〈論文〉

Biomethanation by Hydrogen-supplemented Mesophilic Anaerobic Digested Sludge

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Abstract

Hydrogen (H_2) addition to anaerobic reactors may enhance conversion of carbon dioxide (CO_2) to methane (CH_4) by hydrogenotrophic methanogens. In this study, the effect of H_2 addition on the biomethanation performance of digested sludge was investigated in batch experiments. Addition of H_2 enhanced the conversion of CO_2 to CH_4. The rate of H_2 consumption in mesophilic digested sludge was 8.9–11.2 mL/(g_{VS}・h). Addition of >60 mL of H_2 (equivalent to a 0.75 atm partial pressure of H_2) increased the pH from 7.5 to 8.5 and the soluble chemical oxygen demand (SCOD) 1.5-fold. Furthermore, the CH_4 yield of digested sludge containing sodium acetate and butyrate revealed that H_2 addition did not influence acetic acid methanogenesis, but inhibited its acetogenesis. Thus, a single addition of H_2 is recommended to promote recovery of acetogenesis.

Our results indicate that H_2 should be added in the later phase of continuous anaerobic digestion, when acetogenesis is almost complete.

Keyword：Hydrogen, biogas, digested sludge, hydrogenotrophic methanogens, acidogenic bacteria
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1. Introduction

Sewage sludge, an organic byproduct discharged from wastewater treatment plants (WWTPs), contains large amounts of organic matter and nutrients[1]. Anaerobic digestion is used to generate bioenergy, in which sludge is transformed into methane (CH_4). As a promising alternative to fossil fuels, CH_4 produced by sludge anaerobic conversion can indirectly reduce greenhouse gas (GHG) emissions[2]. However, the complex floc structure and low level of biodegradable organic matter in sludge lead to a longer retention time and a lower CH_4 yield from anaerobic digestion of sludge. To accelerate hydrolysis and enhance the biogas yield, mechanical, thermal, and chemical sludge pretreatment technologies have been developed[3].

The main components of biogas produced by anaerobic digestion of sludge are CH_4 (40–75%) and carbon dioxide (CO_2; 25–60%). Although biogas yield can be improved by these pretreatment technologies, its utility is limited by the low CH_4 content. Thus, CH_4-rich biogas (biomethane) is needed. Biomethane (CH_4>90%) is a potential alternative to natural gas and gasoline[4]. Methods for removing CO_2 from biogas include water washing, pressure swing adsorption, and polyglycol adsorption[5]. However, the widespread use of these technologies is hampered by their cost and requirement for addition of chemicals and use of high pressures.

Biological conversion of CO_2 to CH_4 by hydrogenotrophic methanogens (Eq. 1) is a focus of research[6]. According to the power-to-gas concept, hydrogen (H_2) can be produced by electrical decomposition of water using excess electricity from renewable energy, including wind and solar power[7].

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (1)$$

During anaerobic digestion, about 30% of the CH_4 is produced directly from H_2/CO_2 by hydrogenotrophic methanogens[8]. It is thus hypothesized that H_2 addition would promote hydrogenotrophic methanogenesis. Previous studies investigated biocatalytic methanation of H_2 and CO_2 by pure hydrogenotrophic methanogens[3, 6, 9]. However, anaerobic digestion is a complex biochemical process catalyzed by several microbial consortia[10]. Syntrophic degradation of fermentation intermediates functions well provided that the hydrogen partial pressure (P_{H2}) remains low enough for H_2 production[11]. Therefore, although added H_2

enhances the biological conversion of CO_2 to CH_4, excess H_2 inhibits biomethanation by anaerobic digested sludge[8]. Additionally, CO_2 consumption could result in an increase in pH, which would inhibit the conversion of CO_2 to CH_4. However, the biomethanation ability of mesophilic anaerobic digested sludge with H_2 addition has not been investigated.

In this study, the biomethanation ability of mesophilic anaerobically digested sludge with H_2 addition was investigated in batch experiments. To determine the optimum quantity and timing of H_2 addition, the effect of H_2 addition on the biomethanation performance of digested sludge was analyzed.

2. Materials and Methods

2.1 Materials

Mesophilic digested sludge was obtained from WWTP A in Kyoto, Japan. The digested sludge was transferred to the laboratory at room temperature, and immediately characterized and used in batch experiments. The characteristics of the digested sludge are shown in **Table 1**.

Table 1 Characterization of the sludge

Parameter	Range
TS (g/L)	10.38～11.1
VS (g/L)	8.24～8.77
pH	7.03～7.38
H (%TS)	5.52～6.16
C (%TS)	37.90～41.18
N (%TS)	5.59～6.36
SCOD (mg/L)	1056～1178

TS, total solids; VS, volatile solids; H, hydrogen; C, carbon; N, nitrogen; SCOD, soluble chemical oxygen demand

2.2 Digestion batch experiments

Batch experiment 1 estimated the rate of H_2 consumption under mesophilic conditions. To 122 mL serum bottles, 42 mL of mesophilic digested sludge was added. The bottles were purged with N_2 (99.5%; Sumitomo Seika Chemicals, Osaka, Japan) for 2 min and sealed with butyl rubber stoppers and aluminum crimps. To the bottles, 0, 40, 60, 80, or 100 mL of H_2 (99.99%; GL Science, Tokyo, Japan) was added to produce initial P_{H2} values of 0, 0.5, 0.75, 1.0, and 1.25 atm; these bottles comprised groups a, b, c, and d, respectively. The bottles were placed in a rotary shaker (BT300 Water Bath Incubator; Yamato Scientific, Co., Ltd, Japan) at 35℃ for 24 h (**Table 2**).

Batch experiment 2 was carried out to investigate the effect of H_2 addition on anaerobic digestion. To 122 mL serum bottles, 42 mL of mesophilic-digested sludge with or without sodium acetate or butyrate was added. The bottles were purged with N_2 for 2 min and sealed with butyl rubber stoppers and aluminum crimps, followed by H_2 injection as in batch experiment 1. The bottles were placed in a rotary shaker at 35℃ with shaking at 100 rpm for 24 h (**Table 3**).

Table 2 Conditions for batch experiment 1-digestion with H_2 addition

Condition	Levels
Volume of H_2 addition (mL)	40, 60, 80, 100
Shaking speed (rpm)	50, 75, 100, 125

Addition of 40, 60, 80, and 100 mL of hydrogen (H_2) is equivalent to 0.5, 0.75, 1.0, and 1.25 atm initial partial pressure of H_2, respectively.

Batch experiment 3 was performed to determine the effect of the method of H_2 addition on CH_4 production. To 122 mL serum bottles, 42 mL of mesophilic-digested sludge with or without sodium acetate or butyrate was added. The bottles were purged with N_2 for 2 min and sealed with butyl rubber stoppers and aluminum crimps, followed by H_2 injection as in batch experiment 1. In group h, H_2 was injected at the beginning of the experiment. In groups e, f, and g, H_2 was injected into the bottles at 3, 6, and 12 h intervals, respectively. The bottles were placed in a rotary shaker at 35℃ with shaking at 100 rpm for 24 h (**Table 4**).

All experiments were performed in triplicate, and digested sludge without H_2 was used as the control. To determine the volume of H_2, a syringe was injected vertically into the bottles. As the gas enters the syringe, the plunger is pushed outward until the internal and external air pressure equalize. The H_2 volume was calculated by multiplying the total volume (volume of gas added and volume of gas in the bottle)

Table 3 Conditions for batch experiment 2-inhibition of anaerobic digestion by H_2 addition

Condition	Levels
Volume of H_2 addition (mL)	0, 20, 40, 80, 100
Sodium acetate (mmol/bottle)	0.446
Butyrate (mmol/bottle)	0.223

Addition of 40, 60, 80, and 100 mL of H_2 is equivalent to 0.5, 0.75, 1.0, and 1.25 atm initial partial pressure of H_2, respectively.

Table 4 Conditions for batch experiment 3-method of H_2 addition

Condition	Levels
Volume of H_2 addition (mL)	0, 20, 40, 80, 100
Addition frequency (h)	Beginning, 3, 6, 12

Addition of 40, 60, 80, and 100 mL of H_2 is equivalent to a 0.5, 0.75, 1.0, and 1.25 atm initial partial pressure of H_2, respectively.

by the concentration of H_2. Because gas-liquid mass transfer is a limiting factor[8], the bottles were placed horizontally.

2. 3　Analytical methods

The soluble chemical oxygen demand (SCOD), and the total solid (TS) and volatile solid (VS) contents of sludge were determined according to standard methods[12]. Gas samples were taken from the bottles using a syringe (20, 30, 50, or 100 mL ; M. S. Surgical, Ahmedabad, India) for analysis of the CO_2, H_2, and CH_4 levels by gas chromatography (GC) (490 Micro GC ; Agilent Technologies, Santa Clara, CA, USA). pH was measured using a pH meter (D-71S ; Horiba, Kyoto, Japan). The C, H, and N contents of the solids in sludge samples were analyzed using an elemental analyzer (Microcorder, JM10 ; J-Science, Kyoto, Japan).

3.　Results and Discussion

No leakage from bottles containing H_2 and water was detected (**Table 5**). The amount of dissolved H_2 can be ignored because of its low solubility in water (18.2 mL/L at 20℃ and 1 atm).

Table 5　Variation in H_2 volume before and after the leakage check experiment

Measured by syringe		Measured by GC	
Beginning (mL)	End (mL)	Beginning (mL)	End (mL)
40	40～42	40.3～40.7	40.1～40.3
60	60～61	60.3～61.2	60.7～61.3
80	80～81	80.0～81.9	79.2～82.6
100	100～102	101.5～103.7	97.8～105.5

3. 1 Rate of H_2 consumption

The rate of H_2 consumption increased with increasing shaking speed (**Fig. 1**), indicating that the rate of H_2 conversion to CH_4 is increased by the enhanced H_2 gas-liquid mass transfer rate. However, increasing the initial P_{H2} did not increase the rate of H_2 consumption (**Fig. 1c** and **d**) because the limiting factor was the microbial activity or CO_2 production rate, not the H_2 gas-liquid mass transfer rate.

A shaking speed of 100 rpm is recommended because the difference in H_2 consumption rate between 75 and 100 rpm was greater than that between 100 and 125 rpm. Complete consumption of 40, 60, 80, and 100 mL of H_2 (initial P_{H2} 0.5, 0.75, 1.0, and 1.25 atm, respectively) required 12, 16, 18, and 20 h, respectively (**Fig. 1c**). The rate of H_2 consumption was 9-11 mL/(g_{vs}・h) under mesophilic conditions.

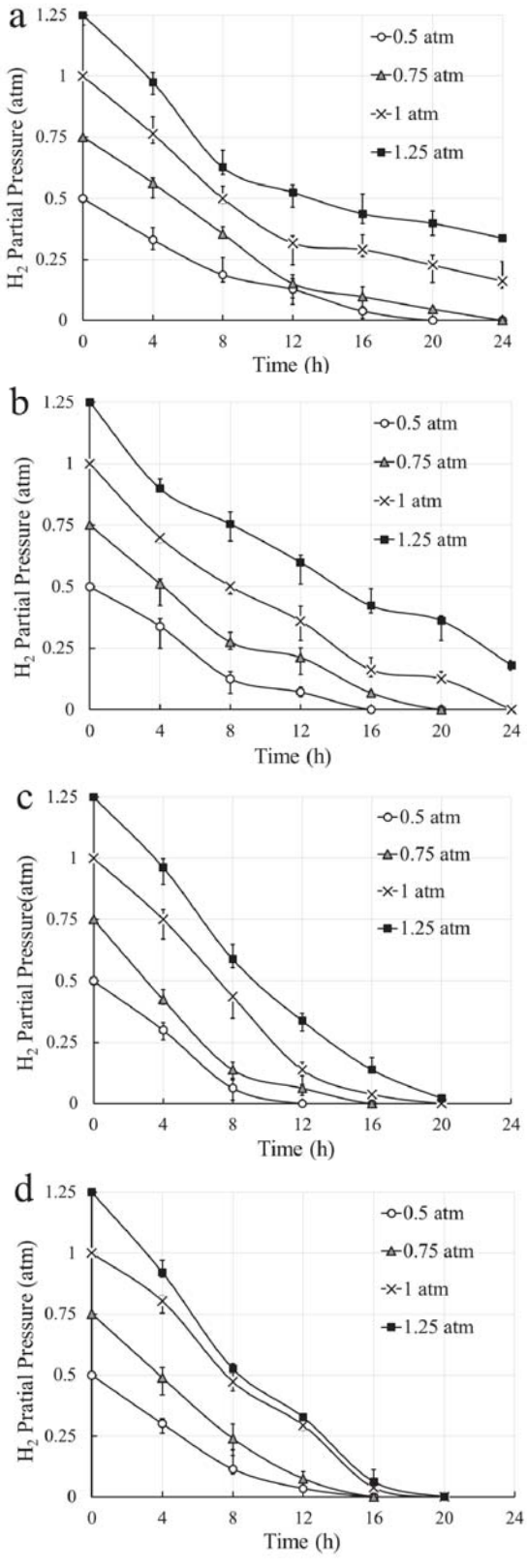

Fig. 1　Variation of hydrogen (H_2) level with shaking speed in batch experiment 1. H_2 consumption with shaking at (a) 50, (b) 75, (c) 100, and (d) 125 rpm. Addition of 40, 60, 80, and 100 mL of H_2 is equivalent to a 0.5, 0.75, 1.0, and 1.25 atm initial partial pressure of hydrogen (H_2), respectively.

No increase in the acetic acid concentration was

observed during anaerobic digestion in any of the groups. This indicates consumption of H_2 by hydrogenotrophic methanogens, or by homoacetogens, to produce acetic acid, which was subsequently converted to CH_4 by methanogens.

3. 2 Effect of H_2 addition on anaerobic digestion

Theoretically, four unit volume of H_2 is converted to one unit volume of CH_4. Thus, CH_4 digestion can be evaluated by determining the decrease in H_2 volume because all of the H_2 was converted to CH_4 [Eqs. (2) and (3)].

$$CH_{4(a)} = CH_{4(a:\,total)} - CH_{4(a:\,H_2+CO_2)} \quad (2)$$

$$CH_{4(a:\,H_2+CO_2)} = H_{2(a)} \div 4 \quad (3)$$

- $CH_{4(a)}$: Volume of CH_4 produced in sludge while volume of initial H_2 is a mL ;
- $CH_{4(a:\,total)}$: Total volume of CH_4 in sludge while volume of initial H_2 is a mL ;
- $CH_{4(a:\,H_2+CO_2)}$: Calculated volume of CH_4 converted from H_2 and CO_2 while volume of initial H_2 is a mL ;
- $H_{2(a)}$: Volume of H_2 added ;
- $CH_{4(0)}$: Volume of CH_4 produced from sludge without H_2 addition.

Total CH_4 production with H_2 addition in the sludge only group was slow in comparison with the calculated volume of CH_4 produced from sludge decreased sharply (**Fig. 2**).

The magnitude of inhibition of digestion can be calculated by Eq. (4) :

$$\text{Inhibition of digestion} = CH_{4(a)} \div CH_{4(0)} \quad (4)$$

The magnitude of inhibition of digestion increased as the volume of H_2 added increased from 20 to 40 mL (initial P_{H2} 0.25 and 0.5 atm, respectively) and reached 100% upon addition of 100 mL of H_2 (initial P_{H2} 1.25 atm).

In sludge and sludge containing sodium acetate, CH_4 production was unaffected by H_2 addition (**Fig. 3**). Therefore, H_2 addition had no effect on acetic-acid methanogenesis.

Addition of ≥40 mL of H_2 (initial P_{H2} 0.5 atm) to sludge and sludge containing butyrate resulted in similar levels of CH_4 production (**Fig. 3**). The lack of inhibition of butyrate degradation upon addition of smaller quantities of H_2 could be due to the bicarbonate buffer system[6] and more rapid consumption of H_2 by hydrogenotrophic methanogens. This would likely result in maintenance of an adequately low pH and dissolved H_2 level for butyrate degradation. In contrast, the degradation of butyrate and sludge was inhibited by addition of ≥40 mL of H_2 (initial P_{H2} 0.5 atm). Therefore, H_2 is not only a substrate for hydrogenotrophic methanogens, but also a metabolic product of acidogenesis. Addition of H_2 to a biogas reactor may increase the P_{H2} value, leading to inhibition of acidogenesis.

Consumption of H_2 and CO_2 by hydrogenotrophic methanogens increases the pH, which may inhibit bacterial activity but not hydrolytic processes. If so, organic matter could be hydrolyzed but could not be acetylated to acetate acid and subsequently to CH_4.

The SCOD increased at initial P_{H2} values of ≥0.75 atm (**Fig. 4**), likely due to the pH (8.06) being outside the optimal range (7.0-7.5) for the growth of aceticlastic methanogens[13]. In this study, the pH of digested sludge was determined by the synergy

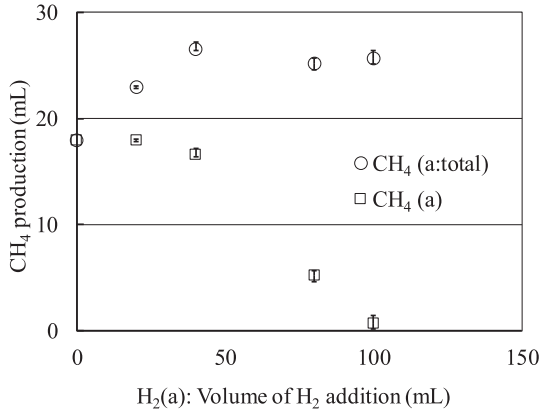

Fig. 2 Variation of methane (CH_4) production with H_2 addition to sludge in batch experiment 2. Addition of 40, 60, 80, and 100 mL of H_2 is equivalent to a 0.5, 0.75, 1.0, and 1.25 atm initial partial pressure of H_2, respectively.

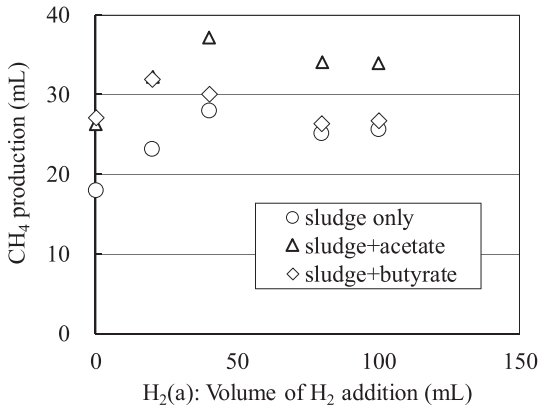

Fig. 3 Variation in total CH_4 production in batch experiment 2. Addition of 40, 60, 80, and 100 mL of H_2 is equivalent to a 0.5, 0.75, 1.0, and 1.25 atm initial partial pressure of H_2, respectively.

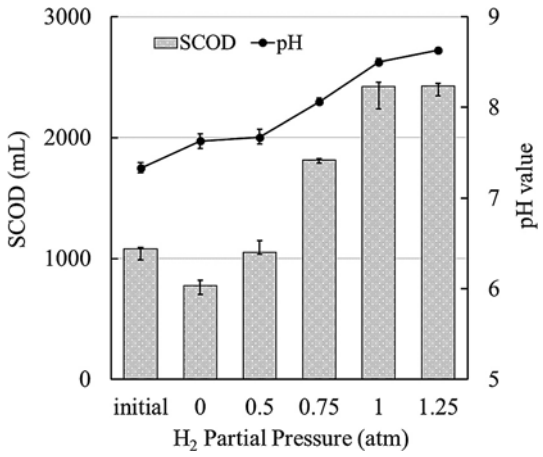

Fig. 4 Variation of soluble chemical oxygen demand（SCOD）and pH in sludge with H_2 addition in batch experiment 1 with shaking at 100 rpm. Original sludge before batch experiment 1. Addition of 40, 60, 80, and 100 mL of H_2 is equivalent to a 0.5, 0.75, 1.0, and 1.25 atm initial partial pressure of H_2, respectively.

between the dissolved CO_2 and the accumulation of volatile fatty acids（VFAs）. Based on Henry's law, the solubility of CO_2 in the liquid phase is proportional to the partial pressure of CO_2 in biogas. Therefore, hydrogenotrophic methanogens consumed the dissolved CO_2 in sludge containing H_2, which also made the CO_2 in the generated biogas dissolve. A high pH also inhibits acetogenesis and methanation, leading to the accumulation of VFAs and a concomitant decrease in pH. However, the pH increases if the dissolved CO_2 concentration is insufficient, which explains the absence of an increase in pH at initial P_{H2} values of <0.5 atm.

3. 3 Effect of the frequency of H_2 addition on CH_4 production

The effect of the frequency of H_2 addition on CH_4 production was investigated in batch experiment 3（**Fig. 5**）. CH_4 production was significantly lower in groups e, f, and g compared to group h. Therefore, frequent addition of H_2 to avoid increasing the P_{H2} does not increase CH_4 production.

At the end of the experiment, H_2 was detected in groups e, f, and g, in the order e＞f＞g. This was due in part to the insufficient reaction time for conversion of H_2+CO_2 to CH_4.

CH_4 production in sludge decreased as the H_2 injection frequency increased（**Fig. 6**）. Therefore, the magnitude of inhibition of acetogenesis increases with increasing H_2 injection frequency. Acetogenesis gradually recovers so long as the P_{H2} value is low enough to enable consumption of H_2. For instance,

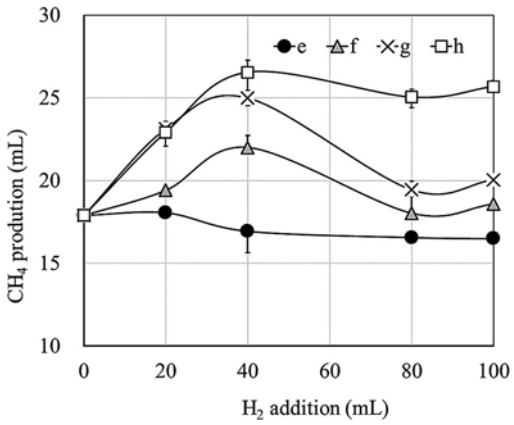

Fig. 5 Total CH_4 volume according to the volume of H_2 injected in batch experiment 3.

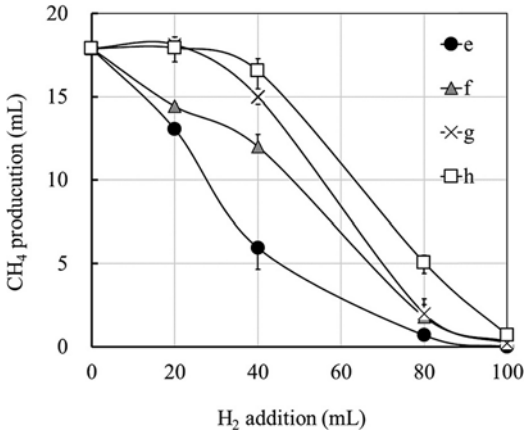

Fig. 6 Calculated CH_4 volume in sludge according to the volume of H_2 injected in batch experiment 3.

degradation of butyrate requires an H_2 concentration of $<10^{-4}$ atm)[13]. Frequent H_2 addition did not promote recovery of acetogenesis because the reaction time was insufficient.

Conclusion

In this study, biomethanation by mesophilic anaerobically digested sludge with H_2 addition was investigated in batch experiments. The H_2 consumption rate was determined by the methanogen activity of digested sludge and the gas–liquid mass transfer rate（shaking speed）; maximum H_2 production occurred at a shaking speed of 100 rpm. The H_2 consumption rate in mesophilic digested sludge was 8.9–11.2 mL/(g_{VS}・h). Addition of ＞60 mL of H_2（H_2 partial pressure of 0.75 atm）resulted in an increase in pH from 7.5 to 8.5 and a 1.5-fold increase in the SCOD. Furthermore, H_2 addition had no influence on acetic acid methanogenesis but inhibited its acetogenesis, which might disrupt the anaerobic digestion of sewage

sludge. Moreover, our results indicate that a single addition of H_2 is optimal for promoting the recovery of acetogenesis.

In conclusion, H_2 should be added in the later phase of continuous anaerobic digestion when acetogenesis is almost complete. Addition of H_2 at the appropriate time not only prevents inhibition of digestion but also enhances the activity of hydrogenotrophic methanogens. The appropriate dose and frequency of H_2 addition need to be determined.

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水素添加による中温消化汚泥のバイオメタネーション特性に関する研究

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概　要

欧州で注目されている“Power to Gas”の概念に基づいて，余剰電力を用いた水の電気分解により得られた水素を嫌気性消化タンクへ添加すれば，水素酸化型メタン生成によるバイオメタネーション反応が生じ，バイオガスに含まれる二酸化炭素（CO_2）がメタン（CH_4）に転換され，バイオガスをアップグレードできる可能性がある。本研究では，まず消化汚泥への水素添加がバイオメタネーション特性へ与える影響をバッチ試験により評価した。その結果，水素の添加により CO_2 の CH_4 への転換が確認された。また，中温消化汚泥の水素消費速度は 8.9–11.2 mL/（gVS・h）程度と見積もられた。しかしながら，60 mL（水素分圧で 0.75 atm）以上，水素を添加した場合 pH が 7.5 から 8.5 まで，溶解性 COD が 1.5 倍まで上昇し，メタン発酵阻害が生じる傾向がみられた。
更に，酢酸ナトリウムと酪酸ナトリウムを消化汚泥に添加した試料を用いた実験結果から，過剰な水素添加は酢酸からのメタン生成には影響せず，むしろ酢酸生成を阻害すること，酢酸生成能を回復させるため，水素は連続して投入しない方が望ましいと考えられた。以上から，連続式の実プラントでは，酢酸生成が完了した後に水素を添加すべきと考察した。

キーワード：水素，バイオガス，消化汚泥，水素酸化型メタン生成，酸生成細菌