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Journal of Hazardous Materials A87 (2001) 11–21

Journal of Hazardous Materials

www.elsevier.com/locate/jhazmat

Toxic effect of sulfur compounds on anaerobic biogranule

Chiu-Yue Lin*, Feng-Yung Chang, Chun-Hao Chang

Department of Hydraulic Engineering, Feng Chia University, Taichung 40724, Taiwan, ROC

Received 10 December 1999; received in revised form 26 March 2001; accepted 27 March 2001

Abstract

The effects of sulfide, sulfite and sulfate on degradation of volatile fatty acid (VFA) in UASB process have been studied by using serum bottle assay and septage–leachate acclimated biogranules. The relative toxicity of the compounds towards methane production and degradation of total VFA varied as $SO_4{}^{2-}$–S > S^{2-} > $SO_3{}^{2-}$–S and $SO_4{}^{2-}$–S > $SO_3{}^{2-}$–S > S^{2-}, respectively. The difference of this order shows the importance of choosing monitoring factor in evaluating the effect of sulfur compounds on a UASB system. For the individual VFA the effects of sulfur compounds depended on the types of VFA. The VFA-degrading activity of anaerobic biogranules was decreased by 50% when 34, 26 and 20 mg of S^{2-}, $SO_3{}^{2-}$–S and $SO_4{}^{2-}$–S were added to each gram of biomass, respectively. A comparison of the toxicity-resistance between two different anaerobic biogranules that acclimatized with septage–leachate mixture and septage was also made.

In the presence of the leachate, the toxicity-resistance of biogranules was not weakened to sulfide and sulfate but was enhanced to sulfite.

Keywords: Toxicity; Sulfide; Sulfite; Sulfate; Methanogenic activity

1. Introduction

An upflow anaerobic sludge blanket bed (UASB) process is a modern anaerobic system. In Taiwan, more than 30 UASB plants are in operation at present for treating industrial wastewater, landfill leachate and pig house wastewater. Landfill leachate contains high concentrations of refractory organics, which adversely affect the biological treatment plants in 230 landfill sites in Taiwan. Sanitary sewer systems serve only 3.5% of the population in Taiwan; in the non-sewer areas, more than 80% of the night soil is treated with septic tanks that produce thousands of tonnes (wet weight) of septage daily. Most of

* Corresponding author. Fax: +886-4-24519746.
E-mail address: cylin@fcu.edu.tw (C.-Y. Lin).

PII: S0304-3894(01)00262-X

the septage is inadequately treated, causing pollution problem islandwide. Therefore, it is proposed to use biological leachate treatment plants to treat the septage and leachate simultaneously.

Sulfur compounds such as sulfate, sulfite and sulfide are well found in some wastewaters discharged from tannen, pulp/paper, sea food processing, edible oil, fermentation and chemical industries. These sulfur compounds may inhibit the normal anaerobic microbes. Studying the effects of sulfur compounds on the UASB system has always been focused on the competition of sulfate-reducing bacteria with methane-producing bacteria [1–3] and little consideration has ever been given to the comparison of the compounds' toxicity. Recently, we have studied the toxicity effects of the sulfur compounds on the anaerobic sludge biogranule obtained from a UASB reactor fed on septage [4]. The leachate contains refractory organics and inorganics and its characteristics are quite different from that of a septage. To successfully perform the UASB systems co-treating septage and leachate, it is necessary to establish some database about the toxicity-resistance of the biogranules.

In this study, we used sulfate, sulfite and sulfide as the toxicants in methanogenic experiments to investigate the effects of these sulfur compounds on the anaerobic biogranule in degrading the volatile fatty acid (VFA). Specifically, the effect of these toxicants in the presence of the leachate was studied.

2. Materials and methods

2.1. Seeding biogranule

The seeding biogranule was acclimated in a laboratory-scale mesophilic (35°C) UASB reactor (internal diameter 14 cm, working volume 13.5 l) treating a mixture of septage and landfill leachate. The septage (the sludge produced in individual onsite wastewater-disposal systems) originated from septic tanks in Taichung City (central Taiwan), having a population of 940,000. The leachate was collected from Taichung City municipal refuse disposal site that was 3-year-old. The septage was settled for 24 h to concentrate the total solids (TS) to prepare the substrate mixture. Settled septage and leachate were mixed in a ratio of 2:1 on a chemical oxygen demand (COD) basis. The substrate mixture of septage and leachate had TS, alkalinity, total COD (TCOD), soluble COD (SCOD), total phosphorus (T-P), total Kjeldahl nitrogen (TKN) and ammonia nitrogen concentrations of 2700, 3700, 14,100, 8300, 55, 1250 and 850 mg/l, respectively. No extra nutrient was added to the substrate mixture. The hydraulic retention time (HRT) was 1.5 days and the acclimation period for the seed biogranule was 4 months. The pH, ORP (oxidation–reduction potential) and volatile suspended solids (VSS, to express the biomass) concentrations of the acclimated granular bed zone sludge averaged 7.0, −360 mV and 28 g/l, respectively. The average granule diameter of the seed biogranules was 0.80 mm. In this bed zone the concentrations of TS, VS, TCOD SCOD, T-P, TKN and NH_3-N were 48,400, 34,100, 47,600, 8300, 35, 900 and 550 mg/l, respectively. The COD recovery for the seed UASB system was 91.2% (CH_4 19.7, VSS 7.2 and effluent COD 64.3%).

2.2. Experiments

Experiments were performed in serum vials with 100 ml working volume. The vials were shaken at 35°C. Substrate, sulfur compounds and distilled water were added into the gassed (65% CO_2 + 35% N_2) vials. The contents were taken every 30 h via a syringe to determine the VFA concentrations. After sampling mixed liquor, for the purpose of measuring methane production, the total gas production and its composition were then measured. The gas volumes were corrected for water–vapor content, assuming vapor-saturated gas, and to standard temperature (0°C) and pressure (760 mmHg) (STP). Each experimental condition was prepared in triplicate. Other experimental details were the same as our previous study [5].

2.3. Analyses

The mixed liquors sampled were centrifuged (900 × g, 15 min) and the supernatants were taken for VFA analysis. VFA and gas composition were analyzed with a gas chromatograph having a flame ionization detector (glass column, 145°C; injection temperature, 175°C; carrier gas, N_2; packing, FON 10%) and a thermal conductivity detector (stainless column, 55°C; injection temperature, 90°C; carrier gas, Ar; packing, Porapak Q, mesh 80/100). Other water quality parameters were measured according the procedures of *Standard Methods* [6].

3. Results and discussion

Methane production and VFA degradation were employed to monitor the inhibition. Methanogenic activity (Am) and VFA degradation activity (Av) were defined to indicate the extent of inhibition of methane production and VFA degradation, respectively. Am and Av was defined as the percentages of methane produced and VFA degraded, respectively, in 30 h by sulfur-dosed seed biogranule against the control [5].

The sulfur concentrations that caused inhibition of the selected activity levels were determined for various sulfur compounds. The concentration at which a sulfur compound caused 50% inhibition (a 50% reduction in methane production over 30 h with sulfur dosage) was defined as C_{50} and used to compare the toxicity of the sulfur compounds. Moreover, the sulfur amount causing a 50% reduction in activity for a unit mass of biogranule was defined as SSL_{50} (specific sulfur loading). According to the present paper, the inhibited degree of methane production or VFA degradation was compared on the basis of C_{50} or SSL_{50} results. The degree was considered to be the same when the variation of these values was calculated to be less than ±5%.

The pH in the serum vials ranged 6.8–7.2 during incubation for all the tested sulfur compounds. Since most methanogenic bacteria activated in a pH range between 6.7 and 7.4. Under anaerobic environments, sulfate is reduced to sulfite and then to sulfide which may form insoluble metal sulfide to be precipitate. The cation concentrations (average, Fe^{3+} 13 mg/l; Mn, Ni, Cu, Zn, Pb $<$ 0.9 mg/l) were very low and their decreasing effect on the precipitation of the tested sulfur compounds was then neglected. Moreover, the seeding sludge is more resistant to the toxicant when it is acclimated with the toxicant. In this study, the mixture substrate used to acclimatize the seeding biogranule contained 240 mg S^{2-}–S/l, 13 mg $SO_3{}^{2-}$–S/l and 67 mg $SO_4{}^{2-}$–S/l. The sulfur concentrations described in this paper were the experimental dosages.

3.1. Effect on methane production

As an example showing the effect of sulfur compounds, Fig. 1 shows the time courses of methane production and sulfate degradation by the biogranule dosed with sulfate. The methane production reduced when sulfate concentration increased. Similar results were achieved for the tests on sulfide and sulfite (Figs. 2 and 3). Fig. 4 indicates that the methanogenic activity decreased with an increasing sulfur concentration. The biogranules' methanogenic activity reduced markedly when the sulfur concentrations were larger than 200 mg S/l. At these sulfur concentrations, the methanogenic activity reduced by 40–50%. Table 1 lists the experimental results for 50% inhibition of methanogenic activity (C_{M50}). These C_{M50} values were read from Fig. 4, which curves had been regressed non-linearly and their coefficients of relationship (R^2) were in the range of 0.97 (sulfite), 0.97 (sulfide) and 0.95 (sulfate). These C_{M50} and SSL_{M50} data of methanogenic activity ranged from 120 to 660 mg S/l and 5–25 mg S/g VSS, respectively The comparison either on a concentration or specific loading basis, indicates that the relative toxicity was $SO_4{}^{2-}$–S > S^{2-} > $SO_3{}^{2-}$–S to the methanogens. Sulfate was the most toxic sulfur tested. Table 1 also includes the C_{M50} and SSL_{M50} values of septage biogranules obtained in our laboratory [7]. The relative changes of C_{M50} and SSL_{M50} were calculated on the basis of septage biogranules'

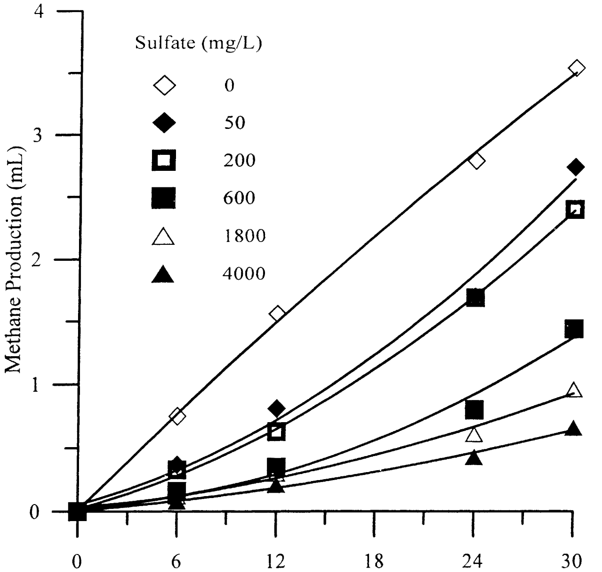

Fig. 1. The time course of methane production after dosing sulfate.

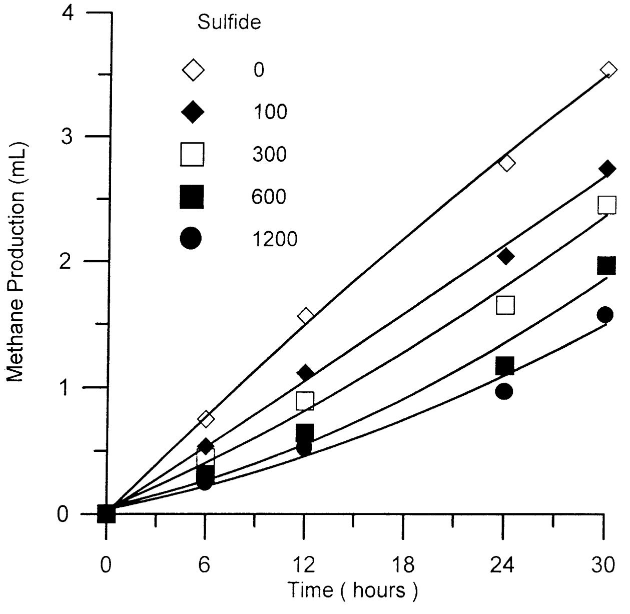

Fig. 2. The time course of methane production after dosing sulfide mg/L.

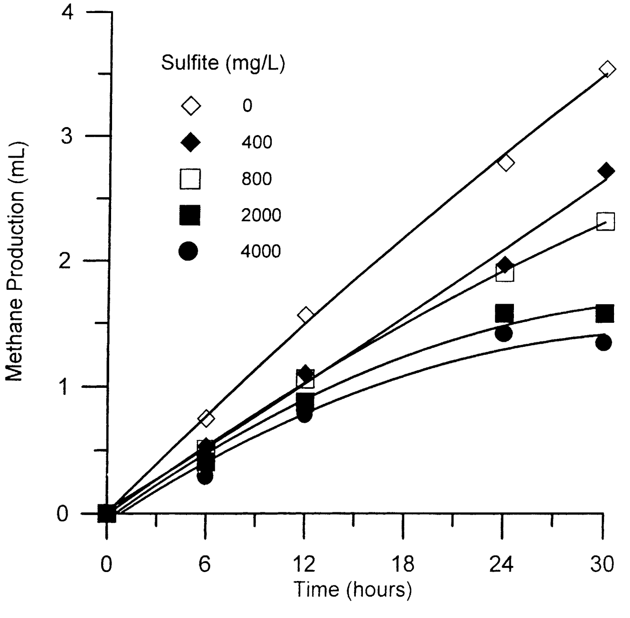

Fig. 3. The time course of methane production after dosing sulfite.

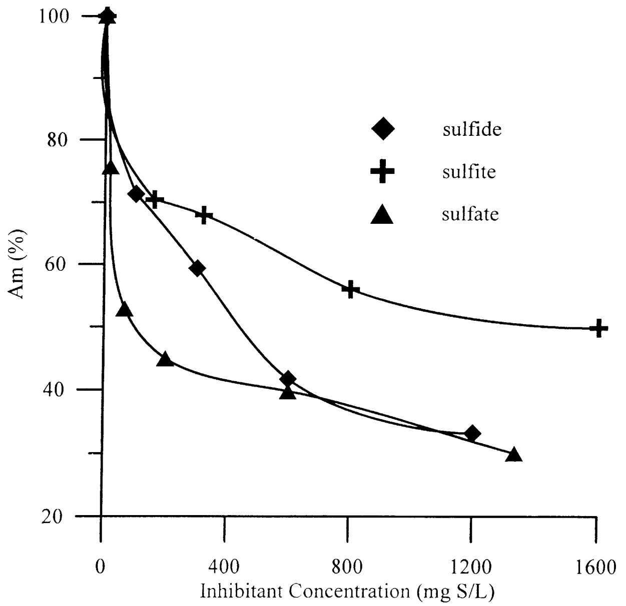

Fig. 4. The methanogenic activity (Am) at various dosed concentrations of sulfur compounds.

values of C_{M50} and SSL_{M50}, respectively. For each tested sulfur compound, consistent magnitudes of these values indicate the reliability of the toxic analysis. Negative values of relative change for sulfide and sulfate indicate that the toxicity of sulfide and sulfate was reduced in the presence of leachate. For sulfite, positive value of relative change showed that the septage–leachate biogranules had higher toxicity-resistance than that of septage biogranules.

3.2. Inhibition of degradation of total VFA

The activity of VFA degradation reduced with increased sulfur concentrations (Fig. 5). For the same activity level, there were different sulfur concentrations that caused inhibition. A similar result was also experienced in an acidogenic experiment [8]. Table 2 lists the experimental results for 50% inhibition of total VFA degradation read from Fig. 4. The reported 50% inhibition in the activity of TVFA degradation in the biogranules fed on septage [4] is also included in the table for comparison. In the present study, the sulfur concentrations that caused a 50% reduction in activity ranged from 530 to 900 mg S/l. These results indicate that the biogranules have different degrees of toxicity-resistance towards the tested sulfur compounds. Because of small variations in the relative change values, the sulfur concentrations that caused 50% inhibition on the degradation activities of the biogranules fed on septage and septage–leachate show that leachate did not affect the toxicity-resistance of the biogranules to sulfide and sulfite. However, a relative change value of +18–25% shows that the septage–leachate biogranules were more toxicity-resistant to

Table 1
Results for 50% inhibition of methanogenic activity (C_{M50}) and specific sulfur loading causing 50% inhibition on activity (SSL_{M50}) and the relative changes of C_{M50} and SSL_{M50} on the basis of septage biogranules' values

Sulfur	Septage–leachate biogranules[a]		Septage biogranules[b]		Relative changes of C_{M50} and SSL_{M50}	
	C_{M50} (mg S/l) (A)[c]	SSL_{M50} (mg S/g VSS) (B)[d]	C_{M50} (mg S/l) (C)	SSL_{M50} (mg S/g VSS) (D)	(A − C)/C (%)	(B − D)/D (%)
S^{2-}	470	18	580	21	−19	−14
SO_3^{2-}–S	660	25	580	21	+14	+19
SO_4^{2-}–S	120	5	280	10	−57	−50
Relative toxicity	SO_4^{2-}–S > S^{2-} > SO_3^{2-}–S		SO_4^{2-}–S > SO_3^{2-}–S ≒ S^{2-}			

[a] This study.
[b] [7].
[c] Sulfur concentration causing 50% inhibition on methanogenic activity.
[d] Specific sulfur loading causing 50% inhibition on methanogenic activity.

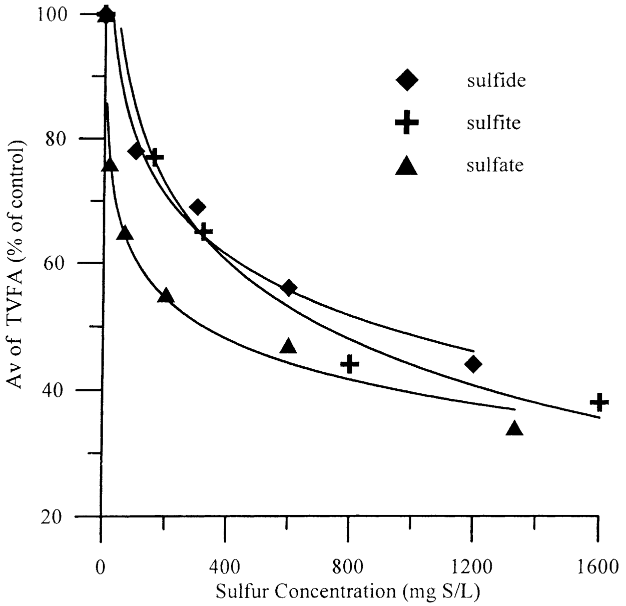

Fig. 5. The degradation activity (Av) of total volatile fatty acids (TVFA) at various dosed concentrations of sulfur compounds.

sulfate than the septage biogranules. This might result from the presence of non-sulfur refractory inorganics in the leachate which the microbes were acclimated. In the leachate, the concentrations of sulfide, sulfite and sulfate were 100, 8 and 15 mg S/l, respectively.

The type of these tested sulfur compounds varies under anaerobic environments. In this study, the degree of inhibition of TVFA degradation among these sulfur compounds was also discussed on the basis of C_{50}. The relative toxicity of sulfur compounds towards the biogranules is in the order SO_4^{2-}–S > SO_3^{2-}–S > S^{2-}. This result is different from that of the on methanogenic activity whose with the toxicity order SO_4^{2-}–S^{2-} > SO_3^{2-}–S. This fact suggests that inhibition of the methane production is not necessarily synonymous with inhibition of TVFA degreadation. Since the micro-organisms responsible for methane production differ from VFA degradation, the difference between these two relative toxicity orders reveals that some organisms within the biogranules might be more severely inhibited by the sulfur compounds than the methanogens. Similar results have been reported in a pulse addition of heavy metals to suspended sludge or granular anaerobic sludge [5,9,10]. The sulfate concentration causing 50% reduction in TVFA degradation is comparable to a reported value for treating a wastewater containing benzoate [11]. In an anaerobic process, the importance of the sulfate presence lies on the fact that sulfate-reducing bacteria play an unexpected role in process failure [12,13]. It has been reported that in a sulfate-reducing digester, 330 mg SO_4^{2-}–S/l of influent had only a sulfate removal of 92% and a COD removal of 62% [1].

TVFA-degrading granules are in stratified structure and contain acidogens and methanogens in different layers. For each of the tested sulfur compounds, same magnitudes of relative

Table 2
Results for 50% inhibition of total volatile fatty acid degradation (C_{A50}) and specific sulfur loading causing 50% inhibition on activity (SSL_{A50}) and the relative changes of C_{A50} and SSL_{A50} on the basis of septage biogranules' values

Sulfur	Septage–leachate biogranules[a]		Septage biogranules[b]		Relative changes of C_{A50} and SSL_{A50}	
	C_{A50} (mg S/l) (A)[c]	SSL_{A50} (mg S/g VSS) (B)[d]	C_{A50} (mg S/l) (C)	SSL_{A50} (mg S/g VSS) (D)	(A − C)/C (%)	(B − D)/D (%)
S^{2-}	900	34	900	32	±0	+6
SO_3^{2-}–S	680	26	660	24	+3	+8
SO_4^{2-}–S	530	20	450	16	±18	+25
Relative toxicity	SO_4^{2-}–S > SO_3^{2-}–S > S^{2-}		SO_4^{2-}–S > SO_3^{2-}–S > S^{2-}			

[a] This study.
[b] [4].
[c] Sulfur concentration causing 50% inhibition on VFA degradation activity.
[d] Specific sulfur loading causing 50% inhibition on VFA degradation activity.

Table 3
Results for 50% inhibition of volatile fatty acid degradation (mg S/l)

Sulfur	Acetic acid	Propionic acid	*iso*-Butyric acid	*n*-Butyric acid	Valeric acid	Caproic acid
S^{2-}	800	1040	800	920	240	260
$SO_3{}^{2-}$–S	660	700	660	660	290	280
$SO_4{}^{2-}$–S	450	430	450	750	120	400

change values (sulfide 0 to +6, sulfite +3 to +8, sulfate +18 to +25%) were obtained on the basis of C_{A50} and SSL_{A50}. This also strongly supports the reliability of toxic analysis. Moreover, the great differences between these relative change values obtained from methane production (Table 1) and TVFA degradation (Table 2) show the importance of choosing monitoring factor in evaluating the effect of sulfur compounds on a UASB system.

3.3. Inhibition of degradation of individual VFA

Degradation of individual VFAs elucidates that whether the VFA-degrading biogranules are inhibited or not by sulfur compounds. The time dependence of individual VFA degradation indicated that sulfur compounds affected the degradation of individual VFA with different degrees (Fig. 5). The biogranules' activities were generally decreased with increasing sulfur dosage, but different degrees of decrease were observed for various VFAs. The results for 50% inhibition of individual VFA degradation are summarized in Table 3. The magnitude of C_{50} (Table 3) for each individual VFA varied with the type of sulfur compounds and in the ranges of 120–1040 mg S/l. For the major intermediate products of anaerobic digestion, i.e. acetic (HAc), propionic (HPr) and butyric (HBu) acids, the C_{50} values of sulfur compounds ranged from 430 to 1040 mg S/l. Moreover, the magnitude of inhibition effect depended on the sulfur compound itself as well as on the individual VFAs. For example, for the degradations of Hac, HPr and *iso*-HBu, the relative toxicity of the tested sulfur compounds relative toxicity was in the order of $SO_4{}^{2-}$–S > $SO_3{}^{2-}$–S > S^{2-}, whereas the relative toxicity was in the order of $SO_3{}^{2-}$–S > $SO_4{}^{2-}$–S > S^{2-} for the degradation of normal HBu. Our previous study on the toxicity-resistance of the septage biogranules has shown a similar result [4].

The reduction of sulfate to sulfite and then to sulfide under anaerobic process and the complicated interaction among these compounds with the presence of leachate organics [14,15] make it difficult to elucidate the inhibitory mechanisms of the tested sulfur compounds. More further investigations will be needed to solve this problem.

4. Conclusions

The effects of sulfide, sulfite and sulfate on methane production and VFA degradation of anaerobic biogranules of anaerobic biogranules were studied using serum bottle assays. Septage–leachate acclimatized seed granule was used and the following conclusions were drawn.

The effects of each sulfur compound depended on the types of VFA involved. The relative toxicity of the sulfur compounds towards methane production and total VFA degradation varied as $SO_4{}^{2-}S > S^{2-} > SO_3{}^{2-}$–S and $SO_4{}^{2-}$–S $> SO_3{}^{2-}$–S $> S^{2-}$, respectively. Therefore, it is important to choose a monitoring factor in evaluating the toxic effects of the sulfur compounds. The presence of the landfill leachate increased the toxicity-resistance of the biogranules. The VFA-degrading activity of anaerobic biogranule was reduced by 50% when individual dosages of 34, 26 and 20 mg of S^{2-}, $SO_3{}^{2-}$–S and $SO_4{}^{2-}$–S were added to each gram of biomass, respectively. For the individual VFA degradation, the effect of sulfur compounds depended on the type of different VFA components.

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