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Analysis of methane production inhibition for treatment of sewage sludge containing sulfate using an anaerobic continuous degradation process

Tae-Young Jeong*, Hyung-Keun Chung*, Sung Ho Yeom, and Suk Soon Choi***,†**

*YIEST, Division of Environmental Engineering, Yonsei University, Wonju 220-710, Korea
**Department of Environmental & Applied Chemical Engineering,
Kangnung National University, Kangneung 210-702, Korea
***Department of Biological and Environmental Engineering, Semyung University, Jecheon 390-711, Korea
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Abstract−The inhibition of methane production in the continuous anaerobic degradation process for the treatment of sewage sludge containing sulfate was investigated. Also, the competition between sulfate-reducing bacteria (SRB) and methane-producing bacteria (MPB) with COD/sulfate ratio was explained in terms of electron flow. The methane production rate was 0.07, 0.13, 0.24, 0.31 and 0.33 l-CH_4 g-COD^{-1} when the initial COD/sulfate ratio was 3.3, 5.0, 6.7, 10 and 20, respectively. The numbers of SRB and MPB were counted after the continuous reactor reached steady state and the two bacteria showed opposite growth behaviors with COD/sulfate ratio. The inhibition by sulfate compounds was found to follow the uncompetitive model and inhibition constants were 24.57 and 87.99 mg l^{-1} for SRB and MPB, respectively. These results can be useful data for the efficient treatment of sewage sludge in a continuous anaerobic degradation process.

Key words: Continuous Anaerobic Degradation, Inhibition of Methane Production, Uncompetitive Model

INTRODUCTION

It is generally known that the reduction of sulfate during the digestion of sludge reduces bioavailable organic contents, which in turn decreases methane production. In particular, unionized hydrogen sulfide (H_2S) was reported to impose toxic effect on various anaerobic bacteria [1-3]. The extent of inhibition over methane producing bacteria (MPB) was related to the concentration of sulfur compounds produced by sulfate-reducing bacteria (SRB) [4]. According to Parkin et al. [5], methane production was inhibited under as low as 50 mg S^{2-} l^{-1} of sulfur compounds in a batch anaerobic digestion. They also reported that methane production was not affected by the sulfur compounds up to 400 mg S^{2-} l^{-1} in a submerged anaerobic filtering process, while it was reduced by 30% at 800 mg l^{-1} of sulfur compounds. Sulfur compounds produced by SRB usually exist as the form of H_2S, HS^-, S^{2-} or gaseous H_2S in aqueous solution and 20% of the total sulfur compounds at pH 7.0 is free H_2S [6]. Kroiss and Wanbnegg suggested that the level of free H_2S in aqueous solution was related to the toxicity toward MPB [7]. Acetoclastic MPB was inhibited by about 50% at 50 mg l^{-1} of free H_2S and was completely inhibited at 200 mg l^{-1} of free H_2S [8]. Recently, pretreatment methods such as thermal sludge treatment have been tried in many researches in order to enhance the efficiency of methane production [9-11]. In a previous study [12], methane production was reduced by 50% in a batch experiment at the COD/sulfate ratio of 11.6. So far, few researches have been performed to analyze methane production in terms of electron flow.

In this study, the concept of "electron flow" was introduced to explain the inhibition of methane production and analyze quantitatively the inhibition by SRB and MPB. The results were applied to the continuous anaerobic degradation containing sulfate for the actual treatment of sludge. Also, the inhibition by sulfate on the two different bacteria was evaluated by kinetic models.

MATERIALS AND METHODS

The waste activated sludge (WAS) serving as a substrate in this study was collected at a sewage treatment facility located at the city of Wonju. After being treated at 120 °C for 30 minutes, the sludge was used in this study. The cell concentration of mixed liquor volatile suspended solid (MLVSS) was adjusted to 8,200 (±200) mg l^{-1} and divided equally to several portions. Sulfate with different concentrations was added to each portion and the inhibition of methane production by SRB and MPB was investigated. The influent COD of waste sewage sludge was about 10,000 mg l^{-1} and initial sulfate concentrations were 500, 1,000, 1,500, 2,000, 3,000 or 5,000 mg l^{-1}, which corresponded to 20, 10, 6.7, 5, 3.3 or 2.0 of COD/sulfate ratio, respectively.

The schematic of the reactor system is depicted in Fig. 1. And the digestion gas from the reactor was circulated into the reactor itself, which continuously mixed and discharged digesting sludge solution. To maintain temperature in the reactor at 35±1 °C, a thermo controller was installed in a thermostat vessel. Operation condition of the reactor is described in Table 1. The composition of emitting gas was analyzed by gas chromatography (GC-14A, Shimadzu, Japan) with a TCD detector, and volatile fatty acid (VFA) was done with another gas chromatography (GC-8A, Shimadzu, Japan) with an FID detector. Total sulfide (TS) and dissolved sulfide (DS) produced by SRB were analyzed according to iodometric method [13]. Sulfate ion (SO_4^{2-}) was analyzed by ion chromatography (DX-120, Dionex, USA). The number of bacteria was counted at steady state ac-

†To whom correspondence should be addressed.
E-mail: sschoi@semyung.ac.kr

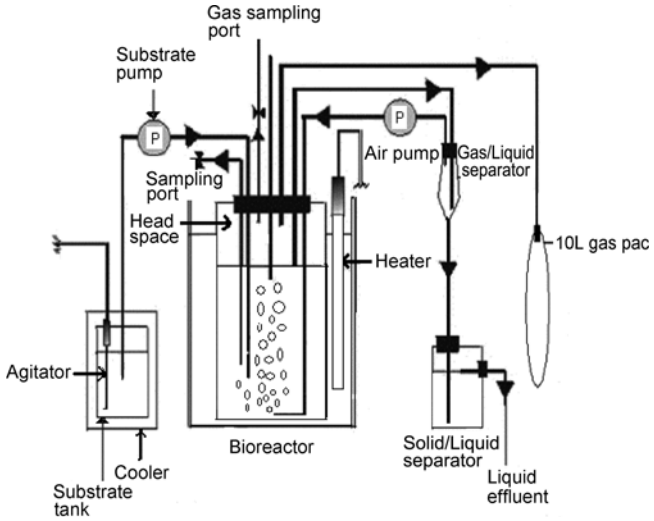

Fig. 1. The experimental equipment of continuous anaerobic sludge degradation.

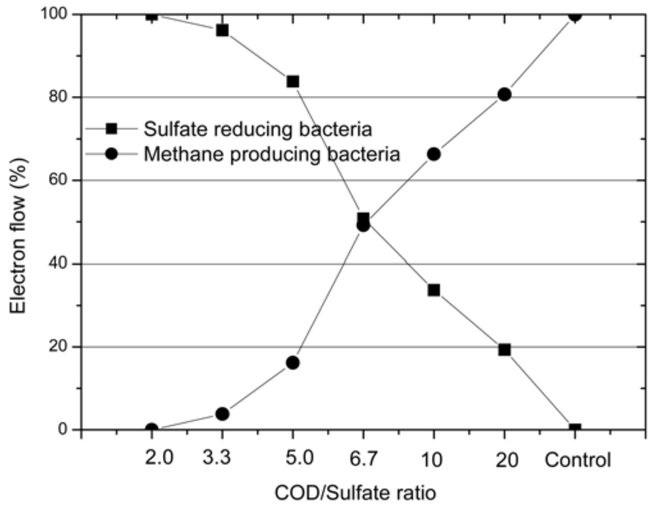

Fig. 2. Electron flow of SRB and MPB by COD/Sulfate ratio.

Table 1. Operation conditions for the continuous anaerobic sludge degradation process (In all experiments, hydraulic retention time was 10 days and temperature was 35±1 °C)

	WAS	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
COD/ Sulfate ratio	0	20	10	6.7	5	3.3	2

cording to most probable number (MPN) method [13]. MPN counting was carried out as follows. Each 1 ml of sample was added to the culture medium where MPB or SRB was growing. Thereafter, they were incubated for 30 days under anaerobic condition. The number of MPB was determined by analyzing gas sample from the culture using gas chromatography (GC-14A, Shimadzu, Japan). The number of SRB was determined by counting the number of black colonies. MPB and SRB in anaerobic culture were reported to grow on acetic acid and hydrogen [14]. Therefore, MPB and SRB growths on either acetic acid or hydrogen were counted separately.

RESULTS AND DISCUSSION

1. Electron Flow of MPB and SRB

Koster et al. suggested that the inhibition of methane production by MPB was closely related to pH, and that MPB was significantly affected by high concentration of unionized H_2S, while it was less influenced by TS [16]. Hilton and Oleszkiewicz reported that SRB, contrary to MPB, was proportionally influenced by the concentration of TS, and that unionized H_2S exhibited significant effect on both SRB and MPB [16].

Isa et al. introduced the electron flow to SRB and MPB in an anaerobic sludge degradation reactor [17,18]. The electron flow was defined as the total COD removed per COD removed by MPB or SRB [17-19]. In the anaerobic digestion where sulfate is abundant, the electron flow of substrates in SRB can be different that in MPB. Fig. 2 shows the electron flows of SRB and MPB with the ratio of COD/sulfate. The electron flow of predominant bacteria tends to change after 6.7 of COD/sulfate ratio. According to Isa et al. [19], the electron flow of MPB decreased from 89% to 66% when the ratio of acetic acid to ethanol was decreased from 5 g COD l^{-1} to 0.5 g COD l^{-1} under high sulfate concentration. This emphasized the importance of high substrate concentration and suggested that MPB, competing with SRB for acetic acid and ethanol, could overcome the inhibition caused by SRB. Mizuno et al. investigated the electron flows of MPB and SRB with butyric acid as a substrate at different COD/sulfate ratios [20]. They found that both MPB and SRB were dependent on the ratio of COD/sulfate, and that MPB exhibited higher electron flow than SRB at higher COD/sulfate ratio. Table 2 shows the competition between SRB and MPB with the ratio of COD/sulfate. The removal efficiency of sulfate was in the

Table 2. Effect of COD/Sulfate ratio on the competition between SRB and MPB

COD/S ratio	SO_4^{2-} removal efficiency (%)	Composition of biogas			COD removal efficiency (%)	Methane rroduction rate (l-CH_4 g-COD^{-1})	% Electron flow	
		% CH_4	% CO_2	% H_2S			SRB	MPB
Control	85.62	68.99	3.34	0	69.37	0.36	100	0
2	35.50	4.12	2.90	2.55	7.15	0	96.23	3.77
3.3	52.12	12.47	3.08	1.36	10.26	0.08	83.77	16.23
5	73.84	21.05	3.47	1.09	30.13	0.13	50.76	49.24
6.7	88.02	47.83	3.07	0.82	65.66	0.24	33.74	66.26
10	90.94	65.67	3.20	0.15	68.30	0.31	19.32	80.68
20	91.53	66.54	3.97	0.07	69.04	0.33	0.02	99.98

range of 35.5-91.53%, and higher COD/sulfate ratio led to higher removal efficiency of sulfate. These results were due to the influent sulfate being used as cell components of SRB and MPB. Additionally, as the ratio of COD/sulfate decreased, the sulfur compounds produced from sulfate caused severe inhibition on both SRB and MPB, which decreased the removal efficiency of sulfate. In addition, the methane production rate was 0.07, 0.13, 0.24, 0.31 and 0.33 l-CH_4 g-COD^{-1} when the initial COD/sulfate ratio was 3.3, 5.0, 6.7, 10 and 20, respectively. No methane was produced at the ratio of 2.0. The removal efficiency of COD, degradation of organic compounds, was as low as 7.15% because all the organic compounds degraded may have been used by SRB.

2. Numeral Variation of SRB and MPB

The MPN method was employed in this study to investigate the change of numbers of SRB and MPB with COD/sulfate ratio in the continuous reactor. The counting of the two bacteria (MPB and SRB) was performed after sample was taken at steady state and incubated at 35 °C for 30 days. Since MPB and SRB were reported to grow on both acetic acid and hydrogen, acetic acid utilizing-MPB and SRB and hydrogen-utilizing MPB and SRB were counted separately. The growth of MPB and SRB with the ratio of COD/sulfate showed opposite behaviors. That is, the number of MPB increased with increasing COD/sulfate ratio, while SRB showed a reverse behavior. The numbers of hydrogen-utilizing and acetic acid-utilizing SRB were 1.2×10^2-3.3×10^{12} number ml^{-1} and 2.3×10^3-4.9×10^{12} number ml^{-1}, respectively, while those of MPB were 1.7×10^1-3.3×10^7 number ml^{-1} and 2.1×10^2-3.5×10^{10} number ml^{-1}, respectively. As shown in Table 3, the dominant species were changed from MPB to SRB at the ratio of COD/sulfate 6.7 and MPB could not grow below 2.0 of COD/sulfate.

3. Analysis of Methane Production Inhibition by Sulfate

The integrative form of the Monod equation is usually used to describe the kinetics of substrate consumption and the equation is shown below.

$$-\frac{dS}{dt}=\frac{kSX}{(K_S+S)} \quad (1)$$

On the basis of the equation, uncompetitive and noncompetitive model equations can be derived for describing sulfate toxicity [21,22] as shown in Eqs. (2) and (3).

Uncompetitive inhibition model:

$$-\frac{dS}{dt}=\frac{kSX}{K_S+S[1+H_2S(aq)]/K_i} \quad (2)$$

Noncompetitive inhibition model:

$$-\frac{dS}{dt}=\frac{kSX}{(K_S+S)(1+[H_2S(aq)]/K_i)} \quad (3)$$

where, k=specific substrate utilization rate, mg COD mg $MLVSS^{-1}\cdot day^{-1}$

Ks=half-velocity constant, mg l^{-1}

X=bacteria concentration, mg l^{-1}

t=time, day

$H_2S(aq)$=inhibition concentration, mg l^{-1}

K_i=inhibition coefficient, mg l^{-1}

To determine the inhibition constant K_i, S/υ versus $[H_2S(aq)]$ was plotted and this yielded the following relationship:

$$\upsilon=\frac{\left[-\frac{dS}{dt}\right]}{X}, \qquad \upsilon_1=\frac{kS_OX_O}{(K_S+S_O)} \quad (4)$$

where, υ represents the reaction rate of anaerobic bacteria in the reactor, defined as the amount of substrate consumed per time. If the noncompetitive model is employed, the plotting $1/\upsilon$ vs $[H_2S(aq)]$ yields the slope of $1/\upsilon_1K_i$ and interception of $1/\upsilon_1$, respectively, while the slope and interaction are $1/kK_i$ and $1/\upsilon_1$, respectively, for the uncompetitive model. Therefore, the inhibition constant, K_i, at different sulfur compound can be determined by using an initial rate of substrate consumption. In this study, sulfur compound $[H_2S(aq)]$

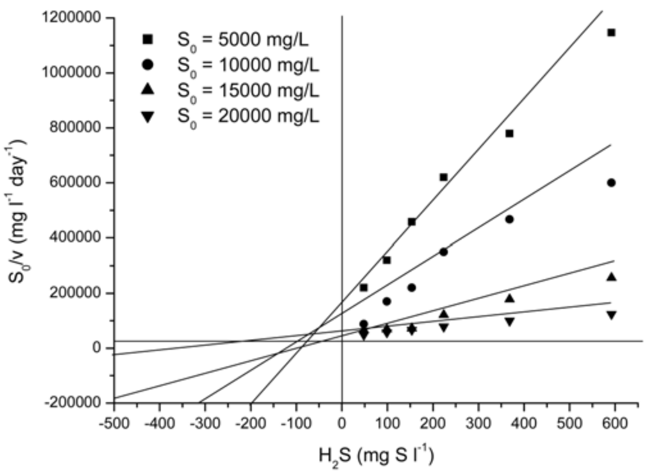

Fig. 3. Determination of inhibition pattern by $H_2S(aq)$ on MPB (S_0: initial substrate, υ: initial reaction rate).

Table 3. The change of populations (SRB and MPB) by the COD/Sulfate ratio

COD/Sulfate ratio	SRB (MPN ml^{-1})		MPB (MPN ml^{-1})		SRB : MPB ratio
	H_2-utilizing	Acetate-utilizing	H_2-utilizing	Acetate-utilizing	
Control	1.2×10^2	2.3×10^3	3.3×10^7	3.5×10^{10}	0.1 : 99.9
2.0	3.3×10^{12}	4.9×10^{12}	0	0	100 : 0
3.3	2.9×10^9	5.5×10^{11}	1.7×10^3	2.1×10^5	99.9 : 0.1
5.0	5.6×10^7	7.9×10^8	2.1×10^7	3.9×10^8	67.3 : 32.7
6.7	4.7×10^7	4.8×10^7	2.5×10^7	5.1×10^8	49.6 : 50.4
10	3.4×10^7	5.6×10^7	3.6×10^7	4.9×10^8	14.6 : 85.4
20	2.2×10^7	2.5×10^5	2.5×10^7	5.5×10^8	3.7 : 96.3

Table 4. Inhibition constant for SRB and MPB due to DS and H_2S

	SRB		MPB	
Reactor	DS, mg l^{-1}	1.25-47.12	H_2S(aq), mg l^{-1}	2-615
Inhibition constant	K_i, mg l^{-1}	24.57	K_i, mg l^{-1}	87.99

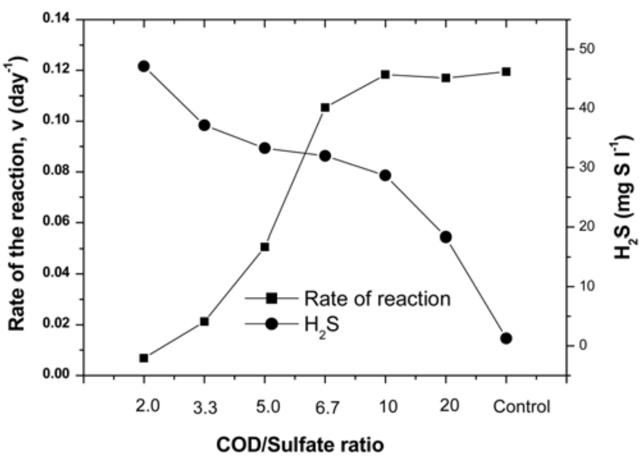

Fig. 4. Relationship of H_2S(aq) and reaction rate with different COD/Sulfate ratio.

produced from different initial sludge concentrations was investigated to determine which inhibition model was applicable for the inhibition of methane production by sulfate. Fig. 3 shows that the inhibition by sulfur compound [H_2S(aq)] toward MPB is close to the uncompetitive one because the line representing initial substrate consumption rate and the slopes with different concentrations of sulfur compounds [H_2S(aq)] intersected on the x-axis [23]. According to Hilton and Oleszkiewicz [9], H_2S(aq) inhibited MPB while DS did SRB. On the basis of this information, K_i was calculated by using various H_2S (aq) and DS values obtained at different COD/sulfate ratios. The inhibition constants of MPB and SRB at different COD/sulfate ratios were calculated using a specific substrate utilization rate (k) and shown in Table 4. The concentration of DS, toward SRB was 1.25-47.12 mg l^{-1} as indicated in Table 4. The concentration of sulfur compound [H_2S(aq)] affecting MPB was 2-615 mg l^{-1}, and the inhibition constants over SRB and MPB were 24.57 and 87.99 mg l^{-1}, respectively.

Fig. 4 shows the relationship between reaction rate with H_2S (aq) produced by the reduction of sulfate at various COD/sulfate ratios. The reaction rate calculated from Eq. (4) was in the range of 0.11-0.006 day^{-1} and the value was almost zero at low COD/sulfate ratio. In addition, the H_2S(aq) produced was in the range of 18.32-47.12 mg l^{-1}. The reaction rate started to decrease dramatically after 6.7 of COD/sulfate ratio as depicted in Fig. 4.

CONCLUSIONS

The inhibition of methane production with COD/sulfate ratio was investigated and sulfate inhibition was evaluated by kinetic models. The competition between sulfate reducing bacteria (SRB) and methane producing bacteria (MPB) with COD/sulfate ratio was well explained in terms of electron flow. The methane production rate was 0.07, 0.13, 0.24, 0.31 and 0.33 l-CH_4 g-COD^{-1} when COD/sulfate ratio was 3.3, 5.0, 6,7, 10 and 20, respectively. The flow of electrons indicating the species of predominant bacteria changed after 6.7 of COD/sulfate ratio. The inhibition by sulfate compounds was found to follow the uncompetitive model and inhibition constants were 24.57 and 87.99 mg l^{-1} for SRB and MPB, respectively.

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