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Sulfur bacteria in sediments of two coastal ecosystems: the Bassin d'Arcachon and the Etang du Prévost, France

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Key words: Sulfur bacteria, MPN counts, vertical distribution, marine sediments, oxygen, sulfide, eutrophication

Abstract

Enumeration of the functional groups of sulfur bacteria was performed in the sediments in the Bassin d'Arcachon, a mesotidal lagoon with strong tidal currents and dominant populations of seagrass (*Zostera noltii*), and in the Etang du Prévost, a shallow lagoon with moderate tidal fluctuations and dominant populations of floating seaweed (*Ulva* sp.). In addition, data were collected on the distribution of oxygen and sulfide at the water-sediment interface during diel cycles. Bacterial enumeration studies revealed highest numbers in the top two cm of the sediments for three functional groups of sulfur bacteria, these being the sulfate-reducing bacteria (SRB), the colorless sulfur bacteria (CSB), and the phototrophic sulfur bacteria (PSB). In both systems high numbers of SRB were encountered, suggesting ample availability of organic matter. A comparison between different sites in each ecosystem showed that sediments overlain by more stagnant water were dominated by PSB, whereas those overlain by more oxygenated water were dominated by CSB. Important factors are the physical forces induced by tidal currents and the degree of daily exchange of water between the lagoons and the sea. These factors may explain the differences observed between the two systems with regard to the development of anoxic conditions, more so than the level of eutrophication. It appears that rooted plants play an important role in the introduction of oxygen into the sediments, thus enhancing the competitive position of CSB compared to PSB. Mini-electrodes studies revealed high concentrations of free sulfide at the inner site of the Etang du Prévost but very low concentrations at the inner station of the Bassin d'Arcachon, which may be explained by the high iron input of the latter, rather than by differences in the rate of sulfide production.

Introduction

In the present study aspects of the sulfur cycle in two eutrophic ecosystems were compared. In the Bassin d'Arcachon, a mesotidal lagoon (i.e. lagoon with a maximum tidal amplitude of 4 m), situated on the French Atlantic coast, tidal currents are strong, resulting in a substantial daily interchange of water with the ocean. On the intertidal flats in this system the eelgrasses *Zostera noltii* Hornem. and *Ruppia* sp. are present in abundance, while the green algae *Monostroma* sp is increasingly colonizing the basin. Decaying seagrass and algae, and faecal products from the abundantly present fauna, provide a rich supply of organic matter to the sediments.

In the second ecosystem studied, the Etang du Prévost, a different situation is encountered. Due to the low tidal amplitude and the relatively small channel (Grau) between the Etang and the Mediterranean the exchange of water is extremely small compared to that observed between the Bassin d'Arcachon and the Atlantic Ocean. The Etang du Prévost receives a heavy load of nutrients from agricultural run-off and sewage. The dominant flora comprises the marine macro alga *Ulva lactuca*, which develops profusely during the growing season. In the water column anoxic conditions occur frequently during this period, when oxygen-consuming processes in the water column exceed the supply of oxygen by production and diffusion. This often coincides with the presence of sulfide in the water column, resulting in suspended elemental sulfur ('white water'). During these periods, blooms of phototrophic sulfur bacteria in the water column are frequently observed (Caumette, 1987). In terms of eutrophication, the Etang du Prévost is considered to be more eutrophic than the Bassin d'Arcachon.

In coastal environments, when high mineralization and respiration rates prevail, oxygen often becomes limiting. Under these conditions a shift to alternative electron acceptors is observed of which sulfate, due to its abundance in the marine environment, is the most important. In anoxic coastal sediments a considerable part of the organic matter (>50%) is mineralized via sulfate reduction by sulfate-reducing bacteria (SRB)(Jørgensen, 1978; Thode Andersen & Jørgensen, 1989). The H_2S produced is extremely toxic for most aerobic organisms; abiotically it can be trapped by iron and precipitated as ferrous sulfide or FeS_2 (pyrite). Sulfide can also be oxidized by two functional groups of sulfur bacteria: the colorless sulfur bacteria (CSB) and the phototrophic sulfur bacteria (PSB). The availability of electron acceptors, such as oxygen or nitrate, is important for the growth of CSB, whereas phototrophic growth of PSB depends on the availability of light. Alternatively, PSB may use oxygen during chemotrophic growth as has been demonstrated for *Thiocapsa roseopersicina* (Bogorov, 1974; De Wit & Van Gemerden, 1987), and other Chromatiaceae (Kämpf & Pfennig, 1980, 1986; Overmann & Pfennig, 1992). Similarly the okenone-containing *Thiocapsa* strain 5811, isolated from the Etang du Prévost has the potential for chemotrophic growth (Caumette et al., 1985).

The abundance of these groups is largely determined by the environmental factors described above. Sulfide oxidation by CSB and PSB is considered to play an important role in these environments, in addition to sulfide removal by FeS formation.

As part of the research programme for the Coastal Lagoon Eutrophication and ANaerobic processes (C.L.E.AN.), a project on the abundance of three functional groups of sulfur bacteria (SRB, CSB, and PSB) in the sediments of the two lagoons was carried. The hypothesis was put forward that in the Etang du Prévost these bacterial groups are more important than in the Bassin d'Arcachon, on the basis of the occurrence of 'white water' and blooms of phototrophic sulfur bacteria. Data were collected on the vertical distribution of oxygen and sulfide in both systems in the sediment and at the water-sediment interface during a full diel cycle. Special attention was paid to the impact of *Zostera* beds on the distribution of oxygen in the sediments and the influence there of on the vertical distribution of CSB.

Materials and methods

Description of systems and sites

The systems under study are both situated in France, the Arcachon Basin (Bassin d'Arcachon) is situated 80 km south-west of Bordeaux at the Atlantic coast, the Prévost lagoon (Etang du Prévost) is situated 40 km south-west of Montpellier on the Mediterranean.

The Bassin d'Arcachon is a triangular-shaped mesotidal lagoon with a total area of 156 km^2. At low tide only 40 km^2 of the basin is covered by water, from which an area of 10 km^2 is used for oyster farming. In the system, the tidal amplitude ranges from 3.90 at spring tide to 2.10 m at neap tide. The Etang du Prévost is a relatively shallow lagoon covering an area of 4.8 km^2 with a water depth ranging from 1.5 to 2 m. In each system two locations were chosen, one relatively close to the channel through which water exchange takes place (the outer station), and one distant from the channel (the inner station). In the Bassin d'Arcachon, the outer station (Station A) was situated on an intertidal flat in the center of the basin where coastal water was well mixed with basin water during each tidal cycle. The sediment at this site consisted of muddy sand. Station B was located in the intertidal area in the eastern part of the basin where little water exchange with coastal waters occurs. Sediment composition at this site was predominantly black mud mixed with some sand. The sampling sites A and B were both situated in eelgrass beds.

In the Etang du Prévost samples were taken at Stations X and 11. Station X was situated on a tidal flat near the channel (Grau) to the Mediterranean. This station was devoid of a well developed flora. The sediment consisted of grey-colored coarse sand. The second sampling site (Station 11) was situated in the western part of lagoon where the exchange of water is minimal. In these sediments (silt mixed with sand) a significant infauna was present, revealed by the large quantities of shells. At this station a dense population of floating algae was encountered, consisting predominantly of *Ulva* sp. Station 11 was continuously submersed and situated at a depth of approximately 50 cm below the water surface.

More detailed information on the different sampling stations is given in the introductory chapter of this volume. For data on seasonal variations the reader is referred to Bourguès et al. (1995) and De Wit et al. (1994).

Enumeration of functional groups, MPN methodology

Data on the vertical distribution of CSB, PSB and SRB were collected in the Bassin d'Arcachon in June and September 1993, and June 1994. The Etang du Prévost was sampled only during the two field campaigns in 1993.

Sediment samples were taken using 3.5 cm diameter stainless steel cores. These cores were either taken directly to the laboratory and processed immediately upon arrival, or kept refrigerated and processed within one day. Based on introductory experiments, cores were sliced in six consecutive layers, corresponding to depth layers of 0–5 mm, 5–10 mm, 10–15 mm, 15–20 mm, 20–30 mm and 30–50 mm. Each slice was suspended in sterilized, filtered and oxygen-free aged seawater (1:4 vol/vol) and subsequently subjected to mild ultrasonic treatment (15 Hz for 30 s, Branson B3 bath) in order to detach the cells from sediment particles and to loosen clumps. The resulting slurry, which was used for the enumeration of all three functional groups of sulfur bacteria, was directly pipetted in the first row of three microtiter plates (12 rows of eight wells, each maximally containing 250 μl). The remaining wells were previously filled with 200 μl of growth medium for the group to be enumerated. Ten-fold dilution steps were used. Microtiter plates for PSB and SRB were incubated in an oxygen-free environment in gas-tight plastic bags (Anaerocult system, Merck) and incubated at room temperature in incandescent light and in darkness, respectively. Medium composition for the enumeration of the different functional groups was as described previously (Visscher et al., 1992a). Growth of PSB was assessed by pigmentation of the wells, growth of CSB was judged from acid formation and turbidity, whereas for the presence of SRB blackening of the growth medium due to FeS precipitation was taken as the criterion. Growth was checked frequently and final scores were obtained after six to ten weeks of incubation. The most probable numbers and 95% confidence limits were calculated according to the method of Klee (1993).

Vertical distribution of oxygen and sulfide

In 1994, data were collected during two field campaigns. During the June campaign attention was focussed on the possibility of sulfide re-oxidation in *Zostera* beds at station B in the Bassin d'Arcachon, while during the August campaign most attention was paid to the measurement of oxygen and sulfide profiles during full diel cycles.

The small-scale sensors for the measurement of oxygen and sulfide were sheathed in stainless steel needles (outer diameter 0.9 mm) in order to prevent damage to the sensors by shells and other small objects. Particularly in the sediments of Station 11 in the Etang du Prévost are shells abundantly present. Details of the construction and calibration of the mini-electrodes are given in Van Gemerden et al. (1989) and Visscher et al. (1991).

During the June 1994 campaign, a platform was installed at station B in an area dominated by eelgrass. At each side of the platform a 0.75 × 2 m area was selected for sampling: in one part *Zostera* leaves were carefully removed without removing the roots, while in the other part *Zostera* plants were left intact. During periods with no overlying water, cores were taken at regular intervals at each side of the platform. Oxygen- and sulfide profiles were recorded at depth intervals of 0.1–1 mm within 15 minutes after sampling, using a custom made nanoAmp- and mV-meter (Electronic Workshop, Biology Department, University of Groningen).

During the August campaign electrode studies were performed using a computer controlled submersible device developed for *in situ* measurements in shallow marine environments (Mechanical and Electronic Workshops, Biology Department, University of Groningen). The software for the interactive control was developed by TiePie Engineering, Jornwerd, The Netherlands. A brief description follows, details of the system will be published separately. On a 1-m high stainless steel frame, an electrode block was mounted, which can be moved horizontally, along an X-and an Y-axis for a distance of 50 cm. Vertically, the maximum range was 30 cm with a minimal resolution of 0.1 mm. Profiles were recorded after a depth sensor, mounted on the electrode block, has detected the sediment surface. Needle electrodes for oxygen, sulfide, pH, redox and temperature (four each) were mounted on the electrode block. Amplifiers and nanoAmp-mV converters were mounted directly on top of the electrodes and millivolt signals sent to an on-land computer for further analysis.

During a diel cycle, measurements were repeated each hour and the horizontal spacing between measurements was 2.5 cm.

Results

Bassin d'Arcachon

Station A

Data on the vertical distributions of sulfur bacteria in the sediments of the Bassin d'Arcachon are presented in Figure 1.

At station A (Figure 1, left-hand panels) CSB were present in relatively high numbers with cell densities up to 10^9 cells cm^{-3} sediment in the upper 5 mm of the sediment. The average cell count in the top two cm was 6×10^8 cm^{-3}, which accounted for 99.5% of the total population of CSB in the 0–5 cm depth layer. In the 30–50 mm layer maximum counts were 2×10^6 cells cm^{-3} sediment. PSB were detected in all depth layers from 0–5 cm, however, numbers were low when compared to CSB. Maximum population densities were found in the 10–15 mm depth layer (3×10^5 cells cm^{-3}), cell densities in the other depth layers were two orders of magnitude lower. The average count in the top two cm of the sediment was 8×10^4 cells cm^{-3}, accounting for 98% of the population from 0–5 cm. At this site the CSB clearly outnumbered the PSB. The same was found during the campaigns in June 1993 and June 1994. For a proper evaluation of functional groups, the average individual cell size of each groups has to be taken into account, for PSB this value is approximately 1 0-times higher than for CSB (Visscher & Van Gemerden, 1993; Van den Ende et al., 1995). At Station A, the total biomass of CSB thus was approximately 100-fold higher than that of the PSB.

Highest population densities of SRB also were found in the 0–2 cm layer (2×10^7 cells cm^{-3}), representing 90% of the population present in the layer of 0–5 cm. In the deeper layers cell numbers decreased to 10^6 cm^{-3}.

Station B

The vertical distribution, as well as the abundance, of the three groups of sulfur bacteria was comparable to Station A, i.e. highest population densities were found in the upper two cm of the sediments (Figure 1, right-hand panels). Maximum cell densities for CSB were found in the 5–10 mm depth layer (7×10^8 cells cm^{-3}), but counts were still 10^6 cm^{-3} in the 30–50 mm layers.

As at Station A, low numbers of PSB were encountered. The average population density in the 0–2 cm layer, containing 70% of the cells in the 0–5 cm layer, was 3×10^3 cells cm^{-3}. As at Station A, CSB dominated over the PSB.

For the SRB, maximum counts were obtained in the 10–15 mm depth layer (6×10^7 cells cm^{-3}). As in Station A, 90% of the population of this group revealed to be present in the 0–2 cm depth layer, numbers close to 10^6 cm^{-3} were observed in the deeper layers.

In June 1994 a comparison was made between the numbers of CSB and PSB inside and outside the *Zostera* beds. In both conditions, the majority of CSB and PSB were present in the upper 20 mm of the sediments, for CSB being 96% and 93%, respectively, and for PSB being 84% and 98%, respectively. For CSB, the average count for the top 20 mm inside the *Zostera* bed was 4.6×10^6 cm^{-3}, and outside the seagrass bed 30.4×10^6 cm^{-3}. For the PSB the corresponding numbers were 2.1×10^3 cm^{-3} and 2.0×10^6 cm^{-3}, respectively. The impact of the seagrass on the abundance of sulfide-oxidizing bacteria is elegantly illustrated by comparing the ratio CSB/PSB on a biomass basis. Outside the *Zostera* beds the contribution of CSB to the total biovolume of the populations of CSB and PSB was 60.8%, inside the *Zostera* beds the corresponding figure was 99.5%. It thus appears that the competitive position of colorless sulfur bacteria was enhanced in seagrass beds.

During the June campaign 1994 data were collected on the profiles of oxygen and sulfide in sediment covered with *Zostera* sp. (Figure 2, left-hand panels) and in an area from which the leaves of *Zostera* were removed while the root system was left intact (Figure 2, right-hand panels). Cores were taken at 2-h intervals, except when the sampling site was submerged. The differences observed between four different electrodes used for each parameter were minimal, except for 18:50 h when one electrode showed much deeper penetration of oxygen than the others. At the surface of the *Zostera*-covered area, oxygen concentrations were maximal in the late afternoon (200–300 μmol l^{-1}), but otherwise showed little variation with time. The depth of oxygen penetration was <5 mm during the night and up to 10 mm during the day. The removal of the *Zostera* leaves resulted in lower maximum oxygen concentrations in the superficial layers, and in much shallower penetration. No clear differences were observed

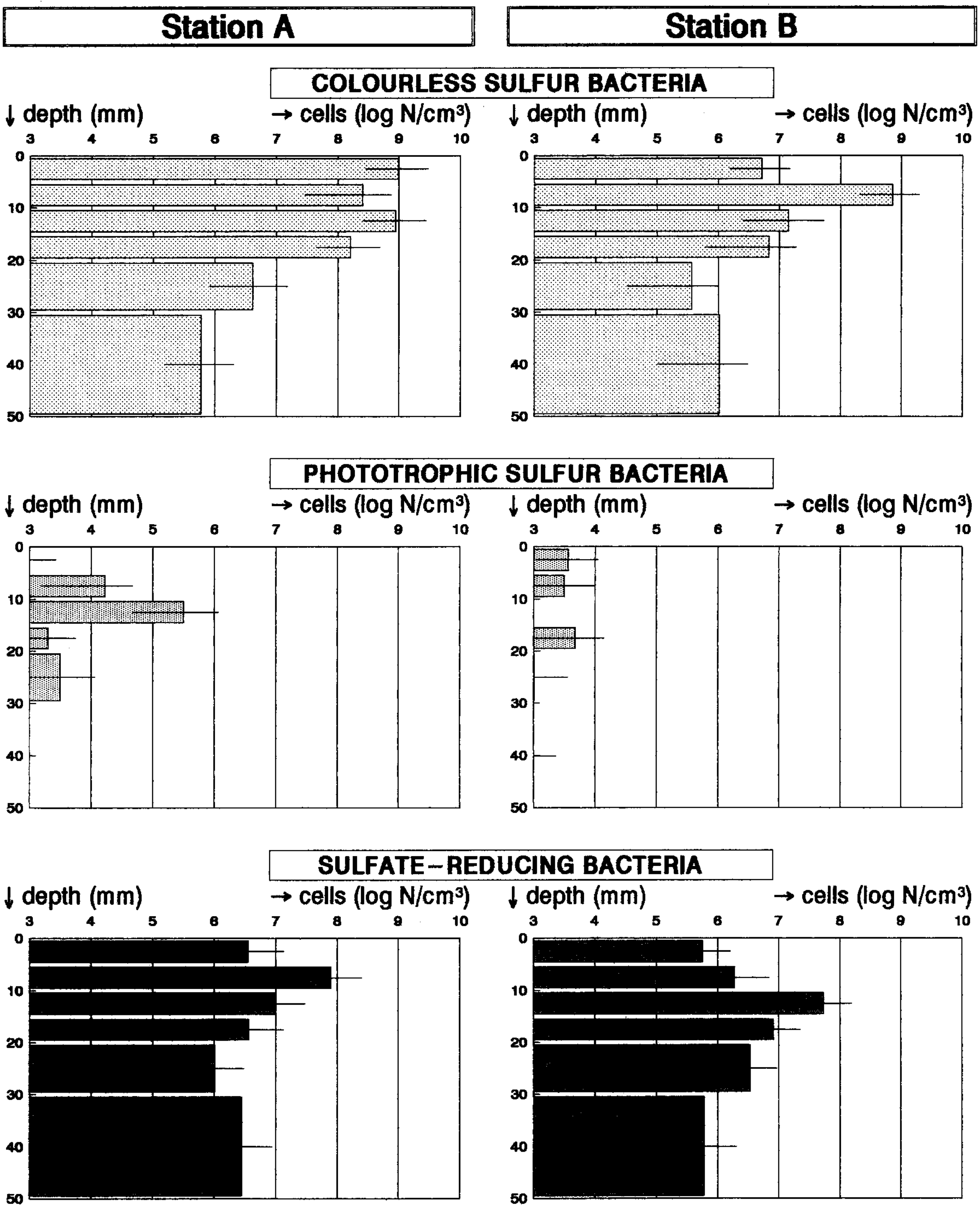

Figure 1. Vertical distribution of sulfur bacteria counted by the MPN-method in the top 50 mm at the outer station (Station A) and the inner station (Station B) in the Bassin d'Arcachon. Cores were collected on September 3^{rd} 1993. Sulfate-reducing bacteria were enumerated with lactate and acetate; colorless bacteria with thiosulfate and anoxigenic phototrophic sulfur bacteria with sulfide and thiosulfate. Error bars indicate 95% confidence intervals after Klee (1993).

at different times, indicating that oxygen production was primarily due to *Zostera* together with the epiphytes removed with the leaves of the latter. It thus appears that sediments with intact *Zostera* plant are more oxygenated than those from which the leaves were removed. Free sulfide could not be detected, neither underneath the *Zostera* beds, nor in the sediment from which the *Zostera* leaves were removed. The mV-signal of the sulfide electrode did not allow calculation of concentrations, nevertheless the trend observed was that sediments without *Zostera* leaves were more reduced than the sediments without *Zostera* leaves (Figure 2).

In August 1994, data were collected at 1-h intervals on the distribution of oxygen and sulfide in the *Zostera* beds during a full diel cycle. Oxygen concentrations, in general, were lower than in June (data not shown). The oxygen concentration in the 10 mm water phase just above the sediments and in between the *Zostera* leaves ranged from 25 to 150 μmol l^{-1}, indicating under-saturation. Maximum oxygen concentrations were observed in the late afternoon, while minimum concentrations were found between midnight and early morning. At all times, concentrations in the sediments gradually decreased to zero. Again, oxygen penetration depth was highly variable and ranged from only a few mm to more then 10 mm. On one occasion, concentrations up to 50 μmol l^{-1} O_2 were observed in the deeper depth layers (8–30 mm) while oxygen in the 6–8 mm was absent. This phenomena, is probably due to the presence of *Zostera* roots. In contrast to measurements performed in September 1993 and June 1994, sulfide could be detected in the sediments of Station B, although at very low concentrations (<6 μmol l^{-1} S^{2-}).

Etang du Prévost

Station X

The vertical distribution of sulfur bacteria at the outer Station X is shown in Figure 3 (left-hand panels).

CSB attained maximum population densities in the upper 3 cm of the sediment with an average density of 10^7 cells cm^{-3}, whereas in the 30–50 mm layer the population density was two orders of magnitude lower. Of the entire population in the top 50 mm, 75% was found in the top 20 mm.

The population of PSB exceeded that observed in the Bassin d'Arcachon and was rather uniformly ($\approx 5 \times 10^4$ cells cm^{-3} sediment) spread over the entire depth layer analyzed.

Although maximum numbers of SRB were found in the upper cm of the sediment (10^6 cells cm^{-3}), a substantial number of viable cells was present in the deeper layers, resulting in an average of 3×10^5 cells cm^{-3} over the entire depth horizon from 0–50 mm.

In August 1994 depth profile data were collected on the distribution of oxygen and sulfide. Oxygen concentrations in the water phase just above the sediment ranged from 75 μmol l^{-1} to 250 μmol l^{-1}. Concentrations were low in the early morning and progressively increased during the day to become maximal in the afternoon. The oxygen penetration depth into the sediments was fairly constant over time. In general, during the day anoxic conditions were observed at depths exceeding 5 mm, during the night oxygen penetration was less. The maximum oxygen concentrations at this site never exceeded 100% saturation levels. During the 24-h period sulfide was detected incidentally at very low concentrations.

Station 11

At Station 11 (Figure 3, right-hand panels), 8×10^7 cells cm^{-3} of CSB were found in the top 5 mm of the sediment. The average number in the top 20 mm was 2×10^7 cells cm^{-3} sediment, accounting for 90% of the viable population of CSB from 0–5 cm.

Relatively high PSB numbers (5×10^7 cm^{-3}) were found in the top 10 mm of the sediment, and 96% of the viable population was enumerated in the top 20 mm. At this location, PSB were more numerous than CSB. When the numbers were transformed to biomass PSB dominated the CSB by a factor of 11. Highest cell densities for the SRB likewise were encountered in the top layers, with a maximum of 6×10^8 cells cm^{-3} sediment in the upper 5 mm. The numbers of SRB showed a remarkable decrease of two to three orders of magnitude between the upper 5 mm and the 5–10 mm layer. The high numbers of SRB in the surface layers of the sediment may explain the formation of 'white water' observed in the days before sampling. If so, it may reflect a faster response of SRB to an increase in substrate availability when compared to the response of CSB and PSB to increased sulfide concentrations.

Free sulfide was clearly present in Station 11, the maximum concentration observed was 6 mmol l^{-1}. The data on oxygen concentrations in the water phase were comparable to those measured at Station X in the Etang du Prévost, however the pattern in the diel cycle

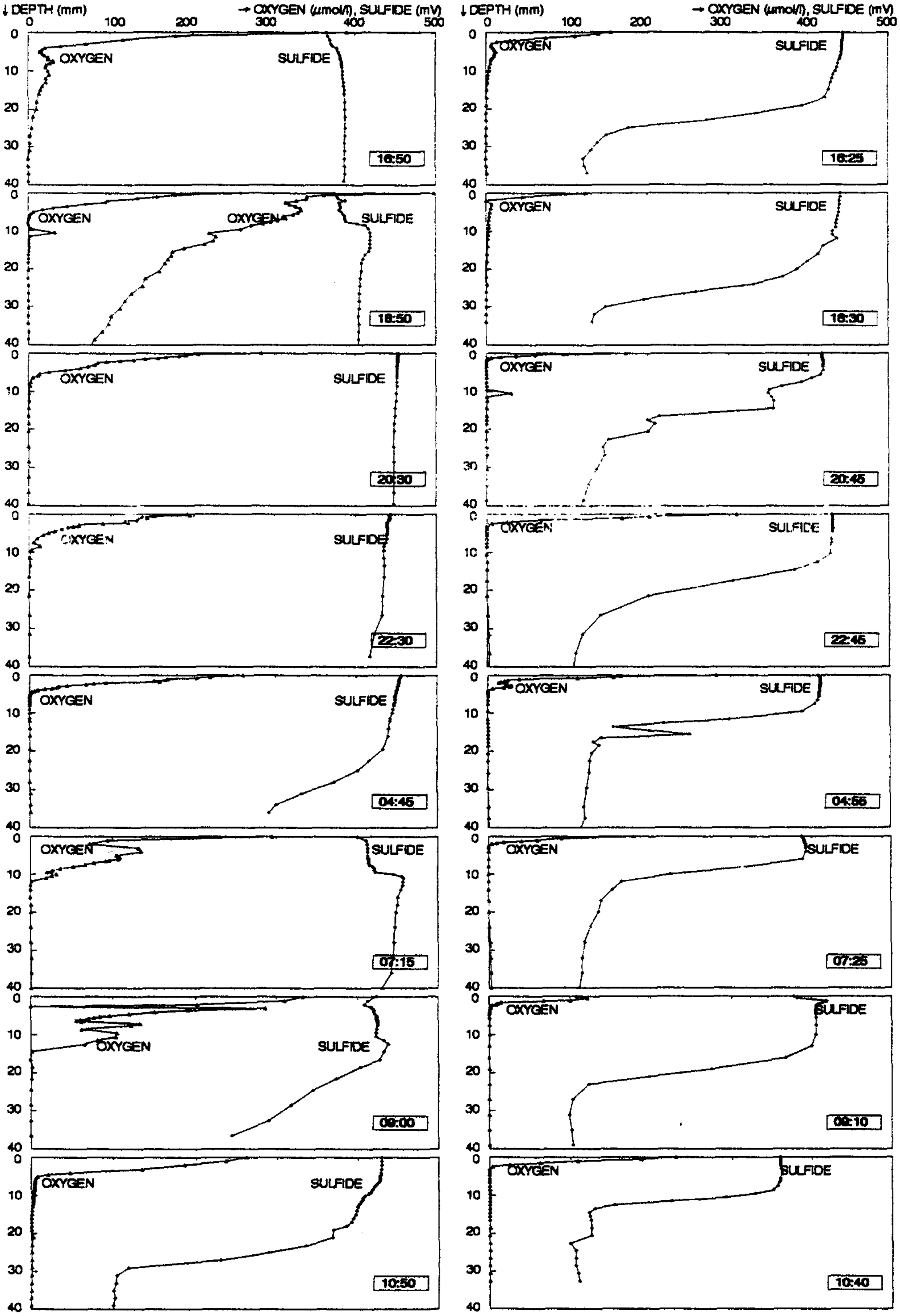

Figure 2. Concentration of oxygen and signal of sulfide needle sensor in cores taken in a *Zostera* bed at different times at Station B in the Bassin d'Arcachon. Left column: intact *Zostera* plants; right column: leaves of *Zostera* cut off on June 2 1994 at 15–16 h. Data were collected on 2–3 June 1994. High tides were at 0:23 h and 13:05 at June 2, and at 1:33 and 14:18 on June 3. Low tides were at 6:19 and 18:49 at June 2, and at 7:23 and 19:54 on June 3.

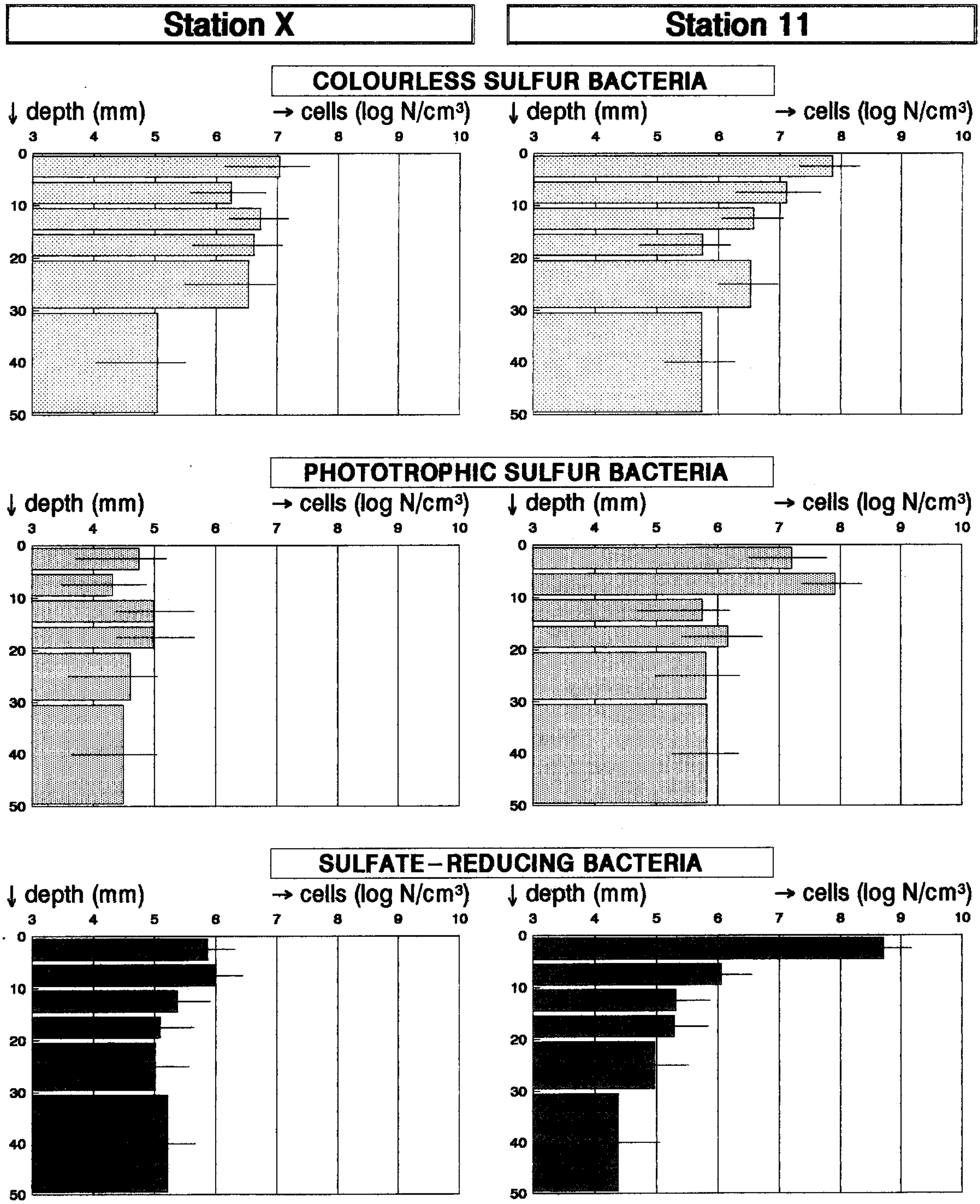

Figure 3. Vertical distribution of sulfur bacteria counted by the MPN-method in the top 50 mm at the outer station (Station X) and the inner station (Station 11) in the Etang du Prévost. Cores were collected on September 6^{th} 1993. Sulfate-reducing bacteria were enumerated with lactate and acetate; colorless bacteria with thiosulfate and anoxigenic phototrophic sulfur bacteria with sulfide and thiosulfate. Error bars indicate 95% confidence intervals after Klee (1993).

was more pronounced. During the night concentrations decreased progressively to reach lowest values in the very early morning ($\approx 10\ \mu\text{mol l}^{-1}$ at 10 mm above the water-sediment interface), while highest oxygen concentrations were observed between 18 and 20 h.

Discussion

Vertical distribution and abundance of sulfur bacteria

Two principal conclusions can be drawn from the data on the vertical distribution of sulfur bacteria. Firstly, the substantial numbers observed for the colorless sulfur bacteria (CSB), the phototrophic sulfur bacteria (PSB), and the sulfate-reducing bacteria (SRB), demonstrate that the sulfur cycle is of major importance both in the Bassin d'Arcachon and the Etang du Prévost. When cell counts were transposed to biomass, it was generally observed that the sum of the biomass of CSB and PSB was roughly proportional to the biomass of the SRB. Secondly, the fact that the bulk of the population of all three groups were found in the surficial layers of the sediments, indicates that the processes primarily take place in the top layers of the sediments.

The high population density of SRB measured at the continuously submerged Station 11 (Etang du Prévost), probably was the consequence of the massive decay of macroalgae in the days before sampling. The observed 'white water' could be the result of sulfur particles, either produced biotically by CSB and/or PSB, or abiotically. However, carbonate precipitation may also have contributed to the formation of 'white water' (Caumette & Baleux, 1980).

The population densities of sulfur bacteria observed in general are high compared to other observations. Caumette (1987) reported maximum numbers of SRB and PSB at an inner station in Etang du Prévost in the order of 10^6 cm^{-3} and 10^5 cm^{-3}, whereas in the inner station of Bassin d'Arcachon PSB counts maximally were 3×10^4 ml^{-1}, respectively. In the Ems-Dollard Estuary maximum numbers of SRB were present just below the oxic/anoxic and accounted to 10^4 colony-forming-unit (Laanbroek & Pfennig, 1981), whereas in the Kattegat the populations of SRB were reported to be no higher than 10^6 cm^{-3} (Jørgensen & Bak, 1991). However, higher numbers were observed in a well developed *Microcoleus*-dominated microbial mat on the island of Texel (The Netherlands), in which the population densities of CSB, PSB, and SRB respectively were 2×10^9 cm^{-3}, 5×10^7 cm^{-3}, and 5×10^3 cm^{-3} (Visscher et al., 1992a; Visscher & Van Gemerden, 1993).

The MPN counts obtained, particularly those of SRB, are generally assumed to be gross underestimates due to the use of highly selective media (Herbert, 1985). Recovery percentages as low as 0.011% have been reported (Gibson et al., 1987), however, a careful choice of conditions, in particular with regard to the composition of the growth medium, resulted in much higher recoveries (Bak & Pfennig, 1991). Further improvements were obtained by using water from the sampling site in the preparation of media (Visscher et al., 1992a).

High numbers of SRB were not only found at the inner station in the Etang du Prévost, but also at the sampling sites in the Bassin d'Arcachon. This may be regarded as indicative for the importance of the input of organic matter. The prominent role of SRB in the mineralization of organic matter is demonstrated by the high rates of sulfate reduction observed in the zones colonized by *Zostera* sp. and *Ruppia* sp. (Finster et al., 1994). No significant accumulation of reduced sulfur compounds was observed, suggesting that most of the sulfide produced by SRB was re-oxidized, either biotically or abiotically. However, the rate of oxygen uptake, as measured by the diffusive flux of oxygen into the sediments, was too low to account for the re-oxidation. In order to explain this discrepancy, Finster et al. (1994) proposed that rooted plants play an important role in the influx of oxygen in these 'anoxic' sediments. In the sediments of stations devoid of rooted plants (e.g. Station 11, Etang du Prévost), bioturbation by bivalves may act as the major way to introduce oxygen into the sediment.

The hypothesis proposed by Finster et al. (1994) may also explain the high numbers of CSB observed at sites colonized by eelgrass (Figure 1), since oxygen is a key environmental factor for the development of CSB.

The simultaneous presence of light and reduced sulfur compounds is favourable for the development of phototrophic sulfur bacteria. The presence of PSB in cell densities upto 8×10^7 cm^{-3} and 3×10^6 cm^{-3} in the sediments of the Etang du Prévost and the Bassin d'Arcachon, respectively, may be explained in this way. The re-oxidation of sulfide without the availability of sufficient oxygen (Finster et al., 1994), could be explained by the presence of phototrophically growing PSB, although growth and activities of PSB in these ecosystems likely are restricted by the limited availability of light. Tidal movements, resulting in resuspension of sediment particles create a shadowing effect. Also the small particle size of the sediment and the presence of high concentrations of FeS (data not shown; Stal et al. 1994) are likely to reduce the light intensity in deeper layers.Light penetration in sediments is restricted to the upper few mm (Jørgensen, 1989; Lassen et al., 1992; Lassen & De Wit, 1994;). At station B, the shadowing effects of dense *Zostera* coverage, results in low photosynthetically available radiation (PAR) in the surface layers of the sediments, however, near infrared radiation was less affected (Lassen et al., 1994). The significance of the very low numbers of PSB (1% of the surface abundance) detected in the deeper layers of the sediments, is marginal. Little is known about the survival characteristics of PSB under the prevailing environmental conditions. Possibly, bioturbation could be responsible for the presence of phototrophic bacteria in deep and dark depth horizons.

Profiles of oxygen and sulfide

Oxygen and sulfide are parameters of crucial importance, either stimulating or inhibiting the development of CSB and PSB. In general, oxygen is required for the proliferation of CSB. Nitrate, the alternative electron acceptor for these organisms, is only present at very low concentrations and, consequently, nitrate plays a minor role (Herbert & Welsh, 1994; Sloth et al., 1994). In addition to oxygen, CSB require sulfide, or another form of reduced sulfur, as electron donor.

CSB and PSB, although competitors for reduced sulfur compounds, might not be expected to thrive in the same habitat since CSB require oxygen, whereas PSB primarily are anoxygenic phototrophs whose pigment synthesis is inhibited by oxygen (De Wit & Van Gemerden 1990a, b). However, when oxygen concentrations show (diel) fluctuations and/or are limiting co-existence is feasible. The co-occurrence of CSB and PSB in these sediments can be explained by the phenomenon that PSB can oxidize intermediate forms of sulfur – such as thiosulfate, elemental sulfur, and poly-sulfide – which are produced by CSB from sulfide upon oxygen limitation (Van Den Ende and Van Gemerden, 1993, 1994). It is evident from the data presented in Figures 1 and 2 that, the conditions required by CSB for growth were met in sediments covered with *Zostera* (Station A and B, Bassin d'Arcachon). These data infer that oxygen was introduced into the sediments via the eelgrass roots.

The oxygen concentrations observed in June 1994 at the surface of the sediments in Station B, were higher then those recorded in August. The community at this site was less healthy in August compared to June, when oxygen saturating conditions were measured at the sediment surface. At the end of the *Zostera* growing season (August–September), oxygen concentrations in the sediment between the *Zostera* leaves were lower, which can be explained by a higher oxygen demand resulting from the accumulation of organic debris and decomposition the *Zostera* leaves. In August–September low oxygen concentrations or even anoxia may be expected, and red colored patches of phototrophic sulfur bacteria have been observed between decaying *Zostera* leaves (P. J. Labourg, pers. comm.).

At Station 11, oxygen concentrations in the water phase were below 25 μmol l^{-1} during the night. At this site the pelagic alga *Ulva*, whose standing crop may exceed 500 g dry weight m^{-2} (Viaroli et al., 1994) constitutes an important source of organic matter, and respiration and mineralization results in a high oxygen demand. Comparable low oxygen concentrations were observed in 1993, and appear to be the result of increased respiration rates, and not decreased rates of primary production (Viaroli et al., 1994).

At station X, variations in oxygen concentration during the diel cycle were less pronounced. The organic matter content of the sediments was low in comparison to site 11 (De Wit et al., 1994). The absence of a dense *Ulva* community and the location adjacent to the exchange channel may be important factors in this respect.

In general, oxygen penetration into the sediments of the Etang du Prévost was limited to the upper 10 mm of the sediments. However, oxygen penetration may extend to depths of 20 mm or more. Deeper oxygen penetration may result from the presence of roots (e.g. *Zostera*), animal burrows, or sea water seeping downwards through the sediments due to tidal movements. Although tidal differences are small in the Etang du Prévost (about 20 cm), the coarse sediment will result in exchange phenomena. In the Etang du Prévost animal burrows may be the most likely explanation. Furthermore the surface structure of the sediments is highly variable in these systems particularly at Station 11 (Etang du Prévost).

The hypothesis put forward by Finster et al. (1994), that *Zostera* plants enrich the superficial layers of sediments with oxygen, is substantiated by the vertical distribution of CSB inside and outside the *Zostera* beds.

Removal of *Zostera* leaves, which prevents transport of oxygen from the leaves to the roots, was found to coincide with reduced concentrations of oxygen in the surface sediment layers, and, more importantly, to result in reduced depth of penetration of oxygen (Figure 2). Although numbers of CSB inside and outside *Zostera* beds were not substantially different, the ratio CSB/PSB was higher inside compared to outside *Zostera* beds. CSB were found to dominate ecosystems covered with *Zostera* (Stations A and B), whereas PSB dominated systems devoid of *Zostera* Beds (Station B and 11).

The high oxygen-uptake rates of these systems (Sloth et al., 1994) are in agreement with the dense populations of CSB, although aerobic heterotrophic organisms can be expected to have contributed. Since the complete oxidation of the sulfide formed by the high sulfate-reduction rates observed did not correspond to the uptake of oxygen, Finster et al. (1994) postulated that sulfide was only partially oxidized, resulting in the temporary accumulation of reduced intermediates.

In this study the hypothesis that cutting of the *Zostera* leaves might limit aerobic respiration by eliminating an important way of introducing oxygen into the sediment was tested. Although the sediment became more reduced free sulfide did not accumulate under these conditions.

Free sulfide, in considerable concentrations, was only observed at Station 11 in the Etang du Prévost, and not in the sediments of Station B in the Bassin d'Arcachon. While little organic matter was found at Station X, Station B was characterized by high organic matter content and high rates of sulfate reduction (De Wit et al., 1994; Finster et al., 1994). At Station 11 (Etang du Prévost) the sulfide concentrations in the sediments were undoubtedly related to the dense *Ulva* populations. Laboratory experiments showed that the mineralization of 1 g dried *Ulva* resulted in the production of approximately 10 mmol sulfide (unpublished).

Comparison between the two ecosystems

On the basis of the population densities of the functional groups of sulfur bacteria, one might expect the inner stations of the Bassin d'Arcachon and the Etang du Prévost (Stations B and 11, respectively) to be comparable with respect to eutrophication levels. Assuming that the number of SRB reflects the availability of substrates for these bacteria, which is substantiated by the numbers of CSB and/or PSB, it can be inferred that the apparent absence of free sulfide in the sediments of the Bassin d'Arcachon cannot be explained by a lower level of eutrophication of the Bassin compared to that of the Etang du Prévost.

A number of explanations can be invoked to explain the differences between the Bassin d'Arcachon and the Etang du Prévost, but it appears that two factors are of major significance. Firstly the strong currents in the Bassin d'Arcachon, due to the large tidal difference between low and high water, as compared to the Etang du Prévost, ensure a thorough mixing of the water column, and as a consequence increased oxygen availability. Secondly, the higher iron concentrations in the Bassin d'Arcachon as compared to the Etang du Prévost, which effectively and efficiently removes free sulfide (Stal et al., 1994) and thus may limit the activities of PSB and CSB.

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