〈論文〉

Use of Exogenous Hydrogen for Thermophilic Digestion of Wastewater Sludge

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Abstract

The feasibility of using hydrogen (H_2) to upgrade biogas during the thermophilic digestion of wastewater sludge was investigated in batch experiments. A high H_2 consumption rate of 32.95～34.13 mL/(g_{VS}・h) was obtained at stirring speed 100 rpm. However, the addition of H_2 inhibited significantly the digestion process. Changing the setting of rectors from horizontal to vertical is an effective method for decreasing the rate of digestion inhibition from 93.70% to 54.24%. Moreover, the required recovery time for methane (CH_4) generation was much long, although digestion process could gradually recover once the hydrogen partial pressure (P_{H2}) become very low. The inhibited digestion from exogenous H_2 was mainly attributed to the increased H_2 concentration in sludge rather than the increased pH or CO_2 depletion. Thus, adding H_2 in the headspace of anaerobic digestor is a feasible method for upgrading biogas as well as avoiding the digestion inhibition.

Key words: hydrogen, digested sludge, methanogens, acid formation bacteria, syntrophic acetate-oxidizing bacteria

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1. Introduction

Biological methanogenesis from wastewater sludge under anaerobic conditions is a highly sustainable waste treatment process due to the conversion of organic content into renewable bioenergy in the form of methane (CH_4) before incineration or landfill treatment. Anaerobic digestion is a complex biological process typically involving hydrolysis, acidogenesis, acetogenesis, and methanogenesis[1]. Complex organic matter (primary polymers of carbohydrates and proteins) is first converted into soluble organic matter, and the hydrolysis products are subsequently fermented to form various intermediated products, such as volatile fatty acids (VFAs), followed by the conversion of these VFAs into acetic acid, hydrogen (H_2), and carbon dioxide (CO_2). Finally, methanogens utilize the acetic acid and H_2/CO_2 as substrates to produce CH_4. **Fig. 1** shows the general pathway of the anaerobic digestion process[1].

Although acetate is one of the methanogenic substrates, H_2 is also an important precursor of hydrogenotrophic methanogens, as it can be used as an electron donor for conversion of CH_4 by reducing CO_2. During anaerobic digestion, about 30% of the CH_4 is produced directly from H_2/CO_2 by hydrogenotrophic methanogens[2]. Furthermore, some studies found that syntrophic acetate oxidation coupled to hydrogenotrophic methanogenesis was a dominant pathway in thermophilic methanogenic reactors[3]. Thus, upgrading biological biogas (primarily CH_4 and CO_2) by adding exogeneous H_2 can remove CO_2 and simulta-

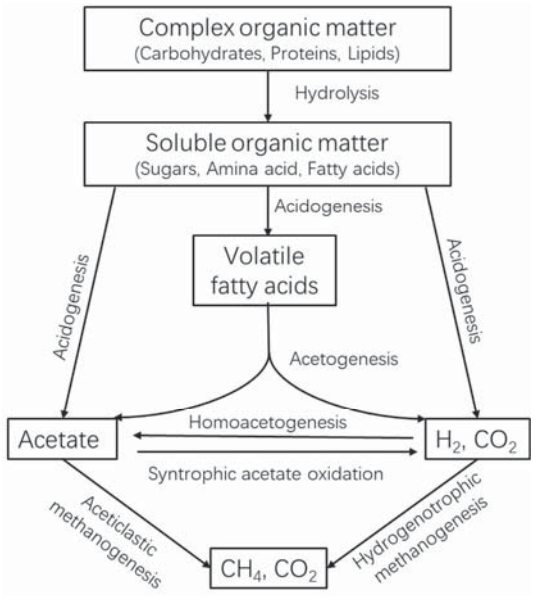

Fig. 1 General pathway of the anaerobic digestion process[1]

neously increase CH_4 yield by accelerating the activity of hydrogenotrophic methanogens.

H_2, an intermediate product of digestion, can be produced from organic waste via biological fermentation, but yields are limited[4]. Hydrogenotrophic methanogens are able to consume an equimolar amount of four times H_2 to CO_2 and generate CH_4 as per Eq. (1).

$$4H_2+CO_2 \rightarrow CH_4+2H_2O,\ \Delta G^0=-131\ \text{kJ mol}^{-1} \quad (1)$$

1 atm, 298 K, pH=7, H_2 partial pressure=1 atm,

However, anaerobic digestion is a complicated biochemical process catalyzed by a range of microorganisms. Therefore, although the addition of H_2 enhanced the biological conversion of CO_2 to CH_4 via hydrogenotrophic methanogens, the associated increase in dissolved H_2 would influence the production of H_2 in the metabolic pathways of bacteria. This is due to thermodynamics, which dictate the feasibility of fatty acid oxidation, such as from propionate (Eq. (2)) and butyrate (Eq. (3)) to acetate via acetogenesis[5].

$$CH_3CH_2COOH+2H_2O \rightarrow CH_3COOH+CO_2+3H_2\ \Delta G^0=+76.1\ \text{kJ mol}^{-1} \quad (2)$$

$$CH_3CH_2CH_2COOH+2H_2O \rightarrow 2CH_3COOH+2H_2\ \Delta G^0=+48.3\ \text{kJ mol}^{-1} \quad (3)$$

1 atm, 298 K, pH=7, H_2 partial pressure=1 atm, 1.0 mol/L of propionate and butyrate

Under standard conditions, these reactions remain endergonic and become feasible only when hydrogen partial pressure (P_{H2}) is maintained below 10 Pa[5]. This extremely low P_{H2} was sustained by interspecies H_2 transfer between acetogens and hydrogenotrophic methanogens. This balance of immediate production and consumption of H_2, and the associated transfer of electrons, is affected by the addition of exogeneous H_2.

Several studies have applied H_2 with the aim of improving biogas quality[4]. One approach is *ex-situ* upgrading in an adjacent external reactor, and another involves injection of H_2 into the reactor (*in-situ* upgrading). In an *ex-situ* system, the initial stages of anaerobic digestion (hydrolysis and acidogenesis) are not present. Thus, reactor stability and performance depend only on sufficient provision of CO_2 (e. g., via a fermentation process), H_2, essential nutrients, and hydrogenotrophic methanogens. However, in an *in-situ* digestion system, H_2 would reduce the biochemical potential of anaerobic digestion. Successful upgrading of biogas processes *in situ* by the addition of H_2 during anaerobic digestion have been reported. Luo's study showed that, in the case of thermophilic co-digestion of manure and acidic whey, the pH of the anaerobic reactor could be maintained below 8.0 with the addition of H_2, which did not inhibit the anaerobic process[6]. In Agneessens's study, when the headspace CO_2 concentration increased above 12%, CH_4 production recovered in a mesophilic co-digestion of sludge and straw, although the pH was still 8.27±0.05. This suggests that the inhibition of hydrogenotrophic methanogens due to a low CO_2 concentration was more pronounced than the effect of the increase in reactor pH[7]. Alfaros's study showed that organic matter removal in a mesophilic upgrading reactor for thickened mixed sludge was not compromised by supplying H_2, or by the high pH level when H_2 was added through a submerged membrane that make H_2 utilized by the biofilm attached on the surface of the membranes[8].

Wastewater sludge, as a by-product of wastewater treatment, can be recovered as energy. There are two methods of recycling wastewater sludge as fuel: carbonization (biochar) and digestion (biogas)[9]. Biochar can be sold to coal fire power plants, but it is very cheap and wastewater treatment plants (WWTPs) sometimes have to pay the transportation costs. To compensate, CH_4 in biogas could supply electricity to WWTPs or even the district grid. Upgrading biogas by the addition of H_2 (e. g., via electrolysis of surplus renewable electricity) would promote energy recovery from sludge and thus indirectly reduce greenhouse gas emissions.

In our pervious study[10], the effect of H_2 addition on mesophilic digestion of wastewater sludge was investigated in batch experiments. For the mesophilic digestion of wastewater sludge, addition of H_2 enhanced the conversion of CO_2 to CH_4; H_2 addition increased pH value and soluble chemical oxygen demand (SCOD); H_2 addition did not influence acetic acid degradation, but inhibited butyric acid degradation which is a substrate of acetogenesis; CH_4 production decreased as the H_2 injection frequency increased if the amount of injection is constant. Inhibition of H_2 can be alleviated if H_2 was added in the later period of a 24-h digestion experiment in that moment acetogenesis is almost complete. But CH_4

production from exogenous H_2 was limited since H_2 consumption rate is low.

Thermophilic digestion has more efficient biogas production capacity compared to mesophilic. The higher heat allows the digestion to operate at greater efficiency, handling more organic material in less time as the rate of bacterial activity increases with temperature. This increased efficiency can reduce digester retention times. But on the other side, thermophilic digestion is more sensitive since its biodiversity of the microbiome is lower. Consequently, the performance of thermophilic digestion with H_2 addition may differ from that of mesophilic digestion.

In this study, batch experiments were carried out to evaluate the H_2 consumption rate and effect of exogenous H_2 on the digestion process. The aim of this work was to determine whether the biogas produced by the thermophilic digestion of wastewater sludge could be upgraded by the addition of H_2.

2. Materials and methods

2.1 Sludge samples

Thermophilic digested sludge collected from a thermophilic digestion reactor in a WWTP（WWTP A）in Osaka City was used as a source of inoculum. The characteristics of the digested sludge are summarized in **Table 1**.

Table 1　Characteristics of thermophilic digested sludge

	Thermophilic digested sludge
TS（g/L）	17.93～18.10
VS（g/L）	12.17～12.25
pH	8.09～8.14
SCOD（mg/L）	2530～2580
Acetate（mg/L）	39.19～40.20
C（%）	31.45～31.46
H（%）	5.27～5.36
N（%）	4.86～4.97

2.2 Batch experiments

The preliminary experiment aimed to determine the CH_4 potential of sludge and optimum time scales for the experiments; The procedure is the same as the previous study[10]: 122-mL serum bottles were used as reactors and 42-mL thermophilic digested sludge was added to each bottle as an inoculum. The bottles were purged with N_2（99.5%; Sumitoto Seika Chemicals）for 2 min and then sealed with butyl rubber stoppers and aluminum crimps. Three groups of reactors were placed horizontally（**Chart 1**）in the rotary shaker（BT300 water bath incubator; Yamato）at 55 ℃ and shaken at 100 rpm.

Chart 1　Serum bottle was were placed horizontally

Batch experiment 1 aimed to estimate the H_2 consumption rate under thermophilic conditions. The reactors with sludge were prepared in the same way as in the preliminary experiment. The bottles were then incubated at 55 ℃ for 1 h to stimulate the microorganisms in the sludge. Then, 80 mL H_2（99.99%; GL Science）was injected into the bottle using a syringe. The bottles were placed horizontally in the rotary shaker at 55 ℃ and shaken at two different speeds（50 and 100 rpm）.

Batch experiment 2 was carried out to investigate the effect of the addition of H_2 on VFA degradation. The reactors with sludge were prepared in the same way as in the preliminary experiment. The bottles were incubated at 55 ℃ for 24 h to decompose the original VFA into sludge. Then, different volumes of H_2 were injected into the bottles. Three conditions were tested（A: 1 mL of water added to the sludge, B: 1 mL of 0.446 mol/L acetic acid added to the sludge, C: 1 mL of 0.223 mol/L butyric acid added to the sludge）. Acetic acid and butyric acid were used in experiment 2 since they are substrate of methanogenesis and acetogenesis, respectively. And the does of acid is excessive. These liquids and H_2（0, 20, 40, 60, 80 mL）were injected using a syringe. The three groups of reactors were placed horizontally in the rotary shaker at 55 ℃ and shaken at 100 rpm.

Batch experiment 3 was carried out to determine how to relieve digestion inhibition due to exogenous H_2; 122 mL serum bottles were used as reactors and 42 mL thermophilic digested sludge was added to each bottle. After that, the bottles were divided into four groups（D, E, F and G）. Propionic acid was used to adjust the pH in group D to 6.55, 7.04, and 7.47. In group E, different volumes（10, 15, 20, and 30 mL）of CO_2 were injected into the bottles. The bottles were purged with N_2 for 2 min and then sealed with butyl rubber stoppers and aluminum crimps, and the bottles were incubated at 55 ℃ for 1 h. Then, 60 mL H_2 was injected into the bottles in groups D and E, which were

placed horizontally in the shaker at 55 ℃ (shaking speed=100 rpm). In group F, 60 mL H_2 was injected into the bottles at the beginning of the experiment, 3, 6, and12 h intervals, respectively. The bottles were also placed horizontally in the shaker at 55 ℃ (shaking speed=100 rpm). In group G, different volumes (40, 60, 80, and 100 mL) of H_2 were injected into the bottles, which were placed vertically in the rotary shaker 100 rpm.

Three experiments were performed in triplicate; bottles containing only digested sludge without H_2 were used as controls.

2.3 Analytical procedures

The soluble chemical oxygen demand (SCOD), total solids (TS) and volatile solids (VS) were determined according to the Standard Methods.[11] Gas samples were taken from the bottles to analyze the CO_2, H_2 and CH_4 contents by micro gas chromatography (micro GC) (490 Micro GC; Agilent Technologies). The pH was measured using a pH meter (D-71S; Horiba). VFAs were measured using high-performance liquid chromatography (HPLC; CDD-10AVP; Shimadzu), Prior to the analysis, the samples were centrifuged at 8,000 rpm for 10 min, and the supernatant was then filtered using a 13-mm-diameter filter (pore size= 0.45 μm) to remove any remaining contaminants. CHN analysis was performed using an elemental analyzer (Micro Corder JM10; J-Science Lab).

3. Results and discussion

In this study, biogas volume is the total gas volume measured by syringe of appropriate range (**Chart 2**).

Chart 2 The gas volume was measured by syringe

The volume of each gas was calculated based on total gas volume and the concentration analyzed by micro GC.

Fig. 2 shows the biogas production of 42 mL sludge at 8, 24, 48, 72 h. The CH_4 production of the digested sludge was almost complete after 24 h and degradable organic compounds were scarce in the samples.

The amount of H_2 in the following H_2 addition experiments was based on the CO_2 yield. The biogas (primarily CH_4 and CO_2) yield of the digestion reactor in WWTP A plant is 0.59～0.73 Nm^3/m^3_{sludge}, and the average CO_2 content is 39.2%.[12] **Table 2** shows the H_2 requirements if all CO_2 is covered with H_2. Under the basis of these values, between 40 and 80 mL of H_2 was added for a sludge volume of 42 mL.

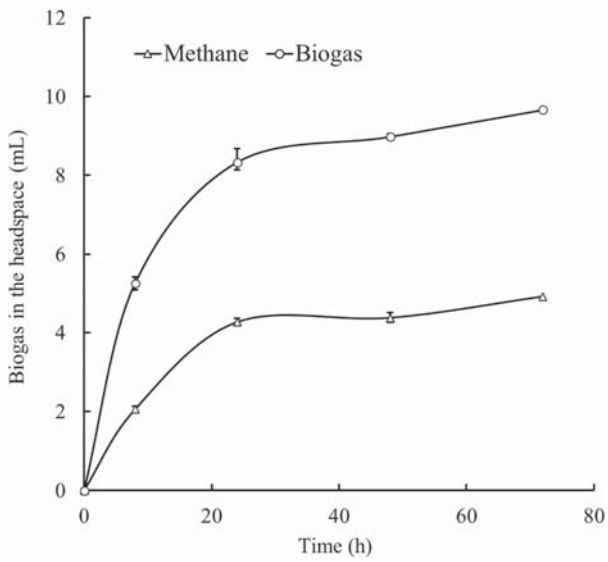

Fig. 2 Biogas productions of 42 mL sludge at 8, 24, 48, and 72 h

Table 2 Requirements for H_2 to react with all of the CO_2

	Co_2 yield ($mL/mL_{sludge}/d$)	H_2 requirement ($mL/mL_{sludge}/d$) [a]	H_2 requirement ($mL/mL_{sludge}/d$) [b]
Min	0.23	0.92	1.56
Max	0.29	1.16	1.80

a: CH_4 from H_2 consumed based on the Sabatier equation (4 mol of H_2 is required to convert 1 mol of CO_2 into CH_4)

b: The amount of dissolved CO_2 in sludge is 0.16 mL/mL_{sludge}, based on the mole fraction of CO_2 in the liquid phase of 0.00131, given that the partial pressure of CO_2 is 40 kPa when the temperature is 55 ℃ [11]

3.1 Hydrogen consumption rate

In experiment 1, 80 mL of H_2 was added to the sludge and digested at different shaking speeds. H_2 uptake was rapid, with 79.83% and 100% of H_2 being consumed within 5 h at 50 rpm and 100 rpm, respectively. The amount of dissolved H_2 can be ignored because the solubility of H_2 in water is very low (18.2

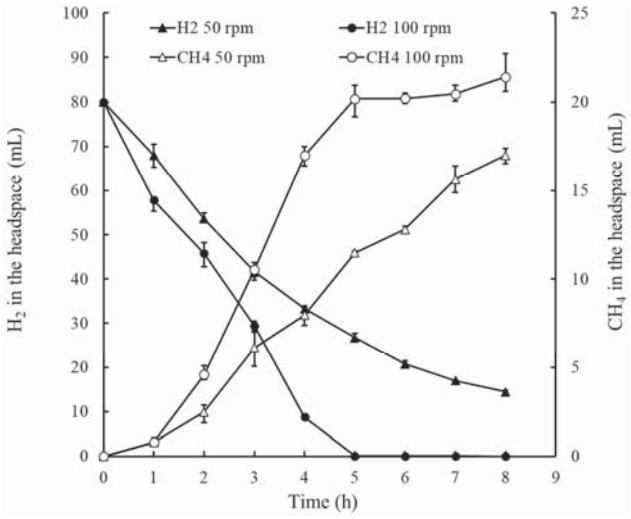

Fig. 3　Variations of H_2 and CH_4 in the headspace with digestion time in batch experiments carried out at 55 ℃, at shaking speeds of 50 and 100 rpm

mL/L, 20 ℃, 1 atm). **Fig. 3** shows that increasing the shaking speed to improve the H_2 gas-liquid mass transfer rate could accelerate the rate of conversion from H_2 to CH_4. The H_2 consumption rate (32.95～34.13 mL/(g_{VS}・h) was evaluated at 100 rpm and a linear downward trend was seen, with microbial activity or CO_2 production rate being the limiting factor, not the H_2 gas-liquid mass transfer rate[14]. Compared to our previous results for mesophilic digestion, in which the H_2 consumption rate was only 9.42～11.22 mL/(g_{VS}・h), thermophilic digestion had a greater ability to upgrade biogas through biological conversion of CO_2 to CH_4 via the addition of H_2.

According to Eq. (1) $4H_2+CO_2=CH_4+2H_2O$, the limiting factor is microbial activity or CO_2 production rate when H_2 is sufficient. Given that a shortage of CO_2 may limit the consumption rate of H_2, another experiment was conducted after 24 h preliminary digestion to produce more CO_2, but a similar H_2 consumption rate was obtained. The result showed that the gas production didn't increase with CO_2 increase. It can indicate that limiting factor of H_2 consumption rate is hydrogenotrophic methanogenic activity when H_2 is sufficient.

3. 2　Effects of exogenous hydrogen on digestion

Two processes can be applied to utilize acetate in anaerobic digestion. The first process is aceticlastic methanogenesis : in this reaction, acetate is cleaved to methyl and carboxyl groups. The methyl group is directly converted to CH_4 via several biochemical reactions, whereas the carboxyl group is oxidized to CO_2[14]. This reaction is not affected by the addition of H_2, because H_2 is neither a product nor a substrate.

Homoacetogens are another group of anaerobes that can use H_2 and CO_2 to form acetate instead of CH_4. When exogenous H_2 is introduced into an anaerobic digestion process, the elevated P_{H2} stimulates homoacetogenesis[15].

In batch experiment 2, no H_2 was observed in group A after 8 h, and little change in acetic acid concentration was seen : Acetic acid concentration was 42.1, 43.0, 41.2, 41.4, ,40.3 mg/L when addition of H_2 was 0, 20, 40, 60, 80 mL, respectively. and ; this indicated that there is no acetic acid accumulation. and the added H_2 had been completely consumed and converted into CH_4. Even if H_2 was consumed by homoacetogenesis, it contributed to acetate production and subsequent acetoclastic CH_4 formation.

The second process is syntrophic acetate oxidation[16]. In this reaction, acetate is converted to H_2 and CO_2 by syntrophic acetate-oxidizing bacteria, as shown in Eq. (4). This reaction requires close cooperation with hydrogenotrophic methanogens. The direct consumption of H_2 by hydrogenotrophic methanogens drives the thermodynamically unfavorable acetate oxidation :

$$CH_3COOH+2H_2O \rightarrow 4H_2+2CO_2\ \Delta G^0= +95\ \mathrm{kJ\ mol^{-1}} \quad (4)$$

1 atm, 298 K, pH＝7, H_2 partial pressure＝1 atm, 1 mol/L of acetate

The increasing H_2 concentration caused by exogenous H_2 inhibits the activity of syntrophic acetate-oxidizing bacteria, resulting in the inhibition of acetic acid degradation. However, the inhibition of exogenous H_2 on syntrophic acetate-oxidizing bacteria could be eliminated upon depletion of H_2 (H_2 was consumed by hydrogenotrophic methanogens).

The results of batch experiment 2 for group B revealed (**Fig. 4**) CH_4 production over the 8-h period following the addition of H_2 and acetic acid after 24 h of preliminary digestion. After 24 h of preliminary digestion, CH_4 production from sludge is rare, so most of the CH_4 produced after 24 h came arose the exogenous H_2 and acetic acid. The CH_4 produced from acetic acid can be calculated by subtracting the CH_4 produced from exogenous H_2 from the total amount of CH_4 produced. No H_2 was observed, indicating that the added acetic acid was utilized during aceticlastic methanogenesis

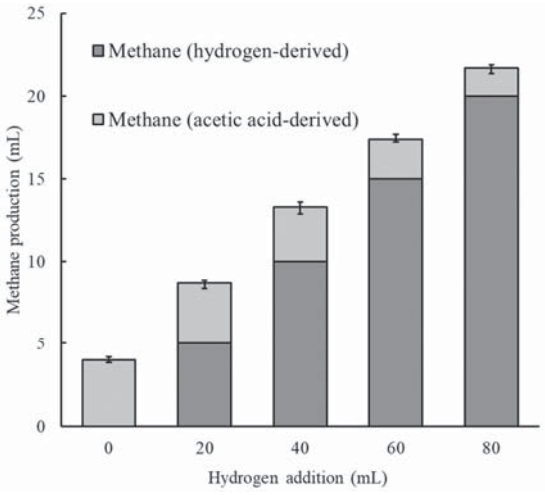

Fig. 4 CH_4 production within an 8-h period with different amounts of added H_2 added in group B (sludge+acetic acid)

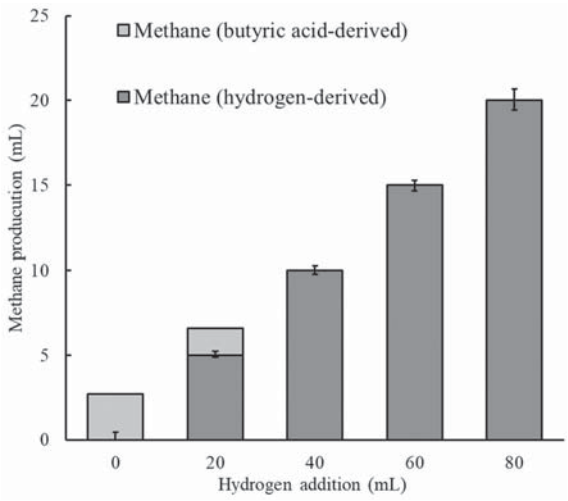

Fig. 5 CH_4 production within an 24-h period with different amounts of H_2 added in group C (sludge+butyric acid)

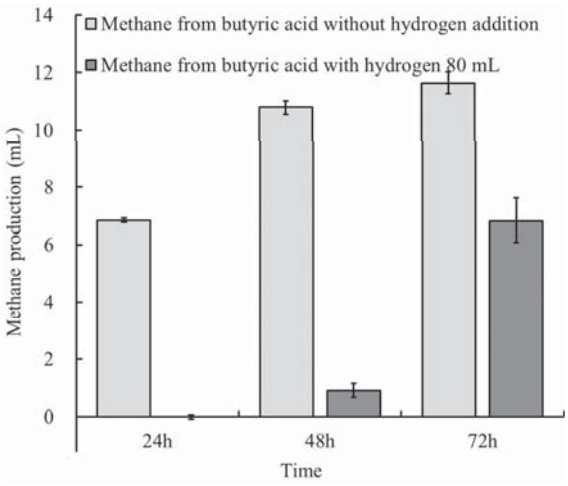

Fig. 6 CH_4 production within a 72-h period with the addition of H_2 and butyric acid in group C (sludge+butyric acid)

or syntrophic acetate oxidation combined with hydrogenotrophic methanogenesis. The production of CH_4 from acetic acid can indicate the degree of degradation of acetic acid.

During an 8-h period, 3.99 mL CH_4 was converted from acetic acid without the addition of H_2, while CH_4 produced from acetic acid decreased to 93.01%, 82.47%, 60.17%, 42.91% when 20, 40, 60, 80 mL H_2 was added, respectively. This indicated that the addition of H_2 could indirectly affect hydrogenotrophic methanogenesis by inhibiting syntrophic acetate oxidation.

We observed CH_4 production in group C after 24 h because butyric acid is more difficult to degrade than acetic acid. After the 24-h preliminary digestion period, most of the CH_4 produced arose from the exogenous H_2 and butyric acid, for the same reason as in group B. Acetic acid converted from butyric acid can be neglected, because it can be utilized to form CH_4 via aceticlastic methanogenesis. The amount of CH_4 produced from butyric acid can be calculated by subtracting the CH_4 produced from exogenous H_2 from the total amount of CH_4 produced. However, according to **Fig. 5**, when 20 mL of H_2 was added, 58.2% more butyric acid was converted into CH_4 compared to the reactor without added H_2. This shows that exogenous H_2 strongly inhibited the degradation of butyric acid, suggesting that H_2 inhibited the acetogenesis process. Adding H_2 to a biogas reactor may cause problems due to an increase in the P_{H2} of the biogas reactor, which inhibits acidogenesis.

When more than 40 mL H_2 was injected, the degradation of butyric acid was completely inhibited. This could be attributed to the higher P_{H2}, higher pH (8.09, 8.41 and 8.59 with the addition 40 mL, 60 mL and 80 mL H_2, respectively), or depletion of CO_2., which rely on CO_2 as a carbon source.

The experimental time was extended to investigate the recovery of digestion following the addition of H_2 (**Fig. 6**) ; 80 mL H_2 was consumed by 42 mL sludge within the first 5 h, but butyric degradation took a long time to recover. Butyric acid started to degrade into CH_4 by the 48^{th} h. During the following 24 h, CH_4 production in group C returned to normal compared to the first 24 h without added H_2 (**Fig. 6**).

To test the hypothesis that low concentrations of CO_2 could inhibit hydrogenotrophic methanogen substrates, in batch experiment 3 the pH was adjusted and CO_2 was added.

3.3 Cause of the digestion inhibition induced by hydrogen addition

The aim of this research was to upgrade biogas in an

anaerobic digester and minimize the inhibition of digestion. There are three possibilities regarding the effect of the addition of H_2 on digestion.

(1) H_2 injection increases pH (because of CO_2 removal) which may limit the methanogenic activity ;
(2) Depletion of CO_2 could inhibit the substrate for hydrogenotrophic methanogens, which relies on CO_2 as a carbon source ;
(3) An increase in dissolved H_2 can subsequently inhibit VFA degradation.

These phenomena were verified in subsequent experiments.

The consumption of H_2 and CO_2 by hydrogenotrophic methanogens could increase the pH. According to Luo, in the case of co-digestion of manure with acidic whey, the pH in the anaerobic reactor with H_2 addition could be maintained below 8.0, which did not inhibit the anaerobic process[6].

Propionic acid was used to adjust the pH in this experiment, because it is more difficult to degrade than acetic and butyrate acid. Different amounts of propionic acid were added to the sludge, and 80 mL H_2 was then injected into the bottles. Bottles containing only sludge and propionic acid, without H_2, were used as controls. **Fig. 7** shows that reducing the initial pH in group D did not relieve the inhibition of CH_4 production.

The pH value could be maintained by supplying CO_2, which also stimulated hydrogenotrophic methanogens to consume H_2 and, subsequently, syntrophic acetate oxidation. However, we observed the same results in group E as in group D, namely that the inhibition was not suppressed.

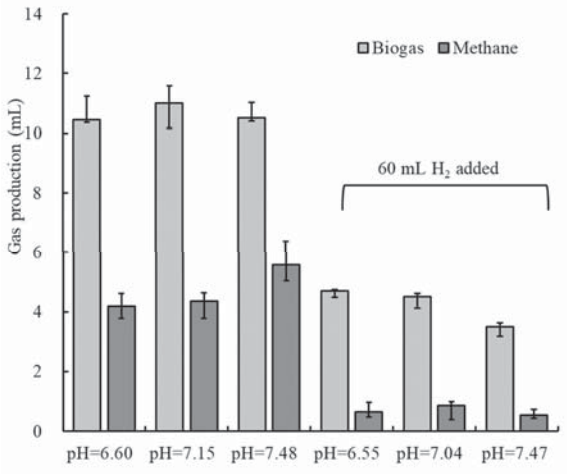

Fig. 7 Group D : biogas production from sludge with pH adjust (initial) after a 24-h digestion period

Under the premise that the dissolved H_2 concentration has an impact on sludge, 122-mL serum bottles (diameter : 40 mm, height : 130 mm), used as reactors, were placed horizontally in the rotary shaker and shaken at 100 rpm to consume H_2 within a short time (80 mL H_2 was consumed by 42 mL sludge in 5 h), because a large gas-liquid interface ensures sufficient mixing of H_2 and sludge. This operation reduces the H_2 consumption time, but all microorganisms, especially VFA-degrading bacteria, are exposed to high concentrations of H_2. Group F was carried out to was performed to determine the effect of P_{H2} on CH_4 production. H_2 was supplied frequently to lower H_2 partial pressure, but it had no effect on gas production even if P_{H2} went down by 1/8 (injection every 3 h). This result was different from that in mesophilic digestion condition in which CH_4 production decreased as the H_2 injection frequency increased if the amount of injection is constant. The possible reason is that difference of the microbial community in mesophilic and thermophilic digestion system.

On the other hand, if only part of the sludge is exposed to H_2, the H_2 can be consumed (80 mL H_2 was consumed by 42 mL sludge in 24 h) such that the degradation of organic matter may proceed without interference. When the serum bottles were placed vertically in the rotary shaker, the small gas-liquid interface allowed only the sludge on the surface to be exposed to H_2 due to the shape of the serum bottles. **Table 3** shows that in group G, CH_4 production from sludge was increased when all the bottles were placed vertically in the rotary shaker and shaken at 100 rpm comparing to the results in Group A which were shaken at the same speed but placed horizontally.

The inhibition rate of digestion has been used in our previous study to describe the performance of digestion with H_2 addition[10].

Theoretically, one-unit volume of CO_2 could convert

Table 3 CH_4 and CO_2 production with different amounts of H_2 after a 24-h digestion period

	Group	H_2 : sludge (mL : mL)	CO_2 (mL)	$CH_{4sludge}$ (mL)	CH_{4total} (mL)	Inhibition (%)
Horizontal	A	0 : 42	8.69	6.56	6.56	0.00
		40 : 42	4.73	1.33	11.33	79.67
		60 : 42	2.96	0.72	15.72	89.04
		80 : 42	1.03	0.41	20.41	93.70
Vertical	G	40 : 42	5.16	4.12	14.12	37.22
		60 : 42	3.07	3.18	18.18	51.61
		80 : 42	1.20	3.00	23.00	54.24

Inhibition is the rate of inhibition of CH_4 production $(1-CH_{4sludge} / 6.56) \times 100$
6.56 (mL) is CH_4 production without H_2 addition

into one-unit volume of CH_4. The amount of CH_4 obtained from sludge digestion with H_2 can be calculated by quantifying the decrease in H_2 volume, because all of the H_2 is converted into CH_4 in accordance with Eqs. (5) and (6):

$$CH_{4(a)} = CH_{4(total,\ a)} - CH_{4(H2+CO2,\ a)} \quad (5)$$

$$CH_{4(H2+CO2,\ a)} = H_{2(a)}/4 \quad (6)$$

$CH_{4(a)}$: volume of CH_4 produced from sludge while the initial volume of H_2 is a mL ;

$CH_{4(total,\ a)}$: total volume of CH_4 in sludge with added a mL H_2 ;

$CH_{4(H2+CO2,\ a)}$: volume of CH_4 arising from the conversion of a mL H_2 ;

$H_{2(a)}$: volume of H_2 ;

$CH_{4(0)}$: volume of CH_4 produced from sludge without added H_2.

The inhibition rate of digestion can be calculated as follows :

$$\text{Inhibition rate of digestion} = CH_{4(a)} / CH_{4(0)} \quad (7)$$

Based on Eq. (7), **Table 3** shows that digestion inhibition in Group G was significantly decreased. A significant increase in CH_4 production was observed (100 mL H_2 was not completely consumed ; not included in the table). The rate of digestion inhibition decreased form 79.67%, 89.04% and 93.70% to 37.22%, 51.61% and 54.24% with the addition of 40, 60, and 80 mL H_2, respectively. On the basis of this result, H_2 should be injected from the middle or above instead of the button of single-phase digestion reactor or through a submerged membrane[17] to reduce sludge exposed to H_2.

4. Conclusion

In this study, the effects of exogenous H_2 on the thermophilic digestion of wastewater sludge from a Japanese treatment plant were investigated in batch experiments. The H_2 strongly inhibited the degradation of butyric acid and mildly inhibited the degradation of acetic acid. As an intermediate, the addition of H_2 inhibited both the acetogenesis process and syntrophic acetate oxidation.

Furthermore, the negative effect of H_2 could not be mitigated by decreasing the pH, adding CO_2, or decrease P_{H2} but was decreased by reducing the size of the contact area between the sludge and H_2 (vertical setting of reactors). The digestion recovered gradually when the P_{H2} was very low. However, the recovery time was much longer than the H_2 consumption time. These phenomena disrupt the anaerobic digestion of wastewater sludge.

From an efficiency and economic point of view, our results suggest that the way of H_2 addition should follow the principle of reducing H_2 exposure such as adding from the middle or above instead of the button or through a submerged membrane in the single-phase digestion reactor. And given the high efficiency of CH_4 converted from H_2 in thermophilic digestion, ex-situ upgrading in an adjacent external reactor is a potential upgrading technology because it can be easily integrated with existing biogas plants while avoiding negative influences of exogenous H_2.

Reference

1) D. J. Batstone, J. Keller, I. Angelidaki, S. V. Kalyuzhnyi, S. G. Pavlostathis, A. Rozzi, W. T. M. Sanders, H. Siegrist and V. A. Vavilin : The IWA Anaerobic Digestion Model No 1 (ADM1). Water Sci Technol, Vol. 45, No. 10, pp. 65-73 (2002)

2) D. G. Mulat, A. J. Ward, A. P. S. Adamsen, N. V. Voigt, J. L. Nielsen, and A. Feilberg : Quantifying contribution of syntrophic acetate oxidation to methane production in thermophilic anaerobic reactors by membrane inlet mass spectrometry. Environmental science & technology, Vol. 48, No. 4, pp. 2505-2511 (2014)

3) M. Blaut : Metabolism of methanogens. Antonie Van Leeuwenhoek, Vol. 66, No. 1-3, pp. 187-208 (1994)

4) I. Angelidaki, L. Treu, P. Tsapekos, G. Luo, S. Campanaro, H. Wenzel, P. G. Kougias : Biogas upgrading and utilization : current status and perspectives. Biotechnol Adv, Vol. 36, pp. 452-466 (2018)

5) S. Fukuzaki, N. Nishio, M. Shobayashi and S. Nagai. Inhibition of the fermentation of propionate to methane by hydrogen, acetate, and propionate. Appl Environ Microbiol, Vol. 56, pp. 719-723 (1990)

6) G. Luo and I. Angelidaki : Co-digestion of manure and whey for in situ biogas upgrading by the addition of H_2 : process performance and microbial insights. Applied Microbiology and Biotechnology Vol. 97, No. 3, pp. 1373-1381 (2013)

7) L. M. Agneessens, L. D. M. Ottosen, N. V. Voigt, J. L. Nielsen, N. de Jonge, C. H. Fischer and M. V. W. Kofoed : In-situ biogas upgrading with pulse H2 additions : The relevance of methanogen adaption and inorganic carbon level. Bioresource technology, Vol. 233, pp. 256-263 (2017)

8) N. Alfaro, M. Fdz-Polanco, F. Fdz-Polanco and I. Díaz : H_2 addition through a submerged membrane for in-situ biogas upgrading in the anaerobic digestion of sewage sludge. Bioresource technology, Vol. 280, pp. 1-8 (2019)

9) Ministry of the Environment, Japan. Solid waste management and recycling technology of Japan. (2012)

10) C. Li, K. Oshita, M. Takaoka, T. Fujimori and T. Kusakabe : Biomethanation by Hydrogen-supplemented Mesophilic Anaerobic Digested Sludge. EICA, Vol. 23, pp. 101-108 (2018)

11） Standard methods for the examination of water and wastewater. 19th edn, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.（1995）
12） Monthly report of digestion tank operation 2016FY provided from WWTP A in Osaka city, Japan（2017）
13） D R. Lide：CRC handbook of chemistry and physics：a ready-reference book of chemical and physical data. CRC press.（1995）
14） G. Luo, S. Johansson, K. Boe, X. Li, Q. Zhou and I. Angelidaki：Simultaneous hydrogen utilization and in situ biogas upgrading in an anaerobic reactor. Biotechnology and bioengineering, Vol. 109, No. 4, pp. 1088-1094（2012）
15） R. Liu, X. Hao, and J. Wei：Function of homoacetogenesis on the heterotrophic methane production with exogenous H_2/CO_2 involved. Chemical Engineering Journal, Vol. 284, pp. 1196-1203（2016）.
16） R. I. Mackie and M. P. Bryant：Metabolic activity of fatty acid-oxidizing bacteria and the contribution of acetate, propionate, butyrate, and $CO_{(2)}$ to methanogenesis in cattle waste at 40 and 60 ℃. Appl Environ Microbiol, Vol. 41, pp. 1363-1373（1981）
17） Alfaro. N, Fdz-Polanco. M, Fdz-Polanco. F, and Díaz, I：H2 addition through a submerged membrane for in-situ biogas upgrading in the anaerobic digestion of sewage sludge. Bioresource technology, Vol. 280, pp. 1-8（2019）.

高温消化下水汚泥への外部からの水素添加の影響

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概　要

本研究は，日本における高温消化下水汚泥に対し，外部から水素を添加した場合のバイオガス生成への影響をバッチ実験より調査した。対象汚泥では，主要なメタン生成古細菌は *Methanothermobacter* であったが，酢酸利用メタン生成菌としての *Methanosaeta* 属の存在量は，わずか 28% であった。

H_2添加は酢酸生成と嫌気性酢酸酸化の両方を抑制し，その後メタン生成に影響を与えた。外因性水素の消化阻害による主な理由は，pH 増加や CO_2減少ではなく，H_2濃度増加であった。水素分圧が非常に低くなると，消化は徐々に回復したが，回復時間は水素消費時間より非常に長くなった。

キーワード：水素，高温消化汚泥，メタン生成古細菌，酸生成細菌，嫌気性酢酸酸化細菌