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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

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A chemostat coculture of the sulfate-reducing bacterium *Desulfovibrio oxyclinae* together with a facultative aerobe heterotroph tentatively identified as *Marinobacter* sp. strain MB was grown under anaerobic conditions and then exposed to a stepwise-increasing oxygen influx (0 to 20% O_2 in the incoming gas phase). The coculture consumed oxygen efficiently, and no residual oxygen was detected with an oxygen supply of up to 5%. Sulfate reduction persisted at all levels of oxygen input, even at the maximal level, when residual oxygen in the growth vessel was 87 μM. The portion of *D. oxyclinae* cells in the coculture decreased gradually from 92% under anaerobic conditions to 27% under aeration. Both absolute cell numbers and viable cell counts of the organism were the same as or even higher than those observed in the absence of oxygen input. The patterns of consumption of electron donors and acceptors suggest that aerobic incomplete oxidation of lactate to acetate is performed by *D. oxyclinae* under high oxygen input. Both organisms were isolated from the same oxic zone of a cyanobacterial mat where they have to adapt to daily shifts from oxic to anoxic conditions. This type of syntrophic association may occur in natural habitats, enabling sulfate-reducing bacteria to cope with periodic exposure to oxygen.

The photic zone of cyanobacterial mats is characterized by extreme shifts in oxygen and sulfide concentrations from oxygen supersaturation during the light period to anoxic, sulfide-enriched conditions in the dark (24). These changes are a challenge for the organisms living in such a habitat. Daily exposure to high oxygen concentrations may be deleterious to obligately anaerobic organisms, such as sulfate-reducing bacteria (SRB) (13, 18, 24). High numbers of SRB were found in the oxic zone of a cyanobacterial mat from the Solar Lake (Sinai) throughout the diurnal cycle (10, 21, 22, 25, 27), indicating that these organisms can deal with exposure to elevated oxygen concentrations of even up to 1.5 mM. Moreover, high rates of sulfate reduction were found in the oxic layers of these microbial mats (4, 5, 9), as well as in microbial mats from Baja California, Mexico (2).

None of the SRB isolated so far can either grow aerobically or reduce sulfate under high concentrations of oxygen. *Desulfovibrio vulgaris* Hildenborough was found to be capable of slow linear aerobic growth under very low concentrations of oxygen. Oxygen concentrations of only 0.07% were toxic to this organism (14). The oxygen-tolerant SRB *Desulfovibrio oxyclinae* isolated in our laboratory (16) was demonstrated to persist in continuous culture gassed with 1% O_2, although flocculation occurred after exposure to oxygen (25).

It was previously demonstrated that SRB in the upper layer of cyanobacterial mats can form close associations with oxygen-scavenging bacteria (12, 26). Such consortia may be responsible, at least in part, for the observed in situ sulfate-reducing activity under aerobic conditions.

In this report, we present the effects of exposure to oxygen on the continuous culture of *D. oxyclinae* from the upper layer of the Solar Lake cyanobacterial mat in defined coculture with the facultatively aerobic heterotroph *Marinobacter* sp. strain MB, isolated from the same niche.

MATERIALS AND METHODS

Growth medium. One liter of the synthetic growth medium was used in all continuous culture experiments and contained the following: NaCl, 50.0 g; KCl, 1.0 g; $MgCl_2 \cdot 6H_2O$, 2.5 g; K_2HPO_4, 0.5 g; NH_4Cl, 1.0 g; $CaCl_2 \cdot 2H_2O$, 0.08 g; resazurine, 0.01 g; vitamin solution, 1 ml; vitamin B_{12} solution, 1 ml; thiamine solution, 1 ml; ascorbate-thioglycolate reducing solution, 10 ml (32); and SL7 mineral solution, 1 ml (1). Sodium sulfate and carbon sources were added as sterile stock solutions to the final concentrations specified below.

Bacterial strains. *D. oxyclinae* strain P1B (DSM 11498) was isolated previously in our laboratory from the oxic zone of the Solar Lake mats (16). *Marinobacter* sp. strain MB was obtained from the same environment (M. V. Baev et al., unpublished data). Comparison of sequences of 16S rDNA genes (positions 1 to 1350) demonstrated its 99% sequence similarity with the type strain *Marinobacter hydrocarbonoclasticus* (11). Unlike the type strain, *Marinobacter* sp. strain MB did not require NaCl for growth.

Continuous culture experiments. Growth experiments were carried out in a 0.45-liter laboratory fermentor (Bioflo; New Brunswick Scientific, Edison, N.J.) equipped with a pH-controlling and oxygen-monitoring device (B. Braun, Melsungen, Germany). pH was maintained at 7.4 ± 0.2 by titration with 1 N HCl and 1 N NaOH. The concentration of dissolved oxygen in the growth vessel was monitored using a polarimetric oxygen electrode (Ingold, Urdorf, Switzerland) calibrated in percent air saturation. Oxygen concentration at 100% air saturation in the chemostat was found to be 166 μM. The growth medium contained 10 mM

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TABLE 1. Growth parameters of the *D. oxyclinae-Marinobacter* sp. mixed culture under various concentrations of oxygen in the incoming gas

O_2 (%)	Mean DO[a] (μM) ± SD	Mean concn in cultural liquid ± SD				Mean protein concn (mg/liter) ± SD	*Marinobacter* sp.	
		Sulfide (μM)	Sulfate (mM)	Acetate (mM)	Lactate (mM)		Mean cell density (10^8 ml^{-1}) ± SD	%
0	0	287 ± 92	3.4 ± 0.4	15.5 ± 0.1	<0.05	30.2 ± 1.5	0.23 ± 0.08	8.5
1	0	108 ± 8	3.3 ± 0.4	15.4 ± 0.2	<0.05	31.6 ± 0.3	0.24 ± 0.02	8.3
2.5	0	122 ± 12	6.3 ± 0.3	11.4 ± 0.3	<0.05	32.1 ± 0.4	1.28 ± 0.05	32.8
5	0	196 ± 2	4.8 ± 1.0	7.2 ± 0.6	<0.05	35.2 ± 0.4	2.09 ± 1.26	36.0
10	8.3 ± 2.0	96 ± 4	6.3 ± 0.7	3.4 ± 0.2	1.5 ± 0.4	85.1 ± 0.9	6.01 ± 0.25	71.5
20	86.8 ± 4.2	44 ± 5	8.6 ± 0.1	<0.05	<0.05	99.9 ± 1.7	5.59 ± 0.07	72.6

[a] DO, residual dissolved oxygen in the growth vessel.

Na_2SO_4 and 15.5 mM sodium lactate. The dilution rate was set at 0.05 h^{-1}, the mixing rate was 200 rpm, and the temperature was maintained at 35°C.

To change the oxygen input to the growth vessel, the following gases were mixed and supplied to the fermentor at a rate of 0.1 liter min^{-1}: oxygen-free nitrogen; 90% N_2, 5% O_2, plus 5% CO_2; and air. During the experiment, the oxygen content in the incoming gas was increased stepwise from zero (nitrogen) to 20% (air). Each continuous culture mode was maintained at least for 5 days, allowing six volume changes of the medium before increasing oxygen input. At the onset of sampling, the chemostat was at steady state, as indicated by the stable cell counts.

To prepare the inoculum for the continuous culture experiment, *Marinobacter* sp. strain MB was grown aerobically in batch culture overnight on 20 mM acetate. *D. oxyclinae* was grown anaerobically in sealed bottles on 20 mM lactate and 20 mM Na_2SO_4 for 2 days. Each culture was harvested by centrifugation, washed with the synthetic medium, and diluted to equal turbidity. The fermentor was then inoculated with equal volumes of cell suspensions of *Marinobacter* sp. strain MB and *D. oxyclinae*. The initial overall cell concentration was 1.5×10^8 ml^{-1}.

Enumeration of *D. oxyclinae* and *Marinobacter* sp. strain MB in the coculture. Total cell numbers in each sample were determined by microscopic counting of at least 500 cells in a Petroff-Hausser chamber. The number of viable sulfate-reducing cells was determined by the most-probable-number (MPN) method in eight replicate fivefold dilutions on Nunclon 96-well plates (Nunc A/S, Roskilde, Denmark). Defined multipurpose medium with ascorbate-thioglycolate reducing solution (31) was supplemented with 50 mg of $FeSO_4 \cdot 7H_2O$ per liter; formation of black FeS precipitate indicated sulfide production. The chemostat sample was diluted 10-fold prior to inoculation. The multiwell plates were incubated in an anaerobic hood. The number of aerobic cells was determined by plating on the synthetic growth medium containing 20 mM sodium acetate as the sole carbon source. The petri dishes were incubated aerobically at 35°C.

For direct microscopic counts of *D. oxyclinae* and *Marinobacter* sp. strain MB, we used the nalidixic acid method described by Kogure et al. (15). Five-milliliter aliquots of the chemostat samples were diluted fivefold with fresh synthetic growth medium containing 20 μg of nalidixic acid (Sigma) ml^{-1} and 20 mM sodium acetate and incubated aerobically overnight at 35°C. Actively growing aerobic cells of *Marinobacter* sp. strain MB became markedly elongated and readily distinguishable from the *D. oxyclinae* cells. Nalidixic acid at 20 μg ml^{-1} was experimentally found not to inhibit cell division of *D. oxyclinae* under anaerobic growth conditions. Elongated cells and total cells were counted microscopically in a Petroff-Hausser chamber.

Measurement of respiration rates. Respiration rates were determined using a reaction chamber described by Cypionka (6). Prior to the measurement, the samples were aseptically washed with sterile medium without carbon and sulfur sources. The resulting thick cell suspension was incubated under nitrogen for 30 min. Protein concentrations in the chamber were determined after the measurement. To determine the rate of aerobic respiration, 100 μl of oxygen-saturated water containing 139 nmol of O_2 was injected in the chamber under excess of lactate, and the rate of oxygen consumption was determined. In the case of *Marinobacter* sp. strain MB, the cell suspension was bubbled with sterile air overnight prior to measurement of respiration rates in order to exhaust oxygen respiration of endogenic carbon sources.

Calculated rates of oxygen consumption and sulfate reduction in the chemostat. The rate of oxygen consumption in the chemostat was calculated from the oxygen saturation as measured in the sterile growth vessel under known oxygen input, and the residual oxygen concentration was determined by the submerged oxygen electrode. Sulfate consumption was calculated from the difference of sulfate concentrations measured in the medium reservoir and in the growth vessel; the sulfate reduction rate was calculated from sulfate consumption and dilution rates.

Analytical procedures. Protein was determined by the Lowry method (19). Elemental sulfur, which interferes with this reaction, was not found in the samples. Sulfate was determined by the turbidimetric method (12a) as modified by H. Cypionka (personal communication), and sulfide was determined by the methylene blue method (3). Organic acids were determined by high-pressure liquid chromatography (29).

DGGE analysis. Denaturing gradient gel electrophoresis (DGGE)-PCR was performed after PCR amplification with primers GM5-F (with GC clamp) and 907-R. PCR products were analyzed in a 30 to 70% denaturing gradient gel as described by Muyzer et al. (23).

RESULTS AND DISCUSSION

In this paper we report for the first time the growth of an SRB, *D. oxyclinae*, in a coculture with *Marinobacter* sp. strain MB under exposure to up to 20% O_2 in the incoming gas and under residual oxygen concentrations of up to 87 μM. Dissolved oxygen was detected in the growth vessel by a submerged oxygen electrode only when the system was bubbled with gas mixtures containing 10 and 20% oxygen. The growth modes observed for the defined coculture of *D. oxyclinae* and *Marinobacter* sp. strain MB can therefore be defined as anaerobic (gassing with N_2), microaerophilic (gassing with 1% to 5% O_2; no dissolved oxygen detected in the growth vessel, resazurine decolorized), and aerobic (gassing with 10 and 20% O_2; residual oxygen detected, resazurine pink). Altogether, the coculture was studied under six different oxygen supply rates (Table 1; Fig. 1). Unlike axenic cultures of *D. oxyclinae*, where gassing with 1% oxygen resulted in flocculation (26), no macroscopic cell aggregates were found in the coculture under any growth mode.

Anaerobic processes. The coculture was grown in a medium containing 15.5 mM sodium lactate and 10 mM Na_2SO_4. After anaerobic batch incubation for 48 h, the total cell number increased from 1.5×10^8 to 6.6×10^8 ml^{-1}. *Marinobacter* sp.

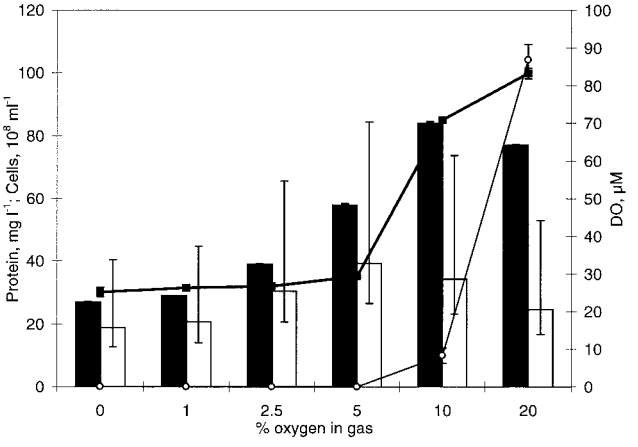

FIG. 1. Protein concentration (■), dissolved oxygen (DO), percent air saturation (○), total cell counts (shaded bars), and MPN counts of *D. oxyclinae* (light bars) in a *D. oxyclinae-Marinobacter* sp. coculture under various oxygen concentrations in the incoming gas. Error bars indicate 95% confidence intervals for MPN and standard deviations for other parameters.

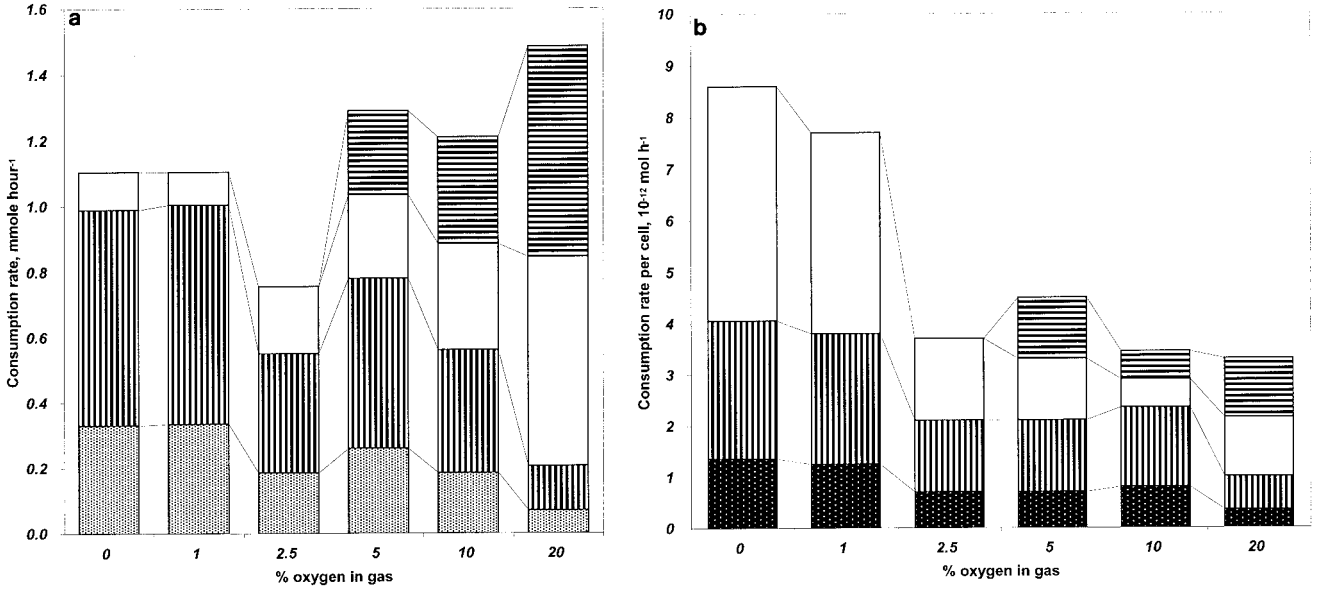

FIG. 2. Calculated rates of substrate consumption. (a) Sulfate reduction (shaded bars), lactate utilization via sulfate reduction (vertically striped bars), lactate oxidation by other pathways (open bars), and complete oxidation of lactate (horizontally striped bars). (b) Rates per cell of sulfate reduction (shaded bars), lactate utilization via sulfate reduction (vertically striped bars), lactate oxidation by other pathways (open bars), and complete oxidation of lactate (horizontally striped bars).

strain MB cells as identified by the nalidixic acid method were found to constitute 11.8% of the total cell number. The medium flow was then set at a dilution rate of 0.05 h^{-1}.

Sulfate reduction was the main process under these conditions, and *D. oxyclinae* was the dominant organism. *D. oxyclinae* oxidized 2 mol of lactate per mol of sulfate reduced. It was therefore possible to estimate the impact of sulfate reduction upon exposure to various levels of oxygen supply (Table 1; Fig. 2).

Although this type strain was described as an aerobic organism capable of nitrate reduction but not of fermentation, only fermentation of glucose was actually tested (11). Therefore, a washout of *Marinobacter* sp. strain MB resulting in an exponential decrease in cell counts could have been expected after the onset of continuous culture. However, the portion of *Marinobacter* sp. strain MB cells in the coculture remained stable throughout the anoxic stage of the experiment at 8.3% (Fig. 3, upper panel). Subsequent experiments demonstrated that the number of culturable cells of *Marinobacter* sp. strain MB in steady-state anaerobic chemostat cocultures also remained constant at $(9.6 \pm 1.1) \times 10^6$ ml^{-1} (Fig. 3, lower panel).

Persistence of *Marinobacter* sp. strain MB under anaerobic conditions resulted in consumption of more lactate than was possible by sulfate reduction alone. Although 6.6 mmol of sulfate was reduced and 13.2 mmol of lactate was oxidized concurrently, all 15.5 mmol of lactate supplied was consumed. An equimolar amount of acetate was found. Since neither nitrate nor Fe(III) was present in the growth medium, the residual 2.3 mmol of lactate was oxidized to acetate by *Marinobacter* sp. MB presumably by fermentation. In the reaction lactate + 2 H_2O = acetate + HCO_3^- + H^+ + 2 H_2, $\Delta G^{0\prime}$ at 1 atm of H_2 is -4 kJ. However, when hydrogen pressure is maintained at 10^{-4} atm, $\Delta G^{0\prime}$ of this process is -49.7 kJ (the values for free energy were taken from reference 28). *Desulfovibrio* spp. are known to be capable of scavenging hydrogen to levels below 10^{-4} atm (32).

If high hydrogen concentrations inhibited fermentative growth of *Marinobacter*, intense purging with an inert gas

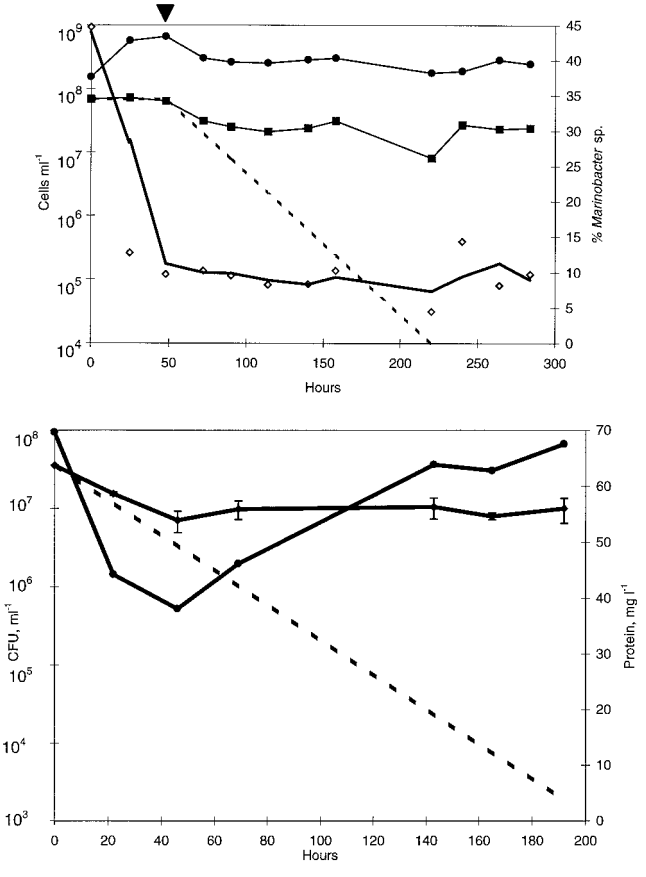

FIG. 3. Persistence of *Marinobacter* sp. strain MB in anoxic chemostats. (Upper) Total cell numbers (●), cells of *Marinobacter* sp. strain MB (■), portion of *Marinobacter* sp. strain MB (◇, bold line), and expected washout of *Marinobacter* sp. strain MB (broken line). The onset of medium flow is marked by an arrowhead. (Lower) Protein concentration (●), CFU of *Marinobacter* sp. strain MB (■), and expected washout of *Marinobacter* sp. strain MB (broken line) from an anaerobic chemostat coculture of *D. oxyclinae* and *Marinobacter* sp. strain MB.

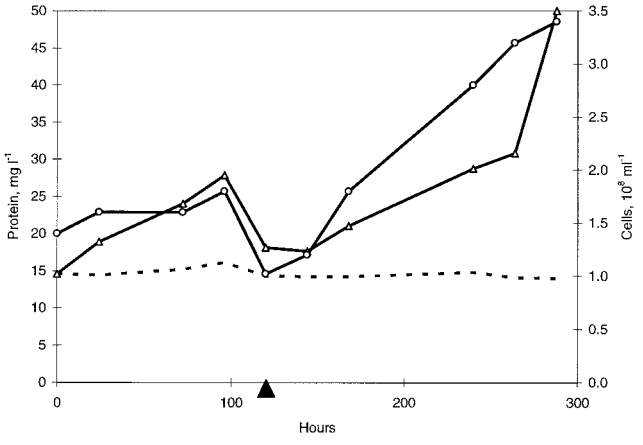

FIG. 4. Anaerobic growth of *Marinobacter* sp. on organic acids. An arrowhead indicates addition of pyruvate. △, protein; ○, microscopic cell counts; broken line, protein concentration of anaerobic cultures in sealed test tubes.

would enable it to grow anaerobically. Indeed, under gassing with nitrogen at the rate of 0.5 1 min^{-1}, *Marinobacter* sp. strain MB grew in an axenic anaerobic culture either with Casamino Acids (data not shown) or with lactate as the sole carbon and energy source (Fig. 4). The expected hydrogen tension under these gassing conditions was 10^{-2} to 10^{-3} atm. Slow linear growth on lactate was maintained for 4 days. When cessation of growth (as evidenced by decreased cell numbers) was observed, pyruvate was added to the growth vessel to 20 mM. Growth was then maintained for an additional 8 days. No growth by fermenting either lactate or pyruvate was observed in closed test tubes without hydrogen removal. When hydrogen was removed by gassing, only very slow linear fermentative growth of *Marinobacter* sp. strain MB occurred in axenic cultures, indicating possible inhibition by hydrogen. *D. oxyclinae* was shown to grow on H_2 plus acetate (16). Under such growth conditions, it used acetate only as a carbon source and therefore consumed it in relatively minor amounts. *D. oxyclinae* can therefore keep the hydrogen level low enough to allow efficient fermentative growth of *Marinobacter*. The cell yield of 3.7×10^7 per mmol of sulfate reduced is close to the yield of 4.1×10^7 cells per mmol of sulfate reduced observed for the axenic culture of *D. oxyclinae* (25). Hydrogen consumption was therefore possibly not coupled to cell growth and protein synthesis. Acetate accumulated in the growth vessel and was not further oxidized under anaerobic conditions.

Microaerophilic processes. Increasing the incoming oxygen concentration to 1% did not result in changes of cell numbers, protein concentration, residual sulfate concentration, and the number of *Marinobacter* sp. strain MB cells. A minor decrease in acetate concentration, possibly the result of complete aerobic oxidation by *Marinobacter* sp. strain MB, was observed, although no increase in the number of *Marinobacter* cells was found. Yet the concentration of dissolved sulfide decreased 2.6-fold compared to the anaerobic mode.

The high rates of gas flow applied in this experiment make quantitative estimations of sulfide production impossible. Yet under these conditions the concentration of dissolved sulfide was less than that observed under anaerobic conditions and even considerably less than those found later under higher oxygen concentrations. The ratio of 35 μmol of residual dissolved sulfide per mmol of sulfate reduced was fairly constant for the two other, more oxic microaerophilic modes, i.e., 2.5 and 5% oxygen in the incoming gas. Assuming that each value represented the portion of sulfide not removed by gassing, we could have expected 235 μM dissolved sulfide instead of the 108 μM actually found. This means that approximately 3.5 mM residual dissolved sulfide was removed from the growth vessel by other processes, most probably by aerobic sulfide oxidation by *D. oxyclinae*; 7 mM oxygen was required for this process. Sulfide was found to be aerobically oxidized by *D. oxyclinae* under low oxygen concentrations of <30 μM (16). The respiration rate determined for this organism under exposure to oxygen was 620 to 700 nmol of O_2 min^{-1} mg of $protein^{-1}$ (26). Assuming equal protein content for cells of *D. oxyclinae* and *Marinobacter* sp. strain MB, the respiration rate required to remove 355 μmol of O_2 h^{-1} was 172 nmol of O_2 min^{-1} mg of $protein^{-1}$. The respiration rate of *Marinobacter* sp. strain MB was found to be 550 to 560 nmol of O_2 min^{-1} mg of $protein^{-1}$. Under such conditions, the two organisms competed for oxygen, and due to the predominance of *D. oxyclinae* it was the main consumer of oxygen. Since the yield of 3.9×10^7 *D. oxyclinae* cells per mmol of sulfate reduced was close to the yield of 3.7×10^7 cells for the anoxic coculture, we conclude that under 1% oxygen in the incoming gas, sulfide oxidation by *D. oxyclinae* was not coupled to cell growth. Oxygen could be used by *D. oxyclinae* also for the aerobic oxidation of hydrogen. However, since fermentative growth of *Marinobacter* sp. strain MB remained at the same level as under anaerobic conditions, we can assume that the rate of hydrogen removal by *D. oxyclinae* did not increase. Aerobic hydrogen oxidation was therefore not an important process.

When gassed with 2.5% oxygen, total cell numbers in the chemostat increased. *Marinobacter* sp. strain MB now constituted 32.8% of the total cell number. Cell numbers of *D. oxyclinae* as determined by the nalidixic acid method did not change, although the viable cell numbers as determined by MPN increased. Sulfate reduction decreased 1.8-fold; the amount of sulfate reduced per cell of *D. oxyclinae* decreased as well. Under this steady-state mode, 26% of acetate produced by anaerobic processes was oxidized completely.

The increased yield of 7.1×10^7 *D. oxyclinae* cells per mmol of sulfate reduced indicated that some process other than sulfate reduction contributed to the growth of this organism. *D. oxyclinae* can perform aerobic oxidation of both sulfide and lactate. Unlike the case for other oxygen-tolerant *Desulfovibrio* species (17), aerobic lactate oxidation by *D. oxyclinae* is a slow process, with a rate of 0.67 nmol min^{-1} mg of $protein^{-1}$ (16). Aerobic sulfide oxidation is therefore more likely to sustain growth. Free-energy gain of this reaction is −828 kJ. *Marinobacter* sp. strain MB oxidized 8.16 mM lactate; half of this amount was oxidized completely. Since lactate fermentation continued, it may be concluded that hydrogen consumption by *D. oxyclinae* persisted via either sulfate reduction or aerobic oxidation. *D. oxyclinae* was demonstrated to be incapable of anaerobic growth on hydrogen plus CO_2 (16). Aerobic H_2 oxidation can occur under low oxygen concentrations. We cannot assess the contribution of this reaction to cell growth of *D. oxyclinae*. Consumption of acetate per *Marinobacter* cell increased 10-fold between 1 and 2.5% oxygen in the incoming gas and remained almost stable at higher oxygen input levels. Increased cell numbers of *Marinobacter* sp. strain MB and complete oxidation of 4.1 mM acetate indicated that under these conditions oxygen was consumed mostly by *Marinobacter* sp. strain MB. The toxic effect of oxygen on *D. oxyclinae* was therefore relieved.

In the presence of oxygen, the concentration of the terminal products of sulfate reduction may decrease. Sulfide can be removed by aeration, while acetate can be oxidized by *Marinobacter* sp. strain MB. Thermodynamically, this can increase

the free-energy gain of sulfate reduction. However, in the case of the concentration ranges observed in the course of this experiment, the overall energy gain will increase only by 10 to 15% as calculated using the values given by Thauer et al. (28). There were only two electron acceptors available in the growth vessel, namely, sulfate and oxygen. For *D. oxyclinae* to maintain its growth rate under aerobic conditions, processes using oxygen were required.

Under gassing with 5% oxygen, over 50% of available sulfate was consumed by sulfate reduction, compared to 37% under gassing with 2.5% O_2. The sulfate reduction rate was 1.4-fold higher than that observed in the previous mode. The concentration of dissolved sulfide was the highest of all of the microaerophilic modes. Sulfate reduction was the main process performed by *D. oxyclinae*. The cell yield of *D. oxyclinae* did not change. All of the lactate was oxidized under these conditions. Acetate concentration in the growth vessel decreased. This implies more pronounced complete aerobic lactate oxidation by *Marinobacter* sp. strain MB. Both cell numbers and protein concentration increased. The portion of *Marinobacter* sp. strain MB cells increased and constituted 36% of the total cell number. The calculated oxygen consumption by *Marinobacter* sp. strain MB increased 2.6-fold.

Aerobic processes. When the chemostat was bubbled with 10% O_2, it became aerobic. Residual dissolved oxygen in the growth vessel was 5 to 8% air saturation, corresponding to 8.3 ± 2.0 μM. Despite a twofold increase in oxygen supply, the calculated oxygen consumption by *Marinobacter* sp. strain MB increased only by 27%. This suggested that certain aerobic processes were performed by *D. oxyclinae*. Both the residual sulfate concentration and the sulfate reduction rate were similar to those observed under 2.5% O_2, although the residual sulfide concentration was only 96 ± 4 μM. The ratio of dissolved sulfide to sulfate consumed at this stage was lower than those observed under higher or lower oxygen flux, indicating that aerobic sulfide oxidation by *D. oxyclinae* occurred concomitantly with sulfate reduction. *D. oxyclinae* remained viable at cell numbers similar to those found under microaerobic conditions at 5% O_2. This indicated that sulfide oxidation was not coupled to growth. *Marinobacter* sp. strain MB numbers increased about threefold to over 70% of the total cell numbers. Total protein also increased significantly and was three times higher than that observed in the anaerobic growth mode. Residual acetate decreased but was still detected at 3.4 ± 0.2 mM. The detection of residual 1.5 mM lactate only under gassing with 10% oxygen is not understood.

A further increase in the oxygen supply by bubbling with air resulted in a dissolved oxygen concentration of 50 to 55% air saturation in the growth vessel. Sulfate reduction still persisted, although the rate of this process decreased markedly to 21% of the rate found under anaerobic conditions. Sulfide was detected in the aerobic chemostat, and 1.4 mM sulfate was reduced. Cell numbers of *D. oxyclinae* decreased slightly, and the MPN counts of this organism were less than those obtained under lower oxygen concentrations but were still higher than the values for the anaerobic culture. No evidence of aerobic sulfide oxidation by *D. oxyclinae* was found. Persistent growth of *D. oxyclinae* at a rate of 0.05 h^{-1} under aeration when sulfate reduction was seriously impaired required the use of the only other available electron acceptor, oxygen. Under these growth conditions, we assume that both sulfate reduction and aerobic respiration were performed concomitantly by *D. oxyclinae*. The physiology of this process is presently being studied.

It was the only gassing mode when both lactate and acetate were oxidized completely. Overall cell numbers decreased somewhat, but total protein level increased. The proportion of *Marinobacter* sp. strain MB did not change significantly compared to the 10% O_2 influx mode.

An attempt was made to examine the changes in the proportion of *D. oxyclinae* and *Marinobacter* sp. strain MB during this experiment by DGGE analysis of PCR-amplified 16S rDNA fragments. DGGE analysis revealed the presence of *D. oxyclinae* in all samples. However, the procedure applied could not detect the presence of *Marinobacter* even in the aerobic stage of the experiment, where this organism was dominant. When this technique was applied to defined cell mixtures with a ratio of *Marinobacter* sp. strain MB to *D. oxyclinae* of 1:100 to 100:1, *Marinobacter* 16S rDNA was detected only when the ratio was 15:1 and higher (data not shown).

Concluding remarks. MPN counts showed that *D. oxyclinae* was present in the fermentor in all of the above-described growth modes. Taking into consideration the inherent inaccuracy of the MPN method (as shown by the 95% confidence intervals in Fig. 1), it may even be concluded that the viable counts of *D. oxyclinae* did not change significantly throughout the experiment; i.e., it maintained a growth rate of 0.05 h^{-1}. Moreover, the slight increase in the numbers of *D. oxyclinae* obtained by MPN can indicate increased viability of *D. oxyclinae* cells under exposure to up to 5% oxygen. Similar results were obtained in the experiment with an axenic culture of *D. oxyclinae* gassed with 1% O_2 (25). Growth of this organism under aeration is attributed at least partially to sulfate reduction. Sulfate reduction was performed by *D. oxyclinae* even under continuous gassing with air.

While *D. oxyclinae* has been shown to be capable of aerobic oxidation of hydrogen, sulfide, and lactate (16) and SRB are known to be able to produce ATP by this pathway (7, 8, 20), no oxygen-dependent growth of this organism was previously reported. *D. vulgaris* was found to be capable of slow linear growth only under very low oxygen levels of less than 0.95 μM (14).

These findings are similar to the results obtained by Gottschal and Szewzyk (12), although the aeration rate of 170 ml of air h^{-1} applied in their experiments corresponded to an oxygen input rate of 25.3 μmol min^{-1}, almost two times less than the lowest rate applied in our experiments. Moreover, unlike *Marinobacter* sp. strain MB, *Vibrio* strain HP1, the facultatively anaerobic component of the coculture, was specifically selected for its pronounced capability for fermentative growth, and glucose was present in the growth medium as a substrate for fermentation. Van den Ende et al. (30) reported syntrophic growth of a marine *Desulfovibrio desulfuricans* and *Thiobacillus thioparus* under up to 5.6% oxygen in the gassing mixture. In the coculture of *D. oxyclinae* and *Marinobacter* sp. strain MB, a more intricate syntrophic relationship occurs. This organism is well adapted to an aerobic environment and could possibly ameliorate its toxic effects on *D. oxyclinae*.

D. oxyclinae and *Marinobacter* sp. strain MB were both isolated from the oxic zone of a cyanobacterial mat where oxygen concentrations vary diurnally from anaerobic conditions in the night to O_2 supersaturation at midday (24). The reported association of the two microorganisms is therefore likely to occur naturally. When enriching for oxygen-tolerant SRB, we observed a stable coculture of *D. oxyclinae* and *Marinobacter* sp. strain MB. Similar association of *D. oxyclinae* and *Arcobacter* sp. was previously described by Teske et al. (26). This paper demonstrates that an SRB which was considered to be an obligate anaerobe grows under aerobic conditions when in coculture with a facultative aerobic heterotrophic bacterium. Such bacterial consortia may enable *D. oxyclinae* to perform sulfate reduction and possibly aerobic respiration under expo-

sure to oxygen concentrations of at least 20%, corresponding to atmospheric air.

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REFERENCES

1. **Biebl, H., and N. Pfennig.** 1981. Isolation of members of the family *Rhodospirillaceae*, p. 267–273. *In* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), The prokaryotes, 2nd ed. Springer-Verlag, New York, N.Y.
2. **Canfield, D. E., and D. J. Des Marais.** 1991. Aerobic sulfate reduction in microbial mats. Science **251:**1471–1473.
3. **Cline, J. D.** 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol. Oceanogr. **14:**454–458.
4. **Cohen, Y.** 1984. Oxygenic photosynthesis, anoxygenic photosynthesis, and sulfate reduction in cyanobacterial mats, p. 435–441. *In* M. G. Klug and C. A. Reddy (ed.), Current perspectives in microbial ecology. ASM Press, Washington, D.C.
5. **Cohen, Y., Y. Helman, and P. Sigalevich.** 1994. Light-driven sulfate reduction and methane emission in hypersaline cyanobacterial mats, p. 421–427. *In* L. J. Stal and P. Caumette (ed.), Microbial mats. NATO ASI Series, per. G, vol. 35. Springer-Verlag, New York, N.Y.
6. **Cypionka, H.** 1994. Sulfate transport. Methods Enzymol. **243:**3–14.
7. **Dannenberg, S., M. Kroder, M. Dilling, and H. Cypionka.** 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.
8. **Dilling, W., and H. Cypionka.** 1990. Aerobic respiration in sulfate-reducing bacteria. FEMS Microbiol. Lett. **71:**123–128.
9. **Fründ, C., and Y. Cohen.** 1992. Diurnal cycles of sulfate reduction under oxic conditions in cyanobacterial mats. Appl. Environ. Microbiol. **58:**70–77.
10. **Fukui, M., A. Teske, B. Aßmus, G. Muyzer, and F. Widdel.** 1999. Physiology, phylogenetic relationships, and ecology of filamentous sulfate-reducing bacteria (genus *Desulfonema*). Arch. Microbiol. **172:**193–203.
11. **Gauthier, M. J., B. Lafay, R. Christen, L. Fernandez, M. Acquaviva, P. Bonin, and J.-C. Bertrand.** 1992. *Marinobacter hydrocarbonoclasticus* gen. nov., sp. nov., a new, extremely halotolerant, hydrocarbon-degrading marine bacterium. Int. J. Syst. Bacteriol. **42:**568–576.
12. **Gottschal, J. C., and R. Szewzyk.** 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.

12a. **Greenberg, A. E., et al. (ed.).** 1971. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, D.C.

13. **Hansen, T. A.** 1994. Metabolism of sulfate-reducing prokaryotes. Antonie Leeuwenhoek **66:**165–185.
14. **Johnson, M. S., I. B. Zhulin, E. R. Gapuzan, and B. L. Taylor.** 1997. Oxygen-dependent growth of the obligate anaerobe *Desulfovibrio vulgaris* Hildenborough. J. Bacteriol. **179:**5598–5601.
15. **Kogure, K., U. Simudu, and N. Taga.** 1979. A tentative direct microscopical method for counting living marine bacteria. Can. J. Microbiol. **25:**415–420.
16. **Krekeler, D., P. Sigalevich, A. Teske, H. Cypionka, and Y. Cohen.** 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.
17. **Krekeler, D., A. Teske, and H. Cypionka.** 1998. Strategies of sulfate-reducing bacteria to escape oxygen stress in a cyanobacterial mat. FEMS Microbiol. Ecol. **25:**89–96.
18. **Le Gall, J., and A. V. Xavier.** 1996. Anaerobes response to oxygen: the sulfate-reducing bacteria. Anaerobe **2:**1–9.
19. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
20. **Marschall, C., P. Frenzel, and H. Cypionka.** 1993. Influence of oxygen on sulfate reduction and growth of sulfate-reducing bacteria. Arch. Microbiol. **159:**168–173.
21. **Minz, D., S. Fishban, S. J. Green, G. Muyzer, Y. Cohen, B. E. Rittmann, and D. A. Stahl.** 1999. Unexpected population distribution in a microbial mat community: sulfate-reducing bacteria localized to the highly oxic chemocline in contrast to a eukaryotic preference for anoxia. Appl. Environ. Microbiol. **65:**4659–4665.
22. **Minz, D., J. L. Flax, S. J. Green, G. Muyzer, Y. Cohen, M. Wagner, B. E. Rittmann, and D. A. Stahl.** 1999. Diversity of sulfate-reducing bacteria in oxic and anoxic regions of a microbial mat characterized by comparative analysis of dissimilatory sulfite reductase genes. Appl. Environ. Microbiol. **65:**4666–4671.
23. **Muyzer, G., A. Teske, C. O. Wirsen, and H. W. Jannasch.** 1995. Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturating gradient gel electrophoresis of 16S rDNA fragments. Arch. Microbiol. **164:**165–172.
24. **Revsbech, N. P., B. B. Jørgensen, T. H. Blackburn, and Y. Cohen.** 1983. Microelectrode studies of the photosynthesis and O_2, H_2S and pH profiles of a microbial mat. Limnol. Oceanogr. **28:**1062–1074.
25. **Sigalevich, P., E. Meshorer, Y. Helman, and Y. Cohen.** 2000. Transition from anaerobic to aerobic growth conditions for the sulfate-reducing bacterium *Desulfovibrio oxyclinae* results in flocculation. Appl. Environ. Microbiol. **66:**5005–5012.
26. **Teske, A., P. Sigalevich, Y. Cohen, and G. Muyzer.** 1996. Molecular identification of bacteria from a coculture by denaturating gradient gel electrophoresis of 16S ribosomal DNA fragments as a tool for isolation in pure cultures. Appl. Environ. Microbiol. **62:**4210–4215.
27. **Teske, A., N. B. Ramsing, K. Habicht, M. Fukui, J. Küver, B. B. Jørgensen, and Y. Cohen.** 1998. Sulfate-reducing bacteria and their activities in cyanobacterial mats of Solar Lake (Sinai, Egypt). Appl. Environ. Microbiol. **64:**2943–2951.
28. **Thauer, R. K., K. Jungermann, and K. Decker.** 1977. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Rev. **41:**100–180.
29. **Tusseau, D., and C. Benoit.** 1987. Routine high-performance liquid chromatographic determination of carboxylic acids in wines and champagne. J. Chromatogr. **395:**323–333.
30. **van den Ende, F. P., J. Meier, and H. van Gemerden.** 1997. Syntrophic growth of sulfate-reducing bacteria and colorless sulfur bacteria during oxygen limitation. FEMS Microbiol. Ecol. **23:**65–80.
31. **Widdel, F.** 1991. The genus *Desulfotomaculum*, p. 1792–1799. *In* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), The prokaryotes, 2nd ed. Springer-Verlag, New York, N.Y.
32. **Widdel, F., and T. A. Hansen.** 1991. The dissimilatory sulfate- and sulfur-reducing bacteria, p. 583–624. *In* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), The prokaryotes, 2nd ed. Springer-Verlag, New York, N.Y.