FEMS Microbiology Ecology 12 (1993) 225–236

Published by Elsevier

FEMSEC 00470

Physiology of purple sulfur bacteria forming macroscopic aggregates in Great Sippewissett Salt Marsh, Massachusetts

Angelica P. Seitz [a], Tommy H. Nielsen [b] and Jörg Overmann [c]

[a] *Department of Molecular and Cell Biology, The University of Connecticut, Storrs, CT, USA,* [b] *Institute of Biological Sciences, Department of Microbial Ecology, University of Aarhus, Aarhus, Denmark, and* [c] *Department of Microbiology, The University of British Columbia, Vancouver, BC, Canada*

(Received 25 January 1993; revision received 20 April 1993, accepted 4 May 1993)

Abstract Purple bacterial aggregates found in tidal pools of Great Sippewissett Salt Marsh (Falmouth, Cape Cod, MA) were investigated in order to elucidate the ecological significance of cell aggregation. Purple sulfur bacteria were the dominant microorganisms in the aggregates which also contained diatoms and a high number of small rod-shaped bacteria. Urea in concentrations of ≥ 1 M caused disintegration of the aggregates while proteolytic enzymes, surfactants or chaotropic agents did not exhibit this effect. This suggests that polysaccharides in the embedding slime matrix stabilize the aggregate structure In addition cell surface hydrophobicity is involved in aggregate formation. The concentration of dissolved oxygen decreased rapidly below the surface of aggregates while sulfide was not detected. The apparent respiration rate in the aggregates was high when the purple sulfur bacteria contained intracellular sulfur globules. In the presence of DCMU, respiration remained light-inhibited. Light inhibition disappeared in the presence of KCN. These results demonstrated that respiration in the aggregates is due mainly to purple sulfur bacteria The concentration of bacteriochlorophyll (Bchl) *a* in the aggregates (0.205 mg Bchl*a* cm^{-3}) was much higher than in the pool sediments but comparable to concentrations in microbial mats of adjacent sand flats. Purple aggregates may therefore originate in the microbial mats rather than in the pools themselves Rapid sedimentation and high respiration rates of Chromatiaceae in the aggregates would prevent the inhibition of Bchl synthesis if aggregates were lifted off the sediment and up into the oxic pool water by tidal currents.

Key words: Purple sulfur bacteria; Aggregate formation; Hydrophobicity; Microelectrodes; Respiration, Sulfur

Introduction

Dense populations of microorganisms, whose activities result in stabilization or precipitation of sediment are termed microbial mats. Where light reaches sulfide-containing layers of stratified sediments, phototrophic sulfur bacteria form dense blooms [1–4] which extend over only a few millimeters depth. The biomass concentration of phototrophic sulfur bacteria in sediment systems is much higher (max. 900 μg Bacteriochlorophyll cm^{-3} [3]) than that in lakes (max. 20.9 μgBchl cm^{-3} [5]).

Correspondence to J. Overmann, Department of Microbiology, University of British Columbia, #300–6174 University Boulevard, Vancouver, BC, Canada V6T 1Z3.

Multi-layered microbial mats in tidal flats of Great Sippewissett Salt Marsh, Cape Cod, Massachusetts, have been studied extensively [1,2]. In past years densely-packed purple bacterial and green-coloured cyanobacterial aggregates with diameters up to 6 mm have been repeatedly observed in pools of the marsh [6] (see Fig. 2A). There is no information available on the origin and physiology of the bacteria in the aggregates or on the ecological significance of aggregation. Isolates from the Sippewissett microbial mats characteristically form cell clumps and attach to organic or inorganic surfaces [7]. It has been suggested that aggregation provides an oxygen shield for microorganisms in the interior of the aggregates [7]. As sulfate-reducing bacteria are present in high numbers in the microbial mat (10^4–10^5 g^{-1} sediment [7]), it seems they might also be present in the aggregates and provide sulfide as an electron donor for anoxygenic photosynthesis.

In order to elucidate the ecological significance of purple sulfur bacterial aggregation we investigated the structure, adhesion mechanism and sedimentation of microbial aggregates, and measured vertical oxygen, sulfide and pH microprofiles in aggregates and the surrounding sediments.

Materials and Methods

Study area

Great Sippewissett Salt Marsh is located at 41°35′N and 41°40′W along the western shore of Cape Cod, Massachusetts, USA. Nineteen small seawater pools on a 60 m wide and 160 m long peninsula in the main tidal channel (Great

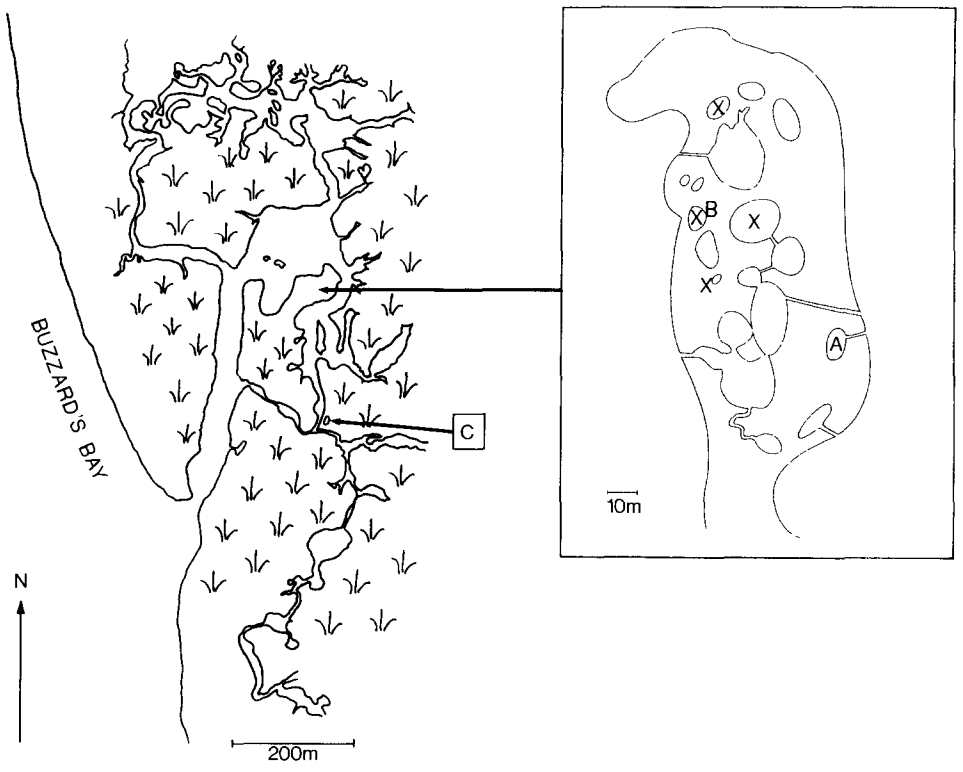

Fig 1 General map of Great Sippewissett Salt Marsh (left) Study sites were a peninsula in Great Sippewissett Creek with 19 tidal pools (insert) and an additional pool 200 m south (pool C) In summer 1992, considerable numbers of purple aggregates were present only in four pools on the peninsula (X) Three pools were chosen for detailed investigation pool A without any, pool B with and pool C with outstandingly high numbers of aggregates

Sippewissett Creek) and a single pool 200 m south were chosen as study sites (Fig. 1). All pools are located in a part of the marsh dominated by dense stands of marsh eelgrass *(Spartina alterniflora)* and are regularly flushed with seawater during high tide. In the summer of 1992 purple aggregates were found in four pools on the peninsula and in extraordinarily high numbers in the pool farthest south.

Collection of samples and field measurements

The study sites were visited a total of five times during June, July and September 1992. Purple aggregates were collected individually with a small sieve or with a Plexiglass tube connected to a flexible Tygon hose that allowed sampling from a distance. Aggregates were transported to the laboratory in glass jars filled with pool water.

Samples of the pool sediments were obtained with 25 cm long, 30 or 50 mm diameter sharpened Plexiglass corers and were kept at ambient temperature. Prior to microelectrode measurements, sediment cores were incubated overnight with a 5 mm overlay of seawater.

Light and epifluorescence microscopy

Sections from the surface or center of the aggregates were cut with a scalpel, transferred to a microscope slide and squashed under a coverslip. Samples were examined with a Zeiss Axioskop photomicroscope at magnifications of 100 ×, 400 × and 1000 ×. For epifluorescence, the filter sets 01 or 18 (excitation wavelengths 365 nm or 390–420 nm) were employed.

Scanning electron microscopy (SEM)

Aggregates were rinsed with seawater and fixed in a solution containing 1.5% formaldehyde, 1.5% glutaraldehyde and 0.1 M cacodylate. After rinsing twice in distilled water, aggregates were transferred to 1% osmium tetroxide, rinsed twice again, dehydrated in ethanol and critical point dried in a Polaron E3000. Specimens were mounted with carbon tape on aluminium holders and subsequently coated with evaporated carbon then with gold in a Polaron E5100 Sputter Coater for 1 min. Scanning electron microscopy was done on a Coates and Welter field emission SEM model HPS50B operated at a voltage of 19.5 kV.

Pigment analysis

Bacteriochlorophyll *a* was determined after cold extraction of the samples in 99.5% acetone (24 h at 4°C in the dark under N_2). Individual aggregates were extracted after grinding in a homogenizer. For extraction of sediment samples, cores were sliced in 2 cm portions and subsamples taken with a 1 cm diameter brass cork borer; all visible bacterial aggregates were removed, the samples centrifuged, and acetone added to the pellets.

Absorbance was measured in a Shimadzu UV-3101 PC spectrophotometer. Pigment concentrations were calculated after Steenbergen and Korthals [8]. Sediment extracts exhibited absorbance maxima at wavelengths between 750 and 766 nm indicating varying mixtures of bacteriochlorophyll *a* (Bchl *a*, maximum at 771 nm) and bacteriopheophytin *a* (Bph *a*, maximum at 748 nm). In order to calculate the concentrations of both pigments, a sample of purple sulfur bacteria was extracted in acetone and one-half of the extract acidified with concentrated HCl to convert Bchl *a* completely to Bph *a*. The absorption spectra of both compounds were recorded and added in different ratios using the computer program SPECTRACALC™ (Galactic Industries Corp.). These calculated spectra were identical to those obtained by mixing subsamples of Bchl *a* with the neutralized Bph *a* extract. The concentrations of both pigments in sediment extracts were then calculated by estimating the proportion Bchl *a*: Bph *a* from the position of the absorbance maximum.

Sedimentation velocity

Sedimentation velocity of microbial aggregates was determined in a graduated cylinder containing seawater at 25°C. Because of the rapid sedimentation of aggregates any error due to temperature-induced convection currents can be neglected in these measurements. All measurements were repeated three times with each aggregate.

Cell aggregation mechanism

The hydrophobicity of the cell surfaces was assessed by the method of Rosenberg [9]. A 3 ml cell suspension was pipetted into 10 ml glass test tubes containing 0.5 ml hexadecane. The gas phase of each tube was flushed with N_2 for 1 min and the tubes sealed with butyl-rubber stoppers. After 10 min of pre-incubation, the assays were stirred vigorously for 2 min. Following phase separation (15 min) the optical density at 650 nm of the lower phase was measured. The hydrophobicity index H (%) was calculated according to:

$$H = 100 \cdot (OD_{650} - OD'_{650})/OD_{650} \quad (1)$$

where OD_{650} and OD'_{650} represent the optical densities of untreated cells and of the lower phase of the hydrophobicity assay, respectively.

Nineteen compounds (see Results) were tested for their capacity to disintegrate the microbial aggregates. Aggregates were transferred to tubes containing the test compounds in N_2-gassed distilled water, and each assay was gassed with N_2 for 30 s, sealed gas-tight with butyl-rubber stoppers and stirred vigorously

Microelectrode measurements

Oxygen, sulfide and pH profiles were measured using microelectrodes [10]. The oxygen electrode had a sensing tip of 20–100 μm and was calibrated in seawater flushed with air or with nitrogen. The sulfide electrode (sensing tip 100 μm) was calibrated at 3.9, 7.8, 15.6, 31.3, 62.5, 125, 250, 500 and 1000 μM total sulfide in 100 mM phosphate buffer (pH 7.2); the mV readings of the sulfide electrode increased log-linearly with sulfide concentrations above 7.8 μM. The pH electrode (sensing tip 30 μm in diameter and 250 μm long) was calibrated in buffers with a pH of 4.0, 7 0 or 9.0. A reference calomel electrode was lowered into the seawater surrounding the microbial aggregate or the seawater overlying the sediment core. Measurements of sulfide and pH were conducted by spacing the tips of both microelectrodes 0.5 mm apart and lowering them simultaneously using a manually driven micromanipulator.

The position of microbial aggregates was fixed by placing them on acid-washed sand in a test tube containing seawater and holding the aggregates with a 4 mm thick agar ring around them In order to create turbulent conditions in the water phase, a constant stream of air was blown on the water's surface with a Pasteur pipet. During all microelectrode measurements sulfide was absent in the seawater surrounding the aggregates. Unless otherwise stated, all microelectrode measurements were conducted in the dark. For illumination a Schott KL 1500 electronic lamp was used.

Apparent respiration rates were calculated from vertical oxygen profiles. Revsbech et al. [12] demonstrated that vertical oxygen profiles can be described by a binominal equation. Therefore binominal equations of the form

$$O_2(x) = a \cdot x^2 - b \cdot x + c \quad (2)$$

were fitted to our data points where x represents depth in the aggregate and a, b and c are coefficients. Correlation coefficients in most cases were > 0.99. Equation (2) is equivalent to the equation given in [12], with $a = R/(2 \cdot D)$. Employing the molecular diffusion coefficient for oxygen of $D = 1.4 \times 10^{-5}$ cm^2 s^{-1} [11], the apparent respiration rate R was calculated according to:

$$R = 2 \cdot a \cdot D \quad (3)$$

Results

Composition of aggregates

Coccoid purple sulfur bacteria (Chromatiaceae) were the dominant microorganisms in the aggregates and were identified microscopically by the purple-pink color of the cells and the presence of intracellular sulfur droplets. Bacteriochlorophyll *a* was the major photosynthetic pigment. Absorption spectra of whole cells (after disintegration of the aggregates, see section: Sedimentation and disaggregation experiments) and acetone extracts exhibited maxima characteristic for bacteriochlorophyll *a* (at 841, 803, 374 nm for whole cells and 770, 577, 358 nm in acetone), chlorophyll a (672 and 664) and carotenoids (522 and 490 nm). The respective concentrations were 0.205 mg Bchl*a* (cm aggregate)$^{-3}$ and 0.039 mg

Chl*a* (cm aggregate)$^{-3}$. The mean diameter of the dominant cells in the aggregates was 1.7 ± 0.2 μm.

Even in squashed preparations of aggregates purple sulfur bacteria were observed only in distinct densely packed clumps held together in the aggregate by a matrix of extracellular slime material (Fig. 2B). Purple sulfur bacterial cells developed a characteristic transient yellow fluorescence when exposed for 2 min to light of a wavelength of 390–420 nm in the epifluorescence microscope. This fluorescence is possibly caused by degradation products of bacteriochlorophyll such as Mg-protoporphyrin IX (monomethyl ester), protoporphyrin IX and Mg-2,4-divinylphaeoporphyrin a_5 monomethyl ester [13]. Yellow fluorescent cells were only observed in the spherical clumps but not in the slime matrix in between. However, the matrix contained a high number of small rod-shaped bacteria and some spirochetes (Fig. 2C). Diatoms were frequently observed in the aggregates (Fig. 2B). Bright red fluorescence also revealed the presence of a considerable number of coccoid chlorophyll *a*-containing cells. No sand grains were detected in the aggregates.

Sedimentation and disaggregation experiments

The sinking velocity of microbial aggregates increased with their radius (Fig. 3). Employing the density ρ (1023 kg m^{-3}) and viscosity, η (0.890×10^{-2} g cm^{-1} s^{-1}) for seawater at 25°C [14] the mean density of the aggregates was calculated from Stoke's law:

$$\rho_a = \rho + v_s \cdot 9n/(2r^2 \cdot g) \qquad (4)$$

where ρ_a is the mean density of the aggregate, v_s the sinking velocity, r the radius of the aggregate, and g the gravitational acceleration. The mean density of aggregates decreased in a hyperbolic fashion with increasing radius.

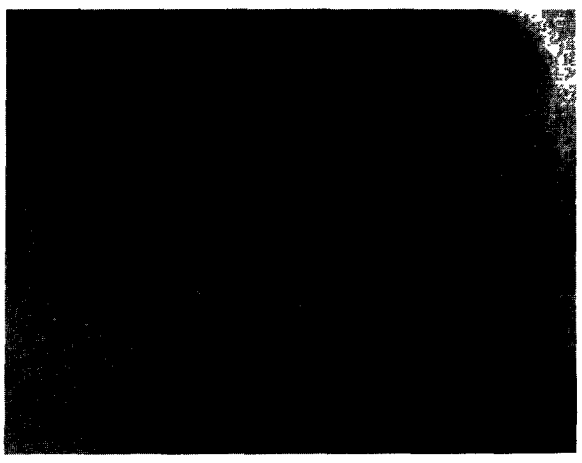

Fig. 2. (A) Macroscopic view of various purple aggregates from Great Sippewissett Salt Marsh. (B) Scanning electron micrograph of the center of an aggregate. Note distinct clumps of spherical purple sulfur bacterial cells held together by extracellular slime matrix. (C) Phase contrast micrograph of slime matrix in a squash preparation of an aggregate. Bar 20 μm.

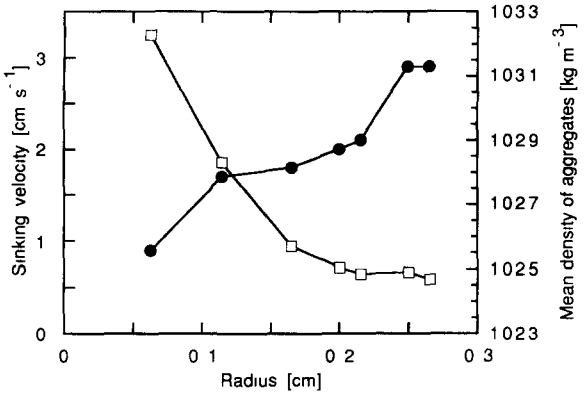

Fig 3 Sinking velocity of microbial aggregates (●) and mean density (□) calculated from Stoke's law for spheres equal in volume to the aggregates

Of all compounds tested, only urea in concentrations of 1 M and above caused disintegration of aggregates. The incubation time required for complete disintegration increased significantly with decreasing urea concentration (Table 1). When urea was removed by dialysis overnight, the cells reaggregated in loose clumps and the hydrophobicity index increased from 15% to 93%. Addition of sugars frequently involved in lectin-mediated adhesion processes, of surfactants, or of proteolytic enzymes did not affect aggregate stability.

Oxygen, sulfide, pH and respiration in aggregates

In order to assess the variability of vertical oxygen profiles at different locations in a single aggregate, four profiles were measured with microelectrodes (Fig. 4). With the exception of one spot (spot 4 in Fig. 4) apparent respiration rates were similar. When the electrode was inserted at spot 4, a slight tilting of the aggregate was observed which may have been the reason for the less steep oxygen profile at this position. Therefore, all subsequent measurements were performed through the center of aggregates. Due to the small dimensions of the microelectrode and the uneven surface of the aggregates it was difficult to determine the exact position where the electrode touched the surface. This might account for the fact that the vertical position of the oxygen gradients varied by 200 μm.

Dissolved oxygen declined rapidly below the surface of aggregates. Sulfide was never detected in the aggregates and the pH decreased only slightly with depth (Fig. 5A). If aggregates were stored in anoxic seawater for several days in the light (200 μmol m^{-2} s^{-1} of a tungsten lamp bulb) their chalky pink outer surface became translucent and its pink color more intense which is typical for sulfur-depleted cultures of Chromatiaceae. After a 3 h incubation in growth medium [15] containing 1 mM sulfide the aggregate's surface regained its refractive chalky appearance and purple sulfur bacterial cells contained sulfur globules again A significantly steeper oxygen profile was measured compared to the sulfur-depleted state (Fig. 5B). The apparent respiration rate increased by a factor of 10.

Table 1

Compounds tested for disintegration of purple microbial aggregates of Great Sippewissett Salt Marsh

Compound	Concentration	Incubation time	Disintegration *
H_2S/HS^- (pH 7 4)	1, 10, 50 mM	4 days	–
D(+)-glucose	200 mM	3 days	–
D(+)-maltose	200 mM	3 days	–
D(+)-mannose	200 mM	3 days	–
D(+)-galactose	200 mM	3 days	–
Lactose	200 mM	3 days	–
L(+)-rhamnose	200 mM	3 days	–
L(+)-arabinose	200 mM	3 days	–
Urea	3–8 M	60–90 min	+
Urea	2 M	2 5 hours	+
Urea	1 M	12–18 hours	+
Urea	1, 10, 100 mM 1, 10, 100 μM	2 days	–
NaSCN	0 5 M	overnight	–
Tween 80	1% v/v	overnight	–
SDS	1% v/v	overnight	–
Triton X-100	4% v/v	overnight	–
Isopropanol	5% v/v	overnight	–
EDTA	100 mM	overnight	–
Lysozyme [a]	2% w/v	overnight	–
Glucuronidase [a]	2% w/v	overnight	–
Proteinase K [b]	1, 10 mg/ml	overnight, 37°C	–
Pronase E [b]	10 mg/ml	overnight, 37°C	–

* – no disintegration, + disintegration of aggregates

[a] incubation buffer· 50 mM Tris (pH 8 0) 50 mM NaCl in distilled water

[b] incubation buffer 10 mM Tris (pH 7 2) in distilled water

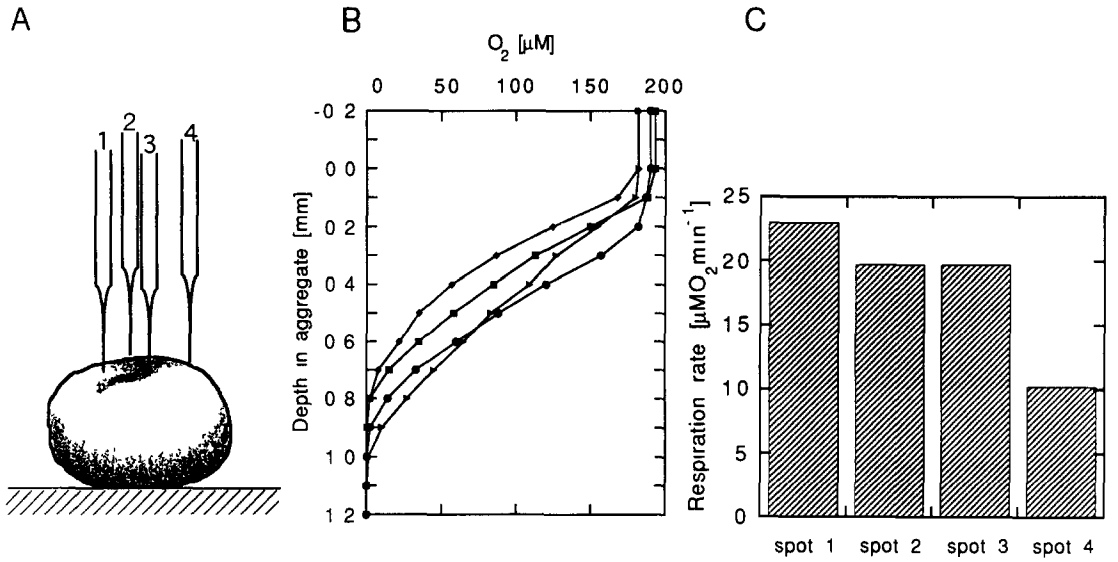

Fig. 4. (A) Four different insertion sites of oxygen microelectrodes in a microbial aggregate, (B) vertical oxygen profiles (● spot 1, ■ spot 2, ♦ spot 3, ▲ spot 4) (C) apparent respiration rates at the different sites

When aggregates were exposed to light (1780 μmol m^{-2} s^{-1} of a Schott KL 1500 lamp), oxygen concentrations in the aggregate increased, reaching a plateau after 10 min. After the light was turned off, concentrations dropped within 3 min to the previous values (Fig. 6). In order to avoid the variation in oxygen profiles observed between parallel measurements, the oxygen concentrations in the presence and absence of light were determined at each vertical position of the microelec-

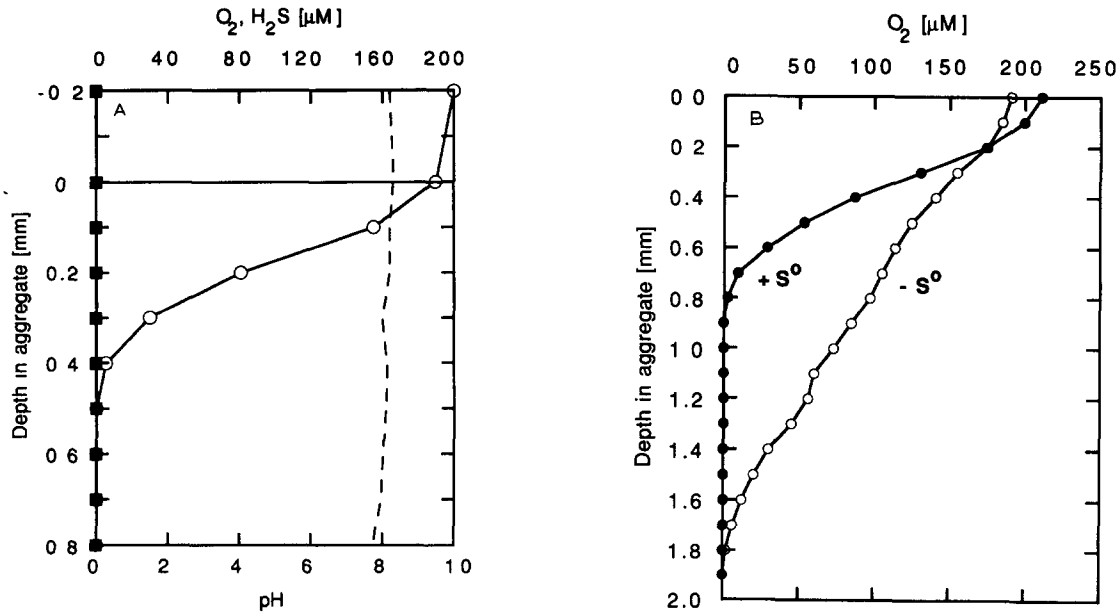

Fig. 5 (A) Vertical profiles of oxygen (○), sulfide (■) and pH (— — —) in a microbial aggregate (B) Vertical oxygen profiles in an aggregate after depletion of intracellular sulfur (○, − S°) and after 3 h of incubation in sulfide-containing growth medium (●, + S°)

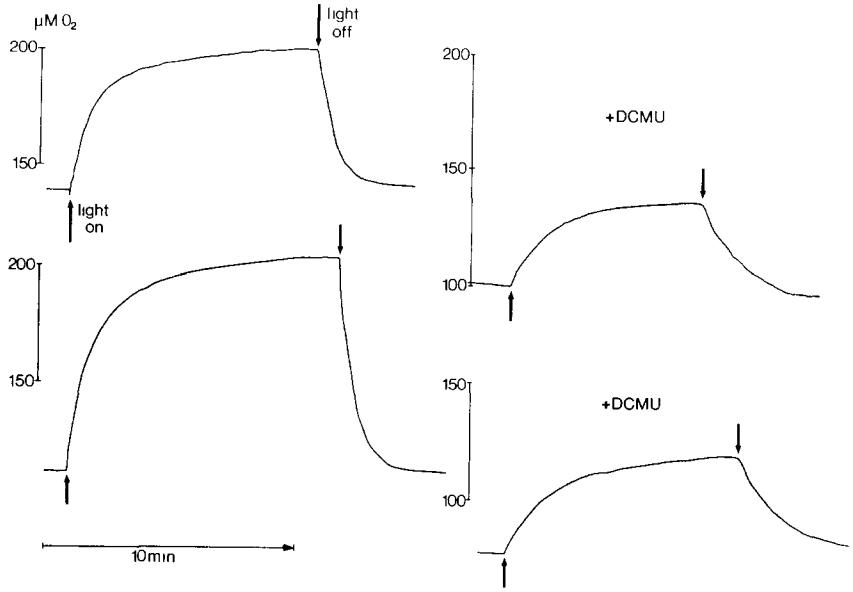

Fig 6 Effect of light (1780 μE m^{-2} s^{-1} tungsten light) on oxygen concentrations 300 μm (upper panels, left and right) and 400 μm (lower panels, left and right) below the surface of a microbial aggregate Left panels untreated aggregate, right panels same aggregate in presence of 60 μM DCMU

trode. The apparent respiration rate calculated from the vertical oxygen profile in the light was zero (Fig. 7). In order to evaluate if the increase in oxygen concentrations in the light (Fig. 6) was caused solely by oxygenic photosynthesis of diatoms and coccoid algae, aggregates were incubated for 60 min in seawater containing 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and the measurements repeated. Even after treatment with 60 and 100 μM DCMU, the concentration of molecular oxygen increased in the light, although to a lower level (Fig. 6). Under these conditions the apparent respiration rate was decreased 39% in the light (Fig. 7). Compared to the untreated aggregate the respiration rate in the dark increased after treatment with DCMU. However, this variation is within the range observed for measurements at different spots of one aggregate (compare Fig. 4C). As oxygen concentrations in the presence and absence of light were compared for each vertical position without moving the microelectrode the change in the oxygen profile upon illumination (either for the untreated aggregate or in the presence of DCMU) has to be attributed to light alone.

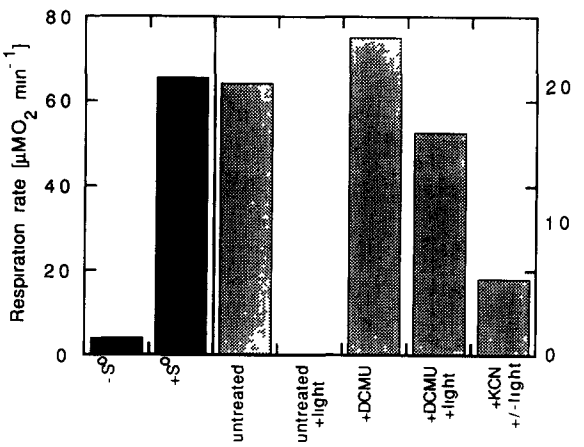

Fig 7 Apparent respiration rates determined in an aggregate before ($-$S°) and after ($+$S°) formation of intracellular sulfur globules (left). Respiration rates measured in a second aggregate after treatment with various inhibitors and light (right)

The light effect completely disappeared after the aggregate had been incubated 20 min in seawater containing 50 μM KCN. Concomitantly, the apparent respiration rate decreased 76% (Fig. 7).

Sediments

The sediment of the marsh pools A, B and C (Fig. 1) contained 1.1–7.8 μg bacteriochlorophyll *a* cm^{-3}, 1.7–8.5 μg bacteriopheophytin *a* cm^{-3} and 7.2–27.1 μg chlorophyll *a* cm^{-3}. The oxic/anoxic boundary was present at 1 mm depth in the sediments of pools A and B. In contrast, the surface of the sediment core obtained from pool C showed low oxygen as well as significant sulfide concentrations despite stirring of the overlying water.

Sandbars in tidal channels adjacent to the pools contained dense populations of purple sulfur bacteria a few mm below the sand surface. When samples from the purple layers were shaken for 10 seconds with an equal volume of seawater, purple fluffy aggregates formed in the supernatant after the sand grains had settled out. Microscopic inspection of these aggregates revealed the presence of spherical clumps similar to the clumps constituting the purple aggregates and very similar to the microcolonies described for different microbial mats [1,4]. The microscopic observations and the comparable Bchl *a* concentrations support the view that the purple aggregates in Great Sippewissett Salt Marsh develop outside the pools in microbial mats and are washed into the pools during high tide rather than being formed in the pools. Gelatinous purple masses have been observed directly on sand flats near main drainage channels of the marsh [7].

Discussion

Physiology of bacteria in the aggregates

The apparent respiration rate of aggregates was significantly increased after the purple sulfur bacterial cells had formed intracellular sulfur globules under anoxic conditions in the light. Numerous Chromatiaceae species respire oxygen at high rates with sulfide, thiosulfate or intracellular sulfur present [16]. This indicates that purple sulfur bacteria in the aggregates contribute significantly to respiration. Large numbers of bacteria were present in the slime matrix of the aggregates. Although the increase of respiration was observed after incubation under conditions which favor intracellular sulfur formation by phototrophic sulfur bacteria, the contribution of chemolithotrophic or heterotrophic bacteria cannot be excluded.

Respiration of phototrophic sulfur bacteria is inhibited by light [17,18] and in this way can be distinguished from respiration of chemotrophic bacteria. But the decrease of apparent respiration rates of untreated aggregates in the light has to be attributed, at least partly, to oxygen evolution by diatoms and other oxygenic phototrophs present in the aggregates. Such oxygenic photosynthesis is inhibited in the presence of 5–10 μM DCMU [2,19]. As light inhibition of respiration was observed even at much higher DCMU concentrations the respiration of Chromatiaceae must be significant in the purple aggregates. In some other phototrophic bacteria the respiration is only partially inhibited by light even if cells are grown photosynthetically [18]. Therefore it seems likely that Chromatiaceae actually contribute more to respiration in the aggregates than the 39% decrease upon illumination in Fig. 7 (+DCMU) indicates. Respiration of Chromatiaceae is inhibited by cyanide in concentrations of 1–7 μM [16]. The complete disappearance of the light inhibition in the presence of 50 μM KCN provides further evidence for the significance of Chromatiaceae in respiration in the aggregates. The low respiration occuring in the presence of KCN may be due to bacteria containing a less sensitive alternative oxidase (inhibited at 500 μM KCN).

Assuming that the increase in respiration (61.4 μmol O_2 l^{-1} min^{-1}, Fig. 7 left) during formation of intracellular sulfur is exclusively due to Chromatiaceae, the specific respiration rate can be calculated from the specific content of Bchl *a* of purple aggregates (0.205 mg cm^{-3}) and the protein specific Bchl *a* content reported for Chromatiaceae (30–90 μgBchl*a* (mg protein)$^{-1}$ [3]). This yielded specific respiration rates of 9.0–27.0 nmol O_2 (mg protein min)$^{-1}$ which are comparable to values determined for sulfur-containing pure cultures of purple sulfur bacteria [16].

Under natural conditions oxygen concentrations in the aggregates are likely to be much lower than during our measurements. When the

water surrounding the aggregate was not stirred during the microelectrode measurements, oxygen concentration decreased from 1 mm above the aggregate to a concentration of only 33 μM at its surface. Thus even if turbulent mixing of the aggregates occurs in the pool water, oxygen concentrations sufficient to fully repress Bchl *a* synthesis (50 μM O_2 [20]) will be present only at the surface of the aggregates.

Aggregate formation is an important feature of natural assemblages of bacteria. The aggregate formation of *Amoebobacter purpureus* in a meromictic salt lake was attributed to the hydrophobic effect involving surface proteins [21]. In contrast, proteolytic enzymes did not disintegrate purple aggregates in Great Sippewissett Salt Marsh. Since the disruption of hydrogen bonding by urea disintegrated the aggregates but detergents, proteases and chaotropic agents had no effect, the slime matrix surrounding the cells most likely contains polysaccharides. Furthermore the significant change in cell surface hydrophobicity after dialysis of urea indicates that the hydrophobic effect is involved in the aggregation of the microorganisms.

Ecological implications

The concentration of bacteriochlorophyll *a* determined for the purple aggregates in Great Sippewissett Salt Marsh is similar to the concentrations reported for multi-layered microbial mats at the same location [2], for microbial mats on the island of Texel [22] and for sulfureta on the Orkney Islands [3]. Compared to the pigment concentrations in the surrounding pool sediment, bacteriochlorophyll *a* is significantly enriched (factor of > 17) while chlorophyll *a* concentrations are only slightly increased (factor of 1.4) in the aggregates. Furthermore, the high level of bacteriopheophytin *a* present in the sediment suggests that a significant amount of purple bacterial biomass in the sediment is in a decayed state and thus cannot be the source of aggregate formation of purple sulfur bacteria. Rather, the aggregates are the source of bacterial pigments in the surrounding pool sediment.

As sulfide was not detected inside the aggregates, sulfide oxidation must take place at a faster rate than sulfide production. The intracellular sulfur globules of the purple sulfur bacteria disappeared if the aggregates were incubated in deoxygenated seawater in the light. Even if sulfide is produced by sulfate reducers inside the aggregates, its oxidation by anoxygenic photosynthesis occurs much faster and purple sulfur bacteria therefore must obtain the electron-donating substrate for photosynthesis from the pool sediment. Only aggregates extending more than 1 mm into the sediment would reach the sulfide-containing zone in pools A and B while sulfide would be readily available even at the sediment surface in pool C. The very dense cover of purple aggregates on the sediment surface of pool C, all exhibiting a highly refractile surface, supports this conclusion. If sulfide is supplied by the surrounding sediment, the deposition of intracellular sulfur globules should occur preferentially in the outer cell layers of the aggregates. When cell aggregates were cut in half a thin refractile outer layer could be distinguished from a more translucent inner part. If only the outer layer of the aggregates contains high amounts of intracellular sulfur droplets, the density of small aggregates should be higher than that of bigger ones and the density of the aggregates should be inversely related to their radius (i.e., directly related to the surface to volume ratio). This conclusion is substantiated by the sedimentation experiments (Fig. 3): the calculated density values could be fitted by the equation

$$\rho_a = 0\ 63/r + 1022.3\ (n = 7,\ r = 0.992).$$

As the maximum depth of the tidal pools was between 10 and 32 cm, purple aggregates mixed into the oxygenated pool water by tidal currents or wind action will settle within 3 to 32 s. During exposure of aggregates to oxic conditions respiration will keep the concentrations of molecular oxygen low; accordingly no inhibition of bacteriochlorophyll synthesis should occur. Purple sulfur bacteria in the aggregates therefore will maintain their photosynthetic capability as long as elemental sulfur is available as intracellular electron donor for respiration. Because their physiological versatility includes respiration, purple sulfur bac-

teria are much better adapted to the microenvironment of aggregates than green sulfur bacteria.

Acknowledgements

This research was conducted as part of the course 'Microbial Diversity' at the Marine Biological Laboratory at Woods Hole, MA during the summer of 1992. We thank the MBL for providing space and facilities to Angelica P. Seitz and Tommy H. Nielsen (students) and Jorg Overmann (instructor). A.P.S. was supported by the NASA Planetary Biology Internship program. J.O. was supported by a fellowship from the Deutsche Forschungsgemeinschaft. Thanks to Jim Romanow of the Electron Microscopy Facility, Department of Physiology and Neurobiology, The University of Connecticut, for the scanning electron microscopy.

References

1 Nicholson, J A.M , Stolz, J.F and Pierson, B K (1987) Structure of a microbial mat at Great Sippewissett Marsh, Cape Cod, Massachusetts. FEMS Microbiol Ecol 45, 343–364

2 Pierson, B , Oesterle, A and Murphy, G.L (1987) Pigments, light penetration, and photosynthetic activity in the multi-layered mats of Great Sippewissett Salt Marsh, Massachusetts FEMS Microbiol. Ecol. 45, 365–376.

3 van Gemerden, H., Tughan, C.S., de Wit, R and Herbert, R.A (1989) Laminated microbial ecosystems on sheltered beaches in Scapa Flow, Orkney Islands FEMS Microbiol Ecol. 62, 87–102

4 van Gemerden, H , de Wit, R , Tughan, C S and Herbert, R A (1989) Development of mass blooms of Thiocapsa roseopersicina on sheltered beaches on the Orkney Islands. FEMS Microbiol Ecol. 62, 111–118.

5 Overmann, J , Beatty, J T , Hall, K.J , Pfennig, N and Northcote, T G. (1991) Characterization of a dense, purple sulfur bacterial layer in a meromictic salt lake Limnol Oceanogr 36, 846–859.

6 Edgar, B (1992) Science writers meet microbes in vivo at Woods Hole ASM News 58, 376–379

7 Gibson, G , Leadbetter, E R and Jannasch, H W (1984) Great Sippewissett Salt Marsh A summary of projects carried out by students in the microbial ecology course of the Marine Biological Laboratory, Woods Hole, during summers 1972–1981 In. Microbial Mats· Stromatolites (Cohen Y., Castenholz R W , Halvorson H O., eds) Alan Liss, New York, pp. 95–100.

8 Steenbergen, C.L.M. and Korthals, H J (1982) Distribution of phototrophic microorganisms in the anaerobic and microaerophilic strata of Lake Vechten (The Netherlands) Pigment analysis and role in primary production. Limnol Oceanogr. 27, 883–895.

9 Rosenberg, M , Gutnick, D. and Rosenberg, E (1980) Adherence of bacteria to hydrocarbons a simple method for measuring cell-surface hydrophobicity FEMS Microbiol Lett. 9, 29–33

10 Revsbech, N P and Jørgensen, B B (1986) Microelectrodes Their use in microbial ecology In· Marshall, K C (ed) Advances in Microbial Ecology, Vol 9, pp 293–352 Plenum, New York

11 Revsbech, N P , Madsen, B., and Jørgensen, B.B (1986). Oxygen production and consumption in sediments determined at high spatial resolution by computer simulation of oxygen microelectrode data Limnol Oceanogr 31, 293–304

12 Revsbech, N P., Jørgensen, B B and Blackburn, T.H. (1980) Oxygen in the seabottom measured with a microelectrode Science 207, 1355–1356

13 Kaiser, G H , Beck, J., von Schutz, J U and Wolf H.C (1981) Low temperature excitation and emission spectroscopy of the photosynthetic bacteria Rhodopseudomonas sphaeroides 'wild-type' strain ATCC 17023. Biochim Biophys. Acta 634, 153–164

14 CRC Handbook of Chemistry and Physics, 64th edition 1983–1984, CRC Press, Boca Raton, Florida

15 Pfennig, N and Truper, H (1989) Anoxygenic phototrophic bacteria, pp 1635–1709. In Bergey's Manual of Systematic Bacteriology Vol 3 Williams and Wilkins

16 Overmann, J. and Pfennig, N (1992) Continuous chemotrophic growth and respiration of Chromatiaceae species at low oxygen concentrations Arch. Microbiol 158, 59–67

17 Oelze J and Weaver, P (1971) The adjustment of photosynthetically grown cells of Rhodospirillum rubrum to aerobic light conditions Arch Microbiol. 79, 108–121.

18 Pierson B K and Castenholz, R W (1974) Studies of pigments and growth in *Chloroflexus aurantiacus*, a phototrophic filamentous bacterium. Arch Microbiol. 100, 283–305.

19 Jørgensen, B B , Kuenen, J.G. and Cohen, Y. (1979) Microbial transformations of sulfur compounds in a stratified lake (Solar Lake, Sinai). Limnol Oceanogr 24, 799–822

20 De Wit, R and van Gemerden, H. (1987) Chemolithotrophic growth of the phototrophic sulfur bacterium *Thiocapsa roseopersicina* FEMS Microbiol Ecol. 45, 117–126.

21 Overmann J and Pfennig N (1992) Buoyancy regulation and aggregate formation in Amoebobacter purpureus from Mahoney Lake FEMS Microbiol Ecol. 101, 67–79

22 Visscher, P.T , Nijburg, J W. and van Gemerden, H (1990) Polysulfide utilization by *Thiocapsa roseopersicina* Arch Microbiol. 155, 75–81.