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RESEARCH ARTICLE

Efficient methane production from petrochemical wastewater in a single membrane-less microbial electrolysis cell: the effect of the operational parameters in batch and continuous mode on bioenergy recovery

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Abstract

The main objective of this study is to evaluate the treatment and simultaneous production of methane from low-strength petrochemical wastewater by single membrane-less microbial electrolysis cells. To achieve this objective, the influence of variables such as applied voltage, operation mode, and hydraulic retention time (HRT) on the performance of the MEC system was investigated over a period of 110 days. According to the obtained results, the maximum COD removal efficiency in the batch mode was higher than which in the continuous mode (i.e. 85.9% vs 75.3%). However, the maximum methane production in the continuous mode was almost 1.6 times higher than which in the batch mode. The results show, COD removal, methane content, and methane production in both operation modes, were enhanced as applied voltage increased from 0.6 to 0.8-1 V. The proportion of methane, methane production rate, and COD removal were increased as HRT decreased from 72 to 48 h, while these values were decreased as the HRT decreased from 48 to 12 h. In continues mode, the energy efficiency had a range of 94.7% to 97.9% with an average of 96.6% in phase III, which almost recovered all of the electrical energy input into the system. These results suggest that single membrane-less microbial electrolysis cell is a promising process in order to the treatment of low-strength wastewater and methane production.

Keywords Microbial electrolysis cell · Treatment · Petrochemical wastewater · Methane production · Bioenergy

Introduction

The petrochemical industry, including petrochemical processing, oil refining, and natural gas production, consumes large amounts of wastewater. Hence, petrochemical industry generates the large water volume with poor biodegradability. The petrochemical wastewater contains oil, heavy metals, salt, sulfide, volatile organic matters, polycyclic aromatic compounds, aliphatic hydrocarbons, organic acids, sulfides, etc. [1, 2]. Various methods are used for petrochemical wastewater

Highlights
- Treatment of petrochemical wastewater by MEC system was investigated.
- The influence of hydraulic retention time (HRT) and applied voltage on the performance of MEC system was investigated.
- The COD removal rate, methane production rate, pH level and content of methane were increased by applied voltage.

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treatment. Physical, biological, and chemical methods can be used for treating petrochemical wastewater. Also, various wastewater pre-treatment methods used by the industry include coagulation, flocculation, adsorption, using membranes, and chemical oxidation. Generally, there are several challenges to remove small suspended oil particles and organic pollution by sole use of physical or chemical technologies. In contrast, biological treatment methods are more cost-effective and sustainable compared to physical and chemical oxidation processes [3]. Biological treatment of petrochemical wastewater can be cost-effective, environment-friendly, and less costly compared to other techniques. For example, in physical method, MBR and active carbon are used for treating petrochemical wastewater. MBR has some disadvantages like high operational and investment costs [2]. There are two types of biological treatment methods, containing aerobic and anaerobic microorganisms. The toxicity of the organic compounds on types of microorganisms is a serious problem during biological treatment [4].

Anaerobic treatment has several advantages over the aerobic processes, which include lower sludge production, less energy needs due to elimination of aeration and generation of methane as an energy source which can be used for temperature control [5, 6]. The anaerobic treatment methods also have disadvantages like poor biodegradability of hydrocarbons by microorganisms [2].

Therefore, in order to overcome these limitations, biological wastewater treatment technologies require suitable processes to enhance the treatment efficiency. Compared with anaerobic treatment, it has been demonstrated that BES could significantly increase the removal rate of hydrocarbons.

Bioelectrochemical systems (BESs) are a novel technology for generating power and simultaneous treatment of wastewaters [7]. In the anode, electrochemically active bacteria oxidize organic substances and generate CO_2, protons and electrons. Exoelectrogenic bacteria transfer the electrons to the anode surface, and the protons are released into the solution. Electrons travel through the external circuit to a cathode [8, 9] .

In the MEC, microbes are biocatalysts that can convert organic matters into $H_2$and value-added chemicals, such as methane, acetate, hydrogen peroxide, ethanol, and formic acid. Thus, MES systems can generate clean and renewable energy. Hence, MES is an alternative technology for conventional treatment method [10]. In MEC systems, because of shortcomings in the hydrogen production, such as low purity, low volumetric efficiency, and difficulty of storage, some scholars evaluated the methane production potential by the use of MEC [11].

Although various methods had been developed to inhibit methane production, the methane production was widely observed in those types of MECs used for hydrogen production. Therefore, that hydrogen generation fails due to methanogenesis after long-term operation of MECs. It was concluded that methane generation might be more suitable than hydrogen production in MECs. In MEC systems, bioelectrochemical production of methane was theoretically produced in two pathways: first, the methane generation by hydrogenotrophic methanogenesis, secondly, electrons and H^+ are directly converted to methane.

The single membrane less microbial electrolysis cell has been a simple architecture with low capital requirements. Recently, single-chambered MECs have been operated for methane production [12]. In this system, electromethanogenesis uses an electric current as the source of the electrons for reducing $CO_2$to CH_4. In cathode, electrons can be transferred either directly or indirectly to the methanogens [13].

The microbial electrolysis cell technology has been widely used to treat various synthetic and real wastewater in recent years such as domestic wastewater [14–16],molasses wastewater [17], saline wastewater [18], hydrothermal liquefied wastewater [19], refinery wastewater [20], food processing wastewater [21].

This study has been conducted on the use of a microbial electrolysis cell to treat low-strength petrochemical wastewater. So, the purpose of this study was to evaluate the simultaneous treatment and generation of methane from petrochemical wastewater by applications of microbial electrolysis cell. Therefore, a MEC system was operated for 110 days to treat petrochemical wastewater, and the objectives of this study were (1) to investigate the effect of two key factors, such as applied voltage, hydraulic retention time (HRT), on the performance of the MEC system, (2) to evaluate the operation mode on the performance of the MEC system.

Material and method

The petrochemical wastewater characteristics

The petrochemical wastewater used in this study was taken from a petrochemical industry located in Isfahan (Iran). The petrochemical wastewater had a chemical oxygen demand (COD) of approximately 490–534 mg/l. The petrochemical wastewater was stored in a cooled room at 4 °C and brought to room temperature before feeding to the MEC. The characteristics of petrochemical wastewater used in this study is shown in Table 1.

Reactor setup MEC

Two single membrane less microbial electrolysis cell were made of polymethyl methacrylate (Fig. 1). The systems were 15 cm long, 15 cm wide and 10 cm deep with a total volume of 2.25 L. The MECs in this work have 0.45 L headspaces and 1.8 L working volume. The inoculum was obtained from an anaerobic digester of Isfahan municipal wastewater treatment

Table 1 The physical chemistry of petrochemical wastewater

Parameter	Concentration (mg/l)
COD	490–534
BOD	316–330
pH	7.3–7.9
Conductivity (ms/m)	1–1.1
Total phosphorus (mg/l)	0–1
Total nitrogen (mg/l)	6.50–8.15
NH_3-N (mg/l)	1–1.7
NO_3-N (mg/l)	4–5.4
NH_4-N (mg/l)	0.80–1.04
NO_2-N (mg/l)	0–0.01
Sulfide (mg/l)	0–1
TSS (mg/l)	212–247

plant (Isfahan, Iran). The MECs were filled with the anaerobic sludge with a mixed liquor suspended solids (MLSS) content of 25,740 mg/l and mixed liquor volatile suspended solids (MLVSS) 11,100 mg/l. The anode (10 cm width × 10 cm length) and cathode (10 cm width × 10 cm length) were held together by plastic screws with electrodes spaced 2 cm apart. Also, a thick piece of polyester cloth was placed between the anode and the cathode to avoid any electrical contact between the two electrodes.

The electrodes were connected to a DC power source for voltage control through titanium wires. The electrical current through the system was monitored and recorded by a data logger (ELR2510, Iran) connected to the electrodes. The influent was pumped with a peristaltic pump (PRP-TN-556, TOOS NANO Company, Iran) into the MEC systems. Biogas was collected in a gas bag which was attached on the top of the MEC systems. The MECs were purged with pure nitrogen gas for 20 min to remove oxygen prior to use. The MECs were fed with petrochemical wastewater, which was mixed with 50 mM phosphate buffer solution (Na_2HPO_4, 4.58 g/L; NaH_2PO_4. H_2O, 2.45 g/L; NH_4Cl, 0.31 g/L; KCl, 0.13 g/L). The temperature of MECs were controlled by a water jacket to maintain the operation at a constant mesophilic temperature of 34 ± 1 °C.

Chemical analyses

Chemical oxygen demand (COD) was measured using standard methods (method 5220, HACH COD system, HACH Company, Loveland, Co) [22]. The pH was measured using a pH meter (model 7440). TSS and sulfide were determined according to the standard methods [23]. The biogas (CH_4,$H_2$and CO_2) were analyzed by a gas chromatography (Sp-3420A, Beijing Beifen Ruili Analytical Instrument CO) equipped with a packed column (Porapack Q, Chrompack, 3 m length, 3 mm ID, stainless steel, Germany) with a thermal conductivity detector and the injection temperature of 140 °C. Nitrogen was used as the carrier gas with a flow rate of 20 $ml\ min^{-1}$. Gas samples were injected with a 250 μL pressure lock gas syringe (SGE, Australia). The biogas production was measured by liquid displacement method.

Biofilm analysis by scanning electron microscopy (SEM)

The sample of carbon cloth (anode) was collected at the end of experiments. The sample was washed three times with 0.1 M phosphate buffer solution (pH 7.4) and fixed in the same buffer solution using 2.5% glutaraldehyde at 4 °C for 4 h, followed by washing three more times with the 0.1 M phosphate buffer solution. The sample was then dehydrated in increasing ethanol concentrations of 50, 70, 90, and 100% (15 min each), and then air-dried. Then, the sample was coated with gold particles (SC7620 Sputter Coater) to increase signal detection and visualized on a Scanning Electron Microscope (Zeiss EVO HD15).

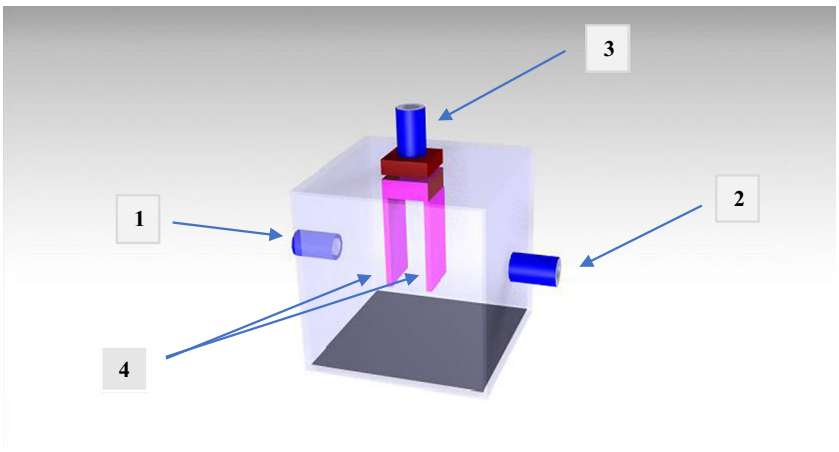

Fig. 1 Schematic diagram of the MEC system. NOTICE: (1) inlet, (2) outlet, (3) gas outlet, and (6) electrodes

Electrochemical calculations

The Coulombic efficiency ($C_{E\%}$) was calculated on the basis of measured coulombs of current compared to the total coulombs from substrate removed (based on COD) as presented in eq. (1,2):

$$C_E = \frac{8I}{Fq\Delta COD} \quad \text{(continues mode)} \tag{1}$$

$$C_E = \frac{8\int_0^{t_b} Idt}{FV_{An}\Delta COD} \quad \text{(batch mode)} \tag{2}$$

where I is the current (A), F is Faraday's constant, q is the volumetric influent flow rate (L/s), and $\Delta COD$is the difference between the influent and effluent COD (g/l), V_{An} is the volume of liquid in the anode compartment [24].

Electric energy supply (W_E) in MEC system was calculated according to the following Eq. (3):

$$W_E = \sum_1^n IE_{ap}\Delta t \tag{3}$$

Where I is the current (A), E_{ap} is the applied voltage, Δt is the time of the experiments [25].

The net chemical energy output (in the form of methane) from the MEC can be calculated as follow [25]:

$$W_{out} = \frac{\Delta t\Delta H_{methane}(V_1 - V_2)}{V_m} \tag{4}$$

Where $\Delta H_{methane}$is the energy content of CH_4 based on the heat of combustion (upper heating value) (891 kJ/mol), Δt is the time of experiments (hour). $V_1$is the accumulative CH_4 production in the MEC system (mL/h), V_2 the accumulative CH_4production in the control system (mL/h) and V_m is molar volume of the gas.

Energy efficiency (η_E) relative to the electric energy supply and energy output was calculated by the following Eq. (5):

$$\eta_E = \frac{W_{out}}{W_E} \times 100 \tag{5}$$

Cathodic gas recovery (r_{CAT}) was calculated as the ratio of moles of methane measured and moles of methane theoretically produced based on current intensity measured, as presented in eq. (6):

$$r_{CAT} = \frac{\eta_{CH_4}}{\frac{\int_{t_0}^{t} I(t)dt}{8F}} \tag{6}$$

where η_{CH_4} is the number of moles of methane measured, calculated according to the ideal gases law knowing the methane volume measured and 8 is the number of moles of electrons per mole of methane [13].

Start-up and operation of MEC systems

This study lasted 110 days and the COD value of the influent petrochemical wastewater was 490–534 mg/l. The experiments were conducted in two MECs (MEC, control MEC). The control MEC was a microbial electrolysis cell, open circuit, with no energy input (control system).

The experimental period was divided into four phases, as was shown in Table 2. The systems were operated into four phases: phase I (start-up) (from 1st day to 30th day), phase II (from 31th day to 54th day) and phase III (from 55th day to 78th day), phase IV (from 79th day to 110th day).

In startup phase, MEC system was started with petrochemical wastewater at HRT of 72 h for one month. At this phase, the MEC system was operated in batch mode under applied voltage 0.6 V, so as to stabilize the biofilm formation and attachment on the electrodes. After the MEC reached to steady state condition, the operation phases were started. The stable performance was achieved after 30 days operation in startup phase, as indicated by the slight variation of COD removal efficiency and current. In phase II, the effect of applied voltage on the performance of MEC system was investigated in batch mode. In this phase, the applied voltage gradually increased from 0.6 to 1 V at HRT of 48 h. In phase III, the wastewater was continuously fed into the MEC at HRT of 48 h, and the effect of the applied voltage at 0.6, 0.8, and 1 V on MEC performance was investigated in terms of COD removal and biogas production and current. In phase IV, the applied voltage was maintained at 1 V and the effect of HRT on MEC performance was evaluated at the HRT of 72, 48, 24 and 12 h within the next 30 days.

Results and discussion

In this study, the performance of a membrane less microbial electrolysis cell for treatment low-strength petrochemical wastewater was evaluated in different condition. In each applied voltage or HRT, each experiment lasted at 4 cycles to ensure that the MEC reached a steady state. The MEC performance was described by using three main parameters, current, methane production, and COD removal. Summary of achieved results shown in Table 3.

Table 2 Operational conditions of MEC system

phase	Operation mode	Interval(day)	HRT(days)	Applied voltage
1	batch	0–30	3	0.6
2	batch	31–54	2	0.6,0.8,1
3	continuous	55–78	2	0.6,0.8,1
4	continuous	79–110	3,2,1,0.5	1

Table 3 Summary of achieved results shown in MEC system

Phase	HRT (day)	Voltage (V)	C_E%	$\eta_{electricity}$	r_{cat}	Methane production (mL)	COD removal (%)	proportion methane (%)
startup	3	0.6	–	–	–	40±1	56	–
batch	2	0.6	43.3	31.8	16.6	55±1	71.1	64.5
batch	2	0.8	53.2	23.2	16.1	58±1	78.5	73.1
batch	2	1	73.8	18	15.6	63±1	85.9	75.3
continues	2	0.6	11.8	97.1	50.8	64±1	58.4	70.1
continues	2	0.8	13.5	94.7	65.6	75±1	64.7	74.7
continues	2	1	11.7	97.9	85.3	79±1	72.6	78.9
continues	3	1	22.9	75.3	65.5	72±1	63.3	83.1
continues	2	1	14.7	70.4	61.5	77±1	75.3	72.4
continues	1	1	16.7	28.7	25	59±1	56	64.4
continues	0.5	1	33.9	23.2	20.4	26 ±1	26.5	45.9

The COD removal

The main function of a MEC system using wastewater as its anode substrate is considered to be pollutant removal; thus, COD removal is a key parameter to evaluate the MEC performance [26]. In the previous studies, bioelectrochemical systems were proved as an effective technology to degrade complex organic substrates. Actually, these systems have a high diversity of biomass in electrode biofilm to increase carbon degradation [10]. At the start-up of the MEC system, the COD removal rate increased from values in the range of 18–20% up to the values in the range of 55–56%, with COD effluent values in the range of 171–179 mg/l, while the COD removal rate at the end of the startup phase in the control system was only 30%. In this phase, the removal rate of COD started to increase by increased production current with time. This is due to biofilm production included exoelectrogenesis on anode which enhances organic matter oxidation (increase COD removal) and electron production (current increment). The previous studies suggested that the COD removal rate in the MEC system was improved during this phase probably due to the formation of biomass on the electrodes [27]. As it presented in Fig. 2 since the third week with methanogen growth on the anode, Methane gas production started to increase which this increment coincided with COD removal and current reduction. Because of partly consumption of generated electron from organic matter oxidation consumed with methanogens, production current was diminished. Jourdin et al. suggests that the high electron consumption and microbial electrochemical activity in MEC may be attributed to significant bioelectrochemical activity through the development of a uniform formed biofilm on the anode [28].

In phase II (batch phase), the MEC was operated in different voltages (0.6, 0.8 and 1 V) at HRT for 48 h. The applied voltage in MEC was gradually increased in these phases from 0.6 up to 1 V. After the stability of MEC in each voltage, the applied voltage was changed. The steady state condition is achieved when the changing the rate of parameters such as COD is impalpable [29]. In the phase II, by increasing the applied voltage COD removal rate increased significantly. According to the result, COD removal rate of 71.1%, 78.5% and 85.9% were achieved in 0.6, 0.8 and 1 V respectively. By contrast, in this phase, the COD removal rate of 51% was achieved without applying the voltage for control system. It seems that increased voltage improves MEC performance due to stimulation of microorganisms by the applied voltage. The development of COD removal rates also showed a similar trend to current profile increases. Also, results showed that increase voltage induced more oxidation of organic matter and followed by increase methanogens activity and methane production.

Following the batch tests, the MEC was operated in continuous mode at the hydraulic retention time of 48 h. In this phase, the COD removal rate of 58.4%,64.7% and 72.6% were achieved in 0.6, 0.8 and 1 V, respectively. In continues mode, the COD removal efficiency was substantially higher than that was obtained by Escapa et al., who achieved the COD removal rate of 10% in membrane-less microbial electrolysis cell for domestic wastewater treatment [15]. Heidrich et al. also reported the achievement of COD removal rate in range of 1.8–63.1% in an MEC, but it was still lower than that obtained within this

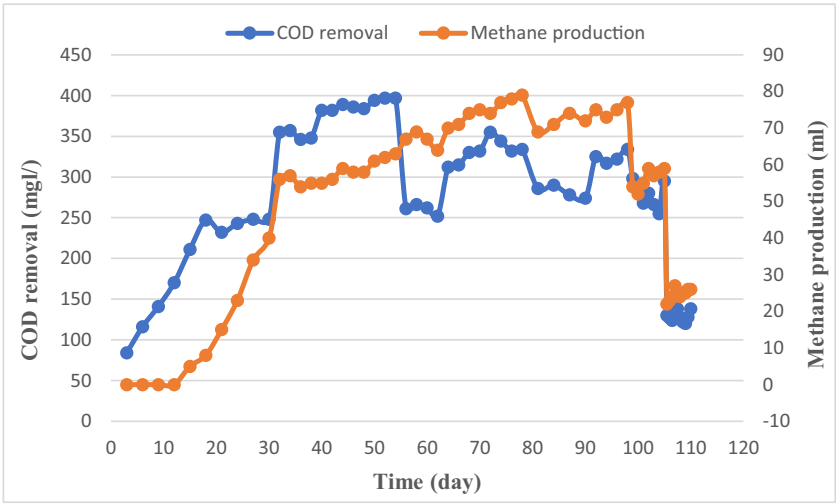

Fig. 2 Performance of the MEC in terms of COD removal and methane production

research [14]. In this phase, the COD removal trend and methane production were similar to the phase II.

As shown in Fig. 2, for phase II and III, COD removal in the MEC system was enhanced as applied voltage increased from 0.6 to 0.8 V and then to 1 V. Generally, the operation mode was considered as one of the most important factors affecting the performance of BES [19, 30]. According to the results, the COD removal in phase batch is significantly better than its performance in continuous phase. In control system, COD removal rate at the end of the third phase in non-applied voltage conditions was around 49%. Results also revealed that in non-applied voltage conditions, operation mode was less effective on COD removal rate. It seems that exogenesis bacteria in batch mode possess more available substrate and activity rather than continuous mode. So, substrate oxidation rate and then COD removal is more than batch mode.

In phase IV, the performance of MEC systems was evaluated at four different HRTs with a constant applied voltage of 1 V. Therefore, MEC was operated in voltage of 1 V at HRTs of 72, 48, 24 and 12 h, while, control system was operated in voltage of 0 V at HRTs of 72, 48, 24 and 12 h. In this phase, in control system, the result shows that lower HRTs resulted in decreased COD removal and relatively more COD concentrations in the effluent wastewater in the control system. The previous studies indicated that this progress could be the result of inhibiting the substrate at lower HRTs (Figs. 3 and 4).

In MEC, decreasing of HRT from 72 to 48 h leads to increased removal rate of COD in MEC. An explanation for this trend is that a decrease in the HRT is associated with an increase in the amount of substrate fed into the reactor. Also, decreasing of HRT from 72 to 48 h leads to increased methane production increased in accordance with COD removal increment. It seems that enhanced organic loading rate leads to increase COD removal rate and then methane production. This trend was not observed at the HRTs of 24 and 12 h, probably because of the partial washout of the methanogenic biomass. So, methane production decreased significantly. The previous studies reported that this trend is the occurrence of the shocked OLR [5].

Electrochemical properties of MEC at different phases

In startup phase, the current in the MEC was below 1 mA in the first 4 days, under a supplied voltage of 0.6 V. During the start-up phase, the current ascended to a peak until it reached its maximum peak on the 21th day. It seems that the formation of biofilms on the electrodes is the main reason for the increase of current in the startup phase. After that, the current decreased since the 21st day. This reduction was due to the production of methane in the system.

Differences in bioelectrochemical behavior were observed during batch and continuous phases. In the batch phase, after applying any voltage, the current reached a maximum value and then the current was reduced. These observations correlate with the reported results by Koch et al. [31]. It seems that the increase of current related to the destruction of complex organic matter into the simple organic matter. Also, the decrease in current occurred due to the consumption of organic materials. In contrast, the current profile in continues mode was a more sustained current throughout the tests.

In phase II (batch phase), the stable current was obtained as 9.4,13.7, and 18.7 mA, on the voltage of 0.6,

0.8, 1.0, respectively at HRT of 48 h. The area of the carbon cloth was 100 cm^2 which meant the stable current density was 1.06, 1.42, 2.04 A/m^2. In phase III, immediately after start-up of the continuous mode operation, the current dropped (from 18.7 to 3.5 mA), but gradually increased with time of operation (4.2 mA). The current increased gradually with the increase of voltage to 4.8, 5.4 and 5.9 mA for 0.6, 0.8 and 0.1 V, respectively.

The results show that the current generation in the batch mode was also higher than the continuous mode in MEC. The batch mode produced the highest current densities, with a maximum current density of 2.46 A/m^2 for the petrochemical wastewater, while, under continuous flow conditions and hydraulic retention time of 48 h, the maximum current density was 0.74 A/m^2. These results were consistent with the COD removal rate in these phases.

In phase IV, when HRT decreased from 72 to 48 h, the current increased gradually from 5.8 to 8 mA, while the current increased significantly as HRT decreased from 48 to 24 h (8 to 12.9 mA) and then from 24 to 12 h (12.9 to 20.6 mA). Current rate increment concord with methane production rate reduction. Results showed that reduction in hydraulic retention time from 48 to 12 h, a lower amount of electron produced by substrate oxidation turn into methane gas. So, the current rate increased significantly.

The coulombic efficiency (C_E) is a key parameter to evaluate the performance of MEC systems. Theoretically, the coulombic efficiency is a parameter to evaluate the fraction of electrons available from organics that ends up as electrical current [32].

In phase II (batch phase), the C_E enhanced from 43.3% to 53.2% and from 53.2% to 73.8% when the applied voltage increased from 0.6 to 0.8 V and from 0.8 to 1 V, respectively. The increase of coulombic efficiency suggested that the anodic oxidation gradually became the main pathway for organic matter destruction during the phase II [32]. In phase II, the maximum C_E was found with a value close to 73.8%. This result indicates that at applied voltage 1 V at least 73.8% of the substrate was biologically recovered as electrons.

The C_E production of the MEC was very low in the phase III, the highest being 13.5% in MEC system with a COD removal of 64.7% and current of 6.7 mA. In continuous phase, the coulombic efficiency increased from 11.8% to 13.5%, and then, decreased from 13.5% to 11.7% when the applied voltage increased from 0.6 to 0.8 V and from 0.8 to 1 V, respectively. In continuous mode, the $C_E$was low. In phases III, anodic coulombic efficiency suddenly decreased and dropped to 11.7% at the end of phase, indicating that the anodic oxidation only contributed to a small part of organic decomposition and methane production at this phase. This result shows that a significant amount of oxidized organic compounds has been converted to methane gas (through the process by methanogens) and hydrogen gas. Also, the previous research reported the presence of methanogens in anode due to low C_E [12, 33].

In phase IV, when the HRT decreased from 72 h to 48 h, C_E declined from 22.9 to 14.7%. These results were related to increased methane production in the system. In the following, decreasing HRT (from 48 to 12 h) is due to the enhanced C_E. This result could be related to increasing OLR and washout of the methanogens from the system.

Cathodic methane recovery (r_{cat}) is the efficiency of converting current to methane. In both phases, the hydrogen was detected at the MEC on all the applied voltages. The results show that cathodic methane recovery in continuous mode was higher than the batch mode. The cathodic methane recovery in batch mode was 16.6%, 16.1% and 15.6% on the applied voltages of 0.6, 0.8 and 1 V, respectively, while, in continuous mode, it was 50.8%, 65.6% and 85.3% on the applied voltages of 0.6, 0.8 and 1 V respectively. Also, in phase IV, its value was 65.5%, 61.5%, 25% and 20.4% at HRTs of 72, 48, 24 and 12 h, respectively.

In phase II, electrical energy input rate increased with applied voltage and ranged from 0.3 Wh at 0.6 V, to 0.98 Wh at 1 V. In phase III, electrical energy input rate increased with applied voltage and ranged from 0.14 Wh at 0.6 V, to 0.29 Wh at 1 V. According to the results, energy efficiency ranged from 18% to 31.8% with an average of 24.3% in batch mode which recovered in about a quarter of the electrical energy input into the system, while its value ranged from 94.7% to 97.9% with an average of 96.6% in phase III. Thus, the input of energy under all applied voltage could be neglected compared to the energy generated from methane and hydrogen, which meant that the experiments at all applied voltage caused a net energy output. In phase IV, its value was 0.46, 0.48, 0.5 and 0.62 Wh at HRTs of 72, 48, 24 and 12 h, respectively. While the amount of energy used is lower than that needed for activated sludge treatment, this energy use was offset by the energy content of biogas [34]. According to the results, the energy efficiency was not significantly correlated with applied voltage during phases II and III. In phase IV, the result shows that lower HRTs resulted in a major decrease of energy efficiency which was related to reducing activity of methanogens and the subsequent methane production. The suitable net energy recovery of MEC performance could substantially lower the treatment cost of petrochemical wastewater (Fig. 3).

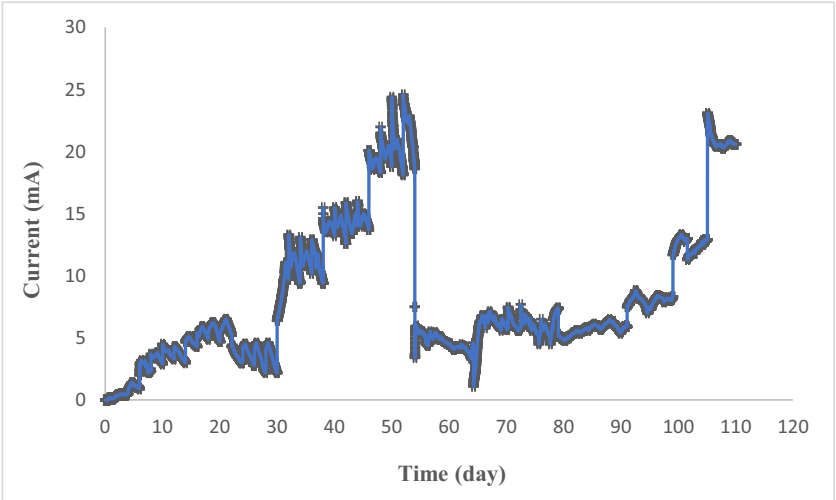

Fig. 3 Current change in MEC system during all phases

The pH level

The pH level is a key parameter which affects the performance of the bioelectrochemical systems. Also, Methanogens are sensitive to changes in pH and the optimal pH range of methanogen activity is 6.8–7.2 [16, 35, 36]. If pH be different from the optimized pH range of methanogens, the methanogenic activity decreased. However, Methanogens grow at a neutral pH range (6–8). Hu et al. was reported that MEC with a mixed culture achieved a maximum biogas production rate of with an suitable current density at pH 5.8–7.00 [37]. While O'Flaherty et al. was reported that above and below the pH range of 7.00–7.50, the growth rates of methanogens could be inhibited [38].

The pH of the effluents was measured throughout experiments. In the startup phase, (days 0–10), the organic content of the petrochemical wastewater was converted to organic acids and the pH dropped to about 6.3. In days 15–30, when the intermediate compounds were converted via methanogenesis bacteria and microbial electrolysis, the pH started to enhance. So that at the end of the startup phase, pH reached to 7.3 ±0.1 in MEC. In the phase II, the pH reached to about 8.1 (0.6 V), 8.3 (0.8 V), 8.6 (1 V), respectively. This suggests that the pH of the MEC increased with increasing of applied voltages. These results seem to be due to the excessive use of hydrogen ion, cathodic reduction of CO_2 or H^+ for producing CH_4 or hydrogen, respectively. It seems that the decrease of methane production can be related to the increase of the pH level [39].

In phase III, the pH level was appropriate for growing methanogens in the MEC system. During this phase, the pH was suitable for growing and overcoming the methanogens in the MEC. In continuous mode, the pH of effluent maintained a value of 7–7.4, throughout the experiments, while, its value was 8.1–8.6 in the batch mode. In continuous phase, the applied voltages can increase the level of pH, maintain the pH at a favorable range, and therefore, enhance the growth of methanogens. A previous study suggests that the applied voltages in the MEC can lead to the pH enhancement, maintaining pH at a favorable range, and therefore, improving the growth of methanogens [40]. Also, the decreasing of HRT can lead to a decline in pH in MEC systems. The pH partially decreased as the HRT decreased from 72 to 48 h (7.4 to 7.3), while the decrease HRT from 48 to 24 and then from 24 to 12 h lead to decline pH to 6.7 and 6.2 respectively. So, the results show that optimal pH for methanogens is between 7 and 7.4.

The production of methane

Methane production was detected after 14 days of operation in MEC. Because of the low growth rate of methanogens at the earlier days (0–15 d), methane production was very low in the startup phase. Thus, most of the hydrogen was produced in the first 5 days of the startup and most of the methane was generated after 21 days. The methane production trend increased rapidly from the 20th day to a maximum of 40 ml around days 30. At the end of the first phase, the methane production rate in the MEC system was more than its rate in the control system. It seems that the biofilm developed on the electrode might help to enhance methane production rate in the MEC.

In the phase II, the methane production was simultaneously increased from 55 ±1 mL to 58 ±1 mL and from 58 ±1 ml to 63 ±1 ml when the applied voltage increased from 0.6 V to 0.8 V and from 0.8 V to 1 V.

By contrast, the maximum cumulative hydrogen production of the MEC system at the applied voltages of 0.6 V, 0.8 V and 1 V were 22 ±1, ml 20 ±1ml and 9 ±1mL at HRT of 48 h, respectively. At the end of this phase, the values of hydrogen, methane, and carbon dioxide were accounted as 11.1%, 75.3% and 5.1% within the biogas produced in MEC at 0.6 V. In phase II, the maximum of methane production in MEC system was almost 1.6 times higher than that of MEC in phase I. Also, this amount was 1.4 times then the maximum of methane production in MEC control reactor in phase II. In phase II, according to the empirical formula (Eq.7), the estimated methane production converted from COD removal Under a temperature of 35 °C and ambient pressure was 137.5, 152.5 and 156.9 mL for each of voltages 0.6, 0.8 and 1 V respectively. The ratio of measured methane production to estimated values was 40%, 38% and 40.1%, respectively. The ratio reached the peak when the applied voltage was 1 V.

In phase III, the methane yield increased from voltage 0.6 V to 1 and reached to a maximum value of 79 ±1 mL at 1 V. The trend of methane yield change was similar to that of the COD removal variation, so that both reached the max on 1 V. In this phase, the maximum methane production rate was 64±1 mL with a hydrogen content of 19.8% and a methane content of 70.1% at an applied voltage of 0.6 V. When the applied voltage increased to 0.8 V, the maximum methane production rate increased to 75±1 mL with a hydrogen content of 15.4% and a methane content of 74.7%. Finally, as the applied voltage increased to 1 V, the maximum methane production rate increased to 79±1 mL with a hydrogen content of 8.3% and a methane content of 78.9%. In phase III, both COD removal rate and methane production were higher when the applied voltage was controlled at 1 V. The estimated methane production converted from COD removal was 99.6, 131.2 and 132 mL for each of voltages 0.6, 0.8 and 1 V respectively. In this phase, the ratio of measured methane production to estimated values was 64.2%, 57.1% and 59.8%, respectively.

In phase IV, the proportion of methane and methane production rate increased as the HRT decreased from 72 to 48 h, which is most likely due to increased loss of organic compounds [15]. The trend of methane production rate variation was similar to the COD removal change, both got to the max at HRT of 48 h. By contrast, the gradual decrease of HRT from 48 to 12 h led to decrease of methane production. The decrease of CH_4 production in the both reactors was because the acidic pH due to the organic acid accumulation under a short HRT. This trend is probably because of the partial washout of the methanogenic biomass. Also, the previous studies suggested that this effect could be a result of substrate inhibition at lower HRTs. During the anaerobic process, when HRT decreased, the methanogenesis process changed into the acidogenesis process. Therefore, hydrogen content is increased. Also, the results show that the low and high organic loading rates were suitable for methanogenesis and hydrogen-producing bacteria, respectively [41]. Actually, both methanogens and exoelectrogens were influenced by the acidic pH caused by higher influent load [42]. However, it seemed methanogens were more sensitive to acidity than exoelectrogens. Based on the results, the hydraulic retention time has a greater effect than the applied voltage on methane production.

In phase IV, the estimated methane production converted from COD removal was 108.3, 132, 116.6 and 54.5 mL at HRTs of 72, 48, 24 and 12 h respectively. The calculations showed that the ratio of measured methane production to estimated values was 66.5%, 58.3%, 50.6% and 36.5%, respectively.

$$1mLCH_4 = \frac{1mmolCH_4}{22.4}\frac{273.15k}{308.15k}\frac{8meqe^-}{mmolCH_4}\frac{8mgCOD}{meqe^-} = 2.53mgCOD \quad (7)$$

Tests performed in the control system revealed that, at all HRTs, comparable amounts of methane were produced in the absence of applied voltages. Operation in open-circuit conditions (control system) was associated with lower CH_4 production rates with a biogas mostly consisted of CO_2.

Considering the results, as the applied voltage increased along with increasing COD removal, the proportion of carbon dioxide decreased, while methane production was increased. Also, the proportion of hydrogen gas changed by the applied voltage. The result shows that in the MEC not only a greater amount of substrate was consumed, but also a significant amount of the CO_2 was diverted to the methane gas production.

In MEC systems, bioelectrochemical production of methane was theoretically continued in two pathways. Firstly, the methane generation through the direct electron transfer mechanism (reaction 2). Secondly, the methane generation by the indirect electron transfer mechanism. In the direct electron transfer mechanism, H^+ is used for the cathodic methanogenesis, which led to the increased pH. In this study, the hydrogen gas was observed in all of the phases in different conditions. The existence of hydrogen in the biogas in all tests suggests that the indirect mechanism is predominant rather than the direct mechanism [16] (4).

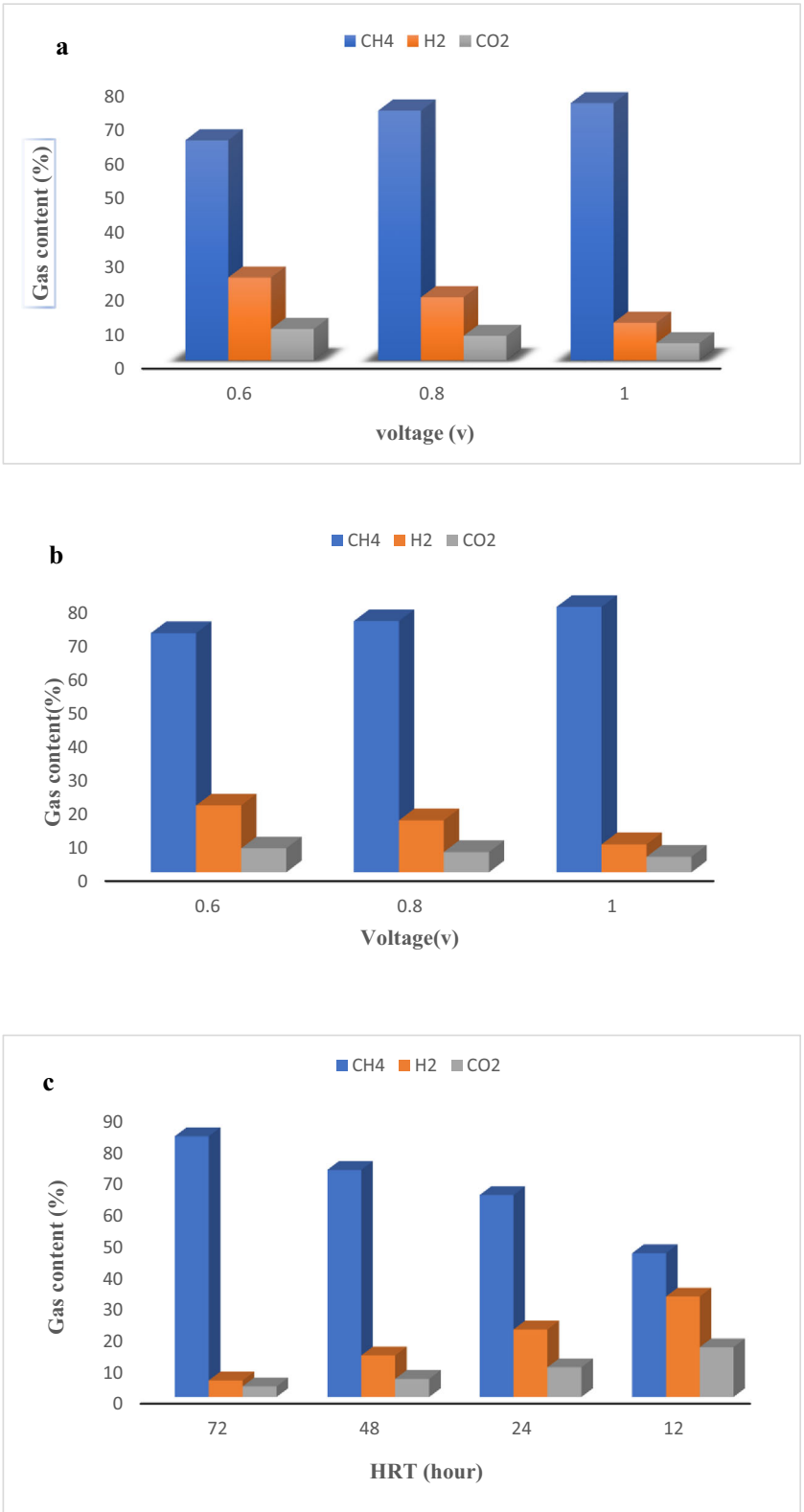

Fig. 4 Changes in the biogas content over the course of MEC performance. Phase II (**a**), phase III (**b**), phase IV (C)

Microorganisms morphology observation by SEM

The biofilm of the anode was analyzed by SEM to characterize its structure and morphology. SEM imaging shows that the surface of the anode carbon cloth includes the formation of a biofilm layer. Figure 5a has demonstrate that biofilm of the anode was visually dense. As shown in Fig. 5a, most of the carbon cloth

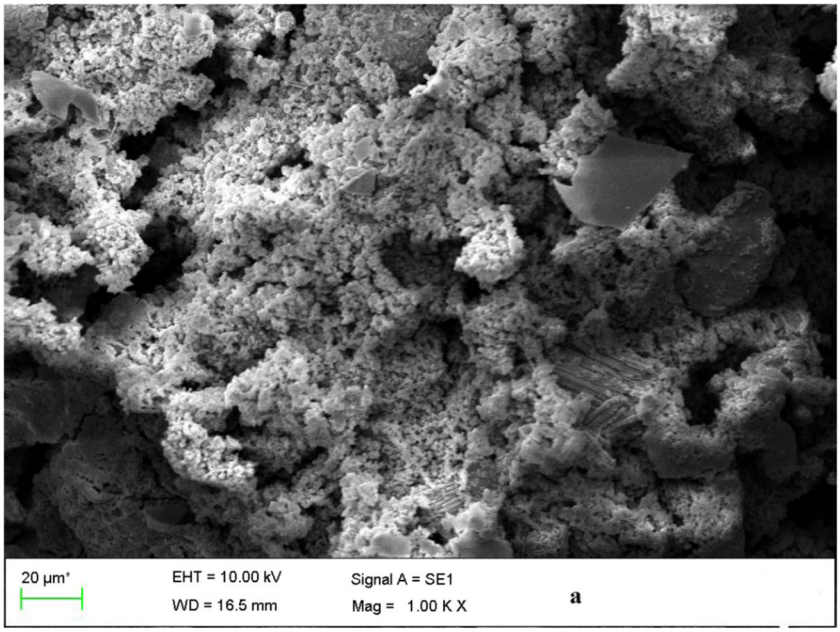

Fig. 5 Photograph of scanning electron microscope of the anodic electroactive biofilm observed after than 110 days of MEC operation. Scale bars are indicated in the bottom left for both pictures

was covered by amorphous layers. The previous study mentioned that the bacterial cells in highly enriched biofilms accumulate as densely packed amorphous layers which completely cover the surface of the anode. [18]. In the present study, anaerobic sludge was used as inoculum source. Therefore, the amorphous structure observed in Fig. 5a could be caused by a microbial biofilm formed due to the long-term operation. Nonetheless, when closely analyzing the pictures with the 1 μm scale bar in Fig. 5.b several rod-shaped structures can be seen. So, this finding indicates that Several distinct morphotypes were present at the anode biofilm.

Conclusions

The influence of several variables such as applied voltage, operation mode, hydraulic retention time on COD removal, methane production rate, the content of methane, current profile, and pH level were determined. In the batch mode, COD removal reached to 85.9% at an HRT of 48 h under an applied voltage of 1 V. While, in continues mode this value reached to 75.3% at an HRT of 48 h under an applied voltage of 1 V. However, the maximum methane production in the continuous mode was almost 1.6 times higher than which in the batch

mode. Considering these results, the increase of applied voltage had a positive effect on COD removal, methane content, and methane production rate. The results also show that the low and high HRTs were suitable for hydrogen-producing bacteria and methanogenesis, respectively. These results suggest that the single membrane-less microbial electrolysis cell is a promising process in order to the treatment of low-strength wastewater and methane production.

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Compliance with ethical standards

Conflict of interest The authors would like to declare that there is no conflict of interest with this research and in the publication.

References

1. Zhang H, He Y, Jiang T, Yang F. Research on characteristics of aerobic granules treating petrochemical wastewater by acclimation and co-metabolism methods. Desalination. 2011;279(1–3):69–74.
2. Yeruva DK, Jukuri S, Velvizhi G, Kumar AN, Swamy Y, Mohan SV. Integrating sequencing batch reactor with bio-electrochemical treatment for augmenting remediation efficiency of complex petrochemical wastewater. Bioresour Technol. 2015;188:33–42.
3. Ebrahimi M, Kazemi H, Mirbagheri S, Rockaway TD. An optimized biological approach for treatment of petroleum refinery wastewater. J Environ Chem Eng. 2016;4(3):3401–8.
4. Hoseinzadeh E, Rezaee A, Farzadkia M. Nitrate removal from pharmaceutical wastewater using microbial electrochemical system supplied through low frequency-low voltage alternating electric current. Bioelectrochemistry. 2018;120:49–56.
5. Arvin A, Peyravi M, Jahanshahi M, Salmani H. Hydrodynamic evaluation of an anaerobic baffled reactor for landfill leachate treatment. Desalin Water Treat. 2016;57(42):19596–608.
6. Arvin A, Peyravi M, Jahanshahi M. Fabrication and Evaluation of anaerobic baffle reactor for leachate treatment of Sari province 2017;19(74):159–171.
7. Jafary T, Daud WRW, Ghasemi M, Kim BH, Jahim JM, Ismail M, et al. Biocathode in microbial electrolysis cell; present status and future prospects. Renew Sust Energ Rev. 2015;47:23–33.
8. Guo X, Liu J, Xiao B. Bioelectrochemical enhancement of hydrogen and methane production from the anaerobic digestion of sewage sludge in single-chamber membrane-free microbial electrolysis cells. Int J Hydrog Energy. 2013;38(3):1342–7.
9. Hoseinzadeh E, Rezaee A, Farzadkia M. Low frequency-low voltage alternating electric current-induced anoxic granulation in biofilm-electrode reactor: a study of granule properties. Process Biochem. 2017;56:154–62.
10. Kadier A, Kalil MS, Abdeshahian P, Chandrasekhar K, Mohamed A, Azman NF, et al. Recent advances and emerging challenges in microbial electrolysis cells (MECs) for microbial production of hydrogen and value-added chemicals. Renew Sust Energ Rev. 2016;61:501–25.
11. Gulhane M, Khardenavis AA, Karia S, Pandit P, Kanade GS, Lokhande S, et al. Biomethanation of vegetable market waste in an anaerobic baffled reactor: effect of effluent recirculation and carbon mass balance analysis. Bioresour Technol. 2016;215:100–9.
12. Sangeetha T, Guo Z, Liu W, Cui M, Yang C, Wang L, et al. Cathode material as an influencing factor on beer wastewater treatment and methane production in a novel integrated upflow microbial electrolysis cell (Upflow-MEC). Int J Hydrog Energy. 2016;41(4):2189–96.
13. Baek G, Kim J, Lee S, Lee C. Development of biocathode during repeated cycles of bioelectrochemical conversion of carbon dioxide to methane. Bioresour Technol. 2017;241:1201–7.
14. Heidrich ES, Edwards SR, Dolfing J, Cotterill SE, Curtis TP. Performance of a pilot scale microbial electrolysis cell fed on domestic wastewater at ambient temperatures for a 12 month period. Bioresour Technol. 2014;173:87–95.
15. Escapa A, San-Martín M, Mateos R, Morán A. Scaling-up of membraneless microbial electrolysis cells (MECs) for domestic wastewater treatment: bottlenecks and limitations. Bioresour Technol. 2015;180:72–8.
16. Moreno R, San-Martín M, Escapa A, Morán A. Domestic wastewater treatment in parallel with methane production in a microbial electrolysis cell. Renew Energy. 2016;93:442–8.
17. Wang Y, Guo W-Q, Xing D-F, Chang J-S, Ren N-Q. Hydrogen production using biocathode single-chamber microbial electrolysis cells fed by molasses wastewater at low temperature. Int J Hydrog Energy. 2014;39(33):19369–75.
18. Carmona-Martínez AA, Trably E, Milferstedt K, Lacroix R, Etcheverry L, Bernet N. Long-term continuous production of H2 in a microbial electrolysis cell (MEC) treating saline wastewater. Water Res. 2015;81:149–56.
19. Shen R, Liu Z, He Y, Zhang Y, Lu J, Zhu Z, et al. Microbial electrolysis cell to treat hydrothermal liquefied wastewater from cornstalk and recover hydrogen: degradation of organic compounds and characterization of microbial community. Int J Hydrog Energy. 2016;41(7):4132–42.
20. Ren L, Siegert M, Ivanov I, Pisciotta JM, Logan BE. Treatability studies on different refinery wastewater samples using high-throughput microbial electrolysis cells (MECs). Bioresour Technol. 2013;136:322–8.
21. Tenca A, Cusick RD, Schievano A, Oberti R, Logan BE. Evaluation of low cost cathode materials for treatment of industrial and food processing wastewater using microbial electrolysis cells. Int J Hydrog Energy. 2013;38(4):1859–65.
22. APhA A. WEF (American public health association, American Water Works Association, and water environment federation). 1998. Standard methods for the examination of water and wastewater 1998;19.
23. Eaton AD, Clesceri LS, Greenberg AE, Franson MAH. Standard methods for the examination of water and wastewater. American public health association. 2005;1015:49–51.
24. Logan BE. Microbial fuel cells. Wiley 2008.
25. Cai W, Liu W, Yang C, Wang L, Liang B, Thangavel S, et al. Biocathodic methanogenic community in an integrated anaerobic digestion and microbial electrolysis system for enhancement of methane production from waste sludge. ACS Sustain Chem Eng. 2016;4(9):4913–21.
26. Yossan S, Xiao L, Prasertsan P, He Z. Hydrogen production in microbial electrolysis cells: choice of catholyte. Int J Hydrog Energy. 2013;38(23):9619–24.
27. De Vrieze J, Gildemyn S, Arends JB, Vanwonterghem I, Verbeken K, Boon N, et al. Biomass retention on electrodes rather than electrical current enhances stability in anaerobic digestion. Water Res. 2014;54:211–21.

28. Jourdin L, Grieger T, Monetti J, Flexer V, Freguia S, Lu Y, et al. High acetic acid production rate obtained by microbial electrosynthesis from carbon dioxide. Environ Sci Technol. 2015;49(22):13566–74.
29. Elreedy A, Tawfik A, Kubota K, Shimada Y, Harada H. Hythane (H2+ CH4) production from petrochemical wastewater containing mono-ethylene glycol via stepped anaerobic baffled reactor. Int Biodeterior Biodegrad. 2015;105:252–61.
30. Ren L, Ahn Y, Hou H, Zhang F, Logan BE. Electrochemical study of multi-electrode microbial fuel cells under fed-batch and continuous flow conditions. J Power Sources. 2014;257:454–60.
31. Koch C, Popiel D, Harnisch F. Functional redundancy of microbial anodes fed by domestic wastewater. ChemElectroChem. 2014;1(11):1923–31.
32. Zhao Z, Zhang Y, Quan X, Zhao H. Evaluation on direct interspecies electron transfer in anaerobic sludge digestion of microbial electrolysis cell. Bioresour Technol. 2016;200:235–44.
33. Van Eerten-Jansen MC, Heijne AT, Buisman CJ, Hamelers HV. Microbial electrolysis cells for production of methane from CO2: long-term performance and perspectives. Int J Energy Res. 2012;36(6):809–19.
34. Cusick RD, Logan BE. Phosphate recovery as struvite within a single chamber microbial electrolysis cell. Bioresour Technol. 2012;107:110–5.
35. Wu T, Zhu G, Jha AK, Zou R, Liu L, Huang X, et al. Hydrogen production with effluent from an anaerobic baffled reactor (ABR) using a single-chamber microbial electrolysis cell (MEC). Int J Hydrog Energy. 2013;38(25):11117–23.
36. Cai W, Han T, Guo Z, Varrone C, Wang A, Liu W. Methane production enhancement by an independent cathode in integrated anaerobic reactor with microbial electrolysis. Bioresour Technol. 2016;208:13–8.
37. Hu H, Fan Y, Liu H. Hydrogen production using single-chamber membrane-free microbial electrolysis cells. Water Res. 2008;42(15):4172–8.
38. O'Flaherty V, Mahony T, O'Kennedy R, Colleran E. Effect of pH on growth kinetics and sulphide toxicity thresholds of a range of methanogenic, syntrophic and sulphate-reducing bacteria. Process Biochem. 1998;33(5):555–69.
39. Feng Y, Zhang Y, Chen S, Quan X. Enhanced production of methane from waste activated sludge by the combination of high-solid anaerobic digestion and microbial electrolysis cell with iron–graphite electrode. Chem Eng J. 2015;259:787–94.
40. Yin Q, Zhu X, Zhan G, Bo T, Yang Y, Tao Y, et al. Enhanced methane production in an anaerobic digestion and microbial electrolysis cell coupled system with co-cultivation of Geobacter and Methanosarcina. J Environ Sci. 2016;42:210–4.
41. Elreedy A, Tawfik A. Effect of hydraulic retention time on hydrogen production from the dark fermentation of petrochemical effluents contaminated with ethylene glycol. Energy Procedia. 2015;74:1071–8.
42. Li Y, Zhang Y, Liu Y, Zhao Z, Zhao Z, Liu S, et al. Enhancement of anaerobic methanogenesis at a short hydraulic retention time via bioelectrochemical enrichment of hydrogenotrophic methanogens. Bioresour Technol. 2016;218:505–11.