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Kinetics of Sulfate and Acetate Uptake by *Desulfobacter postgatei*

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The kinetics of sulfate and acetate uptake was studied in the sulfate-reducing bacterium *Desulfobacter postgatei* (DSM 2034). Kinetic parameters (K_m and V_{max}) were estimated from substrate consumption curves by resting cell suspensions with [^{35}S]sulfate and [^{14}C]acetate. Both sulfate and acetate consumption followed Michaelis-Menten saturation kinetics. The half-saturation constant (K_m) for acetate uptake was 70 μM with cells from either long-term sulfate- or long-term acetate-limited chemostat cultures. The average K_m value for sulfate uptake by *D. postgatei* was about 200 μM. K_m values for sulfate uptake did not differ significantly when determined with cells derived either from batch cultures or sulfate- or acetate-limited chemostat cultures. Acetate consumption was observed at acetate concentrations of ≤1 μM, whereas sulfate uptake usually ceased at 5 to 20 μM. The results show that *D. postgatei* is not freely permeable to sulfate ions and further indicate that sulfate uptake is an energy-requiring process.

Acetate is a quantitatively important intermediate in the anaerobic mineralization of organic matter (20, 25, 30, 31, 38, 40). Acetate does not usually accumulate in anaerobic sediments because it serves as a major energy substrate for both sulfate-reducing bacteria (SRB) and methane-producing bacteria (MPB). Ecological studies show that SRB outcompete MPB for acetate (and H_2) when sulfate is present at nonlimiting concentrations for the SRB (11, 19, 23, 37). The basis for sulfate inhibition of methanogenesis is not fully understood, and several mechanisms have been proposed (2, 19, 26, 37). One such mechanism is that SRB have more efficient uptake systems than MPB for substrates utilized by both.

In situ concentrations of acetate in anaerobic sediments are low, usually in the lower micromolar range (3, 26, 42), which indicates that natural populations of acetate utilizing SRB and MPB have developed efficient uptake systems for acetate. However, SRB competing with MPB for acetate will not possess their maximum capacity for acetate uptake if they are simultaneously limited by sulfate. The half-saturation constant (K_m) for sulfate uptake by SRB is, therefore, a key parameter for predicting the outcome of substrate competition between SRB and MPB, particularly in freshwater environments where sulfate concentrations are significantly lower (usually 100- to 1,000-fold) than in marine environments. Despite the significance of acetate as an intermediate fermentation product, SRB able to oxidize acetate to CO_2 have only recently been isolated (35, 36). Acetate-oxidizing SRB of the genus *Desulfobacter* appear to be widespread in marine and brackish water sediments (13, 17, 36) and could, therefore, be important organisms in anaerobic carbon mineralization in these environments.

This paper reports a detailed study of substrate uptake kinetics in the SRB *Desulfobacter postgatei*. Experiments were carried out with resting cell suspensions by using ^{14}C and ^{35}S radiotracer techniques. The ability of *D. postgatei* to adapt its uptake system in response to various nutrient levels was investigated with long-term acetate- and sulfate-limited chemostat cultures. The apparent half-saturation constant (K_m) for sulfate uptake determined in SRB is reported for the first time.

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MATERIALS AND METHODS

Organism, media, and growth conditions. *D. postgatei* (strain 2ac9, DSM 2034) was kindly provided by F. Widdel, University of Konstanz, Federal Republic of Germany.

The bacteria were grown in a mineral salts medium (M1 medium) with acetate as the sole organic substrate. The following stock solutions were used to prepare the medium (grams per liter of distilled water): (solution A) KH_2PO_4 (27.2); (solution B) Na_2HPO_4 (35.6); (solution C, mineral salts solution) NH_4Cl (60), NaCl (157), $CaCl_2 \cdot 2H_2O$ (22), and $MgCl_2 \cdot 6H_2O$ (119); (solution D) $NaHCO_3$ (84) (CO_2 saturated and autoclaved under CO_2 atmosphere); (solution E) trace element solution according to Zehnder et al. (41); (solution F) Na_2SeO_3 (0.02); (solution G) vitamin mixture according to Wolin et al. (39); (solution H) $Na_2S \cdot 9H_2O$ (240.2); and (solution I) resazurin (1.0). One liter of M1 medium was prepared with 20 ml of solution A, 47 ml of solution B, and 1 ml each of solutions F and I and was made up with distilled water, 6.8 g of $CH_3COONa \cdot 3H_2O$, and 1.4 g of Na_2SO_4 to 930 ml. This solution and solutions C and E were flushed and evacuated three times in a gassing manifold (1) with anaerobic gas composed of 20% CO_2 plus 80% N_2 and autoclaved under 1.2 atm (ca. 122 kPa) of the gas mixture. Vitamin solution G was filter (0.2 μm) sterilized and stored under N_2. Bicarbonate stock solution D was saturated with anaerobic CO_2 gas and autoclaved in a closed serum bottle under a CO_2 atmosphere.

After cooling of the medium, 1 ml each of trace element solution E and vitamin mixture G, 22 ml of mineral salts solution C, and 2 ml of sulfide solution H in 50 ml of bicarbonate solution D were added aseptically by syringe transfer. Finally the gas phase was brought to 1.5 atm (ca. 152 kPa) with 20% CO_2–80% N_2; the pH of the medium was 7.0 to 7.2. Sodium dithionite ($Na_2S_2O_4$) was occasionally added to a final concentration of 0.1 mM (36) to shorten the lag phase in batch cultures, but it was not required for growth.

Batch cultures were routinely grown in 40 ml of medium in

115-ml serum vials with black butyl rubber stoppers (Bellco Glass, Inc., Vineland, N.J.) or in 100 ml of medium in 250-ml serum flasks with rubber septums and metal screw caps which allowed syringe injections. Cells were incubated in the dark at 30°C without shaking. With sulfate as the electron acceptor, an average growth yield of 4.3 g (dry weight) per mole of acetate oxidized was obtained (μ_{max} = 0.03 h^{-1}; generation time [t_d] = 21 to 29 h).

General anaerobic techniques. Strict anaerobic and aseptic conditions were maintained throughout the handling of cell suspensions in all experiments, including those with isotopes. The anaerobic techniques used were essentially the syringe methods of Macy et al. (22).

Chemostat cultures. Continuous cultures were grown under either acetate limitation (M1 medium: 10 mM acetate plus 50 mM sulfate) or sulfate limitation (M1 medium: 50 mM acetate plus 10 mM sulfate) in an all-glass growth vessel with a culture volume of 2.4 liters (Schmizo, Zofingen, Switzerland). The pH in the chemostat was kept constant at 7.2 by keeping the system under a slight overpressure with a 30% CO_2–70% N_2 gas mixture. Since some adhesion of cells on the walls of the growth vessel occurred, thereby invalidating conventional continuous culture theory (34), kinetic parameters could not be accurately determined by using equations 9 or 14 in the article by Herbert et al. (7). Instead, substrate kinetic parameters (K_m and V_{max}) were determined by using radiotracer techniques with cell suspensions removed from steady-state, substrate-limited chemostat cultures. Unless stated otherwise, data reported in this study for chemostat cultures pertain to a dilution rate (D) of 0.01 h^{-1}. *D. postgatei* did not grow well at dilution rates below 0.01 h^{-1} when sulfate was limiting.

Growth rate determination. Growth in batch cultures was followed by measuring the optical density of bacterial suspensions at 578 nm (light path = 1 cm) in an Eppendorf photometer. A few crystals of sodium dithionite were added to the cuvette to prevent the resazurin in the medium from turning pink during measurement.

Cell yields and enumeration of cells. Cultures of *D. postgatei* (usually 60 ml) were harvested and centrifuged for 30 min at 17,000 × *g* in a Heraeus Christ refrigerated centrifuge (Cryofuge 20-3) at 10°C. The pellet was washed twice with washing solution and finally concentrated in 2 or 3 ml of washing solution. The washing solution contained (grams per liter of distilled water): NaCl, 12; $MgCl_2 \cdot 6H_2O$, 10; KH_2PO_4, 0.5; and Na_2HPO_4, 1.3. The content of organic carbon was determined in acidified portions of the concentrated cell suspension with a C,H,N-Analyzer (model 185; F & M Scientific Corp., Avondale, Pa.). Dry weights were obtained by assuming a dry cell mass carbon content of 50%.

Cell numbers were obtained in a Petroff-Hausser counting chamber after appropriate dilution of the original sample in washing solution.

Chemical analysis. Acetate was determined with a Shimadzu (model/RIA) gas chromatograph equipped with a flame ionization detector. The 2.1-m glass column (outer diameter, 5 mm; inner diameter, 2.6 mm) was packed with Chromosorb W AW, mesh 80-100, coated with 10% SP-1200 and 1% H_3PO_4 (Supelco, Inc.). Nitrogen was used as carrier gas at a flow rate of 40 ml min^{-1}. The flow rates of H_2 and air were set at 50 and 450 ml min^{-1}, respectively. The temperatures of the injection port and column were 200 and 115°C, respectively. Before analysis, 1 ml of filtered culture medium was acidified with 100 μl of concentrated formic acid, and 2-μl samples were injected into the gas chromatograph.

Sulfide was determined photometrically by using the methylene blue method of Pachmayer (F. Pachmayer, Ph.D. thesis, University of Munich, Munich, Federal Republic of Germany 1960).

Sulfate was determined by suppressor-type ion chromatography with a Dionex AutoIon System 12 Analyzer (Dionex Corp., Sunnyvale, Calif.) equipped with a conductivity detector. The eluent was $NaHCO_3$-Na_2CO_3 (3.0 mM/2.4 mM; pH 10.4) with a flow rate of 1.7 ml min^{-1}. Two serial connected separation columns (250 by 3 mm [inner diameter]) were used for analysis. The system was maintained at 25°C.

[^{35}S]sulfate measurements of sulfate consumption in resting cell suspensions. Excess sulfate had to be removed from batch-grown cultures and in particular from cells grown under acetate limitation (i.e., sulfate surplus) before use for $^{35}SO_4^{2-}$ uptake experiments. This was done as follows. Cells (late-exponential phase when batch grown) were harvested anaerobically by centrifugation. The cells were resuspended and washed twice with reduced sulfate-free growth medium and finally transferred to sterile 115-ml serum vials sealed with black-lip butyl stoppers (Bellco) crimped with an aluminum seal. The serum vial was then aseptically pressurized to 1.4 atm (ca. 142 kPa) with 20% CO_2–80% N_2 and stored (≤6 h) at 3°C until use. Cells grown under sulfate limitation in the chemostat were transferred directly (i.e., without washing) to sterile serum vials and likewise pressurized. From this point on the experimental procedure was the same irrespective of prior culture conditions. All cultures were preincubated at 30°C for 1 h. The reaction was started by injecting sterile anaerobic solutions of unlabeled sulfate (final concentration, 100 to 500 μM) and carrier-free $H_2{}^{35}SO_4$ (10 to 60 μCi) and incubating the vial at 30°C in the dark in a shaking water bath. Sulfate consumption was followed by removing 1 ml of culture after various incubation times with a nitrogen-flushed syringe. These samples were quickly injected into 0.5 ml of alkaline zinc acetate solution in a centrifuge tube, diluted with an appropriate amount of anaerobic distilled water, mixed well, and centrifuged. After centrifugation, 1.0 ml of clear supernatant containing the $^{35}SO_4^{2-}$ radioactivity was carefully removed and mixed with 10 ml of Insta-Gel (Packard Instrument Co., Inc., Downers Grove, Ill.) scintillation cocktail. Thorough testing showed that the supernatant was free of both cells and $Zn^{35}S$. The $^{35}SO_4^{2-}$ radioactivity was determined in a BETAmatic I (Kontron AG, Zurich, Switzerland) liquid scintillation spectrometer by using external standard ratio quench correction. The counting efficiency was 90%.

[^{14}C]acetate measurements of acetate consumption in resting cell suspensions. The kinetics for acetate consumption were measured in cell suspensions, sampled from both sulfate- and acetate-limited chemostats. Acetate-limited chemostat cultures were used directly (i.e., without washing). However, sulfate-limited chemostat cultures were harvested by centrifugation and washed twice in reduced acetate-free medium before being used for the experiments. In each case the cell suspensions were transferred to 115-ml sterile serum vials, pressurized to 1.4 atm (ca. 142 kPa) with 20% CO_2–80% N_2 and stored (≤6 h) in the dark at 3°C until use. (The storage of chemostat-grown cells at 3°C did not have any effect on either the K_m and V_{max} for acetate or on sulfate.) After preincubation (1 h at 30°C), unlabeled sodium acetate (final concentration, 300 to 600 μM) and 5 to 10 μCi of [1-^{14}C]acetate were added with a syringe to start the experiment. The experimental culture was then incubated at 30°C in the dark in a shaking water bath. At appropriate time intervals, 1-ml samples were removed anaerobically and filtered (SM 11306 membrane filters, 0.2 μm, 13-mm diameter, Sartorius, Göttingen, Federal Republic of Germany),

and the filters were washed with 1 ml of 20 mM sodium acetate solution. The combined filtrate was then acidified (pH <2) and purged for 10 min with air to remove the $^{14}CO_2$ formed. After neutralization, a 0.5-ml sample of filtrate was mixed with 10 ml of Insta-Gel (Packard) in a scintillation vial, and the [^{14}C]acetate radioactivity was determined by liquid scintillation counting.

Kinetic constants. Kinetic constants (K_m and V_{max}) for uptake were determined from progress curves for sulfate and acetate utilization by using an integrated solution of the Michaelis-Menten equation (5). Incubation times were kept as short as possible (usually ≤3 h) by adjusting the density (and thus the V_{max}) of the experimental cultures to eliminate interference from growth. Bacterial densities used in ^{35}S and ^{14}C radioisotope experiments varied between 28 and 85 mg of cells (dry weight) liter^{-1}. This corresponded to an average bacterial cell number of about 5×10^8 cells ml^{-1}. Separate determinations of K_m and V_{max} with the same batch of cells were highly reproducible (less than 10% deviation between replicate samples).

Gases, chemicals, radiochemicals. The CO_2-N_2 gas mixtures (20%-80% or 30%-70%), N_2, and CO_2 were high-purity gases purchased from Carba Gas (Zürich, Switzerland). All gases were passed through an Oxisorb (Messer Griesheim, Düsseldorf, Federal Republic of Germany) oxygen scrubber before use. All chemicals were of analytical quality. Carrier-free $H_2{}^{35}SO_4$ (43 Ci mg^{-1}) was obtained from New England Nuclear Corp. (Boston, Mass.), and [1-^{14}C]sodium acetate (40 to 60 mCi mmol^{-1}) was from Amersham Corp. (Arlington Heights, Ill.).

RESULTS

Acetate kinetics. Acetate consumption by cell suspensions of *D. postgatei* followed Michaelis-Menten kinetics (data not shown). Kinetic constants determined for acetate uptake are shown in Table 1. Thus, *D. postgatei* did not change its kinetic parameters for acetate uptake whether it grew with 40 mM acetate (i.e., sulfate limitation) or with 0.1 mM acetate (i.e., acetate limitation) in the chemostat. The V_{max} values in Table 1 correspond to specific acetate uptake rates (and hence sulfate uptake rates of 8×10^{-15} mol cell^{-1} day^{-1}, which is of the same order of magnitude as those reported for other species of SRB (12). When the specific activity of acetate was varied in dilute suspensions of *D. postgatei*, it was shown that this bacterium was able to deplete the acetate in the medium below 1 μM.

Sulfate kinetics. A typical progress curve for sulfate utilization by a batch-grown culture of *D. postgatei* is shown in Fig. 1A. Sulfate utilization showed Michaelis-Menten hyperbolic dependence (Fig. 1B), and this was also the case when sulfate uptake was measured in cell suspensions of sulfate- and acetate-limited chemostat cultures (results not shown). The calculated half-saturation constant (K_m) for the experiment shown in Fig. 1 was 145 μmol of SO_4^{2-} liter^{-1}, and the V_{max} was 352 μmol of SO_4^{2-} liter^{-1} h^{-1} (4.2 mmol h^{-1} g [dry weight]$^{-1}$).

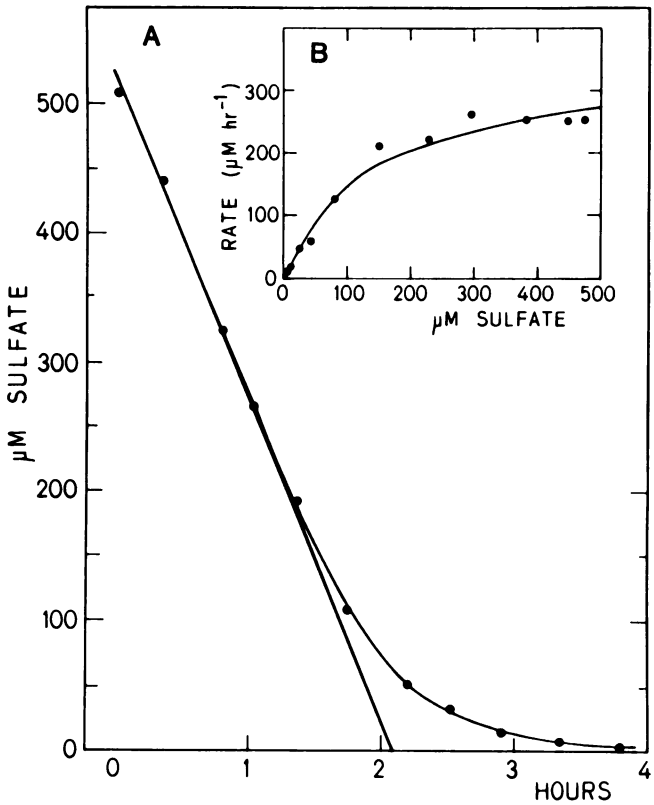

FIG. 1. (A) Time course of sulfate consumption by a batch-grown culture of *D. postgatei*. A K_m of 145 μmol liter^{-1} and a V_{max} of 352 μmol liter^{-1} h^{-1} were calculated by using the integrated rate equation of Halwachs (5). (B) Plot of sulfate uptake rate ($-dS/dt$) against sulfate concentration. $-dS/dt$ versus S values (●) were determined graphically from the progress curve in Fig. 1A. The curve corresponds to the Michaelis-Menten equation [$v = V_{max}(S/K_m + S)$] with a V_{max} of 352 μM h^{-1} and a K_m of 145 μM.

TABLE 1. Apparent K_m and V_{max} values for uptake of acetate by *D. postgatei* measured with [1-^{14}C]acetate[a]

Culture conditions[b]	K_m (μM)	V_{max} (mmol h^{-1} g [dry wt]$^{-1}$)
SLC	64	3.1
ALC	77	3.2

[a] All experiments were conducted in M1 medium containing 50 mM Na_2SO_4 (pH 7.2) at 30°C.

[b] SLC, Long-term sulfate-limited chemostat culture; ALC, long-term acetate-limited chemostat culture. The dilution rate (D) was 0.02 h^{-1}.

To test whether *D. postgatei* had the same K_m for sulfate uptake when grown at high and low concentrations of sulfate, kinetic experiments were carried out with cell suspensions from both sulfate- and acetate-limited chemostat cultures. The results from a series of kinetic experiments with cultures of different origin are summarized in Table 2. From the data in Table 2, it appears that there was no significant difference in the K_m for sulfate uptake between batch- and chemostat-grown cells under the conditions tested. The specific maximum uptake rate (V_{max}), although subject to slightly more variation than the K_m values, did not show any significant trend either.

Residual sulfate concentrations. During measurement of $^{35}SO_4^{2-}$ uptake kinetics in *D. postgatei*, an interesting phenomenon was observed. Sulfate consumption as measured with $^{35}SO_4^{2-}$ always stopped when sulfate in the medium approached a concentration which was ca. 10 to 40 times lower than the apparent K_m for sulfate uptake. The existence of this threshold concentration for sulfate uptake was observed in both batch- and chemostat-grown cultures with highly different specific activities of sulfate (see Table 3). Threshold concentrations for sulfate uptake were variable (Table 3) and probably dependent on the physiological

TABLE 2. Apparent K_m values for uptake of sulfate by *D. postgatei* measured with [^{35}S]sulfate[a]

Culture conditions[b]	Avg K_m[c] (μM)	Observed range of K_m (μM)
SLC (D = 0.01 h^{-1})	212	156–330
ALC (D = 0.02 h^{-1})	220	120–316
BC	170	145–200

[a] All experiments were conducted in M1 medium with 50 mM acetate, pH 7.2, at 30°C.
[b] SLC, Long-term sulfate-limited chemostat culture; ALC, long-term acetate-limited chemostat culture; BC, batch-grown culture; D, dilution rate.
[c] Average from at least two experiments.

state of the bacteria, since prolonged energy starvation caused the threshold concentration to increase. Thorough testing ruled out the possibility that the often high threshold concentrations for sulfate uptake were due to methodological errors, e.g., incomplete separation of the [^{35}S]sulfide and the [^{35}S]sulfate radioactivities, and this was further confirmed by chemical analysis of the cell-free medium. That the residual radioactivity, present in the cell-free medium when sulfate uptake stopped, was indeed $^{35}SO_4^{2-}$ and not some radioactively labeled sulfur-containing organic compound excreted by *D. postgatei* could be demonstrated in experiments like the one reported in Fig. 2. The time course started with the addition of tracer and unlabeled sulfate to a final concentration of 750 μM. At point B, sulfate consumption stopped, and the sulfate concentration remained constant at 18 μM for about 24 h. When carrier-free $^{35}SO_4^{2-}$ was added at point C to increase the specific activity of the low extracellular sulfate concentration, sulfate consumption was not observed. At point E, nonlabeled sulfate was added to give a final concentration of 87 μM and sulfate uptake resumed, but it again ceased at 17 μM. At point G, additional nonlabeled sulfate was added to a final concentration of 2,836 μM, which caused a drastic decrease in the $^{35}SO_4^{2-}$ radioactivity of the medium.

DISCUSSION

The apparent K_m of about 200 μM sulfate for *D. postgatei* determined here is the first in vitro sulfate half-saturation constant reported for an SRB. Sulfate uptake usually stopped at 5 to 20 μM sulfate. Therefore, it is unlikely that *D. postgatei* plays an important role in carbon mineralization in eutrophic lake sediments, in which sulfate usually becomes depleted within the uppermost centimeters of the sediment (9, 10, 28, 37). This may also be true for oligotrophic lakes, even though sulfate concentrations in these sediments are considerably higher than in the surface sediments of eutrophic lakes (21).

Earlier studies on the kinetics of sulfate reduction in slurries of freshwater sediments indicated K_m values for sulfate reduction of 50 to 100 μM sulfate (9, 28). In marine sediments considerably higher K_m values of 1 to 5 mM sulfate were obtained (J. T. Westrich, Ph.D. thesis, Yale University, New Haven, Conn., 1983). Recent studies with sediment slurries indicate that both marine and freshwater sediments contain coexisting populations of SRB which differ significantly with respect to their half-saturation constants (i.e., K_m) for sulfate. However, the populations have adapted their sulfate uptake systems to the in situ sulfate concentrations. Thus, the highest K_m for sulfate uptake measured in freshwater sediment was much lower than the highest K_m measured in marine sediments (B. B. Jørgensen and K. Ingvorsen, unpublished data).

Few studies have dealt with sulfate uptake in bacteria, and most of these studies were on sulfate uptake in connection with assimilatory sulfate reduction (for references see reference 27). The mechanisms of sulfate uptake in SRB are not known, but Furusaka (4) and Littlewood and Postgate (18) found evidence that *Desulfovibrio* species are not freely permeable to sulfate ions. The results obtained in this study agree with these earlier findings, and they further indicate that metabolic energy is involved in sulfate uptake (cf. reference 32). Apparently, sulfate must enter the cytosol to become reduced, and one may hypothesize that when sulfate uptake operates at about 0.05 to 0.09 V_{max} (depending on prior growth conditions), the cells are no longer able to divert enough energy to the sulfate transport system and sulfate uptake stops. As in the genus *Desulfovibrio* (24, 33), the enzymes involved in the dissimilatory reduction of sulfate to sulfide in *D. postgatei* are probably cytoplasmic.

An apparent K_m for acetate by *D. postgatei* of 70 μM was obtained in this study, and dilute suspensions of this bacterium depleted the acetate concentration to below 1 μM. Apparent K_m values of 0.5 to 0.7 mM and 3.0 to 5.0 mM acetate have been reported for the two acetotrophic MPB *Methanothrix soehngenii* (8, 41) and *Methanosarcina barkeri* (26, 29), respectively. Schönheit and co-workers (26) determined an apparent K_m of 230 μM acetate for batch-grown cultures of *D. postgatei*, which is a factor of three higher than our K_m. This difference is most likely due to differences in growth conditions and experimental methods, and it shows that kinetic constants should be interpreted with considerable care. Therefore, K_m values for acetate uptake have only been published for two nonmarine isolates of acetotrophic MPB (8, 26, 29, 41) and the acetate-oxidizing SRB *D. postgatei* (26; this study), a marine or brackish water isolate. In any case, the kinetic data available to date support the general hypothesis that SRB have lower apparent K_m values for acetate (and H_2) than MPB (2, 16, 26, 37).

D. postgatei did not either lower its K_m or increase its overall uptake capacity (V_{max}/K_m) when cultivated under long-term conditions with either sulfate or acetate limitation. It is known that some bacteria respond to substrate-limited growth in a chemostat by increasing their specific substrate uptake capacity (6, 15). This may be accomplished by

TABLE 3. Threshold concentrations for sulfate uptake measured with [^{35}S]sulfate in cultures of *D. postgatei*

Culture conditions[a]	Initial sulfate concn (μM)	Specific radioactivity of sulfate (kdpm μmol^{-1})	Threshold concn[b] (μM)
BC	10,000	2.279	15
BC	10,000	2.279	36
BC	195	3.017	2
BC	512	1.508	≤3
SLC	147	2.898	10
SLC	156	13.024	15
SLC	705	0.936	18
ALC	531	4.347	4
ALC	326	5.937	6

[a] BC, Batch-grown culture; SLC, long-term sulfate-limited chemostat culture; ALC, long-term acetate-limited chemostat culture.
[b] The threshold concentration for sulfate uptake is defined as the minimum extracellular sulfate concentration below which sulfate uptake does not occur. The criteria used to establish a threshold concentration were (i) that the $^{35}SO_4^{2-}$ radioactivity in the cell-free medium remained constant for 24 h and (ii) that upon addition of nonradioactive sulfate, consumption resumed, causing a concomitant decrease in $^{35}SO_4^{2-}$ in the medium.

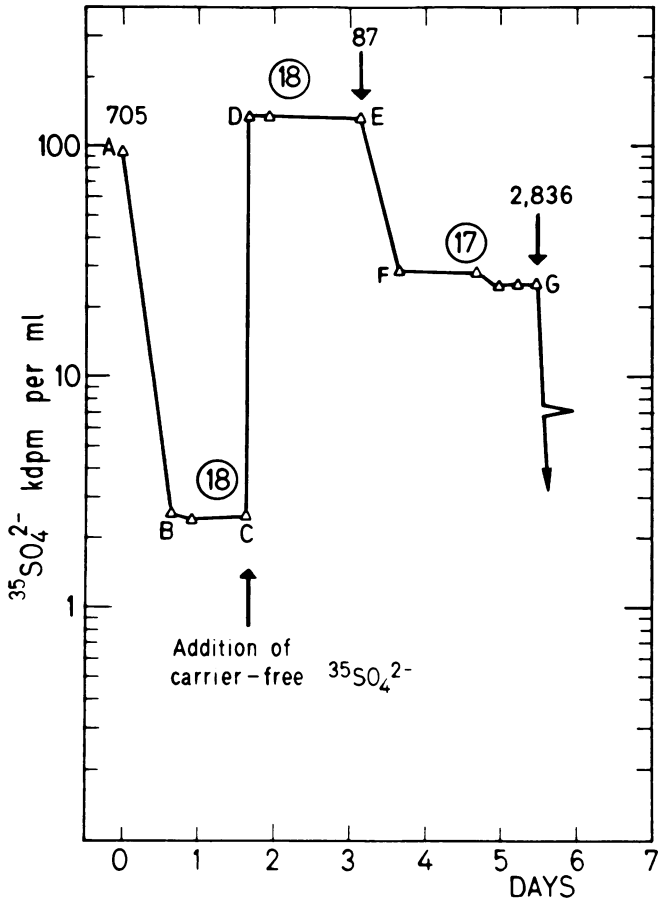

FIG. 2. Demonstration of threshold concentration for sulfate uptake in a culture of *D. postgatei*. The sulfate concentrations (in μM) at the three plateaus (B-C, D-E, F-G) are given within the circles. Carrier-free $^{35}SO_4^{2-}$ was added to the culture at point C (first arrow). At points E and G (indicated by arrows) the sulfate concentration was increased to 87 and 2,836 μM, respectively, by the addition of nonlabeled sulfate (see text for details). The cell suspension contained 40 mM acetate, pH 7.2, at 30°C.

changing either V_{max} or K_m to maximize the V_{max}/K_m ratio. The V_{max}/K_m ratio (or clearance rate) is a better parameter than the K_m for assessing the scavenging ability of a bacterium at very low substrate concentrations (14). This would be true, for instance, for substrate concentrations $\leq 0.02\ K_m$, where uptake is essentially a first-order reaction. This pertains especially to acetate, which is present in very low concentrations in anaerobic marine sediments (3, 42).

Thus, it seems that either (i) *D. postgatei* belongs to the group of bacteria which do not increase their clearance rate (V_{max}/K_m) at low dilution rates (cf. reference 6) or (ii) lower dilution rates than those used here are needed to cause *D. postgatei* to change its uptake kinetic parameters (which is less likely). Most of the substrate kinetic parameters determined in this investigation were obtained with chemostat cultures of *D. postgatei* grown at dilution rates (D) of 0.3 μ_{max} (sulfate limitation) and 0.6 μ_{max} (acetate limitation). It is possible that the low energy yield of acetate oxidation with sulfate ($\Delta G^{\circ\prime} = -57$ kJ mol^{-1}) puts a constraint on the ability of *D. postgatei* to adapt its uptake systems.

The K_m values determined in this study for sulfate and acetate in *D. postgatei* are in agreement with earlier findings which indicate that this bacterium is widespread in anaerobic sediments of brackish and marine waters (13, 17, 36). In these environments, sulfate will normally be present at saturation concentrations, and our results show that under such conditions *D. postgatei* would be a good scavenger of acetate. In situ concentrations of acetate measured in unpolluted sulfate-rich sediments are indeed very low (e.g., 2 to 70 μM; reference 3) and are often below the limits of detection (42), whereas higher concentrations of acetate are generally found in sediments low in sulfate (26, 42). It is puzzling that acetate-oxidizing SRB, morphologically similar to *D. postgatei*, are present in freshwater sediments but can only be enriched from these habitats if the freshwater enrichment medium is supplemented with NaCl and $MgCl_2$ (36). However, once isolated, these salt-requiring strains can adapt and grow in normal freshwater media, although the conditions in such media appear to be suboptimal (36; K. Ingvorsen, unpublished findings). Thus, the SRB responsible for the high acetate-oxidizing potential of eutrophic lake sediments (20, 37) have yet to be identified. To be competitive with acetotrophic MPB in freshwater sediments, these SRB should possess efficient sulfate uptake systems with K_m values at least an order of magnitude lower than the K_m of 200 μM found for *D. postgatei* in this study.

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