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Contributed Paper

Methane Production from Acidic Effluent Obtained from Hydrogen Fermentation Process of Food Waste Using Continuous Stirred Tank Reactor

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ABSTRACT

This study investigated the effect of hydraulic retention time (HRT) on methane production from the acidic effluent of a hydrogen fermentation process of food waste using a continuous stirred tank reactor (CSTR). The CSTR was operated at 35 ± 3°C with HRTs of 12, 10, 8 and 6 days (d). The hydrogen content, methane yield (MY) and methane production rate (MPR) were increased with a decrease in HRT from 12 to 10 d. At the HRT of 10 d, the maximum methane content of 71.3%, MY of 155 mL CH_4/g-COD and MPR of 4629.1 mL CH_4/L d were achieved. However, a further decrease in HRT from 10 to 8 and 6 d resulted in a decrease of methane content, MY and MPR. The main soluble metabolite products obtained at the HRT of 10 d were butyric and acetic acids. The maximum energy production rate obtained at the optimum HRT of 10 d was 185.31 kJ/L d.

Keywords: biogas production, continuous stirred tank reactor (CSTR), methane

1. INTRODUCTION

Anaerobic digestion (AD) has gained popularity because of its efficiency in the conversion of organic waste, such as food waste, sewage sludge, animal waste and livestock waste, into an energy carrier. The organic waste is converted to methane by a series of biological reactions that include hydrolysis, acidogenesis, acetogenesis and methanogenesis [1]. The advantages of AD include reduction of chemical oxygen demand (COD) of organic waste, pathogens and organic waste odors. Importantly, this process produces hydrogen and methane as energy carrier in which methane is a popular energy carrier that accounting for 2-3 % of the total primary energy

demand in EU countries [4].

In our previous study [5], the continuous hydrogen fermentation from food waste by anaerobic mixed cultures was conducted in which a maximum hydrogen yield of 261 mL H_2/g-volatile solid $(VS)_{added}$ was obtained. During the hydrogen production process, a large amount of acidic effluent containing volatile fatty acids (VFAs) such as butyric and acetic acids was generated. This effluent had a high COD value which could cause environmental problems upon disposal. In order to reduce the COD of this acidic effluent and to obtain the energy carrier i.e., methane, the conversion of the acidic effluent of the hydrogen fermentation process to methane via the methanogenesis stage of AD was conducted.

A continuous stirred tank reactor (CSTR) is commonly used to produce methane due to its highly nonlinear behaviors and wide operating ranges [6]. The keys to achieving a successful methane production performance [1-4] include temperature, substrate concentration, microorganisms, pH, type of substrate, hydraulic retention time (HRT) and reactor configuration. Among these factors, the HRT is the most important parameter because of its substrate uptake efficiency [7].

With regards to the above information, the objective of this research was to optimize the HRT for achieving the maximum methane production efficiency from the acidic effluent of food waste using a CSTR.

2. MATERIALS AND METHODS

2.1 Feedstock

Acidic effluent was obtained from the CSTR reactor used to produce bio-hydrogen from food waste with an initial concentration of 2.54 g-volatile solid (VS)/L-substrate at the optimum HRT of 60 h (equivalent to organic loading rate (OLR) of 0.042 g-VS/L-substrate.h) [5]. The composition of the acidic effluent is shown in Table 1. The pH of the acidic effluent was 5.0. The acidic effluent was stored at -4°C in a refrigerator prior to use.

Table 1. The chemical characteristics of acidic effluent from hydrogen fermentation process of food waste.

Parameter	Concentration (mg/L)
Total chemical oxygen demand (tCOD)	23,000
Butyric acid	3105.9
Acetic acid	2293.2
Propionic acid	239.4
Lactic acid	107.9
Ethanol	661.5

2.2 Microorganisms Preparation

Anaerobic seed sludge was obtained from the full-scale anaerobic digester of an upflow anaerobic sludge blanket (UASB) reactor that belonged to a brewery company in the Northeastern part of Thailand. The UASB is used to produce methane from the wastewater of the beer production process. The pH and volatile suspended solid (VSS) concentration of the sludge were 6.8 and 7.4 g/L, respectively.

2.3 CSTR Operation

CSTR was made from plastic with a total

volume and working volume of 5.0 L and 3.5 L, respectively. The setup of the CSTR is shown in Figure 1. The CSTR contains 2.0 L of seed inoculum and 1.5 L of the acidic effluent. The headspace of the reactor was flushed with nitrogen gas for 15 min to create an anaerobic condition. The reactor was operated at a temperature of 35±3°C.

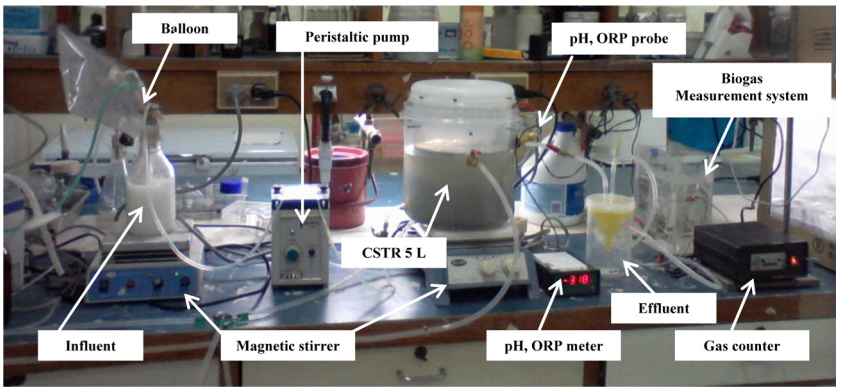

Figure 1. CSTR setup for methane production.

The CSTR was initially operated at a HRT of 12 d which was based on the previous report that the HRT used in methane production is generally 5 times greater than HRT used in hydrogen production [8]. Since the acidic effluent used as the substrate in this study was obtained from the CSTR operated at the optimum HRT of 60 h (5 d) [5], therefore the HRT used in methane production was 300 h (12d). Subsequently, the HRT was shortened to 10, 8 and 6 d by changing the volumetric feeding rate when the steady state of each HRT was reached. The steady state was justified when there was less than 10% variation of biogas production, methane content, methane yield (MY) and methane production rate (MPR). The CSTR was continuously stirred at 120 rpm using a magnetic stirrer. The optimum HRT was determined by the maximum MPR, MY, and methane content.

During operation, gaseous samples, liquid samples, temperature, oxidation-reduction potential (ORP), and pH were recorded. The pH value of the fermentation broth was monitored using a pH meter (pH 190 series, Eutech Instruments, Singapore) and was manually adjusted to 5.0 by adding 2 mol/L NaOH or 2 mol/L of HCl to the influent in order to maintain the pH inside the reactor. The ORP was monitored using an ORP meter (ORP 190 series, Eutech Instruments, Singapore) to ensure that the methane production was carried out under anaerobic condition in which the ORP should be less than -200 mV. Liquid samples were collected to determine the concentrations of soluble metabolite products (SMPs) i.e., VFAs and alcohol (ethanol, butanol).

2.4 Analytical Methods

Biogas composition was measured using a gas chromatograph (GC-2014, Shimadzu) equipped with a thermal conductivity detector (TCD) and a 2 m stainless column packed with shin carbon (50/80 mesh). The GC-TCD condition was set according to the method of Fangkum and Reungsang, 2010 [9].

To carry out the VFAs analysis, the liquid samples were first centrifuged at 6,000 rpm for 10 min. The supernatant was

further filtered through a 0.45 mm nylon acetate membrane and acidified with 0.2 mol/L oxalic acid. The resulting filtrate was further analyzed for VFAs and alcohol concentrations using high performance liquid chromatography (HPLC) (Shimadzu LC-10AD) equipped with an Aminex HPX-87H column and ultraviolet (UV) and refractive index (RI) detectors. HPLC operation conditions were set according to Fangkum and Reungsang, 2010 [9]. VS and VSS were measured according to the procedures described in standard methods [10].

3. RESULTS AND DISCUSSION

3.1 Methane Production in CSTR

The time course profile of methane content, MPR, MY and HRT are depicted in Figure 2. The operational conditions during the fermentation process are summarized in Table 2. Results indicate that HRT has an influence on methane content, MPR and MY. Initially, methane content and MPR were increased with a decrease in HRT from 12 to 10 d (Figure 2). MY was also increased from 106 to 155 mL CH_4/g-COD (Figure 2). A decrease in HRT resulted in an increase in the organic loading rate (OLR), which has a correlation with an increase in substrate concentration. At an appropriate range of substrate concentration, the microorganisms could effectively utilize substrate for their growth and activity as well as for the production of methane. Thus, a maximum methane content, MY and MPR were obtained. A further decrease in HRT to lower than 10 d resulted in a significant decrease in methane content and MPR (Figure 2). MY was significantly decreased from 155 mL to 105 CH_4/g-COD with a decrease in HRT from 10 to 6 d (Figure 2). At a short HRT of 8 to 6 d (i.e., high substrate feeding rate), the methanogenic bacteria can be washed out from the continuous process. Therefore, in order to prevent the biomass washout in the continuous methane fermentation, a recirculation system should be implemented.

In this study, the optimum HRT for maximizing methane content, MY, MPR was 10 d. At the optimum HRT, the maximum methane content, MY and MPR were 71.3%, 155 mL CH_4/g-COD and 4629.1 mL CH_4/L d, respectively. At the optimum HRT, the substrate concentration and flow rate in the fermentation system were suitable for growth and microbial activity, which resulted in a high methane production, MY and MPR. It is worth noting that the maximum methane yield of 155 mL CH_4/g-COD is only 44% of a 350 mL CH_4/g-COD theoretical yield. Therefore, the improvement of methane production such as an addition of Ni and Fe in methane fermentation process [11], an improvement of acidogenesis phase to increase the acetic acid production as well as a blocking the pathways of ethanol, lactic acid and propionic acid production should be conducted. Fe and Ni are the trace elements for the co-enzyme F420 responsible for methane production [11]. Acetic acid is the main substrate that subsequently be converted to methane by acetoclastic methanogen in the methanogenesis phase of AD. Production of ethanol and lactic acid does not yield methane while propionic acid inhibits the methanogenic bacteria [12]. More discussions on the SMPs distribution and low methane yield can be found in section 3.2.

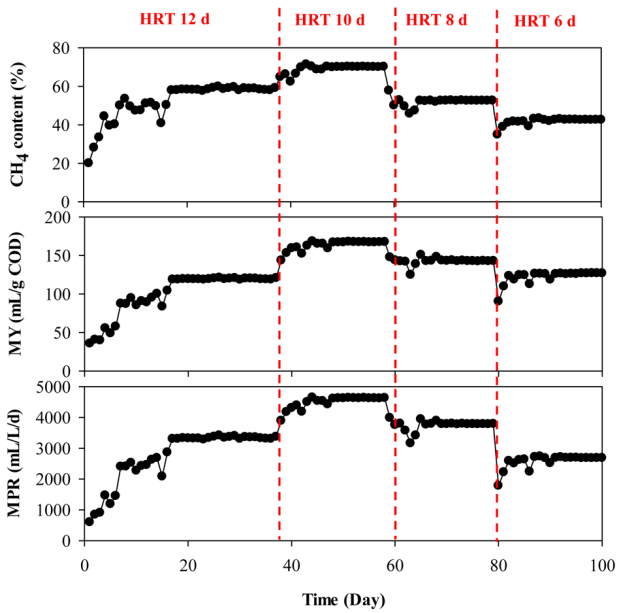

Figure 2. Performance of CSTR reactor: (a) methane content; (b) methane yield and (c) methane production rate.

Table 2. Operational and environmental parameters during methane fermentation process.

Operation days	HRT (days)	Temperature (°C)	pH	ORP (mV)
1-36	12	35	7.0	- 326
37-59	10	35	7.0	- 379
60-79	8	35	7.0	- 421
80-100	6	35	7.0	- 410

3.2 SMPs

Table 3 shows a summary of SMPs concentrations under a steady state condition at each HRT. The concentration and compositions of SMPs at different HRT is a key to describe the performance of reactor. The SMPs ranged from 816.5 to 1910.1 mg/L (Table 3). The total VFAs and SMPs reduced with a decrease in HRT from 12 to 10 d but were increased when the HRT was decreased to lower than 10 d (Table 3). A decrease of HRT from 12 to 10 d resulted in an increase in the consumption of butyric acid, which correlated with the highest MY and MPR obtained at the HRT of 10 d (Figure 2). However, further decreasing the HRT from 10 to 6 d resulted in a decrease in butyric and acetic acids consumption, which correlated with a low MY and MPR.

The distribution of SMPs concentrations were various at different HRT. In all ranges of HRTs, butyric acid, followed by acetic acid, was the main SMPs. Ethanol was found as a minor metabolite with a small amount of propionic and lactic acids, (Table 3). The results in Table 3 indicated that the main metabolic products for the methane production process were acetic and butyric acids. A low concentration of butyric and acetic acids were obtained at the optimum HRT (10 d), which indicated an efficient

methane production from VFAs, since butyric and acetic acids degradation was in general positively correlated to methane production as shown in Eq (1) and (2), respectively. In the Eq (1), the aceticlastic methanogens convert the methyl and carboxyl group of acetate to CH_4 and CO_2 while butyric acid was converted to acetic acid by acetate oxidizing bacteria (Eq. 2). Subsequently, acetic acid was converted to CH_4 by aceticlastic methanogens (Eq.1). Thus, Eq (1) and (2) reveal that 1 mol of acetic and butyric acids can be converted to 1 and 2 mols of methane by methanogenic bacteria [13].

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (1)$$
$$CH_3CH_2CH_2COOH \rightarrow 2[CH_3COOH] \quad (2)$$

These results correlated with the theoretical results, which explained that 70% of methane production in the AD process was obtained from the activity of acetotrophic methanogenic bacteria and less of 30% was obtained from the activity of hydrotrophic methanogenic bacteria [14-15]. A decrease of HRT from 12 to 10 d resulted in an increase in acetic acid concentration, which disagrees with the methane production result (Table 3 and Figure 2). This may be due to an acetogenic bacteria, contained in the substrate, degrading ethanol into acetic acid during the fermentation process [15-16]. A high ethanol concentration observed at the HRTs of 12, 8 and 6 d could be the reason for the low methane production obtained. However, ethanol does not normally inhibit the methanogenic bacteria directly but the previous research has shown that the fermentation process that contained some acidogenic bacteria can convert substrate to ethanol and VFAs [15-16]. The production of ethanol and VFAs indicated that the dominant microorganisms in these HRT were acidogenic bacteria, which resulted in the low methane production [15-16]. The highest concentration of lactic acid (65.2 mg/L) was observed at the HRT 6 d, which implied that lactic acid bacteria could grow and were more active than at the other HRT. The production of lactic acid in the fermentation system lowered the methane production efficiency. The presence of lactic acid could destroy the structure of the sludge granules and further cause the decrease in specific methanogenic activity [17]. The inhibition of lactic acid production can be conducted by adding itaconic acid [18] and using the proton-suicide technique with NaBr and $NaBrO_3$ [19].

At the HRT of 8 and 6 d, an increase in lactic acid concentration correlated with an increase in propionic acid concentration. This result might be due to the lactic acid, which is usually considered to be a precursor of propionic acid during the anaerobic digestion [20-21]. The propionic acid is an undesirable intermediate product in the methanogenesis process [22]. This is because in the AD process, the methanogenesis of propionate was slower, when compared with acetate and butyrate. This is due to the fact that, according to the Gibb's free energy, the conversion of propionate to acetate does not occur until the hydrogen partial pressure is less than 10^3 Pa. However, in practical, the hydrogen partial pressure during the AD process usually exceeds 10^3 Pa [23]. Consequently, the conversion of propionate to acetate is slow or does not occur. In addition, propionic acid inhibits the methanogenic bacteria [12]. Therefore, in order to increase the methane production, it is necessary to avoid the presence of lactic and propionic acids in the AD process.

Table 3. Profiles of metabolites and VFAs in the hydrogenogenic effluent from CH_4-CSTR.

Parameter	Initial	HRT 12 d	HRT 10 d	HRT 8 d	HRT 6 d
Butyric acid (mg/L)	3105.9	279.5	341.6	559.1	683.3
Acetic acid (mg/L)	2293.2	160.5	310.6	496.9	652.2
Propionic acid (mg/L)	239.4	31.1	52.8	68.3	77.6
Lactic acid (mg/L)	107.9	8.6	40.3	55.9	65.2
Ethanol (mg/L)	661.5	445.5	71.2	307.5	431.8
Total VFAs (mg/L)	5746.4	479.8	745.3	1180.2	1478.3
Total SMPs (mg/L)	6407.9	925.3	816.5	1487.7	1910.1

3.3 COD Distribution

Methane production, metabolite production and COD balance are shown in Table 4. The COD balance at the optimum HRT of 10 d was 37.97% error. This error may be due to the microorganism utilized substrate to form the biomass which accounted for approximately 15% of the initial substrate concentration [24]. Left of 22.97% error might have been caused by the microorganisms converting the substrate to other VFAs such as formic acid, valeric acid and hexanoic acid, and to the solvents such as propanol and butanol [16]. Methane is the major product in the fermentation process (53% distribution). The minor metabolites are butyric and acetic acids (Table 4). The highest COD distribution in the methane production indicated that the microorganisms efficiently degrade the acidic effluent obtained from the hydrogen fermentation process into methane. The energy production rate was calculated based on the MPR, density of methane (0.72 mg/ml) and heating value of the methane (55.6 kJ/g), respectively. The MPR obtained at the various HRT (12, 10, 8 and 6 d) were 3374.9, 4629.1, 3936.2 and 2693.7 mL CH_4/L d, respectively. Therefore, energy production rate at the various HRT are as follows:

$[(3374.9 \times 0.72 \times 55.6)/1000] = 135.10$ kJ/L d. (HRT = 12 d)

$[(4629.1 \times 0.72 \times 55.6)/1000] = 185.31$ kJ/L d. (HRT = 10 d)

$[(3936.2 \times 0.72 \times 55.6)/1000] = 157.57$ kJ/L d. (HRT = 8 d)

$[(2693.7 \times 0.72 \times 55.6)/1000] = 107.83$ kJ/L d. (HRT = 6 d)

The maximum energy production rate was 185.31 kJ/L d, which coincides with the maximum MPR obtained. The variation of HRT resulted in the variation of MPR and also affected the energy production rates. The results indicated that the optimization of HRT for methane production was the key factor in obtaining the maximum energy production rate and efficient methane production performance.

Table 4. Methane production, metabolites production and COD balance at the end of fermentation (HRT 10 days).

Products	Concentration (mg/L)	Concentration (g-COD/L)	COD distribution (%)
COD consumption	14207.00	14.207	-100.00
Methane	1901.46	7.59	53.40
Butyric acid	341.60	0.62	4.38
Acetic acid	310.60	0.33	2.34
Propionic acid	52.80	0.08	0.56
Lactic acid	40.30	0.04	0.30
Ethanol	71.20	0.15	1.05
Balance			-37.97

4. CONCLUSIONS

HRT plays an important role in the production of methane using CSTR. The results show that an HRT of 10 d was suitable for methane production from the acidic effluent obtained from a hydrogen fermentation process in which a maximum methane content (71.3%), MY (155 mL CH_4/g-COD) and MPR (4629.1 mL CH_4/L d) were obtained. The main metabolite products were butyric and acetic acids. At the optimum HRT the maximum energy production rate was 185.31 kJ/L d.

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