ELSEVIER

FEMS Microbiology Ecology 40 (2002) 47–54

FEMS MICROBIOLOGY Ecology

www.fems-microbiology.org

Growth and chemosensory behavior of sulfate-reducing bacteria in oxygen–sulfide gradients

Andrea M. Sass [a], Andrea Eschemann [a], Michael Kühl [b], Roland Thar [b], Henrik Sass [a], Heribert Cypionka [a,*]

[a] *Institute for Chemistry and Biology of the Marine Environment, University of Oldenburg, D-26111 Oldenburg, Germany*
[b] *Marine Biological Laboratory, University of Copenhagen, Strandpromenaden 5, DK-3000 Helsingør, Denmark*

Received 1 November 2001; received in revised form 10 January 2002; accepted 11 January 2002

First published online 18 April 2002

Abstract

Growth and chemotactic behavior in oxic–anoxic gradients were studied with two freshwater and four marine strains of sulfate-reducing bacteria related to the genera *Desulfovibrio, Desulfomicrobium* or *Desulfobulbus*. Cells were grown in oxygen–sulfide counter-gradients within tubes filled with agar-solidified medium. The immobilized cells grew mainly in the anoxic zone, revealing a peak below the oxic–anoxic interface. All tested strains survived exposure to air for 8 h and all were capable of oxygen reduction with lactate. Most strains also oxidized sulfide with oxygen. *Desulfovibrio desulfuricans* responded chemotactically to lactate, nitrate, sulfate and thiosulfate, and even sulfide functioned as an attractant. In oxic–anoxic gradients the bacteria moved away from high oxygen concentrations and formed bands at the outer edge of the oxic zone at low oxygen concentration ($< 5\%$ O_2 saturation). They were able to actively change the extension and slope of the gradients by oxygen reduction with lactate or even sulfide as electron donor. Generally, the chemotactic behavior was in agreement with a defense strategy that re-establishes anoxic conditions, thus promoting anaerobic growth and, in a natural community, fermentative production of the preferred electron donors of the sulfate-reducing bacteria. © 2002 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: chemotaxis; aerotaxics; oxic-anoxic interface, gradient; *Desulfovibrio*; *Desulfomicrobium*; *Desulfobulbus*

1. Introduction

The sulfate-reducing bacteria can be divided into two physiological groups: species that oxidize organic substrates incompletely to acetate, and species that oxidize organic substrates completely to CO_2 [1]. Incompletely oxidizing species generally grow much faster than the complete oxidizers. Their preferred substrates are simple primary fermentation products like hydrogen, lactate or ethanol, which due to sedimentation or oxygenic photosynthesis are formed at highest rates close to oxic–anoxic interfaces in sediments or microbial mats. Many of the sulfate reducers that are found near such oxic–anoxic interfaces or even in the oxic layers belong to incompletely oxidizing species of genera like *Desulfovibrio*, *Desulfomicrobium* or *Desulfobulbus* [2–10]. The only completely oxidizing species found to be abundant in the oxic zone of microbial mats was the filamentous species of the genus *Desulfonema* [7,11].

Most of the known sulfate reducers within the mentioned genera are well adapted to cope with oxygen. They survive temporary oxygen contact, and even have various mechanisms to reduce oxygen [12–14]. Furthermore, they show behavioral responses to oxygen, such as migration, aggregate formation and band formation [15]. Bands can be observed in cell suspensions during short-term experiments [16,17] as well as in growth experiments in oxic–anoxic gradients [18,19]. Aerobic growth, however, is poor, unless – like in their natural habitats or in co-culture experiments [9,20–24] – aerobic bacteria are present.

The situation is more complicated by the fact that sulfate-reducing bacteria do not only form sulfide, but also oxidize it, if oxygen (in some cases also nitrate or nitrite) as electron acceptor is available. For some sulfide reduc-

* Corresponding author. Tel.: +49 (441) 798-5360; Fax: +49 (441) 789-3583.
E-mail address: cypionka@icbm.de (H. Cypionka).

0168-6496/02/$22.00 © 2002 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.
PII: S0168-6496(02)00180-0

ers, sulfide is even the best electron donor for the reduction of oxygen [25]. During sulfide oxidation elemental sulfur is formed as an intermediate, which may precipitate depending on the conditions [26]. The presence of particles is also known to influence the oxygen tolerance of sulfate-reducing bacteria [27,28].

In our previous studies on oxygen-dependent behavior of cell suspensions we had kept the assays sulfide-free [17]. Here we report on the behavior of sulfate reducers belonging to the genera *Desulfovibrio*, *Desulfomicrobium* and *Desulfobulbus* in artificial sulfide–oxygen counter-gradients. By use of different strains and by studying growth over several days and short-term behavior in cell suspensions a more defined overall picture of the behavior in natural environments is achieved.

2. Materials and methods

2.1. Bacterial strains

Experiments were carried out with six strains of sulfate-reducing bacteria, two freshwater and four marine strains, from the culture collection of our laboratory. The freshwater strains were *Desulfovibrio desulfuricans* CSN (DSM 9104) [29] and *Desulfomicrobium norvegicum* Norway (DSM 1741), formerly *Desulfovibrio desulfuricans* Norway 4 [30]. The marine strains were *Desulfovibrio oxyclinae* N13 [4], *Desulfomicrobium* sp. strains Sal and AcI.2 (related to *Desulfomicrobium apsheronum*) [10], and *Desulfobulbus* sp. strain 86FS1 (DSM 13871).

2.2. Cultivation

Bacteria were grown in the following medium after Widdel and Bak [31] (components in g l^{-1}): KH_2PO_4 0.2, NH_4Cl 0.25, KCl 0.3, $CaCl_2 \cdot 2H_2O$ 0.15, $MgCl_2 \cdot 6H_2O$ 0.5. The marine medium additionally contained 20 g l^{-1} NaCl, and an increased amount of $MgCl_2 \cdot 6H_2O$ (3 g l^{-1}). The medium was supplemented with a trace element solution (SL10), a solution of selenite and tungstate [31], and 0.25 mg l^{-1} of the redox indicator resazurin. The components were dissolved in double-distilled water, autoclaved and cooled under nitrogen. The medium was buffered with a $NaHCO_3$ solution (final concentration 2.5 g l^{-1}), autoclaved separately in a tightly sealed bottle. Bicarbonate and a filter-sterilized vitamin solution [32] were added to the medium after cooling. The pH of the freshwater medium was adjusted to 7.0 with sterile HCl, that of the marine medium to 7.3. A few milligrams of sterile sodium dithionite were added until the resazurin in the medium turned colorless, indicating that the medium was oxygen-free. The substrates for cultivation were Na_2SO_4 (10 mmol l^{-1}) and sodium lactate (20 mmol l^{-1}), prepared as stock solutions (1 mol l^{-1}) and autoclaved separately.

2.3. Preparation of oxygen–sulfide counter-gradients

Oxygen–sulfide counter-gradients were prepared in glass tubes (300 mm length, 35 mm diameter) sealed with rubber stoppers at both ends. Anoxic mineral medium was reduced with sodium dithionite and solidified with 1% (w/v) washed agar (Scharlau, Barcelona, Spain). The tubes were filled with warm medium and inoculated with freshly grown cultures of sulfate-reducing bacteria adjusted to a cell density of about 10^6 cells ml^{-1}, and immediately cooled in ice water. The upper rubber stoppers were pierced with sterile needles (0.9 mm diameter) that were plugged with cotton to prevent contamination. The tubes were stored overnight at 4°C to let air diffuse into the upper centimeters of the medium, then they were further incubated at room temperature (20°C). The headspace of the tubes was flushed with a water-saturated mixture of air and carbon dioxide (80%/20%). The incubation time was 1 day for *D. desulfuricans* CSN, *D. norvegicum* Norway and *Desulfomicrobium* sp. strain Sal and 2 days for the other strains. Oxygen–sulfide counter-gradients developed due to sulfate reduction occurring in the lower part of the tubes. The pH in the tubes remained stable. At least four replicates were prepared for each strain. Cellulose or sediment particles were added as solid phase to half of the replicates (1% w/v). The sediment particles were prepared from Wadden Sea sediment by heating to 500°C for 4 h and subsequent washing with concentrated hydrochloric acid and with water. The size of the particles ranged from < 2 μm to > 500 μm, as measured with a laser particle sizer (Analysette 22, Fritsch, Idar Oberstein, Germany).

2.4. Measurement of sulfide, oxygen and cell densities in the gradient tubes

Concentration profiles of oxygen and sulfide were measured by means of needle electrodes (Microscale Measurements, Den Haag, Netherlands) [33]. Calibration of the ion-sensitive sulfide electrode was performed in nitrogen-saturated phosphate buffer [34], the salinity of which was adjusted to that of the medium. Calibration of the oxygen electrode was performed in air-saturated salt solutions.

The agar medium was sliced into 5 mm thick layers after electrode measurements. Approximately 1 cm^3 of the slices, undisturbed by the electrodes, was cut out, weighed and added to 8 ml of a 4% glutardialdehyde solution. The agar was broken up by pressing the suspension through a needle of 0.6 mm diameter.

An aliquot of the suspension was stained with DAPI [35] and filtered through a Nucleopore filter of 0.2 μm pore width (Millipore, Bedford, MA, USA).

2.5. Measurement of oxygen consumption

Rates of oxygen consumption by washed cells were measured by means of an oxygen electrode (Bachofer,

Reutlingen, Germany) in a multi-electrode chamber [36]. The cells were suspended in a nitrogen-saturated salt solution, which consisted of 350 mM NaCl (pH 7.3) for marine strains and of 150 mM KCl (pH 7) for freshwater strains. Both salt solutions were buffered with 3-*N*-morpholinopropanesulfonic acid (10 mM). The oxygen concentrations in the chamber never exceeded 30% air saturation during all experiments.

2.6. Observation of aerotaxis

Suspensions of washed cells were brought into microslide capillaries (100×10×1 mm, VitroCom, Mountain Lakes, NJ, USA). For the *Desulfovibrio* strains a sodium phosphate buffer (10 mmol l^{-1}) with EDTA (0.1 mmol l^{-1}) was used, which was supplemented with 350 mmol l^{-1} NaCl for *D. oxyclinae* N13. The buffer for the other strains consisted of the respective anoxic medium used for cultivation. Sodium lactate (10 mmol l^{-1}) was used as electron donor in all media to sustain oxygen consumption by the sulfate-reducing bacteria. The redox indicator resazurin (100 μmol l^{-1}) indicated presence of oxygen. Cell suspensions were filled into the capillaries with a gas-tight syringe, all steps were performed quickly to minimize penetration of air into the cell suspension. The capillaries were sealed with parafilm on both ends. After the decolorizing of the resazurin 10 μl of oxygen gas was placed into the middle of the capillary by means of a Hamilton syringe. Formation of clear zones and rings of higher turbidity were documented on a dark background with a digital camera (Camedia, Olympus, Tokyo, Japan).

2.7. Measurements of sulfide and oxygen in microslide capillaries

Oxygen and sulfide concentrations in the vicinity of the oxygen bubble in microslide capillaries were determined with microelectrodes with tip diameters of < 10 μm and with long slender shafts to facilitate their use within the narrow capillaries. The oxygen microelectrode was a Clark-type with a guard cathode [37], the hydrogen sulfide microelectrode was of the new amperometric type [38,39]. More detailed descriptions of the experimental set-up and calibration procedures are presented elsewhere [17,40].

2.8. Survival of sulfate-reducing bacteria after oxygen exposure

To study the survival of sulfate-reducing bacteria after oxygen exposure 500-ml bottles were filled with 100 ml of medium. The caps of the bottles contained a gas-tight rubber septum. The media were inoculated with freshly grown cells adjusted to a density of 10^6 cells ml^{-1} and the headspace of the flasks was immediately purged with a mixture of nitrogen and carbon dioxide (80/20%) to remove air. After 30 min the flasks were flushed with a water-saturated mixture of air, nitrogen and carbon dioxide (25/55/20%), resulting in an oxygen concentration of 5% (v/v) in the headspace corresponding to ~65 and ~60 μmol O_2 l^{-1} in freshwater and marine medium, respectively. The flasks were shaken during the whole experiment to ensure a homogeneous oxygen distribution in the medium. Four replicates were carried out for each strain, two replicates with sediment particles (1% w/v) and two without particles. In an anaerobic chamber, subsamples were taken of each flask with a sterile syringe and subjected to a dilution series (10-fold dilutions, three replicates) in microtiter plates (Greiner, Kremsmünster, Austria). The media in the plates were as described above, but supplemented with $FeSO_4$ (0.5 mmol l^{-1}) to detect sulfide formation via a black FeS precipitate. The plates were sealed with a sterile foil and incubated at room temperature (20°C) for 4 weeks. Only wells with a visible cell pellet were considered positive. Viable counts were calculated after the most probable number (MPN) method [41].

2.9. Chemotaxis experiments

Chemotaxis was studied by measuring accumulation of cells in microcapillaries [42]. A freshly grown culture was diluted to 5×10^7 cells ml^{-1} in mineral medium and filled into bottles. Microcapillaries (Brand, Wertheim, Germany) were filled with supplemented medium and entered into the cell suspension. The supplements were sulfate, sulfide, lactate or nitrate (10 mmol l^{-1}). After incubation for 1 h at 30°C the capillaries were removed and their content mixed with formaldehyde. The cell count per capillary was then determined microscopically in a counting chamber.

3. Results

3.1. Growth in oxygen–sulfide counter-gradients

In agar medium with oxic–anoxic gradients the sulfate-reducing bacteria grew in the anoxic zone (Fig. 1), while in the oxic zone no or only poor proliferation occurred. Most of the strains (*Desulfomicrobium* strain Sal, *D. norvegicum*, *D. oxyclinae*, and *D. desulfuricans*) grew preferentially directly below the oxic–anoxic interface, as indicated by a peak in cell number, often accompanied by a peak in sulfide concentration. Such peaks in cell number were observed in all replicates performed. Towards the deeper anoxic zones cell counts and sulfide concentrations increased, too. With *Desulfomicrobium* strain AcI.2 no distinct peak was found, with *Desulfobulbus* sp. strain 86FS1 no growth was observed during the experiment.

In the microscope microcolonies of up to several hundred cells were observed. This showed that the cells remained immobile in the agar medium. Macroscopically

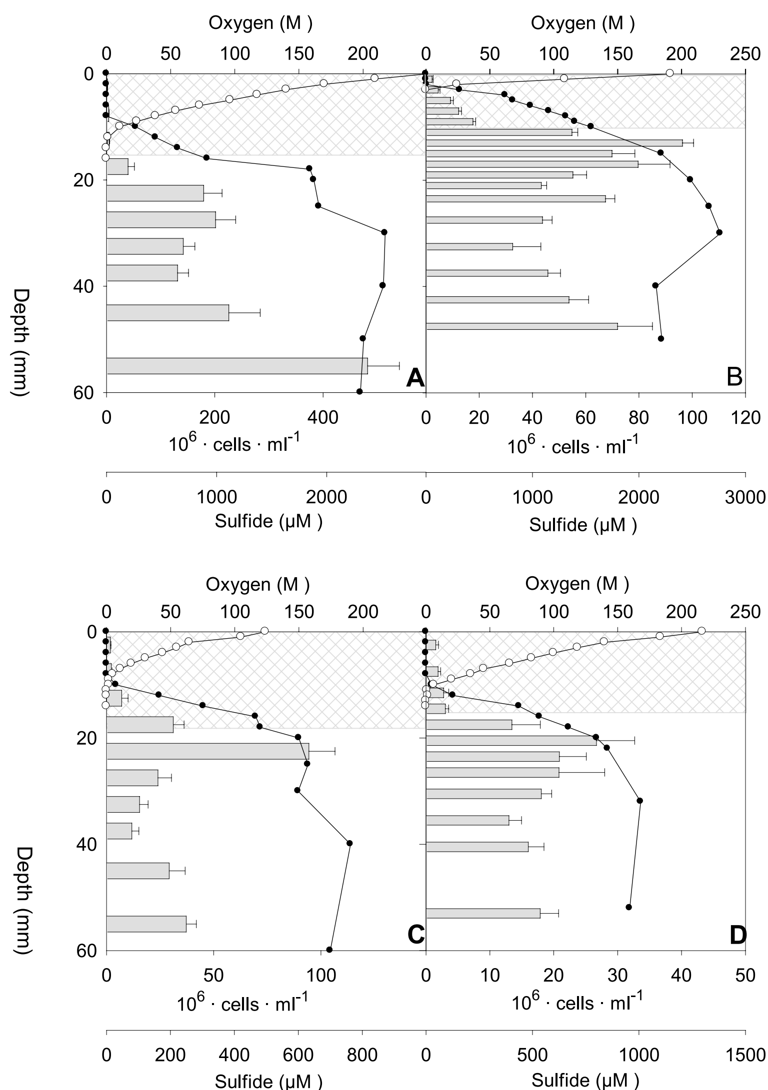

Fig. 1. Growth of sulfate-reducing bacteria in oxygen–sulfide counter-gradients. A: *D. norvegicum* Norway. B: *Desulfomicrobium* sp. Sal with cellulose 0.5% (w/v). C: *D. oxyclinae* N13 with sediment particles. D: *D. desulfuricans* CSN. ○: oxygen; ●: sulfide; bars: cell count with S.E.M.; hatched area: pink color of the redox indicator resazurin.

no colonies could be observed in the relatively short incubation time of 1 or 2 days. Longer incubation times were unfavorable due to migration of the oxic–anoxic interface and due to sulfur precipitation.

In replicates with sediment particles (1% w/v) cell counts and sulfide concentrations were generally increased, but particles had no influence on the overall growth pattern. As before, no growth occurred in oxic zones, and a peak in cell number was observed directly below the oxic–anoxic interface. With *Desulfomicrobium* strain Sal, cellulose powder was tested as alternative solid substratum. This also had no visible influence on the growth pattern. Control experiments without cells showed that particles did not alter the oxygen penetration into the medium.

3.2. Survival of oxygen exposure and oxygen reduction

The sulfate-reducing bacteria survived exposure to an atmosphere containing 5% oxygen over 8 h without heavy loss in viability (Fig. 2). After 2 days, however, the viable counts had decreased by three to five orders of magnitude. Strains belonging to the Desulfovibrionaceae showed initial growth, which ceased after two or three doublings (Fig. 2A–E). The presence of particles notably elevated the survival rate only with *Desulfomicrobium* sp. strain AcI.2 (Fig. 2C).

Aggregate formation upon oxygen exposure was observed with most strains (Table 1). The aggregates, except those of *Desulfobulbus* sp. strain 86FS1, disintegrated if the suspensions were shaken vigorously. Strain 86FS1 formed aggregates also in anaerobic cultures.

All studied strains reduced oxygen with lactate, and with the exception of *D. desulfuricans* CSN all strains oxidized sulfide with oxygen (Table 1). Three of the strains consumed oxygen by sulfide oxidation with higher rates than with lactate as electron donor.

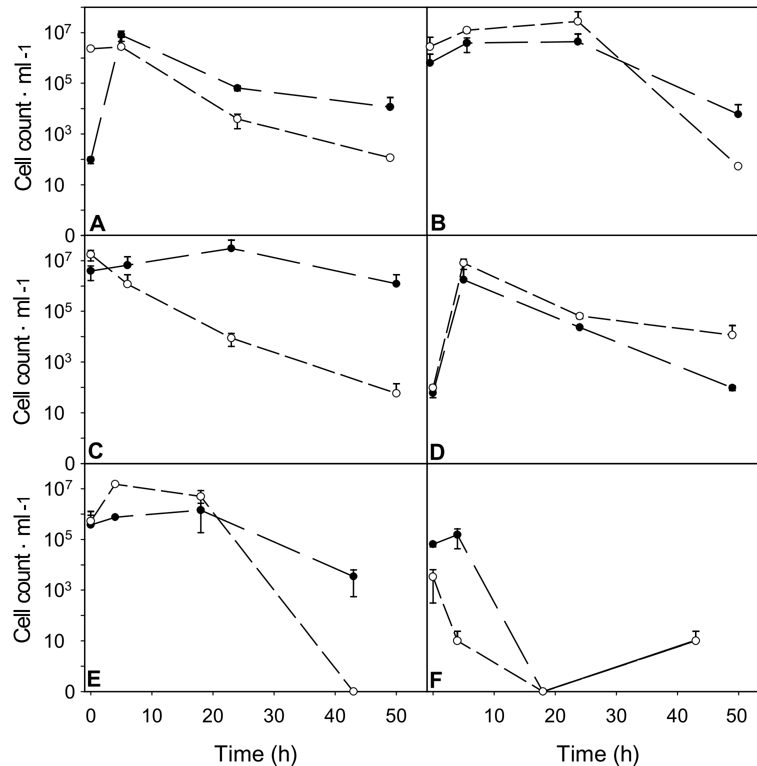

Fig. 2. Survival of oxygen exposure. A: *D. norvegicum* Norway. B: *Desulfomicrobium* sp. Sal. C: *Desulfomicrobium* sp. strain AcI.2. D: *D. oxyclinae* N13. E: *D. desulfuricans* CSN. F: *Desulfobulbus* sp. strain 86FS1. Cell numbers were determined by the MPN method as described in Section 2. ○: replicates without sediment particles; ●: replicates with sediment particles; all values represent the mean of two replicates. Error bars indicate S.D.

3.3. Chemotaxis and band formation in oxygen gradients

The sulfate-reducing bacteria showed chemotactic responses to oxygen and a range of other compounds. Cells of *D. desulfuricans* accumulated up to fivefold (within 1 h) in capillaries containing various electron acceptors, lactate or sulfide (Fig. 3). Sulfate was the most effective attractant, followed by sulfite and thiosulfate.

Aerotactical responses towards oxygen were studied in microslide capillaries. Upon placement of a small oxygen bubble (5 μl) into anoxic cell suspensions an oxic zone developed. The bacteria slowed down the expansion of this zone by aerobic respiration as already reported for sulfide-free systems with *D. desulfuricans* CSN and *D. oxyclinae* N13 [17]. Lactate was required to achieve an aerotactic response. Strains with low motility showed no band formation. Cell suspensions of *Desulfomicrobium* sp. Sal never showed aerotactic responses. *Desulfobulbus* strain 86FS1 revealed negative aerotaxis (Table 1). *D. norvegicum* additionally formed a conspicuous bacterial band at the edge of the oxygen penetration zone. Sometimes in a zone on the far side of the bacterial band the cell suspension was less dense than in the rest of the capillary, showing that bacteria moved into the band even from the an-

Table 1
Physiological responses of sulfate-reducing bacteria to oxygen

	O_2 reduction rate with lactate (nmol O_2 min^{-1} mg^{-1} protein)	O_2 reduction rate with sulfide (nmol O_2 min^{-1} mg^{-1} protein)	Motility[a]	Negative aerotaxis	Band formation in gradients	Flocculation upon O_2 exposure[b]
D. norvegicum Norway	4	50	+	+	+	n.d.
Desulfomicrobium sp. Sal	4	77	(+)	−	−	−
Desulfomicrobium sp. AcI.2	38	3	(+)	−	−	+
D. oxyclinae N13	9	199	+	+[c]	+	n.d.
D. desulfuricans CSN	119	0	+	+[c]	+	+
Desulfobulbus sp. 86FS1	27	3	(+)	+	−	(+)

n.d.: not determined.
[a](+): <1% motile cells, +: at least 50% motile cells.
[b]Suspensions of about 10^8 cells ml^{-1} were exposed to 1% oxygen in the headspace of a flask (100 ml suspension and 400 ml headspace), the suspension was continuously shaken during the experiment.
[c]Eschemann et al. [17].

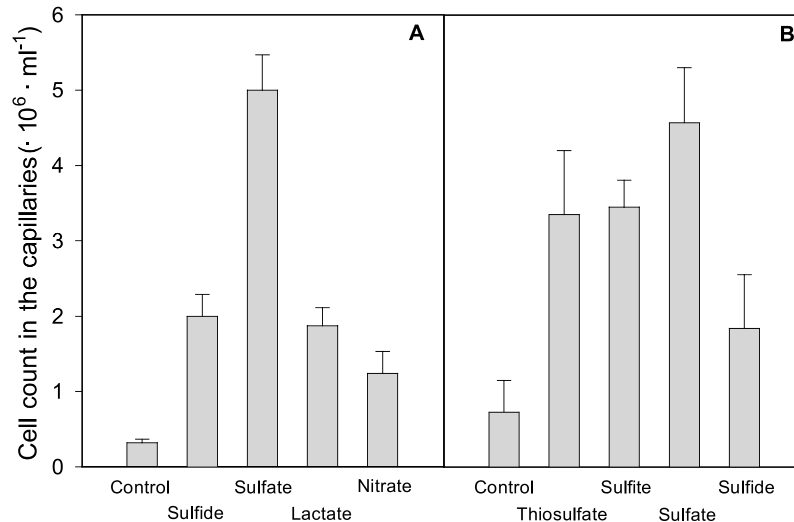

Fig. 3. Chemotaxis of *D. desulfuricans* CSN. A: Comparison of different electron acceptors and donors. B: Comparison of different sulfur compounds; the concentration of attractants was always 10 mM, incubation proceeded for 1 h at 30°C, cells were cultivated on lactate (20 mM) and sodium sulfate (10 mM); values are means of four measurements. Error bars indicate S.D.

oxic zone, as described earlier for *D. desulfuricans* CSN and *D. oxyclinae* [17].

Sulfide and oxygen profiles around oxygen bubbles were measured in suspensions of *D. oxyclinae* N13 and *D. desulfuricans* CSN. The strains formed sulfide within the anoxic part of the assay, and formed bands at the edge of the oxic zone (Fig. 4). The inner edge of the bacterial bands was positioned at an oxygen saturation of about 5%. Towards the outer edge of the bacterial band the oxygen concentration decreased to zero, and oxygen did not reach the medium outside the bands. In suspensions with *D. oxyclinae* N13 the oxygen and sulfide gradients showed little overlap, and sulfide was completely oxidized with the oxygen diffusing from the bubble (Fig. 4B). In suspensions

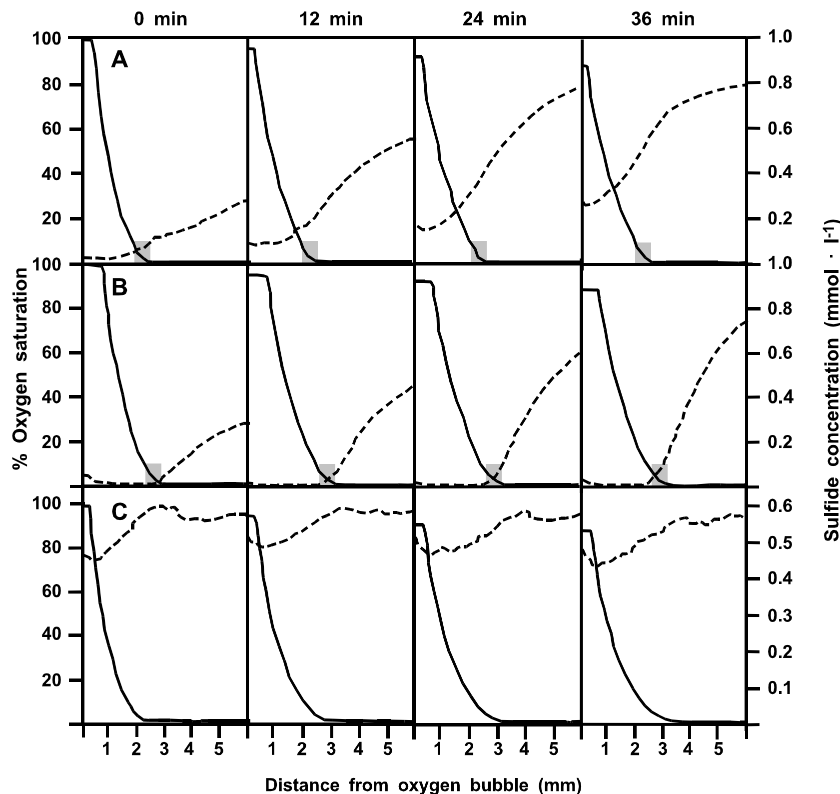

Fig. 4. Profiles of oxygen and sulfide around an oxygen bubble in suspensions of sulfate-reducing bacteria. A: *D. desulfuricans* CSN (5 mM lactate, 5 mM sulfate). B: *D. oxyclinae* N13 (10 mM lactate, 5 mM thiosulfate). C: Control experiment (2 mM sulfide); gray boxes indicate the location of bacterial bands. The cell suspensions had a density of about 0.5 mg protein per ml.

of *D. desulfuricans*, which did not oxidize sulfide with oxygen (as mentioned above), sulfide diffused into the oxic zone as far as to the oxygen bubble (Fig. 4A).

4. Discussion

4.1. Growth in oxygen–sulfide counter-gradients

Growth and liquid cell suspension experiments in our study refer to different aspects of the relations of sulfate-reducing bacteria to oxygen. In the growth experiments, the cells were immobilized in agar (higher concentrations than in previous studies [18,19]) in order to obtain a homogeneous initial cell density and to prevent chemotactic responses. Some of the strains studied showed initial growth in the oxic zones (Fig. 1). However, a growth peak was observed below the oxic–anoxic interface, in accordance with repeated observations in natural mats or sediments [3,5,7]. Our results indicate that this profile is not necessarily due to feedback reactions in complex communities. The reason for maximum growth near the interface could be the formation of intermediate sulfur compounds that can be reduced with a higher energy yield than sulfate [18,43].

In some cases, the addition of particles had a stimulating effect on growth of the bacteria in the gradient tubes and enhanced the survival of oxygen exposure in some strains. These effects have been observed with different physiological groups of bacteria, including sulfate reducers [28,44,45]. However, the presence of particles did not basically change the capacity for growth in the presence of oxygen. The interactions of aerobic and sulfate-reducing bacteria in continuous co-cultures [21–24] still remain to be elucidated.

Desulfomicrobium sp. strain AcI.2, which did not respond chemotactically towards oxygen, showed a distinctly higher survival rate after oxygen exposure when sediment particles were present in the media than without particles (Fig. 2). This strain, related to *D. apsheronum*, was isolated from a sandy sediment in the intertidal zone of the Wadden Sea and formed aggregates with sand grains in pure cultures [10].

4.2. Chemotactic responses

The sulfate-reducing bacteria in cell suspensions responded chemotactically to their typical electron acceptors and donors, but also to sulfide (Fig. 3). The presence of 12 genes for potential chemoreceptor proteins had already been concluded from genetic analyses of *Desulfovibrio vulgaris* [46,47]. Here we could demonstrate for the first time a corresponding behavior. Interestingly, with the exception of oxygen all compounds tested functioned as attractants, although some of them (e.g. sulfide and nitrate) in natural environment might indicate different situations and thus might be counteracting.

Oxygen, if present, appears to govern the chemotactic response. At high concentration it acts as a repellent. However, band formation at low oxygen concentrations indicates a more complex regulatory network that is not yet fully understood. The overall behavioral patterns resemble a fire-brigade strategy [12]: the bacteria avoid high oxygen concentrations, but they stay at the outer edge of the oxic zone (Fig. 4). They cooperate in oxygen removal by respiration, and finally re-establish anoxic conditions. For this activity electron donors are required, and even sulfide can be used by some strains for this purpose.

The sharp oxygen and sulfide gradients formed in the bands of *D. oxyclinae* prove that the joint effort of the bacteria can design their microenvironment with high efficiency, and that they are not dependent on aerobes in order to obtain favorable conditions in their surroundings.

Although an autecological model study, our investigation can help to explain observations made in natural environments with complex communities. Many incompletely oxidizing sulfate-reducing bacteria show oxygen tolerance and even limited growth under oxic conditions. However, they in principle remain anaerobes. Their electron donors are formed by fermentations under anoxic conditions only, and their behavior appears to be directed primarily to the removal of oxygen, which also prevents oxidation of their substrates by aerobic competitors.

Acknowledgements

This work was supported by grants from the Deutsche Forschungsgemeinschaft and the Danish Natural Science Research Council (M.K., Contract 9700549). We thank Tobias Sperr and Anni Glud for excellent technical assistance.

References

[1] Widdel, F. and Hansen, T.A. (1992) The dissimilatory sulfate- and sulfur-reducing bacteria. In: The Prokaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications (Balows, A., Trüper, H.G., Dworkin, M., Harder, W. and Schleifer, K.H., Eds.), pp. 583–624. Springer, New York.

[2] Ramsing, N.B., Kühl, M. and Jørgensen, B.B. (1993) Distribution of sulfate-reducing bacteria, O_2, and H_2S in photosynthetic biofilms determined by oligonucleotide probes and microelectrodes. Appl. Environ. Microbiol. 59, 3840–3849.

[3] Risatti, J.B., Capman, W.C. and Stahl, D.A. (1994) Community structure of a microbial mat: the phylogenetic dimension. Proc. Natl. Acad. Sci. USA 91, 10173–10177.

[4] Krekeler, D., Sigalevich, P., Teske, A., Cypionka, H. and Cohen, Y. (1997) A sulfate-reducing bacterium from the oxic layer of a microbial mat from Solar Lake (Sinai), *Desulfovibrio oxyclinae* sp. nov. Arch. Microbiol. 167, 369–375.

[5] Sass, H., Cypionka, H. and Babenzien, H.-D. (1997) Vertical distribution of sulfate-reducing bacteria at the oxic-anoxic interface in sediments of the oligotrophic Lake Stechlin. FEMS Microbiol. Ecol. 22, 245–255.

[6] Sass, H., Berchtold, M., Branke, J., König, H., Cypionka, H. and Babenzien, H.-D. (1998) Psychrotolerant sulfate-reducing bacteria from an oxic freshwater sediment, description of *Desulfovibrio cuneatus* sp. nov. and *Desulfovibrio litoralis* sp. nov. Syst. Appl. Microbiol. 21, 212–219.

[7] Minz, D., Fishbain, S., Green, S.J., Muyzer, G., Cohen, Y., Rittmann, B.E. and Stahl, D.A. (1999) Unexpected population distribution in a microbial mat community: sulfate-reducing bacteria localized to the highly oxic chemocline in contrast to a eucaryotic preference for anoxia. Appl. Environ. Microbiol. 65, 4659–4665.

[8] Sahm, K., MacGregor, B.J., Jørgensen, B.B. and Stahl, D.A. (1999) Sulphate reduction and vertical distribution of sulphate-reducing bacteria quantified by rRNA slot-blot hybridization in a coastal marine sediment. Environ. Microbiol. 1, 65–74.

[9] Bade, K., Manz, W. and Szewzyk, U. (2000) Behavior of sulfate-reducing bacteria under oligotrophic conditions and oxygen stress in particle-free systems related to drinking water. FEMS Microbiol. Ecol. 32, 215–223.

[10] Wieringa, E.B.A., Overmann, J. and Cypionka, H. (2000) Detection of abundant sulphate-reducing bacteria in marine oxic sediment layers by a combined cultivation and molecular approach. Environ. Microbiol. 2, 417–427.

[11] Teske, A., Ramsing, N.B., Habicht, K., Fukui, M., Küver, J., Jørgensen, B.B. and Cohen, Y. (1998) Sulfate-reducing bacteria and their activities in cyanobacterial mats of Solar Lake (Sinai, Egypt). Appl. Environ. Microbiol. 64, 2943–2951.

[12] Cypionka, H. (2000) Oxygen respiration by *Desulfovibrio* species. Annu. Rev. Microbiol. 54, 827–848.

[13] Brune, A., Frenzel, P. and Cypionka, H. (2000) Life at the oxic-anoxic interface: microbial activities and adaptations. FEMS Microbiol. Rev. 24, 691–710.

[14] Lemos, R.S., Gomes, C.M., Santana, M., LeGall, J., Xavier, A.V. and Teixeira, M. (2001) The 'strict' anaerobe *Desulfovibrio gigas* contains a membrane-bound oxygen-reducing respiratory chain. FEBS Lett. 496, 40–43.

[15] Krekeler, D., Teske, A. and Cypionka, H. (1997) Strategies of sulfate-reducing bacteria to escape oxygen stress in a cyanobacterial mat. FEMS Microbiol. Ecol. 25, 89–96.

[16] Johnson, M.S., Zhulin, I.B., Gapuzan, M.-E.R. and Taylor, B.L. (1997) Oxygen-dependent growth of the obligate anaerobe *Desulfovibrio vulgaris* Hildenborough. J. Bacteriol. 179, 5598–5601.

[17] Eschemann, A., Kühl, M. and Cypionka, H. (1999) Aerotaxis in *Desulfovibrio*. Environ. Microbiol. 1, 489–494.

[18] Cypionka, H., Widdel, F. and Pfennig, N. (1985) Survival of sulfate-reducing bacteria after oxygen stress, and growth in sulfate-free oxygen-sulfide gradients. FEMS Microbiol. Ecol. 31, 39–45.

[19] Marschall, C., Frenzel, P. and Cypionka, H. (1993) Influence of oxygen on sulfate reduction and growth of sulfate-reducing bacteria. Arch. Microbiol. 159, 168–173.

[20] Gottschal, J.C. and Szewzyk, R. (1985) Growth of a facultative anaerobe under oxygen-limiting conditions in pure culture and in co-culture with a sulfate-reducing bacterium. FEMS Microbiol. Ecol. 31, 159–170.

[21] Wimpenny, J.W.T. and Abdollahi, H. (1991) Growth of mixed cultures of *Paracoccus denitrificans* and *Desulfovibrio desulfuricans* in homogeneous and in heterogeneous culture systems. Microb. Ecol. 22, 1–13.

[22] Sigalevich, P. and Cohen, Y. (2000) Oxygen-dependent growth of the sulfate-reducing bacterium *Desulfovibrio oxyclinae* in coculture with *Marinobacter* sp. strain MB in an aerated sulfate-depleted chemostat. Appl. Environ. Microbiol. 66, 5019–5023.

[23] Sigalevich, P., Meshorer, E., Helman, Y. and Cohen, Y. (2000) Transition from anaerobic to aerobic growth conditions for the sulfate-reducing bacterium *Desulfovibrio oxyclinae* results in flocculation. Appl. Environ. Microbiol. 66, 5005–5012.

[24] Sigalevich, P., Baev, M.V., Teske, A. and Cohen, Y. (2000) Sulfate reduction and possible aerobic metabolism of the sulfate-reducing bacterium *Desulfovibrio oxyclinae* in a chemostat coculture with *Marinobacter* sp. strain MB under exposure to increasing oxygen concentrations. Appl. Environ. Microbiol. 66, 5013–5018.

[25] Dannenberg, S., Kroder, M., Dilling, W. and Cypionka, H. (1992) Oxidation of H_2, organic compounds and inorganic sulfur compounds coupled to reduction of O_2 or nitrate by sulfate-reducing bacteria. Arch. Microbiol. 158, 93–99.

[26] Fuseler, K., Krekeler, D., Sydow, U. and Cypionka, H. (1996) A common pathway of sulfide oxidation by sulfate-reducing bacteria. FEMS Microbiol. Lett. 144, 129–134.

[27] Laanbroek, H.J. and Geerligs, H.J. (1983) Influence of clay particles (illite) on substrate utilization by sulfate-reducing bacteria. Arch. Microbiol. 134, 161–163.

[28] Fukui, M. and Takii, S. (1990) Survival of sulfate-reducing bacteria in the oxic surface sediment of a seawater lake. FEMS Microbiol. Ecol. 73, 317–322.

[29] Cypionka, H. (1989) Characterization of sulfate transport in *Desulfovibrio desulfuricans*. Arch. Microbiol. 152, 237–243.

[30] Miller, J.D.A. and Saleh, A.M. (1964) A sulphate-reducing bacterium containing cytochrome c_3 but lacking desulfoviridin. J. Gen. Microbiol. 37, 419–423.

[31] Widdel, F. and Bak, F. (1992) Gram-negative mesophilic sulphate reducing bacteria. In: The Prokaryotes (Balows, A., Trüper, H.G., Dworkin, M., Harder, W. and Schleifer, K.H., Eds.), pp. 3353–3378. Springer, New York.

[32] Balch, W.E., Fox, G.E., Magrum, L.J., Woese, C.R. and Wolfe, R.S. (1979) Methanogens: reevaluation of a unique biological group. Microbiol. Rev. 43, 260–296.

[33] Revsbech, N. and Jørgensen, B.B. (1986) Microelectrodes: their use in microbial ecology. Adv. Microb. Ecol. 9, 293–352.

[34] Cypionka, H. (1986) Sulfide-controlled continuous culture of sulfate-reducing bacteria. J. Microbiol. Meth. 5, 1–9.

[35] Porter, K.G. and Feig, Y.S. (1980) Use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. 25, 943–948.

[36] Cypionka, H. (1994) Sulfate transport. Methods Enzymol. 243, 3–14.

[37] Revsbech, N.P. (1989) An oxygen microsensor with a guard cathode. Limnol. Oceanogr. 34, 474–478.

[38] Kühl, M., Steuckart, C., Eickert, G. and Jeroschewski, P. (1998) A H_2S microsensor for profiling biofilms and sediments: application in an acidic lake sediment. Aquat. Microb. Ecol. 15, 201–209.

[39] Kühl, M. and Steuckart, C. (2000) Sensors for in situ analysis of sulfide in aquatic systems. In: In Situ Monitoring of Aquatic Systems (Buffle, J. and Horvai, G., Eds.), pp. 121–159. Wiley, Chichester.

[40] Thar, R. and Kühl, M. (2001) Motility of *Marichromatium gracile* in response to light, oxygen and sulfide. Appl. Environ. Microbiol. 67, 5410–5419.

[41] de Man, J.C. (1977) MPN tables for more than one test. Eur. J. Appl. Microbiol. 4, 307–316.

[42] Fröstl, J.M. and Overmann, J. (1998) Physiology and tactic response of the phototrophic consortium '*Chlorochromatium aggregatum*'. Arch. Microbiol. 169, 129–135.

[43] van den Ende, F.P., Meier, J. and van Gemerden, H. (1997) Syntrophic growth of sulfate-reducing bacteria and colorless sulfur bacteria during oxygen limitation. FEMS Microbiol. Ecol. 23, 65–80.

[44] Fukui, M. and Takii, S. (1994) Kinetics of sulfate respiration by free-living and particle-associated sulfate-reducing bacteria. FEMS Microbiol. Ecol. 13, 241–248.

[45] Taylor, G.T. and Gulnik, J.D. (1996) Enhancement of marine bacterial growth by mineral surfaces. Can. J. Microbiol. 42, 911–918.

[46] Fu, R., Wall, J.D. and Voordouw, G. (1994) DcrA, a *c*-type heme-containing methyl-accepting protein from *Desulfovibrio vulgaris* Hildenborough, senses the oxygen concentration or redox potential of the environment. J. Bacteriol. 176, 344–350.

[47] Deckers, H.M. and Voordouw, G. (1994) Identification of a large family of genes for putative chemoreceptor proteins in an ordered library of the *Desulfovibrio vulgaris* Hildenborough genome. J. Bacteriol. 176, 351–358.