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Biogas Production Performance Based on Carbon Number and Double Bond Count of Long-chain Fatty Acids

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Abstract

Purpose: The objective of this study was to investigate the high-efficiency anaerobic digestion of organic wastes with high fat content. Specifically, the analysis focused on biogas production performance with a focus on carbon number and the double bond count of the long-chain fatty acids (LCFAs), which are hydrolysis products of triglycerides. **Methods:** Experiments were performed under mesophilic anaerobic conditions with a feed-to-microorganism ratio (F/M) of 1.0. Biogas production performance was analyzed through biogas production patterns, lag-phase, and the time required for 90% biogas production (T90). **Results:** Biogas production increased when the content of unsaturated LCFAs (containing relatively large numbers of carbon atoms) increased. In substrate containing LCFAs with four or more double bonds, although the initial lag-phase in biogas production was shortened, development of a three-step lag-phase resulted in decreased biogas production. These results suggest that high rates of anaerobic digestion are possible when the LCFAs have high unsaturated fatty acid content with three or fewer double bonds. **Conclusions:** When various types of LCFAs are digested anaerobically, biogas production performance can be improved if the unsaturated fatty acid content and number of double bonds are optimized for maximum production.

Keywords: Anaerobic digestion, Biogas production, Carbon number, Double bond, LCFAs

Introduction

In accordance with the London Convention, the dumping of organic substances into oceans has been prohibited since 2012, but organic waste continues to be produced and must be treated (Kim and Yoon, 2007). Anaerobic digestion is an effective process for the treatment of organic waste because it is environmentally friendly and can generate alternative energy in the form of methane gas (Ministry of Environment, 2014). However, biogas production performance varies considerably depending on the relative composition of six major components (carbohydrates, proteins, fats, moisture, crude fiber, and ash) in the digested organic material (Monnet, 2003). Generally, organic wastes have various composition ratios depending on the season and region. Materials with higher fat content can be difficult to effectively treat and the production of biogas can be hindered (Kim and Kim, 2017; Kafle and Kim, 2013). Nonetheless, fat remains attractive for anaerobic digestion because it can theoretically produce biogas with higher methane content compared to protein and carbohydrate-rich substrates (Alves et al., 2009).

Triglycerides, which account for a majority of the fat content in organic waste, are hydrolyzed to form glycerol and long-chain fatty acids (LCFAs) (Batstone et al., 2002). LCFAs, which have chain lengths greater than 12 carbons, are expected to be suitable for anaerobic digestion because of their theoretically high methane production per mass unit. However, LCFAs are toxic toward methane-producing bacteria, hindering biogas production, and resulting in low digestion efficiency (Kim, 2000). LCFAs have a chemical composition and structure similar to those of the lipid components of cell walls of anaerobic

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organisms, and can be easily dissolved in cell walls (Zeikus, 1977). Furthermore, dissolved LCFAs can adversely affect the transference and protection functions of microorganisms, which degrades anaerobic digestion performance (Zeikus, 1977). Martinez et al. (2016) reported that LCFAs adsorbed into biomass interfere with the degradation of the substrate, which contributes to the initial lag-phase in biogas production. Demeyer and Henderickx (1967) reported that increased amounts of unsaturated LCFAs (with chain lengths of 18 carbons) hampered methane production because the increased double bond content inhibited the activity of methane-producing bacteria.

Although previous studies have investigated the effects of LCFAs on anaerobic digestion, most have focused on individual LCFAs. Most organic materials contain a mixture of various LCFAs, and mixed LCFAs exhibit more complex effects on biogas production than individual molecules (Lalman and Bagley, 2002). Therefore, it is necessary to investigate the influence of

Table 1. Characteristics of the materials used in this study.

Materials	TS, (%)	VS, (%)	VS/TS	pH
Inoculum	1.17	0.43	0.37	8.45
Sunflower Oil	100.00	99.49	1.00	
Lard	100.00	99.83	1.00	
Butter	99.84	99.82	1.00	
Castor Oil	99.82	99.79	1.00	
Safflower Oil	100.00	99.45	1.00	
Perilla Oil	100.00	99.77	1.00	
Salmon Oil	99.98	98.53	1.00	

TS, Total Solid; VS, Volatile Solid.

Table 2. Fatty acid composition of the substrates used in this study.

Carbon number: Double bond	Content of Fatty acid (%)						
	Sunflower Oil[1]	Lard[2]	Butter[2]	Castor Oil[2]	Safflower Oil[1]	Perilla Oil[1]	Salmon Oil[2]
C4:0			3.0				
C6:0			2.0				
C10:0			3.0				
C12:0			4.0				
C14:0		2	12.0		0.5		3.0
C16:0	5.7	27	26.0	1.5	5.5	7.0	11.0
C18:0	3.0	11	11.0	1.5	2.3	2.0	4.0
C20:0	1.3				0.2		
C22:0	0.8						
C24:0							
Saturated Fatty acid	10.8	40.0	61.0	3.0	8.5	9.0	18.0
C16:1		4	3.0				5.0
C18:1	22.2	44	28.0	94.0	15.3	13.0	25.0
C18:2	66.2	11	2.0		76.0	14.0	5.0
C18:3	0.3					63.5	5.0
C18:4							2.0
C20:1							
C20:4							7.0
C20:5							5.0
C22:4							2.0
C22:5							7.0
C22:6							17.0
Unsaturated Fatty acid	87.7	59.0	33.0	94.0	91.3	90.5	80.0
Total Fatty acid	98.5	99.0	94.0	97.0	99.8	99.5	98.0

[1] chempro (https://www.chempro.in/fattyacid.htm); [2] Gunstone, 1996;

mixed LCFAs in organic materials on the biogas production process. In general, LCFAs are classified by their carbon number and number of double bonds, which significantly influence biogas production performance (Alves et al., 2009). This study investigated biogas production performance in terms of the carbon number and double bond count of the LCFAs in the high-efficiency anaerobic digestion of fat.

Materials and methods

Experimental materials

Swine manure was collected from a 4000-acre pig farm located in Green Village, Gwangju Metropolitan City (Jeolla-do, Rep. Korea). The material was then digested anaerobically in a lab-scale continuous digester under mesophilic conditions at 36.5℃. A total of 7 refined oils consisting of 100% fat were purchased from a supermarket and used as experimental substrates. The properties of these oils are listed in Table 1, and their fatty acid compositions are presented in Table 2. Each experiment was performed in triplicate and the mean values were reported.

Batch test set-up and design

A batch anaerobic digester (Fig. 1) was used in this study. The total volume of the anaerobic digestion tank was 1.3 L with a liquid volume of 0.8 L. The feed-to-microorganism ratio was set to 1.0 and was applied equally to all experiments. Pure nitrogen, an inert material, was injected into the digester for longer than 2 min to create an oxygen-free anaerobic environment, and the container was subsequently sealed with a silicone rubber stopper. All anaerobic digesters were placed in an incubator maintained at 36.5°C. The produced biogas was measured in 24 h intervals and the digesters were stirred manually for 2 min before each measurement.

The experiments were designed to examine the effects of carbon number and number of double bonds in the LCFAs. In the first experiment, the biochemical methane potential (BMP) was measured to determine biogas production performance as a function of numbers of carbon atoms in the fatty acids. In general, the carbon chain lengths in saturated fatty acids are shorter than those in unsaturated fatty acids (Rustan and Drevon, 2005). Therefore, it was hypothesized that the carbon number would decrease as the saturated fatty acid content increased. Thus, an experiment was designed to consider three concentrations of saturated fatty acids, 11wt%, 40wt%, and 61wt% (Table 3). Sunflower oil, lard, and butter, with carbon numbers of 17.914, 17.293, and 15.661, respectively, were used as experimental materials.

In the second experiment, BMP was measured to determine the biogas production performance as a function of the number of double bonds in the constituent fatty acids. To eliminate the effects of saturated fatty acids, the experimental materials contained unsaturated fatty acid contents of ≥80% and exhibited differences in double bond numbers. The following experimental materials were used: castor oil (highest count of 1 double bond in fatty acids), safflower oil (highest count of 2 double bonds), perilla oil (highest count of 3 double bonds), and salmon oil (highest count of 4–6 double bonds) (Table 4).

Biogas measurement and analytical methods

The biogas production during the anaerobic process was measured at 24 h intervals using a pressure meter (WAL-BMP-Test system type 3150, Wal, Germany). The amount of biogas accumulated in the head space of the batch digester was determined in accordance with the standard temperature and pressure (STP, 0°C, 1 atm), and Eq. (1) was used to obtain the cumulative biogas and methane production curves. A biogas analyzer (Biogas 5000, Geotech, England) was used to measure the amounts of methane (CH_4), carbon dioxide (CO_2), oxygen

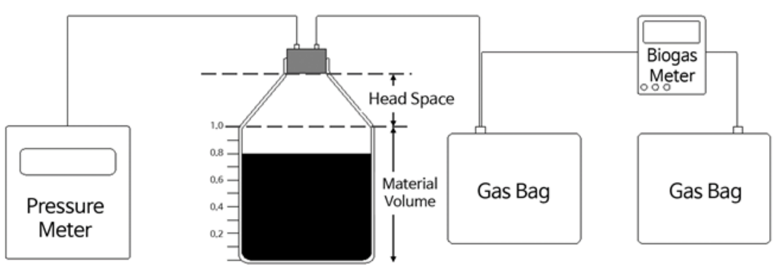


Figure 1. Batch test set up

Table 3. Experimental design for the first experiment.

Substrate	F/M	Liquid Volume (L)	Fatty Acid (wt%)		Carbon number (% weight)
			Saturated	Unsaturated	
Sunflower Oil[1]	1	0.8	11	88	17.914
Lard[2]	1	0.8	40	59	17.293
Butter[2]	1	0.8	61	33	15.661

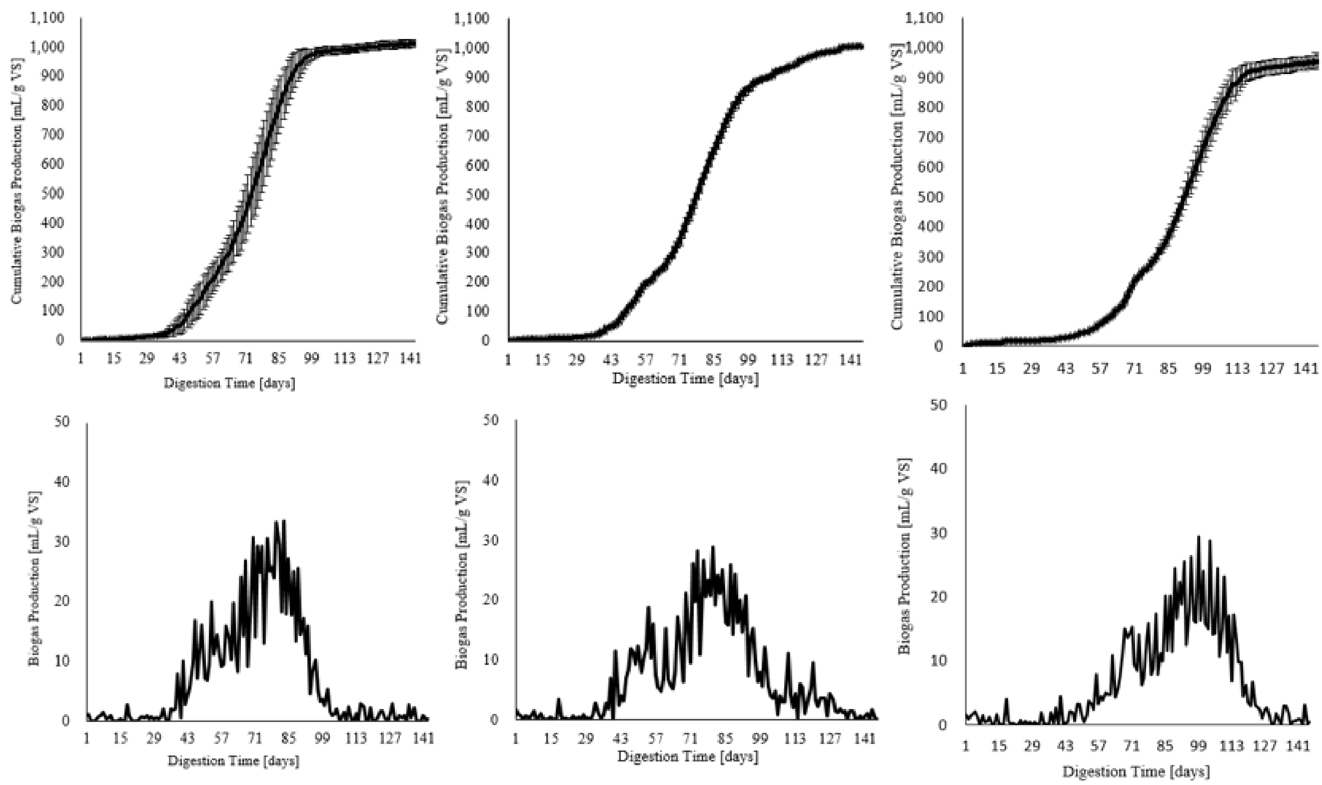
Table 4. Experimental design for the second experiment.

Substrate	F/M	Liquid volume (L)	Unsaturated fatty acid (wt%)	Double bond contents of unsaturated fatty acid (%)					
				1	2	3	4	5	6
Castor Oil[1]	1	0.8	94	94			0	0	0
Safflower Oil[2]	1	0.8	91	15	76	0	0	0	0
Perilla Oil[2]	1	0.8	91	13	14	64	0	0	0
Salmon Oil[3]	1	0.8	80	38	6	6	14	15	21

(O_2), and hydrogen sulfide (H_2S) produced. Standard methods (Clesceri et al., 1998) were used to measure the total solid (TS) and volatile solid (VS) (Clesceri et al., 1998).

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

where V_B is the biogas volume (L), P_i is the initial pressure in the reactor head space (mbar), P_f is the final pressure in the reactor head space after 24 h (mbar), V_H is volume of the head space (L), C is the molar volume (22.41 L/mol), R is the universal gas constant (83.14 L mbar/K/mol), and T is the temperature (K).



(a) Sunflower Oil (b) Lard Oil (c) Butter Oil

Figure 2. Cumulative biogas yield and biogas production rate for the substrates with carbon numbers (a) 17.914, (b) 17.293, and (c) 15.661

Carbon number calculation

The carbon number represents the average number of carbon atoms in the triglyceride. To calculate this number, Eq. (2), as described by Rhein et al.(2007), was used in this study:

$$\text{Carbon number of fatty acid} = a \times b \quad (2)$$

where a is the fatty acid content (% weight) and b is number of carbon atoms in the fatty acid.

Lag-phase determination

The value of the lag-phase (λ) was calculated based on the modified Gompertz model using data obtained from the BMP experiments (Kafle and Kim, 2012). A nonlinear regression analysis was performed using SPSS software (IBM SPSS statistics 23, 2016) and Eq. (3):

$$G(t) = G_m \times \exp[-\exp\left\{\frac{R_{\max} \times e}{G_m}(\lambda - t) + 1\right\}] \quad (3)$$

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

Results and discussion

BMP experiments in relation to the carbon number of LCFAs (experiment 1)

The results of the BMP experiments as a function of the carbon numbers of the LCFAs are shown in Figure 2. The cumulative biogas production shows a single-step lag-phase with initial lag-phase for all three substrates tested. The initial lag-phases of the three substrates were reasonably long at 37–39 d (Table 5), which can be attributed to the toxicity of the LCFAs produced by the anaerobic hydrolysis of fat (Cirne et al., 2007). However, after the initial lag-phase, smooth gas production was evident, exhibiting a single-step pattern.

The T90 of experiment 1, i.e., the time required for 90% biogas production, ranged between 91 and 110 d and the lag-phase was 13–27 d (Table 5). Butter exhibited a longer lag-phase compared to the other substrates because of the high toxicity of lauric acid (C12:0), which has a chain length of 12 carbons and is a major LCFA component in butter (Koster and Cramer, 1987). It has been previously reported that lauric acid (C12:0) is the most toxic LCFA and interferes with methane production from acetate (Koster and Cramer, 1987). Furthermore, its adverse effects are far more severe when present in combination with other LCFAs (Michael, 2003).

The cumulative biogas production was highest at 1012.76 mL/g VS with sunflower oil, which has the highest carbon number at 17.914. Butter, with the lowest carbon number of 15.661, yielded cumulative biogas production of 937.23 mL/g VS (Table 5), which is consistent with the report of Mulka et al. (2016). This suggests that biogas production increases with increasing carbon number of the substrate, i.e., biogas production increases with the carbon number of the LCFA.

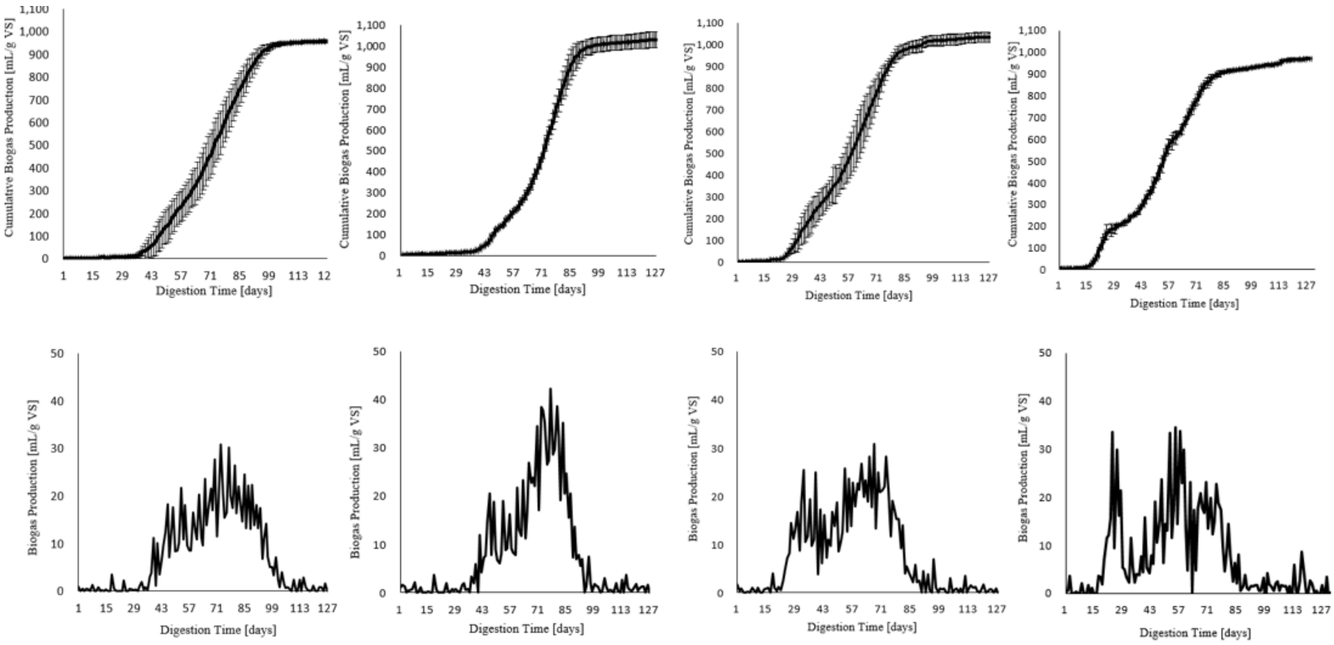
BMP experiments as a function of the number of double bonds of LCFAs (experiment 2)

Figure 3 shows the cumulative and daily biogas production obtained from the BMP experiments as a function of the numbers of double bonds of the LCFAs. For daily biogas production, a second lag-phase was

Table 5. Biogas yield, methane content, and lag-phase duration for experiment 1.

Parameters	Units	Sunflower Oil	Lard	Butter
Carbon number	% weight	17.914	17.239	15.661
F/M		1	1	1
Cumulative biogas yield	mL/g VS	1012.76 (±13)	1003.88 (±5)	937.23(±33)
Methane contents	%	78.1(±2)	79.8(±1)	80.7(±4)
Methane yield	mL/g VS	790.97	801.10	756.342
T90	days	91	109	110
Initial lag-phase	days	39	37	37
Lag-phase (λ)*	days	13	16	27

Value are expressed as mean (standard deviation, n=3).
* calculated lag phase (λ) except the initial lag-phase.



(a) Castor Oil (b) Safflower Oil (c) Perilla Oil (d) Salmon Oil

Figure 3. Cumulative biogas yield and biogas production rate for substrates with double bond count maxima of (a) 1, (b) 2, (c) 3, and (d) 4-6

observed as the numbers of double bonds increased. However, all substrates other than salmon oil showed that this phase did not last long, resulting in a single-step lag-phase. Conversely, salmon oil displayed a three-step lag-phase, showing three separate delays in the overall lag-phase. These results suggest that more that 50% of fatty acids in salmon oil contain 4 or more double bonds.

Castor and safflower oils exhibited an initial lag-phase of 23–25 d, whereas the initial lag-phase of the perilla and salmon oils were relatively short at 16–17 d (Table 6). This is likely because of the characteristics of the unsaturated LCFAs contained in the various oils. Nocak and Carlson (2017) reported that for unsaturated LCFAs, the LCFAs with longer carbon chains and higher numbers of double bonds are initially decomposed. Therefore, salmon oil decomposed before the other oils because its constituent fatty acids have chain lengths of up to 24 carbons and up to 6 double bonds.

Cumulative biogas production was highest at 1032.73 mL/g VS for perilla oil, and the methane content of the produced gas was also the highest at 81.0%. Salmon oil, with the highest carbon number of the substrates tested, showed a smaller cumulative biogas production of 970.52 mL/g VS (Table 6). This indicates that substrates

Table 6. Biogas yield, methane content, and lag-phase duration for the second experiment.

Parameters	Units	Castor Oil	Safflower Oil	Perilla Oil	Salmon Oil
Double bonds		1	2	3	4-6
Carbon number	% Weight	17.969	17.958	17.859	18.857
F/M		1	1	1	1
Cumulative biogas yield	mL/g VS	956.95(±6)	1028.48(±38)	1032.73(±22)	970.52(±40)
Methane contents	%	78.9(±4)	79.8(±3)	81.0(±2)	79.2(±0)
Methane yield	mL/g VS	755.36	820.38	836.17	768.65
T90	days	92	86	79	81
Initial lag-phase	days	25	23	18	17
Lag-phase (λ)*	days	15	11	25	30

Values are expressed as mean (standard deviation, n=3)
*Calculated lag-phase (λ) except initial lag-phase.

with unsaturated fatty acid content of ≥80% are more significantly affected by the number of double bonds in the fatty acids compared to the number of carbon atoms.

The results of the second experiment demonstrated that fatty acids with ≤3 double bonds showed smooth gas production after the initial lag-phase, suggesting that double bonds had minimal effect on biogas production. Conversely, fatty acids with ≥4 double bonds showed different results. Although the period of the initial lag-phase decreased, the appearance of a three-stage anaerobic curve suggested decreased biogas production.

Conclusions

In this study, various experimental methods were used to investigate biogas production performance from fatty substrates. Fat exists as a mixture of various fatty acids, and its effects on biogas production performance can change depending on the specific composition of the fatty acid mixture. To investigate the anaerobic digestion of fat, the biogas production performances of LCFAs were analyzed in relation to the carbon number and double bond count of the constituent components.

BMP experiments, which were designed to consider the carbon numbers of the LCFAs, showed a single-step lag-phase with an initial lag-phase of 36–37 d. This suggested that LCFAs produced through the hydrolysis of fat are toxic to the anaerobic bacteria, resulting in a long initial lag-phase. The cumulative biogas production was highest at 1012.76 mL/g VS for sunflower oil, which had the highest carbon number (17.914) of the oils tested. Butter, which has the smallest carbon number (15.661) of those tested, had the lowest cumulative biogas production of 937.23 mL/g VS. Therefore, biogas production increases as the LCFA carbon number increases.

The results of the BMP experiments designed to consider the numbers of double bonds of the LCFAs showed that castor, safflower, and perilla oils (with double bond counts of ≤3) exhibited a single-step lag-phase. Conversely, salmon oil (with double bond counts of ≥4) exhibited a three-step lag-phase (i.e., three instances of a lag-phase). This suggests that LCFAs with ≤3 double bonds had a minimal effect on biogas production performance after the initial lag-phase. However, fatty acids with ≥4 double bonds decreased the duration of the initial lag-phase period, but the three-step lag-phase suggested that overall biogas production was reduced.

When various fatty acids are digested anaerobically, improved biogas production capacity can be accomplished by optimizing the content of unsaturated fatty acids and the double bond count for high-efficiency digestion. Therefore, even organic waste with high fat content can be conditioned by mixing with other organic waste to adjust the unsaturated fatty acid content and number of double bonds. Thus, high-efficiency anaerobic digestion of fatty acids is possible when the content of unsaturated fatty acids is high and the effects of the lag-phase are reduced with higher contents of fatty acids with ≤3 double bonds.

Conflict of Interest

The authors have no conflicting financial or other interests.

Acknowledgements

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