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# Biogas potential and methanogenic community shift in in-situ anaerobic sewage sludge digestion with food waste leachate additions

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## Abstract

The objective of this study was to determine methane yields (MY) of organic wastes in biogasification facilities according to the mixing ratio of food waste/food waste leachate and sewage sludge. One biogasification facility that treated sewage sludge only was compared with three biogasification facilities treating sewage sludge and food waste. The theoretical MY was derived based on analyses of carbohydrate, fat, and protein to examine the efficiency of the biogasification facility. The average actual MY was  $0.424 \text{ Sm}^3\text{CH}_4/\text{kg}$  volatile solids, which corresponded to 83.7% of theoretical MY. In the case of combined anaerobic digestion (CD) mixing with food waste/food waste leachate, inhibitory factors (volatile fatty acids [VFAs], total nitrogen [TN], and organic matter contents) showed the tendency to have relatively higher values in CD facilities than in the biogasification facility treating sewage sludge only. Mean concentrations of VFAs and TN in the anaerobic digester effluent, and the organic loading rate were 406 mg/L, 3,721 mg/L, and 1.62 kg volatile solids/ $\text{m}^3$  day, respectively. The influence of anaerobic digester effluent was in charge of 10% within the influent environmental loading rate from the sewage treatment plants associated with the biogasification facilities. Analyses of the microbial community showed that a remarkable change in the structure of methanogens was directly related to different MY in each plant. In particular, *Methanoculleus* and *Methanosaeta* increased with an increasing ratio of food waste/food waste leachate to sludge, while *Methanococcus* and *Methanosarcina* decreased. In conclusion, CD showed steady operational conditions and high efficiency of MY by injecting food waste/food waste leachate into the anaerobic digester. It met the current criteria for integrated treatment of organic waste in biogasification facilities in South Korea.

**Keywords:** Methane yield, Sewage sludge, Anaerobic digestion, Metagenome, Volatile solids

## Introduction

Due to social aspects such as population growth, urbanization, and industrialization, the amount of organic wastes (containing sewage sludge and food waste) has

been increasing annually in South Korea [1, 2]. In 2014, 10,112 ton/d of sewage sludge was generated, with an annual growth rate of 4.6% during the previous decade. Food waste was produced at a rate of 13,697.4 ton/d which accounted for 27.4% of municipal waste in Korea [2, 3]. Ocean dumping and direct landfill of organic waste have been prohibited since 2012 in accordance with policy formulation [4–6]. Among land-based treatment methods including landfill and incineration, biogasification is a technology that can produce methane gas as a renewable energy through an anaerobic digestion

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process, and is a novel response to the current situation [7, 8].

Biogasification facilities in sewage treatment plants in South Korea have a low anaerobic digestion efficiency (only 54.2%) compared to those in developed countries such as Europe due to relatively low influent concentrations with a low enrichment rate, a lack of knowledge on operating technology by process flow, and inadequate management [9, 10]. In 2010, the Ministry of Environment (South Korea) promoted a master plan named 'energy-independent project to develop biogasification efficiency and utilize sewage sludge as biomass' [11]. In particular, sewage treatment without an anaerobic digestion tank is reviewed with treatment of food wastes. This involves establishment of anaerobic digestion in a sewage treatment plant for co-digestion with food wastes additions [12, 13].

In 2016, the number of biogasification facilities in South Korea that could treat organic wastes was increased by two (2.3% increment) compared to the previous year (90 facilities). The total treatment capacity of biogasification facilities was 59,204 ton/day, which was an increase of 4.7% from the previous year. Among facilities for treating organic wastes, sewage sludge biogasification facility showed a high increase rate compared to other facilities for treating organic wastes. However, the average annual operation rate of biogasification facilities was only 32% [14].

The amount of biogas produced in sewage sludge biogasification facilities was 6.6 m<sup>3</sup>/ton, which was considerably lower than that of food waste (111.6 m<sup>3</sup>/ton) and food waste leachate (50.5 m<sup>3</sup>/ton) [15, 16]. Combining the low concentration of sewage sludge with the

high concentration of food wastes, the disadvantage of each organic waste is complemented. Thus, the operation efficiency of anaerobic digestion is improved and stable operation is achieved.

Therefore, the objective of this study was to accumulate in situ facility data on the effect of co-digestion of sewage sludge and food waste. The influence of water quality was also investigated in regard to the wastewater loading impact of re-circulated water after co-digestion with food wastes in the wastewater treatment plant. Metagenome analysis was performed to determine the effect of biogasification on co-digestion of sewage sludge and food waste in South Korea.

## Materials and methods

### Target biogasification facilities and sampling method

One sewage sludge biogasification facility (Busan, 35° 07' 33.5'' N 129° 06' 53.8'' E), and three combined anaerobic digestion facilities (Seoul [37° 34' 38.6'' N 126° 49' 33.3'' E], Bucheon [37° 32' 43.5'' N 126° 45' 55.9'' E], and Ulsan [35° 27' 39.4'' N 129° 21' 28.3'' E]) were selected for this study. Sampling was carried out at the inlet and outlet of anaerobic digestion. Among the samples, effluent samples from the anaerobic digester were kept frozen for accuracy of microbial analysis. Remaining samples were refrigerated immediately after collection. Table 1 presents fundamental information regarding the four biogasification facilities which were selected for this investigation.

### Analysis methods of physicochemical properties

Volatile solids (VS) were determined by 'Loss on ignition/volatile solids and total organics-gravimetry (ES 06301.1b)' and 'Humidity and total solid-gravimetry (ES

**Table 1** Outline of target biogasification facilities

Facility	Treatment materials <sup>c</sup>	Type of digestion	Mixing ratio (SS:FW(L))	Design capacity of digester (ton/day)	Volume of digester (m <sup>3</sup> )	HRT (day)	Organic loading rate (kg <sub>VS</sub> /m <sup>3</sup> day)	pH
SD <sup>a</sup>	Sewage sludge (rWWTSS, eWWTSS)	Mesophilic, single stage	–	932	21,000	28	1.38	7.9 (±0.1)
CD <sup>b1</sup>	Sewage sludge (rWWTSS, eWWTSS), food waste leachate	Mesophilic, single stage	0.965:0.035	7680	179,988	39	0.84	8.2 (±0.1)
CD2	Sewage sludge (rWWTSS), food waste leachate	Mesophilic, single stage	0.87:0.13	4205	82,776	27	1.40	8.1 (±0.3)
CD3	Sewage sludge (rWWTSS), food waste	Mesophilic, single stage	0.37:0.63	430	14,000	23	2.88	8.0 (±0.3)

<sup>a</sup> SD Anaerobic digestion facility treating sewage sludge only

<sup>b</sup> CD: Combined anaerobic digestion facility treating sewage sludge and food waste (leachate)

<sup>c</sup> rWWTSS Raw wastewater treatment sewage sludge, eWWTSS Excess wastewater treatment sewage sludge

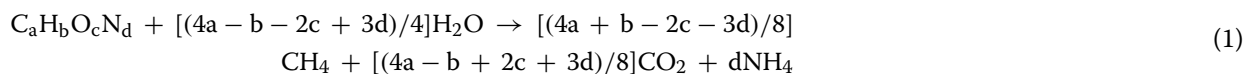
06303.1)' of the Korean Waste Standard Examination [17]. Chemical oxygen demand (COD) by chromium (COD<sub>Cr</sub>) was analyzed according to the 'Titrimetric method (ES 04315.3b) of the Korean Official Test Water Pollution Standard [18]. Volatile fatty acids (VFAs), which is one of the impediment factors, and the observation index in the anaerobic digestion system, were analyzed by titration methods proposed in the Biogas Technical Guideline for Biogasification facilities in Germany [19]. In the titration method, samples were centrifuged at 10,000g and were reacted with sulfuric acid (0.1 N H<sub>2</sub>SO<sub>4</sub>) to measure VFA concentrations in the samples. Total nitrogen (TN) in samples was determined using the official testing method with respect to water pollution processes (ES 04363.1a) [18]. Nutrients including carbohydrate, protein, and fat were analyzed by the Korean Food Standard Codex [20].

#### Calculation of sewage sludge treatment plant input loading rate

The impact of the input loading rate of food waste in recycled water in the sewage sludge treatment plant was calculated as the ratio of the influent loading rate (biological oxygen demand [BOD], COD, suspended solids [SS], TN, and total phosphorus [TP]) of food waste in the recycled water and the influent loading rate in the sewage sludge treatment plant. Various factors were necessary to calculate the input loading rate of recycled water from the anaerobic digestion system. Data for each factor was collected during a field survey and included the sewage disposal plant capacity, design concentrations of input sewage, and flux and concentration of recycled water.

#### Theoretical methane yield

According to various literature reviews, methane and carbon dioxide are generated from organic wastes such as food waste and sewage sludge in anaerobic conditions. The theoretical MY of organic wastes is defined for specific compositions of microorganism substrates. Angelidaki and Sanders [21] and Tchobanoglous et al. [22] suggested Eq. (1) for the calculation of the theoretical MY. This equation is derived assuming that total organic materials were converted to CO<sub>2</sub> and CH<sub>4</sub> with H<sub>2</sub>O as an external source.



Here, a, b, c, and d are the molecular amounts of carbon, hydrogen, oxygen, and nitrogen, respectively. In this study, the organic formula described above was expressed on a molar basis using the results of nutrient content

analyses. The theoretical MY representative of a standard status (0 °C, 1 atm) was calculated following Eq. (2).

$$\begin{aligned} &\text{Theoretical methane gas production (STP L} \cdot \text{CH}_4/\text{g} \cdot \text{VS)} \\ &= 22.4\{[(4a + b - 2c - 3d)/8]/[12a + b + 16c + 14d]\} \end{aligned} \quad (2)$$

#### Practical MY

In this study, the following field data were used to estimate the actual MY of target facilities; input treatment amount of organic waste (ton/day), VS contents (%) of the inlet and outlet of the anaerobic digestion system, biogas production (m<sup>3</sup>), and biogas composition (%) especially methane. The field data were mainly selected from the normal operating data for the 12-month period from 2015–2016.

#### Metagenome analysis

The samples collected from each full-scale anaerobic digestion plant were extracted and purified using UltraClean Soil DNA Kit (Mo Bio Laboratory Inc., USA) and UltraClean Microbial DNA Isolation Kit (Mo Bio Laboratory Inc., USA) according to the manufacturer's instructions. A 20 ng aliquot of each DNA sample was sampled and injected for PCR reaction. The 16S universal primers 27F (5' GAGTTTGATCMTGGCTCAG 3') and 800R (5' TACCAGGGTATCTAATCC 3') for bacteria; and Arc8f (5'-TTCCGGTTGATCCYGCCGGA-3') and Arc519r (5'-TTACCGCGGCKGCTG-3') for archaea, were used for the 16 s rRNA genes amplification [23, 24]. The Fast Start High Fidelity PCR System (Roche, Switzerland) was utilized for PCR under three steps: 94 °C for 3 min for 35 cycles at 94 °C for 15 s, 55 °C for 45 s and 72 °C for 1 min, and a final elongation step at 72 °C for 8 min. After PCR, products were processed using a miseq system (Illumina, CA, USA) by commercial company (Macrogen, Korea). Operational taxonomic units (OTUs) were then trimmed and identified by using QIIME software.

## Results and discussion

#### Operation factors of target biogasification facilities

To determine the operation factors of target biogasification facilities, VS, COD<sub>Cr</sub>, total nutrient contents, and VFAs were analyzed. Table 2 shows the removal efficien-

cies and concentrations of VS and COD<sub>Cr</sub> in the target digestion system.

VS contents in biogasification facilities were 2.07% in SD and 5.21% in CD. COD<sub>Cr</sub> concentrations were

**Table 2** Operation status of biogasification facilities

Seasons <sup>a</sup>	SD <sup>b</sup>				CD <sup>c</sup>			
	VS (%)	DRE <sup>d</sup> of VS (%)	COD (mg/L)	DRE of CODcr (%)	VS (%)	DRE of VS (%)	COD (mg/L)	DRE of CODcr (%)
WIN-in	1.28	42.56	14,108	35.90	5.40	62.81	45,963	52.62
WIN-out	0.74	–	9044	–	2.01	–	21,777	–
SPR-in	2.78	44.32	49,488	78.72	5.49	62.08	70,116	66.22
SPR-out	1.55	–	10,532	–	2.08	–	23,685	–
SUM-in	1.87	17.11	27,638	18.18	5.73	71.86	57,629	55.39
SUM-out	1.55	–	22,613	–	1.61	–	25,708	–
AUT-in	2.34	50.85	41,205	26.71	4.19	54.93	53,876	59.21
AUT-out	1.15	–	30,200	–	1.89	–	21,974	–
Average-in	2.07	38.71	33,110	39.88	5.21	62.92	56,896	58.36
Average-out	1.25	–	18,097	–	1.90	–	23,286	–

<sup>a</sup> WIN: Winter, SPR: Spring, SUM: Summer, AUT: Autumn

<sup>b</sup> SD: Anaerobic digestion facility treating sewage sludge only

<sup>c</sup> CD: Combined anaerobic digestion facility treating sewage sludge and food waste (leachate)

<sup>d</sup> DRE: Degradation efficiency of anaerobic digester

33,110 mg/L in SD and 56,896 mg/L in CD. In each season, the organic material concentration of CD was higher than that of the SD using sewage sludge as the substrate. Especially, the lowest concentration of input substrate in winter was 14,108 mg/L as CODcr and 1.28% as VS. VS and CODcr of input substrate in biogasification facilities tended to increase in the order of winter < summer < autumn < spring. In terms of removal efficiency, the VS and CODcr fluctuations in SD were large. On the contrary, the seasonal removal efficiency did not change significantly by treatment sewage sludge with high organic matter together.

The nutrient contents in samples are presented in Fig. 1. The mean contents of nutrients in SD were 1.62 g/100 g (44.9%) as protein, 0.92 g/100 g (25.5%) as fat, and 1.07 g/100 g (29.6%) as carbohydrate. In the case of the CD input substrate, the mean weights of nutrients were 1.80 g/100 g (40.1%) as protein, 1.48 g/100 g (33.0%) as fat, and 1.20 g/100 g (26.9%) as carbohydrates.

The mean total contents of nutrients in samples were 3.60 g/100 g in SD and 4.48 g/100 g in CD. As a result of the degradation efficiency depending on mixing with food wastes, fat and carbohydrate were degraded by 31.74% and 37.44%, respectively, compared to SD. Especially, the protein removal efficiency was increased by approximately 1.5 times compared to that of fat and carbohydrate.

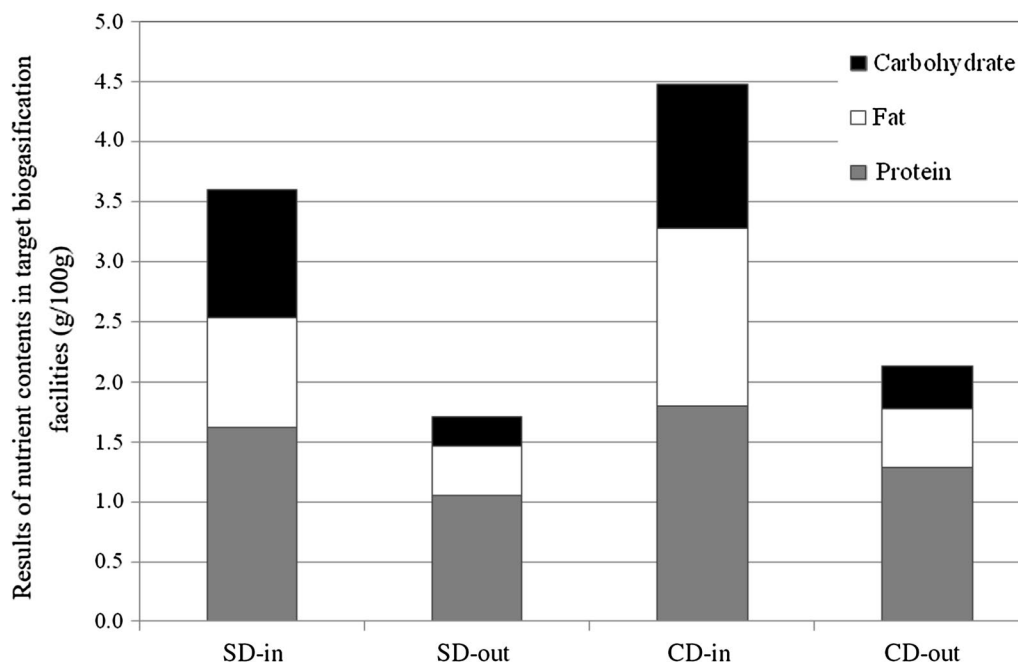
VFAs and TN are the main factors that ensure that decomposition process of organic materials proceeds stably inside the anaerobic digester. When high VFAs and TN concentrations accumulate in the anaerobic digester,

VFAs act as an inhibitory substance which decrease methanogens activity and the decomposition rate of organic matter during hydrolysis and acidification procedures [21]. VFAs concentrations in the target anaerobic digester effluent were 352 mg/L in SD and 424 mg/L in CD, which means that the removal efficiency of organic matter inside the anaerobic digester was lower than that of the CD system due to the low input of VFAs in SD.

Nitrogen content is one of the evaluation factors related with associated wastewater treatment following an anaerobic digestion system. The TN concentration in the CD was 4359 mg/L in the anaerobic digestion effluent and 1747 mg/L in the final wastewater. These concentrations are approximately 2.4 times and 1.3 times higher, respectively, than the TN concentration in the sewage sludge digestion system.

#### Impact loading rate into the sewage sludge treatment plants

Anaerobic digester effluent is discharged as wastewater and dehydrated sludge cake through the dewatering system in the biogasification facility. Especially, the wastewater which is the final effluent of the anaerobic digestion system, is transferred to the grit chamber or the first settling reservoir of the sewage treatment plant, which is located near the biogasification facility. According to the technical design and operation guidelines of biogasification facilities in Korea [25], it suggested that anaerobic digester effluent should be within 10% of influent environmental loading rate in sewage treatment plants.



**Fig. 1** Results of nutrient contents in target biogasification facilities. \*SD: Anaerobic digestion facility treating sewage sludge only. \*CD: Combined anaerobic digestion facility treating sewage sludge and food waste (leachate)

Table 3 shows the impact of anaerobic digestion effluent on environmental loading input in the sewage treatment plant. Five water quality factors (i.e., biochemical oxygen demand [BOD], COD, suspended solid [SS], TN, and TP) were collected and reconstructed the operation field data of the target facilities. The influences of anaerobic digester effluent in SD were 0.7% in BOD, 0.6% in COD, 0.7% in SS, 6.2% in TN, and 4.5% in TP. On the other hand, the mean influences in CD were 1.3% in BOD, 1.0% in COD, 1.4% in SS, 6.5% in TN, and 4.5% in TP. The effects of the load factor on food waste addition were 0.6%, 0.4%, and 0.7% in BOD, COD, and SS, respectively, and 0.3% in TN and 0% in TP.

#### Theoretical MY

The theoretical MY was calculated based on the postulation that input organic wastes were 100% decomposed during the anaerobic digestion procedure. In this study, MY was estimated by mass per composition and molar ratio of nutrients (protein, fat, and carbohydrate) according to Angelidaki and Sanders [21]. The specific theoretical MY and characteristics of substrate components are described in Table 4.

Table 5 presents the calculation results of the theoretical MY in the target biogasification facilities. The theoretical MY of each nutrient were 0.496  $\text{Sm}^3\text{CH}_4/\text{kg VS}$  as protein, 1.014  $\text{Sm}^3\text{CH}_4/\text{kg VS}$  as fat, and 0.415  $\text{Sm}^3\text{CH}_4/\text{kg VS}$  as carbohydrate. Based on the nutrients analysis

results and the data in Table 3, the mean mass and molar ratios (protein: fat: carbohydrate) were 0.40:0.32:0.27 and 0.64:0.07:0.30, respectively. The mean potential theoretical MY by the nutrient compositions was 0.507  $\text{Sm}^3\text{CH}_4/\text{kg VS}$ . The theoretical MY was estimated to be 0.496  $\text{Sm}^3\text{CH}_4/\text{kg VS}$  in the case of anaerobic digestion of sewage sludge only. However, the same value for the combined anaerobic digesters was 0.510  $\text{Sm}^3\text{CH}_4/\text{kg VS}$ , which showed no difference from the SD.

#### Actual MY

The actual MY in biogasification facilities were calculated based on dry gas and standard conditions at 0 °C and 1 atm and are shown in Table 6. The mean actual MY based on VS in target biogasification facilities was 0.424  $\text{Sm}^3\text{CH}_4/\text{kg VS}$ . Depending on whether sewage sludge and food waste (leachate) were mixed, the actual MY in CD was 0.478  $\text{Sm}^3\text{CH}_4/\text{kg VS}$ , which is 1.8 times greater than that of SD.

Compared with the theoretical values shown in Table 4, the actual MY in each facility was quite different in accordance with the substrate status. In the case of SD, the actual MY was 0.262  $\text{Sm}^3\text{CH}_4/\text{kg VS}$ , which accounted for 52.7% of the theoretical value in the SD facility. In contrast, the actual MY corresponded with theoretical values by up to 93.7% by increasing the ratio of food waste (leachate) in the organic substrate. These results demonstrate that mixing the input substrate with

**Table 3 Impact on environmental loading of dewatered wastewater on nearby sewage treatment plants**

Facility	SD	CD1	CD2	CD3	Avg	Min	Max
Capacity of sewage disposal plant (1000 m <sup>3</sup> /d)	340	1630	680	250	725	250	1630
Design concentration of input sewage (mg/L)							
BOD	125	174	180	160	160	125	180
COD	110	94	160	150	129	94	160
SS	147	133	180	180	160	133	180
TN	38	37	40	50	41	37	50
TP	5	4	5	6	5	4	6
Design influent loading rate of input sewage (kg/d) (A)							
BOD	42,500	283,620	122,400	40,000	122,130	40,000	283,620
COD	37,400	153,220	108,800	37,500	84,230	37,400	153,220
SS	49,980	216,790	122,400	45,000	108,543	45,000	216,790
TN	12,920	60,310	27,200	12,500	28,233	12,500	60,310
TP	1700	6846	3400	1375	3330	1375	6846
Recycle water flux after anaerobic digestion (m <sup>3</sup> /d)	900	4600	1742	600	1961	600	4600
Mixing ratio of food waste (leachate) in organic materials (%)	–	4	26	63	31	4	63
Concentration of recycle water (mg/L)							
BOD	322	307	1129	1450	802	307	1450
COD	262	226	523	1205	554	226	1205
SS	373	386	1250	1750	940	373	1750
TN	895	408	1056	2012	1093	408	2012
TP	85	93	52	102	83	52	102
Influent loading rate of recycle water (kg/d) (B)							
BOD	290	1410	1967	870	1134	290	1967
COD	236	1037	911	723	727	236	1037
SS	336	1776	2178	1050	1335	336	2178
TN	806	1878	1840	1207	1433	806	1878
TP	77	428	91	61	164	61	428
Percentage of recycle water to influent loading rate (B/A)*100(%)							
BOD	0.7	0.5	1.6	2.2	1.3	0.5	2.2
COD	0.6	0.7	0.8	1.9	1.0	0.6	1.9
SS	0.7	0.8	1.8	2.3	1.4	0.7	2.3
TN	6.2	3.1	6.8	9.7	6.5	3.1	9.7
TP	4.5	6.2	2.7	4.5	4.5	2.7	6.2

high-nutrient food waste (leachate) is more efficient for anaerobic microorganisms such as acid-producing bacteria and methanogenic bacteria inside the anaerobic digester.

**Table 4 Specific theoretical methane yield and characteristics of substrate components**

Nutrients	Chemical formula	Mass of 1 mol (g/mole) <sup>b</sup>	Methane yield (Sm <sup>3</sup> CH <sub>4</sub> /kgVS)	CH <sub>4</sub> content (%) <sup>b</sup>
Carbohydrate	(C <sub>6</sub> H <sub>10</sub> O <sub>5</sub> ) <sub>n</sub>	162	0.415	50
Protein <sup>a</sup>	C <sub>5</sub> H <sub>7</sub> NO <sub>2</sub>	113	0.496	50
Fat	C <sub>57</sub> H <sub>104</sub> O <sub>6</sub>	884	1.014	70

<sup>a</sup> It assume that nitrogen is converted to NH<sub>3</sub>

<sup>b</sup> Angelidaki et al. [22]

**Metagenome analysis**

To reveal how the microbial structure differed among four full-scale anaerobic digestion plants in terms of the response to the effect of different feedstock compositions, microbial community analysis was conducted by a next generation sequencing technique (NGS). A total of 86,309 high-quality sequence reads and 387 OTUs in bacteria and 185 OTUs in archaea with similarity cutoffs of 3% were obtained (data not shown). The result of alpha

**Table 5 Theoretical methane yield in target biogasification facilities based on the nutrient contents**

Sample <sup>a</sup>	Mass per composition in samples (g/100)			Molar ratio <sup>b</sup>	Mass ratio	Theoretical methane yield (Sm <sup>3</sup> CH <sub>4</sub> /kgVS)
	Protein	Fat	Carbohydrate			
SD1	1.26	0.70	0.82	0.66: 0.04: 0.30	0.45:0.25:0.30	0.496
CD1	2.06	1.15	0.64	0.78: 0.05: 0.17	0.54:0.30:0.16	0.511
CD2	1.09	1.68	0.98	0.55: 0.11: 0.34	0.29: 0.45: 0.26	0.524
CD3	2.01	1.62	1.92	0.56: 0.06: 0.38	0.36: 0.29: 0.35	0.496
Average	1.61	1.28	1.09	–	–	0.507

CD: Combined anaerobic digestion facility treating sewage sludge and food waste (leachate)

<sup>a</sup> SD: Anaerobic digestion facility treating sewage sludge only

<sup>b</sup> The average molar ratio by components of food waste extracted in biogasification facilities was applied

**Table 6 Actual methane yield based on VS in target biogasification facilities**

Sample	Methane yield (Sm <sup>3</sup> CH <sub>4</sub> /kgVS <sub>in</sub> <sup>b</sup> )	Methane yield <sup>b</sup> (Sm <sup>3</sup> CH <sub>4</sub> /kgVS <sub>rem</sub> <sup>c</sup> )
SD <sup>a</sup>	0.10	0.262
CD1	0.22	0.423
CD2	0.30	0.538
CD3	0.34	0.475
Average	0.24	0.424

CD: Combined anaerobic digestion facility treating sewage sludge and food waste (leachate)

<sup>a</sup> SD: Anaerobic digestion facility treating sewage sludge only

<sup>b</sup> VS<sub>in</sub>: based on values analyzed from sample of inlet

<sup>c</sup> VS<sub>rem</sub>: based on values calculated from removed VS

diversity analysis showed higher microbial diversity in samples of CD1, with 94 bacterial and 42 archaeal OTUs in comparison to samples from SD (77 bacterial and 39 archaeal OTUs), showing that both bacterial and archaeal diversity of co-digestion were higher than in single digestion anaerobic digestion. Besides, 101 OTUs of bacteria and 49 OTUs of archaea in CD2 and 115 OTUs of bacteria and 55 OTUs of archaea in CD3 were observed. This phenomenon indicates that as more food waste was added to the anaerobic digestion plants, the microbial structure became more diverse.

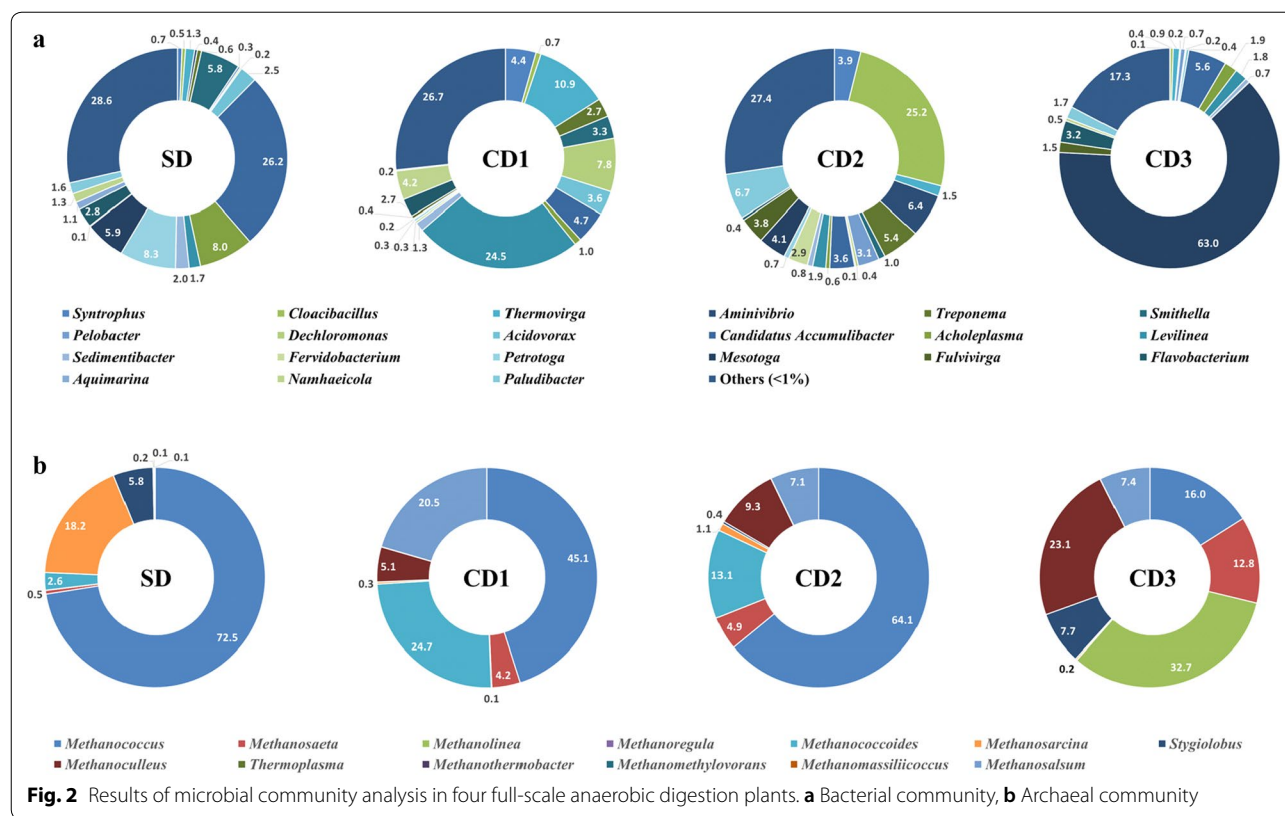
Figure 2 shows the different core bacteria existing in the four full-scale anaerobic digestion systems. The taxonomic assignment in SD showed that the vast majority of the bacterial community belonged to the genus *Candidatus Accumulibacter* (26.2%), *Petrotoga* (8.3%), and *Acholeplasma* (8.0%) as shown in Fig. 2a. As food waste was added as a co-substrate, those members decreased while increases in other bacterial members were observed; however, those patterns were irregular in each plant. The relative abundances of *Levilinea* and *Thermovirga* increased in CD1, accounting for 24.5% and 10.9%, respectively. In CD2, genus belonging

to *Cloacibacillus* (25.2%), *Aminivibrio* (6.4%), and *Treponema* (5.4%) became the vast majority of the bacteria. Meanwhile, genera *Mesotoga* (63.0%) was the predominant genera in CD3.

In general, changes in the community of methanogenic archaea is directly related to the MY in each anaerobic digestion plant. Figure 2b shows a more remarkable change in the archaeal structure at the genus level, and different patterns in the relative abundance were observed in each plant compared to the results of the bacterial taxonomic assignment. There was an apparent decrease in the relative abundance of genera belonging to *Methanococcus* (45.1%, 64.1%, and 16.0% in CD1, CD2, and CD3, respectively), which was 72.5% in SD. The second-largest sequences belonging to *Methanosarcina* in SD also decreased in all CDs, but varied from 0.2–1.1%. *Methanosarcina* are known as the most metabolically versatile methanogens and can produce CH<sub>4</sub> by means of both hydrogenotrophic and acetoclastic pathways. A high abundance of *Methanosarcina* are often related to an adaptation response to stress such as fluctuations in substrate, or accumulation or shock loading of organic acids [26]. Instead, sequences belonging to *Methanosaeta* and *Methanoculleus* increased with the increasing ratio of food waste to sludge, occupying 4.2% and 5.1% in CD1, 4.9% and 9.3% in CD2, and 12.8% and 23.1% in CD3, respectively. It has been reported that when the carbohydrate composition increased in feedstock, the anaerobic digestion community was dominated by *Methanoculleus* and *Methanosaeta* [27]. In particular, *Methanosaeta* have been reported as efficient methanogens which exclusively use acetate as a substrate for CH<sub>4</sub> production, and have been observed at low acetate concentrations [28].

#### Comparative studies of MY of sewage sludge and food waste (leachate)

Table 7 presents the research results of MY in various preliminary studies in South Korea. Kim et al., Byun



**Table 7** Comparative other studies on biogasification of sewage sludge and food waste (leachate)

Sample	Methane yield (Sm <sup>3</sup> CH <sub>4</sub> /kgVS)	Temp (°C)	HRT (day)	C/N ratio	Removal efficiency of VS (%)	Reference
Municipal wastewater sludge (1)	0.232	35	24	6.7	–	Kim et al. [29]
Municipal wastewater sludge (2)	0.181	35	24	5.0	–	
Wastewater sludge with food waste leachate	0.396	35	24	8.3	–	
Sewage sludge with food waste leachate (1:9)	0.233	55	30	6.0	72.5	Lee et al. [7]
Sewage sludge with food waste leachate (3:7)	0.298	55	30	7.4	84.3	
Sewage sludge with food waste leachate (5:5)	0.344	55	30	9.4	89.0	
Sewage sludge	0.180	35	30	–	47.6	Byun et al. [30]
Sewage sludge with food waste leachate (5:5)	0.223	35	30	–	63.4	
Sewage sludge	0.186	35	–	–	35.4	Kim et al. [31]
Sewage sludge with food waste (0.87:0.13)	0.201	35	–	–	39.9	
Sewage sludge only	0.262	Mesophilic	28	7.3	38.7	This study
Sewage sludge mixing with food waste leachate	0.478	Mesophilic	30	9.1	59.6	

et al., and Kim et al. determined the MY with or without the addition of food waste leachate [29–35]. Especially, Kim et al. [29] examined the C/N ratio of input substrate for evaluating the impact on methane production. Inherent MY of sewage sludge in previous research have been shown in the range of 0.180~0.232 Sm<sup>3</sup>CH<sub>4</sub>/kg VS at 35.4~47.6% VS degradation efficiency.

Kim et al. [31] estimated the feasibility of co-digestion of sewage sludge and food waste. Combined anaerobic digestion of sewage sludge and food waste enhanced the MY and COD removal efficiency by approximately 1.1 times compared to that of anaerobic digestion of sewage sludge only. Lee et al. [35] carried out a biochemical methane potential test under thermophilic temperature conditions (55 °C). At a substrate ratio of



up to 5:5, MY was increased from 0.233 Sm<sup>3</sup>CH<sub>4</sub>/kg VS to 0.344 Sm<sup>3</sup>CH<sub>4</sub>/kg VS, exhibiting a 1.5 times improvement in results. Comparing the MY in this study with existing case studies, it can be seen that the overall flow was similar in terms of factors such as MY, removal rate, and the C/N ratio.

#### Acknowledgements

This work was supported by a grant from the National Institute of Environmental Research (NIER), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIER-2016-01-01-038).

#### Authors' contributions

J-SB, Y-MY, S-KS, D-JL and D-CS designed and conducted the experiment as well as wrote the manuscript. D-JL and D-CS inspired the overall work and revised the final manuscript. All authors helped prepare the manuscript. All authors read and approved the final manuscript.

#### Funding

Not applicable

#### Availability of data and materials

All data is available in the main text.

#### Competing interests

The authors declare that they have no competing interests.

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Received: 4 August 2020 Accepted: 12 September 2020

Published online: 26 September 2020

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