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# Competition for Sulfate and Ethanol Among *Desulfobacter*, *Desulfobulbus*, and *Desulfovibrio* Species Isolated from Intertidal Sediments

HENDRIKUS J. LAANBROEK,†* HARM J. GEERLIGS, LOLKE SIJTSMA, AND HANS VELDKAMP

*Laboratory of Microbiology, University of Groningen, 9751 NN Haren, The Netherlands*

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Competition for sulfate and ethanol among *Desulfobacter*, *Desulfobulbus*, and *Desulfovibrio* species isolated from estuarine sediments was studied in energy-limited chemostats. *Desulfovibrio baculatus* was the most successful competitor for limiting amounts of sulfate and ethanol, followed by *Desulfobulbus propionicus*. The success of *Desulfovibrio baculatus* was dependent on the availability of sufficient iron. Of the three species studied, *Desulfobacter postgatei* was the least successful competitor for limiting amounts of sulfate. Although stimulating the growth of *Desulfobacter postgatei*, addition of Ca-saturated illite particles to culture media did not affect the outcome of competition for sulfate. Thus, under sulfate limitation acetate accumulated. This phenomenon was briefly discussed in relation to the flow of electrons during anaerobic mineralization in marine and estuarine sulfate-limited sediments.

In natural anaerobic sediments organic matter is degraded by a community of physiologically different bacteria (15). In these environments sulfate or carbon dioxide are quantitatively the most important electron acceptors. The flow of electrons in anaerobic sediments is determined by the amount of sulfate available for oxidation of the organic compounds. In sulfate-rich, little-polluted marine or estuarine sediments, most of the electrons flow to sulfate, producing sulfide (8, 10, 19, 26, 30). In marine or estuarine environments with a relative shortage of sulfate, such as the sediments of salt marshes, seagrass beds, mussel beds, or places of waste water disposal, many of the electrons flow to carbon dioxide with concomitant production of methane (6, 11, 18, 21). So, in sediments heavily loaded with organic matter, the sulfate-reducing bacteria regularly encounter sulfate limitation. This will lead to competition for sulfate and to accumulation of fermentation products which become available for the methanogenic population. Since the latter can only use acetate and hydrogen directly for the production of methane (17), the competition for sulfate among the sulfate-reducing population strongly influences the composition of the methanogenic population and, hence, the flow of electrons through the anaerobic sediments. When fermentation products other than acetate or hydrogen accumulate, the presence of a hydrogen-producing, acetogenic population is indispensable (4).

Bacteria belonging to the sulfate-reducing genera *Desulfobacter*, *Desulfobulbus*, and *Desulfovibrio* occur simultaneously in the anaerobic intertidal sediments of the Ems-Dollard estuary (14) in which large amounts of waste water rich in readily degradable organic matter are discharged (5), leading to a temporary shortage of sulfate. Competition among *Desulfobacter*, *Desulfobulbus*, and *Desulfovibrio* species was studied in sulfate-limited chemostats. Since the three species also have a common ability to use ethanol as an electron donor for the reduction of sulfate (13, 14), competition for ethanol among these strains was also studied in an ethanol-limited chemostat.

* Corresponding author.
† Present address: Delta Institute for Hydrobiological Research, 4401 EA Yerseke, The Netherlands.

Since the oxidative capacity of *Desulfobacter postgatei* was stimulated by the presence of illite particles (13) and sulfate-reducing bacteria of the genus *Desulfovibrio* are extremely sensitive to iron limitation (23), the additional effects of Ca-saturated illite and ferro ion concentration on the outcome of competition were included in the present study.

## MATERIALS AND METHODS

**Bacterial strains.** *Desulfobacter postgatei* D.A41 (DSM 2553), *Desulfobulbus propionicus* NS.P31 (DSM 2554), and *Desulfovibrio baculatus* H.L21 (DSM 2555) were isolated from anaerobic intertidal sediments (salinity, 3 to 23‰) of the Ems-Dollard estuary located at the border between The Netherlands and Germany (14). Stock cultures were maintained in completely filled screw cap bottles at 4°C or in anaerobically sealed ampules at −80°C after freezing in liquid nitrogen.

**Cultivation conditions.** The following basal medium was used for growth of the organisms (components in grams per liter): $KH_2PO_4$, 0.2; $MgCl_2 \cdot 6H_2O$, 2.0; NaCl, 10; $NH_4Cl$, 0.3; $CaCl_2 \cdot 2H_2O$, 0.15; KCl, 0.3; $NaHCO_3$, 2.52; yeast extract, 0.1; resazurin, 0.001; trace elements solution (1 ml/liter) and vitamin solution (1 ml/liter). The trace elements solution contained the following components (per liter): 4.0 ml of 12.5 N HCl, 2,000 mg of $FeCl_2 \cdot 4H_2O$, 70 mg of $ZnCl_2$, 100 mg of $MnCl_2 \cdot 4H_2O$, 190 mg of $CoCl_2 \cdot 6H_2O$, 17 mg of $CuCl_2 \cdot 2H_2O$, 24 mg of $NiCl_2 \cdot 6H_2O$, 36 mg of $Na_2MoO_4 \cdot 2H_2O$, 39 mg of $Na_2SeO_3 \cdot 5H_2O$, and 49 mg of $Na_2WO_4 \cdot 2H_2O$. The vitamin solution contained (milligrams per liter): biotin, 10; nicotinic acid, 100; β-aminobenzoic acid, 50; thiamine, 100; pantothenic acid, 50; pyridoxamine, 250; and cobaltamine, 50. The vitamin solution was filter sterilized (0.2 μm) and aseptically added to sterile basal medium. The stock solution of $NaHCO_3$ (84 g/liter) was separately autoclaved in a closed bottle containing an atmosphere of $CO_2$. Ethanol, sodium acetate, sodium propionate, and sodium sulfate were aseptically added from sterile 1 M stock solutions to final concentrations indicated in the experiments described. The ethanol stock solution was also filter sterilized (0.2 μm).

Cultures were grown in energy-limited chemostats flushed with anaerobic gas composed of 80% $N_2$–20% $CO_2$ to keep the sulfide concentration below 3.0 mM. All incubations were done at a dilution rate of 0.02 $h^{-1}$, a pH of 7.0, and a temperature of 30°C.

Before competition, pure cultures were grown under the same growth-limiting conditions in chemostats. Competition was followed by direct microscopic counts and by measuring changes in concentrations of volatile fatty acids.

**Illite experiments.** Illite predominantly saturated with calcium was kindly supplied by A. van Diest, Agricultural University, Wageningen, The Netherlands. The clay particles were heat-sterilized (30 min at 120°C) at a concentration of $2 \times 10^9$ particles per ml and aseptically added to the cultures to a final concentration of $1 \times 10^7$ particles per ml.

**Determination of molar growth yields.** Cultures were centrifuged for 20 min at 10,000 × *g* in a Sorvall super-speed RC2-B centrifuge at room temperature. The pellets were washed twice with an NaCl solution (5 g/liter) and finally suspended in $CO_2$-free water. The content of organic carbon in the samples was determined with a Beckman 915A carbon analyzer connected to a Beckman 865 infrared analyzer.

**Chemical analyses.** Absence of sulfate was qualitatively demonstrated by addition of a $BaCl_2$ solution (5 g/liter) to acidified culture liquid. The limit of detection was 100 μM.

Sulfide was quantitatively determined by the method of Pachmayer as described by Trüper and Schlegel (27). Ethanol was analyzed with a Packard 427 gas chromatograph equipped with a flame ionization detector. A 2-m glass column (2-mm inside diameter) was filled with Porapack Q (Waters Associates Inc., Milford, Mass.), mesh 100 to 120. The flow rate of the carrier gas nitrogen was set at 30 ml/min. The temperatures of injection port, column, and detector were 200, 180, and 250°C, respectively. The inlet pressures of $H_2$ and air were set at 1 kp/$cm^2$. Culture liquid (2 μl) was directly injected into the column. The limit of detection was 10 μM.

Volatile fatty acids were analyzed with a Pye Unicam 104 gas chromatograph equipped with a flame ionization detector. A 2-m glass column (2-mm inside diameter) was filled with Chromosorb W AW, mesh 80 to 100, coated with 10% SP-1000–3% $H_3PO_4$ (Chrompack Nederland B.V., Middelburg, The Netherlands). The flow rate of the carrier gas nitrogen was set at 30 ml/min. The temperatures of injection port, column, and detector were 180, 120, and 170°C, respectively. The flow rates of $H_2$ and air were set at 15 and 250 ml/min, respectively. Volatile fatty acids were determined by thoroughly mixing 1.0 ml of culture liquid, 4.0 g of NaCl, 0.1 ml of 18 N formic acid, and 1.0 ml of diethyl ether. The ether layer (2 μl) was injected into the column. The limit of detection was 0.1 mM.

## RESULTS

**Isolation and growth characteristics of sulfate-reducing bacteria.** *Desulfobacter postgatei* D.A41 was isolated from brackish sediments (salinity, 23‰) and did not grow in freshwater medium. A minimum salinity of 10‰ was required for its cultivation. Pure cultures were obtained via dilution series from sediment samples in agar shake tubes containing acetate and sulfate as energy sources. Isolated colonies were further purified in a similar way. Besides acetate, only ethanol could be used as an electron donor. *Desulfobulbus propionicus* NS.P31 was isolated from less saline (3‰) sediments but grew even better in marine and more brackish media. *Desulfobulbus propionicus* NS.P31 was isolated in a similar way as *Desulfobacter postgatei*, using agar shake tubes containing propionate plus sulfate as energy sources. This strain could use many electron donors for sulfate reduction, including ethanol, and was even able to ferment L-lactate and ethanol in the presence of $CO_2$ to acetate and propionate (12). *Desulfovibrio baculatus* H.L21 was isolated from brackish sediments (salinity, 23‰) but also grew in freshwater medium. *Desulfovibrio baculatus* H.L21 was isolated as described for *Desulfobacter postgatei*, using agar shake tubes containing L-lactate plus sulfate as energy sources. Like *Desulfobulbus propionicus* NS.P31, *Desulfovibrio baculatus* could use a wide range of electron donors for sulfate reduction, including ethanol, but unlike the other sulfate-reducing strains, it could also use elemental sulfur as an electron acceptor for growth. *Desulfovibrio baculatus* lacked desulfoviridin, a common pigment of other *Desulfovibrio* species.

A brackish medium (salinity, 14‰) was chosen for all the experiments described, and a small amount of yeast extract was added as an additional carbon source and chelating agent, although the three strains grew well without yeast extract. Maximal specific growth rates on ethanol plus sulfate were determined by the method of Jannasch (7) in washout experiments in a chemostat at pH 7.0 and 30°C; they were 0.13, 0.08, and 0.10 $h^{-1}$ for *Desulfobacter postgatei* D.A41, *Desulfobulbus propionicus* NS.P31, and *Desulfovibrio baculatus* H.L21, respectively. Growth rates determined in closed screw cap bottles were 50 to 70% lower for all strains studied. With *Desulfobulbus propionicus* NS.P31, linear growth was observed in batch cultures. The lower growth rates in batch cultures were probably due to higher sulfide concentrations.

The strains were studied separately in an ethanol-limited chemostat with excess sulfate at a dilution rate of 0.02 $h^{-1}$, a pH of 7.0, and a temperature of 30°C (Table 1). This dilution rate was chosen to obtain naturally low substrate concentrations. However, at this dilution rate, *Desulfobacter postgatei* D.A41 was unable to use all of the ethanol offered, and the acetate intermediately produced was only partly oxidized to $CO_2$. Addition of Ca-saturated illite particles to the culture resulted in complete utilization of the ethanol, and almost all of the acetate intermediately produced was oxidized again. The increased amount of oxidized acetate was also reflected in a higher cell yield. Carbon determinations

TABLE 1. Characteristics of *Desulfobacter postgatei* D.A41, *Desulfobulbus propionicus* NS.P31, and *Desulfovibrio baculatus* H.L21[a]

| Strain | s[b] (mM) | Y[c] (g of cell carbon per mol of ethanol) | Cell no.[d] | Acetate/ethanol ratio |
|---|---|---|---|---|
| *Desulfobacter postgatei* D.A41 | 2.8 | 3.0 | $6.7 \times 10^{12}$ | 0.65 |
| *Desulfobulbus propionicus* NS.P31 | 0.0 | 1.0 | $4.8 \times 10^{12}$ | 0.97 |
| *Desulfovibrio baculatus* H.L21 | 0.0 | 1.5 | $19.4 \times 10^{12}$ | 0.95 |

[a] Grown anaerobically in an ethanol-limited chemostat with excess sulfate at a dilution rate of 0.02 $h^{-1}$, a pH of 7.0, and a temperature of 30°C. The medium in the reservoir contained 20 mM ethanol plus 20 mM sulfate in the case of *Desulfobulbus propionicus* NS.P31 and *Desulfovibrio baculatus* H.L21, and 40 mM sulfate in the case of *Desulfobacter postgatei* D.A41.

[b] Steady state ethanol concentration in the culture.

[c] Molar growth yield.

[d] Cell number per mole of ethanol utilized.

were not possible in the presence of clay particles. *Desulfobulbus propionicus* NS.P31 and *Desulfovibrio baculatus* H.L21 used all of the ethanol offered but were unable to oxidize the acetate produced. Their molar growth yields on ethanol are, in consequence, low compared with *Desulfobacter postgatei* D.A41. The cell size of *Desulfovibrio baculatus* H.L21 was small compared with the other strains, and hence, the cell number per mole of ethanol utilized was relatively high. Addition of ferrochloride (2 g/liter) to the ethanol-limited cultures had no effect on substrate utilization or growth yield.

**Competition for sulfate between *Desulfobacter postgatei* and *Desulfobulbus propionicus*.** *Desulfobacter postgatei* and *Desulfobulbus propionicus* were grown separately in continuous cultures with excess sulfate at a dilution rate of 0.02 $h^{-1}$, a pH of 7.0, and a temperature of 30°C and with growth-limiting amounts of acetate and propionate, respectively. After reaching steady state, both cultures were mixed, and by simultaneously lowering the sulfate concentration in the medium reservoir, sulfate limitation was introduced (Fig. 1A). Propionate was rapidly utilized, whereas acetate accumulated. After 5 days, no sulfate and propionate were detectable in the culture, and the acetate concentration was 17.5 mM. The following conversions may have been responsible for this observation. For *Desulfobulbus propionicus* NS.P31 the equation is 10 propionate + $7.5SO_4^{2-} \rightarrow$ 10 acetate + $10CO_2$ + $7.5S^{2-}$, and for *Desulfobacter postgatei* D.A41 the equation is 2.5 acetate + $2.5SO_4^{2-} \rightarrow 5CO_2$ + $2.5S^{2-}$; for both species together the equation is 10 propionate + $10SO_4^{2-} \rightarrow$ 7.5 acetate + $15CO_2$ + $10S^{2-}$. The 10.0 mM acetate produced by *Desulfobulbus propionicus* plus the 10 mM acetate from the medium reservoir minus the 2.5 mM acetate used by *Desulfobacter postgatei* would give the acetate concentration observed in the culture. Obviously, *Desulfobulbus propionicus* consumed sulfate faster than *Desulfobacter postgatei* and eventually became propionate-limited, leaving excess sulfate to *Desulfobacter postgatei*. After 14 days, sulfate limitation was released, and acetate disappeared slowly. Addition of excess propionate to a propionate-plus sulfate-limited mixed culture resulted in washout of the acetate-oxidizing species (data not shown).

Addition of Ca-saturated illite particles to the mixed culture at a final concentration of $10^7$ particles per ml had a significant effect; after removal of the sulfate limitation, acetate was more rapidly oxidized (Fig. 1B).

Competition for ethanol between *Desulfobacter postgatei* D.A41 and *Desulfobulbus propionicus* was not studied since these strains are microscopically indistinguishable, and fatty acid analyses alone would not give a decisive answer about the outcome of competition.

**Competition for sulfate and ethanol between *Desulfobacter postgatei* and *Desulfovibrio baculatus*.** Competition for ethanol in an ethanol-limited chemostat with excess sulfate at a dilution rate of 0.02 $h^{-1}$, a pH of 7.0, and a temperature of 30°C resulted in coexistence of both species (Fig. 2A). Directly after mixing the two pure cultures, the cell percentage of *Desulfobacter postgatei* decreased from 74 to 16%. However, acetate utilization increased, and at the moment when all of the acetate intermediately produced was oxidized again, the cell percentage of *Desulfobacter postgatei* remained at a constant level. The coexistence obtained thus was based on complete consumption by *Desulfobacter postgatei* of acetate produced from ethanol by *Desulfovibrio baculatus*. Additional utilization of some ethanol by *Desulfobacter postgatei* cannot entirely be excluded. When the competition experiment was started with a low cell percentage of *Desulfobacter postgatei* (12%), the acetate-oxidizing strain washed out and the acetate accumulated (data not shown).

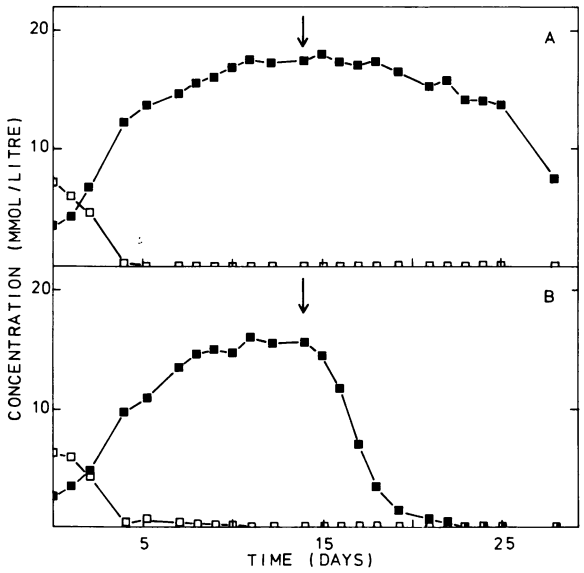


FIG. 1. Competition for sulfate between *Desulfobacter postgatei* D.A41 and *Desulfobulbus propionicus* NS.P31 in an anaerobic energy-limited chemostat at a dilution rate of 0.02 $h^{-1}$, a pH of 7.0, and a temperature of 30°C. The medium fed into the chemostat contained 10 mM acetate, 10 mM propionate, and 10 mM sulfate. After 14 days the sulfate concentration in the medium reservoir was increased to 40 mM. A, No addition of Ca-saturated illite particles; B, $10^7$ Ca-saturated illite particles per ml of culture. Symbols: ■, acetate; □, propionate

When Ca-saturated illite particles were included in the medium (Fig. 2B), a rapid utilization of acetate and a high level of cell percentage of *Desulfobacter postgatei* were observed. Again, utilization of some ethanol by *Desulfobacter postgatei* cannot entirely be excluded.

Introduction of sulfate limitation in the absence of Ca-saturated illite particles by lowering the sulfate concentration in the medium reservoir resulted in an accumulation of acetate and a concomitant decrease of the cell percentage of *Desulfobacter postgatei* (Fig. 2C). Part of the ethanol in the medium reservoir was replaced by acetate which therefore was present directly at the start of the experiment. However, *Desulfovibrio baculatus* was apparently the better competitor for limiting amounts of sulfate. After 15 days no sulfate and ethanol were detectable in the culture, and the acetate concentration measured was 4.5 mM. This acetate concentration may be explained by the following conversions. For *Desulfovibrio baculatus* H.L21 the equation is 10 ethanol + $5SO_4^{2-} \rightarrow$ 10 acetate + $5S^{2-}$, and for *Desulfobacter postgatei* D.A41 the equation is 15 acetate + $15SO_4^{2-} \rightarrow 30CO_2$ + $15S^{2-}$; for both species together the equation is 10 ethanol + 5 acetate + $20SO_4^{2-} \rightarrow 30CO_2$ + $20S^{2-}$. The excess acetate in the reservoir, 5 mM, would give the acetate concentration measured in the culture. Additional utilization of some ethanol by *Desulfobacter postgatei* could not be excluded.

**Competition for sulfate and ethanol between *Desulfobulbus propionicus* and *Desulfovibrio baculatus*.** Competition for limiting ethanol concentrations obtained in an ethanol-limited chemostat with excess sulfate at a dilution rate of 0.02 $h^{-1}$, a pH of 7.0, and a temperature of 30°C, between *Desulfobulbus propionicus* and *Desulfovibrio baculatus* resulted in

coexistence of both species (Fig. 3A). The ethanol from the reservoir was completely used and quantitatively converted to acetate. The cell percentage of *Desulfobulbus propionicus* varied between 63 and 75%. Owing to the large difference in cell volumes between both species, the biomass percentage of *Desulfobulbus propionicus* is even larger. Addition of ferrochloride (2 g/liter) after 13 days resulted in a slow decrease of the cell percentage of *Desulfobulbus propionicus*. However, after 14 days with extra iron in the culture, *Desulfobulbus propionicus* was still detectable in the culture.

In the absence of extra iron, sulfate limitation was obtained in the mixed culture by lowering the sulfate concentration in the medium reservoir. At the same time, part of the ethanol in the reservoir was replaced by propionate. Introduction of sulfate limitation resulted in an accumulation of propionate and a concomitant decrease of the cell percentage of *Desulfobulbus propionicus* (Fig. 3B). The cell percentage of *Desulfobulbus propionicus* reached a more or less constant level of 6%, the amount of ethanol was below the limit of detection (10 μM), and the acetate and propionate concentrations became constant at ca. 14.0 and 6.0 mM, respectively. These concentrations might have been the results of the following conversions. For *Desulfovibrio baculatus* H.L21 the equation is 10 ethanol + $5SO_4^{2-} \rightarrow$ 10 acetate + $5S^{2-}$, and for *Desulfobulbus propionicus* NS.P31 the equation is 4 propionate + $3SO_4^{2-} \rightarrow$ 4 acetate + $4CO_2$ + $3S^{2-}$; for both species together the equation is 10 ethanol + 4 propionate + $8SO_4^{2-} \rightarrow$ 14 acetate + $4CO_2$ + $8S^{2-}$. This acetate concentration and the excess propionate in the reservoir, 6.0 mM, would give the volatile fatty acid concentration measured in the mixed culture. Utilization of some ethanol by *Desulfobulbus propionicus* could not entirely be excluded. In pure culture studies, *Desulfobulbus propionicus* preferred ethanol to propionate under sulfate-limiting conditions (data not shown).

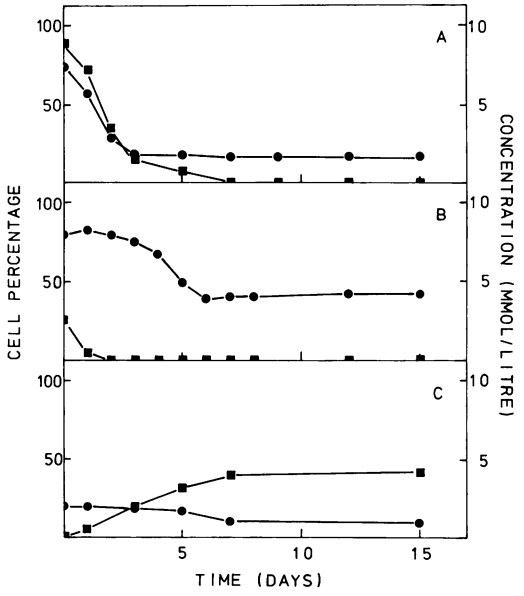


FIG. 2. Competition for sulfate or ethanol between *Desulfobacter postgatei* D.A41 and *Desulfovibrio baculatus* H.L21 in an anaerobic energy-limited chemostat at a dilution rate of 0.02 $h^{-1}$, a pH of 7.0, and a temperature of 30°C. A, The medium in the reservoir contained 20 mM ethanol plus 40 mM sulfate, and no Ca-saturated illite particles were added to the culture. B, The same medium as in A, but $10^7$ particles of Ca-saturated illite were added per ml culture. C, The medium fed into the chemostat contained 10 mM ethanol and 10 mM acetate plus 20 mM sulfate. Symbols: ●, cell percentage of *Desulfobacter postgatei* D.A41; ■, acetate

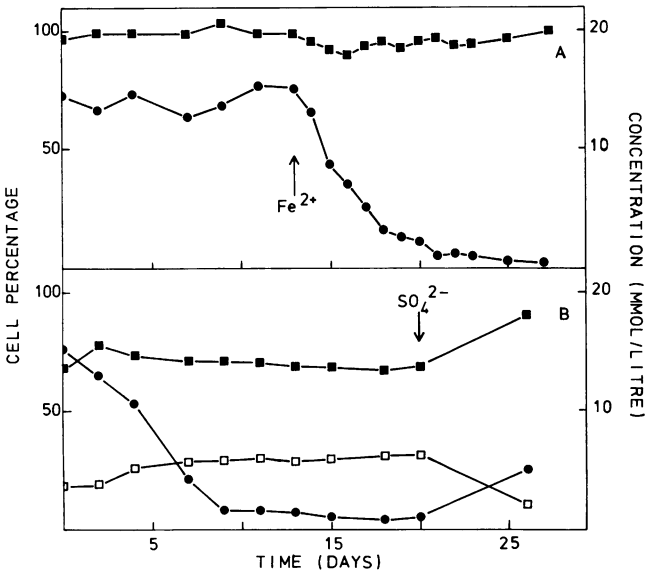


FIG. 3. Competition for sulfate or ethanol between *Desulfobulbus propionicus* NS.P31 and *Desulfovibrio baculatus* H.L21 in an anaerobic, energy-limited chemostat at a dilution rate of 0.02 $h^{-1}$, a pH of 7.0, and a temperature of 30°C. A, The medium fed into the culture contained 20 mM ethanol plus 40 mM sulfate. After 13 days ferrochloride (2 g/liter) was added to the culture. B, The medium in the reservoir contained 10 mM ethanol, 10 mM propionate, plus 8 mM sulfate. After 20 days the sulfate concentration was increased to 40 mM. Symbols: ●, cell percentage of *Desulfobulbus propionicus* NS.P31; ■, acetate; □, propionate.

Removal of the sulfate limitation resulted again in an increase of the cell percentage of *Desulfobulbus propionicus* and a stimulation of the oxidation of propionate to acetate.

## DISCUSSION

Of the three strains studied, *Desulfovibrio baculatus* was the best competitor for limiting amounts of sulfate and ethanol provided that excess iron was present. In competition for L-lactate among *Desulfovibrio baculatus* and two fermentative species, *Veillonella alcalescens* and *Acetobacterium* sp., the former species was also the best competitor when sufficient sulfate and iron were available (H. J. Laanbroek, H. J. Geerligs, A. A. C. M. Peijnenburg, and J. Siesling, Microb. Ecol., in press). The predominance of *Desulfovibrio* species in enrichment experiments in energy-limited chemostats with excess sulfate and iron (unpublished data) shows that the results obtained with pure cultures of *Desulfovibrio baculatus* also holds for other members of the genus in anaerobic sediments. The ability of *Desulfovibrio baculatus* to utilize elemental sulfur may be of additional advantage in sediments in which free sulfur is available (2). *Desulfovibrio* species are very dependent on iron for the reduction of sulfate (23). Sulfide production during growth could easily lead to iron limitation. It is still questionable whether iron limitation also occurs in natural, energy-limited sediments. The sulfate-reducing bacteria abundantly present in anaerobic microniches in the aerobic layers of the sediment (9) especially may take advantage of the iron-leaching activity of aerobic, sulfide-oxidizing bacteria (20).

*Desulfobulbus propionicus* held an intermediate position between *Desulfovibrio baculatus* and *Desulfobacter postgatei* with respect to competition for sulfate. *Desulfobulbus* species are the dominant propionate-oxidizing, sulfate-reducing bacteria in marine and estuarine sediments (14, 29). In this type of sediment, propionate is a significant substrate for sulfate-reducing bacteria (6, 25). In freshwater sediments, in which propionate may also be an important fermentation product (16), at least 50% of this fatty acid is degraded by sulfate-reducing bacteria (24). Degradation of propionate in the absence of sulfate would require the presence of a hydrogen-producing, acetogenic bacterium like *Syntrophobacter wolinii* (3). In the absence of sulfate, propionate conversion to methane and carbon dioxide proceeded in semicontinuous enrichments inoculated with samples of intertidal sediments of the Ems-Dollard estuary. However, attempts to isolate the bacterium responsible for propionate oxidation failed (unpublished results). *Desulfobulbus propionicus* is not able to grow as a hydrogen-producing, acetogenic bacterium in syntrophic cultures with a methanogen (F. Widdel, Ph.d. thesis, Georg-August-Universität, Göttingen, Federal Republic of Germany, 1980). So, for growth on propionate, *Desulfobulbus propionicus* is dependent on the presence of sulfate. However, unlike other sulfate-reducing genera, *Desulfobulbus* is not dependent on the presence of oxidized sulfur compounds for growth on alcohols or lactate (12, 29). Owing to the ability to use acetate plus $CO_2$ as an electron acceptor for growth, it can grow fermentatively. The ability to grow on $H_2$, acetate plus $CO_2$ without sulfate, however, is from a questionable ecological point of view as was discussed before (12).

Of the three strains studied, *Desulfobacter postgatei* was the least successful competitor for limiting amounts of sulfate and ethanol. Of all three sulfate-reducers it does, however, have the highest maximum specific growth rate in the presence of excess ethanol and sulfate. Although, it was clearly stimulated by the presence of Ca-saturated illite particles, these clay particles did not have a decisive influence on the outcome of competition in the chemostat. Stimulation of substrate utilization by *Desulfobacter postgatei* in the presence of clay particles was observed before in batch cultures (13). The characteristic ability of *Desulfobacter* species is the utilization of acetate in the presence of sulfate (28). Compared with other acetate-oxidizing sulfate-reducing genera, *Desulfobacter* has a very limited range of usable substrates, but growth on acetate plus sulfate was often much better (F. Widdel, Ph.d. thesis). Enumerations in estuarine sediments, using anaerobic agar shake tubes, yielded only *Desulfobacter* species (14). Acetate is a major substrate for sulfate-reducing bacteria in marine sediments (1, 11, 22, 25, 30). The absence of methane formation from acetate in marine sediments with sufficient sulfate (18, 19, 25) shows that sulfate limitation is usually not encountered by acetate-oxidizing, sulfate-reducing bacteria in these sediments. However, a relative shortage of sulfate caused by a high input of organic matter may result in the production of methane from acetate in coastal and estuarine sediments (11, 14, 18). Since *Desulfobacter postgatei* was a bad competitor for limiting amounts of sulfate, a relative shortage of sulfate would have consequences for the flow of electrons through the anaerobic mineralization process. Under these sulfate-limiting conditions, acetate would be degraded by methanogenic bacteria, whereas other fermentation products could still be used by sulfate-reducing bacteria. The possibility of a shift in sulfate reduction from acetate to more reduced fermentation products in the presence of limiting amounts of sulfate, was also considered by Gunnarsson and Rönnow (6) and Mountfort et al. (19) in relation to in situ experiments in coastal and estuarine sediments. Care must be taken to extrapolate the results of our competition experiments to field conditions, in particular to environments other than brackish estuaries. The chosen parameters, such as temperature, pH, salinity, free sulfide concentration, and sulfate limitation, are not unrealistic for the brackish part of the Ems-Dollard estuary for at least part of the year (H. G. J. Schröder, personal communication). With respect to *Desulfobacter postgatei*, further investigation should reveal whether affinity for sulfate is affected by the salinity of the growth medium. At the salinity applied (14‰) the organism grew very well, however, and even showed the highest maximum specific growth rate of all three strains in the presence of excess ethanol and sulfate.

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