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Evaluation of the Degradation of Carbohydrate-based Material During Anaerobic Digestion for High-efficiency Biogas Production

Min-Jee Kim[1], Sang-Hun Kim[1]*

[1]Department of Biosystems Engineering, College of Agriculture and Life Sciences, Kangwon National University, 1, Gangwondaehak-gil, Chuncheon, 24341, Republic of Korea

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Abstract

Purpose: In this study, the potential for biogas production, degradation rates, and lag-phase of diauxic growth of carbohydrate-based material, which is one of the proximate compositions, were investigated. **Methods:** This study was conducted using starch as a carbohydrate-based material. In experimental condition 1, the biogas potential of carbohydrate-based material was measured. In experimental condition 2, the effect of feed to microorganism ratio (F/M ratio) on lag-phase of diauxic growth from carbohydrate-based material was tested. Biochemical methane potential tests were performed at five different feed to microorganism ratios (0.2, 0.4, 0.6, 0.8, and 1.0) under mesophilic conditions. The biogas production patterns, lag-phase, total volatile fatty acids to total alkalinity ratio (TVFA/TA ratio), and time required for 90 percent biogas production were used to evaluate biogas production based on the biochemical methane potential tests. **Results:** In experimental condition 1, unlike previous studies, biogas was produced in the TVFA/TA ratio ranging from 1.131 to 2.029 (approximately 13–19 days). The methane content in the biogas produced from the digesters was 7% on day 9 and increased rapidly until approximately day 27 (approximately 72%). In experimental condition 2, biogas yield was improved when the feed to microorganism ratio exceeded 0.6, with an initial lag-phase. **Conclusions:** Even if the TVFA/TA ratio was greater than 1.0, the biogas production was processed continuously, and the CO_2 content of the biogas production was as high as 60%. The biogas yield was improved when the F/M ratio was increased more than 0.6, but the lag-phase of carbohydrate-based material digestion became longer starting with high organic loading rate. To clarify the problem of the initial lag-phase, our future study will examine the microbial mechanisms during anaerobic digestion.

Keywords: Anaerobic digestion, Biogas production, Proximate composition, Carbohydrate-based material, Batch test

Introduction

The increasing demand for renewable energy sources and reuse of wastes requires better technological solutions for energy production (Fernandes, 2010). Anaerobic digestion is a series of biological processes in which microorganisms break down biodegradable material in the absence of oxygen, which is an effective waste treatment method for pollution control and energy recovery, such as treating agricultural and industrial wastes containing easily biodegradable substances (Yuan and Zhu, 2016; Kim et al., 2017).

Although the co-digestion of organic waste produces more biogas, the complex and variable characteristics of co-substrates may inhibit the microbial communities that drive this process, thereby causing system failure (Ohemeng-Ntiamoah and Datta, 2018). These inhibitors of biogas production are influenced by the amount of added organic materials, pH of the digester, toxic substances, and anaerobic condition carbon to nitrogen ratio as well as the proximate composition (moisture, crude ash, crude protein, ether extract, crude fiber, and nitrogen-free extracts) of the initial organic materials (Walker et al., 2009). However, many studies have limited these inhibitory effects to environmental factors

***Corresponding author:** Sang-Hun Kim
Tel: +82-33-250-6492; Fax: +82-33-255-6406
E-mail: shkim@kangwon.ac.kr

as pH, ammonia, and carbon to nitrogen ratio (Cazier et al., 2015; Yuan et al., 2016). Key parameters, such as lipids, proteins, and carbohydrates, which are precursors of inhibitory compounds, such as long-chain fatty acids and ammonia, have not been widely analyzed (Ohemeng-Ntiamoah and Datta, 2018).

Kafle and Kim (2013) reported that biogas production is not consistent and results in the formation of high-carbohydrate and high-fat by-products, which are caused by a lag-phase of diauxic growth, one of the biogas production patterns. These biogas production patterns follow diauxic growth, which shows two phases of growth patterns or reactions caused by the involvement of rapidly used substrates into slowly used substrates by inhibiting enzyme biosynthesis (VDI 4630, 2006). Kim and Kim (2017) suggested that organic waste with approximately 40% fat content can be mixed with >10% carbohydrate-based organic waste to improve biogas production. Although the proximate composition may affect biogas production, few studies (Labatut et al., 2011; Sun et al., 2014) have conducted detailed characterizations of their content in substrates for anaerobic digestion. It is necessary to understand the characteristics of each proximate composition to produce high-efficiency biogas through anaerobic co-digestion.

The purpose of this study was to examine the effect of the carbohydrate-based material on the performance of biogas production. Specifically, the effects of biogas production and degradation rates of carbohydrates, which are part of the proximate composition, were investigated and the lag-phase of diauxic growth was defined.

Materials and methods

Experimental materials

Swine manure collected from Gwangil farm in Anseong City, Republic of Korea, was processed with the anaerobic treatment in a continuous digester at a mesophilic temperature (36.5℃) and was used as the digestive inoculum for this study. Starch soluble (CAS-9005-84-9) was used as the carbohydrate-based material. The characteristics of the microbial inoculum and carbohydrate-based material are shown in Table 1. Each experiment was repeated three times to provide mean values.

Experiment design and batch set-up

The batch test was done under different conditions and two experiments were performed with experimental condition 1 and experimental condition 2. The design for each experiment is shown in Table 2. In experimental condition 1, the biogas potential of carbohydrate-based material was measured. In experimental condition 2 the effect of feed to microorganism ratio (F/M ratio) on

Table 1. Characteristics of inoculum and carbohydrate-based material

	TS (%)	VS (%)	VS/TS	pH
Inoculum 1	1.44 (0.02)	0.50 (0.02)	0.349 (0.02)	9.15
Inoculum 2	1.39 (0.03)	0.48 (0.02)	0.347 (0.02)	9.05
Starch soluble	89.17 (0.05)	85.55 (0.04)	0.993 (0.04)	

*TS: Total Solid; VS: Volatile Solid; TS/VS: Total solid and volatile solid ratio

Table 2. Summary of experimental design

	Feedstock	Substrate loading (gVS·L^{-1})	F/M	Volume (L)	Temperature (℃)	Stirring	Remark
Experimental condition 1	Starch	5.1	4.0	5.0	36.5	2min/time	For the first 4 days, proceed continuous test, and then batch.
Experimental condition 2	Starch	1.0	0.2	0.8	36.5	2min/day	
		1.9	0.4	0.8	36.5	2min/day	
		2.9	0.6	0.8	36.5	2min/day	
		3.8	0.8	0.8	36.5	2min/day	
		4.8	1.0	0.8	36.5	2min/day	
	Blank 1	-	-	0.8	36.5	2min/day	

lag-phase of diauxic growth from carbohydrate-based material was tested. Feed to microorganism ratio (F/M ratio) was calculated based on the initial VS of the substrate and inoculum. In experiment 2, six bottles were used to identify the cause of the initial lag-phase, two bottles were decomposed each time for liquid parameter analysis at 7, 14, and 35 days.

A single-stage continuous process under experimental condition 1 was performed in an 8.0 L Continuous Stirred-Tank Reactor (CSTR) with a 5.0-L working volume. The reactor was installed inside a temperature-controlled chamber (36–38℃) and fed once a day using a peristaltic pump. An equivalent volume of digester content was discharged prior to feeding. The biogas was collected in a gas collector by the water displacement method. In the continuous test, the daily gas yield was divided by daily VS input to the digester. The reactor was stirred (2 min hourly) by circulating the produced biogas using the peristaltic pump. In this case, the reactor was inoculated on day 0 with 5.0 L of digested sludge (inoculum), and anaerobic conditions were created by flushing the headspace with nitrogen gas. After confirming that the biogas was produced during the first 4 days, the supply of feed was stopped and the batch experiment was conducted.

A batch digester under experimental condition 2 was employed to analyze the amount and components of the biogas production (Fig. 1). A vial of 1.3 L was used in the study, and actual volume of 0.8 L was used (El-Mashad and Zhang, 2010). The F/M ratio was calculated based on the initial VS of the substrate and inoculum. After adding the required amounts of inoculum and substrate, each digester was filled with tap water to maintain a designated volume. The digester was flushed with 100% N_2 for 2–3 min before sealing. The experiments were conducted in an incubator to maintain the batch digester at medium temperature 36.5℃, and the digester was stirred for two minutes before measuring the amount of biogas produced every 24 h for smooth gas production.

Biogas measurement

This study measured methane emissions during the anaerobic incubation period, and the temperature inside the digester and headspace pressure was calibrated by using equation (1) to obtain cumulative methane production curves. The daily biogas production of each digester was determined by the volume of biogas produced, which was calculated from the volume and pressure in the headspace of the digester (El-Mashad and Zhang, 2010). A WAL-BMP-Test system pressure gauge (3150, WAL Mess-und Regelsysteme GmbH, Oldenburg, Germany) was used to measure the gas production in the digester (Owen et al., 1979). A biogas analyzer (Biogas 5000, Geotechnical Instruments Ltd., Leamington Spa, UK) was used for the measurement of methane (CH_4), carbon dioxide (CO_2), oxygen (O_2), and hydrogen sulfide (H_2S), and a gas chromatograph (GC-2014, Shimadzu Corp., Kyoto, Japan) was used for periodic calibrations. APHA (1998) were used to measure the TS and VS. Biogas and methane yields were calculated by dividing the cumulative biogas and methane volume by the amount of substrate (feed) VS initially added to each digester in the case of batch test.

$$V_B = \frac{(P_f - P_i) \times V_H \times C}{R \times T} \tag{1}$$

where V_B = biogas volume (L), P_i = initial pressure in the reactor headspace (mbar), P_f = final pressure in the reactor headspace after 24 h (mbar), V_H = headspace

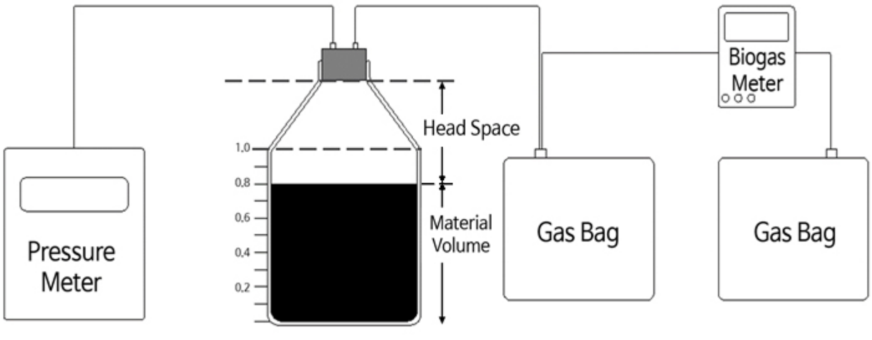


Figure 1. Batch test set-up for biogas potential test (Kim and Kim, 2017).

volume (L), C = molar volume (22.41 L/mol), and R = universal gas constant (83.14 L mbar/K/mol).

Analytical analysis methods

Chemical analyses including total solid (TS), volatile solid (VS), NH3-N, pH, and total chemical oxygen demand (TCOD) were measured using standard methods (APHA, 1998). TVFA (total volatile fatty acids), TA (total alkalinity) and TVFA/TA ratio were determined using the Nordmann method (Nordmann, 1977; Voß et al. 2009). The samples were centrifuged at 3,000 rpm for 30 minutes and titrated using a Titrator DL15 device (Mettler Toledo). A 5 mL sample was diluted with 50 mL deionized water and then titrated with a standard 0.1N H_2SO_4. The TVFA and TA were calculated by using empirical equations (2) and (3) respectively (Nordmann, 1977).

$$\mathrm{TVFA} = [((\mathrm{ConB} - \mathrm{ConA}) \times 20\,\mathrm{mL/EF} \times 1.66) - 0.15] \times 500\ [\mathrm{mg/L acetate}] \quad (2)$$

$$\mathrm{TA} = \mathrm{ConA} \times 250 \times 20\,\mathrm{mL/EF}\ [\mathrm{mg/L\ CaCO_3}] \quad (3)$$

where ConA = mL of 0.1N H_2SO_4 consumed by the sample to reach a pH of 5.0, ConB = mL of 0.1N H_2SO_4 consumed by the sample to reach a pH of 4.4, and EF = extracted fluid volume (sample volume in mL).

Lag-phase (λ) determination

The data obtained from the methane production potential experiment were used to derive the lag-phase using equation (4), which was modified using the Gompertz model (Kafle et al., 2012). Nonlinear regression analyses were conducted using equation (4) and the statistical package for the social sciences (SPSS) program (SPSS, 2015).

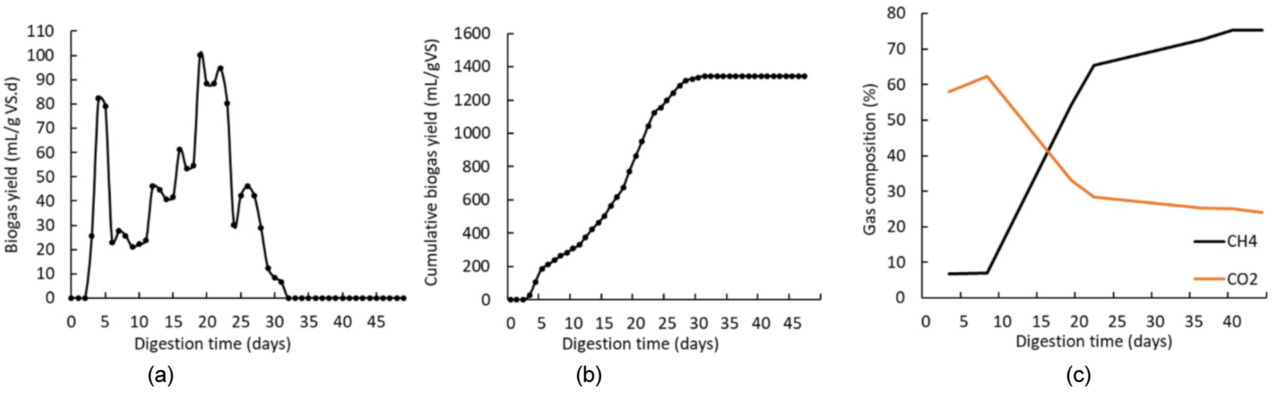
$$\mathrm{G(t)} = G_m * exp - \exp[\frac{R_{max} * e}{G_m}(\lambda - t) + 1] \quad (4)$$

where G(t) is the cumulative methane yield (mL/gVS added), G_m is the methane production potential (mL/gVS added), R_{max} is the maximum methane production rate (1/day), t is the time (days), λ is the lag-phase (days), and e is 2.718282.

Results and Discussion

Biogas yield and biogas production rate of carbohydrate-based materials (Experiment 1)

The biogas yield (mL/gVS added), biogas production rate (mL/gVS/day), and methane content for carbohydrate-based material in experimental condition 1 are shown in Figure 2. Biogas production started on day 3 of digestion. After 4 days (from 5 days of digestion), the biogas production decreased for 7 days, continuously increased



(a) (b) (c)

Figure 2. (a) Biogas production rate; (b) Cumulative biogas yield; (c) Methane content during anaerobic digestion with carbohydrate-based material

Table 3. Biogas yield, methane content, and T90 from the carbohydrate-based material

Parameters	Substrate loading (g VS/L)	F/M*	Cumulative Biogas yield (mL/gVS)	Methane contents (%)	Methane yield (mL/gVS)	T90 (days)
Starch	5.1	4.0	1342	72.5	973	27

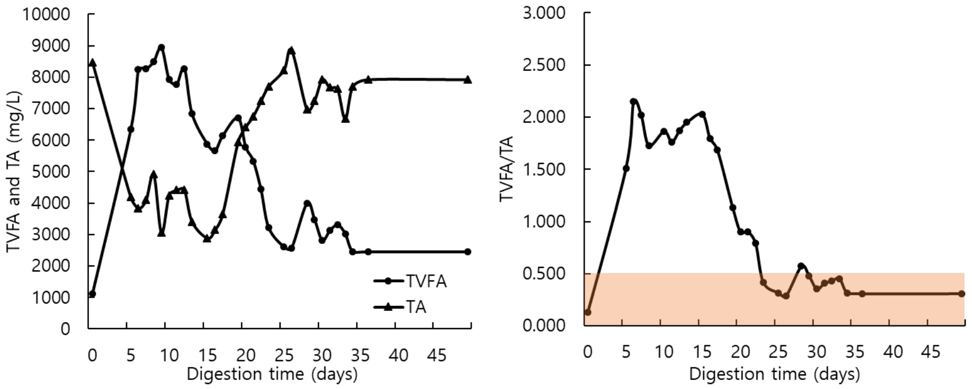
*F/M: Feed to microorganism ratio (g VS substrate added/g VS inoculums added).

until 19 days of digestion, and then began to decline (Fig. 2(a)). The peak value of daily biogas production rates was calculated to be 100 mL/gVS/day after 20 days of digestion. The 90% total biogas yield was achieved within 27 days of digestion (Table 3) and gas production had nearly ceased by 32 days of digestion (Fig. 2(b)). The methane content in the biogas produced from the digesters was 7% on day 9 and increased rapidly until approximately day 27 (approximately 72%), after which it remained nearly constant throughout the remaining test period (Fig. 2(c)). Production of CO_2 (approximately 63%), without accompanying methane production in the hydrolysis step, which is the initial stage of anaerobic digestion, is a sign of rapid fermentation (Parawira et al., 2008).

The TVFA, TA, and TVFA/TA ratio characteristics of the digester contents under experimental condition 1 are shown in Figure 3. TA was rapidly decreased until 7 days of digestion, while sharp increases were observed for TVFA and TVFA/TA, but increased until 7, 8 days of digestion (Fig. 3(a)). At a TVFA/TA ratio of 2.147, reduced biogas production was observed. The accumulation of initial TVFA is caused by high organic matter supply. If a large amount of substrate is injected in a short period of time, there is an imbalance between the acid forming stage and methane forming stage. As a result, accumulation of VFA and decrease of pH can be induced (Kim et al., 2014). The TVFA/TA ratio is one of the most important monitoring parameters of the anaerobic process. It has been reported that a TVFA/TA ≤ 0.4 is the safe range for anaerobic digestion, with a low risk of acidification, and that destabilization of the digester is observed at a TVFA/TA ratio of approximately 0.4–1.0 (Switzenbaum et al., 1990; Hernández et al., 2014; Wilawan et al., 2014). However, in contrast to previous studies, we found that biogas was produced at TVFA/TA ratios of 1.131 to 1.870 (approximately 13–19 days) (Fig. 3(b)). Hernández et al. (2014) reported that carbon dioxide and hydrogen, which are decomposition products in the acid-forming stage, are mainly produced at a TVFA/TA ratio ≥ 1.0. Here, we found that biogas composition at approximately 13 days was 53% of CO_2 and 28% of CH_4. At 19 days of digestion, the TVFA was reduced from 6711 to 2448 mg/L and the TVFA/TA ratio decreased from 1.870 to 0.308.

Effect of F/M ratio on biogas yield from carbohydrate-based material (Experiment 2)

The biogas and methane yield (mL/gVS added) from a



(a) (b)

Figure 3. Performance of carbohydrate-based material: (a) TVFA and TA; (b) TVFA/TA ratio

Table 4. Biogas yield, methane content, and T90 from the carbohydrate-based material at different F/M ratios

Substrate loading (gVS/L)	F/M*	Cumulative Biogas yield (mL/gVS)	Methane contents (%)	Methane yield (mL/gVS)	T90 (days)	Lag-phase (days)
1.0	0.2	120	45.5	55	30	0.57
1.9	0.4	344	51.9	179	30	5.26
2.9	0.6	456	63.2	288	30	3.05
3.8	0.8	755	63.5	459	34	3.79
4.8	1.0	1007	63.5	639	38	3.36

*F/M: Feed to microorganism ratio (g VS substrate added/g VS inoculums added).

mixture of carbohydrate-based material at the F/M ratio are shown in Table 4. The average biogas yields from the digesters operated at 0.2 to 1.0 F/M were 120 to 1007 mL/gVS after 50 days of digestion (Fig. 4). Fig. 5 shows the biogas production rate at five different F/M ratios. At F/M rations was 0.2, the biogas production was less than other ratios The T90 of experimental condition 1, i.e., the time required for 90% biogas production, ranged between 30 and 38 days and the lag-phase were 0.6 to 5.3 days (Table 4). When the F/M ratio was greater than 0.6, biogas production began immediately above 17 mL/gVS/day on the first day. The biogas production rate continuously increased until approximately day 5, and then started to rapidly decline. When the F/M ratios were 0.8, and 1.0, the peak value of daily biogas production rates was calculated to be 54 mL/g VS/daymL·gVS·day, and 75 mL/g VS/day mL·gVS·day at 5 days of digestion. Biogas composition (CO_2 and CH_4) from the carbohydrate-based material is shown in Fig. 6. When the F/M ratios were 0.6, 0.8, and 1.0, the initial biogas component produced more CO_2 than CH_4, and CH_4 production was greater than that of CO_2 after 15 days; anaerobic digestion stabilized as methane content exceeded 60% after approximately 25 days. As the initial CO_2 content was high, hydrolysis is considered as faster than the low F/M ratio (F/M ratio lower than 0.6). In general, when the anaerobic process has a CH_4 content of 60%, the phase is considered as stabilized (Kafle et al., 2012). When the F/M ratio was 0.2, the final CH_4 content was approximately 45% and showed low efficiency in anaerobic digestion.

The TVFA, TA, and TVFA/TA ratio characteristics of the digester contents under experimental condition 2 are shown in Table 5. When the lag-phase appeared after 14 days (Fig. 5(d) and 5(e)), the TVFA/TA at F/M ratios of 0.8 and 1.0 was 0.176 to 0.210. This range has been shown to be stable in anaerobic digestion. The lag-phase may be caused by the difference in growth rates of the two substrates. When Archaea grow in a medium containing both glucose and lactose, the cells use glucose preferentially until glucose is exhausted. A lag-phase appears for enzyme synthesis using lactose (McBrien and Moses, 1968; Essenberg and Hall, 1980). One mole of glucose is converted to two moles of lactate during anaerobic glycolysis. No CO_2 is produced in this pathway.

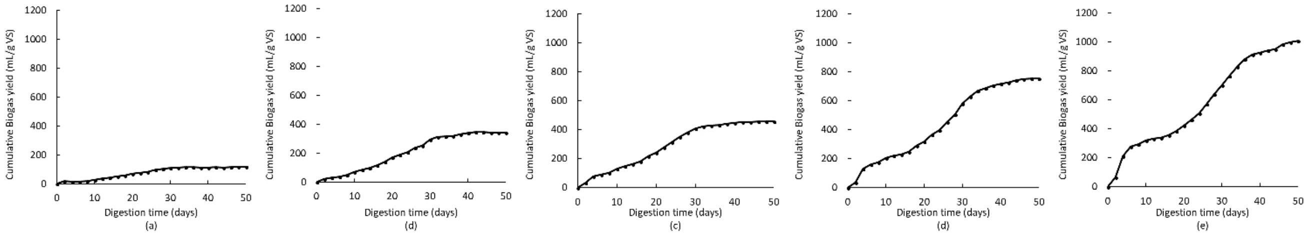


Fig 4. Cumulative biogas yield from carbohydrate-based material (a) F/M = 0.2; (b) F/M = 0.4; (c) F/M = 0.6; (d) F/M = 0.8; (e) F/M = 1.0

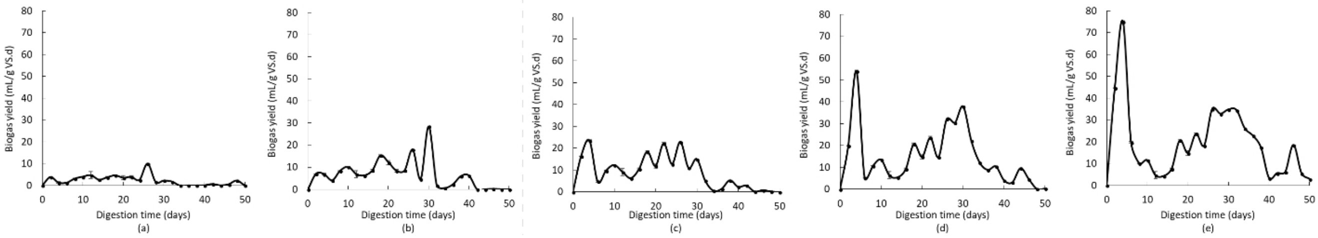


Fig 5. Biogas production rate from carbohydrate-based material (a) F/M = 0.2; (b) F/M = 0.4; (c) F/M = 0.6; (d) F/M = 0.8; (e) F/M = 1.0

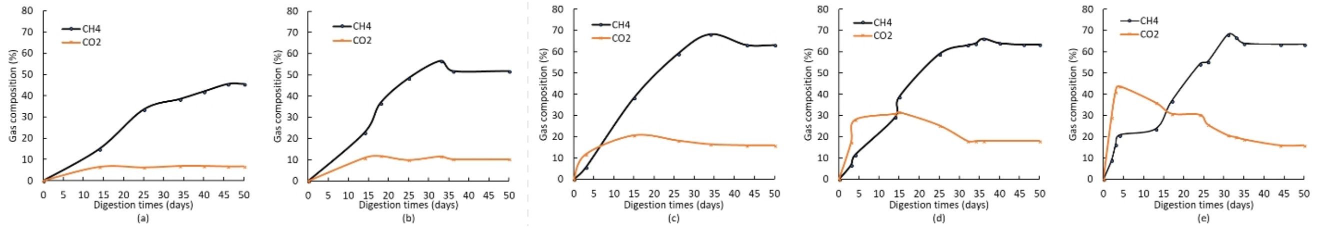


Fig 6. Biogas composition from carbohydrate-based material (a) F/M = 0.2; (b) F/M = 0.4; (c) F/M = 0.6; (d) F/M = 0.8; (e) F/M = 1.0

Table 5. Comparison of TVFA, TA, and TVFA/TA ratio depending on the different F/M ratios

F/M*	TVFA (mg/L)					TA (mg/L)					TVFA/TA ratio				
	Initial	7 days	14 days	35 days	Finish	Initial	7 days	14 days	35 days	Finish	Initial	7 days	14 days	35 days	Finish
0.2	737 (63)	1223 (92)	957 (-)	705 (90)	673 (73)	10269 (111)	11158 (93)	10687 (-)	12958 (114)	10568 (483)	0.072	0.110	0.090	0.054	0.064
0.4	771 (50)	1881 (42)	1107 (-)	628 (218)	752 (50)	10746 (504)	10559 (176)	10426 (-)	10763 (100)	10540 (25)	0.072	0.178	0.106	0.058	0.071
0.6	906 (32)	2142 (152)	1910 (-)	834 (195)	804 (87)	11472 (162)	10230 (222)	10474 (-)	10419 (125)	10942 (122)	0.079	0.209	0.182	0.080	0.073
0.8	846 (114)	2120 (41)	1817 (305)	728 (108)	745 (28)	11089 (194)	10047 (255)	10298 (600)	10889 (46)	11015 (63)	0.077	0.211	0.176	0.067	0.070
1.0	719 (80)	2299 (300)	2139 (59)	1133 (522)	767 (28)	10306 (26)	10043 (152)	10198 (112)	10617 (46)	11035 (40)	0.070	0.229	0.210	0.107	0.070

However, CO_2 can be produced during various fermentation processes (such as alcohol, propionate, and acetate fermentation) of pyruvic acid as an intermediate of carbohydrate metabolism (Baynes and Dominiczak, 2018). In this study, the initial CO_2 production was higher than CH_4 production for this reason.

Conclusion

In this study, the potential for biogas production, degradation rates, and lag-phase of diauxic growth of carbohydrate-based material, which is one of the proximate compositions, were investigated. In general, anaerobic digestion was stable when the TVFA/TA ratio was less than 0.4, but in this study even if the TVFA/TA ratio was greater than 1.0, the biogas production was processed continuously, and the CO_2 content of the biogas production was as high as 60%. The CO_2 were produced by the activation of hydrogenotrophic methanogens in the methanogenic stage. Although methane could be produced more by acetotrophic methanogens than hydrogenotrophic methanogens, at the earlier stage, we have to investigate the effects of activation of two kinds of methanogens.

At the F/M ratio of 0.6, the lag-phase of carbohydrate-based materials digestion became shorter with high biogas production. The biogas yield was improved when the F/M ratio was increased more than 0.6, but the lag-phase of carbohydrate-based material digestion became longer starting with high organic loading rate. One of the reasons of the initial lag-phase may be by carbohydrate metabolism, which is the difference in growth rates of the glucose and lactose. To clarify the problem of the initial lag-phase, our future study will examine the microbial mechanisms during anaerobic digestion.

The data obtained from this study provides a basis for designing methods for co-digestion of various organic materials.

Conflict of Interest

The authors have no conflicting financial or other interests.

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