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Changes of Bacterial Communities in an Anaerobic Digestion and a Bio-Electrochemical Anaerobic Digestion Reactors According to Organic Load

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Abstract: Bacterial communities change in bulk solution of anaerobic digestion (AD) and bio-electrochemical anaerobic digestion reactors (BEAD) were monitored at each organic loading rate (OLR) to investigate the effect of voltage supply on bacterial species change in bulk solution. Chemical oxygen demand (COD) degradation and methane production from AD and BEAD reactors were also analyzed by gradually increasing food waste OLR. The BEAD reactor maintained stable COD removal and methane production at 6.0 kg/m^3·d. The maximum OLR of AD reactor for optimal operation was 4.0 kg/m^3·d. pH and alkalinity decline and volatile fatty acid (VFA) accumulation, which are the problem in high load anaerobic digestion of readily decomposable food wastes, were again the major factors destroying the optimal operation condition of the AD reactor at 6.0 kg/m^3·d. Contrarily, the electrochemically activated dense communities of exoelectrogenic bacteria and VFA-oxidizing bacteria prevented VFAs from accumulating inside the BEAD reactor. This maintained stable pH and alkalinity conditions, ultimately contributing to stable methane production.

Keywords: bio-electrochemical anaerobic digestion (BEAD); bacterial communities; bulk solution; organic loading rate; food waste

1. Introduction

As the demands for bioenergy to replace fossil fuel continues to increase, anaerobic digestion (AD) investigation becomes more important in order to produce biogas (methane) out of organic wastes [1–3]. General AD reaction comprises hydrolysis, acid production, and methane production and consists of relationship among various microorganism metabolisms. The balance of reaction rates among each stage of AD is the key determining the stable operation of AD [4]. High-concentrated organic wastes such as food wastes frequently cause overload in the reactor because they consist of easily decomposable carbohydrates featuring acidity (pH level under 5.0) [5]. For this reason, when food wastes are processed at a higher OLR (organic loading rate), an imbalance among hydrolysis, acidogenesis, acetogenesis, and methanogenesis may occur in the AD reactor. In turn, this causes frequent accumulation of volatile fatty acids (VFAs), and pH and alkalinity decrease, which largely affects the AD performance [6,7].

Bio-electrochemical anaerobic digestion (BEAD) could solve these problems of AD by supplying voltage to electrodes installed inside the AD reactor because supplied voltage could accelerate electron transfer between organic oxidation and methane reduction. Recently, interest in this new technology is increasing, and a wide range of research is currently in process [8–10]. One noticeable feature of BEAD is its bio-electrochemical reaction which solves the conventional problems in AD at high OLR such as the accumulation of VFAs and, pH and alkalinity decline, and low microorganism activities. These studies also report that it contributes to fast stabilization of methanogenesis [11–13]. Feng et al. [14]

reported that a voltage supply improves the activity of electro-active microorganisms (EAM) in bulk solution in the BEAD reactor. Park et al. [15] explained that BEAD induces species change of microorganisms in bulk solution and its bio-electrochemical reactions decompose accumulated VFAs fast. Thus, it ultimately stabilizes each step of AD and increases the rate of methane production. Feng et al. [16] further reported that the community structure of electroactive bacteria in bulk sludge became richer than the bio-film of the up-flow bio-electrochemical anaerobic reactor; this improves the electron delivery efficiency of bulk solution, consequently increasing the organic waste removal efficiency and methane production. In other words, the voltage supply induces species change and activity increase of microorganisms in the BEAD reactor. This enables specific electrochemically active EAMs to decompose VFAs fast and increases the rate of methane production [3,13]. An interesting point is that voltage supply largely contributes to species change in BEAD bulk solution together with microorganisms on the electrode surface which was once widely investigated as a central research subject. EAMs activated in bulk solution could reduce the electrode surface area in the BEAD technology and may provide greater efficiency and economy for practical application. In sum, existing studies imply that voltage supply can induce species change in microorganisms in BEAD bulk solution; this eliminates problems such as VFA accumulation and pH decrease for treating high load food wastes, thus allowing stable methane production. H^+ and e^- released from bio-anode or bulk solution are rapidly reduced on bio-cathode or bulk solution by indirect and direct methane production. Its reductive reaction could prevent pH decrease by bio-electrochemical mechanisms, but rapid VFAs removal by bacterial microorganism is not clearly investigated to enhance methane production in bio-electrochemical anaerobic digestion with high organic loading rate (OLR). Most of BEAD researches have also emphasized biofilm microorganisms attached on the electrodes or methanogens in bulk solution. Therefore, bacterial communities change in a bulk solution according to OLR should be studied to provide more information of BEAD mechanism and to identify effects of voltage on bacterial communities in a bulk solution.

This study analyzed bacterial communities change in bulk solution of AD and BEAD reactors by gradually increasing the OLR of food wastes to investigate the effect of voltage supply on bacterial species change in bulk solution. Operation performances of AD and BEAD reactors were also analyzed to compare with results of bacterial community change.

2. Materials and Methods

2.1. Set-Up and Operation Conditions

2.1.1. Reactors and Electrodes Set-Up

Acryl cylinder reactors with 25 L of total volume (diameter 280 mm, and height 410 mm) and 20 L of working volume were manufactured to compare the efficiency of AD and BEAD. An agitator operated with 100 rpm was installed in the reactors for maintaining homogeneous condition. A valve for biogas release was installed at the upper part of the reactors, and the biogas produced from reactor was captured with a 50 L gas bag (Tedlar gas bag). Valves for sludge injection and release were installed at the upper and lower part of the reactors respectively. The reactors were operated in the sequencing batch reactor (SBR) mode. A total 6 sets of electrodes were installed in the BEAD reactor for electrochemical reaction. Anode and cathode in each electrode set (W150 × H300 mm, area: 0.045 m^2) are spaced with a minimum gap (1 mm) to ensure fast substance delivery between electrodes. A piece of nonwoven fabric was placed to form a sandwich in order to prevent short circuit caused by the contact between anode and cathode. The electrodes were primarily made of graphite carbon, and the surface of anode was coated with Ni, and the surface of cathode was coated with Ni, Cu, and Fe in order to increase the activity of electrochemical reactions such as electrical conductivity and current density. 0.3 V was supplied to the electrodes using a DC power supply. Detailed reactors configurations are described in Figure 1.

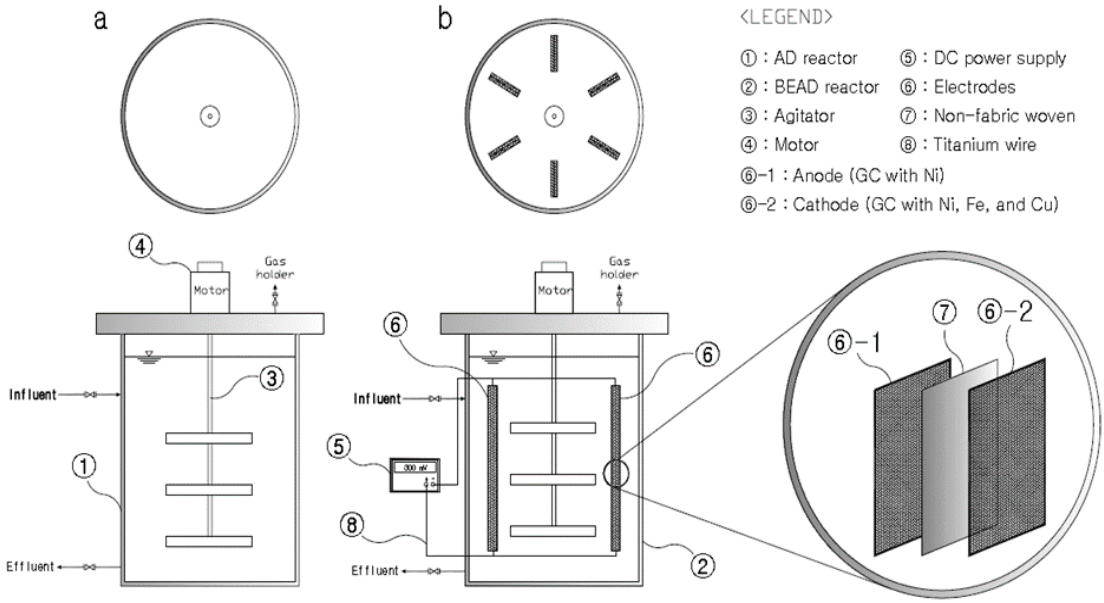

Figure 1. Detailed configuration of anaerobic digestion (AD) (**a**) and bio-electrochemical anaerobic digestion (BEAD) (**b**) reactors.

2.1.2. Operational Conditions

The OLR was gradually increased in the operation of the two reactors from 2 (S1, 0–364 d), to 4 (S2, 365–598 d), and 6 (S3, 599–657 d) kg-chemical oxygen demand (COD)/m^3·day, as shown in Table 1. They were operated in a temperature maintain chamber with temperature fixed at 35–37 °C. Inoculum sludge was sampled from the full scaled mesophilic anaerobic digestion reactor in the food waste to energy facility based in C city and its pH, total solid (TS), and volatile solid (VS) were 7.41%, 1.8%, and 1.4%, respectively. Food wastes arriving at the same facility were used for substrate injection. The pH, total COD (TCOD), soluble COD (SCOD), TS, and VS of food waste used as substrate were 3.8 ± 0.7, 114.2 ± 11.8 g/L, 82.4 ± 7.1 g/L, 10.3 ± 1.1%, and 8.7 ± 0.8%, respectively. Substrate was injected and released once a day in sequencing batch reactor (SBR) in terms of OLR.

Table 1. The operational parameters of AD and BEAD reactors. OLR: organic loading rate; S: stage; HRT: hydraulic retention time; GC: graphite carbon; Ni: nickel; Fe: iron; Cu: copper; SBR: sequencing batch reactor.

Parameters		S1	S2	S3
OLR (kg-COD/m^3·d)		2	4	6
HRT (d)			20	
Temperature (°C)			35–37	
Operation time (d)		1–364	365–598	599–657
Inoculated sludge		Anaerobic sludge from food waste to energy facility		
Voltage (V)			0.3	
Electrodes materials	Anode		GC coated with Ni	
	Cathode		GC coated with Ni, Fe, and Cu	
Operation type			SBR	

2.2. Bacterial Communities Analysis

2.2.1. Sampling of Sludge and Extraction of DNA

Sludge for analyzing bacterial community was sampled from bulk solution of AD and BEAD reactors at end of each stage of S1, S2, and S3, respectively. DNA extraction was conducted

using the FastDNA SPIN kit for Soil (MP Biomedical, LLC, Santa Ana, CA, USA) according to the manufacturer's instructions.

2.2.2. Polymer Chain Reaction (PCR) Amplification and Illumina Sequencing

PCR amplification and Illumina sequencing were conducted at Chunlab, Inc. (Seoul, Korea) which is a domestic institution specialized in microbial analysis. Detailed analysis methodology was same with previous study of Park et al. [17].

2.2.3. Method of MiSeq Pipeline

Clustering of sequence data and alpha diversity analysis were also conducted at Chunlab, Inc. (Seoul, Korea) based on microbial community analyze methods of previous study [17].

2.3. Measurements

pH was analyzed by pH auto meter (Orion 420A$^+$, Thermo Sientific, Waltham, MA, USA) and COD was analyzed by methods of closed reflux colorimetric chrome after solid-liquid separation using a centrifugal machine (Hanil, MF-80, Gimpo, South Korea). Dissolved water quality items were filtered using 1.2 μm GF/C (Whatman, GF/CTM, USA) for analysis. Biogas captured using the gas bag (Tedlar Bag, 100 L, South Korea) was quantitatively and qualitatively analyzed using gas chromatography (GC) (GOW-MAC, series 580, Bethlehem, PA, USA) featuring a thermal conductivity detector (TCD). Voltage was supplied into BEAD reactor by using DC power supply (TOYOTECH, DP30-05TP, Fermont, CA, USA). Volatile fatty acids (VFAs) was measured using liquid chromatography (LC) (Younglin, SDV50A, South Korea) with an absorbance detector (UV725S). Other items were analyzed using standard methods (APHA, 1915).

3. Results and Discussion

3.1. pH and Alkalinity

pH and alkalinity changes during the total operation period (S1–S3) are shown in Figure 2. pH and alkalinity declined at the S1 start-up stage in the AD reactor. Na_2CO_3 0.1M solution was injected to maintain alkalinity to restore the optimal operation condition [13]. pH and alkalinity stabilized about 100 days later and reached the steady state, but these two parameters fell to 6.0 and 4.2 g/L as $CaCO_3$, respectively, at OLR 6.0 kg/m^3·day at S3. The additional alkalinity supply could not increase pH and alkalinity at S3. Conversely, in the BEAD reactor, the pH and alkalinity decrease was not observed at the start-up stage. Initial pH 7.70 did not decrease but continuously increased to 8.31 during each period S1–S3; and alkalinity was maintained at a start-up stage around 7585 mg/L as $CaCO_3$ and it continuously increased up to 13,939 mg/L as $CaCO_3$ at S3 stage. This indicates that the BEAD reactor can quickly stabilize pH and alkalinity during the initial operation and maintain the pH stably at a high OLR [10]. Alkalinity continued to increase as the OLR did, because the alkalinity might be produced followed by methane production [17]. The maximum OLR of food wastes in the AD reactor was 4.0 kg/m^3·day. pH and alkalinity declined again at the OLR 6.0 kg/m^3·day, and the continuous alkalinity addition could not improve pH and alkalinity at all. The anaerobic digestion of high load food wastes suffers frequent VFA accumulation and low pH. In this condition, it is not easy to ensure operational stability, and it is hard to expect stable treatment efficiency [18]. In particular, the degree of imbalance among the hydrolysis, acidogenesis, and methanogenesis stages is considerably great in the single-stage AD reactor. It was thus not suitable for high load treatment of food wastes which are high biodegradable [5]. However, the BEAD reactor maintains stable pH and alkalinity even at a higher OLR of 6.0 kg/m^3·day. This suggests that BEAD is capable of treatment of high load food wastes [15,19]. Simply put, voltage supplied to the BEAD reactor cleared the imbalance among hydrolysis, acidogenesis, and methanogenesis of food wastes, and maintained the pH stable at higher loading operation.

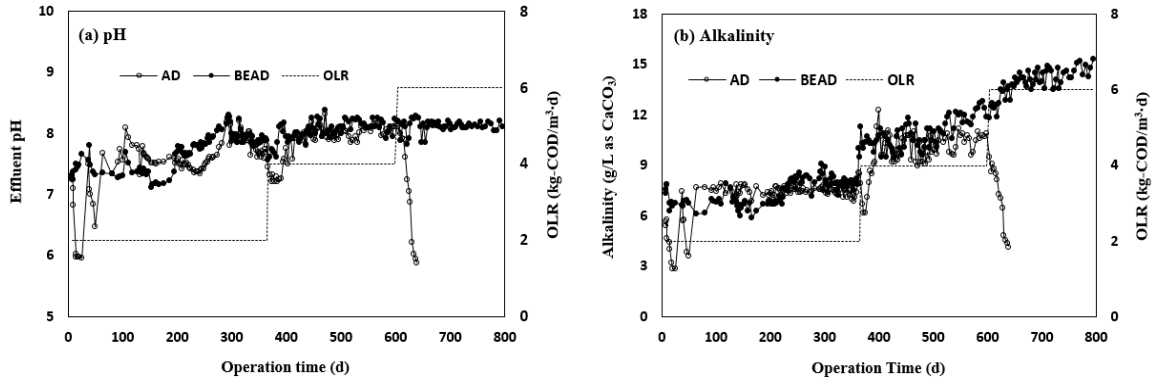

Figure 2. pH and alkalinity changes in AD and BEAD reactors at each OLRs stage.

3.2. COD Removal, Methane Production, and VFAs Concentration

COD removal efficiency during the entire operation period is shown in Table 2. The COD removal efficiency at S1–S3 in the AD reactor was 46.0 ± 17.3%, 65.6 ± 4.3%, and 48.2 ± 19.2%, respectively. In the AD reactor, pH and alkalinity declined at the initial start-up stage. In this period, COD removal efficiency decreased to 9.8%, and it continuously increased after about 100 days by addition of alkalinity. It finally stabilized to reach the COD removal efficiency similar to the BEAD reactor after 270 days [13]. The COD removal efficiency in the AD reactor rapidly dropped at S3, and the additional alkalinity supply could not in significantly recover the performance of COD removal efficiency. The BEAD reactor showed stable COD removal efficiency from the initial operation (S1) more than 60%, and it maintained during the entire operation stage. The COD removal efficiencies at each S1–S3 were 60.8 ± 7.2%, 70.6 ± 2.9%, and 73.5 ± 3.0%, respectively.

At the start-up of S1 stage, the AD reactor could not convert organic matters to methane due to the low pH and alkalinity. Methane was produced approximately after 100 days and it gradually increased (Table 2) to reach the steady state after 270 days, showing the similar methane production to BEAD reactor. Although there was a gap between the methane production in the AD and BEAD reactors at the initial S2 stage, the methane production in AD converged to the BEAD reactor after 25 days operation at S2. At S3 stage, however, methane production rapidly decreased. This suggests that 4.0 kg/m^3·day is the maximum OLR of food wastes in the AD reactor. It also demonstrated that the temporary alkalinity supply did not significantly influence on methane production recovery [13]. On the other hand, stable methane production occurred in the BEAD reactor from the initial operation at S2, and it proportionately increased as the OLR increased. Although the methane production was slightly low at each initial operation stage, it recovered fast to reach the steady state. The methane production in the BEAD reactor at each S1–S3 was 15.7 ± 4.6, 35.0 ± 3.9, and 52.6 ± 6.3 L/d. The BEAD reactor demonstrated a stable COD removal and methane production at OLRs up to 6.0 kg/m^3·day. This is because the voltage supply might improve the organic removal and methane production efficiencies through bio-electrochemical reactions. Additionally, the results suggest that voltage supply contributes to stable methane production at high organic load operation with food wastes [12,20,21].

The VFA concentration provides good information to assess the rates of hydrolysis and acidogenesis in the AD and BEAD reactors. The accumulation of VFAs is an important indicator to evaluate the reactor efficiency as it is an inhibitor of microorganic metabolism [22,23]. The concentration of volatile fatty acids (VFAs) in the AD and BEAD reactors at each operation stage (S1–S3) is shown in Table 3. In the AD reactor, VFA concentration in the initial operation accumulated up to 7394 ± 1152 mg/L. The VFAs accumulation in the AD reactor reduced pH and alkalinity, inhibiting methane production [24]. Although VFA accumulation at S2 disappeared after 270 days operation, it recurred at S3 (maximum 7915 mg/L). This means that the accumulated organic acids were not quickly converted to methane at each initial stage and they were accumulated in the AD reactor [25]. On the contrary, VFAs were quickly decomposed through the bio-electrochemical reaction in the BEAD reactor. Therefore, the acids

did not accumulate, and the TVFAs concentration recorded 2566 ± 964, 3083 ± 234, and 3874 ± 232 mg/L, respectively at each S1–S3 stage. During the entire operation period, VFA concentration in the BEAD reactor increased as OLR did. Drastic accumulation of VFAs did not occur, which could maintain pH and alkalinity conditions for methane production. In the BEAD reactor, electrochemical reactions might accelerate the organic acid decomposition and methane conversion. This also maintained the balance between acid and methane production even at high organic load of food wastes, by quickly clearing VFA produced in a BEAD reactor.

Table 2. Chemical oxygen demand (COD) removal efficiency and methane production in AD and BEAD reactors at each OLRs stage.

Items	AD Reactor			BEAD Reactor		
	S1	**S2**	**S3**	**S1**	**S2**	**S3**
COD removal efficiency (%)	46 ± 17.33	69 ± 4.27	48 ± 19.18	61 ± 7.17	71 ± 2.93	73 ± 2.95
Methane production (L/day)	10 ± 6.39	31 ± 5.90	23 ± 19.12	16 ± 4.59	35 ± 3.87	53 ± 6.32

Table 3. VFAs (acetic acid, propionic acid, and butyric acid) concentrations in AD and BEAD reactors at each OLRs stage. AD: anaerobic digestion; BEAD: bio-electrochemical anaerobic digestion; VFAs: volatile fatty acids.

Items	AD Reactor			BEAD Reactor		
	S1	**S2**	**S3**	**S1**	**S2**	**S3**
VFAs (g/L as $CaCO_3$)	3.24 ± 1.71	3.44 ± 0.46	5.85 ± 1.96	2.57 ± 0.96	3.08 ± 0.23	3.87 ± 0.23
Acetic acid (g/L as $CaCO_3$)	0.66 ± 0.20	0.84 ± 0.19	0.69 ± 0.14	0.59 ± 0.13	0.74 ± 0.18	1.04 ± 0.12
Propionic acid (g/L as $CaCO_3$)	0.99 ± 0.50	1.17 ± 0.18	1.92 ± 0.50	0.84 ± 0.36	1.08 ± 0.17	1.36 ± 0.15
Butyric acid (g/L as $CaCO_3$)	1.58 ± 1.07	1.43 ± 0.29	3.24 ± 0.86	1.14 ± 0.52	1.26 ± 0.17	1.48 ± 0.19

3.3. Bacterial Community Analysis

3.3.1. Bacterial Communities at S1 Stage

A preliminary study confirmed that classes *Clostridia* and *Bacteroidia* were dominant bacterial communities in both AD and BEAD at S1. Dominance of *Clostridia* and *Bacteroidia* in the AD reactor was 49.3% and 38.2%, respectively, and 56.6% and 28.0%, respectively in the BEAD reactor [3]. In particular, *EU358683_s* sp. accounted for 11.0% of species in the AD reactor. These species belong to *Bacteroidia* which produces acetate, lactate, formate, and propionate by fermenting carbohydrate, protein, and peptone [26,27]. In BEAD, *HM107102_s sp.* were the dominant species accounting for 7.4%. It belongs to *Bacteroidia* of the family *Porphyromonadaceae* together with *EU358683_s* sp. The *Porphyromonadaceae* family produces acetate, and propionate from diverse amino acids. Depending on the species, some of them produce succinate during the sugar fermentation process [28].

3.3.2. Bacterial Communities at S2 Stage

The analysis results of bacterial communities in AD and BEAD reactor bulk solution at S2 are shown in Figure 3. *Defluviitoga tunisiensis* sp. was the most dominant in the AD reactor recording 45.3%. *FN436026_s* sp. and *HQ183800_s* sp. were 10.5% and 5.6%, respectively. *Defluviitoga tunisiensis* sp. is known to produce acetate or H_2 and CO_2 through the fermentation of carbohydrates in mesophilic and thermophilic AD [29,30]. *FN436026_s* sp. belongs to the family *Porphyromonadaceae*, and is a kind of bacterium that grows through sugar hydrolysis and acid production in mesophilic AD reactor. *HQ183800_s* sp. belongs to the genus *Syntrophaceticus*, and oxidizes acetate in mesophilic AD reactor. This acetate-oxidizing bacterial species produces H_2 and CO_2, and grows in symbiosis with H_2-utilizing methanogens [31–33]). These bacterial communities decomposed the abundance of carbohydrates and sugar in food wastes into H_2 and CO_2 in AD reactor during S2 (4.0 kg/m^3·day). This prevented from the accumulation of VFAs in the reactor, contributing to maintain pH and alkalinity stable at S2.

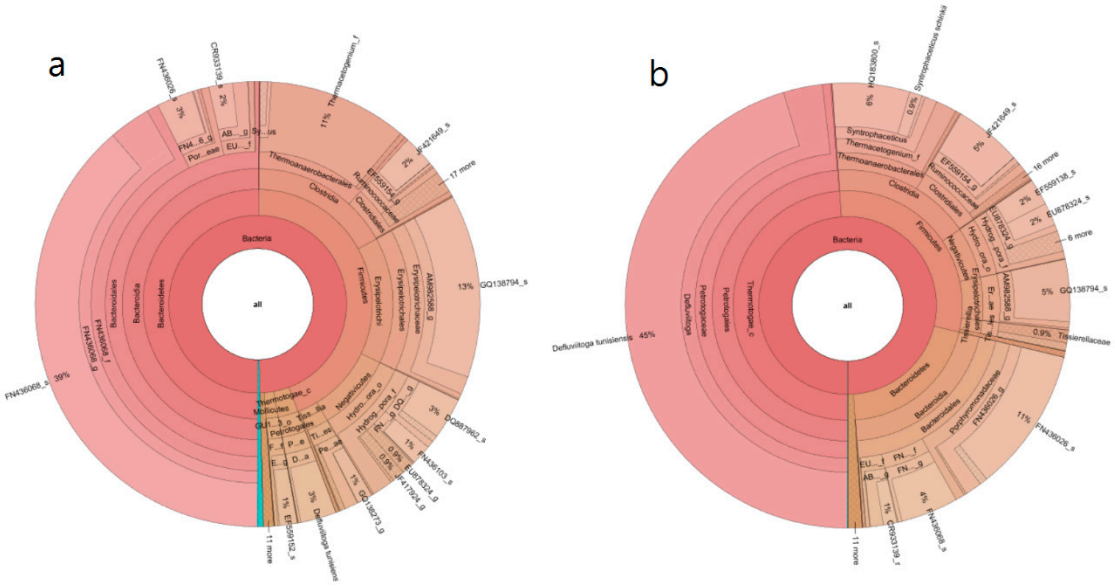

Figure 3. Results of bacterial communities analysis in AD (**a**) and BEAD (**b**) reactors at the end of S2 (4.0 kg/m^3·d).

In the BEAD reactor, the dominant species was *FN436068_s* sp. of 38.7%, followed by *GQ138794_s* sp., and *Thermacetogenium_f_uc_s* sp. of 13.3% and 10.1% respectively. *FN436068_s* sp. is a bacterium of the class *Bacteroidia*, and is known to be an exoelectrogenic species dominated in anode and bulk solution in various BEAD studies. This species oxidizes various VFAs into acetate, H_2 and CO_2 [34,35]. *GQ138794_s* sp. of the family *Erysipelotrichaceae* is known to oxidize glucose and other diverse carbohydrates into small molecular organic acid or H_2 [36]. *Thermacetogenium_f_uc_s* sp. of the class *Clostridia* is one of the representative exoelectrogenic bacteria together with Bacteroidia, and is capable of oxidizing diverse organic matters and VFAs [37].

In sum, the ratio of bacteria growing with carbohydrates and sugar decomposition was high in the AD reactor. These bacterial communities contributed stable operation of AD reactor and also prevented from VFAs accumulation and pH decrease at S2 stage. In BEAD reactor, exoelectrogenic bacteria as well as the bacterial communities growing with carbohydrate decomposition appeared as a main species. At the S2 stage, different bacterial species were contributed to the stable operation in AD and BEAD reactors. Especially, the voltage supply in the BEAD reactor could improve the community of exoelectrogenic bacteria which could accelerate decomposition rate of organic and VFAs. These results show that the voltage leads to changes of bacterial communities and increases electrochemically activated bacterial communities in the bulk solution of BEAD reactor. Interestingly, despite the different bacterial communities in bulk solution of AD and BEAD reactors, both of two reactors were successfully operated at S2 stage. VFAs-oxidizing bacteria dominated only in BEAD reactor may contribute to stable operation compared to AD at a higher OLR.

3.3.3. Bacterial Communities at S3 Stage

The analysis results of microorganism communities at S3 in AD and BEAD reactor bulk solution are shown in Figure 4. Approximately, 2000 of various bacterial communities were observed in the AD reactor bulk at S3 stage (OLR: 6 kg/m^3·day) after the methane production and the COD removal were stopped (657 days after operation). *Nitrospira defluvii* sp. (2.9%) and *Ttrichococcus flocculiformis* sp. (2.5%) were dominant. De Vrieze et al. [38] reported that communities of *Nitrospira* and *Trichococcus* increased in the AD reactor with high VFA and ammonium nitrogen concentrations. This is likely because the growth environment for bacteria was destroyed by the VFA accumulation and pH reduction at a high OLR (S3, 6.0 kg/m^3·day), triggering the reproduction of various bacteria present in the substrate. In other words, optimum conditions for stable operation and for methane production were destroyed

at a high OLR (S3) because most of bacterial communities related with organic degradation were washed out from AD reactor and various spoilage bacterial communities in substrate were maintained in AD reactor

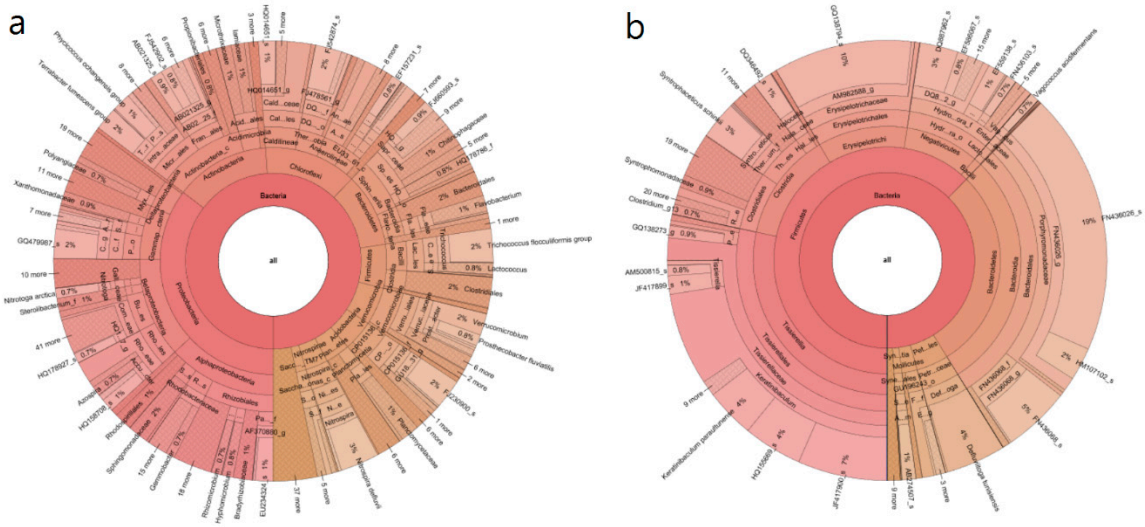

Figure 4. Results of bacterial communities analysis in AD (**a**) and BEAD (**b**) reactors at the end of S3 (6.0 kg/m^3·d).

On the other hand, *FN436026_s* sp. was dominant 19.3% in the BEAD reactor, followed by 10.5%, 6.8%, and 6.6% of *GQ138794_s* sp., *Keratinibaculum_uc* sp., and *JF417900_s* sp. *FN436026_s* sp. is known as an exoelectrogenic bacterium of the class *Bacteroidia* in various existing BEAD studies [30,39]. Class *Bacteroidia* is well-known as fermentative and exoelectrogenic bacteria and is possible to survive in extreme pH conditions [34,40]. Kong et al. [34] and Zhao et al. [40] reported that class *Bacteroidia* played a critical role in hydrolyzing and fermenting organic matters. The dominant ratio of *GQ138794_s* sp. decreased compared to S2 (13.3%), but it was the second most dominant species equally as in S2. The role of *GQ138794_s* sp. of the family *Erysipelotrichaceae* is the same as previously described. *Keratinibaculum_uc* sp. and *JF417900_s* sp. both belong to the genus *Keratinibaculum*. These two VFAs-oxidizing bacterial species grow using various VFAs including acetic acid, propionic acid, butyric acid, isobutyric acid, and isovaleric acid as energy sources in an anaerobic condition of 30–65 °C and pH 6.0–10.5 [17]. One interesting point is that the bacterial community structure of species of the genus *Keratinibaculum* largely increased to 21.6% compared to S2 (<1.0%). This means that the increase of these bacteria species may decompose organic acids that could be frequently accumulated inside the reactor as the OLR rises; and this, in turn, prevented from pH and alkalinity decrease, contributing to stable methane production. Among the bacteria species analyzed, the classes *Bacteroidia* and *Clostridia* recorded 27% and 6.8%, respectively, were slightly lower than at S2 (approximately 46% and 13.5%) but exoelectrogenic bacteria were equally dominant with voltage supply. Rapid hydrolysis and acidogenesis were accelerated by exo-electrogenic fermentative bacteria, then produced VFAs was rapidly decomposed by VFAs-oxidizing bacteria. These dominant microbial communities in BEAD bulk solution may contribute rapid hydrolysis and VFAs oxidation to maintain optimum condition for stable operation and methane production.

In brief, external voltage supply improved the community of exoelectrogenic and VFAs-oxidizing bacteria in the BEAD reactor bulk solution, allowing fast decomposition of VFAs which might be frequently accumulated inside the BEAD reactor at high OLRs.

4. Conclusions

Whereas the BEAD reactor maintained stable COD removal and methane production at 6.0 kg/m^3·day, the AD reactor's maximum OLR for optimal operation was only up to 4.0 kg/m^3·day. pH

and alkalinity decline and VFA accumulation are the major problems in high load anaerobic digestion of readily decomposable food wastes. These two problems are also major factors destroying the optimal condition for AD reactor at 6.0 kg/m^3·day. In the BEAD reactor, the bio-electrochemically activated dense community of exoelectrogenic bacteria and VFAs-oxidizing bacteria prevented VFAs accumulation with voltage supply. Rapid hydrolysis and acidogenesis were accelerated by exo-electrogenic fermentative bacteria, then produced VFAs was rapidly decomposed by VFAs-oxidizing bacteria. In conclusion, bio-electrochemical reactions might maintain pH and alkalinity conditions, which ultimately contributing to stable methane production.

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