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# Microbial communities change in an anaerobic digestion after application of microbial electrolysis cells

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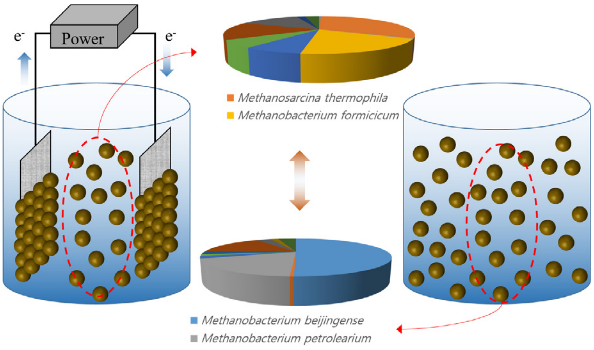
## HIGHLIGHTS

- Improving the generation of renewable sources of energy.
- Anaerobic digester and anaerobic digester combined with microbial electrolysis cells.
- Archaeal communities between the AD and AD-MEC.
- Increased methane production using activated microbial communities.

## GRAPHICAL ABSTRACT

Changes in the structure of microbial communities were observed in the bulk sludge of conventional anaerobic digester and anaerobic digester combined with microbial electrolysis cells (MECs). According to the results, there was a difference in the dominant species of the two reactors, where anaerobic digester combined with a MECs activates microbial communities associated with acetoclastic methanogens, thereby resulting in increased methane production.



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## ABSTRACT

Microbial electrolysis cells (MECs) are being studied to improve the efficiency of anaerobic digesters and biogas production. In the present study, we investigated the effects of electrochemical reactions in AD-MEC (anaerobic digester combined with MECs) on changes in the microbial communities of bulk sludge through 454-pyrosequencing analysis, as well as the effect of these changes on anaerobic digestion. *Methanobacterium beijingense* and *Methanobacterium petrolearium* were the dominant archaeal species in AD, while *Methanosarcina thermophila* and *Methanobacterium formicicum* were dominant in AD-MEC at steady-state. There were no substantial differences in dominant bacterial species. *Clostridia* class was more abundant than *Bacteroidia* class in both reactors. Compared to AD, AD-MEC showed a 40% increase in overall bacterial population, increasing the removal of organic matters and the conversion of volatile fatty acids (VFAs). Thus, the MEC reaction more effectively converts organic matters to VFAs and activates microbial communities favorable for methane production.

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*Abbreviations:* AD, anaerobic digester; SCOD, soluble chemical oxygen demand; CuPc, copper phthalocyanine; SHE, standard hydrogen electrode; FePc, iron phthalocyanine; TCOD, total chemical oxygen demand; MECs, microbial electrolysis cells; VFA, volatile fatty acid; MFCs, microbial fuel cells; VS, volatile solids.

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## 1. Introduction

There is a growing need for renewable source of energy to decrease the use of fossil fuels, which affect global warming (McKendry, 2002). Various methods can be used to generate different forms of renewable energy, including wind power, solar heat, water power, and biogas from anaerobic digesters (Johansson et al., 1992). Anaerobic digestion is widely used for bioenergy production from various organic matters with sludge reduction and stabilization (Guo et al., 2013). Anaerobic digestion consists of a series of reactions that proceed over the course of various stages involving diverse microbial communities. The process of anaerobic digestion comprises three stages: hydrolysis, acidogenesis, and methanogenesis (Alexiou et al., 1994).

The final stage of anaerobic digestion (i.e., methane production) involves microorganisms that can be divided into two groups: acetoclastic methanogens (which split acetate into methane and carbon dioxide) and hydrogenotrophic methanogens (which use hydrogen as an electron donor and carbon dioxide as an electron acceptor to produce methane). The chemical reactions of the two microbial groups are provided in detail as follows (Lise et al., 2008):

$$\text{Acetoclastic methanogenesis}: CH_3COOH \rightarrow CH_4 + CO_2 \qquad (1)$$

$$\text{Hydrogenotrophic methanogenesis}: CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \qquad (2)$$

However, anaerobic digestion has several operating problems owing to the slow growth rate of methanogenic archaea, the low removal efficiency of organic matters, the increased hydraulic retention time (HRT), its prerequisite for high temperatures, and the accumulation of volatile fatty acids (VFAs) within the reactor (Hori et al., 2006).

To solve these problems, many techniques—using various physical and chemical preprocessing methods—have been developed to improve the efficiency of hydrolysis, which is a rate-determining step in anaerobic digestion reactions. However, most of these techniques have inherent disadvantages, such as high initial investment and operating costs, increased energy expenses, etc., and require further studies to overcome these shortcomings (Li et al., 2012).

Currently, several new technologies including microbial electrolysis cells (MECs) have been considered to improve the efficiency of the conventional anaerobic digesters. MECs is a technology related to microbial fuel cells (MFCs). While MFCs produces an electric current from the microbial decomposition of organic matters, MECs is the process to increase biogas from organic matters by applying an electric current for microorganisms. (Sun et al., 2015).

MECs supply low voltage (0.2–0.8 V) to the anaerobic digester for the bioelectrochemical reaction, where exoelectrogenic bacteria decompose organic matters and emit an electron at the anode (Eq. (3)), which moves to the cathode in a closed circuit and is consumed, thereby producing $CH_4$ (Eq. (4)) and $H_2$ (Eq. (5)). The corresponding theoretical potentials, which are indicated next to the equations (Logan et al., 2008), are given as follows:

(SHE: standard hydrogen electrode)

$$\text{Anode}: C_2H_4O_2 + 2H_2O \rightarrow 2CO_2^- + 8H^+ + 8e^-$$
$$E^0 = -0.28\ \text{V vs SHE} \qquad (3)$$

$$\text{Cathode}: CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O$$
$$E^0 = -0.24\ \text{V vs SHE} \qquad (4)$$

$$2H^+ + 2e^- \rightarrow H_2 \quad E^0 = -0.42\ \text{V vs SHE} \qquad (5)$$

Consequently, in MECs methane can be produced via two different reaction mechanisms. First reaction mechanism is related to electrochemical reaction, $CO_2$ is reduced to $CH_4$ reacted with protons and electrons. The other mechanism is the hydrogenotrophic methanogenesis reaction, which can improve methane production rate and yield compared to the conventional anaerobic digester (Villano et al., 2013).

In this regard, the microorganisms associated with MECs, such as *Clostridium*, *Geobacter*, and *Shewanella* spp. were found on anode, while *Methanosarcina* and *Methanobacterium* spp. were the dominant archaea on cathode(Sun et al., 2015). They are not remarkably different from those microorganisms in the process of conventional anaerobic digestion.

Generally, the process of methane production in anaerobic digestion is composed of 72% acetoclastic methanogenesis and 28% hydrogenotrophic methanogenesis (Jeris and McCarty, 1965). In MECs, additional amounts of methane could be produced electrochemically at the cathode by combining $CO_2$ with hydrogen ions and electrons generated at the anode, as shown in Eq. (3) (Villano et al., 2013).

However, Tian et al. (2016) observed no difference in the composition of biogas between conventional anaerobic digester and anaerobic digester combined with MECs. It is presumed that the MECs reaction increases the activity of biological methane production instead of electrochemical methane production. In addition, Feng et al. (2015) observed a difference in microbial communities between a MECs reactor operated by supplying a voltage of 0.3 V for anaerobic digestion, and a conventional reactor without voltage supply. It has been reported that voltage supply can increase the activity of archaeal communities and increase the production of acetate by inducing the diversity of bacterial communities. In addition, according to Sun et al. (2015), methane yield increased concomitantly with changes in microbial communities in the MECs. This finding shows that MECs affect not only electrochemical reactions, but biological reactions as well (Sun et al., 2015).

Most microbial research on the MECs has been focused on the microbes living on the surface of anodes and cathodes, concentrating on the mechanisms of electron transfer between electrodes and the microbial cell (Chae et al., 2010; Kiely et al., 2011; Omidi and Sathasivan, 2013). In this study, we investigated the effects of the electrochemical reactions on methane production and changes in the microbial communities in the bulk sludge, both in anaerobic digester (AD) and anaerobic digester combined with MECs (AD-MEC) under the same operating conditions, with food waste leachate as a substrate.

## 2. Materials and methods

### 2.1. Reactors configuration

The reactor consists of a cylindrical structure (280 mm diameter × 410 mm height) made of acrylic material, with a 25 L total reactor volume and an effective volume of 15 L (Fig. 1). DC power (0.3 V) was supplied to the single-chamber AD-MEC (Fig. 1a) through 6 sets of electrodes to investigate changes in the microbial communities. Conventional anaerobic digestion was carried out in the control reactor (Fig. 1b), operated on the same conditions in the absence of electrodes.

The anode (150 mm × 300 mm, area: 0.045 $m^2$) and the cathode (150 mm × 300 mm, area: 0.045 $m^2$) supplied voltage for the microbial electrochemical reactions consisted of 6 sets in the reactor. Each electrode is composed of graphite mesh coated with Ni to increase the electrical conductivity (Song et al., 2014). For the cathode, a complex metal catalyst solution was prepared by dissolving 30.125 g $MnSO_4 \cdot H_2O$, 19.75 g $KMnO_4$, 0.5684 g iron phthalocya-