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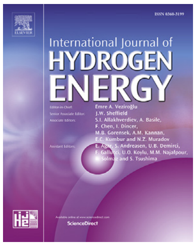

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Optimizing the operating temperature for microbial electrolysis cell treating sewage sludge

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ABSTRACT

Temperature is a very important parameter affecting the performance of microbial electrolysis cell (MEC). Generally, the activity of methanogens can be improved by operating at higher temperature, while electrochemically active bacteria (EAB) have the highest activity at around 30 °C. In this study, batch tests were performed to investigate the effect of temperature on the methane production and organic matter removal of MEC treating sewage sludge. As the temperature increased, the pH and alkalinity of digestate at the end of each cycle increased from 7.72 and 2055.5 mg $CaCO_3$/L (at 30 °C) to 8.62 and 2804.9 mg $CaCO_3$/L (at 40 °C) possibly due to the improved activity of methanogens. The VSS removal increased linearly from 35.1% to 45.8% by increasing the temperature from 30 °C to 40 °C, while COD removal was not significantly affected (<5%). The maximum methane yield and current density were 139.2 ± 11.2 L CH_4/kg VSS_{re} and 1.63 ± 0.11 A/m^3 at the temperature of 35 °C, which were higher than those obtained at 30 °C (136.6 ± 10.9 L CH_4/kg VSS_{re}, 1.54 ± 0.04 A/m^3) and 40 °C (107.7 ± 10.3 L CH_4/kg VSS_{re}, 1.23 ± 0.16 A/m^3). The current density generated from anode dropped by 23.5% when the operating temperature increased from 35 °C to 40 °C. These results indicate that the higher temperature of over 40 °C can inhibit the activity of EAB on the anode. In terms of the relationship between methane yield and current density, the higher current production could also enhance MPY owing to the improved electrochemical reaction (direct electron transfer from the cathode to the biofilm).

Introduction

Sewage sludge is a byproduct of wastewater treatment process and causes a lot of environmental problems due to concentrating heavy metals and potentially pathogenic organisms [1]. The sewage sludge production has increased continuously with the construction of new wastewater treatment plant (WWTP) and stringent environmental standards [2]. The reduction and stabilization of sludge, therefore, has become a significant challenge around the world, as the treatment and disposal of sludge accounts for up to 60% of operating cost of a plant [3].

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Anaerobic digestion (AD) is commonly employed to reduce the amount of sludge, stabilize the sludge, kill pathogens and recovery energy in the form of methane [1]. The methane production during AD process is divided into two steps which are hydrolysis/acidogenesis and methanogenesis [4]. In the first step, hydrogen, carbon dioxide and volatile fatty acids (VFAs) are produced from organic materials via biochemical reactions, and then the methanogens utilize mainly H_2/CO_2 and acetic acid to form methane and carbon dioxide in the second step. The temperature of AD was maintained at mesophilic (35 °C) or thermophilic (55 °C) temperature [5]. Even though the higher temperature can promote metabolic rates, specific growth rates, and rates of the pathogen destruction, mesophilic AD is more widely used compared to thermophilic AD owing to low energy intensity and high stability [6]. However, AD has been faced with several obstacles such as a demand for high thermal energy, long hydraulic retention time (over 20 days), low removal rate of organic matters [7].

Microbial electrolysis cell (MEC) has gained wide interest as a versatile device that can produce biogas (H_2, CH_4) or chemicals from various waste organic materials. The organic matter removal rate and methane production rate using MEC have been significantly enhanced by adding a small voltage, compared to AD [2]. In an MEC, electrochemically active bacteria (EAB) oxidized organic matter contained in organic waste, produce electrons and carbon dioxide. Electrons were transferred to the cathode through an external circuit, while protons diffuse to the cathode through the electrolyte. In cathode compartment, the byproducts released by bacteria are consumed to generate hydrogen or methane gas depending on cathode potential via electro-biochemical reaction [8,9].

Methane production using MECs can occur through two routes. The first pathway is by acetoclastic methanogens that can convert acetate to methane, the second pathway is by hydrogenotrophic methanogens based on following equations [9].

$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O \quad E^{0'} = -0.24\ V\ vs\ NHE \tag{1}$$

$$2H^+ + 2e^- \rightarrow H_2 \quad E^{0'} = -0.41\ V\ vs\ NHE \tag{2}$$

The required energy for methane production by hydrogenotrophic methanogenesis (eq (2)) can theoretically be higher than that needed via acetoclastic methanogenesis (eq (1)) under standard conditions (pH 7, 1 atm) due to the possibility of using more negative cathode potentials [10]. Thereby, methane is produced mostly from acetate (70%) in AD, which requires at least 3–5 days at mesophilic temperature [11]. In MEC with an external voltage, hydrogenotrophic methanogenesis that is known to convert hydrogen gas and carbon dioxide to methane in less than one day can be promoted, which means MEC allow to reduce the hydraulic retention time compared to mesophilic AD [11,12]. The methane production in single chamber MECs was mainly associated with the current generation and hydrogen production, and the rate of it could be catalyzed by EAB [8,9].

Temperature is one of the critical parameters affecting the activity of microorganism that leads to changing the performance of MEC such as current density, biogas production, and organic removal. The current density by EAB on anode can increase temporarily exposing at temperatures above 35 °C shortly but generally, tend to decrease in the long-term [13]. Kyazze et al. reported the optimum temperature of a two chamber MEC fed with acetate was determined to be about 30 °C. The current generation and biogas (H_2, CH_4) production decreased at the temperature of under 25 °C or above 40 °C on account of the lower activity of EAB [14]. According to the results of another research, the maximum current density generated from a single chamber MEC was obtained at the temperature of 29–31 °C and also the COD reduction showed a similar tendency in the system [15].

Methanogens that produce methane via biochemical reaction in final step have a different behavior with temperature variation compared to EAB. Previous researchers demonstrated that the activity of methanogens under thermophilic condition increased by 1.6–1.8 times than under mesophilic condition [16,17]. For this reason, it is known that the performance of AD increases with an increasing temperature due to the faster metabolic rate of the microorganisms. For example, biogas production from thermophilic AD fed with fruit and vegetable waste was higher than one from psychrophilic and mesophilic ADs by 144% and 41%, respectively [18]. Similarly, the thermophilic AD performed better in terms of methane yield compared to the control mesophilic AD with the OLR rate of 2.8–3.7 kg VS/m^3/d [5].

The purpose of this research is to find out optimal temperature for both EAB and methanogens in MECs fed with sewage sludge as substrate. Two reactors equipped with two-pairs of graphite felt electrode were simultaneously set up at the temperature variation of 30, 35, 40 °C to investigate the effect of temperature on the variations of digestate, current generation, and methane production. The current density passing through a circuit connected to the electrodes was recorded during experimentation in order to assess the portion of methane produced by electrochemical reaction compared to overall methane production. To determine if the methane produced in MEC was from a bioelectrochemical reaction of the electrode or from the other pathway, the total charge was calculated from the current and compared to the balance of electrons recovered in methane gas based on equation (1).

Materials and methods

Substrate and inoculum

Raw sludge was obtained from the J Wastewater Treatment Plant in Jinju, South of Korea. Nondegradable solid matter in raw sludge was removed with a standard sieve (10 mesh) and the remaining sludge stored at 4 °C, which was used as the substrate. The seed sludge was collected from another MEC fed with sewage sludge reactor that had operated at 30 °C for 4 months. The raw sludge was mixed with the seed sludge with a ratio of 7:3 during experimentation without any additional chemicals, but with a ratio of 5:5 at the beginning of operation (30 days) to reduce the adaptation period for anaerobe. The characteristics of raw sludge and seed sludge were shown in Table 1.

Table 1 – Characteristics of the raw sludge and seed sludge.

	Raw sludge	Seed sludge
pH	6.3 ± 0.1	6.3 ± 0.1
Alkalinity (mg $CaCO_3$/L)	637.4 ± 13.9	2704.4 ± 17.0
Total suspended sludge (g/L)	21.5 ± 0.1	20.0 ± 1.4
Volatile suspended sludge (g/L)	17.1 ± 0.4	15.8 ± 0.1
Total chemical oxygen demand (g/L)	29.2 ± 2.9	26.8 ± 0.1
Soluble chemical oxygen demand (g/L)	1.1 ± 0.1	1.9 ± 0.8

Construction and operation of MEC

Two pairs of graphite felt electrodes (30 mm width, 90 mm length; Morgan, UK) were inserted into a cylindrical acrylic MEC reactor (170 mm diameter, 200 mm length) with an effective volume of 2.5 L (Fig. 1). The electrode spacing between anode and cathode was 16 mm and they were connected to DC power source or the resistance with a stainless wire. A gas collector filled with acidic saline water (under pH 2) was used to measure the volume of biogas. A voltage of 0.3 V was applied across each pair of electrodes using a power source. The reactors were operated at three different conditions (30, 35 and 40 °C) and stirred at 100 rpm to mix well. After the stabilization period for two months, the reactors were operated for 6 days for a cycle and purged using nitrogen gas (99.99%) for 30 min to remove oxygen at the beginning of every cycle. All experiments were performed in fed-batch mode and in duplicate.

Experimental measurements and calculations

TCOD, SCOD, TSS, VSS were analyzed according to the Standard Methods for the Examination of Water and Wastewater [19]. The pH and alkalinity of initial and final sludge samples were measured with a pH meter (Sevencompact S220, Mettler Toledo, Switzerland). For the measurement of SCOD and VSS, the sludge samples were filtered through a 0.45 um pore size cellulose membrane filters. The filtrate and residue on the filter were analyzed for SCOD and VSS, respectively.

The volume and composition of biogas were analyzed 5 times a cycle (6 days) at the same time. The biogas composition was obtained using gas chromatography (Series 580, GowMac Instrument Co., USA) equipped with a thermal conductivity detector (TCD) and a 1.8 m × 3.2 mm stainless-steel column packed with porapack Q (80/100 mesh SS). Nitrogen was used as the carrier gas with a flow rate of 30 mL/min and the temperature of the injector, oven, and detector were 80, 50, and 90 °C, respectively.

The indicators such as current density and energy recovery could be used as an evaluation index for the performance of MECs. Current density from electrode was determined using Ohm's law $I_V(A/m^3) = E/(R_{ex} \times V)$ where, E is the voltage generated from MECs over a 10 Ω resistance was recorded every 30 min using a digital multimeter (Keithley 2700, USA), and R_{ex} is a value of external resistance and V the working volume of reactor. The electric energy (W_E) supplied to MEC was calculated using following equation (3)

$$W_E(kJ) = \sum_{1}^{n} \left(IE_{ap}\Delta t - I^2 R_{ex} \Delta t \right) \tag{3}$$

where E_{ap} is the applied voltage from the external power, and Δt is the interval time for the measurement of voltage during a cycle. The energy recovery (η_{E+S}) is the rate of the energy content of recovered methane and the total energy content supplied to the reactor as;

$$\eta_{E+s} = \frac{W_{CH_4}}{W_E + W_S} \tag{4}$$

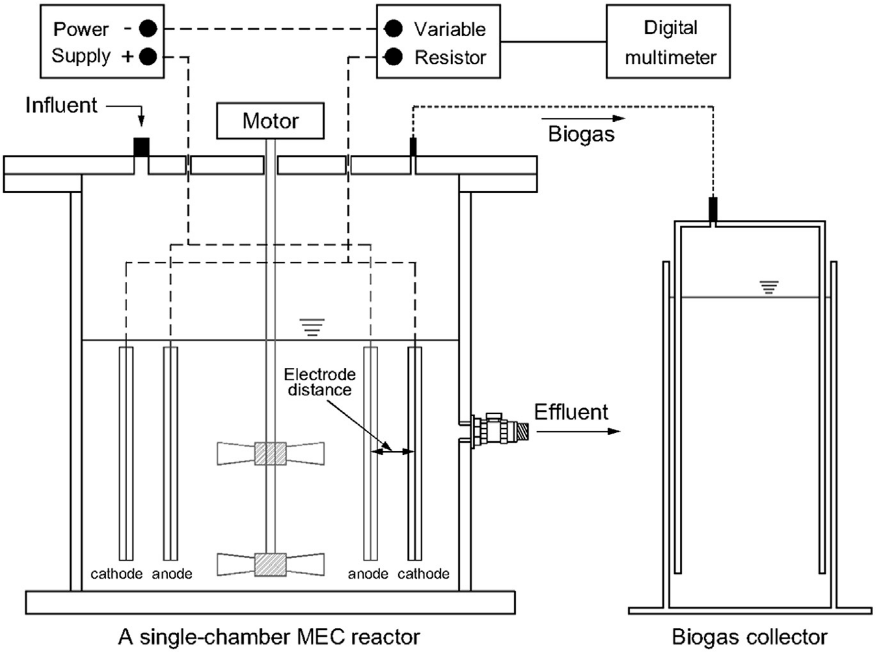

Fig. 1 – Schematic diagrams of a single-chamber microbial electrolysis cell.

where W_{CH_4} is calculated as $W_{CH_4}(\mathrm{kJ}) = \Delta H_{CH_4} \times n_{CH_4}$; where ΔH_{CH_4} is the heat of combustion of methane (890.8 kJ/mol) and n_{CH_4} is the amount of produced methane in moles (CH_4 production (L)/22.4 (L)) [11]. W_S is calculated as $W_S(\mathrm{kJ}) = \Delta H_S \times n_S$; where ΔH_S is the heat of combustion of sewage sludge (385.6 kJ/mol) refer to Shizaz and n_S is the amount of total COD removal in moles (($COD_{in} - COD_{out}$) (g)/32 (g)) [20]. To evaluate electrode efficiency for methane production via bioelectrochemical reactions, the theoretical methane production was calculated with the number of electrons passed through a circuit between electrodes by the following equation (5)

$$CH_{4(\mathrm{electrode})} = \frac{\sum I\Delta t/F}{n} \times 22.4 \qquad (5)$$

where F is the Faradays constant (96,485 C/mol). n is the number of electrons to form methane with carbon dioxide [21].

Results and discussion

pH and alkalinity variation

The pH variation after the fermentation is shown in Fig. 2. The pH increase of the digestates at 40 °C was higher than that at 30 °C and 35 °C, and the pH reached at about 7.79 ± 0.10 (30 °C), 8.06 ± 0.03 (35 °C) and 8.44 ± 0.26 (40 °C) at the end of the cycle, respectively. The component that influences on the pH of digestate is the concentration of VFAs produced during AD [6]. This seemingly resulted from the improvement of methanogens activity and electrode efficiency which could consume VFAs and protons, specifically on acetic acid produced during hydrolysis/acidogenesis process [2,4]. Lau and Fang showed that the pH of the effluent from the AD treating cattle sludge decreased when the temperature varied from 55 °C to 37 °C, resulting from the accumulation of VFAs [22,23]. The similar results also reported that acetic, propionic, and butyric acid concentration in the effluent increased by 3.7, 3.5, and 2.5 times, respectively, when temperature decreased from 35 °C to room temperature [24]. Moreover, the consumption rate of VFAs and H^+ could be accelerated by the enhanced cathodic reduction of CO_2 or H^+ (reaction 1, 2) to produce CH_4 or H_2 in MECs, since the activity of methanogens and hydrogen producing bacteria enriched in the biocathodes was improved by increasing temperature [1,25].

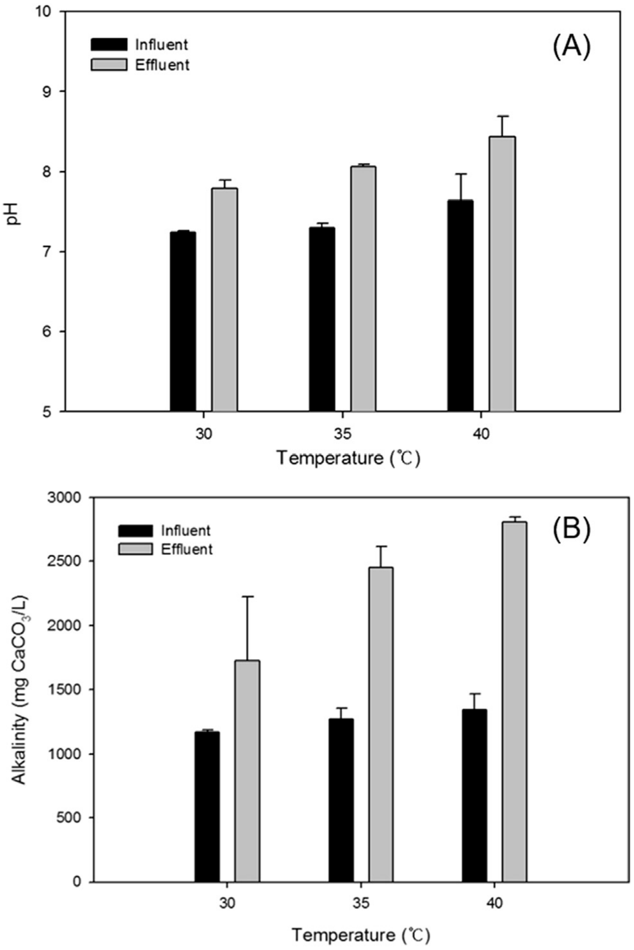

Fig. 2 – The variation of (A) pH and (B) alkalinity of the digestate in MECs with different operating temperature.

The alkalinity of the initial sludge mixture ranged 1170–1340 mg $CaCO_3$/L. After anaerobic digestion, the highest alkalinity level was 2804.9 ± 43.6 mg $CaCO_3$/L at 40 °C, that was 36.5%, 14.4% higher than that at 30 °C (1728.9 ± 495.1 mg $CaCO_3$/L) and 35 °C (2451.1 ± 165.7 mg $CaCO_3$/L), respectively (Fig. 2). In AD, the alkalinity of sludge increased by the production of bicarbonate that is the main form of dissolved carbon dioxide in the range between pH 6.5 and 10.0 [26]. The solubility of carbon dioxide in water is enhanced with the higher partial pressure and pH value, which may be achieved by the furious activity of methanogens that can convert from H^+ contained in sludge to methane and supply a large amount of carbon dioxide into biogas [6]. For these reasons, the activity of methanogens in MEC could be enhanced by the increasing temperature.

COD and VSS removal

Chemical oxygen demand (COD) and volatile suspended solid (VSS) were analyzed to evaluate the effect of temperature on the reduction of organic matter in sludge. The maximum COD and VSS removal were 44.2 ± 2.8% and 45.8 ± 1.1% at the operating temperature of 40 °C, respectively (Fig. 3). In MEC system, COD and VSS removal could be improved by enhancing the activity of anaerobes attached on the electrode surface, which participated in removing organic matters [27]. However, the COD removals at 30 °C and at 35 °C were 40.9 ± 2.5 and 39.1 ± 0.2%, which meant that the MEC performance in terms of the COD removal was less dependent on the temperature from 30 to 40 °C and this trend was consistent with that of the results reported by Omidi and Sathasivan. Anaerobic bacteria in MEC fed with acetate as carbon source shown the similar activity at the temperature from 30 to 35 °C, while it severely decreased below 30 °C conditions [15]. The VSS removal obtained at 40 °C increased by 10.7 and 5.9% compared to 30 and 35 °C, respectively. The increase of temperature in anaerobic fermentation improves the biodegradation of the complex organic matter through providing environmental for the thermophilic bacteria which have the outstanding ability to utilize several carbon sources to grow

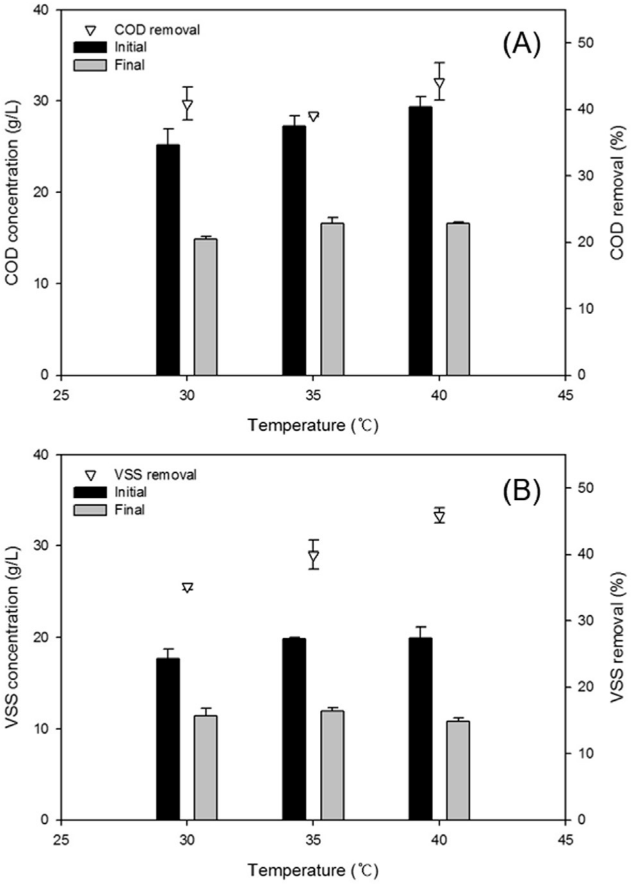

Fig. 3 – The concentration of (A) COD and (B) VSS of initial and final sludge and organic matter removal.

than mesophilic bacteria [18,28]. In addition, the activities acidogenic bacteria and methanogenic bacteria were 1.8 and 1.6 times higher under thermophilic conditions compared to mesophilic conditions [16,17]. It should be noted that organic matter degradation efficiency was influenced by operating temperature resulting in the activities of anaerobes in a reactor and on the electrode surface.

Current and methane production

The electric current generated from two sets of electrodes in the MEC was measured for 36 days (total 6 cycles) to obtain the maximum volume-based current density that was the highest mean value of over 6 h in each cycle (Fig. 4). After stabilization period (about two months), the produced current density (sum of the results from electrode 1 and 2) reached 1.54 ± 0.04 A/m^3 at the temperature of 30 °C, and slightly increased by 5.8% (1.63 ± 0.11 A/m^3) when the temperature of MEC varied to 35 °C. However, the sudden drop of current generated from one of the electrodes was observed after 11 h at the first cycle of the 40 °C temperature test, resulting in the decrease of current density (1.24 ± 0.26 A/m^3). The average current density for the last cycle (40 °C) seriously decreased by 37% from 0.71 ± 0.06 A/m^3 to 0.23 ± 0.03 A/m^3 compared to 35 °C. To figure out if this temperature shock effected on the current generation in the MEC temporarily or permanently, the recovery test was carried out at 35 °C for a week after the last cycle, but the performance of electrode 2 was not recovered (data not shown).

Geobacter and *Shewanella* species are known as representative EAB can transfer electrons from inside of the cell to electrode via nano-wire in MEC system. Their activities were enhanced with increasing temperature from 4 °C to 35 °C, leading to the increase of current density in accordance with the results from this [12,24,29]. However, the current density obtained in a MEC fed with artificial wastewater rapidly decreased at the long term operation over 40 °C, gradually started recovering under mesophilic condition [14]. Although the bio-anode function declined over 40 °C due to the decreased activity of EAB on the anode surface, it might be reversible by providing sufficient time and other favorable conditions for EAB.

The methane content in biogas ranged from 57% to 67% during whole operating times. The temperature variation between 30 °C and 35 °C did not significantly affect the methane content in biogas (<5%). The methane content at 40 °C (66–67%) was 8–10% higher than the other conditions due to the higher pH value of digestate (Fig. 2) [26]. The maximum methane production based on working volume (2.5 L) was 1.11 ± 0.07 m^3 CH_4/m^3 at 35 °C, and it was 30% and 13% higher than that at 30 °C (0.85 ± 0.11 m^3 CH_4/m^3) and 40 °C (0.98 ± 0.01 m^3 CH_4/m^3), respectively (Fig. 5). The methane yield at 35 °C was also the highest (104.2 ± 11.2 L CH_4/kg COD_{re}, 139.2 ± 0.6 L CH_4/kg VSS_{re}) compared to other two conditions. However, the methane yield obtained at 30 °C and 40 °C showed the different tendency with methane production. The methane yield at 30 °C (82.1 ± 0.2 L CH_4/kg COD_{re}, 136.6 ± 10.9 L CH_4/kg VSS_{re}) was 6.5% (based on kg COD_{re}) and 26.9% (based on kg VSS_{re}) higher than the results at temperature of 40 °C (77.1 ± 7.2 L CH_4/kg COD_{re}, 107.7 ± 10.3 L CH_4/kg VSS_{re}), while the methane production was opposite of the methane yield. Methanogenesis could be conducted by methanogens via largely two pathways, which were acetoclastic (with acetate) and hydrogenotrophic methanogenesis (with hydrogen and carbon dioxide) [2]. Generally, methanogens in anaerobic digestion convert from intermediate products (acetate, formate, and hydrogen) produced by acidogenic and syntrophic bacteria to methane [6]. In MEC system equipped with electrodes, the methane seemed to be produced through interaction between EAB and methanogens, since the electrons and hydrogen, which were produced by EAB or electrochemical reaction, could be directly transferred to methanogens [21,30]. Methanogens were sensitive to temperature fluctuations and its activity tended to be improved with increasing temperature [5,24]. The inhibition of methane production below 35 °C was due to the lower activity of acetoclastic methanogens that accounted for 70% of total methane production in anaerobic digestion [18]. Under thermophilic condition (55 °C), the activity of hydrogenotrophic methanogens, such as *Methanobacterium* species, increased and enhanced the methane content in biogas and methane production from organic waste [28]. As shown Fig. 4, the activity of EAB was hindered over 40 °C. Methane production rate rapidly increased from 2.69 mL CH_4/day to 12.9 mL CH_4/day when the operating temperature changed from 30 °C to 40 °C. Nevertheless, it was not maintained too long and finally

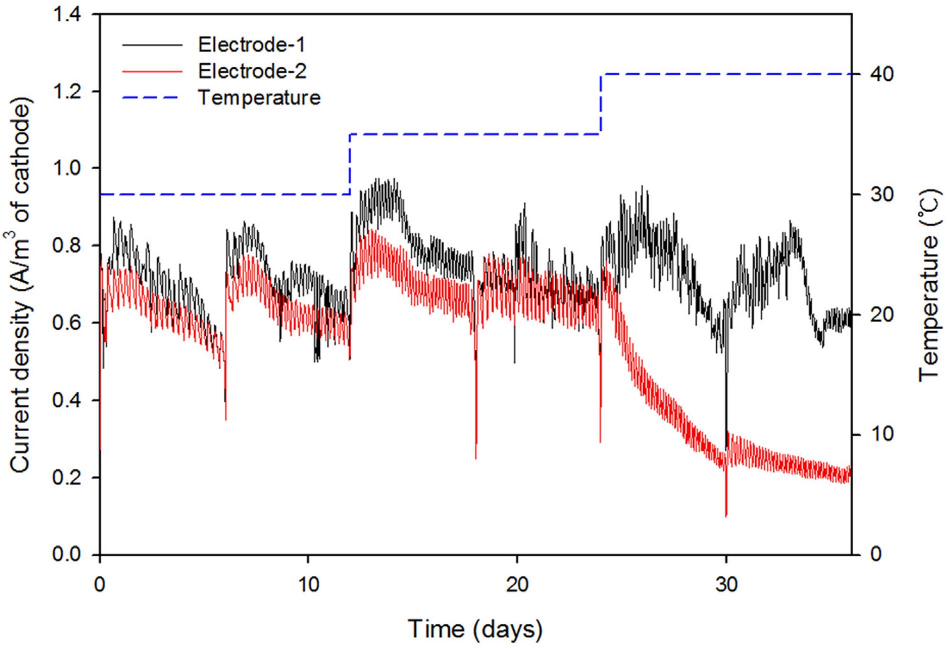

Fig. 4 – **The current densities generated from two pairs of electrodes inserted in MECs during methane fermentation.**

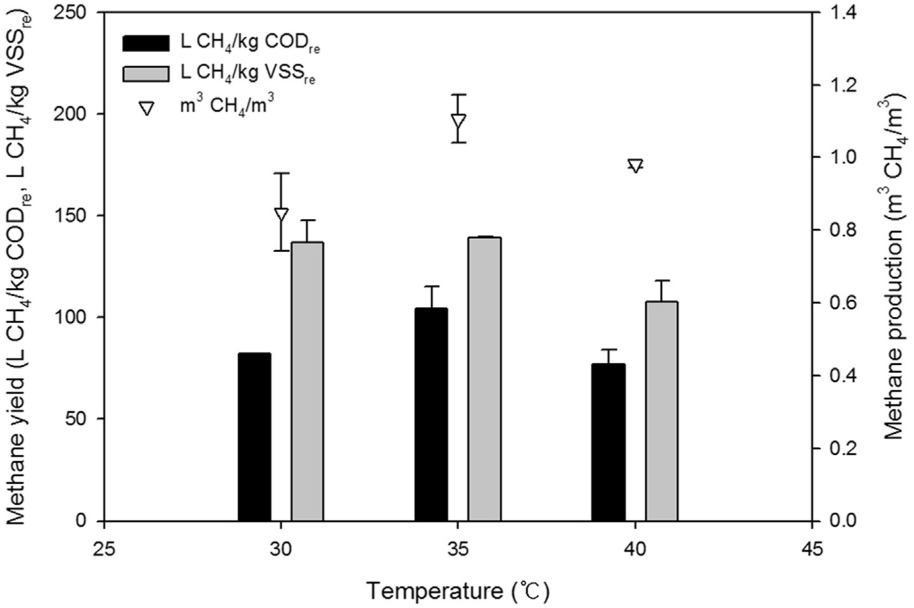

Fig. 5 – **Methane yield and production from sewage sludge using MECs operated at the temperature of 30, 35 and 40 °C.**

reached to 1.94 mL CH_4/day owing to the drop of bioelectrode performance [14]. By the same token, although the activity of methanogens increased with higher temperature, the methane production and yield at the temperature of 40 °C were lower than those obtained at 35 °C due to the decrease of EAB activity [14].

Impact evaluation of electrochemical reaction and energy recovery

The theoretical methane production calculated by current and energy recovery (based on heat energy) was used to evaluate the performance of MEC. Bio-electrochemical system, such as MFC and MEC, has attempted to improve methane production rate and energy recovery on the AD, and $CH_{4(electrode)}$ was mainly utilized as a performance assessment [21]. The methane could be produced by (1) electrochemical reaction or (2) biochemical reaction by anaerobic bacteria. In this study, the proportion of $CH_{4(electrode)}$ was only 2–3% of the entire methane production during the experiment. The energy content of decomposed substrate, the recovered methane, and the supplied electric energy shown in Table 2. Energy recovery after fermentation reached 27.0, 34.3, and 25.4% for 30 °C, 35 °C, and 40 °C, respectively. Even though the heat energy content of methane

Table 2 – Energy content of the removed substrate, the recovered methane, and the supplied electric power and energy recovery obtained in MECs operated at three different temperature (30, 35 and 40 °C).

Temperature (°C)	W_S (kJ)	W_{CH_4} (kJ)	W_E (kJ)	$\eta(E+S)$ (%)
30	311.6 ± 39.7	84.3 ± 10.6	0.48 ± 0.1	27.0
35	320.8 ± 15.1	110.0 ± 6.6	0.50 ± 0.1	34.3
40	385.0 ± 31.9	97.5 ± 1.0	0.4 ± 0.1	25.4

produced at 40 °C was 15.7% higher than that 30 °C, energy recovery was slightly low. According to these results, the operation of MEC under inappropriate condition can cause energy loss by anaerobic fermentation without methane production.

Conclusions

This study showed that operating temperature has some significant effect on the performance of single-chamber membrane-free MEC equipped with bioelectrodes for sewage sludge treatment. The pH value and alkalinity of the digestates increased due to enhanced activity of methanogens under the higher temperature condition. VSS removal was enhanced by around 5.9–10.7% with increasing operating temperature, while COD removal of the MEC was not substantially influenced among three temperature conditions. The current density produced by anaerobes on the anode surface was inhibited at the temperature of 40 °C, which led to the decrease of the methane yield in MECs. Although the organic matter reduction in MECs operated at 40 °C was higher than the other conditions, the maximum methane production and yield were gained at 35 °C. These results showed that the temperature of 35 °C is the optimal condition for methane production from sewage sludge using MEC, since electrode efficiency for current generation might be started to drop above 35 °C.

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