〈研究発表〉

Modeling Sulfide Inhibition on Methanogen's Decay

Thi Oanh Phung[1)], Meng Sun[1)], Mitsuharu Terashima[1)] and Hidenari Yasui[1)]

[1)] Faculty of Environmental Engineering, The University of Kitakyushu
(1-1 Hibikino, Wakamatsu Ward, Kitakyushu, Fukuoka 808-0135 Japan, E-mail:Hidenari-yasui@kitakyu-u.ac.jp)

Abstract

This study is focused on the biocidal effect of sulfide inhibition on methanogenic biomass. Using the methanogen-enriched cultures obtained from the digestate of the anaerobic digester in the wastewater treatment plant, Kitakyushu, Japan, batch inhibition tests were performed for 6 days to analyze the dynamic change of the living microorganism concentrations under varied sulfide concentrations. When the cultures were exposed by high sulfide without addition of methanogenic substrates, the methanogenic archaeal concentration were considerably reduced. Higher sulfide concentration in the system resulted in higher decay of the biomass. Besides, to collect the additional data to examine the impact of unionized H_2S on methanogen decay, another batch test was performed with addition of sulfide at varying pH conditions. These experimental results are almost identical, it seems that the methanogen was inhibited by total sulfide, not only unionized H_2S gas.

キーワード：methanogen, sulfide inhibition, anaerobic process
原稿受付 2023. 6. 29　　**EICA**: 28(2・3) 117--120

1. Introduction

Anaerobic processes are typically used for the treatment of waste sludge and high-strength organic wastes. It involves the decomposition of organic matter and inorganic matter in the absence of oxygen[1)]. It can reduce organic pollution from the liquid outputs of homes, industry, and agriculture, while potentially offsetting the use of fossil fuels. In anaerobic digestion, the acid-forming and the methane-forming microorganisms differ widely in terms of physiology, nutritional needs, growth kinetics, and sensitivity to environmental conditions. Failure to maintain the balance between these two groups of microorganisms is the primary cause of reactor instability. A wide variety of wastewater sources have been reported to be inhibitory in anaerobic digestion processes. According to the literature, the inhibitors commonly present in anaerobic digesters include in low pH, ammonia, sulfide, light metal ions, heavy metals, and organics, etc. There have been numerous studies on the effect of inhibitors on anaerobic digestion, but the majority of them have focused on reducing methane gas production while ignoring biomass decomposition[2,3)]. For these reasons, it would be necessary to investigate the impact of inhibitors on the decay of methanogenic biomass. This study focuses on the biocidal effect of sulfide on the decomposition of two types of methanogens, namely hydrogenotrophic and acetoclastic methanogens.

2. Materials and methods

This study utilized a mixed culture of methanogens that was obtained from a mesophilic anaerobic digester located at the Hiagari municipal wastewater plant in Kitakyushu, Japan. Two types of methanogens were enriched with synthetic influent consisting of either acetate buffer or formate buffer to grow acetoclastic methanogen and hydrogenotrophic methanogen respectively.

After more than 3 months of operation, from the main reactors, sludge was extracted to conduct the batch incubation. Each 100 ml of the sludge was transferred to 120-ml bottle that purged with pure nitrogen for a minute to create an oxygen-free environment. The bottles were then treated with sulfide inhibitors with varying concentrations at 0 mgTS/L, 100 mgTS/L, 200 mgTS/L, 300 mgTS/L and 400 mgTS/L in triplicate for each batch test and the pH was fixed at about 7 (Set I). Besides, the second batch test using the same procedure was performed with addition of 200 mg/L total sulfide was exposed to the system at pH 6, 7 and 8 (Set II) in

order to examine the impact of unionized H_2S on methanogen decay. The experiments were placed in a respirometer at 35℃ and incubated for six days without the addition of substrate and nutrients.

This research was conducted according to the method described in SUN, et al., (2020)[4]. The LIVE/DEAD® BacLight™ cell viability kit (L-7012, Molecular Probes, USA) were found as a novel and useful kit for differentiating between viable cells and deceased cell[5]. During six days of incubation time, 5 ml of sludge from each bottle was extracted every day to examine the living cell area changes along with time. To observe the living cell area, a fluorescent microscope equipped with a bandpass filter cube (GFP-B: excitation wavelength=460-500 nm; emission wavelength=515-560 nm) was used. A total of 30 photos of green fluorescent were taken per glass slide at different places. The procedure is performed triplicated for each sample. After that, the green fluorescent cell images with 200 magnifications (10x20) were analysed using QuickGrain software (Inotech, Japan) then the results of living cell area per field were estimated. Next step, the datasets were statistically analyzed using a commercial data analysis software- Igor pro (Wavemetric, USA). By using this method, the dataset of specific decay rate was estimated for each batch experiment, performed with prediction interval and 95% of the confident interval in order to optimize the experimental data.

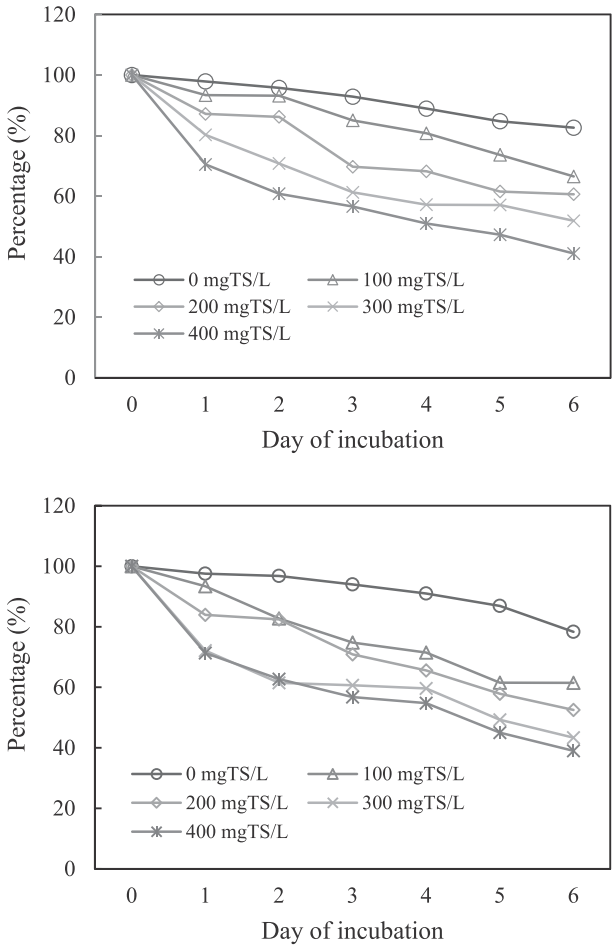

Fig. 1 The proportion of methanogens whose biomass decays in response to sulfide exposure (Upper- hydrogenotrophic methanogen, lower- acetoclastic methanogen).

3. Results and discussion

3.1 Effect of sulfide inhibition on biomass decay

In the initial phase, the living cell area estimated from the fluorescent cell area per microscope field was counted to be about 3000 μm^2 /field (about 400 fluorescent plots per field) for both the formate-fed system and acetate-fed system. After six days of the batch experiment, in case no sulfide was exposed, the green fluorescent cell area was reduced by 17% in formate system and 22% in the acetate system, respectively. These values are significantly decreased at the sludge with addition of sulfide inhibition. At the end of the experiment, the living cell area of formate-utilizing microorganisms were reduced by 34% at 100 mgTS/L, 39% at 200 mgTS/L, 48% at 300 mgTS/L and 59% at 400 mgTS/L, respectively (**Fig. 1**). These experimental results are slightly increased at methanogen biomass enriched from the acetate-fed system. The biomass concentration was reduced by 39% at the end of the batch tests with 100 mg/L total sulfide, this value reached 47% at 200 mg/L, 57% at 300 mg/L, and 61% at 400 mg/L, respectively (**Fig. 1**). After 24 hours of exposure to an inhibitor in batch experiments with high sulfide concentrations (above 300 mgS/L), the concentration of living biomass decreased rapidly. It was estimated to drop by about 20-30% which is as fast as the decay of microorganisms in normal conditions after six days.

3.2 Impact of unionized H_2S on biomass decay

To investigate the effect of unionized H_2S on the decomposition of methanogen, batch experiments with sulfide exposure at varying pH levels were conducted. In aqueous environment, sulfide presents at 3 main species including unionized H_2S, HS^- and S^{2-}. Each specie concentration is dependent on the pH of the environment. The concentration of unionized H_2S is estimated approximately 90% of the total sulfide in an environment with a pH of about 6. It will decrease to approximately 50% of the total sulfide concentration at

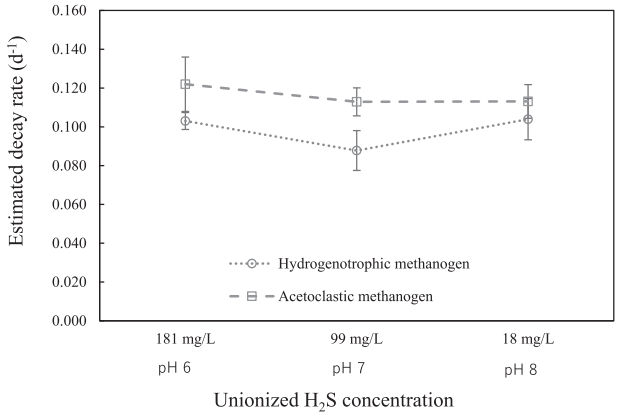

Fig. 2 Compare the estimated decay rates of hydrogenotrophic and acetoclastic methanogen when exposed to 200 mg/L of total sulfide at various pH values.

pH 7 and 10% in a pH 8 environment. Under an exposure of 200 mgTS/L, the specific decay rate of hydrogenotrophic methanogen was estimated to be $0.1 \pm 0.006\ d^{-1}$ at pH 6, $0.09 \pm 0.006\ d^{-1}$ at pH 7 and $0.1 \pm 0.002\ d^{-1}$ at pH 8, respectively. Slightly higher specific decay rate was obtained in batch experiments with methanogenic biomass enriched from acetate-fed system comparing to format-fed system. It was estimated at about $0.12 \pm 0.011\ d^{-1}$, $0.11 \pm 0.012\ d^{-1}$ and $0.11 \pm 0.007\ d^{-1}$ for experiment at pH 6, pH 7 and pH 8, respectively (**Fig. 2**). The values of both methanogens are nearly identical, suggesting that the methanogen was inhibited by total sulfide and not just unionized H_2S. Therefore, the decay rate of methanogen can be modeled based on the concentration of total sulfide.

3. 3 Modeling sulfide inhibition on methanogen's decay

The specific decay rate of methanogens was estimated based on the first order decay function. Under the normal condition, decay rate of methanogen biomass enriched from formate culture was estimated about $0.038\ d^{-1}$ and $0.037\ d^{-1}$ for acetate culture. It tends to increase as sulfide concentrations rise. Specifically, the specific decay rate of formate-utilizing organisms increased to $0.074 \pm 0.01\ d^{-1}$ at biomass containing 100 mgTS/L, $0.09 \pm 0.007\ d^{-1}$ at 200 mgTS/L, and $0.127 \pm 0.013\ d^{-1}$ at 300 mgTS/L, respectively. This demonstrates that, depending on the inhibitor concentration, sulfide can accelerate bacterial degradation by two to three times. The deleterious effect of sulfide was also observed in the acetate system. The specific decay rate was estimated at about $0.083 \pm 0.015\ d^{-1}$ at the sample incubated by 100 mg/L total

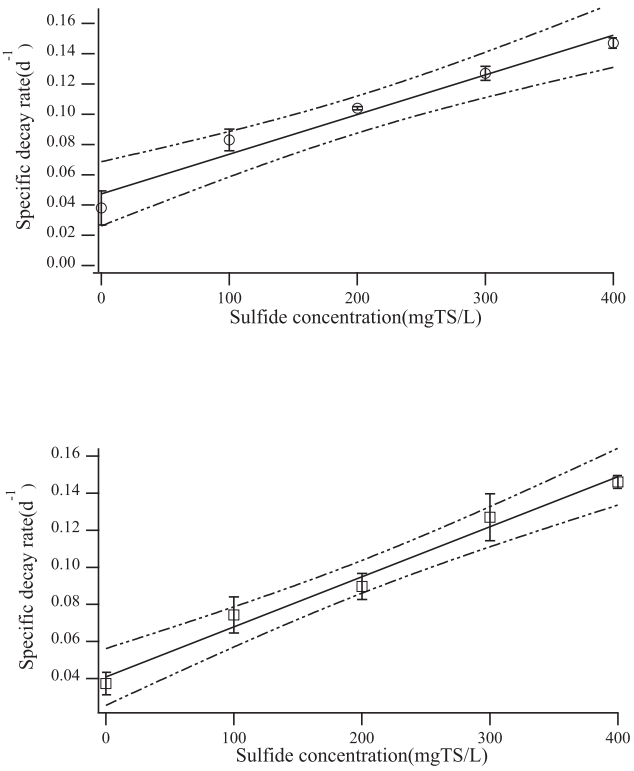

Fig. 3 The estimated decay rates of methanogens when exposed to sulfide (Upper- hydrogenotrophic methanogen, lower-acetoclactic methanogen).

sulfide, which was increased 2 times compared with the normal specific decay rate. It reached $0.104 \pm 0.018\ d^{-1}$ at 200 mg/l, $0.126 \pm 0.02\ d^{-1}$ at 300 mgTS/L, respectively. And especially, the specific decay rate jumped to $0.147 \pm 0.035\ d^{-1}$ at 400 mgTS/L, almost 4 times higher than normal conditions. It seems that hydrogenotrophic methanogen is more resistant to environmental changes and toxicity than acetoclastic methanogen. These experimental results demonstrated the correlation between specific decay rate and sulfide concentration (**Fig. 3**).

4. Conclusion

When the cultures were exposed to high sulfide without addition of methanogenic substrates, the methanogenic archaeal concentration was considerably reduced in both cultures. It seemed that unionized H_2S is not the main factor which may kill methanogens although it was considered that this species is a strong inhibitor since it can penetrate the cell membrane and inhibit to metabolic activities of microorganisms. The decay rates of living methanogen have been modeled based on the sulfide concentration-dependent function. The specific decay rate was accelerated when high sulfide concentration was

present, indicating an exogenous decay took place due to poisoning by sulfide inhibition.

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