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Effects of supplement additives on anaerobic biogas production

Minsoo Kim*, Dan Li*, Okkyoung Choi, Byoung-In Sang**, Pen Chi Chiang***, and Hyunook Kim*,†**

*Department of Environmental Engineering, University of Seoul, 163 Seoulsiripdae-ro, Dongdaemun-gu, Seoul 02504, Korea
**Department of Chemical Engineering, Hanyang University, 222 Wangshimni-ro, Seongdong-gu, Seoul 04763, Korea
***Graduate Institute of Environmental Engineering, National Taiwan University, Taiwan
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Abstract−Anaerobic digestion (AD) converts biomass to biogas. However, its performance is often affected by the nutrient condition of AD substrate. In this study, a few substrate supplements were selected to promote the biogas production; MgO, $FeCl_3$, and cellulase were selected based on the result from elemental analyses of the biomass. The potential impact of the additives on AD process was evaluated by performing a series of biochemical methane potential (BMP) tests. BMP reactors with the substrate with one of the selected additives (i.e., MgO of 380 mg Mg L^{-1}, $FeCl_3$ of 88 mg Fe L^{-1} or cellulase of 25 mg L^{-1}) exhibited higher microbial activity; 5-15% more biogas production was observed, compared to the blank. Microbial community analysis showed that different additives resulted in proliferation of different microbial species. Therefore, it was decided to add the mixture of the three additives to the biomass. Addition of the mixed additive resulted in 22% more gas production.

Keywords: Anaerobic Digestion, Biochemical Methane Potential (BMP) Test, Additives, Microbial Activity, Biogas Production

INTRODUCTION

The anaerobic digestion (AD) process can recover energy from organic wastes and animal manure. Therefore, this process has been applied in wastewater treatment plants (WWTPs) and organic waste recycling facilities. However, the AD process is slow and requires a long reaction time for degrading organic matters and producing energy, biogas. It is also difficult to maintain the system at a stable condition to produce a large amount of biogas, especially when toxic by-products accumulate in a reactor during fermentation [1]. Therefore, ongoing management and a good knowledge of the AD process is required.

In Korea, main feedstocks for an AD process are comprised of household food waste (FW), swine slurry (SS), and activated sludge (AS) from WWTPs. Direct disposal of FW into landfills has been banned in Korea since 2005 [2]. As a result, a separate collection/recycle program for food waste has been enforced. Additionally, SS has caused a significant odor pollution and posed a threat to the environment. The ban on ocean dumping of AS in 2012 has further increased the cost of sludge disposal [3,4].

Biogas is produced through AD, in which organic matter is degraded through stepwise conversion processes: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Therefore, the operational plan should be carefully designed to enhance activity of anaerobes belonging to different communities at each step. To promote an AD process for biogas production, factors affecting its performance such as substrate, temperature, and pH should be well-controlled [5,6]. Some organic wastes are unbalanced in element compositions such as carbon, nitrogen, sulfur, and trace elements. In addition, some substrates contain a large amount of toxins (e.g., butyric acid, free ammonia, hydrogen sulfide), which can accumulate during the AD process and can result in a low biogas production or system shut-down [7]. To enhance the performance and biodegradation of solid wastes, additional processes, such as heaters for digesters and grinders for organic matter, have been installed [8,9]. However, these additional facilities demand more operation cost as well as higher capital cost and large space.

Therefore, supplemental additives for promoting biogas production have attracted more attention. Additives are added to improve the AD process performance by supplying deficient nutrients or by reducing impacts from toxins in feed [10,11]. In general, supplements for the AD process include mineral additives and biological enzymes [12]. Mineral additives are also sub-divided into macronutrients and micronutrients. Macronutrients act as a buffer and compensate essential components for microbial growth, for example, nitrogen and phosphorous. In general, micronutrients are supplements for trace elements, such as Fe, Ni, and Co [10]. Often, these elements are fed in excess for other purposes. For example, H_2S, which is produced as a by-product during the AD process, can inhibit the growth of methanogens. One approach to control H_2S toxicity is to add iron, as it reacts with H_2S to form a salt precipitate (i.e., FeS). Similarly, magnesium can reduce the accumulation of ammonia in the mixed liquor through magnesium-ammonium-phosphate (MAP) crystallization [13].

Impacts of micronutrient additives on the AD process producing biogas have been evaluated [14-16]. Iron addition could make the environmental condition more favorable for biogas production by decreasing the oxidation-reduction potential (ORP) [17,18]. Co,

†To whom correspondence should be addressed.
E-mail: h_kim@uos.ac.kr

Ni, Zn, and Mo also have been shown very effective in promoting methane production even at a very low concentration [19]. A few biological enzymes are also reported to increase hydrolysis of organic compounds and methanogenic activity [12,20]. So far, studies on supplemental additives have focused mainly on their effects on the AD process performance in terms of biogas production. Few studies have been performed to develop the mixture of different additives for practical field application.

Anaerobes degrade substrates using enzymes in both intracellular and extracellular pathways [21]. In fact, the chemical composition of organic waste is complicated; it contains a large variety of particulate, colloidal, and soluble substrates. Each enzyme can degrade only few specific substrates. Therefore, diverse enzymes are required to ensure complete degradation of substrates that are present in the feed organic waste. However, it is difficult to maintain a stable and diverse microbial community and sufficient enzymes during the AD process. If enzymes are insufficiently available, AD can be limited. Therefore, if necessary, a supplemental enzyme can be provided for promoting biodegradation of substrate and increasing methane production [22-24]. However, the performance of an AD process might be deteriorated if an additive is supplied in excess [25]. Therefore, the amount and kinds of supplemental additives should be carefully designed to achieve improved gas production, depending on the substrate fed to an AD system [26].

In this study, elemental additives and biological enzymes were selected and added to batch AD reactors. Then, their influence on methanogenic activity was evaluated by performing biochemical methane potential (BMP) tests. The applied organic waste for the BMP test was a mixture of AS, FW, and SS.

Nowadays, more WWTPs anaerobically digest AS for volume reduction and biogas production. However, low C/N ratio and deficiency of essential nutrients, FW and SS, are used together. Even so, FW and SS have high content of nitrogen and sulfur. It makes the inhibition of microbial growth by accumulated by-products as free ammonia and sulfide during AD process. These by-products could be controlled by adding of Mg and Fe supplements [25,27]. Also, FW contains a large amount of lignin, cellulose and lipid, which could be affected for microbial activity of methanogens and disintegration of organic compounds. Therefore, AD of FW are required to enforce the methanogen activity and primary degradation steps for biogas production by using biological enzyme such as cellulase, lipase and protease [28].

We selected Mg and Fe supplements to control toxicity from free ammonia and hydrogen sulfide. Cellulase was used to promote hydrolysis of cellulose and lignin materials in our feedstock. Microbial community analysis was also performed to understand dominant anaerobic species in each batch reactor supplemented with a different additive. Based on the effects of individual additive to the AD performance, an additive mixture consisting of Mg, Fe, and cellulase was designed to reduce toxicity of free ammonia and hydrogen sulfide and to increase the organic degradation rate in this study.

Finally, a comparative evaluation was performed with the additive mixture and commercially available additives and the feasibility of the developed additive mixture for field application was evaluated. The result indicated that the combined additives, selected based on substrate characteristic, were effective in promoting the activity of anaerobes and increasing methane production.

MATERIALS AND METHODS

1. Organic Wastes and Inoculum

In this study, organic waste consisting of a mixture of FW, SS, and AS was used as the substrate for BMP tests. FW and SS were obtained from an organic waste treatment facility and a nearby livestock farm in Wonju, Korea. AS and digested sludge (used as inoculum) were collected from a local WWTP in Seoul, Korea. Upon collection, the samples were stored at 4 °C.

2. Additives for Batch Experiments

To select appropriate additives for the BMP tests which were performed in this study, the composition of feed substrate was determined. The ideal nutrient ratio of C : N : P : S for the AD process is known as 600 : 15 : 5 : 3 [29]. N, P, and S in substrate are converted to ammonia, phosphate, and sulfide during the AD process. Thereof, ammonia and sulfide are, if present in excess, toxic for methanogens [7]. In general, SS and FW contain large amounts of N and S [30] and produce high amount of ammonia and sulfide while being anaerobically digested. Therefore, MgO and $MgCl_2$ were selected as a Mg supplement to remove ammonia from the solution in the BMP reactors via magnesium ammonium phosphate (MAP) crystallization [13]. $FeCl_2$ and $FeCl_3$ were selected as a Fe supplement; they can remove sulfide from the solution by forming iron complex/precipitation [25]. Lastly, cellulose (Sigma-Aldrich, Saint Louis, USA) was used as a biological enzyme to boost biodegradation of substrate and accelerate methane production.

Dose of each supplemental additive was determined by content of N, P, and S in the mixed substrate, which was analyzed before each BMP test. The elemental analysis result showed that N and S content of the substrate was high enough to cause toxicity AD process. Therefore, our purpose for adding additives to substrate was to reduce the potential toxicity of N and S in substrate and to promote biodegradation efficiency of organics. By comparing ideal elemental composition of AD substrate in the literature [29] and result of elemental analysis, excess amount of N, P, and S was calculated as shown in Eqs. (1), (2) and (3).

$$N_e = TS \times \mu_N - TS \times \mu_C \times (15/600) \quad (1)$$

$$P_e = TS \times \mu_P - TS \times \mu_C \times (5/600) \quad (2)$$

$$S_e = TS \times \mu_S - TS \times \mu_C \times (3/600) \quad (3)$$

N_e, P_e and S_e are concentration of excess N, P and S in substrate, respectively. TS is total solid content of substrate. μ_C, μ_N, μ_P, and μ_S are fractions of C, N, P and S in the substrate.

Additionally, it was assumed that most N, P, and S in substrate would exist as NH_4^+/NH_3, PO_4^{3-}, and HS^-, respectively. Fe^{3+} preferentially reacts with sulfide to precipitate as $FeS_{(s)}$. Mg^{2+} easily reacts with PO_4^{3-} and NH_4^+ to form MAP (i.e., $MgNH_4PO_4$); phosphorous content is always limiting the MAP process. Once formed, FeS and $MgNH_4PO_4$ do not dissociate to produce sulfide and ammonia in AD process, so toxicity from them can be reduced.

According to Eqs. (4) and (5), the dose of Fe and Mg for substrate subjected to a BMP test was calculated as shown in Eqs. (6)

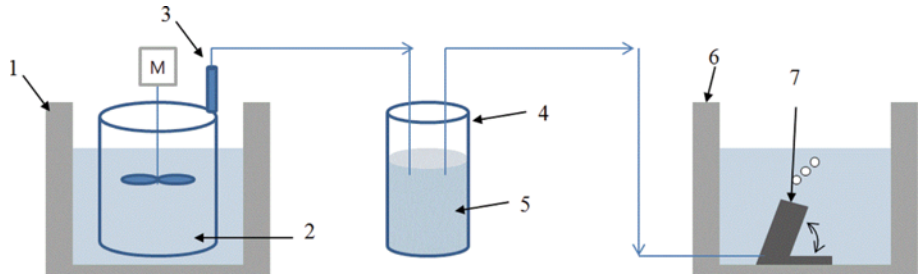

Fig. 1. Schematic diagram of the batch experiment.
1. Water-batch thermostat 3. Vent for biogas 5. 3.0 M NaOH solution (CO_2-fixation) 7. Barorecepter (flow cell)
2. Anaerobic digester (glass bottle) 4. Buffer reactor 6. Water batch package

and (7).

$$2\,Fe^{3+}+3\,HS^{-}\rightarrow 2\,FeS_{(s)}+S+3\,H^{+} \quad (4)$$

$$Mg^{2+}+NH_4^{+}+HPO_4^{2-}\rightarrow MgNH_4PO_{4(s)}+H^{+} \quad (5)$$

$$C_{Fe}=S_e\times(112/96) \quad (6)$$

$$C_{Mg}=P_e\times(24/31) \quad (7)$$

C_{Fe} and C_{Mg} are the dose of complementary Fe and Mg in a BMP test. In this study, Fe and Mg doses were calculated as 88 and 380 mg L^{-1}, respectively.

3. Experiment Setup for BMP Tests

Each BMP test was carried out in a 600 mL glass bottle at 35 °C in a thermostatic bath. Biogas production was monitored by an automatic methane potential test system as shown in Fig. 1. A bottle was filled with 400 mL substrate, a mixture of FW, SS, and AS at a ratio of 1 : 3 : 2, and with 20 mL seeding sludge. The mixture was stirred by a mixer at 120 rpm, and oxygen was removed by purging N_2 gas before starting the BMP test. During the BMP test, biogas produced in the BMP bottle flowed through a bottle with 3 M NaOH solution for CO_2-fixation and a flow cell in another water bath for flow measurement. The BMP test was terminated when biogas production rate reached at a limit of 5 mL d^{-1}. A more detailed method of the BMP test has been reported elsewhere [31,32]. Sampling was performed in the beginning and at the end of each BMP test.

4. Analytical Methods

Water quality parameters including total and soluble chemical oxygen demand (tCOD, sCOD), total solids (TS), volatile solids (VS), total Kjeldahl nitrogen (TKN), total ammonium nitrogen (NH_4^++NH_3), total phosphorus (TP), and alkalinity were measured according to Standard Methods [33]. The soluble water quality parameters were measured after a sample was filtered using a GF/C filter with a pore size of 1.0 μm (Whatman, Maidstone, UK). The solution pH was measured using a pH meter (Orion Benchtop, Thermo Fisher Scientific, Waltham, MA, USA). The element analysis for C, H, O, N, and S was carried out using an atomic absorption spectrophotometer (AA-6200, Shimadzu, Kyoto, Japan). All samples were dried in an oven at 105 °C for 24 h before the elemental analysis. Theoretical methane yield was calculated using Eq. (8) and Eq. (9) based on the determined element composition [27,34]. The biodegradation potential was calculated by comparing the theoretical methane yield and the cumulative methane yield observed in the BMP test, as shown in Eq. (10) [35].

$$C_aH_bO_cN_dS_e+(a-0.25\,b-0.5\,c+0.75\,d+0.5\,e)\,H_2O \rightarrow(0.5\,a+0.125\,b-0.25\,c-0.375\,d-0.25\,e)\,CH_4 +(0.5\,a-0.125\,b+0.25\,c+0.375\,d+0.25\,e)\,CO_2+d\,NH_3+e\,H_2S \quad (8)$$

$$BMP_{theo}=22.4\times(0.5\,a+0.125\,b-0.25\,c-0.375\,d-0.25\,e)/(a+b+16\,c+14\,d+32\,e)\ (NM^3\,kg^{-1}\,VS_{add}) \quad (9)$$

$$BD_{CH4}=(BMP_{exp}/BMP_{theo})\times100\% \quad (10)$$

BMP_{exp}: Biochemical methane potential from batch experiment
BMP_{theo}: Theoretical methane potential based on elemental analysis result
BD_{CH4}: Biodegradation potential

Volatile fatty acids (VFA) were measured using a gas chromatograph with a flame ionization detector (GC 2010, Shimadzu, Kyoto, Japan). The column used in this study was SH-Rtx-Wax with 30 m length×0.25 mm inner diameter×0.25 μm thickness (Shimadzu, Kyoto, Japan). The operating conditions were as follows: helium gas was used as a carrier gas; detector temperature was 250 °C; injection volume was 5 μL. Oven temperature was programmed to be initially held at 120 °C for 2 min, raised at 10 °C min^{-1} to 150 °C, at 5 °C min^{-1} to 180 °C, and at 10 °C min^{-1} to 240 °C, and held for 5 min.

Trace elements such as Mg, Fe, Mo, Ni, Co, Mn, and Ni were selected based on metal composition of the substrate, which was measured by an inductively coupled plasma-atomic emission spectrometer (ICP-AES; ICPE-9800, Shimadzu, Kyoto, Japan).

5. Microbial Community Analysis

5-1. DNA Extraction and Pyrosequencing

Total genomic DNA was extracted using a Power Soil DNA Kit (MoBio Inc., Carlsbad, USA) following the manufacturer's instructions, and it was stored at −20 °C. To confirm the composition of microbial communities, amplification of the V3-V4 region of the bacterial 16S rRNA gene, which is highly variable was performed for each sample using 341F and 805R primers. Samples were amplified for pyrosequencing using forward and reverse fusion primers. A forward primer was constructed with a (5′-3′) Nextera consensus (TCGTCGGCAGCGTC) and a sequencing adaptor (AGATGTGTATAAGAGACAG); the final forward primer selected for the bacterial diversity assay was 341F one (CCTACGGGNGGCWGCAG) [36]. A reverse fusion primer was also constructed with a (3′-5′) Nextera consensus (GTCTCGTGGGCTCGG) and

the sequencing adaptor; the finally designed reverse primer for the bacteria diversity assay was 805R one (GACTACHVGGGTATCTA-ATCC) [36]. Amplifications were conducted in 25 μL reaction mixtures including Dr. MAX DNA Polymerase (Doctor Protein Inc, Seoul, Korea), 1 μL of each 5 μM primer, and 1 μL of template. The polymerase chain reactions were carried out under the following conditions: initially held at 95 °C for 3 min, 25 cycles at 95 °C for 30 s, at 55 °C for 30 s, and at 72 °C for 30 s, one cycle at 72 °C for 5 min, and held at 4 °C. The DNA sequencing was performed by ChunLab, Inc. (Seoul, Korea) using an Illumina/MiSeq platform, following the manufacturer protocol [37].

5-2. Biodiversity Analysis and Phylogenetic Classification

Following the bioinformatics procedures described by Fadrosh et al. [36] and Jeon et al. [38], DNA sequences were proceeded and analyzed. Raw sequencing reads from different reactor samples were classified by barcode sequences that were included in the PCR primers. Short sequences (less than 300 base pairs (bp)) or the sequences that included more than two ambiguous bases (Ns) were removed before analysis. Primer, linker, and barcode sites were then trimmed by pairwise alignment. Target genes were searched using the EzTaxon-e database. Non-target genes that showed no matches in the 16S rRNA gene database were discarded. Chimeric sequences were detected using the BLAST program. Taxonomic assignment is a similarity search used to identify the taxonomy of the contig sequences. This step was performed by comparing the sequence reads against the EzTaxon-e database using a combined method of BLASTN searches and pairwise similarity comparisons. Finally, species identification was conducted according to their similarity values.

The diversity and species richness indices were calculated by setting the cutoff value for assigning a sequence to a species-level phylotype to at least 97% similarity. The overall phylogenetic distances between communities of the BMP test samples were estimated using the Fast UniFrac calculation [39].

RESULTS AND DISCUSSION

1. Water Quality Characteristics of the Three Substrates

Characteristics of substrates (i.e., FW, SS, and AS) used in this study are shown in Table 1. As expected, FW was rich in VFAs. According to the elemental analysis, all substrates, especially, SS and AS had N, P, and S all in excess; their content was much higher than the theoretical requirement. Therefore, it was expected that ammonia, phosphate, and sulfide could be accumulated during the AD process and biogas production could be affected. The amounts of Mg and Fe supplements were determined based on the analyzed characteristics of the mixed substrate consisting of FW, SS, and AS at a ratio of 1 : 3 : 2. In this study, before the BMP test, both elemental analysis (C, N, P and S) and water quality analysis (i.e., TKN, TP and TS) were carried out with the mixed substrate. Based on the result from the element analysis, μ_C, μ_N, μ_P and μ_S were calculated 52.4%, 4.9%, 1.4% and 0.4%, respectively. By comparing the ideal element ratio and the element-analysis result, the excess N, P and S were calculated using Eqs. (1) to (3); the excess N_e, P_e, and S_e were calculated 1,813, 501, and 75 mg L^{-1}, respectively. Thus, the required dosage (concentration, mg L^{-1}) of Mg (C_{Mg}) and Fe (C_{Fe}) was calculated 388 and 88 mg L^{-1}, respectively, which was equivalent to MgO of 647 mg L^{-1} and $FeCl_3 \cdot 6H_2O$ of 425 mg L^{-1}.

In addition, VFAs in FW and SS were notably high, as shown in Fig. 2. Acetic and propionic acids are readily transformed to methane during AD process. On the other hand, certain fatty acids, such

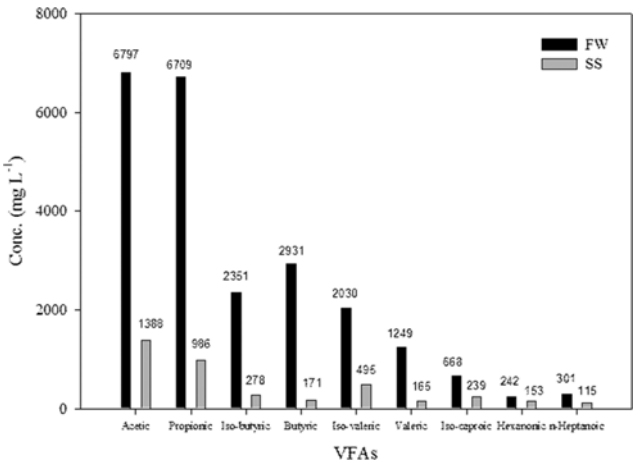

Fig. 2. VFAs of FW and SS.

Table 1. Characteristics of the three substrates

Parameter	Food Waste (FW)	Swine Slurry (SS)	Activated Sludge (AS)
pH	4.9	7.7	7.4
Total solids (mg L^{-1})	106,530	85,630	22,850
Volatile solids (mg L^{-1})	88,960	62,530	14,160
$tCOD_{cr}$ (mg L^{-1})	112,400	82,520	17,280
$sCOD_{cr}$ (mg L^{-1})	43,610	7,360	124
TKN (mg L^{-1})	3,265	4,123	1,320
NH_4^+-N (mg L^{-1})	1,288	3,514	406
TP (mg L^{-1})	752	979	342
TVFA (mg L^{-1})	23,280	3,990	35
Total sulfur (mg L^{-1})	127	678	104
Ratio of C : N : P : S	600 : 31.2 : 7.2 : 1.44	600 : 53.3 : 12.5 : 9.1	600 : 81.6 : 21.1 : 6.7

Table 2. ICP-AES analysis data of each substrate (unit: mg kg TS^{-1})

	Mg	Cu	Fe	Mo	Zn	Ni	Se	Co	Mn	W
FW	860	11	170	1.2	52	2.9	0.2	3.9	90	0.1
SS	3,200	67	2,780	9.8	90	16	0.5	5.2	170	0.2
AS	4,900	83	3,680	7.1	100	10	0.9	8.7	420	0.2
Recommended range	100-1,500	10-80	750-5,000	0.05-16	30-400	4-30	0.05-4	0.4-10	100-1,500	0.1-30

as iso-butyric and iso-valeric acids, inhibit methanogenic activity, being used as an indicator for failure of an AD process; these acids are not easily converted to methane. For FW, iso-butyric and iso-valeric acid concentrations were 2,351 and 2,030 mg L^{-1}, respectively. Such high concentrations of iso-butyric and iso-valeric acids might have an adverse impact on methanogenic activity. Kanokwan (2006) observed inhibited methane production when iso-butyric and iso-valeric acids were more than 15 mg L^{-1} [40]. On the other hand, SS contained relatively low amounts of iso-butyric and iso-valeric acids. VFAs in AS were much lower than those in SS.

According to the result from the ICP-AES analysis (Table 2), most mineral metals and trace elements of substrates were in a normal concentration range [41]. This result demonstrates that these substrates are rich in those trace elements and mineral elements, and it is not necessary to feed more of these trace elements for promoting methane production.

2. Methane Yields by Supplying Different Additives

2-1. Influence of Additive Addition to Methane Production

As shown in Fig. 3, additives led to an increase of BMP values and methane yields, which proves that elemental composition adjustment and microbial enzymes could be used to promote the performance of an AD process.

Both $MgCl_2$ and MgO showed higher methane yields (2,900 and 2,825 mL, respectively) and BMP values (658 mL g VS^{-1}). MgO was selected as an Mg additive in the further BMP tests as its alkaline nature would be beneficial for stabilizing the system pH during the AD process as shown in Table 3 [12]. $FeCl_2$ and $FeCl_3$ also showed similar results, being 639 and 642 mL g VS^{-1}, respectively. $FeCl_3$ was selected as the Fe additive in the further BMP tests since it is an oxidant and is more stable than $FeCl_2$ [12,42]. These additives could be applied with ease in practice.

The BMP bottle with cellulase added showed a high BMP value;

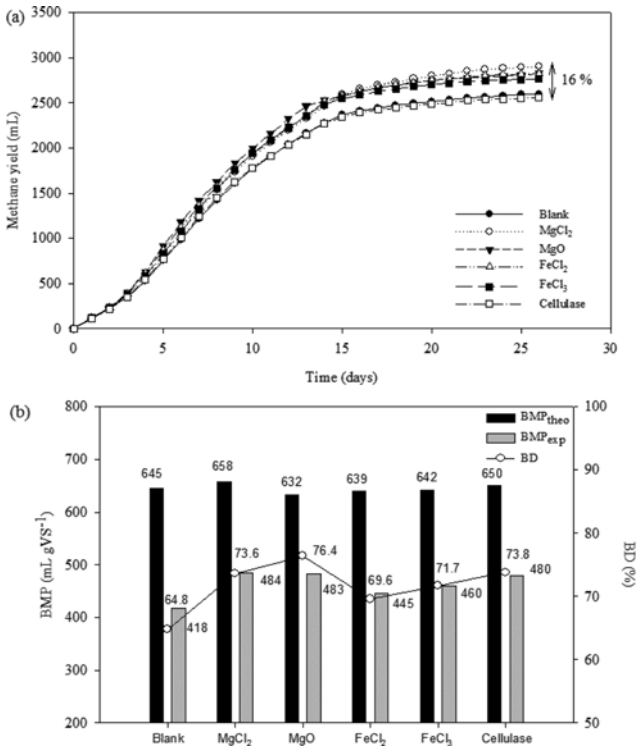

Fig. 3. (a) Methane yields and (b) BMP values for different supplemental additives.

the enzyme improved the primary degradation step first and then methanogen activity. Food waste contains a large amount of residual food, which contains cellulose, lignins, lipids, and proteins. In this case, the addition of an enzyme could speed up the initial degradation step (or hydrolysis), resulting in more effective bio-

Table 3. Digestion efficiencies for applied different additives

	$MgCl_2$	MgO	$FeCl_2$	$FeCl_3$	Cellulase
Methane yield (mL)	2,900	2,825	2,822	2,762	2,555
VS removal rate (%)	26	25	27	26	23
tCOD removal rate (%)	40	41	42	39	37
BMP_{theo} (mL g VS^{-1})	658	632	639	642	650
Acetic acid removal rate (%)	97	98	97	97	94
Propionic acid removal rate (%)	91	87	90	92	89
Butyric acid removal rate (%)	90	96	87	90	92
pH control	Neutral	Alkaline	Acidic	Acidic	No effect
Redox property	Stable	Stable	Reductant, unstable	Oxidant & stable	No effect

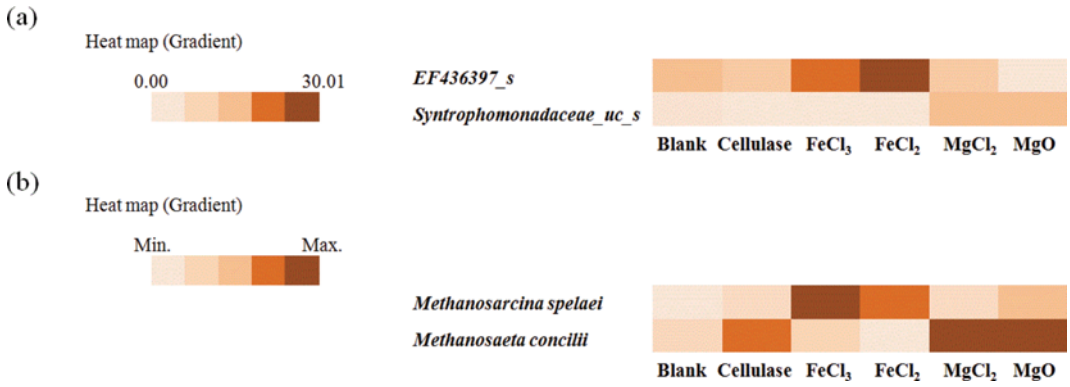

Fig. 4. (a) Major bacterial species and (b) major methanogens for different supplemental additives.

degradation; sCOD was changed from 4,013 to 345 mg L^{-1} during BMP test period [43]. Enzymes can promote hydrolysis of most of the organic macromolecules to small-molecules which could be utilized by anaerobes [28]. Although enzymes might promote degradation of organic substrates, accumulation of ammonia which can inhibit activity of methanogens can be resulted in. In this case, the rate of increase for ammonia was 36% by cellulase addition. On the other hand, the ammonia in case of blank was increased by 49%. Therefore, ammonia is accumulated less if enzymes are added, so it might be better to apply cellulase with other additives able to reduce toxicity of ammonia and sulfide. Therefore, MgO and $FeCl_3$ were selected and added to AD reactors along with cellulase as ingredients of a combined additive to improve the gas production in this study.

2-2. Difference of Microbial Community

The microbial community analysis was performed to understand the effect of the additives on the development of microbial community during batch experiments, as shown in Fig. 4. Microbial species consisting of a microbial community in samples treated with different additives were differentiated. As shown in Fig. 4(a), different bacterial species appeared in the batch reactors according to the applied additives. Increased biogas production was observed when MgO and $MgCl_2$ were added, which was coincident with the increase of *Clostridium* (*Syntrophomonadaceae_uc_s*). The microorganisms in this family use carboxylic acid as an energy source to produce short-chain volatile fatty acid (VFAs) such as formic acid and hydrogen [44]; methanogens can produce CH_4 using the VFAs [44]. Especially, when $FeCl_2$ and $FeCl_3$ were added to substrate, the population of *Clostridium* (*EF436397_s*) was observed to increase. *Clostridium* is a genus of acidogenic hydrogen-producing bacteria [45].

Both products made by *Clostridium* can be consumed by *archaeum* of methanogens (such as *Methanosarcina spelaei* and *Methanoaseta concilli*) as substrates, resulting in methane production as shown in Fig. 4(b). The batch reactors treated with $FeCl_2$ or $FeCl_3$ showed proliferation of *Methanosarcina spelaei*, which produce CH_4 using H_2 and CO_2 [46].

Lastly, in this study, *Methanoaseta concilli* was shown when $MgCl_2$, MgO and cellulase were added to substrate; therefore, the methane potential of the reactor might be promoted by the increased population of methanogenic archaeum.

In short, the following conclusion can be drawn from the result. Different microorganisms producing methane via different pathways can develop depending on the additive added. Therefore, a mixture of MgO, $FeCl_3$, and cellulase was added to substrate of BMP reactors in the present study in order to investigate how much the BMP of the batch system is improved.

3. Comparison between Commercial Products and Mixed Additives

The feasibility of the mixture of additives in field application was studied by comparing BMPs of substrates treated with the mixture of MgO, $FeCl_3$, and cellulose and the commercially-available products. Three commercial products were purchased: Neutral-Protease (Protease, Shenzhen Hengsheng Biological Technology Co.

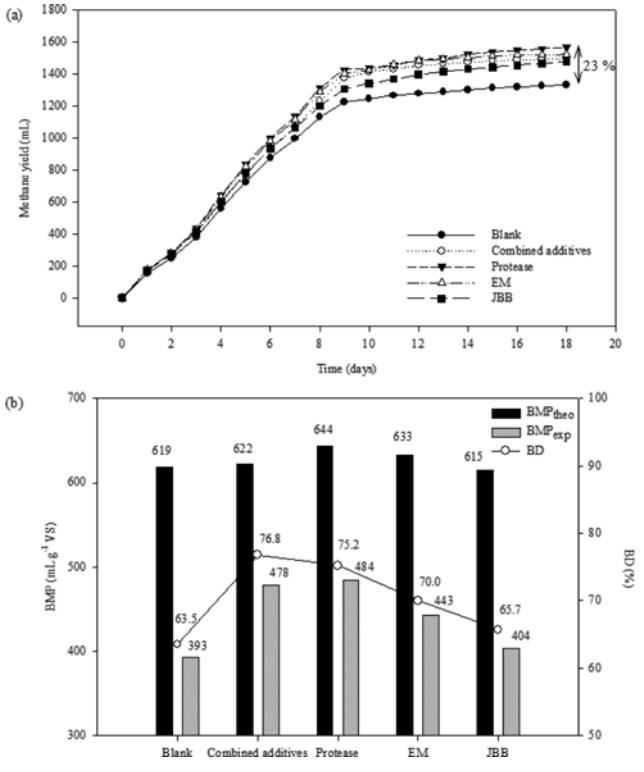

Fig. 5. (a) Methane yield, (b) BMP value and biodegradation of combined additives and different commercial products.

Ltd., China), Effective Microorganism Pure Culture (EM, Anhui Guangyu Biotechnology Co. Ltd., China), and Jinbaobei Biogas Additive (JBB, Beijing Healthhead Science & Technology Co. Ltd., China). Based on manufacturer's advertisement, the protease product can cause rapid protein hydrolysis and amino acid production. The EM and JBB products are argued to contain active methanogens to produce biogas. These commercial products and the combined additives made in this study were tested together in batch experiments and evaluated by the BMP test.

As shown in Fig. 5, the batch reactor with the combined additive showed 22% more biogas production compared to the blank. Among the three commercial products, the protease product showed the highest digestion efficiency (23%) with the BMP value of 484 mL g VS^{-1} and 75% biodegradation value. The combined additive showed the BMP value of 478 mL g VS^{-1} and 77% biodegradation value, confirming that it could improve the biogas production as much as a commercial one, by compensating unbalanced nutrients of feed substrate.

CONCLUSIONS

We evaluated the effect of addition of different additives, which were composed of MgO, $MgCl_2$, $FeCl_2$, and $FeCl_3$, cellulase on biogas production using mixed organic wastes composed of FW, SS, and AS. A series of BMP tests were conducted with the organic substrate with different additives which were considered to be able to reduce inhibition caused by excess ammonia and sulfide in mixed liquor. In addition, cellulase was selected as an enzyme additive for promoting the degradation of cellulosic matters, as FW has large amounts of lignin and cellulose. The BMP values differed for added additives. The amount of biogas produced by the reactor treated with either of $MgCl_2$ or MgO was 15% higher than that of the blank.

The batch reactors treated with $FeCl_2$ or $FeCl_3$ also showed higher BMP values than the control; more biogas production could be observed in the reactor with $FeCl_3$ added. Thus, $FeCl_3$ was selected for the present study. Microbial community analysis revealed that different additives might promote growth of different microorganism groups. For example, the additives like MgO and $FeCl_3$ showed improved activity of hydrogen producing bacteria, such as *Syntrophomonadaceae* and *Clostridium*; therefore, biogas production could be increased easily through the conversion of hydrogen generated by these bacteria to methane. Cellulase was observed to promote the growth of *Methanosaeta concilii*; as a result, the biogas production was enhanced.

Lastly, MgO, $FeCl_3$ and cellulose were applied as a mixture. The reactors treated with the combined additive showed a higher cumulative methane yield than the control; the BMP value and biodegradation efficiency of reactors were higher than those with the commercial products. Therefore, the combined additive can be used for promoting biogas production in practice. From this study, we demonstrated that anaerobic biogas production can be promoted simply by adding substrate supplements which can reduce the indigenous toxicity of substrates. Therefore, further research should be pursued to identify inhibitory effects of substrate on an AD and to develop substrate supplements to negate the inhibitory effect.

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REFERENCES

1. M. Murto, L. Björnsson and B. Mattiasson, *J. Environ. Manage.*, **70**, 2 (2004).
2. B. S. Lee, S. C. Nam and W. Namkong, *J. Korea Soc. Waste Manage.*, **28**, 6 (2011).
3. D. H. Kim, H. S. Shin and S. E. Oh, *J. Korea Soc. Waste Manage.*, **25**, 8 (2008).
4. S. Lee, Y. S. Yoon, J. G. Kang, K. H. Kim and S. K. Shin, *J. Korea Org. Resour. Recycl. Assoc.*, **24**, 1 (2016).
5. Y. G. Chen, S. Jiang, H. Y. Yuan, Q. Zhou and G. W. Gu, *Water Res.*, **41**, 30 (2007).
6. K. Wang, J. Yin, D. Shen and N. Li, *Bioresour. Technol.*, **161**, 395 (2014).
7. Y. Chen, J. J. Cheng and K. S. Creamer, *Bioresour. Technol.*, **99**, 10 (2008).
8. C. Dumas, G. S. G. Damasceno, A. Barakat, H. Carrère, J. P. Steyer and X. Rouau, *Ind. Crops. Prod.*, **74**, 450 (2015).
9. H. Carrere, G. Antonopoulou, R. Affes, F. Passos, A. Battimelli, G. Lyberatos and I. Ferrer, *Bioresour. Technol.*, **199**, 386 (2016).
10. A. Schattauer, E. Abdoun, P. Weiland, M. Plöchl and M. Heiermann, *Biosyst. Eng.*, **108**, 1 (2011).
11. I. A. Nges and L. Björnsson, *Biomass Bioenergy*, **47**, 62 (2012).
12. M. S. Romero-Güiza, J. Vila, J. Mata-Alvarez, J. M. Chimenos and S. Astals, *Renew. Sust. Energy Rev.*, **58**, 1486 (2016).
13. Y. Liu, S. Kumar, J. H. Kwag and C. Ra, *J. Chem. Technol. Biotechnol.*, **88**, 2 (2013).
14. B. Demirel and P. Scherer, *Biomass Bioenergy*, **35**, 3 (2011).
15. W. Zhang, L. Zhang and A. Li, *Water Res.*, **84**, 266 (2015).
16. J. L. Linville, Y. Shen, R. P. Schoene, M. Nguyen, M. Urgun-Demirtas and S. W. Snyder, *Process Biochem.*, **51**, 9 (2016).
17. Y. Liu, Y. Zhang, X. Quan, Y. Li, Z. Zhao, X. Meng and S. Chen, *Chem. Eng. J.*, **192**, 179 (2012).
18. Y. Zhang, Y. Feng, Q. Yu, Z. Xu and X. Quan, *Bioresour. Technol.*, **159**, 297 (2014).
19. T. Schmidt, M. Nelles, F. Scholwin and J. Pröter, *Bioresour. Technol.*, **168**, 80 (2014).
20. W. Parawira, *Crit. Rev. Biotechnol.*, **32**, 2 (2012).
21. M. H. Gerardi, *The microbiology of anaerobic digesters*, Wiley, Canada (2003).
22. J. Ariunbaatar, A. Panico, G. Esposito, F. Pirozzi and P. N. Lens, *Appl. Energy*, **123**, 143 (2014).
23. J. C. Frigon, P. Mehta and S. R. Guiot, *Biomass Bioenergy*, **36**, 1 (2012).
24. S. Yu, G. Zhang, J. Li, Z. Zhao and X. Kang, *Bioresour. Technol.*, **146**, 758 (2013).
25. K. Möller and T. Müller, *Eng. Life Sci.*, **12**, 3 (2012).

26. V. Facchin, C. Cavinato, F. Fatone, P. Pavan, F. Cecchi and D. Bolzonella, *Biochem. Eng. J.*, **70**, 71 (2013).
27. Y. Li, L. Feng, R. Zhang, Y. He, X. Liu, X. Xiao, X. Ma, C. Chen and G. Liu, *Appl. Biochem. Biotechnol.*, **171**, 1 (2013).
28. V. Sonakya, N. Raizada and V. C. Kalia, *Biotechnol. Lett.*, **23**, 18 (2001).
29. K. Fricke, H. Santen, R. Wallmann, A. Huttner and N. Dichtl, *Waste Manage.*, **27**, 30 (2007).
30. S. L. Chiu and I. M. Lo, *Environ. Sci. Pollut. R.*, **23**, 24 (2016).
31. V. Cabbai, M. Ballico, E. Aneggi and D. Goi, *Waste Manage.*, **33**, 7 (2013).
32. K. Koch, Y. B. Fernández and J. E. Drewes, *Bioresour. Technol.*, **186**, 173 (2015).
33. APHA, *Standard Methods for Examinations of Water and Wastewater*, Am. J. Public Health, Washington, D.C. (2005).
34. W. C. Boyle, *Energy recovery from sanitary landfills—a review*, Microb. Energ. Convers. Pergamon Press, Oxford, U.K. (1976).
35. F. Raposo, V. Fernández-Cegrí, M. A. De la Rubia, R. Borja, F. Béline, C. Cavinato, G. Demirer, B. Fernández, M. Fernández-Polanco, J. C. Frigon, R. Ganesh, P. Kaparaju, J. Koubova, R. Méndez, G. Menin, A. Peene, P. Scherer, M. Torrijos, H. Uellendahl, I. Wierinck and V. de Wilde, *J. Chem. Technol. Biotechnol.*, **86**, 8 (2011).
36. D. W. Fadrosh, B. Ma, P. Gajer, N. Sengamalay, S. Ott, R. M. Brotman and J. Ravel, *Microbiome*, **2**, 1 (2014).
37. H. C. Shin, D. H. Ju, B. S. Jeon, O. Choi, H. W. Kim, Y. Um, D. H. Lee and B. I. Sang, *PloS One*, **10**, 12 (2015).
38. Y. S. Jeon, S. C. Park, J. Lim, J. Chun and B. S. Kim, *J. Microbiol.*, **53**, 1 (2015).
39. M. Hamady, C. Lozupone and R. Knight, *ISME J.*, **4**, 1 (2010).
40. B. Kanokwan, *Online monitoring and control of the biogas process*, Ph.D. Thesis, Inst. Environ. Resour., Technical University of Denmark (2006).
41. M. B. Osuna, M. H. Zandvoort, J. M. Iza, G. Lettinga and P. N. L. Lens, *Environ. Technol.*, **24**, 5 (2003).
42. L. Zhang, J. Keller and Z. Yuan, *Water Res.*, **43**, 17 (2009).
43. R. Binner, V. Menath, H. Huber, M. Thomm, F. Bischof, D. Schmack and M. Reuter, *Biomass Convers. Biorefin.*, **1**, 1 (2011).
44. F. Ali Shah, Q. Mahmood, M. Maroof Shah, A. Pervez and S. Ahmad Asad, *Scientific World J.*, **2014**, 1 (2014).
45. C. E. Manyi-Loh, S. N. Mamphweli, E. L. Meyer, A. I. Okoh, G. Makaka and M. Simon, *Int. J. Environ. Res. Public Health*, **10**, 9 (2013).
46. L. Ganzert, J. Schirmack, M. Alawi, K. Mangelsdorf, W. Sand, A. Hillebrand-Voiculescu and D. Wagner, *Int. J. Syst. Evol. Microbiol.*, **64**, 10 (2014).