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THE ROLE OF SULFATE REDUCTION IN METHANOGENIC DIGESTION OF MUNICIPAL SEWAGE SLUDGE

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Relationship between methanogenesis and sulfate reduction in anaerobic digestion of municipal sewage sludge was investigated. The density of methanogenic bacteria in the sludge was estimated to be at the order of 10^4–10^6 cells/ml. The density of sulfate reducing bacteria was at the order of 10^5 colony forming units/ml, while the concentration of sulfate in the sludge was low (<0.2 mM). Addition of sulfate to the sludge markedly enhanced sulfate reduction without significantly affecting methanogenesis. In the sludge supplemented with sulfate, both methanogenesis and sulfate reduction were significantly enhanced upon the addition of H_2. In the presence of exogenous H_2, inhibition of methanogenesis or that of sulfate reduction resulted in enhancement of sulfate reduction or that of methanogenesis, respectively. The addition of acetate markedly enhanced methanogenesis but did not affect sulfate reduction, and the addition of propionate markedly enhanced both methanogenesis and sulfate reduction. Degradation of propionate essentially depended on sulfate reduction, and acetate accumulated in response to the propionate degradation when methanogenesis was inhibited. In conclusion, in the sludge, acetate was used only in methanogenesis, and H_2 was used in both methanogenesis and sulfate reduction. Sulfate reduction degraded propionate to acetate and enhanced electron flow to methanogenesis.

Methanogenesis and sulfate reduction are terminal steps in the anaerobic degradation of organic matter. These reactions are known to compete with each other for electron donors, i.e., H_2 and acetate, in various environments. In the presence of sulfate at available levels, sulfate reduction generally dominates over methanogenesis due to kinetic and thermodynamic properties (*1*, *5*, *6*, *9*, *10*). Beside this, an inhibitory effect of sulfide, the end-product of sulfate reduction, on anaerobic biogas production is known. Thus, sulfate reduction is generally thought

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to be obstructive to the methanogenic digestion.

In anaerobic digestion of municipal sewage sludge, however, sulfate reduction apparently does not compete with methanogenesis, even when sulfate reduction was significantly enhanced by adding sulfate to the sludge (*17*). A similar relation between methanogenesis and sulfate reduction was found in anaerobic digester slurry of animal waste (*12–14*). Thus, the compatible relationship between methanogenesis and sulfate reduction seems to be characteristic of nutritionally rich environments such as sewage sludge and animal waste.

In the present study, effects of sulfate reduction on methanogenesis in anaerobic digestion of municipal sewage sludge were investigated to determine the relationship in the utilization of electron donors between methanogenesis and sulfate reduction. (This research was partly presented at the FEMS Symposium, Microbiology and Biochemistry of Strict Anaerobes Involved in Interspecies Hydrogen Transfer, held in Marseille, France, September 1989.)

MATERIALS AND METHODS

Municipal sewage sludge sample. Sewage sludge was sampled from an anaerobic digester in the Wastewater Treatment Center at Tsuruoka in Japan. The sludge sample was stored at 7–10°C in 500-ml polyethylene bottles sealed tightly with caps until use. The COD_{Mn} of the sludge sample was about 3,200 ppm and the pH was about 8.2. In general, only acetate (2 mM or less), but no other volatile fatty acid, was detected. The sludge contained sulfate up to about 0.2 mM, but sulfide at about 1.5 mM.

Anaerobic incubation of sewage sludge sample. The sludge was incubated at 30°C under N_2 gas for 24 h. Then, 10 mM Na_2SO_4, 20 mM volatile fatty acid (sodium acetate or sodium propionate) and/or inhibitors (0.0005% (v/v) chloroform and/or 5 mM Na_2MoO_4) were added, and 10 ml portions of the sludge were distributed into test tubes (18 × 180 mm) under N_2 gas. In experiments with H_2 as the substrate, H_2/N_2 (40/60, v/v) mixed gas was substituted for the headspace gas. The tubes were sealed with butylrubber double stoppers and incubated at 30°C on a reciprocal shaker.

Enumeration of methanogenic bacteria and sulfate reducing bacteria. Methanogenic bacteria were enumerated by the three-tubes most probable number (MPN) technique using a medium of Balch et al. (*2*) with H_2 or acetate as the substrate. Calcium acetate (10 g/*l*) was substituted for sodium acetate (2.5 g/*l*) as the acetate source. Sulfate reducing bacteria were enumerated by the anaerobic roll tube method using a medium (*17*) with H_2, acetate, propionate or lactate as the electron donor.

The sludge was diluted under N_2 gas immediately after the sampling with a dilution medium (*16*). Aliquots (0.3 ml) of a series of dilutions were inoculated to 10 ml of the enumeration medium in culture tubes (18 × 180 mm) under N_2 gas or H_2/N_2 mixed gas. Tubes were tightly sealed with butylrubber stoppers, and

incubated at 30°C.

Headspace of culture tubes was filled with H_2/N_2 (80/20, v/v) mixed gas in place of N_2 gas for the enumeration of methanogenic bacteria on H_2. Calcium acetate was added to the medium and the headspace was filled with N_2 gas for the enumeration on acetate.

For the enumeration of sulfate reducing bacteria on H_2, the headspace was filled with H_2/N_2 (40/60, v/v) mixed gas. The medium amended with sodium salt of acetate, propionate or lactate at 20 mM was used and the headspace was filled with N_2 gas for the enumeration on the fatty acids. Black colonies were counted as those of sulfate reducing bacteria.

Analytical methods. Gases (CH_4, CO_2 and H_2) and volatile fatty acids were analyzed by gas chromatography, as described previously (*15*). Gas samples taken from the headspace of incubation tubes with a pressure lock syringe (Precision Sampling Co.) through the stopper were injected into the gas chromatograph (Hitachi 163). Sludge samples for the analysis of volatile fatty acids were deproteinized and injected into the gas chromatograph (Hitachi 263). Sulfate was separated by high pressure liquid chromatography using a Hitachi 655 Liquid Chromatogram with a column packed with an anion exchange resin (Hitachi 2710-SA-IC), and measured with a conductivity detector (Hitachi L-3700). A mobile phase composed of 5% (v/v) isopropanol, 2% (v/v) glycerol and 0.75 mM potassium biphthalate was used. COD_{Mn} and sulfide were measured as described previously (*17*).

RESULTS

Effects of sulfate addition on methanogenesis and sulfate reduction in the sludge

Methanogenic bacteria and sulfate reducing bacteria in the sludge were enumerated. As presented in Table 1, MPN of methanogenic bacteria was estimated to be 7.17×10^4/ml or 1.60×10^6/ml with H_2 or acetate as the substrate, respectively. While sulfate concentration in the sludge was not more than 0.2 mM (data not shown), viable counts of sulfate reducing bacteria were 3.10×10^5–6.45×10^5 CFU (colony forming units)/ml in media with H_2, acetate, propionate and lactate. This indicated that relatively large population of sulfate reducing bacteria inhabited the

Table 1. Enumeration of methanogenic bacteria and sulfate reducing bacteria in the sludge.

Substrate	Methanogenic bacteria (MPN/ml)	Sulfate reducing bacteria (Colony forming units/ml)
H_2	7.17×10^4	6.45×10^5
Acetate	1.50×10^6	4.89×10^5
Propionate	N.D.	3.20×10^5
Lactate	N.D.	3.10×10^5

N.D.: Not determined.
Sulfate concentration in the sludge sample was below the detection limit.

Table 2. Distribution of electrons to methanogenesis and sulfate reduction in the anaerobic digestion of sewage sludge.

Incubation[a]		MG	SR	COD-equivalent (ppm)		Distribution of electrons[b] (%)			Intermediary products[c] (mM)
Inhibitor	Sulfate	(mmol/l)		MG	SR	MG	SR	MG+SR	
No addition	−	26.7	0	1708.8	0	100	0	100	
	+	26.6	3.76	1702.4	240.6	99.6	14.1	113.7	
Chloroform	−	2.9	0	185.6	0	10.8	0	10.8	A (9.3), P (1.8), B (0.3), iV (0.4), H_2 (0.24)
	+	3.5	5.75	224.0	368.0	13.1	21.5	34.6	A (11.7), V (0.2), iV (0.4)
Na_2MoO_4	−	26.0	0	1664.0	0	97.4	0	97.4	
	+	28.0	0.07	1792.0	4.5	104.9	0.3	105.2	
Chloroform	−	2.6	0	166.4	0	9.7	0	9.7	A (6.4), P (1.5). B (0.4), H_2 (0.30)
$+Na_2MoO_4$	+	2.6	0.08	166.4	5.1	9.7	0.3	10.0	A (6.1), P (1.7), B (0.5), iV (0.5), H_2 (0.42)

Methanogenesis (MG) and sulfate reduction (SR) in sewage sludge in 8 days of incubation are shown. Values are averages of duplicate experiments.

[a] Concentrations of chloroform and sodium molybdate were 0.0005% (v/v) and 5 mM, respectively. −: Sulfate was not added; +: 10 mM sodium sulfate was added.

[b] Reducing equivalent used in methanogenesis in 8 days of incubation without the addition of inhibitors and sulfate was taken as 100%.

[c] Concentrations of acetate (A), propionate (P), butyrate (B), valerate (V), and isovalerate (iV) after 8 days of incubation are presented. H_2 was transiently evolved, and the maximum values observed after 4 days of incubation are presented.

sludge together with methanogenic bacteria in spite of the low sulfate concentration.

Table 2 shows effects of sulfate addition on the distribution of reducing equivalents to methanogenesis and sulfate reduction in the sludge. Reducing equivalents distributed to methanogenesis and sulfate reduction were calculated according to Isa et al. (*4*), as previously described (*14*). In the sludge supplemented with sulfate, sulfate reduction markedly proceeded without significantly affecting methanogenesis. Total amount of electrons used in both methanogenesis and sulfate reduction increased by the amount equivalent to that used in sulfate reduction, when compared with the amount used in methanogenesis without sulfate addition. Distribution of reducing equivalents to sulfate reduction or methanogenesis significantly increased by inhibiting methanogenesis or sulfate reduction, respectively.

In accordance with the previous report (*17*), without sulfate addition, intermediary products, in particular, H_2, acetate and propionate markedly accumulated when methanogenesis was inhibited. In the sludge with 10 mM sulfate, however, acetate solely accumulated when only methanogenesis was inhibited, and other volatile fatty acids and H_2 accumulated together with acetate when both methanogenesis and sulfate reduction were inhibited. The accumulation of intermediary products occurring upon the inhibition of methanogenesis and/or sulfate reduction suggested that these intermediary products, in particular, H_2 and acetate, propionate were used as substrates in methanogenesis and/or sulfate reduction in the sludge.

Methanogenesis and sulfate reduction during incubation with H_2

To examine effects of exogenous H_2 on methanogenesis and sulfate reduction, the sludge was incubated under the H_2/N_2 mixed gas (Table 3). Exogenous H_2 promoted methanogenesis in the sludge without sulfate by more than 40% and both

Table 3. Distribution of electrons to methanogenesis and sulfate reduction and H_2 consumption during incubation with H_2.

Incubation		MG	SR	Distribution of electrons (%)			H_2 consumed
Headspace	Sulfate	(mmol/*l*)		MG	SR	MG+SR	(mmol/*l*)
N_2	−	12.0	0	100	0	100	
	+	11.2	6.6	93.3	55.0	148.3	
H_2/N_2	−	17.3	0	144.2	0	144.2	29.9
	+	15.1	8.8	125.8	73.3	199.1	42.3

Methanogenesis (MG) and sulfate reduction (SR) in 8 days of incubation of sewage sludge under the headspace with N_2 or H_2/N_2 (40/60, v/v) gas. The initial amount of H_2 was 55.4 mmol/*l* of the sludge. Reducing equivalent used in methanogenesis in 8 days of incubation under N_2 gas without sulfate addition was taken as 100%. Values are averages of duplicate experiments. −: Sulfate was not added; +: 10 mM sulfate was added.

methanogenesis and sulfate reduction in the sludge with 10 mM sulfate by about 20% or more, when compared with those under N_2 gas. Sulfate addition caused decrease in methanogenesis by about 20% in the presence of exogenous H_2.

As also shown in Table 3, 30–40 mmol/*l* of H_2 was consumed during 8 days of incubation with H_2. More H_2 was consumed in the sludge with sulfate than in the sludge without sulfate. About 10 mmol/*l* of CO_2 was evolved in 8 days of incubation under N_2, but no CO_2 was evolved during incubation with H_2 (data not shown).

Effects of chloroform and/or molybdate on methanogenesis and sulfate reduction in the presence of exogenous H_2 are examined (Fig. 1). Without sulfate addition, 23.1 mmol/*l* of CH_4 was evolved in 8 days of incubation. In the sludge with 10 mM sulfate, 19.2 mmol/*l* of CH_4 was evolved and 5.6 mmol/*l* of sulfate was

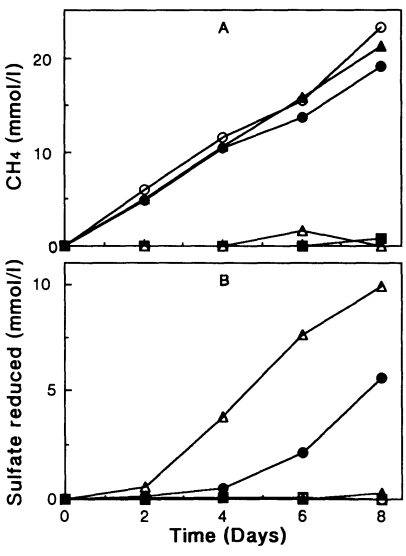

Fig. 1

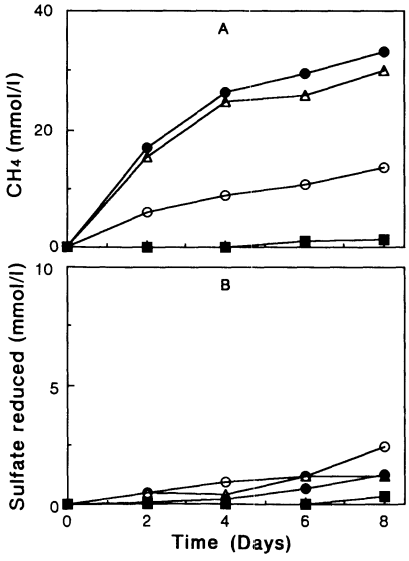

Fig. 2

Fig. 1. Methanogenesis (A) and sulfate reduction (B) in sewage sludge during incubation with H_2.

The sludge supplemented with 10 mM sulfate was incubated with 0.0005% chloroform (○), 5 mM molybdate (▲) or 0.0005% chloroform plus 5 mM molybdate (■), or without inhibitors (●) under H_2/N_2 (40/60, v/v) mixed gas. The sludge was also incubated under the mixed gas without the addition of sulfate and inhibitors (□). Values are averages of duplicate experiments.

Fig. 2. Methanogenesis (A) and sulfate reduction (B) in sewage sludge during incubation with acetate.

The sludge supplemented with 10 mM sulfate was incubated with 20 mM acetate (○), 20 mM acetate plus 5 mM molybdate (●), 20 mM acetate plus 5 mM molybdate and 0.0005% chloroform (■), or without addition of acetate and inhibitors (□). Values are averages of duplicate experiments.

Table 4. Effects of inhibitors on H_2 consumption and concentrations of volatile fatty acids during incubation with H_2.

Incubation[a]		H_2 consumed[b] (mmol/l)	Volatile fatty acids (mM)[c]	
Inhibitor	Sulfate		Acetate	Propionate
No addition	+	55.0	1.6	0
No addition	−	49.5	4.1	0
Chloroform	+	34.0	9.2	0
Na_2MoO_2	+	49.7	1.4	0
Chloroform + Na_2MoO_2	+	30.6	13.8	2.7

Sewage sludge was incubated with or without inhibitors and sulfate under the headspace with H_2/N_2 (40/60, v/v) gas. Values are averages of duplicate experiments.

[a] Concentrations of chloroform and sodium molybdate were 0.0005% (v/v) and 5 mM, respectively. −: Sulfate was not added; +: 10 mM sodium sulfate was added.

[b] The initial amount of H_2 was 55.0 mmol/l of the sludge.

[c] Concentrations of volatile fatty acids after 8 days of incubation are presented. Volatile fatty acids other than acetate and propionate were not detected.

reduced in 8 days. Inhibition of sulfate reduction by molybdate resulted in slight enhancement of methanogenesis to the level of that without sulfate addition, and, vice versa, inhibition of methanogenesis by chloroform resulted in enhancement of sulfate reduction. The amount of sulfate reduced in 8 days reached 9.9 mmol/l, when methanogenesis was inhibited.

Table 4 shows H_2 consumption during the incubation period. H_2 at about 55 mmol/l was completely exhausted in 8 days of incubation with 10 mM sulfate in the absence of inhibitors. The inhibition of methanogenesis or sulfate reduction resulted in the decrease of H_2 consumption to about 60% or 90%, respectively. However, a significant amount of H_2 (30.6 mmol/l) was consumed even when both methanogenesis and sulfate reduction were inhibited. After 8 days of incubation, the amounts of CO_2 evolved were only 0.25 mmol/l or less in all the sludge (data not shown).

Concentrations of volatile fatty acids after 8 days of incubation with H_2 are also shown in Table 4. Acetate accumulated during incubation in the presence or absense of sulfate and inhibitors. In particular, acetate concentration after 8 days reached about 9.2 mM when methanogenesis was inhibited and 13.8 mM when both methanogenesis and sulfate reduction were inhibited. Propionate significantly accumulated when both methanogenesis and sulfate reduction were inhibited.

Methanogenesis and sulfate reduction during incubation with acetate

Methanogenesis and sulfate reduction in the sludge supplemented with about 10 mM sulfate during incubation with about 20 mM acetate are shown in Fig. 2. Without the addition of volatile fatty acids, 13.6 mmol/l of CH_4 was evolved and 2.4 mmol/l of sulfate was reduced in 8 days of incubation. The addition of acetate markedly enhanced methanogenesis but not sulfate reduction, in particular, in the first 4 days of incubation. About 30 mmol/l of CH_4 was evolved in 8 days of

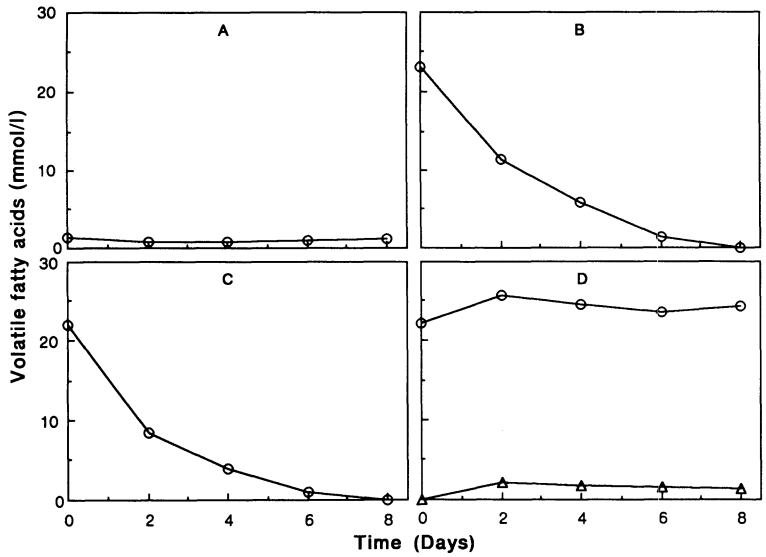

Fig. 3. Changes in concentrations of volatile fatty acids in sewage sludge during incubation with acetate.

The sludge supplemented with 10 mM sulfate was incubated with 20 mM acetate (B), 20 mM acetate plus 5 mM molybdate (C) or 20 mM acetate plus 5 mM molybdate and 0.0005% chloroform (D), or without addition of acetate and inhibitors (A). Only acetate (○) and propionate (△) were detected. Values are averages of duplicate experiments.

incubation with acetate. The addition of molybdate inhibited sulfate reduction but slightly enhanced methanogenesis, and the addition of chloroform almost completely blocked methanogenesis in the sludge with acetate. A few amounts of H_2 (0.1 mmol/*l*) accumulated when both sulfate reduction and methanogenesis were inhibited, and 1.9–3.0 mmol/*l* of CO_2 were evolved after 8 days of incubation with or without acetate and/or inhibitors (data not shown).

Changes in concentrations of volatile fatty acids during incubation with acetate are shown in Fig. 3. Without the addition of volatile fatty acids, volatile fatty acids other than acetate at low concentrations were not detected in the sludge over the incubation period. In the sludge amended with acetate, 23.0 mmol/*l* of acetate was almost completely exhausted in 6 days without being affected by sulfate reduction. Acetate concentration did not decrease, but slightly increased when methanogenesis was blocked.

Methanogenesis and sulfate reduction during incubation with propionate

The addition of propionate markedly enhanced both methanogenesis and sulfate reduction (Fig. 4). In the sludge amended with propionate, methanogenesis proceeded at a constant rate, and 37.5 mmol/*l* of CH_4 was evolved in 8 days. The

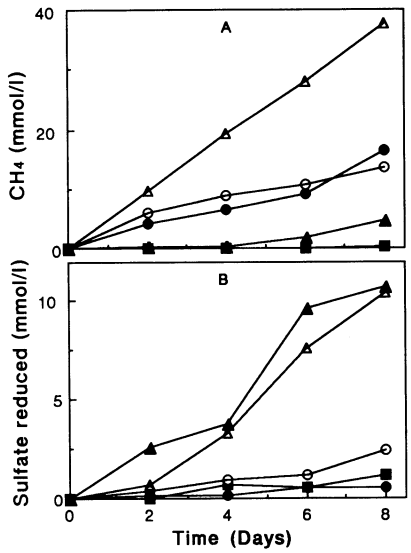

Fig. 4. Methanogenesis (A) and sulfate reduction (B) in sewage sludge during incubation with propionate.

The sludge supplemented with 10 mM sulfate was incubated with 20 mM propionate (△), 20 mM propionate plus 5 mM molybdate (●), 20 mM propionate plus 0.0005% chloroform (▲), or 20 mM propionate plus 5 mM molybdate and 0.0005% chloroform (■). The sludge supplemented with 10 mM sulfate was also incubated without addition of propionate and inhibitors (○). Values are averages of duplicate experiments.

amount of sulfate reduced in 8 days of incubation with propionate reached 10.9 mmol/*l*. The addition of molybdate to the sludge amended with propionate not only markedly inhibited sulfate reduction, but also depressed methanogenesis to the level of that in the sludge without the addition of volatile fatty acids. The inhibition of methanogenesis in the sludge amended with propionate caused marked enhancement of sulfate reduction. In the presence of chloroform plus molybdate, both methanogenesis and sulfate reduction were blocked. A small amount of H_2 was evolved when both sulfate reduction and methanogenesis were inhibited, and 1.2–3.8 mmol/*l* of CO_2 were evolved after 8 days of incubation with propionate in the presence or absence of inhibitors (data not shown).

Changes in concentrations of volatile fatty acids during incubation with propionate are shown in Fig. 5. More than 20 mmol/*l* of propionate was consumed in 8 days in the absence of inhibitors. The propionate consumption was almost completely depressed when sulfate reduction was blocked by molybdate. Propionate was consumed and marked acetate accumulation occurred, when methanogenesis was inhibited. The acetate concentration reached 20.6 mM after 8 days. When both methanogenesis and sulfate reduction were blocked, about 5 mmol/*l* of acetate accumulated after 8 days, while propionate was not degraded.

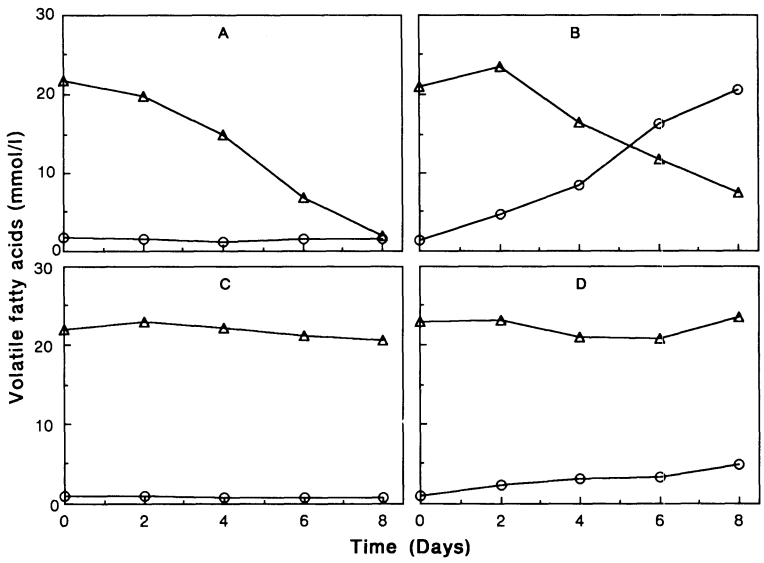

Fig. 5. Changes in concentrations of volatile fatty acids in sewage sludge during incubation with propionate.

The sludge supplemented with 10 mM sulfate was incubated with 20 mM propionate (A), 20 mM propionate plus 5 mM molybdate (C), 20 mM propionate plus 0.0005% chloroform (B), or 20 mM propionate plus 5 mM molybdate and 0.0005% chloroform (D). Only acetate (○) and propionate (△) were detected. Values are averages of duplicate experiments.

DISCUSSION

In anaerobic digestion of municipal sewage sludge, as well as in anaerobic digester slurry of animal waste, sulfate reduction apparently does not compete with methanogenesis (*12–14, 17*), while sulfate reduction is generally known to compete with methanogenesis in various natural environments (*1, 3, 6–8, 11, 18, 20*). The compatible relationship between sulfate reduction and methanogenesis seems to be characteristic of nutritionally rich environments such as sewage sludge and digester slurry of animal waste.

Since sludge samples used in the present study contained sulfate only at low concentrations, methanogenesis was thought to be the sole reaction responsible for the terminal step of electron flow in the anaerobic digestion, unless sulfate was not added. A large population of sulfate reducers, however, inhabited the sludge. Thus, in the sludge amended with sulfate, sulfate reduction together with methanogenesis could serve as the electron sink.

In accordance with the previous study (*17*), the inhibition of methanogenesis and/or sulfate reduction in the sludge with or without sulfate characteristically caused marked accumulation of H_2 and volatile fatty acids. The accumulation of

intermediary products suggested that these compounds, in particular, H_2, acetate and propionate were used as substrates in methanogenesis and/or sulfate reduction in the sludge. Thus, the present study aimed at investigating the relationship in substrate utilization between methanogenesis and sulfate reduction in more detail.

The present study showed that both methanogenesis and sulfate reduction were significantly enhanced upon the addition of H_2. In the presence of exogenous H_2, the inhibition of methanogenesis resulted in enhancement of sulfate reduction and the inhibition of sulfate reduction resulted in enhancement of methanogenesis. These results well documented the competition for H_2 between methanogenesis and sulfate reduction in the sludge. Results also showed that significant amounts of H_2 were consumed even when both methanogenesis and sulfate reduction were blocked. In the presence of exogenous H_2, CO_2 was evolved only slightly, if at all, and acetate accumulated significantly. At least a portion of the consumed H_2 seemed to be used for the reduction of CO_2 to acetate.

The addition of acetate markedly enhanced methanogenesis but did not affect sulfate reduction, while sulfate reducing bacteria were enumerated at a relatively high density on the medium with acetate. We isolated bacteria from black colonies formed on the medium inoculated with the sludge diluted to 10^{-4} and tested their substrate utilization. All the tested colonies grew on the medium with H_2 or lactate, and acetate-utilizers were not specified (data not shown). It seemed that H_2 and lactate-utilizing sulfate reducers formed colonies using the substrate other than acetate, which was derived from 0.1% yeast extract contained in the medium. Thus, taking account of the fact that acetate accumulated in the sludge with or without sulfate when methanogenesis was blocked, the result indicated that acetate was almost used in methanogenesis but not in sulfate reduction. The enumeration of methanogens and sulfate reducers suggested that the acetate-utilizing methanogens occupied more than 95% of the population of methanogens in the sludge and that the density of H_2-utilizing sulfate reducers was estimated to be about 10 times higher than that of H_2-utilizing methanogens. It seemed that in the sludge with sulfate acetate served as the main source of CH_4.

Upon the addition of propionate, both methanogenesis and sulfate reduction were markedly enhanced in the sludge with sulfate. Degradation of propionate essentially depended on sulfate reduction, and acetate accumulated in response to the propionate degradation when methanogenesis was blocked. These results indicate that propionate is degraded to acetate by sulfate reduction. It is notable that methanogenesis enhanced by propionate addition proceeded at a linear rate. Degradation of propionate to acetate by sulfate reduction may have limited the enhancement of electron flow to methanogenesis.

In conclusion, in the sludge, acetate is used only in methanogenesis but H_2 is used in both methanogenesis and sulfate reduction. Sulfate reduction degrades propionate to acetate and rather enhances electron flow to methanogenesis.

It is known that longer-chain volatile fatty acids are in general degraded anaerobically by H_2-producing acetogenic bacteria under the syntrophic association

with H_2-consuming bacteria such as methanogens and sulfate reducers. Present results, however, indicated that sulfate reducers played an essential role as the propionate degrader in the sludge. Some species of sulfate reducing bacteria using propionate as the electron donor have been isolated (*19*). What kinds of sulfate reducing bacteria play the role as the propionate degrader in the sludge is an interesting problem remaining unsolved. We have established enrichment cultures originated from the sewage sludge, in which degradation of propionate, i.e., the sole carbon and energy source, is solely dependent on sulfate reduction. The enrichment cultures contain at least three morphologically different bacteria. We are now trying to isolate the propionate degrader from the cultures.

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