Linking process performances and core microbial community structures in anaerobic membrane bioreactor with rotatory disk (ARMBR) system fed with high-strength food waste recycling wastewater

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GRAPHICAL ABSTRACT
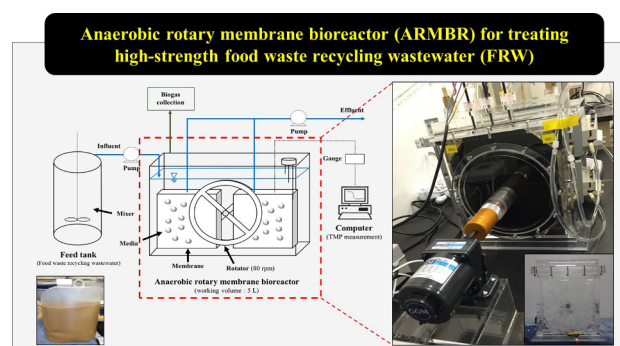
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ABSTRACT

This study first evaluated the process performances and microbial community structures of anaerobic rotary membrane bioreactor (ARMBR) fed with food waste recycling wastewater (FRW). Three identical ARMBRs were operated under different organic loading rate (OLR) conditions (1.5, 3.0, and 6.0 kg COD m^{-3} d^{-1}) after the same start-up periods. The start-up performances and archaeal community structures differed among the ARMBRs, probably due to the sudden OLR shock. After the start-up, bio-methane was stably produced until the end of the operational period, with all of the ARMBRs showing > 95% COD removal efficiency. *Methanosaeta* spp. was the predominant methanogen; diverse hydrogenotrophic methanogens co-existed. *Bacteroidetes*-like bacteria and *Candidatus* Cloacamonas acted as major fermentative bacteria producing acetate or hydrogen for the growth of methanogens. The results suggest that our ARMBR system can be a promising option to manage high-strength organic wastewater such as FRW.

1. Introduction

Food waste recycling wastewater (FRW) management is a critical issue in Korea, because a huge amount of FRW is annually discharged with various organic and inorganic pollutants (Lee et al., 2016). FRW is an excellent substrate for anaerobic fermentation, because it contains easily biodegradable organic compounds and mineral nutrients (Shin et al., 2015). Recently, anaerobic membrane bioreactors (AnMBRs) have attracted as a promising technology to treat organic wastewaters such as FRW, because it simultaneously enables bio-energy production (i.e., methane gas) and achieves high effluent quality (Dvořák et al., 2015). However, AnMBR systems often encounter membrane fouling problem. For this reason, numerous studies have been performed to develop fouling control strategies such as biogas sparging, liquid circulation, and membrane optimization (Jeong et al., 2017a; Lin et al., 2013). Our previous study newly proposed an anaerobic rotary membrane bioreactor (ARMBR) system as a novel AnMBR system to improve fouling mitigation (Kim et al., 2014). In the ARMBR system, rotary disk promotes the collision between membrane surface and sponge media, thereby mitigating fouling. In the previous study, the ARMBR system was stably operated without membrane replacement and washing during synthetic wastewater management, which suggested that using ARMBR system can be a feasible option for treating high-strength organic wastewaters. However, its application on real organic wastewater management has so far been limited.

Organic loading rate (OLR) is one of the significant operational factors determining the process performance and stability of the AnMBR system. Although higher OLR is generally beneficial to larger methane production, excess OLR causes poor process performance and severe fouling problems (Mao et al., 2015). For this reason, many previous studies have reported the different proper OLR conditions with the kinds of target organic wastewater. For example, AnMBR systems treating low-strength municipal wastewater could be operated at ~12.5 kg COD m^{-3} d^{-1} of input OLR (Ozgun et al., 2013). In contrast, AnMBR systems treating high-strength organic wastewaters have been operated at relatively lower OLR ranges, compared with AnMBRs treating low-strength organic wastewaters. It has been reported that an AnMBR treating landfill leachates achieved 90% of COD removal efficiency at < 6.3 kg COD m^{-3} d^{-1} (Zayen et al., 2010). In another study, an AnMBR failed at only 4.8 kg COD m^{-3} d^{-1} of OLR during food wastewater treatment; the authors suggested that the food wastewater contained high content of biodegradable organics, which accelerated system imbalance (He et al., 2005). For high-strength FRW, the treatment feasibility has recently been verified in AnMBR systems. However, they were operated only under the lower range of OLRs (i.e., 2.6–3.3 kg COD m^{-3} d^{-1}) and input COD concentrations (i.e., 1692–2583 mg L^{-1}) (Cho et al., 2018; Jeong et al., 2017b). In addition, the information in the effects of OLR conditions on process performance has still been limited in AnMBR system treating FRW. Therefore, it is necessary to evaluate the process performance of ARMBR at higher substrate conditions and variations of OLR.

Anaerobic methane production is performed by a series of biochemical reactions between hydrolytic/fermenting bacteria and methanogenic archaea. It has been suggested that the functional microbial community structure depends on a variety of process parameters such as reactor configuration, substrate type, OLR, and temperature (Narihiro et al., 2015; Shin et al., 2016). Therefore, understanding the core microbial community structure at given environmental condition is important to improve the process functionality. In particular, OLR is a significant operational parameter determining the microbial community in anaerobic processes (Narihiro et al., 2015). In fact, it has been reported that the predominant microbial communities significantly differed with OLR conditions in an upflow anaerobic sludge blanket (UASB) (Zhang et al., 2018). In an AnMBR treating swine manure, major methanogen was changed from *Methanosaeta* spp. to *Methanosarcina* spp. in accordance with an increase of OLR (Padmasiri et al.,

2007). It has also been reported that OLR increase had remarkable effects on the methanogenic community. The predominant community was changed from *Methanobacterium* to *Methanosaeta* in an AnMBR treating kitchen waste slurry (Xiao et al., 2017). These previous studies imply the functional microbial communities can also be differentiated with OLR conditions in AnMBR systems. In addition, since AnMBR can retain more diverse species than other anaerobic processes such as UASB and continuous stirred-tank reactor (CSTR), which can lead to the unique microbial community structures. It has been reported that higher diversity was discovered in an AnMBR compared to a CSTR-type anaerobic reactor (Greses et al., 2018). However, the information in microbial community structures of ARMBR has been limited so far.

AnMBR in food waste digestion is a promising way to produce energy and high quality effluent. In addition, it facilitates to prevent biomass wash-out, which can be beneficial to enhance process stability. However, its application on high-strength food waste management is still challenge due to severe fouling problem, and thereby the fouling mitigation strategy needs to be developed. Therefore, this study evaluated the ARMBR system which was designed to alleviate membrane fouling for managing high-strength FRW. The process performances and microbial community structures were assessed in three FRW feeding ARMBRs operated under different high OLR conditions (1.5, 3.0, and 6.0 kg COD m^{-3} d^{-1}). To the best of our knowledge, this is the first work applying the ARMBR system to manage real organic wastewater. For this purpose, three identical ARMBRs were set up, and operated for 185 d including start-up periods (53 days). The process performances were evaluated based on the organic removal, methane production, and filtration efficiencies. Microbial community structures during start-up and steady-state periods were analyzed using high-throughput next-generation sequencing method.

2. Materials and methods

2.1. ARMBR set-up and operational conditions

Three identical laboratory-scale ARMBRs (5 L working volume) were operated for 185 d. The ARMBRs were designed with reference to our previous study (Kim et al., 2014) (Fig. 1). Each ARMBR was composed of a submerged membrane and two rotary disks. Polypropylene fabric ball-type media of 8 mm diameter was filled with a 7% (v/v) of packing ratio. The rotational speed of rotating disk was set at 80 rpm. A flat sheet polyethersulfone fabric membrane (pore size: 0.2 um, effective area: 0.0252 m^2) was used in this study (G-brane, LG, Korea). Intermittent suction was performed to alleviate the fouling problem (5 min suction – 5 min relaxation). Biogas produced in the reactors was collected using Tedlar bag. The seeding sludge for the ARMBRs was collected from a full-scale anaerobic digestion (AD) plant treating sewage sludge. The mixed liquor suspended solids (MLSS) concentration of the collected seeding sludge was 24,767 ± 751 mg L^{-1}. The AD sludge was inoculated in each ARMBRs with 8,000 mg MLSS L^{-1}. The FRW was fed into the ARMBRs in semi-continuous modes (5 min feeding – 5 min non-feeding). The characteristics of FRW used as substrate is listed in Table 1. Influent pH was adjusted to 7.4 using 3 N sodium hydroxide and sodium bicarbonate; as a result, the pH was stably maintained at > pH 7.2 in all the ARMBRs. Any membrane cleaning was not conducted throughout the operational periods. The operational temperature was set to 30 °C using a temperature control incubator.

The ARMBRs were operated with the same start-up regime; the OLR increased with increasing input COD concentrations during the start-up periods for achieving the stabilized ARMBRs treating highly concentrated FRW (30,000 mg COD L^{-1}). In this study, the COD removal efficiency (> 95%) and methane content (> 50–60%) were used as indicators for determining the steady-state period. During the steady-state periods, higher OLR conditions were achieved with reducing hydraulic retention time (HRT) for ARMBR 2 and ARMBR 3 (Table 2). In
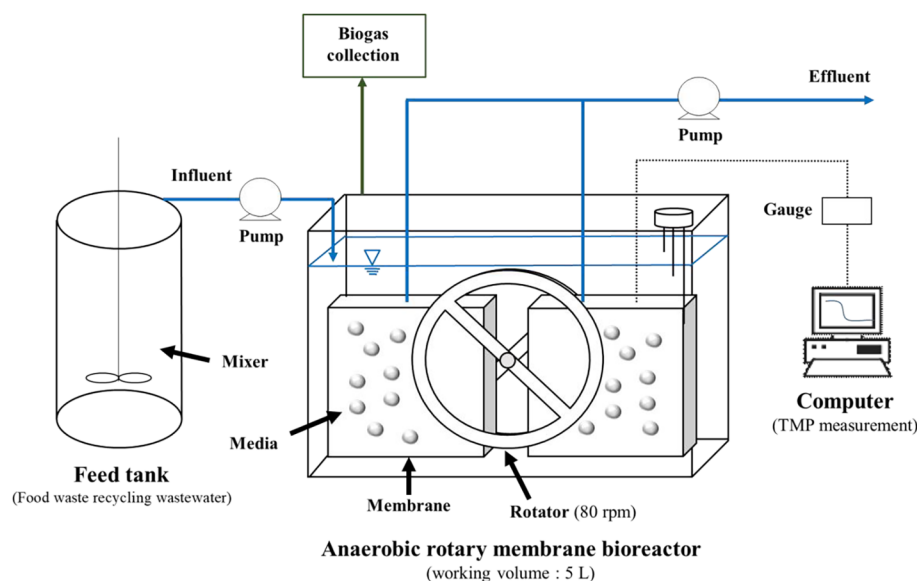
Fig. 1. Schematic diagram of the anaerobic rotary membrane bioreactor (ARMBR) tested in this study.

Table 1
Characteristics of FRW used in this study.

Parameters	Concentration (mg L^{-1})
pH	3.92 ± 0.23 (After pH adjustment: 7.47 ± 0.12)
TCOD	30,125 ± 1,662
TOC	8462 ± 999
T-N	777 ± 107
NH_4^+-N	142 ± 13
T-P	153 ± 32
SO_4^{2-}	65 ± 20
TCOD : T-N : T-P	196.9 : 5.1 : 1.0

addition, the OLR conditions subsequently increased with monitoring the process performances to minimize the shock loading. After the start-up period (53 days), the ARMBRs were operated at 1.5 kg COD m^{-3} d^{-1}. The OLR conditions were controlled in each ARMBR. For ARMBR 1, the OLR condition was maintained at 1.5 kg COD m^{-3} d^{-1} until the end of the operational period, while for ARMBR 2, the OLR increased to 3.0 kg COD m^{-3} d^{-1} at Day 106 (i.e., the HRT was reduced from 20 day to 10 day). For ARMBR 3, the OLR subsequently increased to 3.0 kg COD m^{-3} d^{-1} (Day 106) and to 6.0 kg COD m^{-3} d^{-1} (Day 112) with decreasing the HRT from 10 day to 5 day. For ARMBR 2 and ARMBR 3, the OLR was increased after the ARMBRs achieved the stable process performances (i.e., COD removal efficiency and methane content) during the steady-state periods. The sludge retention time (SRT) was maintained at 100 day. Table 2 summarizes the operational conditions of each ARMBR.

2.2. Physico-chemical analysis

Liquor samples were collected from the ARMBRs for physico-chemical analysis. The MLSS concentration was measured by standard methods (APHA-AWWA-WEF., 2005). The concentrations of total chemical oxygen demand (TCOD), total phosphorus (T-P), ammonia, and sulfate were measured using commercial Hach kits (Product No. 2125915: COD, Product No. 2767245: T-P, Product No. 2606945: ammonia, and Product No. 1206599: sulfate). The pH was measured using a pH meter (HQ40d, Hach, USA). The total organic carbon (TOC) and total nitrogen (T-N; sum of organic nitrogen, ammonia, nitrite, and nitrate) concentrations were analyzed using TOC and TN analyzer (Shimadzu, TOC-L and TNM-L, Kyoto, Japan), respectively. The biogas volume was measured using water substitution method. The contents of

methane (CH$_4$), carbon dioxide (CO$_2$), and dinitrogen gases (N$_2$) were measured using gas chromatography (GC) 7890A (Agilent Technologies, USA) equipped with thermal conductivity detector (TCD) and Restek PC8779 stainless steel packed column (Restek, USA).

2.3. Microbial community analysis using MiSeq platform sequencing

Mixed liquor in the ARMBRs was collected during the start-up and steady-state periods for microbial community analysis. Sample information in sequencing analysis is summarized in Supplementary information. Genomic DNA was extracted from the sludge samples using FastDNA spin kit for soil (MP Bio 101, CA, USA). The extracted DNA samples were submitted to Macrogen Inc. (Seoul, Korea) for high-throughput sequencing. The sequencing was performed by Illumina Miseq sequencing platform. The 16S bacterial and archaeal sequences were amplified using a universal primer; PRK 341F (5′-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGR BGC ASC AG-3′) and PRK 806R (5′-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGG ACT ACY VGG GTA TCT AAT-3′) (Takahashi et al., 2014). The raw sequences were processed using Mothur v. 1.39.5. The sequences (> 500 base pairs) were filtered out using screen command. Chimeric sequences were removed using the UCHIME algorithm. The refined sequences were clustered into operational taxonomic units (OTUs) based on the sequence similarity (≥97%). The sequences were matched with the SILVA reference database (SILVA 132) to identify the microbial species (Quast et al., 2013). The sequences have been deposited in Sequence Read Archive of NCBI database (BioProject: PRJNA521840). Statistical analyses, clustering analysis and non-metric multidimensional scaling (NMDS), were performed using PC-ORD v.5.0, MjM software (Gleneden Beach, OR) to compare microbial community structure along with operational conditions. The major genera discovered with either average relative abundance > 1.0% or peak relative abundance > 5.0% in each ARMBR were used for the statistical analyses.

3. Results and discussion

3.1. Process performances of the ARMBRs

3.1.1. Process performances during the start-up periods

The start-up of the three ARMBRs was performed with the same operational strategy to stabilize the ARMBRs treating high-strength

Table 2
Process performances of the anaerobic rotary membrane bioreactors (ARMBRs) at the start-up and various organic loading rate (OLR) conditions.

	ARMBR 1				ARMBR 2		
	Start-up periods			OLR1.5	Start-up periods		
Elapsed time (d)	0–40	40–50	50–53	53–185	0–40	40–50	50–53
HRT (d)	1.25	20	20	20	1.25	20	20
Flux (LMH)	6.61	0.41	0.41	0.41	6.61	0.41	0.41
OLR[1]	1.26	0.11	0.55	1.5	1.26	0.11	0.55
INF COD[2]	1,577 ± 8157	2,553 ± 498	11,106 ± 2,876	30,125 ± 1,662	1,577 ± 157	2,553 ± 498	11,106 ± 2,876
EFF COD[3]	467 ± 271	97 ± 67	39 ± 5	436 ± 206	439 ± 225	67 ± 51	37 ± 8
$COD_{removed}$[4]	71.3 ± 15.5	95.4 ± 4.3	99.7 ± 0.1	98.6 ± 0.6	72.9 ± 12.8	96.7 ± 3.4	99.7 ± 0.1
CH_4 yield[5]	NA	NA[6]	15.7 ± 4.8	114.9 ± 60.0	NA	NA	24.7 ± 0.8

	ARMBR 2		ARMBR 3					
	OLR1.5	OLR 3	Start-up periods			OLR1.5	OLR 3	OLR 6
Elapsed time (d)	53–106	106–185	0–40	40–50	50–53	53–106	106–112	112–185
HRT (d)	20	10	1.25	20	20	20	10	5
Flux (LMH)	0.41	0.82	6.61	0.41	0.41	0.41	0.82	1.65
OLR[1]	1.5	3.0	1.26	0.11	0.55	1.5	3.0	6.0
INF COD[2]	30,200 ± 2,292	30,093 ± 1,306	1,577 ± 157	2,553 ± 498	11,106 ± 2,876	30,200 ± 2,292	30,165 ± 136	30,091 ± 1,353
EFF COD[3]	410 ± 374	722 ± 635	166 ± 215	83 ± 66	36 ± 4	524 ± 572	846 ± 207	1,329 ± 1,708
$COD_{removed}$[4]	98.6 ± 1.4	97.6 ± 2.1	89.8 ± 13.1	95.9 ± 4.4	99.7 ± 0.1	98.2 ± 2.0	97.2 ± 0.7	95.6 ± 5.8
CH_4 yield[5]	109.8 ± 68.3	140.5 ± 43.2	NA	NA	113.9 ± 62.6	259.3 ± 118.6	267.0 ± 59.3	265.5 ± 56.8

[1] OLR; organic loading rate (kg COD m^{-3} d^{-1}).
[2] INF COD; influent COD concentration (mg L^{-1}).
[3] EFF COD; effluent COD concentration (mg L^{-1}).
[4] $COD_{removed}$; COD removal efficiency (%).
[5] CH_4 production yield (L $CH_4 \cdot kg^{-1}$ $COD_{removed}$).
[6] NA; not analyzed.

FRW (Table 2). After the start-up periods (Day 53), all the ARMBRs achieved high COD removal efficiency and stable methane production. In general, AnMBR systems require relatively short start-up period, compared to conventional AD systems (Lin et al., 2013). However, the AnMBR system treating high-strength organic wastes can take prolonged start-up period. For example, the AnMBRs treating bamboo industry wastewater containing high content organics (17,160 mg COD L^{-1}) took 85 days to achieve the start-up (Xia et al., 2016). In another previous study, the COD removal efficiency reached 81% in an AnMBR treating synthetic wastewater after 56 days of start-up periods (Akram and Stuckey, 2008).

In this study, the organic removal performance during the start-up periods was differentiated among the ARMBRs (ARMBR 1, 2 vs. ARMBR 3). For ARMBR 3, the COD removal efficiency was quickly increased to 96.3% within 15 days, while for ARMBR 1 and ARMBR 2, it reached a similar level (> 95%) after 35 days. The lower organic removal performance of the two ARMBRs (ARMBR 1 and ARMBR 2) might be due to the unforeseen feeding error. In this study, the ARMBR 3 was stably operated in semi-continuous mode (5 min feeding – 5 min non-feeding), whereas the feeding control systems of the other ARMBRs (ARMBR 1 and ARMBR 2) were stopped for 24 h during the start-up. It has been reported that prolonged feeding-starvation cycle can cause a sudden increase of hydrogen and acids contents, which may adversely affect the AD performance (Martinez-Garcia et al., 2009). The TMP of the three ARMBRs reached 30–35 kPa during the initial operational periods (Day 0 to Day 40); then it was significantly decreased to < 5 kPa after the HRT increase (1.25 day to 20 day). In summary, after the start-up periods, the ARMBRs were successfully stabilized to treat high-strength FRW.

3.1.2. Process performances in various OLR conditions

After the start-up, the ARMBRs were operated under the different OLR conditions (1.5, 3.0, and 6.0 kg COD m^{-3} d^{-1}) (Table 2). The process performances were evaluated in terms of the organic removal, methane production, and filtration (TMP), and compared among the ARMBRs. First, all the ARMBRs showed high COD removal efficiencies (> 95%) throughout the operational periods, which indicates that the ARMBR system successfully treated high-strength FRW, despite the high OLR condition (6.0 kg COD m^{-3} d^{-1}) (Table 2). It has been reported that a submerged AnMBR treating tannery wastewater achieved 90.1% COD removal efficiency at 6.0 kg COD m^{-3} d^{-1} OLR condition (Umaiyakunjaram and Shanmugam, 2016). A recent previous study

Fig. 2. The variations of *trans*-membrane pressure (TMP) performances in the anaerobic rotary membrane bioreactors (ARMBRs).

investigated the process performance in an anaerobic ceramic membrane bioreactor (AnCMBR) to co-manage FRW and domestic wastewater (DWW); 98.3 ± 1.0% of COD removal efficiency was observed at 2.95 kg COD m^{-3} d^{-1} OLR (Jeong et al., 2017b). Therefore, it could be suggested that the organic removal efficiency of our ARMBR system is comparable with the AnMBRs treating high-strength organic wastewaters.

Similar to the organic removal efficiency, the methane gas was stably produced in all the ARMBRs until the end of the operational periods. The average methane yield was measured as 114.9 ± 60.0 L $CH_4 \cdot kg^{-1} COD_{removed}$ for ARMBR 1 (OLR 1.5), 140.5 ± 43.2 L $CH_4 \cdot kg^{-1} COD_{removed}$ for ARMBR 2 (OLR 3.0), and 265.5 ± 56.8 L $CH_4 \cdot kg^{-1} COD_{removed}$ for ARMBR 3 (OLR 6.0) (Table 2). The methane yield values were lower than the stoichiometric theoretical value (350 L $CH_4 \cdot kg^{-1} COD_{removed}$). It has been suggested that long SRT reduces the ratio of the substrate converted to methane, which can decrease methane yield (de la Rubia et al., 2006; Parawira et al., 2006). The ARMBRs were operated under long SRT condition (100 day), which might result in the low methane yield. The methane production performance in this study was comparable with the previous AnMBR studies. It has been reported that the methane yield was 130 L $CH_4 \cdot kg^{-1} COD_{removed}$ in an AnMBR treating bamboo industry wastewater operated under 6.0 kg COD m^{-3} d^{-1} of OLR (Xia et al., 2016). Another previous study reported that an AnMBR managing meat processing wastewater achieved 180 L $CH_4 \cdot kg^{-1} COD_{removed}$ of methane yield at 3.2 kg COD m^{-3} d^{-1} input OLR. (Galib et al., 2016). In addition, a methane yield of 210 ± 10 L $CH_4 \cdot kg^{-1} COD_{removed}$ was reported for the AnCMBR co-managing FRW and DWW (Jeong et al., 2017b).

The COD mass balance of the three ARMBRs was assessed based on gaseous methane, gaseous carbon dioxide, permeate COD, and sulfate reduction. As a result, those compositions were determined by 31.6, 7.9, 1.4 and 0.1% in the ARMBR 1 while they were measured by 39.6, 9.9, 1.6 and 0.1% in the ARMBR 2. For the ARMBR 3, the compositions were 74.4, 18.6, 2.1 and 0.1%. Unknown compositions could be regarded as sum of dissolved methane, grown biomass, and accumulated organic matters (Galib et al., 2016; Jeong et al., 2017b). The small portion of the influent COD was utilized to sulfate reduction, which was consistent with a previous study of the mesophilic AnCMBR co-managing FRW and DWW (Jeong et al., 2017b). Interestingly, the methane yield of ARMBR 3 was higher than that of the other ARMBRs (ARMBR 1 and ARMBR 2), despite having the same input OLR condition. For example, at OLR 1.5 kg COD m^{-3} d^{-1}, the methane yield was 114.9 ± 60.0 L $CH_4 \cdot kg^{-1} COD_{removed}$ (ARMBR 1), 109.8 ± 68.3 L $CH_4 \cdot kg^{-1} COD_{removed}$ (ARMBR 2), and 259.3 ± 118.6 L $CH_4 \cdot kg^{-1} COD_{removed}$ (ARMBR 3), respectively. In this study, the three ARMBRs were identically set-up and operated with the same strategies, except for the abrupt loading fluctuation (ARMBR 1 and ARMBR 2). Therefore, it can be hypothesized that the poor start-up performance decreased the methane production performance during the steady-state operational period. However, the reasons for the lower methane production performance of ARMBR 1 and ARMBR 2 are still unclear. Future works need to be performed to elucidate the relationship between the start-up and steady-state performances.

The TMP results showed that the filtration performance of the ARMBRs was stably maintained until Day 106 (Fig. 2). However, for the ARMBR 2 and ARMBR 3, the TMP gradually increased along with the process operation. At the final point (Day 185), the TMP was measured by 20 kPa (ARMBR 1), 36 kPa (ARMBR 2), and 38 kPa (ARMBR 3), respectively (Fig. 2). It has been reported that higher OLR induces the rapid increase of TMP in MBR system (Johir et al., 2012). In general, potential inhibitors such as ammonia are related to the release of extracellular polymeric substance (EPS) in anaerobic processes. It has been reported that EPS production increased in an expanded granular sludge bed (EGSB) reactor, as residual ammonia concentration increased to 1000 mg L^{-1} (Liu et al., 2015). In this study, the residual total nitrogen (T-N) concentration (sum of the organic nitrogen, ammonia, nitrite, and nitrate) was measured with a range of 670–900 mg
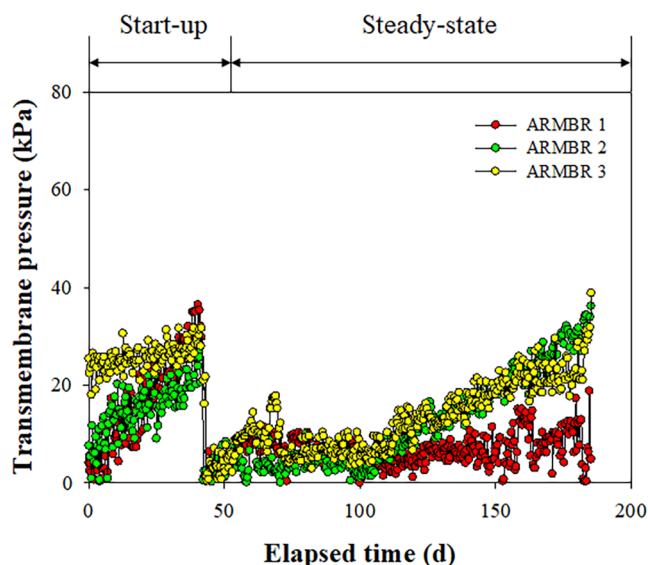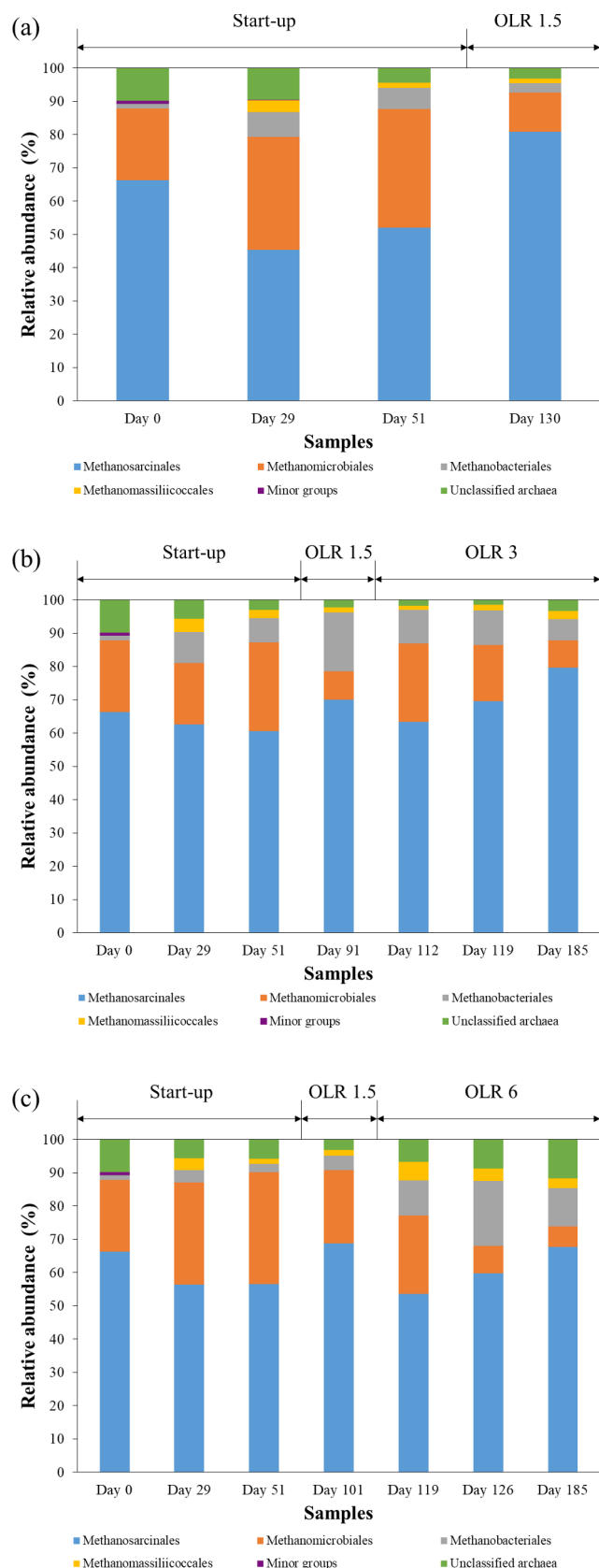
Fig. 3. Archaeal relative abundance at order level in the anaerobic rotary membrane bioreactors (ARMBRs); (a) ARMBR 1, (b) ARMBR 2, and (c) ARMBR 3.
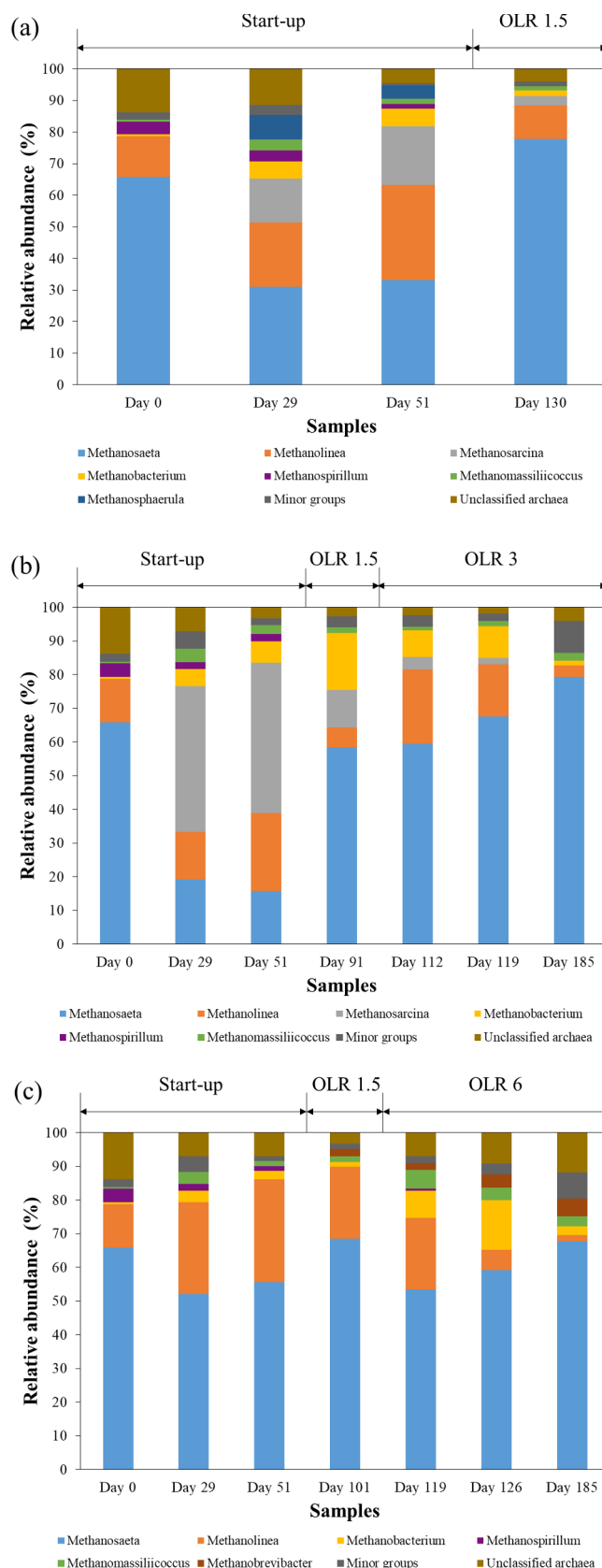
Fig. 4. Archaeal relative abundance at genus level in the anaerobic rotary membrane bioreactors (ARMBRs); (a) ARMBR 1, (b) ARMBR 2, and (c) ARMBR 3.

L^{-1} in all the ARMBRs. The residual T-N might promote to EPS production, which could aggravate membrane fouling in the ARMBRs.

Nevertheless, the ARMBRs were operated for long-term period with stable organic removal and methane production performances, without significant fouling problem. In other words, there was no significant biofilm thickness, which implies that most of the biomass was in suspension phase. However, longer operation may lead to biofilm formation on membrane surface, which can decrease water permeability. During MBR operation, the spatial and temporal distribution of various foulants is changed over TMP development. For example, soluble microbial products (SMP) are major foulants for initial stage of fouling (before TMP jump), while EPS-proteins are play important role for late stage of fouling (after TMP jump). For this reason, understanding the fouling characteristics over TMP variation would be important to develop fouling control strategy (Meng et al., 2017). In addition, membrane resistance is another important indicator addressing membrane fouling (Meng et al., 2017; Ozgun et al., 2013). Further study should be performed to characterize membrane fouling including EPS, SMP, membrane resistance, etc. in the ARMBR systems. Taking all of the results together, the ARMBRs were successfully operated at high OLR condition, which suggests that the ARMBR system can be a promising option to manage high-strength FRW.

3.2. Archaeal community structures

A total of 425,804 archaeal sequences were obtained from sixteen samples (see Supplementary information). The archaeal communities with either average relative abundance > 1.0% or peak relative abundance > 5.0% were presented at order (Fig. 3) and genus levels (Fig. 4). Two statistical analyses, NMDS and clustering analysis, were performed to investigate the microbial clustering patterns. The 2D plot and clustering dendrogram show that the archaeal community structures were distinctly divided with two groups (First node: R1-29d, R1-51d, R2-29d, R2-51d vs. other samples) (see Supplementary information), indicating the different archaeal communities were predominant in both ARMBR 1 and ARMBR 2 during the start-up periods. In addition, the final archaeal community structures of the ARMBRs were grouped in a subnode, implying the major archaeal communities became similar in the ARMBRs operated under the different OLR conditions during the steady-state periods.

3.2.1. Archaeal community structures during the start-up periods

At the beginning, the three ARMBRs were inoculated with the same AD sludge. For this reason, the archaeal community structure of the sludge was regarded as an initial community at Day 0 in each ARMBR. In the seeding sludge, Methanosarcinales (MSL; 66.4%) and Methanomicrobiales (MMB; 21.5%) were observed as the predominant methanogenic orders (Fig. 3). At genus level, one aceticlastic methanogen (AM), Methanosaeta (65.8%) belonging to MSL, and two hydrogenotrophic methanogens (HMs), Methanolinea (12.9%) and Methanospirillum (4.0%) were measured as major methanogens.

During the start-up periods, the ARMBRs showed similar archaeal community pattern at order level. The relative abundance was as follows: MSL > MMB > Methanobacteriales (MBT) > Methanomassiliicoccales (Fig. 3). However, the archaeal community structure differed at the genus level among the ARMBRs (ARMBR 1, 2 vs. ARMBR 3). For example, at Day 51, Methanosarcina newly appeared as the predominant methanogen in the two ARMBRs, ARMBR 1 (18.6%) and ARMBR 2 (44.6%), whereas it was not detected as the major methanogen in ARMBR 3. In addition, the relative abundance of Methanosaeta were lower in ARMBR 1 (33.2%) and ARMBR 2 (15.9%), compared to that of ARMBR 3 (55.7%) (Fig. 4). These methanogenic community differences would be due to the presence (ARMBR 1 and ARMBR 2) or absence (ARMBR 3) of abrupt loading fluctuation described in the previous chapter. Both methanogens belong to the order MSL. Methanosaeta is a representative acetate-utilizing methanogen, while Methanosarcina can use various kinds of substrate for growth.

Methanosaeta which has high substrate affinity prefers low substrate conditions, whereas Methanosarcina usually became predominant in high substrate conditions, because of its low substrate affinity (Cho et al., 2018; Demirel and Scherer, 2008). Moreover, it has been reported that Methanosarcina was detected as a major methanogen in an unstable codigester (Demirel and Scherer, 2008). In addition, according to a previous study, Methanosarcina became predominant in anaerobic digesters that had undergone pulse OLR shock (Steinberg and Regan, 2011). Therefore, the unforeseen OLR shock could result in the different archaeal communities for the three ARMBRs during the start-up periods.

3.2.2. Archaea community structures in various OLR conditions

After the start-up period, the archaeal community structures were analyzed in each ARMBR to investigate the effects of OLR conditions on methanogenic communities. At order level, similar to the start-up period, order MSL was the most dominant with 67.7–80.1% of relative abundance for all the ARMBRs. However, for two orders of HMs, MMB and MBT, their relative abundance varied with the OLR conditions. For order MMB, the relative abundance was measured as 11.7% (ARMBR 1-OLR 1.5), 8.2% (ARMBR 2-OLR 3.0), and 6.1% (ARMBR 3-OLR 6.0) at each final point, whereas for order MBT, it was determined to be 2.9% (ARMBR 1-OLR 1.5), 6.4% (ARMBR 2-OLR 3.0), and 11.5% (ARMBR 3-OLR 6.0), respectively (Fig. 3). The results imply that order MBT prevailed over MMB at higher OLR condition in our ARMBR system. It has been assumed that Methanomicrobiales spp. has higher substrate affinity than Methanobacterium spp. belonging to MBT (Sakai et al., 2009). In another previous study, order MBT outcompeted order MMB at shorter HRT condition in a mesophilic farm-scale AD plant; the opposite result was observed at longer HRT condition (Cho et al., 2013).

At the genus level, different archaeal community structures were observed at the end of the start-up periods (Day 51). However, after the start-up, the archaeal community structure of the ARMBRs became similar. In particular, Methanosaeta became predominant in all the ARMBRs, even though the OLR conditions differed from 1.5 to 6.0 kg COD $m^{-3} d^{-1}$. The results indicate that Methanosaeta mainly led to methane production in the ARMBRs managing high-strength FRW. Methanosaeta has been detected as a predominant methanogen in stably operated AD systems including food waste digestion processes (Wang et al., 2018). It has been reported that Methanosaeta became predominant in a mesophilic AD reactor treating food waste with > 80% of relative abundance (Guo et al., 2014). From anther previous study, Methanosaeta was measured as the most predominant methanogen in a stably operated full-scale UASB plant treating food wastewater (Lee et al., 2017). Taken together, high abundance of Methanosaeta could contribute to achieve the stable methanogenic performance in the ARMBRs.

Although Methanosaeta favors low substrate condition, some previous studies have reported its predominance in anaerobic processes operated under high OLR condition. It has been reported that the relative abundance of Methanosaeta increased to > 82% with OLR increment in an AnMBR performing co-management of food waste and urban wastewater (Zamorano-Lopez et al., 2018). In another previous study, an AnMBR was operated for co-managing cheese whey and cattle slurry. Methanosaeta increased from 37.5% to 62.4% with increasing OLR to 8.4 kg COD $m^{-3} d^{-1}$ (Ribera-Pi et al., 2018). In addition, Methanoseta became predominant in another AnMBR process used for the management of high-strength kitchen waste slurry with increasing OLR from 4.7 to 9.3 kg COD $m^{-3} d^{-1}$, probably because the AnMBR was stably kept to promote the growth of Methanoseta (Xiao et al., 2017). In this study, all the ARMBRs were operated at high OLR conditions (> 1.5 kg COD $m^{-3} d^{-1}$), after the long stabilization periods. For ARMBR 2 and ARMBR 3, the OLR increased subsequently with monitoring the organic removal efficiency. In addition, the T-N concentration was < 900 mg L^{-1} in the ARMBRs, which might be negligible to inhibit the activity of Methanosaeta. It has been recently reported that 1.5 g L^{-1} total ammonia nitrogen (TAN) had no significant inhibitory
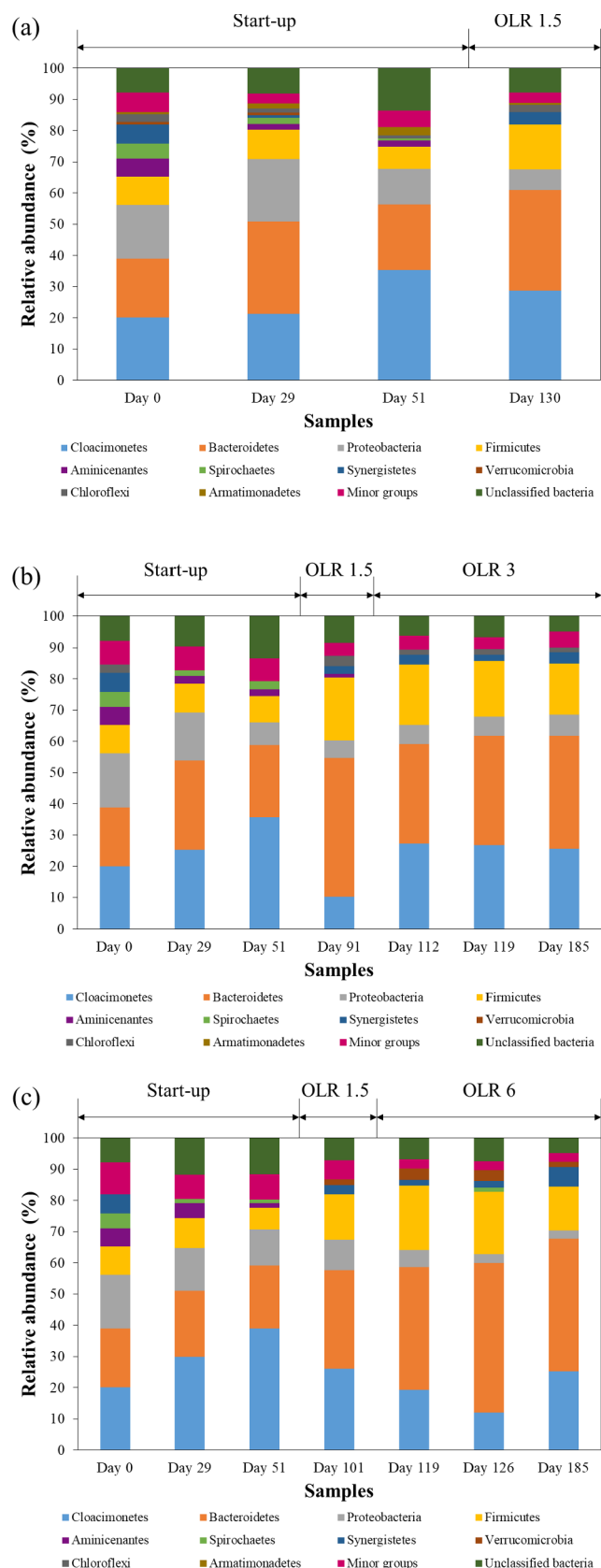
Fig. 5. Bacterial relative abundance at phylum level in the anaerobic rotary membrane bioreactors (ARMBRs); (a) ARMBR 1, (b) ARMBR 2, and (c) ARMBR 3.
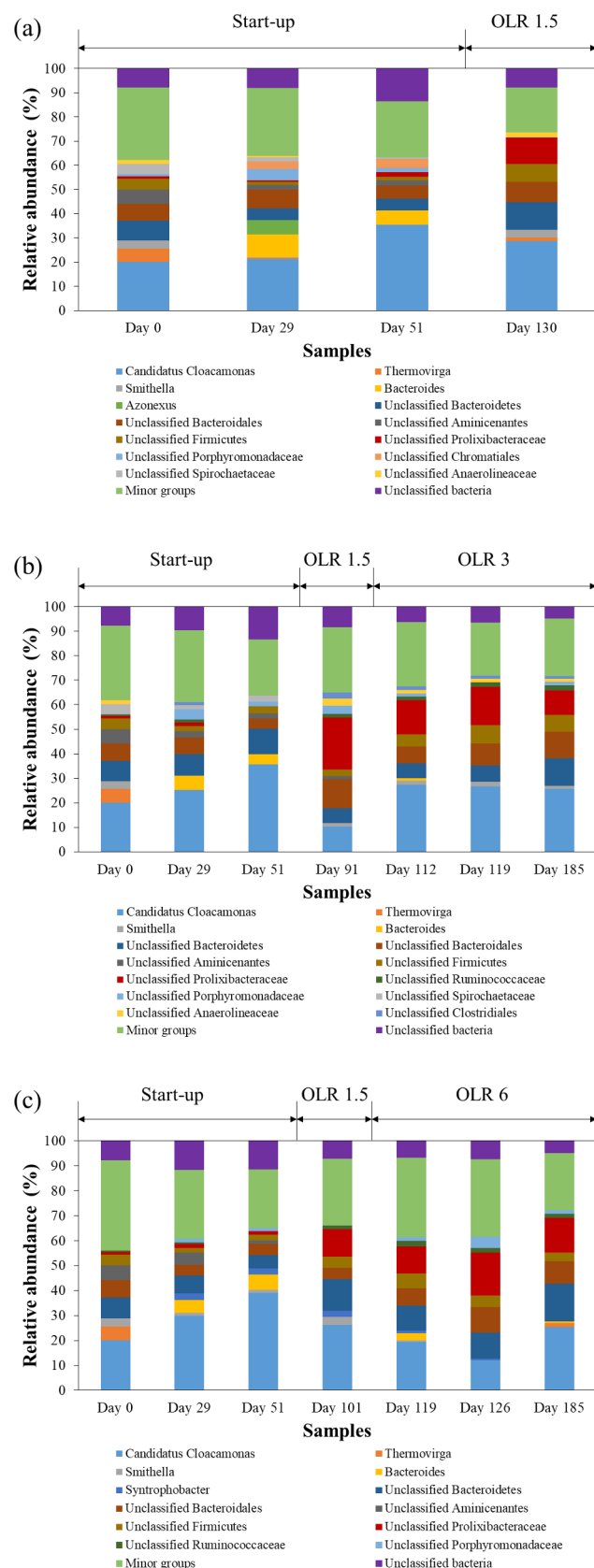
Fig. 6. Bacterial relative abundance at genus level in the anaerobic rotary membrane bioreactors (ARMBRs); (a) ARMBR 1, (b) ARMBR 2, and (c) ARMBR 3.

effect on specific methanogenic activity of *Methanosaeta concilii* (Lee and Hwang, 2019). Another previous study has reported that *Methanosaeta* spp. was the most predominant methanogen in a full-scale UASB reactor operated under 1.0–1.5 g L^{-1} of TAN (Lee et al., 2017). Therefore, the operational strategies (long start-up periods and stepwise increase of OLR) and low T-N concentration would encourage the predominance of *Methanoseata*. In this study, the methane yield was differentiated among the ARMBRs (Table 2), even though the predominant methanogenic communities were similar. It has been suggested that organic carbon is mainly used to biological synthesis (anabolism) under non-stabilized condition rather than methane production (catabolism), which leads to low methane yield (Michaud et al., 2005; Parawira et al., 2006). As described in previous chapter, the ARMBR 1 and ARMBR 2 were undergone by unforeseen feeding shock during the start-up period, which might promote to anabolic pathway. Future research should consider the effects of loading shock on methane yield and physiological characteristics of methanogens.

In addition, various HMs such as *Methanolinea*, *Methanobacterium*, *Methanobrevibacter*, *Methanomassiliicoccus*, and *Methanoculleus* were measured as the Top 5 methanogens at the final point (the sum of their relative abundance was ~13% in all of the ARMBRs), indicating that methane production was also carried out by hydrogenotrophic pathway. This might be attributed to long SRT, one of the unique characteristics of the AnMBR system, allowing the growth of the diverse methanogens. It has been reported that AMs and HMs were co-predominant in the mesophilic AnCMBR system managing FRW (Cho et al., 2018). In general, HMs have high resistance to environmental factors such as abrupt loading fluctuation and toxic matters over AMs (Cho et al., 2017; Lee et al., 2016). Therefore, the co-existence of HMs and AMs might contribute to enhance the process stability of the ARMBRs.

3.3. Bacterial community structures

A total of 3,948,133 sequences were generated from sixteen samples. The bacteria communities with either average relative abundance > 1.0% or peak relative abundance > 5.0% were presented at phylum (Fig. 5) and genus levels (Fig. 6). The statistical results show that the bacterial community structures were distinctly divided with two groups (First node: R1-29d, R1-51d, R2-29d, R2-51d, R3-29d, R3-51d vs. other samples) (see Supplementary information), indicating the different bacterial communities were predominant during the start-up periods in all the ARMBRs. In similar to archaeal community structure, the final bacterial community structures of the ARMBRs were grouped in a sub-node, implying the major bacterial communities became similar in the ARMBRs operated under the different OLR conditions.

3.3.1. Bacterial community structures during the start-up periods

In the seeding sludge, *Cloacimonetes* (20.1%), *Bacteroidetes* (18.8%), *Proteobacteria* (17.3%), *Firmicutes* (9.1%), and *Synergistetes* (6.1%) were detected as major bacterial phyla (Fig. 5). At the genus level, two identified bacteria, *Candidatus* Cloacamonas (20.1%) and *Thermovirga* (5.5%), and three unclassified bacteria were found as the Top 5 genera (Fig. 6).

During the start-up periods, the ARMBRs showed similar bacterial community patterns at the phylum level; *Cloacimonetes*, *Bacteroidetes*, *Proteobacteria*, and *Firmicutes* were commonly predominant in all of the ARMBRs at Day 51. These bacterial phyla have been discovered in an anaerobic system treating organic wastewater (Antwi et al., 2017). In a previous study, the same phyla were predominant in a UASB system for the treatment of municipal wastewater (Quek et al., 2017). It has been reported that *Cloacimonetes*, *Bacteroidetes*, *Proteobacteria*, *Firmicutes*, *Chloroflexi*, and *Ignavibacteriae* were major bacterial phyla in a mesophilic AnMBR treating activated sludge (Zheng et al., 2018). The major bacterial genera were also similar among the ARMBRs at Day 51, unlike the trends in the methanogenic community result that *Methanosarcina* rapidly increased in ARMBR 1 and ARMBR 2, but not in ARMBR 3

(Fig. 4). In a previous study, the bacterial community structure was not shifted in an AD process with changes in OLR (i.e., 1–6 kg VS m^{-3} d^{-1}), because the anaerobic hydrolytic/acidogenic bacteria have strong resistance to process disturbance (Li et al., 2016). The results suggests that the bacterial communities were not significantly affected by the abrupt loading fluctuation in ARMBR 1 and ARMBR 2.

3.3.2. Bacterial community structures in various OLR conditions

After the start-up, the bacterial community structures of the ARMBRs became similar along with the process operation. For example, the same bacterial phyla, *Bacteroidetes*, *Cloacimonetes*, *Firmicutes*, *Proteobacteria*, and *Synergistetes*, were commonly predominant at the final point in each ARMBR (Fig. 5). These phyla are regarded as fermentative bacteria that can degrade macromolecular organics like carbohydrates, proteins, and lipids (Narihiro et al., 2015). In particular, phylum *Bacteroidetes* became predominant in all the ARMBRs, and their relative abundance increased to 32.2, 36.2, and 42.4% in ARMBR 1, ARMBR 2, and ARMBR 3 (Fig. 5). *Bacteroidetes* have been frequently discovered in an anaerobic process; they enable the production of propionate and acetate from proteins and carbohydrates (Narihiro et al., 2015). Therefore, it could be suggested that *Bacteroidetes*-like bacteria functioned as significant acetate producers that symbiotically grew with *Methanosaeta* spp. in the ARMBRs.

Similar to the phylum results, the major bacterial genera were the same in all the ARMBRs. For example, three unclassified genera belonging to phylum *Bacteroidetes* which are regarded as potential acetate producers were predominant at the final point in the ARMBRs. Interestingly, *Ca*. Cloacamonas belonging to phylum *Cloacimonetes* was the most predominant genus in all the ARMBRs with > 25% of relative abundance. In anaerobic processes, syntrophic bacteria play an important role for reducing metabolic intermediates which can inhibit methanogenic activity or supplying substrate on methanogens. In particular, for anaerobic processes treating high-strength organic wastewaters such as food wastes, syntrophic relationship among the functional microbial communities is closely related to stable process performances (Wang et al., 2018). It has been reported that *Syntrophomonas* spp. (a syntrophic fatty-acid-oxidizer) and *Pelotomaculum* spp. (a syntrophic propionate oxidizer) were detected in an anaerobic digester using food waste as substrate (Guo et al., 2014). It has been also reported that several syntrophic bacteria including *Gelria* which can produce H_2 was predominant in a thermophilic AD process treating FRW (Lee et al., 2016). *Ca*. Cloacamonas which was the most abundant in the ARMBRs is also syntrophic fermentative bacteria (Fig. 6). This bacterial genus can produce acetate, hydrogen, and carbon dioxide from various organic materials such as amino acids, lactate, succinate, and propionate (Pelletier et al., 2008). The genus *Ca*. Cloacamonas oxidizes propionate to acetate; the reaction is thermodynamically favorable under low hydrogen pressure, which implies acetate production can be promoted by the mutual growth of hydrogen scavenger such as HMs (Pelletier et al., 2008). In this study, some HMs co-existed with AMs in the ARMBRs (Fig. 4); the HMs encouraged the growth of *Ca*. Cloacamonas by consuming the produced hydrogen, which eventually contributed to increase of *Methanosaeta* spp. in the ARMBRs. Therefore, *Ca*. Cloacamonas could act as a key substrate supplier to both AMs and HMs as well as a major co-worker enhancing the process stability of the ARMBRs.

In addition, the ARMBRs showed similar bacterial community structures at the final point, indicating the OLR conditions did not differentiate the bacterial communities. In a previous study, bacterial community structure was not shifted significantly in a mesophilic fluidized bed reactor (FBR)-UASB hybrid system stably operated under change of OLR from 2.0 to 6.0 kg COD m^{-3} d^{-1} (Kundu et al., 2013). It has also been reported that genus *Kosmotoga* belonging to phylum *Thermotogae* was consistently predominant in all OLR conditions of 4.7 to 9.3 kg COD m^{-3} d^{-1} in an AnMBR treating high-strength kitchen waste slurry (Xiao et al., 2017). Therefore, it could be suggested that

the input OLR conditions tested in this study would not be high enough to lead to variations in the bacterial community structure in the ARMBRs. Various syntrophic bacteria such as *Bacteroidetes*-like bacteria and *Ca.* Cloacamonas spp. were predominant in all the OLR conditions.

3.4. Implications

This study evaluated the process performances and microbial community structures in the ARMBRs fed with high-strength FRW. As a result, the ARMBRs were successfully operated at high OLR condition (6.0 kg COD m^{-3} d^{-1}) without significant fouling problem during long-term operation. The microbial community structures among the three ARMBRs became similar along with process operation. *Methanosaeta* spp. which is acetate utilizing methanogen was predominant while *Ca.* Cloacamonas which is syntrophic bacteria acted as a major co-worker of methanogen.

The energy balance of the ARMBRs were calculated as described in a previous study (Kim et al., 2014). The energy demand was determined as 11.4 kwh m^{-3} for ARMBR 1, 5.7 kwh m^{-3} for ARMBR 2, and 2.9 kwh m^{-3} for ARMBR 3. The produced electrical energy was evaluated by 10.2 kwh m^{-3} for ARMBR 1, 12.4 kwh m^{-3} for ARMBR 2 and 22.9 kwh m^{-3} for ARMBR 3 (see Supplementary information). The net energy production was highest in the ARMBR 3, indicating the higher OLR operation can be beneficial in terms of energy balance. Martin et al., (2011) evaluated energy balance for eight different submerged type AnMBR systems. For their review paper, the energy production was increased from 0.62 to 34.8 kwh m^{-3} as the wastewater strength increased from 0.24 to 10 kg COD L^{-1}. The energy demand of the AnMBRs was ranged from 0.03 to 5.7 kwh m^{-3} (Martin et al., 2011). In the previous studies, biogas sparging was mainly used as fouling control strategy. Periodical backwashing and chemical cleaning (5% NaOCl solution) were also applied for fouling control (Martin et al., 2011). It could be suggested that the net energy production of the ARMBR system was comparable with the previous studies which used other fouling control methods.

In the ARMBR system, the rotary disk alleviates membrane fouling by promoting the collision between membrane surface and sponge media. This fouling control strategy may be useful when biogas sparging is unfavorable. In particular, high energy demand can be required to biogas sparging for managing high-strength organic wastewaters such as food waste, molasses, slaughterhouse, leachates, and liquid dairy manure because they have high suspended solid concentrations or viscosity. This study verified the feasibility of the ARMBR system for managing the high-strength FRW (30,000 mg COD L^{-1}). Therefore, the ARMBR system can be a promising option to manage high-strength organic wastewaters including FRW. Future works, such as membrane fouling characteristics, additional membrane cleaning method, and process optimization, should be performed for the practical application of ARMBR system.

4. Conclusions

The ARMBRs successfully treated FRW at high OLR condition (6.0 kg COD m^{-3} d^{-1}) with averaged COD removal of 95.6 ± 5.8% and methane production of 259.3 ± 118.6 L CH$_4$·kg^{-1}COD$_{removed}$), which suggests that our ARMBR system can be a promising option to treat high-strength FRW. *Methanosaeta* spp. was determined as the most dominant methanogen at steady-state periods under all of the OLR conditions (1.5–6.0 kg COD m^{-3} d^{-1}). *Bacteroidetes*-like bacteria and *Ca.* Cloacamonas spp. became predominant in the ARMBRs. They acted as key fermentative or syntrophic bacteria to facilitate methane production.

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Appendix A. Supplementary data

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