Pergamon

Wat. Sci. Tech. Vol. 38, No. 4–5, pp. 513–520, 1998.
IAWQ

0273–1223/98 $19·00 + 0·00

PII: S0273-1223(98)00552-6

BIOLOGICAL SULFATE REMOVAL IN AN ACIDOGENIC BIOREACTOR WITH AN ULTRAFILTRATION MEMBRANE SYSTEM

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ABSTRACT

The biological sulfate removal in the acidogenic bioreactor with an ultrafiltration membrane system was investigated at 35°C. Sucrose was used as the sole organic substrate. The sulfate concentration in the substrate ranged from 0 to 600mgS·l^{-1}. The chemostat reactor was operated to compare with the membrane bioreactor. The fouling phenomenon caused by FeS precipitate was observed at higher concentration of sulfate. However, it was possible to continuously operate the membrane bioreactor by cleaning the membrane. The efficiency of sulfate removal by sulfate reduction reached about 100% in the membrane bioreactor, and 55 to 87% of sulfide was removed from the permeate by the membrane filtration. The composition of the metabolite was remarkably changed by the change in sulfate concentration. When the sulfate concentration increased, acetate and 2-propanol significantly increased while *n*-butyrate and 3-pentanol decreased. The sulfate-reducing bacteria play the role as acetogenic bacteria consuming volatile fatty acids and alcohols as electron donors under sulfate-rich conditions. The results show that the acidogenensis and sulfate reduction simultaneously proceed in the membrane bioreactor.

KEYWORDS

Acidogenesis; membrane bioreactor; permeate flux; sulfate reduction; sulfate removal; sulfide removal

INTRODUCTION

Anaerobic treatment of industrial wastewater containing a significant amount of sulfate, such as molasses wastewater, has some problems causing the failure of the treatment process. Under anaerobic conditions, sulfate is utilized as an electron acceptor and reduced into hydrogen sulfide by sulfate-reducing bacteria (SRB) (Postgate, 1984). SRB can utilize various substrates, such as alcohols, fatty acids, dicarboxylic acids, and aromatic compounds (Hansen, 1992). As a result of sulfate reduction, a large amount of hydrogen sulfide is produced. It is well known that hydrogen sulfide causes significant damage to treatment facilities. Moreover, the inhibition of the growth of methane-producing bacteria (MPB) by high concentration sulfide will result in process failure. The toxicity of the various sulfide species to microorganisms may be different (Okabe *et al.*, 1992). In particular, free hydrogen sulfide (f-H_2S) is considered to be toxic to MPB and SRB because f-H_2S passes through the cell membrane (Reis *et al.*, 1992). Parkin *et al.* (1990) reported that a f-H_2S of 60-70 mgS·l^{-1} resulted in process failure in acetate- and propionate-fed chemostats. On the other hand, SRB will compete with MPB and H_2-producing acetogenic bacteria (HPAB) for compounds such as H_2, acetate, propionate and butyrate during anaerobic treatment of sulfate-containing wastewater. The competition between SRB and MPB for acetate and H_2 has been extensively investigated by several workers in both natural environments (lake sediments) and artificial systems (anaerobic reactors) (Winfrey and Zeikus, 1977; Abram and Nedwell, 1978; Schönheit *et al.*, 1982; Kristjansson *et al.*, 1982; Yoda *et al.*, 1987; Parkin *et al.*, 1990). According to their results, SRB effectively compete with MPB for both substrates, and the predominance of SRB will cause a significant decrease in methane production (Szewzyk and Pfennig, 1990; Uberoi and Bhattacharya, 1995; Maillacheruvu *et al.*, 1996; Li *et al.*, 1996). The mechanisms of complex interaction among SRB, MPB and HPAB has been suggested in recent studies. In particular, the competition between SRB and MPB has been investigated by many researchers. Therefore, it is indispensebly important for the successful treatment of sulfate-rich wastewater to elucidate the mechanism of the interaction among SRB, MPB and HPAB, and the toxicity of hydrogen sulfide to anaerobic bacteria.

From an engineering point of view, some previous studies have shown the improved processes for the anaerobic treatment of sulfate-rich wastewater (Buisman *et al.*, 1990; Buismann and Lettinga, 1990). Reis *et al.* (1988) showed the application of the two-phase anaerobic process to molasses wastewater. The two-phase anaerobic process has some advantages:(a) sulfate removal by sulfate reduction in acidogenic phase, (b) removal of sulfide as FeS precipitate before the methanogenic phase, (c) separation of sulfate reduction from the methanogenic phase. The sulfate removal in the acidogenic phase is very important for treating the sulfate-rich wastewater by the two-phase anaerobic process. However the complete separation of sulfate reduction from the methanogenic phase has not been reported in the previous studies.

The objectives of this study is to investigate the sulfate and sulfide removals and the characteristics of sucrose degradation in the acidogenic bioreactor with the ultrafiltration membrane system, and to suggest on the feasibility of using a membrane bioreactor for the treatment of sulfate-rich wastewater.

MATERIALS AND METHODS

Seed sludge

The seed sludge was collected from the primary settling tank in the municipal sewage treatment plant located in Sendai, Japan. The inoculum was acclimatized to the synthetic substrate containing sucrose as the sole carbon source for six months in the anaerobic continuous culture at 35°C.

Membrane bioreactor

The experimental apparatus is shown in Figure 1. The ultrafiltration membrane unit used was a Toso cassette system (Toso, UF-LMD II, Japan) with polysulfone flat membranes with a nominal molecular weight cutoff of 3.0 x 10^6 daltons. The total membrane surface area was 200 cm^2. The fluid velocity and pressure were maintained at 1.0 $m \cdot s^{-1}$ and 100 kPa, respectively. The total volume of culture in the membrane bioreactor system was 760 mL. The culture was completely stirred by a magnetic stirrer. The culture was drawn by a peristaltic pump (Masterflex model PA-26A) via a submerged glass tube. After passage across the ultrafiltration membrane, the culture was returned to the bioreactor via a submerged glass inlet in order to reduce foaming within the bioreactor. The culture inlet pressure was monitored by a pressure gauge. The rates of feed medium addition and permeate removal were manually set to be equal. The volume of permeate was measured by collecting samples over timed intervals via the filtrate sampling point and was set to predetermined values by manipulation of the time setting of the filtrate pump. When the permeate decreased, the surface of the membrane was cleaned by recirculating the solution of sodium hydroxide and phosphoric acid. In order to measure the volume of biogas produced, the bioreactor was connected to a biogas collection cylinder placed in an acidic saturated salt solution of NaCl with 2% sulfuric acid. The membrane bioreactor was installed in a temperature-controlled chamber maintained at 35°C. The synthetic substrate was prepared daily and stored in a substrate reservoir maintained at 4°C. The substrate was continuously injected into the bioreactor with a peristaltic pump. The sludge retention time (SRT) was maintained at five days and controlled by a peristaltic pump. The hydraulic retention time (HRT) was maintained at twenty-four hours and controlled by measuring the volumes of permeate and drain. On the other hand, the chemostat reactor described by Mizuno *et al.* (1994) was operated at an HRT of 24 h in comparison with the membrane bioreactor. The membrane bioreactor was continuously operated for each sulfate concentration. When the removal efficiencies of sucrose and sulfate and the biogas production rate were constant for more than one month, the operation of the membrane bioreactor was regarded as in a quasi steady-state condition.

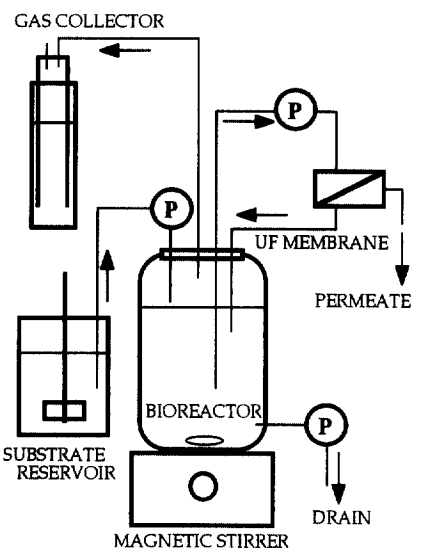

Fig. 1. Experimental apparatus of the membranebioreactor

Substrate

The membrane bioreactor and the chemostat reactor were fed with a medium containing (per liter) the following: NH_3HCO_3 , 1900 mg; K_2HPO_4, 125 mg; $MgCl_2 \cdot 6H_2O$, 60 mg; $FeSO_4 \cdot 7H_2O$, 13 mg; $FeCl_2 \cdot 4H_2O$, 180 mg; $Na_2CO_3 \cdot 10H_2O$, 1800 mg; $CoCl_2 \cdot 6H_2O$, 1.3 mg; $MnCl_2 \cdot 4H_2O$, 1.3 mg; KI, 1.3 mg; $Na_2MoO_4 \cdot 2H_2O$, 0.3 mg; H_3BO_4 ,0.3 mg; $NiCl_2 \cdot 6H_2O$, 0.3 mg; $ZnCl_2$, 0.3 mg. Sucrose (Wako Tokyo, Japan) was used as the organic substrate at a concentration of 5,000 mgCOD$\cdot l^{-1}$, and sodium sulfate was used as the sulfate source. Sulfate concentration in the substrate was changend between 0 (control) and 600 mgS$\cdot l^{-1}$. A trace sulfur source for the growth of miccroorganisms was added as $FeSO_4 \cdot 7H_2O$ into the substrate under control condition.

Analytical methods

Sulfate concentration was analyzed with an ion chromatograph (Shimadzu CDD-6A) equipped with a Shim-pack IC-A3 column with a guard precolumn; the column temperature was 40°C, the mobile phase was 8 mM *p*-aminobenzoic acid + 3.2 mM Bis-tris, and the flow rate was 1.2 ml/min. Methane, carbon dioxide and hydrogen were measured with a gas chromatograph (Shimadzu GC-8A) equipped with a thermal conductivity detector and a 1.5-m stainless column filled with activated carbon. The temperatures of the injection port and column were 140°C and 120°C, respectively. Helium was used as the carrier gas at a pressure of 1.5 kg/cm^2 for the analysis of methane and carbon dioxide. For the analysis of hydrogen, nitrogen was used as the carrier gas at a pressure of 1.5 kg/cm^2. Total sulfide and dissolved sulfide were colorimetrically measured using the spectrophotometric methylene-blue method (APHA 1992). Free-hydrogen sulfide (f-H_2S) concentration was calculated from the pH value and the dissolved sulfide concentration (Isa *et al.*, 1986). For the analysis of hydrogen sulfide, the gas produced from the bioreactor 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-S was then determined by the spectrophotometric methylene-blue method (APHA 1992). Volatile fatty acids (VFAs) were determined with a gas chromatograph (Shimadzu GC-8A) equipped with a flame ionization detector and a 1.5-m (5-mm inside diameter) glass column filled with Greensorb. The temperatures at the injection port and column were 190°C and 170°C, respectively. Helium was used as the carrier gas at a pressure of 1.5 kg/cm^2, in addition, hydrogen and air were used at a pressure of 0.6 kg/cm^2. Alcohols were determined with a gas chromatograph (Shimadzu GC-8A) equipped with a flame ionization detector and a 2.5-m (5-mm inside diameter) glass column filled with Gaskuropack 54 (60/80). The temperatures at the injection port and column were 210°C and 180°C, respectively. Helium was used as the carrier gas at a pressure of 1.5 kg/cm^2, in addition, hydrogen and air were used at a pressure of 0.6 kg/cm^2. The sucrose concentration was colorimetically measured by the method described by Dubois *et al.* (1956). The MLVSS concentration was analyzed according to Standard Methods (APHA 1992).

RESULTS

Permeate flux during continuous operation

Figure 2 shows the time course of permeate flux during the continuous operation of the membrane bioreactor system. Fouling phenomenon was observed during operation. In particular, fouling frequently occurred at a sulfate of 200 mgS$\cdot l^{-1}$. At a sulfate of 0 mgS$\cdot l^{-1}$, no significant fouling was observed. When the permeate flux decreased to an unacceptable level, the surface of the membrane was cleaned by passing the solutions of NaOH and H_3PO_4. A thin layer of FeS was observed on the surface of the membrane. In spite of the high concentrations of sulfide, it was possible to continuously operate the membrane bioreactor by cleaning the membrane.

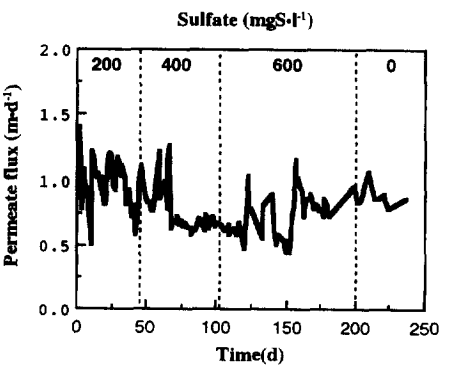

Fig.2. Permeate flux of membrane bioreactor

Sulfate removal and sulfide concentration

Table 1 summarizes the performance of the membrane bioreactor compared with that of the chemostat reactor. The MLVSS in the membrane bioreactor was two-to-six fold higher than that in the

Table 1. Comparison of performance between membrane bioreactor and chemostat reactor

Sulfate ($mgS \cdot l^{-1}$)		pH	MLVSS ($mg \cdot l^{-1}$)	Sucrose removal (%)	Sulfate removal (%)	Sulfide ($mgS \cdot l^{-1}$)		
						TS	DS	f-H_2S
0	MB	6.4	2750	100	100	105	3.8	2.8
	Chemostat	5.9	1300	100	100	ND	ND	ND
200	MB	6.3	2810	100	99.8	86.5	31.1	24.0
	Chemostat	6.4	903	99.2	70.6	64.5	52.5	38.2
400	MB	6.8	4460	100	99.9	132	48.3	24.9
	Chemostat	6.7	682	99.2	85.7	113	106	60.6
600	MB	7.0	4350	100	92.3	386	150	84.9
	Chemostat	6.6	825	93.9	74.7	260	110	69.8

MB: membrane bioreactor, TS: total sulfide, DS: dissolved sulfide, ND: not detectable

chemostat reactor. Sucrose was almost removed both in the membrane and chemostat reactors. However, the sulfate removal in the chemostat reactor was lower than that in the membrane bioreactor. The sulfide concetration in the membrane bioreactor was higher than that in chemostat reactor. In particular, f-H_2S increased up to 85 $mgS \cdot l^{-1}$ in the membrane bioreactor. Sulfide was also detected at a sulfate of 0 $mgS \cdot l^{-1}$ in the membrane bioreactor. Figure 3 shows the sulfide removal by the membrane system. The sulfide removal was in the range of 55 to 87%. The f-H_2S concentration in the permeate increased with increasing sulfate concentration. At a sulfate of 600 $mgS \cdot l^{-1}$, f-H_2S increased up to 61 $mgS \cdot l^{-1}$.

Effect of sulfate reduction on the biogas production

Figure 4 shows the effect of sulfate reduction on the biogas production rate and the biogas composition of the membrane bioreactor. The biogas was composed of carbon dioxide, methane and hydrogen sulfide. Hydrogen was not detected during the experiment. The total gas production rate drastically decreased with increasing sulfate concentration. The total gas production rate was reduced to 32% at a sulfate of 400 $mgS \cdot l^{-1}$. Forty-two percent methane at a sulfate of 0 $mgS \cdot l^{-1}$ significantly dropped to the undetectable level at a sulfate of 600 $mgS \cdot l^{-1}$. Although the high concentration of biomass was maintained by the ultrafiltration membrane system, the methane production was completely inhibited. On the other hand, hydrogen sulfide was increased up to 2.8% at a sulfate of 600 $mgS \cdot l^{-1}$.

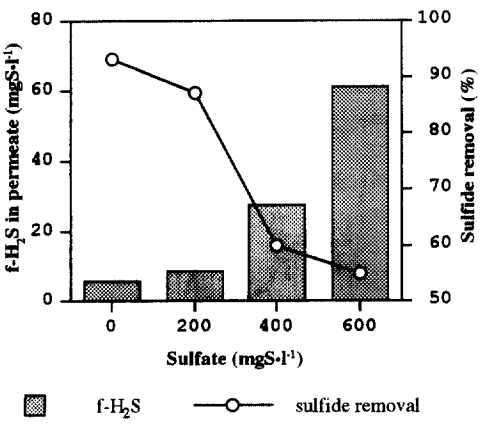

Fig. 3. Sulfide removal by membrane and f-H_2S in pearmeate

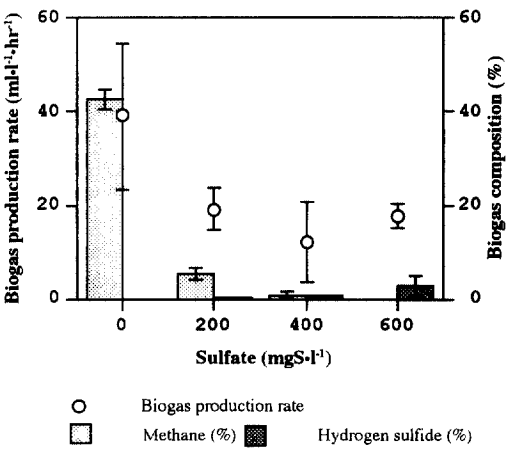

Fig. 4. Effect of sulfate reduction on the biogas production rate and the biogas composition

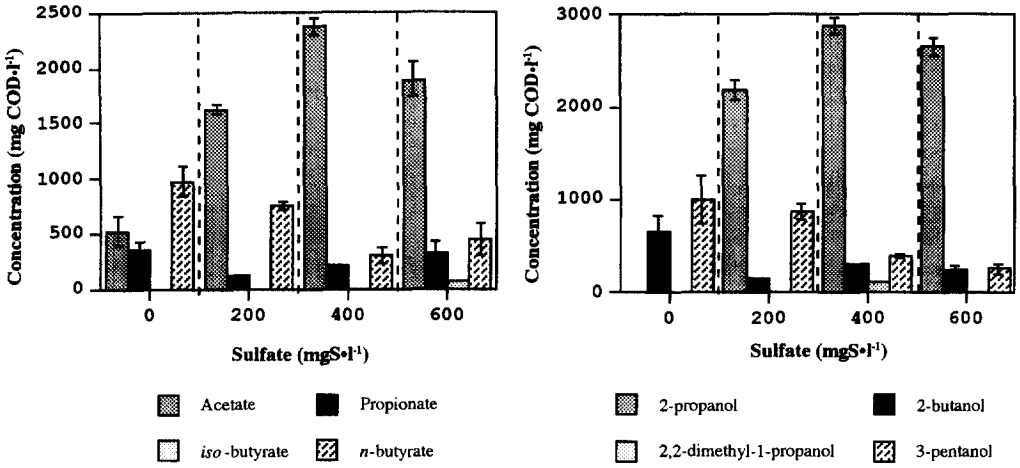

Fig. 5. The effect of sulfate reduction on the composition of metabolites

Effect of sulfate reduction on the composition of metabolites

Figure 5 shows the effect of the sulfate reduction on the composition of metabolites. Acetate, propionate and *n*-butyrate were the main products as volatile fatty acid. Valerate and capronate were not detected during the experiment. 2-propanol, 2-butanol and 3-pentanol were the main products as alcohols and ethanol was not detected during the experiment. The concentrations of acetate and 2-propanol dramatically increased with increasing sulfate concentration, while the concentrations of *n*-butyrate and 3-pentanol decreased. Acetate, which was an important electron donor for MPB and SRB, was accumulated under sulfate-rich condition.

DISCUSSION

The focus of our research was to develop an alternative to the conventional process for treating sulfate-rich wastewater. To accomplish our objective, we investigated the use of an acidogenic bioreactor with an ultrafiltration membrane system.

Performance of the membrane bioreactor

In general, the membrane and membrane modules are subjected to fouling during the continuous operation. Fouling can be caused by any species which interacts chemically or phisically with the membrane. In the bioreactor treating various wastewaters, organic, inorganic and biological metabolites will cause the fouling phenomenon. In this study, the fouling was probably due to the production of FeS precipitation, which was not removed by the scouring action of this cross-flow operation. When the permeate flux decreased to an unacceptable level, a black layer was observed on the surface of the membrane. The permeate flux could be easily regenerated by cleaning with solutions of NaOH and H_3PO_4, suggesting that the fouling was the main factor decreasing permeate flux. The sulfate removal of the membrane bioreactor was greater than that of the chemostat reactor at higher concentrations of sulfate because the biomass in the membrane bioreactor was two-to-six fold higher than that in the chemostat reactor. Sulfate was almost removed by sulfate reduction while the sulfide removal by the membrane was in the range of 55 to 87 %. It was suggested that the membrane bioreactor has more advantage which removes sulfide from the permeate. Molinari (1995) reported that the sulfide recyclable was 55 to 60% on the tannery process with the ultrafiltration membrane system. However, soluble sulfide (H_2S, HS^-) passed through the membrane was contained in permeate suggesting that the inhibition of methane production may be caused in the methanogenic reactor of the two-phase process. Therefore, it is important to remove the sulfide by the membrane in the acidogenic phase. The result indicates that the membrane bioreactor functions so as to maintain of high concentration biomass, remove sulfate by SRB and remove sulfide from the permeate by the membrane system.

Sulfide inhibition

Okabe *et al.* (1992) reported that sulfide inhibition of SRB probably occured when sulfide species (f-H_2S, HS^-, and S^{2-}) combine with the iron of the cytochrome and other essential iron-containing compounds in the cell, causing the electron transport systems to cease activity. In particular, f-H_2S has been thought to be the major toxic form of sulfide because f-H_2S can pass through the cell membrane (Reis *et al.*, 1992). On the other hand, f-H_2S was also an inhibitor for MPB (Parkin *et al.*, 1990). Therefore, the pH of the system, which determines the distribution of sulfide species, would be a key factor in the inhibition of sulfate reduction and methane production. In this study, the f-H_2S of 85 mgS•l^{-1} at a sulfate of 600 mgS•l^{-1} was higher than that of 65 mgS•l^{-1} which reportedly inhibited acetoclastic methanogenesis (Parkin *et al.*, 1990). At higher concentrations of f-H_2S, The significant accumulation of acetate suggested the inhibition of acetate-consuming MPB. Methanogenesis from $H_2 + CO_2$ would be inhibited by f-H_2S because methane cannot be detectable in the gas phase. On the other hand, no significant inhibition was observed on sulfate removal while other organic matters, except for acetate, would be consumed as electron donors of sulfate reduction. Reis *et al.* (1992) reported that the growth inhibition of SRB occurred at an H_2S of 547 mgS•l^{-1}. McCartney and Oleszkiewicz (1991) reported the complete inhibition of sulfate reduction by f-H_2S of 230 mgS•l^{-1}. In this study, therefore, no inhibition of sulfate reduction was caused by f-H_2S. The effect of f-H_2S on the acidogenesis was little known in previous studies. However, in order to develop the process where acidogenesis and sulfate reduction proceed simultaneously, it is important to evaluate the sulfide inhibition of acidogenesis. Hilton and Oleszkiewicz (1988) reported that almost 90% of lactose was utilized at a f-H_2S in excess of 900 mgS•l^{-1} at a retention time of 228 h. Shin *et al.* (1992) reported that glucose utilization was retarded when the sulfide was over 800 mgS•l^{-1}. No inhibition of sucrose utilization was observed at an f-H_2S of 85 mgS•l^{-1} in this study. The sensitivities of SRB and acidogens to f-H_2S would much lower than that of MPB to f-H_2S. The results show that acidogenesis and sulfate reduction will simultaneously be proceeded in the membrane bioreactor. On the other hand, MPB is very sensitive to f-H_2S, suggesting that methanogensis would be excluded from the membrane bioreactor in spite of a long SRT supporting the growth of MPB.

Effect of sulfate reduction on substrate degradation

In general, the role of SRB on the anaerobic ecosystem can be described as follows:(1) acetate consumer, (2) hydrogen consumer , (3) sulfate-reducing acetogen and (4) nonsulfate-reducing acetogen. However, it has not been reported that SRB directly utilizes sucrose as an electron donor. The production of molecular hydrogen is one of the characteristics of acidogenesis (Zoetemeyer *et al.*, 1982). Under sulfate-rich conditions, hydrogen would be the main electron donor of SRB at a short HRT of 2-to-10 h in the acidogenic reactor (Mizuno *et al.*, 1998). Therefore, hydrogen cannnot be detected in the gas phase, suggesting that hydrogen produced from acidogenesis was completely consumed as an electron donor of sulfate reduction. Acetate is an more important substrate for MPB because it is the precursor of about 70% of the methane production and is also the main electron donor for sulfate reduction (Mah *et al.*, 1978; Laanbroek and Pfennig, 1981). The ability of SRB to out-compete MPB for acetate was reported (Schönheit, 1982). However, the study on the bioreactors showed that MPB was a good competitor for SRB (Yoda *et al.*, 1987; Parkin *et al.*, 1990). In this study, methane in the gas phase was 42% at a sulfate of 0 mgS•l^{-1} indicating that acetate and hydrogen would be converted to methane by MPB. However, the methane percent drastically dropped to an undetectable level under sulfate-rich conditions, while acetate was significantly accumulated. It was indicated that the growth inhibition of the acetate-consuming MPB was caused by the high concentration of f-H_2S. In addition, the accumulation of acetate was observed in spite of the under sulfate-rich conditions, suggesting that acetate was not a main electron donor for sulfate reduction in the membrane bioreactor. Therefore, it is assumed that SRB will out-compete AB because of their better growth kinetic properties. Weigant *et al.* (1986) have shown that syntrophic propionate utilizers have extremely low growth rates. Maillacheruvu *et al.* (1996) reported propionate fermenters were very sensitive to sulfide toxicity. Therefore, it is suggested that the syntrophic oxidizers cannot utilize organic matters in spite of the presence of hydrogen-consuming SRB. However, syntrophic oxidation has less advantage compared with acetogenesis by SRB because *n*-butyrate and 3-pentanol were higher than that under sulfate-rich conditions. It is well known that SRB function as incompletely oxidizing SRB converting VFAs and alcohols to acetate under sulfate-rich conditions. In addition, SRB has a significant thermodynamic advantage over the syntrophic oxidizers.

From the results presented here, the substrate degradation pathway can be proposed in Figure 6. Under sulfate-rich conditions, hydrogen produced from acidogenesis was completely utilized by SRB, and then volatile fatty acids and alcohols were converted into acetate by incompletely oxidizing SRB. Several studies have shown that SRB play the

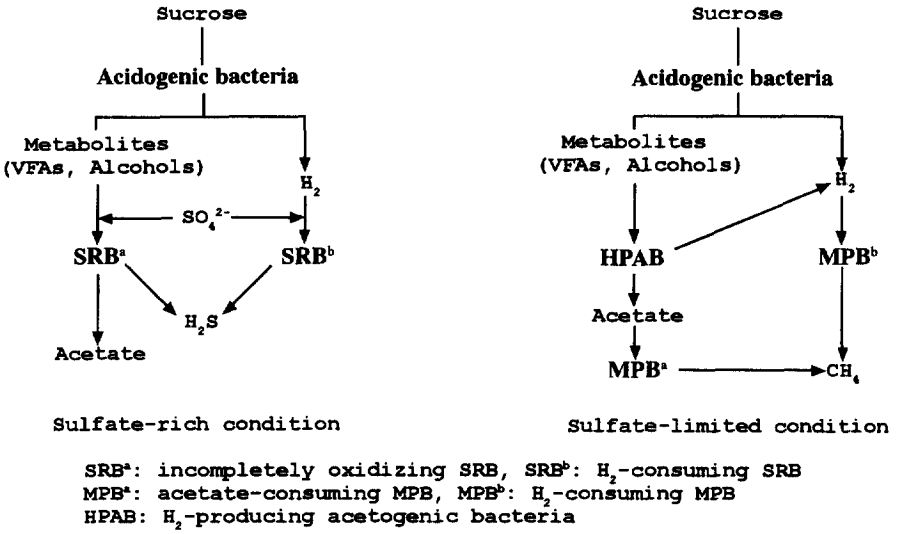

Fig. 6. The effect of sulfate reduction on the pathway of sucrose degradation

role of sulfate-reducing acetogen (Wu *et al.*, 1991; Visser *et al.*, 1993; Mizuno *et al.*, 1994; Maillacheruvu *et al.*, 1996). On the other hand, the syntrophic oxidizers cannot contribute to the decomposition of organic matters, such as propionate, butyrate and alcohols. It is suggested that SRB play a role as hydrogen consumer and sulfate-reducing acetogen. Under sulfate-limited conditions, HPAB utilize organic matter with hydrogen-consuming MPB as hydrogen scavenger, and then acetate would be consumed by acetate-consuming MPB. Hydrogen-consuming MPB would convert H_2 and CO_2 to CH_4.

From the engineering point of view, these results indicate that an acidogenic bioreactor with an ultrafiltration membrane system will operate as a sulfate removal reactor in which acidogenesis and sulfate reduction simultaneously proceed.

CONCLUSIONS

Based on the previous results and discussion, the following conclusions can be made:

1. The ultrafiltration membrane system plays as the three roles: (a) to maintain of a high concentration of biomass, (b) to improve the sulfate removal efficiency, (c) to remove sulfide from permeate.
2. In the membrane bioreactor, the sulfate removal (92%) at a sulfate of 600 $mgS \cdot l^{-1}$ was greater than that of the chemostat reactor (75%).
3. Acidogenesis and sulfate reduction simultaneously proceeded in the membrane bioreactor.
4. Decomposition of metabolites was observed under sulfate-reducing conditions. In particular, the concentrations of acetate and 2-propanol daramatically increased, suggesting that SRB play the role of incompletely oxidizing SRB.
5. Acetate was not a main electron donor for sulfate reduction.

ACKNOWLEDGEMENT

The authors wish to acknowledge the experimetal support of Mr. R. Serizawa, a graduate student in Department of Civil Engineering, Graduate School of Engineering, Tohoku University.

REFERENCES

APHA (1992). Standard Methods for the Examination of Water and Wastewater, 16th edn. American Public Health Association, Washington, D.C.

Abram J.W. and Nedwell D.B. (1978). Hydrogen as a substrate for methanogenesis and sulphate reduction in anaerobic saltmarsh sediment. *Arch. Microbiol.*, **117**, 93-97.

Buisman C.J., Wit B. and Lettinga G. (1990). Biotechnological sulphide removal in three polyurethane carrier reactor: stirred reactor, biorotor reactor and upflow reactor. *Water Res.*, **24**, 245-251.

Buisman C.J. and Lettinga G. (1990). Sulphide removal from anaerobic waste treatment effluent of a papermill. *Water Res.*, **24**, 313-319.

Dubois M., Gilles K.A., Hamilton J.K., Rebers P.A. and Smith F. (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, **28**, 350-356.

Hansen T.A. (1992). Carbon metabolism of sulfate-reducing bacteria. 21-40. In Odom J.M. and Singleton R., Jr. (editors) The sulfate-reducing bacteria: contemporary perspectives. Springer-Verlag.

Hilton B.L. and Oleszkiewicz J.A. (1988). Sulfide-induced inhibition of anaerobic digestion. *J. Environ. Eng.*, **114**, 1377-1391.

Isa Z., Grusenmeyer S. and Verstraete W. (1986). Sulfate reduction relative to methane production in high-rate anaerobic digestion: technical aspects. *Appl. Environ. Microbiol.*, **51**, 572-579.

Kristjansson J.K, Schonheit P. and Thauer R.K. (1982). Different Ks values for hydrogen of methanogenic bacteria and sulfate reducing bacteria: an explanation for the apparent inhibition of methanogenesis by sulfate. *Arch. Microbiol.*, **131**, 278-282.

Laanbroek and Pfennig N. (1981). Oxidation of short-chain fatty acids by sulfate-reducing bacteria in freshwater and in marin sediments. *Arch. Microbiol.*, **128**, 330-335.

Li Y.Y., Lam S. and Fang H.H.P. (1996). Interactions between methanogenic, sulfate-reducing and syntrophic acetogenic bacteria in the anaerobic degradation of benzoate. *Water Res.*, **30**, 1555-1562.

Mah R.A., Smith M.R. and Baresi L. (1978). Studies on acetate-fermenting strain of *Methanosarcina*. *Appl. Environ. Microbiol.*, **35**, 1174-1184.

Maillacheruvu K.Y. and Parkin G.F. (1996). Kinetics of growth, substrate utilization and sulfide toxicity for propionate, acetate, and hydrogen utilizers in anaerobic systems. *Wat. Environ. Res.*, **68**, 1099-1106.

McCartney D.M. and Oleszkiewicz J.A. (1991). Sulfide inhibition of anaerobic degradation of lactate and acetate. *Water Res.*, **25**, 203-209.

Mizuno O., Li Y. Y. and Noike T. (1994). Effects of sulfate concentration and sludge retention time on the interaction between methane production and sulfate reduction for butyrate. *Wat. Sci. Tech.*, **30**(8), 45-54.

Mizuno O., Li Y. Y. and Noike T. (1998). The behavior of sulfate-reducing bacteria in acidogenic phase of anaerobic digestion. *Water Res.*, **32**, 1626-1634.

Molinari R. (1995). Application of membrane separation techniques to the treatment of tanneries wastewaters. 101-122. In Caetano A., De Pinho M.N., Drioli E. and Muntau H. (editors) Membrane technology: applications to industrial wastewater treatment, Kluwer Academic Publishers, Netherlands.

Okabe S., Nielsen P.H. and Characklis W.G. (1992). Factors affecting microbial sulfate reduction by *Desulfovibrio desufuricans* in continuous culture: limiting nutrients and sulfide concentration. *Biotechnol. Bioeng.*, **40**, 725-734.

Parkin G.F., Lynch N.A., Kuo W.C., Van Keren E.L. and Bhattacharya S.K. (1990). Interaction between sulfate reducers and methanogens fed acetate and propionate. *Research J. WPCF.*, **62**, 780-788.

Postgate J.R. (1984). The sulphate reducing bacteria., Cambridge University Press, Cambridge, G.B.

Reis M.A.M., Gonçalves L.M.D. and Carrondo M.J.T. (1988). Sulfate reduction in acidogenic phase anaerobic digestion. *Wat. Sci. Tech.*, **20**(11/12), 345-351.

Reis M.A.M., Almeida J.S., Lemos P.C. and Carrondo J.T. (1992). Effect of hydrogen sulfide on growth of sulfate reducing bacteria. *Biotechnol. Bioeng.*, **40**, 593-600.

Schönheit P., Kristjansson K. and Thauer R.K. (1982). Kinetic mechanism for the ability of sulfate reducers to out-compete methanogens for acetate. *Arch. Microbiol.*, **132**, 285-288.

Szewzyk R. and Pfennig N. (1990). Competition for ethanol between sulfate-reducing and fermenting bacteria. *Arch. Microbiol.*, **153**, 470-477.

Uberoi V.U. and Bhattacharya S.K. (1995). Interaction among sulfate reducers, acetogens, and methanogens in anaerobic propionate systems. *Wat. Environ. Res.*, **67**, 330-339.

Visser A., Beeksma I., van der Zee F., Stams A.J.M. and Lettinga G. (1993). Anaerobic degradation of volatile fatty acids at different sulphate concentrations. *Appl. Microbiol. Biotechnol.*, **40**, 549-556.

Weigant W.M., Hennink M. and Lettinga G. (1986). Separation of the propionate degradation to improve the efficiency of thermophilic anaerobic treatment of acidified wastewaters. *Water Res.*, **20**, 517-524.

Winfrey M.R. and Zeikus J.G. (1977). Effect of sulfate on carbon and electron flow during microbial methanogenesis in freshwater sediments. *Appl. Environ. Microbiol.*, **33**, 275-281.

Wu W-M., Hickey R. F. and Zeikus, J. G. (1991). Characterization of metabolic performance of methanogenic granules treating brewery wastewater : role of sulfate-reducing bacteria. *Appl. Environ. Microbiol.*, **57**, 3438-3449.

Yoda M., Kitagawa M. and Miyagi Y. (1987). Long term competition between sulfate-reducing and methane-producing bacteria for acetate in anaerobic biofilm. *Water Res.*, **21**, 1547-1556.

Zoetemeyer R.J., van den Heuvel J.C. and Cohen A. (1982). pH influence of acidogenic dissimilation of glucose in an anaerobic digestor. *Water Res.*, **16**, 303-311.