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Evaluation of relationship between biogas production and microbial communities in anaerobic co-digestion

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Abstract−Anaerobic co-digestion (ACD) has been used to treat various organic wastes because nutrient balance in the feed can be improved by mixing different organics. Until now, the correlation between characteristics of feedstocks and biogas production by ACD has been studied mainly in terms of biochemical methane potential. It has been rarely tried to understand the co-digestion process in terms of microbial community development. This study aimed to evaluate the performance of batch anaerobic digestion (AD) reactors fed with activated sludge (AS), swine slurry (SS) and food waste (FW) individually or in a mixture of the three wastes (FW : SS : AS=1 : 3 : 2). The AD reactors fed with the mixture showed better performance than those fed with a single substrate. Microbial communities of the batch AD reactors fed with a single substrate or the mixture were analyzed and the result was related to the performance of the AD reactors.

Keywords: Anaerobic Digestion, Biochemical Methane Potential (BMP) Test, Co-digestion, Organic Waste, Microbial Community

INTRODUCTION

Due to the global environmental issues like climate change and energy shortage, a policy of recycling organic wastes as an alternative energy source is being implemented in many countries. Typical organic wastes produced in Korea are activated sludge (AS), swine slurry (SS) and food waste (FW); in 2014, AS of 9,872 ton/d, SS of 175,269 ton/d, and FW of 13,190 ton/d were produced. Before ocean-dumping of organic wastes was officially banned in Korea in 2013 conforming to the London Convention [1,2], a significant amount had been disposed of in open ocean waters each year. After 2013, however, a variety of waste management policies were introduced to control waste generation, promote its recycle, and avoid its landfill [3]. Among them, extraction of energy from organic wastes has attracted attention from the public.

Recovering energy from waste (waste-to-energy) is an effective method to reduce greenhouse gas emission, since it eventually allows us to consume less fossil fuel. The anaerobic digestion (AD) process is greatly appealing as it anaerobically converts organic waste to biogas, an energy source.

To maintain an AD system at a stable condition, the microbial activity of the system should be well managed. Since the AD process consists of four steps, hydrolysis, acidogenesis, acetogenesis, and methanogenesis, a variety of microbial species are involved and interact between each other [4]. Especially, the microbial activity of the system is directly affected by environmental factors such as the partial pressure of hydrogen, temperature, pH, and nutrients [5,6]. To maintain high microbial activity, organic waste as a substrate should be fed to the AD process at an optimal condition. One approach is the anaerobic co-digestion (ACD) process, in which different feedstocks are digested together [7].

As indicated above, the biogas production efficiency of an anaerobic digester depends on the characteristics of a feedstock and the microbial community of the digester [8,9]. In Korea, ACD has been applied for degrading organic wastes such as FW, SS and AS together. Among these feedstocks, FW is considered as a good carbon source since its carbon content is high. During the AD of FW, however, the system pH becomes acidic, resulting in the inhibition of methanogen activity. Therefore, FW should be used along with other wastes to prevent the mixed liquor pH of an AD process from decreasing [10,11]. In contrast, SS is characterized by a high water content and a buffer capacity. Therefore, if it is fed into an AD process along with FW, the system pH will not drop and a stable methane production can be achieved [12]. AS is produced as a byproduct during wastewater treatment. Although AS does not contain much organic carbon compared to FW or SS, it has a high buffering capacity and a variety of vital elements. Therefore, AS is often digested along with other feedstocks containing a higher organic content, e.g., FW, and SS [13-15].

For a successful ACD, the optimal mixing ratio of selected feedstocks should be determined in advance through biochemical methane potential (BMP) tests [5,16]. Conventionally, BMPs are evaluated and correlated with characteristics of feedstocks under ACD and with their biodegradation or conversion to methane. For

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a better understanding of the mechanism of ACD, however, it is necessary to obtain more detailed information about biochemical reactions occurring during the co-digestion of a mixture of different feedstocks and the microbial community structure that is responsible for biogas production.

Our objective was to identify the optimal mixing ratio of organic wastes for stable ACD and promoting biogas production. FW, SS and AS alone and mixtures of them at different mixing ratios were used as a feedstock for ACD. A series of BMP experiments were performed; the amounts of produced biogas and reduced organic materials were investigated. In addition, to better understand the result of the BMP test fed with the wastes individually and the mixture of them with different mixing ratios, the microbial community of the mixed liquor obtained from each BMP test was also analyzed.

MATERIALS AND METHODS

1. Seeding Sludge and Organic Wastes

The seeding sludge and AS used in this study were collected from an anaerobic digester and a return sludge line in the J Wastewater Treatment Plant (WWTP), Seoul, respectively. FW and SS were obtained from the Gangwon Bioenergy in Wonju city, Korea. Right after samples were collected, they were transported to the laboratory in a cooler. On arrival, these samples were stored in a refrigerator at 4 °C. They were filtered with a 4 mm mesh screen before being used in a BMP test.

2. Experimental Setup for BMP Tests

BMP tests were performed with an automatic BMP tester (AMPTS II, Bioprocess Control, Lund, Sweden). The system consists of 15 serum bottles of 500 mL each with a working volume of 400 mL. Once samples were placed in the bottles along with seeding sludge, the bottles were sealed and their headspace was purged with N_2 gas. During the test period, each sample was continuously stirred at 120 rpm.

Once a BMP test started, the automatic tester continuously monitored and recorded the amount of methane produced in each BMP reactor, while CO_2 was absorbed by 3 N NaOH buffer solution. The data of produced biogas were automatically adjusted to the one at the standard state condition (i.e., temperature of 0 °C and pressure of 1 bar). A BMP test was stopped once the methane production rate fell below 5 mL/day. More in-depth information regarding the BMP test method can be found elsewhere [17]. All the BMP tests were performed in triplicate. In this study, the optimal ratio of FW to SS to AS for the ACD was determined by the BMP tests.

3. Measurement of Waste Characteristics

In the beginning of and at the end of each BMP test, an aliquot of mixed liquor was collected from each BMP bottle and its total and soluble chemical oxygen demand (tCOD, and sCOD), total solids (TS), volatile solids (VS), total Kjeldahl nitrogen (TKN), NH_4^+, total phosphorus (TP) and total alkalinity (Alk) were analyzed according to the Standard Methods [18].

tVFA was analyzed by a gas chromatograph with a flame ionization detector (GC 2010, Shimadzu, Kyoto, Japan). The column used in this study was the SH-Rtx-Wax column (30 m length×0.25 mm inner diameter×0.25 μm thickness (Shimdazu, Kyoto, Japan)).

Elements in different feeds were analyzed using a Thermo 1112 Series Flash EA (NC Soil Analyzer, Rome, Italy) [19]. Before elements of a sample were analyzed, the sample was dried at 105 °C for 24 hr.

The theoretical methane potential was estimated using Buswell's equation (Eq. (1)) with the result from the element analysis of a sample [20].

$$\begin{aligned} C_aH_bO_cN_dS_e + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4} + \frac{e}{2}\right)H_2O \\ \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right)CH_4 \\ + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} + \frac{e}{4}\right)CO_2 + dNH_3 + eH_2S \end{aligned} \quad (1)$$

If all the organic materials in the feed are stoichiometrically transformed to CH_4 and CO_2, the theoretical methane yield (BMP_{theo}) can be calculated using Eq. (2) as follows:

$$BMP_{theo} = 22.4 \times \frac{4a + b - 2c - 3d - 2e}{8 \times (a + b + 16c + 14d + 32e)} \ (NM^3/KgVS_{add}) \quad (2)$$

Lastly, using the measured BMP_{exp} and calculated BMP_{theo}, the anaerobic biodegradability of a given substrate (BD_{CH_4}) can be obtained (Eq. (3)) [21]:

$$BD_{CH_4} = BMP_{exp} \div BMP_{theo} \times 100\% \quad (3)$$

4. Microbial Community Analysis

4-1. DNA Extraction and Pyrosequencing

DNA was extracted from mixed liquor using a Soil DNA Isolation Kit (MoBio Inc., Carlsbad, CA, USA) following the manufacturer's instruction. Extracted DNA was stored at −20 °C until analysis. Amplification of the V3-V4 region of bacterial 16S rRNA gene was performed for each sample using the 341F and 805R primer. To detect methanogen species, Arch519F (CAGCCGCCGCGGTAA) and Arch934R (GTGCTCCCCCGCCAATTC) were utilized as a methanogen specific primer [22,23].

Samples were amplified for pyrosequencing using a forward and reverse fusion primer. The forward primer was constructed with the (5′-3′) Nextera consensus (TCGTCGGCAGCGTC), a sequencing adaptor (AGATGTGTATAAGAGACAG), and an appropriate forward primer selected for bacterial diversity assay (341F: CCTACGGGNGGCWGCAG) [24]. The reverse fusion primer was also constructed following Fadrosh et al. [24] using the (5′-3′) Nextera consensus (GTCTCGTGGGCTCGG), the sequencing adaptor, and a reverse primer for bacterial diversity assay (805R: GACTACHVGGGTATCTAATCC). Amplification was conducted in a 25 μL reactor with Dr. MAX DNA Polymerase (Doctor Protein, Seoul, Korea), 1 mL of 5 mM primer, and 1 L of template. The reaction was carried out with the following thermal program: held at 95 °C for 3 min, then 25 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, followed by one cycle at 72 °C for 5 min and held at 4 °C. DNA sequencing was performed at ChunLab, Inc. (Seoul, South Korea) using an Illumina/MiSeq platform, according to the manufacturer's protocol.

4-2. Biodiversity Analysis and Phylogenetic Classification

Raw sequencing reads from different samples were classified by

barcode sequences that are included in the PCR primers. Short sequences (<300 bp) or sequences with more than two ambiguous bases (Ns) were removed before analysis. Then, primer, linker, and barcode sites were trimmed by a pairwise alignment. Non-target genes that did not show any match with 16S rRNA gene sequences in the EzTaxon-e database were also discarded. Chimeric sequences were detected using the BLAST program [25]. The taxonomic assignment was performed by comparing the sequence reads against the EzTaxon-e database, using a combined method of the BLASTN search and the pairwise comparison for similarity. Then, the species diversity of a sample was determined based on their similarity values. The species diversity and richness indices were calculated by setting the cutoff value of assigning a sequence to a species-level phylotype at ≥97% similarity. The overall phylogenetic distance between communities of the samples under BMP tests was estimated using the Fast UniFrac calculation [26].

RESULTS AND DISCUSSION

1. Characteristics of Seeding Sludge and Organic Wastes

The characteristics of the seeding sludge and organic wastes used in this study are summarized in Table 1. FW was characterized by a low pH (pH 4.6) and a high COD value (150 g/L). On the other hand, the pH of SS was high (pH 7.7) probably due to the higher NH_4^+ content (3.1 mg N/L). Lastly, AS was characterized by the neutral pH (pH 6.9) and a low COD value (12 g/L). As shown in the table, the characteristics of these three wastes are quite different. However, if materials with different characteristics are mixed, deficient elements of one material can be provided by other materials. By the same token, if FW, SS, and AS are mixed and co-digested, each waste may provide nutrients which are deficient in the other wastes, so the nutrient balance of digested materials as a whole is improved and results in a higher biogas production.

2. BMP Values and Composition Changes of Single and Mixed Substrates

A series of BMP tests were conducted with FW, SS, and AS individually and mixtures of two or three substrates at different ratios. As shown in Fig. 1, the methane yield of most samples rapidly increased from the first day and then decreased gradually. Of the BMP testers fed with single wastes, the one with FW produced gas only for the first five days. It did not produce any gas for the rest of the incubation period. As reported in the literature, the activity

Table 1. Characteristics of seeding sludge and substrates used in this study

Parameter	Seeding sludge	FW	SS	AS
pH	7.7 (±0.2)	4.6 (±0.4)	7.7 (±0.1)	6.9 (±0.4)
Total solids (%)	2.6 (±0.3)	10.1 (±1.4)	4.6 (±0.6)	1.1 (±0.2)
Volatile solids (%)	1.6 (±0.2)	8.7 (±0.8)	3.2 (±0.4)	0.6 (±0.1)
$tCOD_{Cr}$ (g/L)	27 (±4.4)	150 (±16.4)	60 (±4.3)	12 (±1.2)
$sCOD_{Cr}$ (g/L)	0.6 (±0.1)	61 (±4.1)	6.4 (±0.7)	0.2 (±0.1)
TKN (g/L)	2.8 (±0.4)	4.5 (±1.3)	5.3 (±0.8)	1.2 (±0.2)
NH_4-N (g/L)	1.3 (±0.2)	1.3 (±0.4)	3.5 (±0.5)	0.4 (±0.1)
TP (g/L)	0.8 (±0.2)	1.1 (±0.3)	0.8 (±0.1)	0.3 (±0.1)
tVFA (g/L)	0.2 (±0.05)	15 (±2.9)	3.1 (±0.4)	<0.1
Alk. (g/L)	8.9 (± 0.6)	0.9 (±0.5)	12 (±1.7)	2.1 (±0.5)

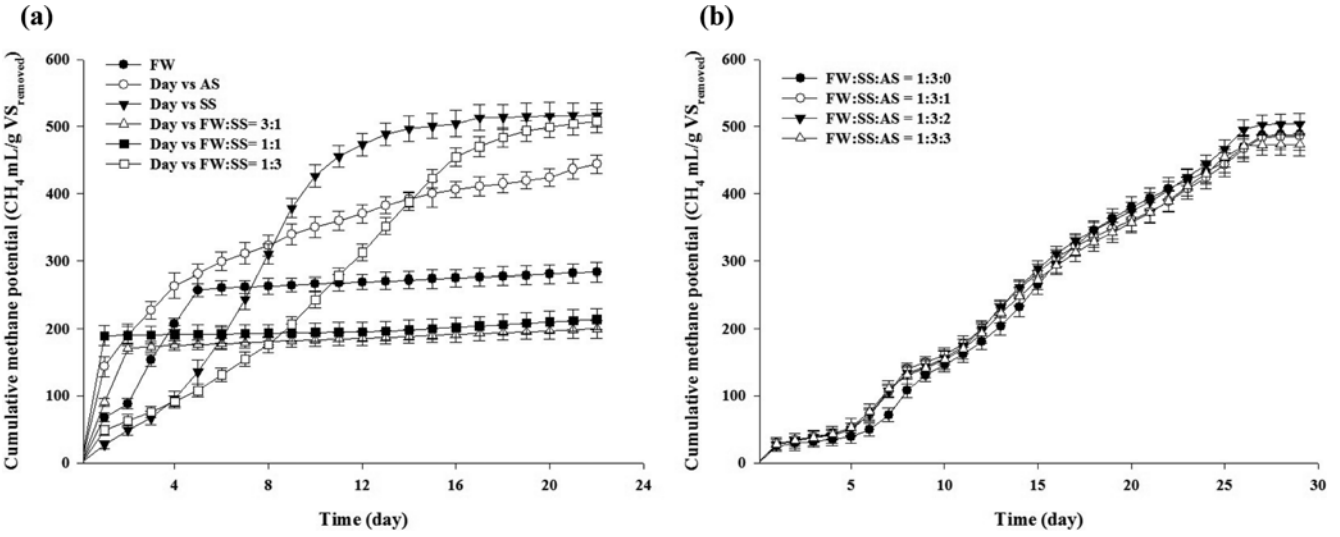

Fig. 1. Cumulative methane yields of single substrate and mixtures of FW and SS at different ratios (a) and of mixtures of FW, SS, and AS at different ratios (b).

Table 2. Initial and final characteristics of BMP samples

Parameter		Single substrate			Mixed substrate					
		FW	AS	SS	FW : SS (3 : 1)	FW : SS (1 : 1)	FW : SS (1 : 3)	FW : SS : AS (1 : 3 : 1)	FW : SS : AS (1 : 3 : 2)	FW : SS : AS (1 : 3 : 3)
pH	Initial	5.2	7.5	7.7	6.2	6.7	7.1	7.6	7.6	7.4
	Final	5.3	7.3	7.6	5.5	5.8	6.5	7.2	7.3	7.2
Total solids	Initial (%)	2.9	1.0	1.7	2.7	2.3	2.1	1.0	1.0	1.0
	Final (%)	2.7	0.9	1.4	2.4	2.3	1.5	0.9	0.9	0.9
	Removal efficiency (%)	6.9	10.0	17.6	11.1	0.0	28.6	10.0	10.0	10.0
Volatile solids	Initial (%)	2.3	0.6	1.1	2.1	1.7	1.5	0.7	0.8	0.8
	Final (%)	2.2	0.5	0.9	1.9	1.6	1.1	0.6	0.6	0.6
	Removal efficiency (%)	4.3	16.7	18.2	9.5	5.9	26.7	14.3	25.0	25.0
$tCOD_{cr}$	Initial (g/L)	40.1	10.9	20.9	32.2	25.3	21.6	14.8	14.9	15.1
	Final (g/L)	37.1	9.8	15.0	30.2	23.0	13.2	11.9	11.6	11.5
	Removal efficiency (%)	7.5	10.1	28	6.2	9.1	39	20	22	24
$sCOD_{cr}$	Initial (g/L)	9.1	0.4	1.3	6.5	3.8	2.7	2.4	2.3	2.4
	Final (g/L)	9.9	0.2	0.5	5.6	1.9	0.3	0.2	0.2	0.2
	Removal efficiency (%)	−8.8	50.0	61.5	13.8	50.0	88.9	91.7	91.3	91.7
TKN	Initial (g/L)	1.9	1.1	2.1	1.9	2.0	2.1	0.9	0.9	1.0
	Final (g/L)	1.6	0.9	1.9	1.7	1.8	2.2	0.9	0.9	1.0
	Removal efficiency (%)	15.8	18.2	9.5	10.5	10.0	−4.8	0.0	0.0	0.0
NH_4-N	Initial (g/L)	0.6	0.4	1.6	0.8	0.9	1.1	0.5	0.6	0.6
	Final (g/L)	1.0	0.5	1.3	1.1	1.2	1.3	0.6	0.7	0.7
	Increase efficiency (%)	66.7	25.0	−18.8	37.5	33.3	18.2	20.0	16.7	16.7
TP	Initial (g/L)	0.5	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.4
	Final (g/L)	0.4	0.3	0.4	0.4	0.4	0.3	0.4	0.4	0.4
	Removal efficiency (%)	20.0	0.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0
Alk.	Initial (g/L as $CaCO_3$)	1.52	3.31	5.45	3.05	3.85	5.11	4.62	4.75	4.77
	Final (g/L as $CaCO_3$)	1.78	4.55	7.85	3.75	5.28	7.69	7.11	7.32	7.16
	Increase efficiency (%)	17.1	37.5	44.0	22.9	37.1	50.5	53.9	54.1	50.1

of methanogens gets inhibited when the system pH is low or when the feedstock contains a high level of sodium (e.g., 2 to 10 g/L) [27,28]. Therefore, in this study, the stopping of methane generation in the bottle with FW containing high organics (about 150 g COD/L) was attributed to the low mixed liquor pH (pH 4.6) (Table 1). As a result, biogas production using FW only was not recommended.

The highest cumulative methane yield (510 mL/g VS) was observed when SS was used as a substrate; the yields were 455 and 265 mL/g VS when AS and FW were used as a substrate, respectively. As shown in Table 1, SS also contained high organic matters (COD of 60 g/L), which resulted in a high methane production in the BMP test. However, SS had a higher alkalinity (12 g/L as $CaCO_3$), which prevented the mixed liquor pH from becoming acidic. If SS is mixed with other substrates with high organics and low alkalinity like FW and digested together, more stable biogas production can be achieved, compared to the case where FW only is digested. Even though the reactor fed with SS alone showed a higher BMP in our study, the co-digestion with FW and SS could be more desirable in practice since FW has more organics that can be converted biogas; the highest COD reduction (i.e., 39%) could be achieved from the BMP bottle fed with a mixture of FW and SS at a ratio of 1 : 3 (Table 2).

To obtain the optimal mixing ratio of FW to SS to AS, a series of BMP tests were first performed with the mixtures of FW and SS; three different mixing ratios were applied (i.e., a ratio of FW to SS=3 : 1, 1 : 1, or 1 : 3). The mixed substrate showed higher pH than that of FW (Table 2). In the BMP tests, the highest methane yield or potential (511 mL/g VS) could be observed from the reactor fed with the FW and SS mixture of 1 : 3. Interestingly, the mixture containing FW and SS at a ratio of 3 : 1 or 1 : 1 did not produce much methane like the case where FW only was used a substrate (Fig. 1(a)). Note that CO_2 was absorbed by a 3 N NaOH buffer solution and was not measured in this specific experiment. From this result, it was again confirmed that methane production and organic stabilization can be limited when the amount of added FW is much higher than the other wastes. It is highly probable that the low pH caused by the high FW content in the two mixtures (Table 2) resulted in the low methane yield.

The BMP tests of mixtures of FW, SS, and AS were also evalu-

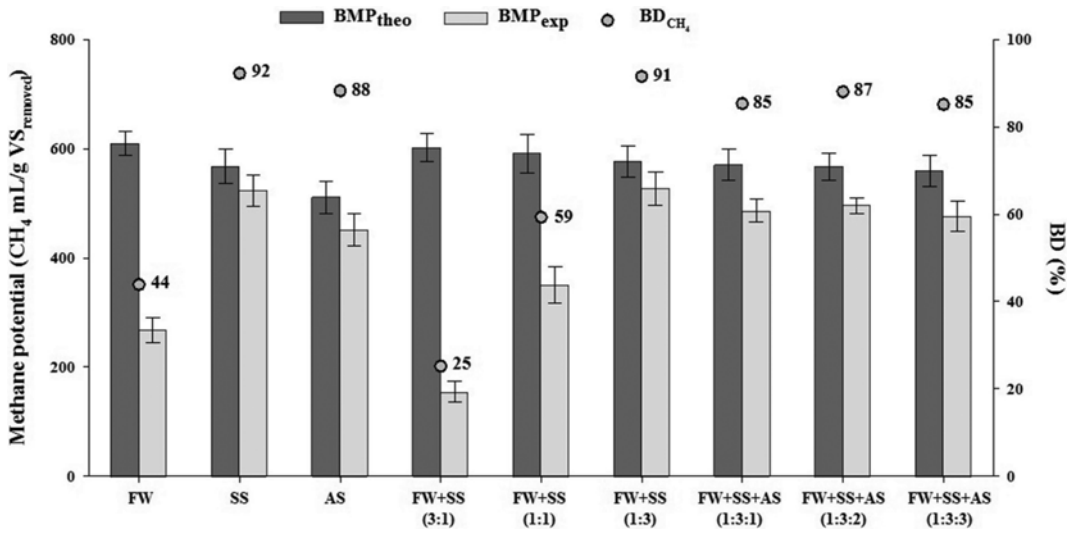

Fig. 2. Comparison of observed (BMP_{exp}) and theoretical (BMP_{theo}) cumulative methane yields and BD_{CH_4}.

ated. Three different mixing ratios were tested: a ratio of FW to SS to AS of 1 : 3 : 1, 1 : 3 : 2, and 1 : 3 : 3 (Fig. 1(b)). In fact, no noticeable difference in methane yields was observed; the yields of these three mixtures were similar. By adding SS to each of the mixtures, the pH values of mixed substrates were all maintained at >7.0, resulting in a relatively higher methane potential than those of substrates with FW only or mixtures with a higher FW content.

Nonetheless, the mixture containing FW, SS, and AS at a ratio of 1 : 3 : 2 showed a slightly higher BD_{CH_4} (Fig. 2), and this mixing ratio was selected and used in the subsequent ACD study.

Changes of water quality parameters were also analyzed during the BMP tests and correlated with gas production potentials of co-digested materials, as shown in Table 2. It is apparent that FW is characterized by low pH (pH 5.2-5.3), while SS and AS have neutral pH (pH 7.3-7.7) during a BMP test. As a result, microbial activity for the FW sample was easily affected by the initial pH than other samples. In the case of SS, it usually has a high organic content that can be potentially converted to methane gas. Note that NH_4^+ in the mixed liquor increased during the BMP test with the mixture of FW and SS (1 : 3), meaning that protein in FW and SS was well hydrolyzed. NH_4^+ concentration of all samples was well below the reported inhibitory level (i.e., 3,000 mg N/L) [29]. In contrast, the BMP test with the mixture of FW and SS of 1 : 1 showed a very similar result to that from the test with FW only: extremely low gas production and TS and VS removal efficiencies. This low methane production and solid reduction could be attributed to the accumulated VFAs in the mixed liquor during BMP tests. In fact, the VFA concentration of the FW used in this study was very high (Table 1).

3. Biodegradability for Different Organic Wastes

The theoretical methane production potential was evaluated by performing the element analysis with different organic wastes and subsequent application of Eqs. (1) and (2). As shown in Table 3, the content of carbon, hydrogen, oxygen, nitrogen, and sulfur in each substrate was plugged into Buswell's equation [20] for estimating theoretical BMPs. Then, the observed BMP values (BMP_{exp}) and theoretical ones (BPP_{theo}) were compared and presented in Fig. 2.

Table 3. Comparison of results from element analysis with different organic wastes

	C (%)	H (%)	O (%)	N (%)	S (%)
FW	59.7	6.3	29.0	4.6	0.3
SS	56.8	6.0	31.5	5.1	0.7
AS	51.5	6.6	35.5	5.6	0.8
FW+SS (3 : 1)	57.4	6.0	30.4	5.7	0.5
FW+SS (1 : 1)	58.0	6.3	29.9	5.5	0.4
FW+SS (1 : 3)	59.0	6.1	29.5	5.3	0.2
FW+SS+AS (1 : 3 : 3)	57.0	6.2	30.8	5.6	0.4
FW+SS+AS (1 : 3 : 2)	56.0	6.4	31.1	5.9	0.6
FW+SS+AS (1 : 3 : 1)	55.8	6.3	31.2	6.1	0.6

For all the substrates, the observed BMP_{exp} values were lower than BMP_{theo}. In addition, the substrates with a higher amount of FW showed much lower methane production than the theoretical estimation (Fig. 2). As explained above, the pH of FW is low by nature, so microbial activity is often inhibited. Contrarily, when FW was mixed with SS and AS, such inhibition was not observed, possibly due to the higher buffering capacity of the latter (Fig. 2). Therefore, it was concluded that ACD is better than AD of a single FW for more and stable gas production.

To better understand the effects of substrate types on methane production, microbial community analysis was conducted with DNA extractions from each BMP reactor. As shown in Fig. 3, there is clear difference between dominating microbial species (at phylum levels) of samples collected from BMP reactors fed with different substrates; for more clear comparison, a colored figure is provided as a supporting information (SI-1). In the case of SS and the FW-SS-AS mixture, the *Firmicutes* appeared to be a dominant phylum, which is known to produce H_2, CO_2 and fatty acid during AD [30]. On the other hand, the dominating phylum identified from the BMP bottle fed with FW only was the *Bacteroidetes*, which

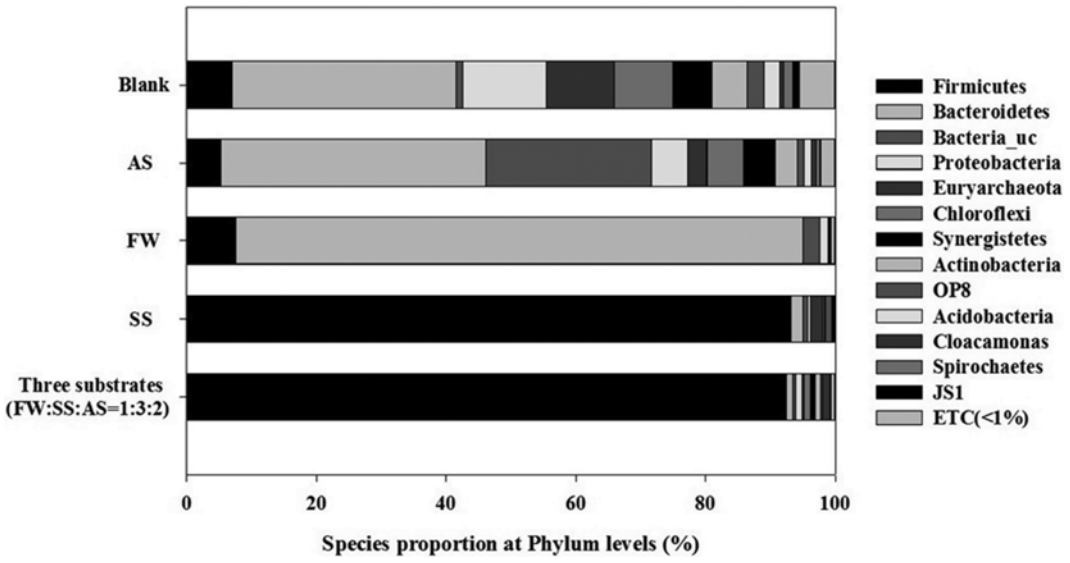

Fig. 3. Comparison of microbial community distributions of BMP reactors fed with different wastes.

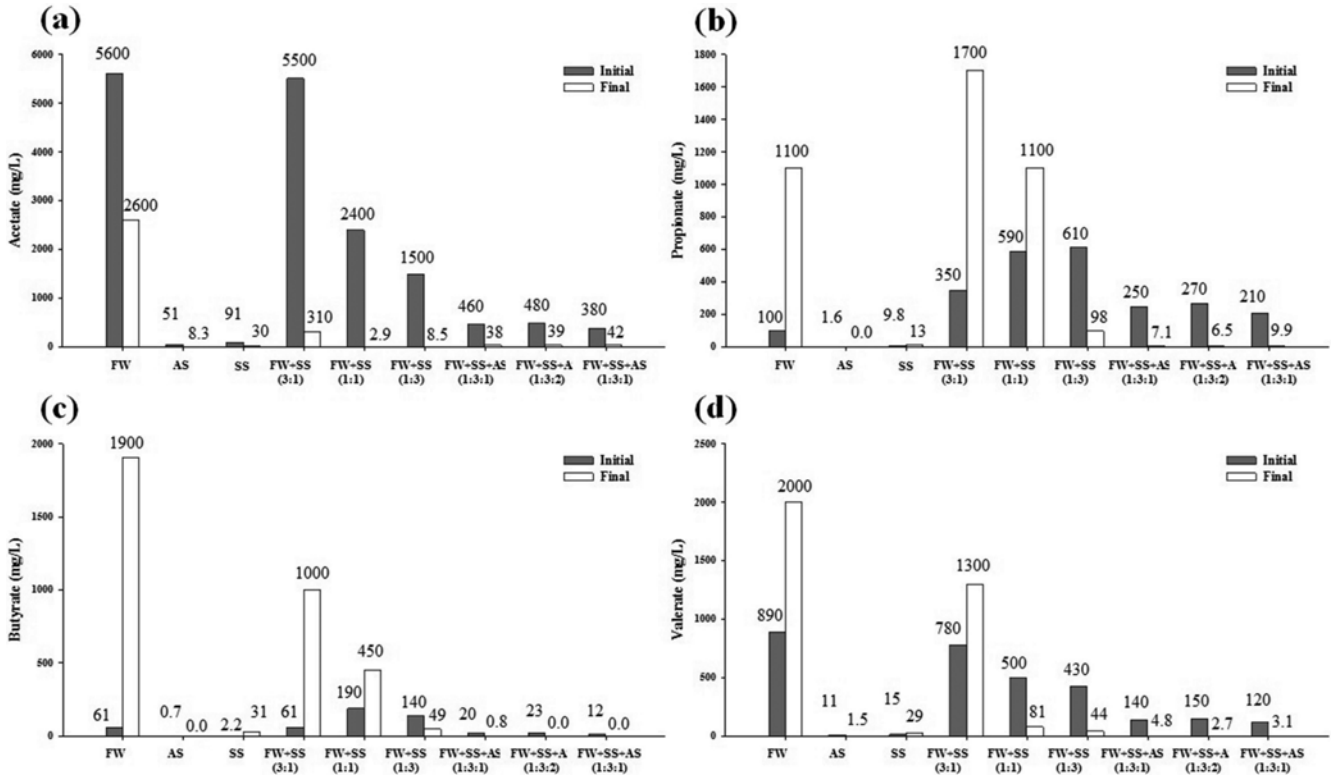

Fig. 4. Comparison of initial and final VFAs (i.e., acetate (a), propionate (b), butyrate (c) and valerate (d)) generated from BMP bottles fed with different wastes.

play a main role in protein degradation. The species are often found as the dominant phyla in ACD of protein-rich wastes [31,32]. Moreover, *Bacteroidetes* produce butyrate and can grow under low pH [33,34]. Therefore, the resistance of the bacterial species against low pH facilitates it to be dominant in the BMP reactors fed with FW only. However, the low pH of FW inhibited further microbial reactions, resulting in accumulation of fatty acids such as propionate, butyrate, and valerate (Fig. 4). In the case of the samples showing *Firmicutes* dominant, fatty acids produced via acidogenesis were used by methanogens.

The proximity of the bacterial community for each waste was compared (Fig. 5). The proximity analysis of the bacterial community structure confirmed that community for SS was certainly different from communities of other samples. The microbial com-

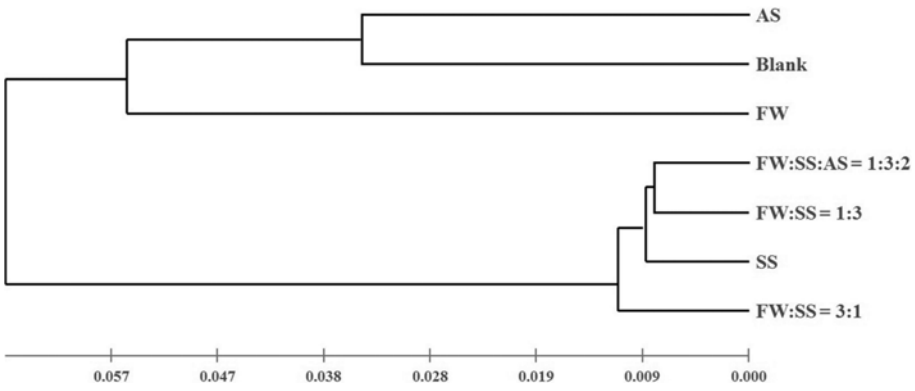

Fig. 5. Proximity of bacterial communities of samples from BMP bottles fed with different wastes.

Table 4. Comparison of major methanogens of BMP reactors fed with different wastes

	Activated sludge	Food waste	Swine slurry	Mixture of three wastes
Methane yield (mL/g VS)	455	265	510	485
Most abundant methanogen		*Methaosaeta concilii*		*Methanosarcina spelaei*
(distribution, %)	33%	27%	52%	31%

munity distributions of the samples from the BMP bottles fed with SS only or the mixtures of FW, SS and AS were similar.

Methanogens were also identified (Table 4). First, *Methanosaeta concilii* were identified as the main methanogenic species in AS, FW, and SS, while the *Methanosarcina spelaei* were in the mixture of FW, SS and AS. The dominance of two methanogens varies depending on the amount of acetate available in the medium. The species *Methanosaeta concilii* have a high affinity for such substrate as acetate. Therefore, the acetate content of AS, FW, or SS determines the abundance of the species [35]. On the other hand, *Methanosarcina spelaei* were identified as dominating methanogens in the BMP reactors fed with substrate mixtures. In fact, the species *Methanosarcina spelaei* are able to grow under a wide pH range (4-10) and at a high salt content (0.05 M NaCl). In addition, their growth rate is higher than that of *Methanosaeta concilii* [36].

The SS slurry collected on Day 0 showed a low acetate concentration (91 mg/L) (Fig. 4(a)). In the sample, *Methanosaeta concilii* were identified as the dominating methanogenic species, which produce methane via acetoclastic methanogenesis. On the other hand, the mixture of FW, SS, and AS contained acetate of 479 mg/L and promoted the growth of *Methanosarcina spelaei* which produce methane via both acetoclastic and hydrogenotrophic methanogenesis [37]. In addition, the species can help other methanogens proliferate by improving the environmental condition; *Methanosarcina spelaei* increase the medium pH by consuming acidogenesis products to produce methane [38,39].

Interestingly, accumulation of butyrate and valerate was observed in the BMP reactor fed with FW as a main substrate (i.e., FW only or a mixture of FW and SS at a mixing ratio of 3 : 1 or 1 : 1) as shown in Fig. 4. Although methanogens can directly use VFAs produced through acidogenesis, they cannot use butyrate and valerate [36]. Therefore, in the case of FW, methanogens could not use the acidogenesis products, since the activity of methanogens was restrained by the low pH. As a result, final concentrations of butyrate, propionate and valerate in the reactor fed with FW were high.

CONCLUSION

The potential of different organic wastes (i.e., FW, SS, and AS) to produce biogas via AD has been evaluated. By a series of BMP tests, the optimal mixing ratio of FW to SS to AS was determined. In addition, the microbial community that had developed in each BMP reactor was analyzed for a better understanding of the BMP test result.

SS showed the highest methane potential of 510 mL/g VS. Interestingly, it was confirmed that *Clostridium* spp. existed as hydrogen-producing bacteria in the BMP reactor, which would provide methanogens with hydrogen as a reducing agent for methane production, when SS only was utilized as a substrate. In addition, the higher buffering capacity of SS was found advantageous since the system could be held at neutral pH, which is favorable for methanogens.

In addition, in the case of the BMP testers with SS or the mixture of three wastes, *Firmicutes* was found as a dominant phylum, which produces H_2, CO_2 and fatty acids. These products are used in methanogenesis of the AD process. On the other hand, in the case of FW, hydrolytic bacteria, i.e., *Bacteroidetes* were more dominant than others, which produce protons and cause the system pH to get lower. As a result, a poor AD performance could result. Therefore, an AD of FW only was not recommended. Instead, FW should be mixed with other substrates that can compensate its buffering capacity or deficient nutrients and be co-digested.

The optimal ratio of FW to SS to AS for the mixed substrate was found to be 1 : 3 : 2. The dominant methanogenic species identified from the batch reactors fed with single substrates were different from those identified from the reactor fed with the FW-SS-AS

mixture. *Methanosaeta concilii* was present as the dominant methanogens in the reactors fed with AS, FW, or SS, while *Methanosarcina spelaei* was in the one fed with the combined wastes. Although *Methanosaeta concilii* only uses acetate for methane production, its activity is inhibited when acetate concentration is over 150 mg/L. On the other hand, *Methanosarcina spelaei* can transform both acetate and H_2/CO_2 to methane and can tolerate external stresses. Thus, ACD of the combined substrate can achieve a high biodegradation for organic waste, and is obviously advantageous compared to an AD of single substrate.

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SUPPORTING INFORMATION

Additional information as noted in the text. This information is available via the Internet at http://www.springer.com/chemistry/journal/11814.

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Supporting Information

Evaluation of relationship between biogas production and microbial communities in anaerobic co-digestion

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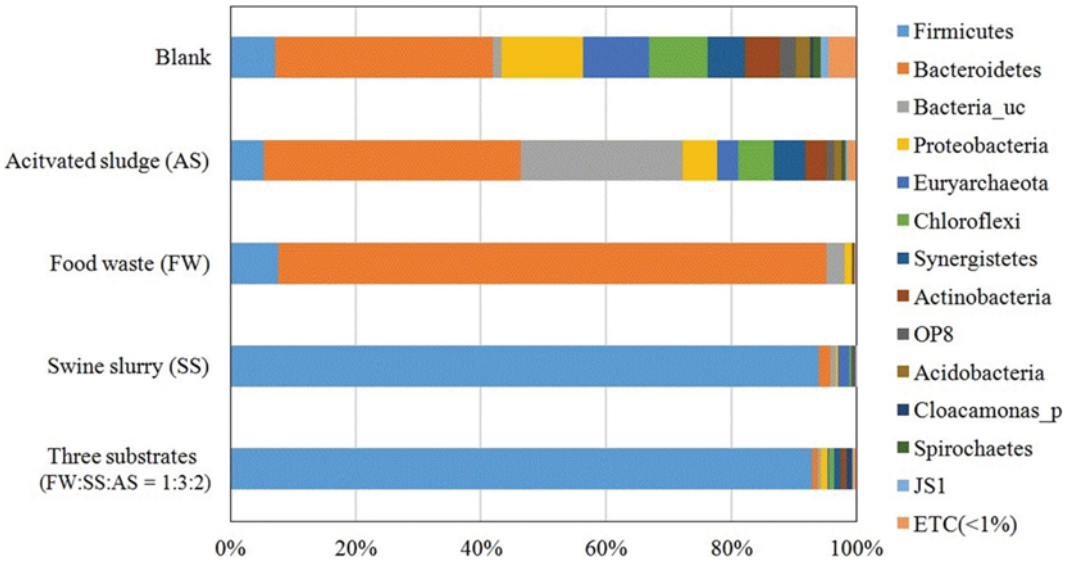

Fig. S1. Comparison of microbial community distributions of BMP reactors fed with different wastes.