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Biomethanation of Sewage Sludge with Food Waste Leachate Via Co-Digestion

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Anaerobic mono- and co-digestion of sewage sludge and food waste leachate (FWL) were performed by assessing methane production and characterizing microbial communities. Anaerobic digestion (AD) of waste activated sludge (WAS) alone produced the lowest methane (281 ml CH_4), but an approximately 80% increase in methane production was achieved via co-digestion of WAS and FWL (506 ml CH_4). There were less differences in the diversity of bacterial communities in anaerobic digesters, while archaeal (ARC) and bacterial (BAC) amounts reflected AD performance. Compared with the total ARC and BAC amounts in the mono-digestion of WAS, the ARC and BAC amounts increased two and three times, respectively, during co-digestion of FWL and WAS. In characterized archaeal communities, the dominant ratio of hydrogenotrophic methanogens in the mono-digestion of WAS approached nearly a 1:1 ratio of the two acetoclastic and hydrogenotrophic methanogens in the co-digestion of FWL and WAS. The ARC/BAC ratio in the digesters varied in the range of 5.9% to 9.1%, indicating a positive correlation with the methane production of AD.

Keywords: Co-digestion, sewage sludge, food waste leachate, biogas, microbial community

Introduction

Conventional wastewater treatment plants (WWTPs) typically utilize biological processes to treat wastewater. Although these processes are effective in reducing pollutants in wastewater, a significant amount of waste activated sludge (WAS) is generated as a by-product that must be disposed of. Anaerobic digestion (AD) has been widely adopted to treat WAS as well as to produce bioenergy. However, the efficiency of AD in treating WAS is limited by the low carbon content and slow biodegradability of WAS [1]. To improve the solubilization of WAS, several thermal and chemical treatments have been applied as a pretreatment in AD systems [2–5]. As the main contributor to municipal solid wastes (MSWs), food waste generated in South Korea amounted to 14,300 tons/day in 2015, accounting for approximately 30% of total MSWs [6]. To treat food waste, AD is an efficient method utilizing food waste characteristics of high organic content and rapid biodegradability [7]. However, mono-digestion of food waste can be inhibited owing to the accumulation of volatile fatty acids (VFAs) as well as lack of essential trace elements such as iron [8].

Co-digestion via the combination of two or more organic wastes is increasingly popular in AD technology [9]. Co-digestion can be one of several suitable options to overcome problems associated with the mono-digestion of WAS and food waste, respectively [10]. Mixed substrates of WAS and food waste ideally should contain balanced C/N ratios, sufficient trace elements, and less inhibitory compounds for AD [11]. Given improved circumstances, anaerobic co-digestion of WAS and food waste can improve digestibility, biogas production, and process stability, providing economic and environmental benefits [7]. Koch *et al.* [12] reported that co-digestion with 10% food waste in a full-scale WWTP increased self-generated energy from 25% to 78%. Liu *et al.* [1] recommended a 1:1 blend ratio of food waste to WAS as the optimal ratio for high solids co-digestion. Moreover, Xie *et al.* [9] observed that co-digestion of primary sludge with food waste resulted in more than twice as

Table 1. Characteristics of selected organic wastes.

Parameters \ Substrates	WAS	FWL	PS
TS (g/l)	38.5 ± 1.3	75.4 ± 1.0	39.7 ± 0.6
VS (g/l)	25.2 ± 1.0	62.8 ± 1.0	27.6 ± 0.7
TCOD (g/l)	59.3 ± 7.1	129.2 ± 1.6	51.1 ± 0.8
SCOD (g/l)	3.0 ± 0.4	83.6 ± 1.6	1.8 ± 0.1
Protein (g/l)	1.0 ± 0.0	26.0 ± 0.8	1.0 ± 0.0

WAS, waste activated sludge; FWL, food waste leachate; PS, primary sludge.

much specific methane yields than with paper pulp reject. Although anaerobic co-digestion of WAS with food waste has been investigated, attention to the events and microbial ecology in co-digestion will help optimize the system. Given that the AD process relies on a balance between functioning groups of microbes, information about the microbial community would be helpful to better understand the interactions of those microbes involved in anaerobic co-digestion systems. In this study, anaerobic co-digestion of sewage sludge (SS) with food waste leachate (FWL) was conducted under mesophilic conditions. To characterize bacterial and archaeal communities in the anaerobic digesters, a combination of different molecular techniques was applied, using denaturing gradient gel electrophoresis (DGGE) for qualitative assay and quantitative polymerase chain reaction (qPCR) for quantitative assay.

Materials and Methods

Biochemical Methane Potential Test

To determine the anaerobic digestibility of organic waste mixed with SS and FWL, biochemical methane potential (BMP) tests were performed for 35 days in 160 ml serum bottles with a working volume of 100 ml. Primary sludge (PS) and WAS were collected from a Gwangju WWTP. FWL was sampled from a food waste treatment facility in Gwangju, South Korea (Table 1). The bottles were initially inoculated with sludge from an anaerobic digester in the WWTP. The inoculum and substrate in mono- and co-digestion of SS and FWL were mixed at a ratio of 1:1 based on the concentration of volatile solids (VS). All BMP tests at 35 ± 1°C and 120 rpm were performed in duplicates.

Chemical Analysis

Total solids (TS), VS, and chemical oxygen demand (COD) were analyzed according to standard methods [13]. Biogas production was measured by a gas chromatography system (HP 5890; HP, USA) equipped with a thermal conductivity detector and helium as a carrier gas [10]. The injector was operated in splitless mode (column flow: 19 ml/min). The temperatures of the oven, injector and detector were 150°C, 150°C, and 180°C, respectively.

Molecular Microbial Analysis

Total DNA from the sludge was extracted and purified using a Nucleo Spin Soil kit (MACHEREY–NAGEL, Germany) according to the manufacturer's protocol. Bacterial communities were analyzed via PCR–DGGE using the primer set BAC338F/805R

Table 2. Detailed information of primers.

Target group	Primers	Sequence	Annealing temperature (°C)
Bacteria	F: BAC338F	ACTCCTACGGGAGGCAG	55.0
	R: BAC805R	GACTACCAGGGTATCTAATCC	
Archaea	F: ARC787F	ATTAGATACCCSBGTAGTCC	
	R: ARC1059R	GCCATGCACCWCCTCT	
Methanobacteriales	F: MBT857F	CGWAGGGAAGCTGTTAAGT	60.0
	R: MBT1196R	TACCGTCGTCCACTCCTT	
Methanococcales	F: MCC495F	TAAGGGCTGGGCAAGT	
	R: MCC832R	CACCTAGTYCGCARAGTTTA	
Methanosarcinales	F: MSL812F	GTAAACGATRYTCGC	
	R: MSL1159R	GGTCCCCACAGWGTACC	
Methanomicrobiales	F: MMB282F	ATCGRTACGGGTTGTGGG	63.0
	R: MMB832R	CACCTAACGCRCATHGTTTAC	

with a GC-clamp as previously described (Table 2) [14]. The PCR protocol was performed as follows: (i) initial denaturation at 95°C for 10 min; (ii) 30 cycles of 95°C for 5 min, 55°C for 30 sec, and 72°C for 30 sec; and (iii) a final extension at 72°C for 10 min. DGGE profiling was conducted using a Dcode Universal Mutation Detection System (Bio-Rad Corp., USA). The PCR product was loaded onto each well of an 8% (w/v) acrylamide gel (acrylamide: bisacrylamide solution, 37.5:1) containing a 30–60% denaturant gradient, where 100% denaturant agent was defined as 7 M urea with 40% formamide. Electrophoresis was performed in 0.5 × TAE buffer for 720 min at 100 V and 60°C (Jang *et al.*, 2013). After electrophoresis, the gel was stained with 0.5× TAE buffer containing SYBR Green I nucleic acid gel stain (1:10,000 dilution; FMC BioProducts, USA) for 15 min and then destained for 30 min with 0.5× TAE buffer. The DGGE gel profile was photographed using a Gel Doc Imaging System with Quantity One software (Bio-Rad Corp.). The DGGE bands were excised directly from the gels with a sterile blade, mixed with 40 µl of deionized water, and incubated overnight at 4°C. Each band eluted into solution (5 µl) was used as a template in a reamplification reaction using the corresponding primers without the GC-clamp. The final products were cloned onto the pGEM-T Easy vector (Promega, Germany). The 16S rRNA gene inserts were sequenced (ABI3730XL DNA analyzer; Applied Biosystems, USA). Database homology searches for these sequences were performed using the BLAST program in the National Center for Biotechnology Information database.

To quantify total bacterial 16S rRNA gene copy numbers, qPCR amplification and fluorescence detection were conducted using an Applied Biosystems 7300 qPCR system (Applied Biosystems, USA) with six primer and probe sets targeting 16S rRNA genes of different microbial groups, the domains Bacteria and Archaea, and the methanogenic orders Methanobacteriales, Methanococcales, Methanomicrobiales, and Methanosarcinales (Table 2) [15]. The qPCR mixture (20 µl) contained 10 µl of TaKaRa SYBR Premix Ex Taq (TaKaRa Bio Inc., Japan), 0.4 µl of each primer (final concentration 0.1 µM), 0.4 µl of 50× ROX reference dye, 20 ng of template DNA, and 6.8 µl of PCR-grade water. The qPCR was conducted on a thermal cycler using the following protocol: (i) 95°C for 10 sec, and (ii) 40 cycles of 95°C for 5 sec, 56°C for 10 sec, and 72°C for 27 sec (fluorescence detection step). All amplifications were performed in duplicates with a non-template control.

Results and Discussion

BMP Tests

Fig. 1 presents the cumulative methane production in mono- and co-digestions of SS and FWL. Compared with mono-digestion of PS (451 ml CH_4) and WAS (281 ml CH_4), mono-digestion of FWL (817 ml CH_4) achieved 1.8- and 2.9-fold higher cumulative methane production, respectively. WAS consists of microbial cells, extracellular polymeric substances, and recalcitrant organics/inorganics from wastewater, resulting in only partial biodegradability of WAS in AD systems [11]. Low energy values of WAS can result in an inefficient AD facility [1]. On the other hand, FWL offers more accessible substrates for microbes, due to high fractions of readily degradable matter in FWL facilitating methane production [9].

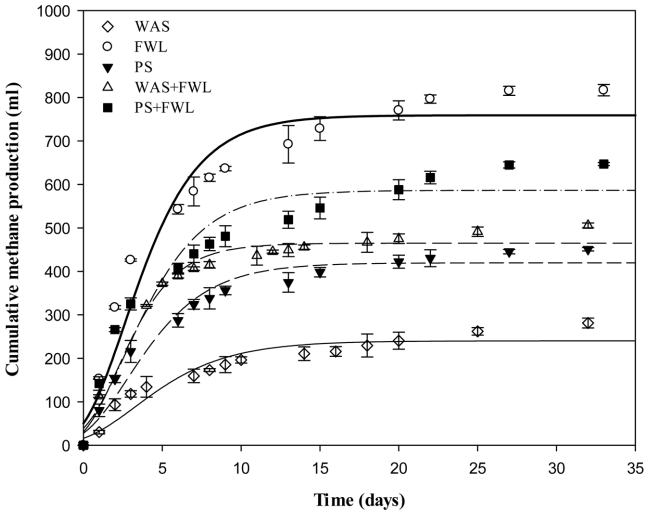

Fig. 1. Cumulative methane production in batch-type anaerobic digestion.
WAS, waste activated sludge; FWL, food waste leachate; PS, primary sludge.

Combining FWL and SS improved methane production compared with the mono-digestion of SS, but the best production was exhibited in AD of mono-FWL (Fig. 1). Compared with AD of WAS alone (281 ml CH_4), an approximately 80% increase was observed in methane production in the co-digestion of WAS and FWL (506 ml CH_4). Co-digestion of PS and FWL (647 ml CH_4) resulted in 40% higher methane production than mono-digestion of PS (506 ml CH_4). These synergistic effects on biogas production during co-digestion of FWL and SS may result from improved C/N ratios as well as additional nutrients and trace elements from different substrates [16, 17]. Enhanced energy recovery via co-digestion of FWL in combination with SS can help WWTPs potentially attain more energy self-sufficiency.

To predict methane yields of the combinations of substrates with SS and FWL in the AD, the values of cumulative methane production were analyzed by the modified Gompertz model, using the following equation:

$$M = P \times \exp\left\{-\exp\left[\frac{R_m \cdot e}{P}(\lambda - t) + 1\right]\right\}$$

where M = cumulative methane production, P = methane production potential, R_m = methane production rate, and l = lag phase time.

According to simulation results based on the modified Gompertz model, mixed substrates of SS and FWL improved both the potentials and rates of methane production. As can be seen in Table 3, AD of WAS with FWL produced approximately 3 times greater R_m than that of WAS alone. Methane production for all BMP tests started with less than 1 day of lag phase.

Microbial Communities

Bacterial communities in the anaerobic digesters were analyzed according to the DGGE results (Fig. 2 and Table 4). The pattern of bacterial DGGE bands did not indicate large differences among the digesters, illustrating less variations in the bacterial community originating from WAS used as an inoculum. The bacterial sequences comprised mostly the phyla Bacteroidetes, Firmicutes, and Actinobacteria (Table 4). The predominant bacterial group, Firmicutes, is known to metabolize a variety of substrates including protein, lipids,

Table 3. Parameters of the Gompertz model obtained from fitting the methane production curve.

Substrates	Parameters				
	P (ml) Maximum methane potential	R_m (ml/day) Maximum methane production rate	λ (day) Lag phase	R^2	p-Value
WAS	240.1	26.9	<0.1 (2.4633E-016)	0.91	< 0.0001
FWL	759.2	106.8	<0.1 (3.4348E-016)	0.94	
PS	419.6	54.8	<0.1 (2.3592E-016)	0.96	
WAS+FWL	465.1	81.1	<0.1 (5.8113E-017)	0.98	
PS+FWL	586.6	79.3	<0.1 (2.1164E-016)	0.91	

WAS, waste activated sludge; FWL, food waste leachate; PS, primary sludge.

Table 4. Phylogenetic affiliation of the 16S rRNA gene sequences from DGGE bands.

Band	Closest species	Accession number	% Similarity	Class/phylum	Allocation				
					WAS	FWL	PS	WAS+ FWL	PS+ FWL
1	*Prolixibacter bellariivorans* strain JCM 13498	NR113041	99	Unclassified/Bacteroidetes					
2	*Syntrophomonas wolfei* subsp. *saponavida* strain DSM4212	NR115849	99	Clostridia/Firmicutes					
3	Uncultured bacterium clone LBAC13	KJ601176	99	Unclassified bacteria					
4	*Bifidobacterium aerophilum*	AY174104	100	Actinobacteria/Actinobacteria					
5	Uncultured bacterium	AB456223	100	Unclassified					
6	Uncultured *Dechloromonas* sp. clone wn87	JQ012310	99	Betaproteobacteria/Proteobacteria					
7	*Acetoanaerobium* sp. WJDL-Y2	KF176997	99	Clostridia/Firmicutes					
8	*Syntrophus* sp.	AJ133796	100	Deltaproteobacteria/Proteobacteria					
9	*Clostridium ultunense* strain Esp	GQ487664	99	Clostridia/Firmicutes					
10	*Clostridium sticklandii* strain DSM 519	NR102880	99	Clostridia/Firmicutes					
11	*Streptomyces* sp. 13(2014)	KJ573803	99	Actinobacteria/Actinobacteria					
12	*Nocardioides* sp. DN36	AB508351	99	Actinobacteria/Actinobacteria					
13	*Lactobacillus panis* strain FQ084	KF418828	100	Bacilli/Firmicutes					
14	Uncultured Bacteroidetes bacterium clone PG-5-1-3-L	EU626571	99	Unclassified/Bacteroidetes					

WAS, waste activated sludge; FWL, food waste leachate; PS, primary sludge.
Grey: present; White: absent.

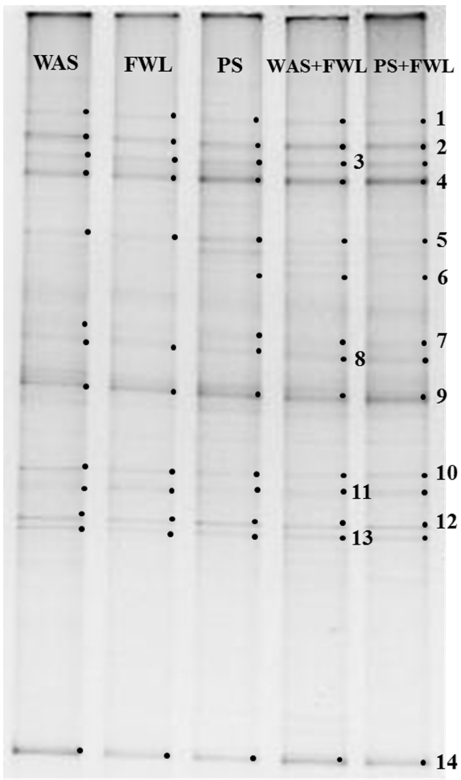

Fig. 2. Bacterial DGGE profiles analyzed from batch anaerobic digesters.
WAS, waste activated sludge; FWL, food waste leachate; PS, primary sludge.

cellulose, sugars, and amino acids, all common constituents of organic wastes. [18]. Within Firmicutes, *Clostridium ultunense* sp. (band 9) is known as a mesophilic bacterium oxidizing acetate in syntrophic association with hydrogenotrophic methanogens [19]. *Syntrophomonas wolfei* corresponding to band 2, has been widely reported as a syntrophic fatty acid oxidizing bacterium in partnership with hydrogenotrophic *Methanoculleus* and *Methanobacterium* methanogens in anaerobic digesters [20, 21]. The *Lactobacillus* genus, affiliated with band 13, is known to produce lactic acid by fermenting sugars [15]. These results support that interaction with syntrophic bacteria and methanogens may represent an important pathway in anaerobic digesters.

Based on 16S rRNA gene concentrations, mono-digestion of FWL indicated both the highest bacterial (4.2×10^8 copies/ml) and archaeal (3.8×10^7 copies/ml) amounts. The lowest archaeal (ARC) and bacterial (BAC) amounts were detected in the mono-digestion of WAS (Fig. 3A), reflecting the substrates' digestibility and methane production. Compared with the total ARC and BAC amounts in the mono-digestion of WAS, the ARC and BAC amounts increased two and three times, respectively, during co-digestion of FWL and WAS, resulting in enhanced performance. The concentration of the acetoclastic Methanosarcinales 16S rRNA gene accounted for approximately 65% of the total methanogens in mono-digestion of FWL (Fig. 3B). This demonstrates that methane formation in the mono-digestion of FWL in our study may be dependent on acetoclastic pathways. In contrast, hydrogenotrophic methanogens were dominant in the mono-digestion of PS and WAS, indicating the concentrations of the two hyrogenotrophic orders Methanomicrobiales and Methanobacteriales as being 60% of the total methanogens. Co-digestion led to changes in the characterized archaeal communities in mono-digestion. The

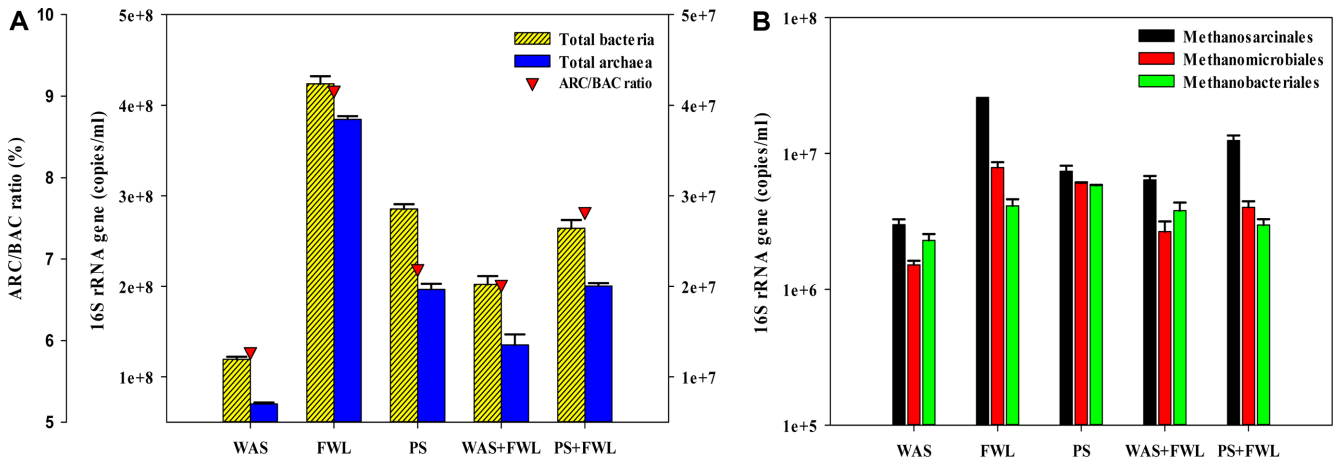

Fig. 3. 16S rRNA gene concentrations of bacteria and archaea and their ratios (**A**) and 16S rRNA gene concentrations of methanogens (**B**) in batch digesters.
BAC: Bacteria; ARC: Archaea; MSL: *Methanosarcinales*; MMB: *Methanomicrobiales*; MBT: *Methanobacteriales*.

ratio of two acetoclastic and hydrogenotrophic methanogens approached nearly 1:1 in the co-digestion of FWL and WAS, but the co-digestion of FWL and PS followed a similar ratio as that in the mono-digestion of FWL. The ARC/BAC ratio in the digesters varied in the range of 5.9% to 9.1% (Fig. 3A). The highest methane was produced in mono-digestion of FWL, where the ARC/BAC ratio was 9.1%. In contrast, mono-digestion of WAS had the lowest ARC/BAC ratio (5.9%), showing the least methane production. Thus, the ARC/BAC ratio may be utilized as a proxy for the performance of AD. A proper proportion of acidogens and methanogens is desirable for controlling VFAs in AD systems [22].

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