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EFFECTS OF SULFATE CONCENTRATION AND SLUDGE RETENTION TIME ON THE INTERACTION BETWEEN METHANE PRODUCTION AND SULFATE REDUCTION FOR BUTYRATE

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ABSTRACT

The effects of sulfate concentration and COD/S ratio on the anaerobic degradation of butyrate were investigated by using 2.0 L anaerobic chemostat-type reactor at 35°C. The study was conducted over a wide range of the COD/S ratio (1.5 to 148) by varying COD concentrations (2500-10000 mg/L) and sulfate concentrations (68-1667 mg-S/L) in the substrate. The sludge retention time at each COD/S ratio was changed from 5 to 20 days. The interaction between methane producing bacteria (MPB) and sulfate-reducing bacteria (SRB) was evidently influenced by COD/S ratio in the substrate. When COD/S ratio was 6.0 or more, methane production was the predominate reaction and over 80% of the total electron flow was used by MPB. At the COD/S ratio of 1.5, SRB utilized over 50% of the total electron flow. A large amount of sulfate reduction resulted in not only the decrease of methane production, but also the rapid increase of the bacterial growth. The degradation pathway of butyrate and the composition of bacterial populations in the reactor were also dominated by COD/S ratio. In sulfate depleted condition, butyrate was degraded to methane via acetate and hydrogen by MPB. On the other hand, butyrate was firstly degraded into sulfide and acetate in sulfate rich conditions by SRB, and the produced acetate was then degraded by acetate consuming MPB and SRB. The methanogenesis from acetate was inhibited by the high concentration of sulfide.

KEYWORDS

Anaerobic degradation, bacterial population, butyrate, competition, electron flow, metabolic activity, methane-producing bacteria, sulfate-reducing bacteria.

INTRODUCTION

Anaerobic biological treatment processes have been widely used for the treatment of various industrial wastewaters since the successful development of a number of high-rate processes, such as the anaerobic filter and the upflow anaerobic sludge blanket reactor (Young and McCarty, 1969 ; Young, 1991; Lettinga

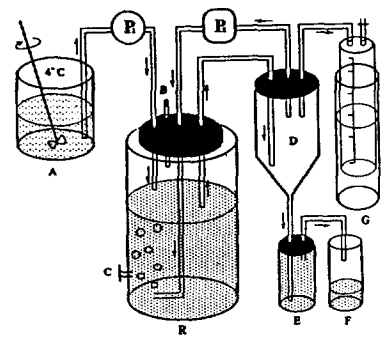
et al, 1980 ; Lettinga and Hulshoff Pol, 1991). However, anaerobic treatment process have some problems which have limited their applications. One typical example is that sulfate, a common substance in industrial wastewater can adversely affect the anaerobic degradation of organic matters. In the presence of sulfate, sulfate-reducing bacteria (SRB) degrade some organic matters by using sulfate as the electron acceptor and as a result, sulfides are produced in the anaerobic treatment process. A small amount of sulfide production can provide the advantageous condition for methane producing bacteria (MPB), because: (a) sulfide can maintain low oxidation-reduction potential in the digester; (b) sulfide is also an important sulfur source for the growth of MPB (Ronnow and Gunnarsson, 1981); (c) some soluble heavy metal ions such as Cu, Ni and Zn which are toxic to anaerobic microorganisms can be precipitated out as insoluble sulfide. On the contrary, high sulfate concentration in wastewaters can cause several problems in the anaerobic treatment process, because: (a) sulfate can be reduced to sulfide which is a strong inhibitor of MPB; (b) sulfide can cause a high oxygen demand in the effluent; (c) hydrogen sulfide, which is being released to the biogas, can cause corrosion problems downstream; (d) the competition between SRB and MPB for organic substrates can decrease the methane production. These problems significantly hinder the application of anaerobic treatment process for the treatment of certain sulfate-rich wastewaters, such as those from citric acid, amino acid, pulp and chemical industries. Therefore, it is important to have a better understanding on the behavior of SRB in the anaerobic process.

It has been reported that SRB have kinetic adventages over MPB for some substrates, especially acetate and hydrogen (Widdel, 1988). In the sulfate-rich culture, SRB can outcompete MPB for hydrogen and acetate, which are the two key intermediates in the anaerobic treatment process (Abram and Nedwell, 1978 ; Schonheit et al, 1982). Several studies (Yoda et al, 1987 ; Isa et al, 1986 : Lovley and Tiedje, 1984; Puhakka et al, 1985) have been focused on the effect of sulfate concentration on the methane fermentation of acetate. Recent microbial studies (Widdel, 1988; Odom and Singleton, 1992) also reported that some species of SRB can oxidize propionate and butyrate by using sulfate as external electron acceptor. However, the competition between MPB and SRB for propionate and butyrate has not been clearly understood yet. So far, no investigation has been conducted to discuss the effect of COD/S ratio on the anaerobic degradation of butyrate.

The purpose of this study is to evaluate the effects of sulfate concentration and sludge retention time (SRT) on the anaerobic degradation of butyrate. The competitions between SRB and MPB at different operational conditions were quantitatively investigated by determining the COD mass balance, electron flow, bacterial population and metabolic activity of each trophic group of bacteria in the reactor. In addition, the role of SRB in the anaerobic treatment process was also discussed.

MATERIALS AND METHODS

Experimental apparatus. Five chemostat-type anaerobic reactors were operated in parallel. A process flow diagram is illustrated in Figure 1. Each reactor was a 3-liter acrylic plastic vessel with working volume of 2-liter. Substrate was fed to the reactor by a peristaltic pump. The content of the reactor was completely mixed by biogas which was being recirculated by a gas pump. In order to measure the volume of gas produced, the reactor was connected to a gas collection cylinder placed in an acidic saturated salt solution which contained NaCl and 2% of sulfuric acid . All the reactors were installed in a constant temperature controlled chamber at 35°C. Since the chemostat-type reactor is a continuous flow and completely mixed tank,



A : Substrate tank, B : Gas sampling port
C : Mixed liquor sampling port
D, E, F : Mixed liquor overflow system
G : Gas collection system
P1 : Feed pump, P2 : Gas recirculation pump, R : Reactor

Fig. 1. Experimental apparatus

the sludge retention time (SRT) can be considered to be equal to the hydraulic retention time under the steady-state condition. In this study, SRT was used to describe the retention time under the steady-state condition. The SRT of each reactor was changed from 20 to 5 days.

Seed sludge and substrate. The original seed sludge was collected from a primary settling tank in the Minamigamo municipal sewage treatment plant, located at Sendai in Japan. After the original seed sludge was inoculated to each reactor, it was acclimatized for 3 months at SRT of 20 days at 35°C by using the substrates shown in Table 1 and Table 2. Table 1 summarizes the chemical composition of the synthetic substrate. Butyric acid was used as the sole carbon source, and sodium sulfate was used as the sulfur source. The experimental conditions of each reactor are given in Table 2. The synthetic substrates were prepared daily and stored in feed tank which maintained at 4°C. Each feed tank was continuously mixed by a magnetic stirrer.

TABLE 1 CHEMICAL COMPOSITION OF SUBSTRATE

Composition	Concentration (mg/L)
$CH_3(CH_2)_2COOH$	*
$(NH_4)_2HPO_4$	700
NH_4Cl	850
KCl	750
$MgCl_2 \cdot 6H_2O$	810
Na_2SO_4	*
$FeCl_2 \cdot 4H_2O$	420
$CoCl_2 \cdot 6H_2O$	2.5
$MnCl_2 \cdot 4H_2O$	2.5
KI	2.5
$Na_2MoO_4 \cdot 2H_2O$	0.5
H_3BO_3	0.5
$NiCl_2 \cdot 6H_2O$	0.5
$ZnCl_2$	0.5

* : see TABLE 2

TABLE 2 EXPERIMENTAL CONDITIONS

	R1	R2	R3	R4	R5
Butyric acid (mg-COD/L)	10000	10000	10000	5000	2500
Sulfate-S(mg-S/L)	68	667	1667	1667	1667
COD/S ratio	148	15	6	3	1.5

Chemical analysis. The amounts of methane, carbon dioxide, and nitrogen in biogas were analyzed by a gas chromatograph (Shimadzu GC-8A) equipped with a thermal conductivity detector and a 1.5 m long stainless column packed with activated carbon. The temperatures of injection port and column were 140°C and 120°C, respectively. Helium of a flow rate of 1.5 kg/cm^2 was used as the carrier gas. For the analysis of hydrogen sulfide, the biogas produced from the reactor was passed into a flask containing 100 ml of zinc acetate solution (40 % w/v) in which hydrogen sulfide was precipitated as zinc sulfide. The amount of zinc sulfide produced was then determined by the spectrophotometric methylene-blue method. The volatile fatty acids (VFA) concentration was determined by a gas chromatograph (Shimadzu GC-8A) equipped with a flame ionization detector and a 1.5 m (length) x 5.0 mm (ID) glass column packed with Greensorb. The temperatures of injection port and column were 190°C and 160°C respectively. Helium of a flow rate of 1.5 kg/cm^2 was used as the carrier gas.

Sulfate and sulfide were analyzed by the turbidimetric method and the spectrophotometric methylene-blue method, respectively. Both dissolved sulfide and mixed liquor volatile suspended solids (MLVSS) which express biomass concentration were measured according to the standard methods.

Enumeration of MPB. The number of each trophic group of MPB was determined by the most probable number method (five tubes). Each enumeration medium contained the following components (per liter) : KH_2PO_4, 0.4 g ; K_2HPO_4, 0.4 g ; NH_4Cl, 1.0 g ; $MgCl_2 \cdot 6H_2O$, 0.21 g ; $NaHCO_3$, 6.0 g ; yeast extract , 0.2 g ; $Na_2S \cdot 9H_2O$, 0.25g ; Cysteine HCl H_2O, 0.5g ; mineral solution 10 ml/L (Li and Noike, 1989) and vitamin solution 10 ml/L (Li and Noike, 1989). Carbon source was 3.0 g/L of sodium acetate for acetate-consuming MPB and H_2-CO_2 (80%/20%) for hydrogen-consuming MPB. The inoculated tubes were incubated at 35°C for one month. The growth of MPB was interpreted on the basis of presence of

methane (over 0.4%) in gas phase of the tube.

Enumeration of SRB. The number of each trophic group of SRB was determined by the most probable number method (five tubes). Each enumeration medium contained the following components (per liter) : Na_2SO_4, 1.0 g ; KH_2PO_4, 0.5 g ; $MgSO_4 \cdot 7H_2O$, 2.0 g ; $CaCl_2 \cdot 2H_2O$, 0.1 g ; NH_4Cl, 1.0 g ; Na_2SO_3, 0.5 g ; $FeSO_4$ $7H_2O$, 0.5 g ; $NaHCO_3$, 6.0 g ; sodium thioglycolate, 0.1 g ; L(+) - ascorbic acid, 0.1 g, biotin 0.1 mg/L and p-aminobenzoic acid 0.05 mg/L. Carbon source was 3.0 g/L of sodium butyrate for butyrate-consuming SRB, 3.0 g/L of sodium acetate for acetate-consuming SRB and H_2-CO_2 (80%/20%) for hydrogen-consuming SRB. The inoculated tubes were incubated at 35°C for one month. The growth of SRB was estimated by the changing of color of medium to black.

Measurement of metabolic activity. The specific methanogenic activity (SMA) and the substrate utilization rate (SUR) of the content in each reactor were measured in serum vials based on the batch experiment (Dolfing and Mulder, 1985; Li and Noike, 1989). The individual substrates used for the SMA and SUR measurements were acetate and H_2-CO_2 (80%/20%).

RESULTS AND DISCUSSION

Sulfate reduction and the inhibition of methanogenesis by sulfide. Table 3 summarizes the main experimental results of VFA degradation and sulfate reduction under the steady-state condition. Under all the operational conditions, butyrate the influent organic substrate, was not detectable or in very low concentration. It was considered that butyrate was completely degraded at each experimental condition. On the other hand, acetate which is a key intermediate in the methane fermentation of butyrate, accumulated to 250 to 694 mg/L in some operational conditions, such as when COD/S ratio was 15 at the SRT of 5 days, COD/S ratio was 6 at the SRT of 5 and 10 days, and COD/S ratio was 3 at the SRTs of 10 and 20 days. Under these conditions, the concentrations of total sulfide and free-H_2S, which were strong inhibitors of methanogenesis,

TABLE 3 SUMMARY OF MAIN OPERATIONAL RESULTS UNDER STEADY-STATE CONDITION

	COD/S	SRT (d) (days)	pH	Sulfate-S (mg/L)	Sulfide-S (mg/L)			Bio gas (%)		VFA (mg/L)		
					TS	DS	Free-H_2S	CH_4	H_2S	HAc	i-HBu	HBu
R1	148	20	7.2	nd	8.3	nd	nd	72.7	0.01	41.0	nd	nd
		10	7.2	3.3	7.6	nd	nd	71.2	0.3	nd	nd	nd
		5	7.0	3.0	7.3	nd	nd	71.6	0.01	192.2	nd	nd
R2	15	20	7.5	20.0	172.7	89.8	17.0	69.5	0.9	nd	53.3	25.7
		10	7.5	6.9	255.8	215.3	37.7	69.0	2.1	113.1	nd	nd
		5	7.4	11.2	394.5	296.4	62.5	66.3	4.4	231.5	nd	nd
R3	6	20	7.5	829.9	372.9	162.2	27.3	67.7	1.4	70.2	nd	58.8
		10	7.4	711.5	393.5	162.1	34.2	66.2	4.8	694.0	nd	nd
		5	7.3	798.4	397.3	270.7	68.1	63.6	8.3	414.2	nd	nd
R4	3	20	7.6	822.8	230.5	114.1	17.8	61.2	1.9	372.2	nd	49.6
		10	7.4	948.2	362.8	299.4	63.1	68.2	2.0	252.0	nd	nd
		5	7.6	1031.9	244.3	114.0	16.4	71.5	2.4	120.9	nd	nd
R5	1.5	20	8.1	822.9	342.3	162.8	7.9	21.1	3.8	nd	nd	39.2
		10	7.9	1060.4	282.7	177.8	13.9	63.3	0.01	98.5	nd	nd
		5	7.8	1027.8	162.2	94.4	9.1	28.8	0.07	100.7	nd	nd

TS : total sulfide, DS : dissolved sulfide, nd : not detectable

were detected as high as 230 to 397 mg/L and 18 to 68 mg/L, respectively. It was considered that the methanogenesis from acetate was inhibited by the high concentration of total sulfide and free H_2S. Table 3 also illustrates that the pH in reactor and biogas composition were influenced significantly by the COD/S ratio and SRT. As the COD/S ratio decreased from 148 to 1.5, the pH value in the reactor increased from 7.2 to 8.1. The highest H_2S content in the biogas reached 8.3% at COD/S ratio of 6 with SRT of 5 days.

Electron flow. The competition between MPB and SRB can be clearly evaluated by the electron flow, because the methane production and sulfate reduction are biological oxidation-reduction reactions. In this study, the electron flow by MPB and SRB was calculated according to the method reported by Isa et al (Isa et al, 1986).

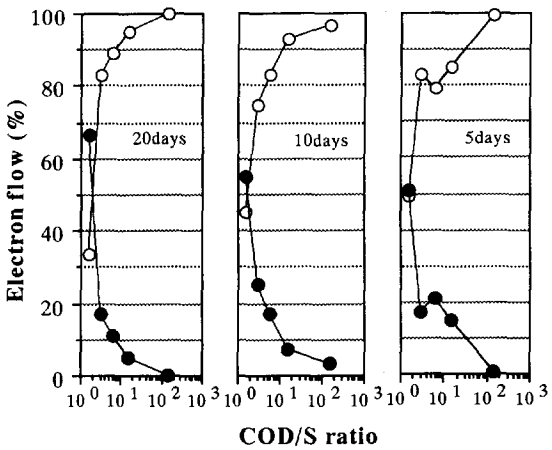


Fig. 2. Effects of COD/S ratio and sludge retention time on the electron flow between sulfate-reducing bacteria and methane producing bacteria

—○— Methane producing bacteria
—●— Sulfate-reducing bacteria

TABLE 4 COD MASS BALANCE UNDER STEADY-STATE CONDITION

	COD/S	SRT	Influent COD	Effluent COD					
		(days)	(%)	CH_4	MLVSS	Sulfide	H_2S	VFA	Recovery (%)
		20	100	85.7	7.4	0.2	0.01	1.8	95.1
R1	148	10	100	82.3	5.0	1.5	1.2	0	90.0
		5	100	87.9	5.0	0.1	0.3	4.1	97.4
		20	100	82.6	8.8	3.5	1.1	1.4	97.4
R2	15	10	100	79.0	11.4	5.1	1.2	1.2	97.9
		5	100	71.1	10.1	7.9	4.4	2.5	96.0
		20	100	72.6	8.1	7.5	1.5	1.8	91.5
R3	6	10	100	65.9	10.1	7.9	5.7	7.4	97.0
		5	100	57.1	11.3	7.9	7.5	4.4	88.2
		20	100	51.8	13.5	9.2	1.6	9.7	85.8
R4	3	10	100	50.9	11.6	14.5	1.4	5.4	83.8
		5	100	57.2	21.3	9.8	1.9	2.6	92.8
		20	100	15.1	26.1	27.4	2.6	2.8	74.0
R5	1.5	10	100	18.9	28.5	22.6	0.2	4.2	74.4
		5	100	13.4	51.3	13.0	0.7	1.1	79.5

Figure 2 illustrates that the electron flow between MPB and SRB depended on the COD/S ratio. At SRT of 20 days, the percentage of electron flow used for MPB decreased from 98% to 34% as the COD/S ratio changed from 148 to 1.5, while the percentage electron flow used for SRB increased from 2% to 67%. On the other hand, the electron flow at the same COD/S ratio was also slightly affected by the SRT. For example, the percentage of electron flow used for SRB at the COD/S ratio of 1.5 increased from 51% to 67% as SRT changed from 20 to 5 days.

COD mass balance and bacterial growth yield. Table 4 illustrates the COD mass balance of each reactor under steady-state condition. Influent COD was recovered from methane, biomass, sulfide, hydrogen sulfide and volatile fatty acids. These products were markedly affected by the COD/S ratio in substrate. When the COD/S ratio was 148, more than 82% of influent COD was converted to methane and only a small amount of COD (less than 3%) was converted to sulfide and hydrogen sulfide. It was clear that methane was the main product in that condition. As the COD/S ratio decreased from 148 to 1.5, methane decreased from 82 to 13 %, while sulfide increased from 1.5 to over 27%. As a result, sulfate reduction became the predominant reaction at the COD/S ratio of 1.5.

As shown in Table 4, the MLVSS increased clearly with decreasing COD/S at each SRT. At COD/S ratio of 148, only about 5% COD was converted into the MLVSS. However, when COD/S ratio was 1.5, the MLVSS yield was over 26% which is 4 times that at COD/S ratio of 148. Since the substrate did not contain any suspended solids, it was considered that the MLVSS exactly represented the biomass present in the

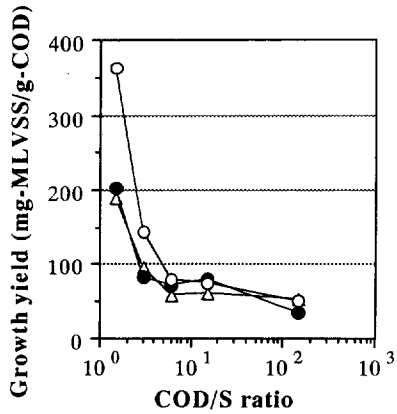


Fig. 3. Effects of COD/S ratio and sludge retention time on the growth yield under steady-state condition

TABLE 5 POPULATIONS OF DIFFERENT METABOLIC GROUPS OF BACTERIA PRESENT IN EACH REACTOR UNDER STEADY-STATE CONDITION

	COD/S	SRT	SRB (MPN/ml)			MPB (MPN/ml)	
		(days)	Butyrate	Acetate	H_2	Acetate	H_2
R1	148	20	2.1×10^{7}	1.8×10^{7}	5.0×10^{2}	4.8×10^{6}	7.9×10^{5}
		10	3.3×10^{7}	3.3×10^{7}	2.4×10^{7}	7.9×10^{6}	1.1×10^{7}
		5	3.3×10^{7}	2.3×10^{7}	4.3×10^{5}	1.7×10^{6}	4.9×10^{6}
R2	15	20	1.1×10^{8}	1.1×10^{8}	4.0×10^{4}	1.3×10^{6}	3.3×10^{3}
		10	2.2×10^{8}	2.4×10^{8}	$<10^{2}$	2.6×10^{6}	2.4×10^{7}
		5	4.9×10^{8}	4.9×10^{8}	3.3×10^{3}	7.9×10^{6}	4.9×10^{6}
R3	6	20	5.9×10^{7}	7.2×10^{7}	3.2×10^{4}	3.1×10^{6}	4.9×10^{2}
		10	1.3×10^{8}	3.3×10^{8}	$<10^{3}$	1.3×10^{6}	1.7×10^{5}
		5	7.9×10^{8}	4.9×10^{8}	9.0×10^{2}	3.3×10^{6}	1.6×10^{6}
R4	3	20	7.0×10^{7}	5.9×10^{7}	9.0×10^{2}	1.4×10^{4}	nd
		10	7.9×10^{7}	7.9×10^{7}	$<10^{3}$	1.3×10^{6}	7.9×10^{3}
		5	3.3×10^{8}	1.7×10^{8}	$<10^{3}$	7.9×10^{4}	2.4×10^{3}
R5	1.5	20	2.4×10^{7}	5.5×10^{6}	3.5×10^{4}	2.6×10^{4}	2.0x10
		10	7.9×10^{6}	1.3×10^{7}	$<10^{2}$	4.9×10^{4}	2.4×10^{3}
		5	1.1×10^{6}	1.3×10^{6}	$<10^{4}$	1.6×10^{5}	1.6×10^{5}

nd : not detectable

reactor. Figure 3 illustrates that the growth yield of biomass at each SRT increased with the decrease of COD/S ratio, especially when COD/S ratio was below 6. These data demostrate that a large amount of sulfate in influent can result in a significant increase of bacterial growth.

Bacterial population. Table 5 summarizes the bacterial population of each trophic group in the reactor under steady-state condition. Populations of butyrate and acetate-consuming SRB were enumerated in the same order of 10^6 to 10^8 MPN/ml in all the reactors, while the bacterial population of acetate-consuming MPB ranged from 10^4 to 10^6 MPN/ml. The counts of butyrate and acetate-consuming SRB were 10 to 100 fold higher than corresponding counts of acetate-consuming MPB. The number of hydrogen-consuming SRB was almost measured in below 10^5 MPN/ml and lower than corresponding counts of hyrdrogen-consuming MPB. The number of hydrogen-consuming MPB decreased from 10^6 to less than 10^3 MPN/ml as the COD/S ratio decreased from 148 to 1.5, but it increased as the SRT decreased.

Metabolic activity. Figure 4 illustrates that the SMA of acetate-consuming and hydrogen-cosuming MPB were evidently affected by the COD/S ratio and the SRT. At the COD/S ratio of 148, both the SMA for acetate and hydrogen were about 20 ml/g-MLVSS/hr under all the SRT conditions. However, the SMA at a same SRT decreased with the increase of sulfate concentration (COD/S ratio decrease) . Particularly, the SMA for hydrogen-consuming MPB decreased rapidly and dropped to be less than 5 ml/g-MLVSS/hr at the COD/S ratio below 6. On the other hand, although the SMA for acetate-consuming MPB also decreased with the increase of sulfate concentration in the substrate, it still stayed at a significant level until the COD/S ratio decreased to 3. Figure 5 illustrates that SMA for acetate-consuming MPB was the main part of the total SUR at the COD/S ratio of greater than 3. So, it can be concluded that SRB did not outcompete MPB for acetate at the COD/S ratio of greater than 3.

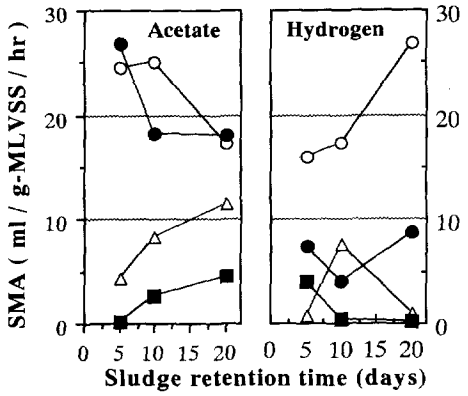


Fig. 4. Specific metanogenic activity under steady-state condition

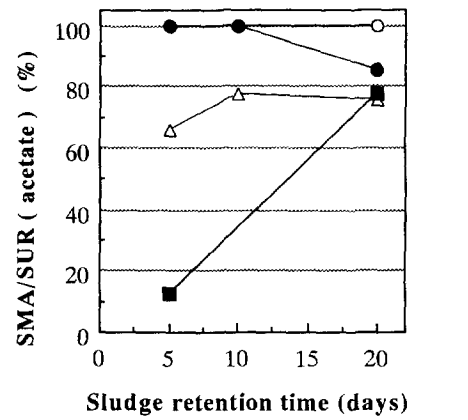


Fig. 5. SMA/SUR (%) for acetate-consuming MPB under steady-state condition

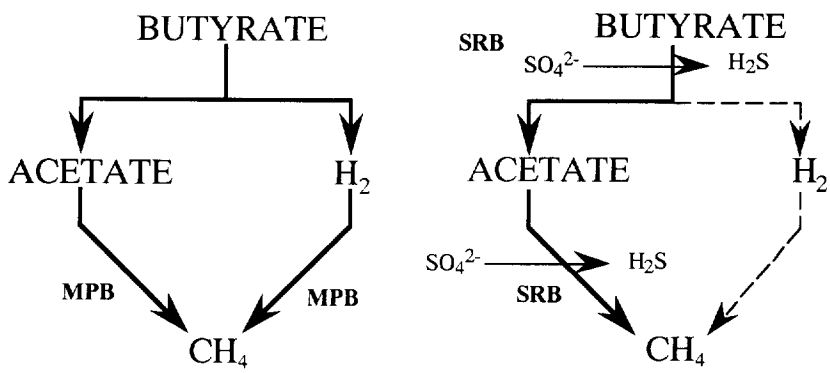
Change in the degradation pathway of butyrate with COD/S ratio. It has been reported that the methane fermentation from butyrate is carried out by two metabolic groups of bacteria (McInerney et al, 1981). Firstly, hydrogen-producing acetogenic bacteria (HPAB), such as *Syntrophomonas wolfei*, *S. sapovarans* and *Syntrophopara bryantii* , degrade butyrate to acetate and hydrogen according to equation (1) shown in Table 6. The produced acetate and hydrogen are then converted into methane by the acetate- and hydrogen-consuming MPB according to the equations (3) and (5) shown in the Table 6. Since equation (1) is thermodynamically unfavorable, the interspecies-hydrogen transfer between hydrogen-producing acetogens and hydrogen consumer is considered to be very important for the degradation of butyrate.

TABLE 6 EQUATIONS AND FREE ENERGY CHANGES FOR REACTIONS IN ANAEROBIC DEGRADATION OF BUTYRATE

Substrate	Bacteria	Equation	Free energy change $\Delta G^{\circ\prime}$ (kJ/reaction)	Equation No.
Butyrate	HPAB	$Bu^- + 2H_2O \rightarrow 2Ac^- + 2H_2 + H^+$	+48.1	(1)
	SRB	$2Bu^- + SO_4^{2-} \rightarrow 4Ac^- + HS^- + H^+$	-55.7	(2)
Acetate	MPB	$Ac^- + H_2O \rightarrow CH_4 + HCO_3^-$	-28.0	(3)
	SRB	$Ac^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^-$	-47.6	(4)
Hydrogen	MPB	$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	-135.6	(5)
	SRB	$4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O$	-151.9	(6)

HPAB : Hydrogen producing acetogenic bacteria
SRB : Sulfate-reducing bacteria
MPB : Methane producing bacteria

In this study, both the population and the SMA of acetate consuming MPB and hydrogen-consuming MPB were high at the COD/S of 148. It is suggested that at the high COD/S ratio, butyrate was degraded into methane via acetate and hydrogen as shown in Figure 6 (a). On the other hand, at the low COD/S ratio (below 3), the populations of hydrogen-consuming SRB and MPB were low (Table 5). In addition, SMA and SUR for hydrogen were also at a low level. These results demostrated that hydrogen was not the important intermediate of degradation of butyrate at low COD/S ratio. So, it is considered that the butyrate-consuming SRB, which was enumerated in the high population level of 10^6 to 10^7 MPN/ml, can outcompete HPAB for the substrate, as equation (2) has a thermodynamical advantage over equation (1) as shown in Table 6. As a result, butyrate was degraded by SRB according to the equation (2). Based on the bacterial population level and activity measurement, the competition between acetate-consuming SRB and MPB ocurred at low COD/S ratio. The degradation pathway in sulfate-rich condition can be summarized as Figure 6 (b).



(a) High COD/S ratio (b) Low COD/S ratio

Fig. 6. Effect of COD/S ratio on the degradation pathway of butyrate
→ : main reaction

The role of SRB in the anaerobic degradation of butyrate. In sulfate-rich condition (COD/S of 1.5), sulfate reduction was the predominant reaction in the reactor. It is considered that the butyrate-consuming SRB incompletely degraded butyrate according to the equation (2) shown in Table 6. However, in sulfate-depleted condition, the butyrate-consuming SRB were also found in the high population levels of 10^7 to 10^8 MPN/ml. These SRB did not reduce sulfate as there was no sulfate source in the substrate. In this case, the butyrate-consuming SRB played an important role in producing hydrogen and acetate from butyrate, as well as the hydrogen producing acetogenic bacteria. It has been reported that *Desulfovibrio* sp. can degrade lactate and ethanol with hydrogen-consuming MPB as hydrogen consumer in the absence of sulfate (Bryant et al, 1977). Chatrain and Zeikus (1986) also reported that lactate consuming SRB and ethanol consuming SRB can enumerated at high levels in sulfate-depleted conditions and they played an important role as hydrogen-producing acetogenic bacteria (Chartrain and Zeikus, 1986).

CONCLUSIONS

Based on the results and disscusion in the previous sections, the following conclusions could be drawn.

1. COD/S ratio in the substrate was an important parameter affecting the competition between SRB and MPB for butyrate. When COD/S ratio was 6.0 or more, methane production was the predominant reaction in the reactor, and over 80% of the total electron donor was utilized by MPB. However, at the COD/S ratio of 1.5, sulfate reduction became the predominant reaction, and over 50% of the total electron donor was utilized by SRB.

2. A large amount of sulfate reduction resulted in not only the decrease of methane production from substrate but also the rapid increase of bacterial growth. In some cases, methanogenesis was inhibited by the high concentration of total sulfide and free hydrogen sulfide produced.

3. The butyrate-consuming SRB was enumerated in high levels of 10^6-10^8 MPN/ml in all the reactors, even at the sulfate depleted condition (COD/S ratio of 148). It is considered that these butyrate-consuming SRB played an important role in producing acetate and hydrogen, as well as the hydrogen-consuming acetogenic bacteria at the low sulfate concentration.

4. The degradation pathway of butyrate and the composition of bacterial population in the reactor were dominated by the sulfate concentration and COD/S ratio. In sulfate-depleted condition, butyrate was degraded to methane via acetate and hydrogen by MPB. On the other hand, in sulfate-rich conditions (COD/S was lower than 1.5) butyrate was firstly converted into sulfide and acetate by SRB, and the produced acetate was then degraded by acetate-consuming MPB and SRB.

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