity of sulfate reduction is found in sediments with low dry weight and high sulfate concentration. Only in lakes Coromies and Negres 1 sulfate concentration the water column (< 1 mM) can act as limiting factor for sulfate-reduction (see Fig. 3). On

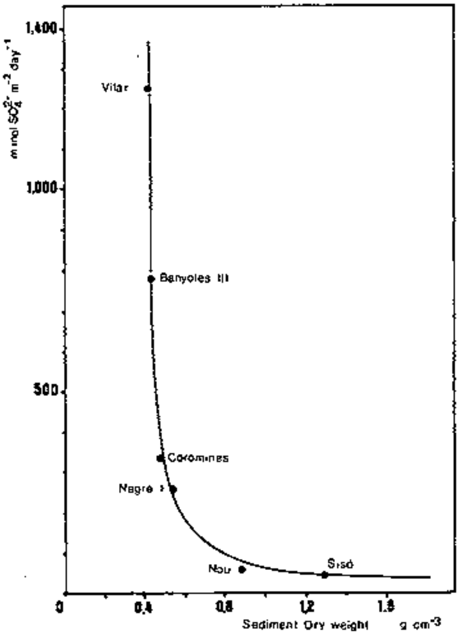

Figure 2. Hyperbolic relastionship between intgrated rate of sulfate reduction and dry weight in anaerobic sediments collected from lakes of the Banyoles karstic area (1979).

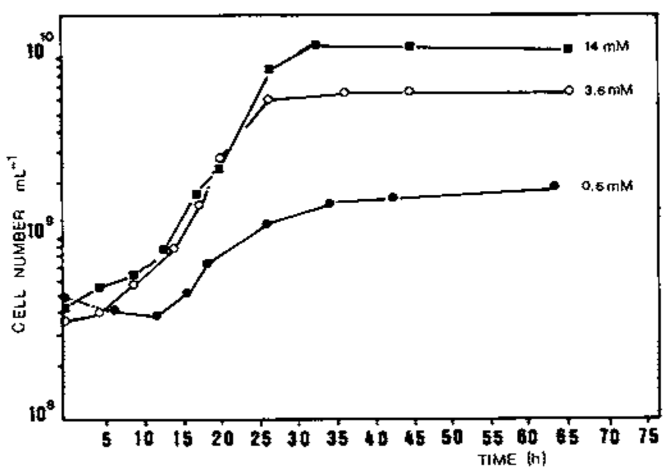

Figure 3. Growth of *Desulfovibrio desulfuricans* isolated from anaerobic sediments of Vilar lake, at different sulfate concentrations in Postgate medium. The kinetics of sulfide production follows the same pattern.

the basis of the results presented here, it is expected that the sediment density is the main limiting factor for sulfate-reduction at values higher than 0.4 $g.cm^{-3}$. Below this values, sulfate concentration and primary production in the water column are threshold factors for sulfate-reduction.

3. Isolation and growth, characteristics of sulfate-reduction bacteria

Sulfate-reducing bacteria were isolated both from the anaerobic sediments and from water samples. *In vivo* absorption spectra of clarified supernatants obtained after sonication of cells, centrifugation and alcali treatment showed the presence of two absorption peaks corresponding to citochrome c3 (420 nm) and desulfoviridin (630 nm). Fluorescence spectra confirmed also the presence of desulfoviridin, suggesting that the microorganisms pertain to genus *Desulfovibrio* (see Fig. 4). Nutritional requirements indicated that the bacteria ara able to growth in the presence of malate or piruvate as the only carbon and energy source and with sulfate as electron acceptor, thus producing sulfide. Therefore it could be determined as *D. desulfuricans* (Krieg & Holt, 1984). Optimal temperature was between 20 to 30 °C. The maximum specific growth rate of 0.179 h^{-1} (duplication time of 3.87 hours) was obtained at 30 °C, 4-14 mM sulfate. The maximum sulfide production activity in

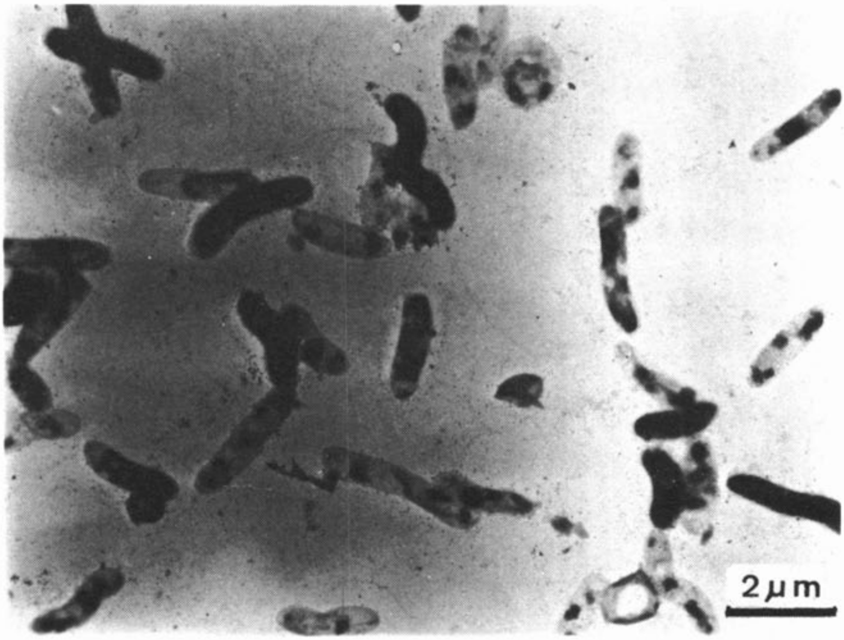

Figure 4. Transmission electron micrography of cells of *Desulfovibrio desulfuricans* in stationary phase of growth. Empty cells and flagella are present.

cultures was of about 2.35 $mmol.-L^{-1}.h^{-1}$. In figure 3 it is shown the effect of sulfate concentration on growth of *D. desulfuricans* isolated from sediments of lake Vilar. Concentrations below 4 mM sulfate are limiting for the development of *D. desulfuricans.*

Both the activity of sulfate reduction and the presence of sulfate-reducing bacteria are consistent with the idea that sulfide in lakes of Banyoles karstic comes from a process of dissimilatory reduction of sulfate.

Aknowlegments

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