(5) EFFECT OF SULFITE ON ANAEROBIC TREATMENT OF EXCESS ACTIVATED SLUDGE
濃縮余剰汚泥の嫌気性消化処理における亜硫酸塩の効果

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ABSTRACT; The effects of sulfite or sulfate on anaerobic digestion of excess activated sludge were investigated at 37 ^{0}C. No inhibition effect was observed up to 4 mM when sulfite was added to the anaerobic digestion adding sulfite system. Biogas production, however, was inhibited by the accumulation of acetate when sulfite was added in the concentration of 8 mM. The optimum concentration of sulfite was 2 mM for the biogas production and 4 mM for the solubilization of VSS of the sludge. The activity of sulfate reducing bacteria was higher when sulfite was added than sulfate was used. In this study, it was confined that sulfite is effective for the solubilization of organic matter.

KEYWORDS: sulfite, sulfate reducing bacteria, solubilization, protein, carbon balance .

1. INTRODUCTION

The anaerobic digestion system has been widely applied to decompose organic pollutants and to produced methane as bioenergy. Sulfate reducing bacteria (SRB) and methane producing bacteria (MPB) have been known to contribute to the degradation of organic materials in water environment syntrophically[1-3]. These microorganisms have been known to be competitive for the available substrates such as volatile fatty acids (VFAs), and hydrogen in waste water and sludge treatment process. SRB utilize the sulfur compounds as electron aceptor and oxidize volatile fatty acids such as butyrate and propionate etc. to acetate[3]. The protein was contained about 50 - 60% in VSS of the excess activated suldge and the solubilization of protein is a reaction limiting step in the anaerobic digestion process[4].

Sulfate reducing bacteria prefer, thermodynamically, sulfite as an electron aceptor to sulfate. When sulfate reducing bacteria reduces sulfate, they need energy such as ATP. The free energies ($G^{0'}$) for the reaction of sulfite, sulfate and elemental sulfur to sulfide were known to be -172,-151 and -28 KJ per reduction, respectively[5-6]. This may means that sulfite is more favorable for SRB than sulfate and elemental sulfur.

On the other hand, sulfitolysis has been frequently used for the cleavage of disulfide bonds in protein [7-8] and applied to the food industry such as the solubilization of protein in corn starch [9]. It postulates that sulfite ion reacts with the disulfide bond of protein selectivelly and then they must be converted to low level compound such as VFAs. Also, the sulfur compounds might be could be improved by

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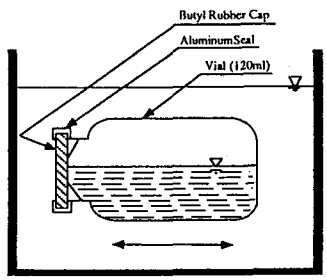
reduced to sulfide by SRB under the anaerobic condition[10]. The efficiency of anaerobic treatment controling the solubilization step, activity of SRB with inorganic sulfur compounds. Therefore it is possible that the solubilization step of protein can be accelerated by the addition of sulfite and it is considerable that both activities of SRB and MPB could be increased. Many studies on anaerobic digestion using sulfate have been reported, but the studies on the effect of sulfite on anaerobic treatment of excess activated sludge were very few. This study was examined the effect of sulfite and sulfate on the decomposition of organic compounds and investigation the competitive relationship between SRB and MPB with respect to the sulfite and sulfate adding system.

2.MATERIALS AND METHODS

2.1 Experimental appratus and process A schematic diagram of the anaerobic batch reactor used in this study is shown in fig. 1. The batch test was performed in 120 ml of vial with working volumes of 80 ml. The vials were operated in a shaking incubator (reciprocating rate: 3.5 x 120 strokes/ min.) at 37 ^{0}C. The experiment started by transfering 40 ml of seed sludge from the acclimation digester into 120 ml of vials in which gas was substituded with nitrogen. The excess activated sludge was added as the substrates which volume was 50 % of the total working volume. Sodium sulfite and sodium sulfate solutions were injected using a syringe according to the intended concentration. The mixture in the viral were sampled every two days for analysis of the water quality and the incubation was conducted for 25 days to determine the effect of sulfite on anaerobic digestion and to compare it with the sulfate adding condition.



Reciprocal Shaker
(37°C. Reciprocation: 35 mm x 100 strokes /min)
Fig. 1. Experimental appratus

2.2 Experimental conditions, seeding materials and wastes

Seed sludge and excess activated sludge were obtained from the mesophilic sewage sludge digester in Shinjiko Toubu sewage treatment plant, Shimane prefecture in Japan. Seed sludge was acclimated under 2 mM of sulfite adding condition in anaerobic digester for more than 4 months at 37 ^{0}C in the laboratory.

Table 1. Physical and chemical characteristics of wastes and experimental conditions.

Materials	CODcr	BOD	TOC	protein	Lipids	Carbohydrate	VS	TS	VS/TS
EAS	38563	16200	15720	13395	8268	2369	25760	32983	0.78
ADS	18720	5212	4726	1873	1156	328	2572	8575	0.3

ADS : anaerobic digestion sludge , EAS: excess activated sludge unit; mg/l

Table 2. Experimental conditions

Run. No.	R-1	R-2	R-3	R-4	R-5	R-6	R-7	R-8	R-9
Con. of SO_3^{2-}	0	2	4	8	16	32			
SO_4^{2-}							2	4	8

unit; mM. Working volume: 80 ml/120ml of serum bottle.
pH was adjusted 7.0 (± 0.2) with HCl or NaOH solutions.
Ratio of ADS (anaerobic digested sludge) to EAS (excess activated sludge) was 50:50 of total working volume

The COD concentrations of seed sludge and excess activated sludge were 18700 mg/l and 38600 mg/l, respectively. The contents of protein, lipids and carbohydrates in the VSS of excess activated sluge were about 52 %, 32 % and 16%, respectively. The phisico- chemical characteristics of excess activated sludge and anaerobic digested sludge summarized in table 1. In this study, sulfite concentrations ranged from zero to 32 mM but the sulfate-S adding system ranged from zero to 8 mM using 1 M of anhydrate sodium sufite (Na_2SO_3) and anhydrate sodium sulfate (Na_2SO_4) standard solutions. Table 2 summarizes the experimental conditions.

2.3 Calculation of the percent electron flow by SRB and MPB

In the anaerobic digestion of media rich in sulfite and sulfate, the substrate electron (in terms of COD) are nomally partitioned between the SRB and MPB. The electron flow by the SRB can be calculated from the following equations which refered from Zaid isa et. al[12]:

(a) By the SRB

Sulfate reduction:

$$3CH_3COO^- + 4SO_3^{2-} \rightarrow 4H_2S + H^+ + 6CO_3^{2-} \quad (1)$$

$$CH_3COO^- + SO_4^{2-} \rightarrow HS^- + 2HCO_3^- \quad (2)$$

$$3H_2 + H_2SO_3 \rightarrow H_2S + 3H_2O \quad (3)$$

$$4H_2 + H_2SO_4 \rightarrow H_2S + 2H_2O \quad (4)$$

COD of the H_2S produced is given by: $H_2S + 2O_2 \rightarrow H_2SO_4$ (5)

From the stoichiometric equation, 1 mole of sulfite or sulfate reduced = 1 mole of H_2S produced, respectively = 2 mole of O_2 (Th-COD) = 64 g of COD. Electron flow by the SRB = moles of sulfite or sulfate S reduced x 64 g = A g.

(b) By the MPB

Methane production:

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (6)$$

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (7)$$

$$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O \quad (8)$$

The COD of the CH_4 produced is given by : $CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$ (9)

thus, 1 mole of CH_4 produced = 2 mole of COD = 64 g of COD. Electron flow by MPB = moles of CH_4 produced x 64 g = B g.

Therefore, percent electron flow by SRB = { A/(A+B)} x 100 (10)

percent electron flow by MPB = { B/(A+B)} x 100 (11)

2.4 Analysis methods

The gas volume was determined by the syringe method and gas production rate was calculated from this result. The methane, carbon dioxide and hydrogen produced were determined by gas chromatograph with thermal conductivity detector (detector; TCD, column; porapack type Q 2000 x ϕ 3 mm, Hitachi

model; 260-30, caria gas; argon). The components of VFAs such as acetate, propionate, n-butyrate, iso-butyrate, formate, pyruvate, valerate and so on were determined by high performance liquid chromatograph (column;Shimpack SCR-101 H, size; 300 mm x ϕ 7.9 mm , UV detector; SPD-6A, pump; LC-6A, Shimazu) using 100 % of $HClO_4$ solution. The samples for the analysis of VFAs were filtered through 2 um of the cellulose acetate filter.

The sulfur compounds such as sulfide (S^{2-}), thiosulfate ($S_2O_3^{2-}$) and sulfite (SO_3^{2-}) were analyzed by ion chromatogrph (UV detector; SPD-6A at 220 nm, column; TSKgel-IC-anion-PW, size; 50 mm x ϕ4.6 mm , pump; SPD-6AV Touso) using 2mM of K_2HPO_4 solution as the mobile phase. The sulfate concentration was determined by ion chromatograph (Shimpack IC-A3, column; TSK-gel IC- anion-PW (50 x ϕ4.6 mm, Shimazu) using 8.0 mM of p-hydroxibenzoic acid and 3.2 mM of bis-tris solution as the mobile phase. To measure the sulfur compounds in the solids, the samples were pretreated by magnesium nitrate oxidizing method[18].

The calculation of hydrogen partial pressure (Pa) was conducted in order to estimate the correlation with the decomposition of VFAs. The hydrogen partial pressure (Pa) was calculated by the partial pressure law of gas using experimental results such as gas volume, components of gas (%), temperature as fellow: Ratio of partial pressure = ratio of molecular numbers = ratio of molcular percentage = ratio of volume (components) and 1 atm is equivalent to 101.3 KPa.

The carbohydrate and lipids were determined by the anthrone method and Kates method, respectively. The protein was determined by the Bio-rad method using the bovine plasma albumin standard. The mixed liquor suspended solids (MLSS) was measured by weighting method [18]. The analysis of total organic carbon was conducted using TOC analyzer (Shimazu TOC-5000). The concentration of COD was analyzed by potasium dichromate method or calculated from TOC using the experimental COD/TOC ratio of each sample when necessary[19].

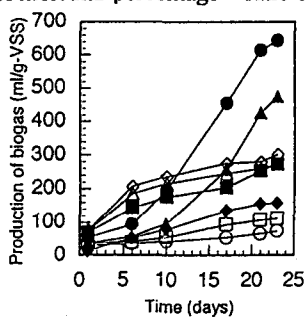


Fig.2. Time course of the biogas production under various adding conditions of sulfite or sulfate in anaerobic digester. sulfite:−■−; control,−●−; 2 mM, −▲−; 4 mM, −◆−; 8 mM, −□−; 16 mM, −○−; 32 mM and sulfate: −△−; 2 mM, −◇−; 4 mM.

3. RESULTS AND DISCUSSION

3.1 Biogas production

Fig. 2 shows the time course of the biogas production at various adding conditions of sulfite and sulfate for 25 days in the batch reactor. In the control system, with out sulfite addition, the biogas 250 ml/g- VSS was produced. In contrast to this, the production of biogas increased two or three fold in the sulfite adding system of 2 M and 4 mM compared with the control system for 25 days. When the sulfite concentration was over 8 mM, the production of biogas decreased and it was lower than that in the control system. These results illustrate that there is no inhibition effect for the methanogenic bacteria until 4 mM of sulfite added and the range of optimum dosage was 2 mM to 4 mM of sulfite. In the higher concentration of sulfite up to 8 mM, however, methane production was inhibited. On the other hand, methane production in the sulfate adding system was similar to the control system although it was slightly higher. Fig. 3 and 4 show the activity of MPB at various adding conditions

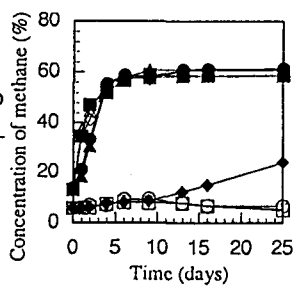


Fig. 3. Comparison of the activity of methane production under various adding conditions of sulfite or sulfate at 37℃. sulfite: −■−; control, −●−; 2 mM,−▲− ; 4 mM, −◆−; 8 mM , −□−; 16 mM, −○− 32 mM and sulfate: −△−; 2 mM, −◇−; 4 mM.

3.4 Effect of sulfite and sulfate concentration on SRB activity

Hydrogen sulfide is a very important ultimate products when SRB reduce sulfur compounds in anaerobic environment. Fig. 7 shows the comparison of the effect of sulfite and sulfate concentration on the activity of SRB in terms of hydrogen sulfide production under various adding conditions. The amount of hydrogen sulfide production was the highest at 4 mM as 38 μmole/ g-VSS in the sulfite adding system and in the sulfate adding system, it was at 8 mM as 24 μmole/ g-VSS. Their values were about 6 folds and 3.5 folds higher than the control system, respectively. The production of hydrogen sulfide was increased with increasing sulfite and sulfate and it was much higher in the sulfite adding system than in the sulfate adding system. This may means that the activity of SRB under conditions of sulfite was higher than that of sulfate[15].

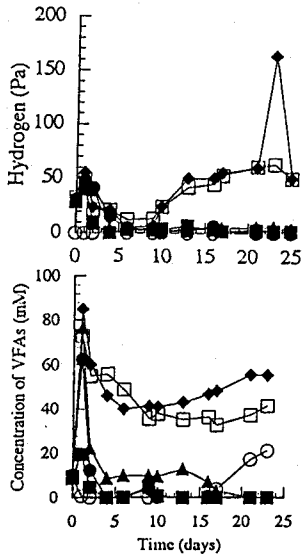


Fig. 6. Relationship between the degradation of VFAs and the hydrogen partial pressure (Pa) under various adding condition of sulfite during the anaerobic digestion. −■−; control, −●−; 2 mM, −▲−; 4 mM, −◆−; 8 mM, −□−; 16 mM, −○−; 32 mM.

3.5 Effect of sulfite concentration on the competitivity of the SRB and MPB

The results in table 3 and fig. 8 reveal that the percent electron flow by SRB at a low concentration of sulfite was much lower (in the control system, 3.3 %) compared with that of the high concentration of sulfite (in 8 mM of sulfite adding system, 82.5%). Simultaneously, the specific yield of methane was higher for the 2 mM and 4 mM sulfite adding systems (332 ml and 229 ml of CH_4 per g of COD removed, respectively) than that of the control system (105 ml of CH_4 per g of COD removed). The percent of sulfite reduction was maximum in the 4 mM sulfite adding system as 87.8 % but it was about 50% in the control system. This may means that almost of the final conversion step flows into the methanozation at low concentrations of the sulfite adding system, but at higher concentration, may SRB played the major role in degradation of organic matter and the activity SRB exhibited the tendency to decrease in sulfite concentration over 8 mM.

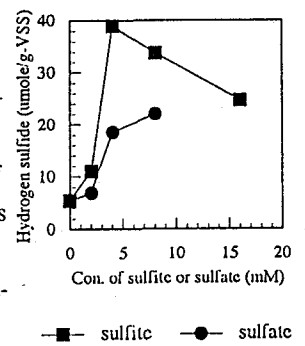


Fig. 7. Comparison of hydrogen sulfide production between the sulfite and sulfate system in batch experiment at 37°C. for 25 days.

3.6 Carbon balance

The determination of carbon balance was conducted by the measurement of initial TOC and final TOC of gas phase and liqueous phase in poresent study. Table 4 summarized the carbon balance at various conditions of sulfite added. The amount of TOC at input was average 68.5 + 0.5 mM. The amount of TOC in the gas phase ranged from 1.4 % to 23.4 % of total organic carbon. The gasification of organic matters was highest as 23.4% of total inputed total organic carbon at 2 mM of sulfite adding system. In contrst to this, much amount of total organic carbon about 26 ~ 32% of total organic carbon was remained in the reactor as VFAsunder the 8 and 16 mM of sulfite adding system. From this result it could considered that the addition of sulfite into anaerobic digestor was good for the solubilization of organic matter than gasification.

of sulfite or sulfate for 25 days. The methane production in control and sulfate systems reached almost 50% within 2 days and more than 55% after 4 days. The pattern of methane production indicated a lag phase till 4 days and methane production was increased more than 60% after 5 days in the sulfite adding condition. These results suggested that toxicity of sulfite for methanogenic bacteria became higher than sulfate than at the initial period of the experiment and MPB exhibited the inhibition effect in sulfite concentration over 8 mM.

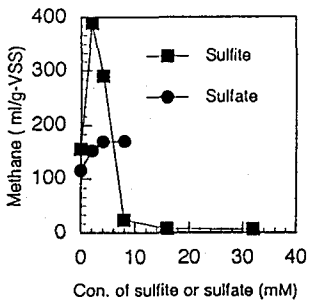


Fig. 4. Effect of sulfite or sulfate on methane production in anaerobic digestion of the excess activated sludge for 25 days at 37°C.

3.2 VFAs production and distribution

Fig. 5. shows the effect of sulfite or sulfate on VFA production and distribution when maximum concentration of VFA was reached at second days. Until 8 mM of sulfite added, the concentration of total VFAs increased with sulfite concentration and was higher than in control system. The components of VFAs in the control system were distributed in order of propionate, 11.5 mM (52.4 %) > acetate, 7.8 mM (35 %) > valerate, 2.3 mM (10.6 %) and small amounts of butyrate and formate. In contrast to this, the distribution was changed in the order of acetate, 40.2 mM (62 %) > formate, 13.7 mM (21.2 %) > propionate, 7.8 mM (12.1) and small amounts of butyrate and *iso*-valerate in the 2 mM of sulfite adding system. In sulfate adding system VFA concentration was low and were distributed similarly to the control system. It was obvious from founded results that solubilization step of organic matter could be faciliated by the addition of sulfite . This solubilization of organic matter may seem to be that the cleavage of disulfide bonds in protein by the addition of sulfite and then acid forming bacyeria or SRB contributed to the degradation of amino acid , to the production of propionate or acetate under anaerobic condition.

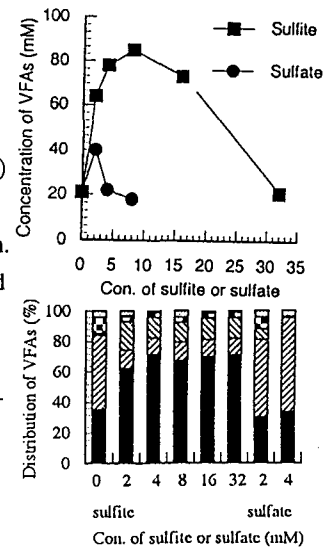


Fig. 5. Effect of sulfite or sulfate on VFA production and distribution of VFAs after 2 days of the incubation in batch experiment at 37°C.

3.3 Relationship between VFAs consumption and hydrgen partial pressure (Pa)

The time course of VFA consumption and hydrogen partial pressure in the sulfite adding system were shown in fig. 6. The hydrogen partial pressure had a tendency to increase with increasing total VFA concentration. At initial stages of the experiment, hydrogen partial pressure increased to more than 50 Pa and then was decreased to less than 20 Pa but in case of 8 mM and 16 mM of the sulfite added, hydro- gen patial pressure was increased again after 10 days of the incubation. At the same time, the total VFA concentration was also increased to more than 60 mM at the initial period of the experiment and then decreased significantly to less than 10 mM within 5 days. In case of 8 mM and 16 mM of the sulfite adding system, however, VFA concentration was decreased from about 80 mM to 40 mM within 8 days. After all, VFA was accumulated with time in higher concentration up to 8 mM as sulfite. Based on these results, it was obvious that hydrogen partial pressure has a correlation with VFA decomposition especially propionate to acetate, in this study. It could be considered that MPB utilyzation of acetate was inhibited by hydrogen partial pressure[11-14].

Table 3. Effect of sulfite on the competitivity of SRB and MPB.

Conditions added SO_3^{2-} (mM)	Reduced SO_3^{2-} (%)	Biogas production ($ml \cdot l^{-1} \cdot d^{-1}$)	composition of biogas (%)			Specific yield of methane	Electron flow (%)	
			CH_4	CO_2	H_2S	$ml\text{-}CH_4 \cdot g\text{-}COD_R^{-1}$	SRB	MPB
0	44.6	130	58	25	0.03	105	3.3	96.7
2	76.0	450	62	37	0.4	332	10.9	89.1
4	87.8	400	55	35	0.8	229	26.3	73.1
8	47.5	60	30	48	1.4	37.5	82.5	17.4
16	7.5	50	18	50	1.6	23.0	74.5	25.5

Notice: $ml\text{-}CH_4 \cdot g\text{-}COD^{-1}_{Removal}$

Table 4. Carbon balance

Conditions Added SO_3^{2-}	Input TOC				Output TOC							Ratio of Output / Input
					Gas phase			Liqueous phase				Loss	
	$VFAs_i$	VSS_i	$others_i$	TOC_i	CH_4	CO_2	G_O	$VFAs_O$	VSS_O	$others_O$	TOC_O		
0	4.60	53.45	10.1	68.15	6.22	0.97	7.2	<0.1	34.21	13.53	47.7	13.25	0.81
(%)	(6.75)	(78.4)	(14.8)	(100)	(9.1)	(1.4)	(11.2)	(0.15)	(50.2)	(19.8)	(69.9)	(19.4)	
2	4.32	53.12	11.4	68.81	13.3	2.88	16.2	<0.1	23.53	12.25	35.7	16.91	0.75
	(6.3)	(77.2)	(16.5)	(100)	(19.3)	(4.1)	(23.4)	(0.14)	(34.2)	(17.8)	(51.8)	(24.5)	
4	4.55	53.78	9.94	68.27	8.78	2.05	10.83	<0.1	19.4	21.56	40.9	16.54	0.77
	(6.7)	(78.9)	(14.6)	(100)	(12.9)	(3.0)	(15.9)	(0.14)	(28.4)	(31.5)	(59.9)	(24.2)	
8	4.38	53.55	10.6	68.49	0.98	0.58	1.56	17.75	24.1	9.51	51.4	15.53	0.77
	(6.4)	(78.2)	(15.4)	(100)	(1.4)	(0.8)	(2.2)	(25.9)	(35.1)	(13.9)	(75.0)	(22.6)	
16	4.72	53.65	10.5	68.82	0.48	0.49	0.97	22.16	30.58	2.32	59.8	8.05	0.88
	(6.8)	(77.9)	(15.9)	(100)	(0.7)	(0.7)	(1.4)	(32.2)	(44.4)	(3.4)	(86.9)	(11.7)	

Unit: mM. Carbon balance: Input C= Output C. Input = $VFAs_i$ + VSS_i + $Others_i$
Output= Gas phase (CH_4 , CO_2) + Liqueous phase ($VFAs_O$,VSS_O ,$Others_O$) + Loss

3.7 Decomposition characteristics of organic matter

The decomposition characteristics of organic matter was estimated in this study. The estimation of decomposition of organic matter was conducted by the measurement of TOC, COD, VSS concentration at the initial and final stage of the experiment. In order to investigate the effect of sulfite concentration on VSS degradation, the components of protein, lipids and carbohydrate were measured. Fig. 9 shows the comparsion of the decomposition efficiencies between the sulfite adding system and sulfate adding system. These result illustrate that the decomposition of VSS in the sulfite adding system was higher than that of control and sulfate adding system, but not so high in TOC and COD removal efficiencies. Fig. 10 shows that the decomposition of protein was increased with increasing sulfite concentrations and it was 2 times higher than lipids and carbohydrate. This shows that, addition of sulfite into the anaerobic digestion process is effective for the solubilization of organic matter.

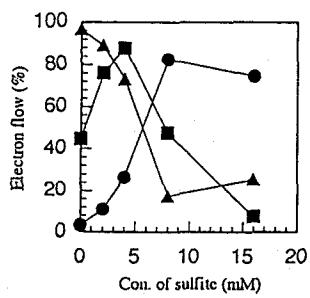


Fig. 8. Effect of sulfite concentration on the competitivity on SRB and MPB in the sulfite adding system at 37°C. for 25 day. —■—; sulfite reduced, —●—; electron flow by SRB, —▲—; electron flow by MPB.

4. CONCLUSION

In the anaerobic digestion of excess activated sludge with adding sulfite, no inhibition effect was observed up to 4 mM of sulfite added, but at higher concentration, the biogas production was in hibited by the accumulation of acetate. Addition of sulfite was effective for the solubilization of organic matter such protein and the optimum concentration of sulfite for the biogas production and the decomposition of VSS were

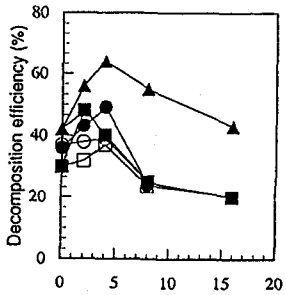


Fig. 9. Comparison of degradation efficiencies of organic matter between sulfite adding system and sulfate adding system under the anaerobic digestion of the excess activated sludge at 37°C. −■−; TOC-SO_3^{2-}, −●−; COD-SO_3^{2-}, −▲−; VSS-SO_3^{2-}.−□−; TOC-SO_4^{2-}, −○−; COD-SO_4^{2-}, −△−; VSS-SO_4^{2-}.

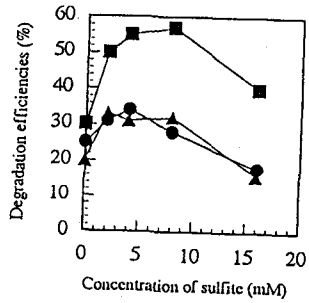


Fig. 10. Effect of sulfite concentration on degradation efficiencies of organic components in excess activate sludge under the anaerobic digestion at 37°C. −■−; protein, −●−;carbohydrate, −▲−; lipids.

both 8 mM. The activity of SRB in the sulfite adding system is higher than that of sulfate adding system and the activity of MPB was higher than that of SRB at sulfite concentrations lower than 8 mM.

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