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Enhancement of Biogas Production in Anaerobic Digestion Using Microbial Electrolysis Cell Seed Sludge

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Abstract: Anaerobic digestion (AD) can produce renewable energy and reduce carbon emissions, but the energy conversion efficiency is still limited in some waste streams. This study tested the effect of applied voltage removal for microbial electrolysis cells (MECs) treating primary sewage sludge. Two MECs were operated in parallel: a MEC-0.3 V with an applied voltage of 0.3 V and a MEC-OCV with open circuit voltage. Both reactors were inoculated with seed sludge originating from a MEC at 0.3 V applied voltage, and three batch cycles were operated for 36 d. The methane production of the MEC-OCV was 3759 mL/L in the first cycle and 2759 mL/L in the second cycle, which was similar (105% and 103%, respectively) to that of the MEC-0.3 V. However, in the third cycle, the methane production of the MEC-OCV (1762 mL/L) was 38.8% lower than that of the MEC-0.3 V (4545 mL/L). The methane contents in the biogas were 68.6–74.2% from the MEC-OCV, comparable to those from the MEC-0.3 V (66.6–71.1%). These results indicate that not only the MEC-0.3V but also the MEC-OCV outperformed AD in terms of methane yield and productivity, and the promotion using MEC-derived inoculum persisted equally with the MEC-OCV for two batch cycles after removing the applied voltage. Therefore, a MEC operation with cycled power supply may be beneficial in reducing the electric energy usage and improving the biogas production performance, compared to conventional AD.

Keywords: bioelectrochemical technology; anaerobic digestion; methane production; primary sewage sludge; open circuit voltage

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1. Introduction

Anaerobic digestion (AD) is a biological process that can reduce the volumetric and stabilization of organic matter in sewage sludge and is the most widely employed method [1–3]. Biogas derived from organic wastes in AD is primarily a mixture of methane (CH_4, 50–75%) and carbon dioxide (CO_2, 25–50%) [4]. Among different waste-to-energy technologies, AD has been highlighted to have lower global warming potential and acidification potential [5]. In addition, AD is regarded as the most sustainable and economical technology compared to technologies such as landfilling (greenhouse gas emissions and odor production), incineration (particulates, carcinogens, dioxin production, etc.), composting (risk for disease dispersal), and dark fermentation (low hydrogen conversion, etc.) for waste treatment and energy recovery [6–9]. Moreover, AD has lower sludge production and operational costs than aerobic wastewater treatment technologies [10]. However, it has several drawbacks, including long hydraulic retention time (~20 days) and low removal efficiencies for chemical oxygen demand (COD) and volatile solids (VS) [11–14]. The slow methanogenesis rate and the imbalance of anaerobic reactions are mainly attributed to the slow growth rate of methanogens, their sensitivity to environmental changes (such as abrupt pH changes, temperature, organic overloading, and high salt concentrations), or the accumulation of volatile fatty acids (VFA) [15–17].

A microbial electrolysis cell (MEC), one of the bioelectrochemical technologies, can overcome several disadvantages of the AD process and produce energy simultaneously [18,19]. Several studies reported that a MEC is considered one of the most promising technologies for bioenergy recovery, as it enables rapid methane production through accelerated hydrolysis compared to conventional anaerobic digestion [19–22]. The methane yield of anaerobic digestion coupled with a microbial electrolysis cell (MEC-AD), with an applied voltage of 0.5–0.8 V for the COD removal rate, caused the carbon recovery rate to increase by 30–230% [23,24]. Zhao et al. reported that adding carbon felt could enhance anaerobic methanogenesis and imposing a small voltage (0.6 V) on the carbon felt further improved methane production. It can be confirmed that more *Geobacter* was enriched in carbon-felt reactors, especially those with a voltage supply [21]. Several studies have shown that there was abundant enrichment in the anode biofilm of the MEC-AD, increasing the methane productivity of waste activated sludge (WAS) by adjusting the microbial community structure [2,20,21,23,24]. Wang et al. showed that the methane yield and methane productivity of raw WAS increased more than that of AD, when the MEC-AD operated in an open-circuit condition, after being domesticated successfully with an applied voltage of 0.8–0.6 V [23].

Nevertheless, only a few studies have investigated a MEC operating in an open-circuit condition. Therefore, it is necessary to investigate how the open-circuit condition in a MEC affects the biogas production of anaerobic digestion. In this study, the impact of utilizing MEC seed sludge (with electrodes) was investigated under power-supplied (MEC-0.3 V) or -unsupplied (MEC-OCV) conditions, by observing the changes in batch cycle time and methane production rate. Three systems, AD, MEC-OCV using pre-adapted sludge from a MEC, and MEC-0.3 V, were compared regarding methane production, stability (pH and alkalinity), and energy efficiency.

2. Materials and Methods

2.1. Sludge Samples

The present study obtained primary sewage sludge and AD seed sludge from a wastewater treatment plant in J city, South Korea. The primary sewage sludge and AD seed sludge were sieved using 800 µm sieve and stored in the refrigerator at 4 °C before use. Before this study, a MEC (0.3 V applied voltage) was operated for 5 months using the primary sewage sludge and the AD seed sludge as the substrate and the inoculum, respectively. Before each batch cycle, all reactors were provided by a seed-to-feed volume ratio of 1:1 (i.e., 50% replacement by fresh substrate) [25]. The characteristics of the primary sewage sludge, the AD seed sludge, and the MEC seed sludge are shown in Table 1.

Table 1. Characteristics of the primary sewage sludge, AD and MEC seed sludges.

Parameter	Primary Sewage Sludge	Seed Sludge	
		AD	MEC
Total solid (TS, g/L)	52.4 ± 14.9	25.7	25.8 ± 1.4
Volatile solid (VS, g/L)	38.9 ± 12.2	17.2	16.6 ± 0.7
Total chemical oxygen demand (TCOD, g/L)	56.1 ± 18.7	20.7	25.8 ± 1.8
Soluble chemical oxygen demand (SCOD, g/L)	1.3 ± 0.6	1.3	1.2 ± 0.4
pH	5.7 ± 0.5	8.3	7.8
Alkalinity (mg/L as $CaCO_3$)	1226.3 ± 605	5945	5124.2 ± 364.7
Total volatile fatty acid (TVFAs, mg/L as HAc)	1307.4 ± 1244	1030	1150.6 ± 453.8

2.2. Reactor Configuration and Operation

The MEC was inoculated with effluent from another MEC and operated at an applied voltage of 0.3 V (named MEC-0.3 V hereafter); the applied voltage of 0.3 V was determined following previous studies [24,26,27] and our own optimization study (unpublished). Another MEC was inoculated with effluent from the MEC and used with no applied voltage of open-circuit potential (named MEC-OCV hereafter). The AD was inoculated

with anaerobic digestion sludge from a wastewater treatment plant in J city. To compare the effect of applying the MEC seed sludge to MEC-OCV (open circuit potential with electrodes), three different reactors, lab-scale AD, MEC-OCV (with electrodes) using pre-adapted sludge from a MEC, and MEC-0.3 V, were operated. The AD was the same as MEC but without electrodes. The detailed configurations of reactors used in the experiment are shown in Figure 1 and Table 2.

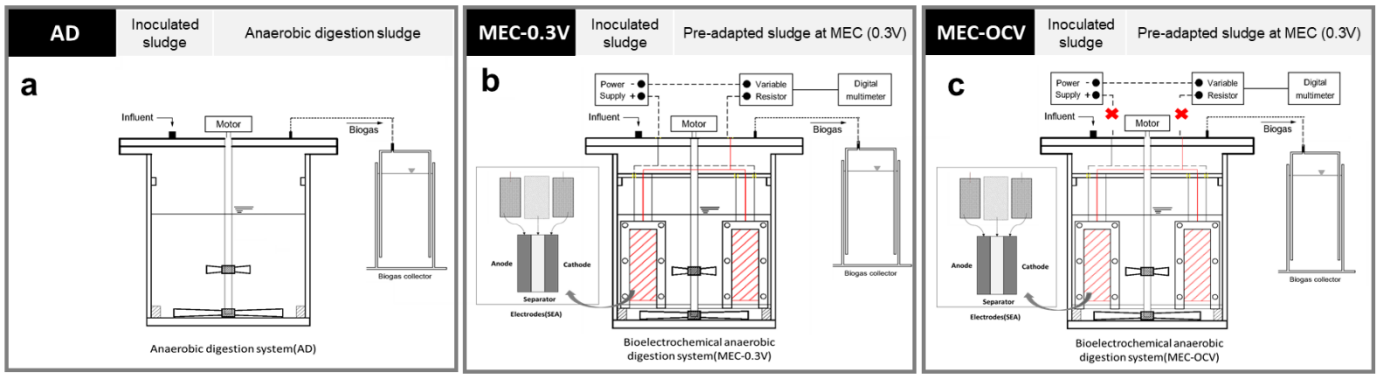


Figure 1. Schematic diagram and operating condition of (**a**) AD, (**b**) MEC-0.3 V, and (**c**) MEC-OCV.

Table 2. Operational conditions in AD, MEC-0.3, and MEC-OCV.

Parameter	AD	MEC-0.3V Cycle	MEC-OCV Cycle
Electrodes	empty	4 sets	4 sets
Voltage (v)	0	0.3	0
Inoculated sludge	Anaerobic digestion sludge	Pre-adapted sludge from an MEC (0.3 V)	Pre-adapted sludge from an MEC (0.3 V)
Substrate	Primary sewage sludge	Primary sewage sludge	Primary sewage sludge
Batch cycles	1 cycle	3 cycles	3 cycles
Operation duration (day)	36	36 (13 + 9 + 12)	36 (13 + 9 + 12)

All reactors were operated under mesophilic conditions (35 ± 3 °C) and stirred at 100 rpm. Before digestion, all reactors were sparged using ultrapure nitrogen gas to remove oxygen for 10 min.

Three batch reactors with 4.6 L of total volume (diameter 170 mm and height 205 mm) and 2.5 L of adequate volume were made of plexiglass. During the operation of batch reactors, biogas production from each reactor was collected using a floating-type gas collector to collect biogas. Anodes and cathodes were made of graphite fiber fabric (GFF) coated with nickel ion using the electrophoretic deposition (EPD) method. After treating with the EPD method, electrodes were assembled with the nonwoven sheet (separator electrode assembly (SEA)) [16]. The separator and electrode assembly (40 mm × 90 mm; area: 0.0036 m^2) was prepared by stacking the anode, a polypropylene nonwoven sheet as a separator, and the cathode. A total of 4 sets of electrodes were installed in the MEC. A titanium wire (0.3 mm) was used in the MEC to connect each electrode to a DC power supply (OPM series, ODA Technologies Co., Incheon, Korea). A voltage of 0.3 V was supplied to each electrode pair [26].

2.3. Analysis and Calculation

During the digestion, the current between the anode and the cathode of the MEC was recorded every 10 min with a multimeter data acquisition system (2700, Keithley Instruments, Inc., Cleveland, OH, USA), by measurement of the voltage across the resistor (10 Ω).

Sludge samples (25 mL) were taken three times a week. Total COD was measured in accordance with standard methods [28]. The pH of sludge samples was measured

using a pH meter (Orion star A121, Thermo, Waltham, MA, USA). The alkalinity and total volatile fatty acid (TVFAs) were determined using the titration method [29]. The sample was titrated to pH 5.1 and 3.5 using 0.1 N sulfuric acid consecutively. TVFAs were then calculated from the volume of consumed sulfuric acid and the volume of the sample taken. Total VFA was used for calculating the ratio of VFA to alkalinity.

Biogas composition was analyzed using a gas chromatograph (GowMac Series 580, Porapak Q 6 ft × 1/8 in stainless steel) equipped with a thermal conductivity detector (TCD). Nitrogen gas (30 mL/min flow rate) was used as carrier gas. The injector, detector, and column temperatures were 90 °C, 80 °C, and 50 °C, respectively [25].

The energy efficiency of the substrate of AD and MEC-OCV were calculated as (Equation (1)) [30]:

$$\eta_S\ (\%) = \frac{W_{CH_4}}{W_S} \quad (1)$$

where $W_{CH_4}\left(= \Delta G_{CH_4} \times n_{CH_4}\right)$ is the energy recovered (kJ/day) as methane (CH_4), calculated from the amount of methane production in moles (n_{CH_4}, mol/day), the Gibbs free energy change of CH_4 oxidation to water and carbon dioxide ($\Delta G_{CH_4} = -818$ kJ/mol) [16]. W_S $(= n_S \times \Delta G_S)$ is the substrate energy content (kJ/day), and n_S is the number of substrate moles removed during a batch cycle based on COD removal (g CODr), based on glucose and the Gibbs free energy change of glucose oxidation to carbon dioxide ($\Delta G_S = -2870$ kJ/mol).

The overall energy efficiency of the MEC-0.3V was calculated from the recovered energy as methane, relative to the sum of the energy contained in the removed substrate and electric energy supplied into the system (Equation (2)) [27]:

$$\eta_{E+S}\ (\%) = \frac{W_{CH_4}}{W_S + W_E} \times 100 \quad (2)$$

where W_E $(= C \times E_{APP})$ is the electrical energy input (kJ/day) to the system, C is the total coulombs calculated by integrating the current over time. E_{APP} (V) is the applied voltage calculated as the difference between the anode and cathode potentials.

The modified Gompertz model is calculated as (Equation (3)):

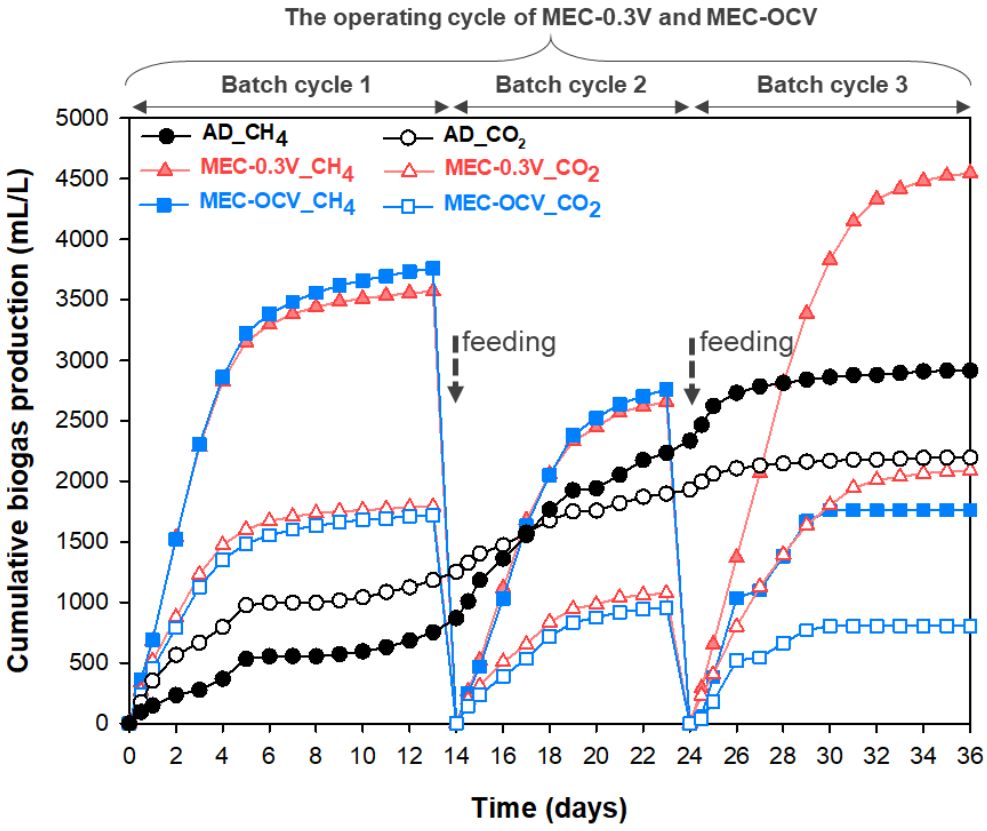
$$P(t) = P \exp\left[-\exp\left[\frac{R_m e}{P}(\lambda - t) + 1\right]\right] \quad (3)$$

where $P(t)$ (mL/g CODr) is cumulative methane production at time t (day), P (mL/g CODr) is predicted methane production potential, R_m (mL/g CODr/day) is the maximum methane production rate, λ (day) is the lag phase, and e is a coefficient of 2.718282 [31].

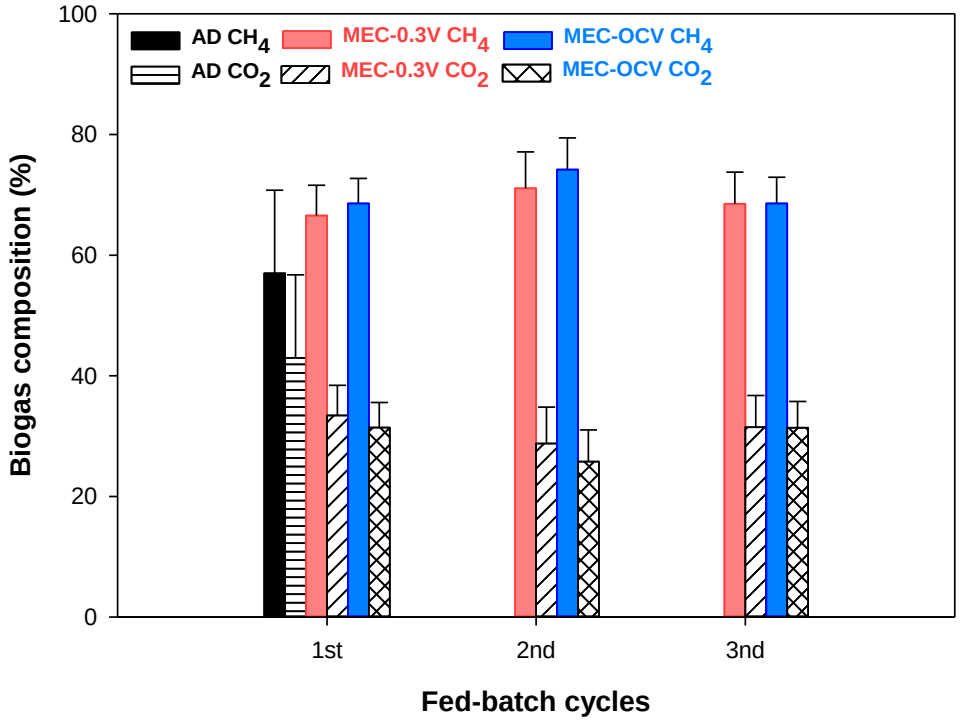
3. Results and Discussion

3.1. Biogas Production

As shown in Figure 2, the cumulative and daily biogas production are presented. The cumulative methane production provides important information for the adaptation and growth of anaerobic microorganisms [22,32]. In the cumulative methane production (volume of methane production/2.5 L of effective volume), the MEC-0.3V produced 3568, 2656, and 4545 mL/L methane, respectively, up to the third cycle (Figure 2a). The methane production of the MEC-OCV was 3759 mL/L in the first cycle and 2759 mL/L in the second cycle, which was similar (105% and 103%, respectively) to that of the MEC-0.3V. In the third cycle, the methane production of the MEC-OCV was dramatically decreased. After the 7th day, the daily methane production stopped. In the third cycle, the methane production of the MEC-OCV (1762 mL/L) was 38.8% lower than that of the MEC-0.3 V (4545 mL/L). The methane production of AD was 2914 mL/L, during just one cycle in the operating period.



(**a**)



(**b**)

Figure 2. Daily biogas production (**a**) and total biogas composition (**b**).

An applied voltage could significantly increase the methane production performance of the MEC-0.3 V. In addition, the MEC-OCV produced methane gas, a similar trend to the MEC-0.3 V in the absence of applied voltage. These results showed that the promoting effect persisted equally after removing the applied voltage. Wang et al. reported that the fermentation bacteria in the MEC-AD (using raw WAS) were effectively enriched under the power supplied and did not lose their metabolic activity or die rapidly in unsupplied conditions [23]. De Vrieze et al. also reported that the lack of difference between the voltage supply and open-circuit systems indicates that the fundamental impact is biomass retention [15]. Therefore, when converted to the open-circuit voltage after being domesticated under the applied voltage, the fermentative bacteria, acidogens, and syntrophic acetogenic bacteria were enriched, so the synergistic effect of syntrophic acetogenic bacteria and hydrogenotrophic methanogens might play a role in maintaining higher efficient methane productivity [23]. These results show that the effectively enriched fermenting bacteria's promoting effect under the voltage supply keeps for up to two cycles in the condition that the voltage is not supplied. However, in the third cycle, methane production decreased as the cumulative volume changes exceeded the working volume. In the case of batch-cycle time, the MEC-OCV and MEC-0.3V were the same at 13 days for the first cycle, 9 days for the second cycle, and 12 days for the third cycle, and AD was the longest at 35 days. The average anaerobic digestion time of 12 days was obtained from the MEC-0.3V and MEC-OCV, which was three times less than that of AD.

The methane content of AD biogas is 57.0%, which is between 55–65%, the commonly known methane composition range of conventional AD [16,33], but the MEC-OCV's is 68.6–74.2%, similar to that of the MEC-0.3V, which shows a composition of 66.6–71.1% (Figure 2b). The increased methane yield and decreased carbon dioxide content in the MEC-0.3V and MEC-OCV compared to those in AD implied that carbon dioxide might be reduced to additional methane. Previous studies have reported that electrochemically active bacteria attached to the anode of the MECs can improve the decomposition of organic matters by consuming VFAs produced during anaerobic digestion and converting e- and H^+ to H_2 through an electrochemical reaction [20,23,34]. Hydrogenotrophic methanogen produced methane, subsequently, through the reduction of CO_2 using H_2.

In the modified Gompertz model, λ, the lag phase, means the minimum time required for methane production [35]. The lag-phase time of AD was 5.68 days, the longest among AD, MEC-0.3 V, and MEC-OCV (Table 3). According to a previous study, the lag-phase time was 3.08 days (using anaerobic sludge and food waste) in the anaerobic digestion reactor, and the lag-phase time of AD was increased in this study [22]. This is considered due to the long hydrolysis time and slow methanogenesis, which are some of the disadvantages of AD [36,37]. It is thought that rapid methane production was achieved by the stable growth of microorganisms involved in hydrogenotrophic methanogenesis of MEC and direct interspecies electron transfer through the electrochemical reaction of microorganisms [23]. The lag-phase time of the MEC-OCV was 1.04 days in the first cycle, 1.15 days in the second cycle, and 1.29 days in the third cycle. This was 0.03 days shorter than that of the MEC-0.3 V in the first cycle, but the lag-phase time increased as the cycle progressed. In the first–second cycles, the lag-phase time in the MEC-0.3 V lasted approximately 1.07–1.01 days, which was very similar to that of the MEC-OCV.

Table 3. Modified Gompertz model parameters.

Parameter	AD	MEC-0.3V			MEC-OCV		
		1st	2nd	3rd	1st	2nd	3rd
λ (day)	5.68	1.07	1.00	1.06	1.04	1.15	1.29
P (mL/g CODr)	157.25	257.21	151.40	187.03	263.08	152.7	79.15
R_m (mL/g CODr/day)	5.25	58.62	31.98	29.82	56.17	31.37	21.33
R^2	0.9837	0.9986	0.9987	0.9992	0.9983	0.9985	0.9846

3.2. pH, Alkalinity, VFAs Concentration, and COD Removal

Anaerobic digestion can be evaluated from the stable variables of fermentation solution, such as pH, alkalinity, and VFA levels, the most important factors in the operation [16,22,38]. At the end of the digestion, the pH was 7.5–7.6 for the MEC-OCV and 7.5–7.8 for the MEC-0.3V. In the first–second cycles, the alkalinity of the MEC-OCV (4120–4550 mg/L) was more stable and higher than that of the MEC-0.3V (3980 mg/L). For this reason, the MEC-OCV tended to increase methane production after 5 days. Overall, all reactors' pH and alkalinity were quite stable during the operation (Table 4). The TVFAs from the MEC-OCV (761–1315 mg/L) were higher than those of the MEC-0.3V (808–917 mg/L). The total VFA from the AD reactor (1317 mg/L) was higher than that of the MEC-0.3V or MEC-OCV. The MEC-0.3V maintained a constant TVFA concentration in all cycles, but the MEC-OCV showed an overall tendency to increase. The ideal value of the total VFA/alkalinity ratio in anaerobic digestion is in the range of 0.1−0.3 [16,22]. This total VFA/alkalinity ratio is an indicator of anaerobic digester stability [39]. In this study, the total VFA/alkalinity ratio for the MEC-OCV (0.19–0.32) was slightly higher than that of the MEC-0.3 (0.09–0.23). All reactors were operated stably during the experimental period.

Table 4. Properties of AD, MEC-0.3, and MEC-OCV.

Parameter	AD	MEC-0.3 V Cycle			MEC-OCV Cycle		
		1st	2nd	3rd	1st	2nd	3rd
pH	7.57	7.49	7.61	7.75	7.57	7.63	7.61
Alkalinity (mg/L as $CaCO_3$)	5100	3980	3980	3965	4550	4120	3975
TVFAs (mg/L as HAc)	1317	917	373	808	1018	1315	761
TVFAs/alkalinity ratio	0.258	0.230	0.094	0.204	0.224	0.319	0.191
COD removal efficiency (%)	38.54	33.24	40.08	48.22	34.01	41.41	43.04

Table 4 shows the COD removal efficiency during operation. After anaerobic digestion, the COD removal efficiency was between 33.2% and 48.2% for the MEC-0.3V and between 34.0% and 43.0% for the MEC-OCV during the operation. The anaerobic digestion system with the MEC-0.3V or MEC-OCV did not influence the extent of COD removal in the first or second cycles. However, COD removal efficiency decreased from 48.2% for the MEC-0.3V to 43.0% for the MEC-OCV in the third cycle.

3.3. Methane Yield

In the first cycle, the MEC-0.3V was 259 mL CH_4/g COD_r, the MEC-OCV was 267 mL CH_4/g COD_r, and the methane yield in the second cycle was 149 mL CH_4/g COD_r in both the MEC-0.3 V and MEC-OCV (Figure 3). At the end of the first and second cycles, the methane yield obtained from the MEC-OCV was similar to that from the MEC-0.3 V. The methane yield of AD was the lowest, with a value of 125 mL CH_4/g CODr. The methane yields of the MEC-0.3 V and MEC-OCV were 1.2–2.1 times higher than that of AD. In previous research, the observed methane yield in the anaerobic biodegradability test was 123–263 CH_4/g COD_r, around 35–75% of its theoretical value (350 mL of CH_4/g CODr for glucose) [40]. The enriched functional microorganisms in the MEC seed sludge enhanced the methane yield of both the MEC-0.3 V and MEC-OCV compared to the AD. These results indicate that not only the MEC-0.3 V but also the MEC-OCV outperformed AD in terms of methane yield and productivity, and the promotion using MEC-derived inoculum persisted equally for the MEC-OCV for two batch cycles after removing the applied voltage. According to the report, the electricigens and acetoclastic methanogens in the anode biofilm and hydrogenotrophic methanogens in the cathode biofilm were enriched under the power supplied [20,23]. The enriched functional microorganism was beneficial to enhancing hydrolysis and acidification, and the promotion did not disappear after stopping the voltage supply [23].

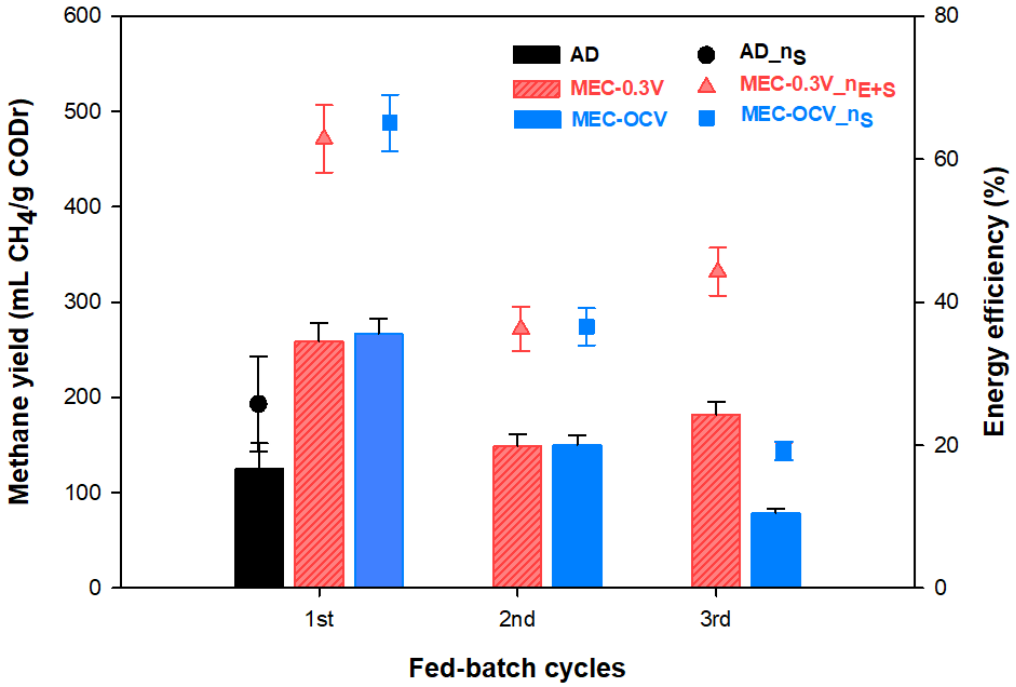


Figure 3. Methane yield and energy efficiency.

In contrast, in the third cycle, the methane yield of the MEC-OCV (79 mL CH_4/g CODr) was 41.2% lower than that of the MEC-0.3 V (182 mL CH_4/g CODr). Likely, the effects of the MEC-derived inoculum diminished with time when no voltage was applied. In this study, 24 days or two batch cycles was the apparent duration of the promotional effects of the MEC-derived inoculum; however, future research is necessary to confirm the duration of this effect under various conditions.

The energy efficiency of the first cycle was 25.8% for AD, 62.8% for the MEC-0.3V, and 65.1% for the MEC-OCV, showing the higher efficiency of MEC-OCV. The energy efficiency of the second cycle was 36.6% for the MEC-OCV and 36.3% for the MEC-0.3V, showing similar efficiencies. The third cycle energy efficiency was 19.2% for the MEC-OCV and 44.3% for the MEC-0.3V, which was 25.1% higher than that of the MEC-OCV. Until the second cycle, the methane production was kept by the fermentative bacteria, acidogens, and syntrophic acetogenic bacteria in the MEC-0.3V seed sludge, showing the same trend as the methane yield [15,23,36]. These energy efficiency values were comparable to the average energy yield of 57.2% for wastewater sludge in the literature [8]. However, the fluctuations of the energy efficiency in this study (Figure 3) should be validated further for confirmation. The economics of biogas production using MEC-OCV could also be studied in the future. Although the prior MEC operation may increase biogas production cost, the overall economic feasibility can be enhanced with elevated energy yield and/or improved sludge reduction [8]. Finally, a future study to optimize various key parameters such as the adaptation methods (applied voltage, adaptation duration, etc.), MEC configuration, reactor operation (type of substrate, feeding scheme, etc.), and the use of valorization systems (production of short- and medium-chain fatty acids rather than biogas) would give insights into the application of the MEC-OCV system [7,41].

4. Conclusions

In this study, the impact of utilizing the MEC seed sludge (with electrodes) was investigated under power-supplied (MEC-0.3V) or unsupplied (MEC-OCV) conditions, by observing the changes in batch-cycle time and methane-production rate. The enriched microorganisms in the MEC seed sludge likely enhanced the methane production of both the MEC-0.3 V and the MEC-OCV. The enhancement was comparable between the MEC-

0.3 V and MEC-OCV during the first two cycles, but the performance of the MEC-OCV decreased in the third cycle after one volume was exchanged. Future studies are necessary to confirm the magnitude and duration of this enhancement and assess the economic feasibility and key parameters of this operation method.

Author Contributions: M.E.L. designed this study, collected the data, and made a draft of the manuscript. Y.A., S.G.S. and J.W.C. analyzed the data and wrote and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest to disclose.

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