Effects of organic strength on performance of microbial electrolysis cell fed with hydrothermal liquefied wastewater

Shen Ruixia[1], Lu Jianwen[1], Zhu Zhangbing[1], Duan Na[1], Lu Haifeng[1], Yuanhui Zhang[1,2], Liu Zhidan[1*]

(1. *Laboratory of Environment-Enhancing Energy (E^2E), and Key Laboratory of Agricultural Engineering in Structure and Environment, Ministry of Agriculture, College of Water Resources and Civil Engineering China Agricultural University, Beijing 100083, China*; 2. *Department of Agricultural and Biological Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA*)

Abstract: Microbial electrochemical technology has drawn increasing attention for the treatment of recalcitrant wastewater as well as production of energy or value-added chemicals recently. However, the study on the treatment of hydrothermal liquefied wastewater (HTL-WW) using microbial electrolysis cell (MEC) is still in its infancy. This study focused on the effects of organic loading rates (OLRs) on the treatment efficiency of recalcitrant HTL-WW and hydrogen production via the MEC. In general, the chemical oxygen demand (COD) removal rate was more than 71.74% at different initial OLRs. Specially, up to 83.84% of COD removal rate was achieved and the volatile fatty acids were almost degraded at the initial OLR of 2 g COD/L·d in the anode of MEC. The maximum hydrogen production rate was 3.92 mL/L·d in MEC cathode, corresponding to a hydrogen content of 7.10% at the initial OLR of 2 g COD/L·d. And in the anode, the maximum methane production rate of 826.87 mL/L·d was reached with its content of 54.75% at the initial OLR of 10 g COD/L·d. Analysis of electrochemical properties showed that the highest open circuit voltage of 0.48 V was obtained at the initial OLR of 10 g COD/L·d, and the maximum power density (1546.22 mW/m^3) as well as the maximum coulombic efficiency (6.01%) were obtained at the initial OLR of 8 g COD/L·d. GC-MS analysis revealed the existence of phenols and heterocyclic matters in the HTL-WW, such as 1-acetoxynonadecane and 2,4-bis(1-phenylethyl)-phenol. These recalcitrant compounds in HTL-WW were efficiently removed via MEC, which was probably due to the combination effect of microbial community and electrochemistry in MEC anode.

Keywords: microbial electrolysis cell, corn stover, hydrothermal liquefaction, recalcitrant wastewater, hydrogen production, organic strength

DOI: 10.3965/j.ijabe.20171003.2879

Citation: Shen R X, Lu J W, Zhu Z B, Duan N, Lu H F, Zhang Y H, et al. Effects of organic strength on performance of microbial electrolysis cell fed with hydrothermal liquefied wastewater. Int J Agric & Biol Eng, 2017; 10(3): 206–217.

1 Introduction

Global energy demands and the foreseeable depletion of fossil fuels have prompted the development of renewable or sustainable green fuels[1,2]. Hydrogen was suggested as one of the most promising energy carrier due to its highest energy content by weight, and its only product is clean water after combustion[3].

In recent years, microbial electrochemical cell (MEC), as a route of hydrogen production, has attracted

Received date: 2016-09-12 **Accepted date:** 2016-12-26

Biographies: Shen Ruixia, PhD candidate, research interest: wastewater treatment, Email: shenruixia@cau.edu.cn; **Lu Jianwen**, PhD candidate, research interest: bio-refinery, Email: jianwenlu2012@126.com; **Zhu Zhangbing**, PhD candidate, research interest: bio-refinery, Email: zhuzhangbing@163.com; **Duan Na**, Senior Engineer, research interest: anaerobic fermentation, Email: **Lu Haifeng**, Associate Professor, research interest: microalgae cultivation, Email: hfcauedu@163.com; **Yuanhui Zhang**, Professor, research interests: bio-refinery, Email: yzhang1@illinois.edu.

***Corresponding author: Liu Zhidan**, Associate Professor, research interest: biorefinery and bioenergy, Mailing address: P.O. Box 67, China Agricultural University, No. 17, Qinghua East Road, Haidian District, Beijing 100083. Tel/Fax: +86-10-62737329; Email: zdliu@cau.edu.cn.

increasing interests because it takes place under mild reaction conditions including ambient temperature, normal pressure and neutral pH[4]. Even, MEC can convert the chemical energy of organic matters to hydrogen energy during the process of substrate oxidation[5]. Moreover, compared to conventional dark fermentation, hydrogen production via MEC has its prominent characteristics, such as efficient conversion of feedstock without the limit of thermodynamics and substrate types, and easy control of hydrogen production[6]. MEC can integrate the multi-functions of microbial-electro-chemical processes, which makes the degradation of complex substances with different characteristics possible[7].

However, the efficiency of hydrogen production via MEC presently is far away from those required for full-scale commercial application. The performance of MEC is constrained by various factors including the configuration of MEC system, characteristics of different substrates, materials of electrode, anode and cathode catalyst, spacing between two electrodes, effective area of proton/cation/anion exchange membrane, anode and cathode electrolytes, organic loading rates (OLRs), applied voltages, reaction temperature, pH and hydraulic retention time (HRT), etc. In the recent years, the researches on the substrates of MEC became a hotspot, especially, kinds of wastewater including industrial wastewater, agricultural wastewater and municipal wastewater. MEC technology can not only produce hydrogen, but also treat various wastewaters, so it became an environmentally friendly technology because of its eco-friendly and eco-energy properties and low cost[8,9]. Up to now, different recalcitrant wastewaters were successfully treated via MEC system[10], such as beer wastewater[11], azo dye wastewater[12], switchgrass pyrolysis-derived aqueous phase[13], industrial and food processing wastewater[14], etc.

Then, in this study we chose hydrothermal liquefied wastewater (HTL-WW) as the feedstock for MEC, which was produced during the hydrothermal liquefaction (HTL) process. HTL is an emerging, promising and attractive thermo-chemical conversion technology for converting wet biomass into valuable petroleum-like biocrude oil, which has a higher energy density than the original biomass[15]. However, in addition to biocrude oil, a large amount of wastewater containing various complex toxic organic matters was also released as a byproduct[16]. If this recalcitrant wastewater was directly discharged to the environment without any treatment, it will probably be harmful to animals, plants, and even human health. Researches into HTL have often focused on the production yield and characteristic of bio-crude oil[17], but little attention has been paid to the treatment of HTL-WW[18]. Some researchers tried to treat the HTL-WW using anaerobic digestion technology[19], which is common method in the treatment of organic wastes, at the same time produce biogas like methane[20,21]. However, it was confirmed that part of the organics in HTL-WW cannot be degraded or was toxic to fermentative microorganisms[21]. Because there were some inhibitory molecules for anaerobic digestion existing in the HTL-WW, like furaldehyde, 5-hydroxymethyl furfural, ketones, phenols and aloxyphenolic[22]. However, there is seldom reported on the treatment of HTL-WW using MEC. Based on the microbial-electro-chemical multi-functions of MEC, our recent study has successfully demonstrated the feasibility of a lab-scale continuous MEC fed with HTL-WW from corn stover as anodic substrate for hydrogen production[23]. Hence, in this context, the initial OLR as one of important factors affecting the performance of MECs, was investigated, ranging from 2 g chemical oxygen demand (COD)/L·d to 10 g COD/L·d. Special purpose of this study was to explore the effect of OLRs on the MEC performance including gases production and wastewater treatment efficiency.

2 Materials and methods

2.1 Characteristics of inoculum, electrolyte and wastewater

The inoculum was anaerobic digested sludge which is from the Xiaohongmen Municipal Wastewater Treatment Plant (Beijing, China). The constituents of the sludge were total solid (TS) of 17.14%, fat of 2.40%, protein of 7.20%, cellulose of 12.33%, hemicellulose of 50.24%, and lignin of 2.48%. In the study, synthetic wastewater

was fed as an electron donor for the initial attachment and acclimation of electrochemical active bacteria on the anode. The component of synthetic wastewater was prepared by method of [24,25]: 16 g peptone/L, 11 g beef extract/L, 3 g urea/L, 3.6 g glucose/L, 2.9 g NaCl/L, 0.4 g $CaCl_2$/L, 0.3 g MgSO4·7H_2O/L, 2.8 g K_2HPO_4/L. The catholyte was phosphate buffered saline (PBS: 50 mmol/L), which contains 0.31 g NH_4Cl, 0.13 g KCl, 2.45 g $NaH_2PO_4·H_2O$, and 4.58 g Na_2HPO_4 per liter. In the anode, there was only wastewater to add without PBS. In addition, the pH of all the influent was adjusted to 7 with NaOH (1 mol/L) prior to using, in order to benefit the growth of exoelectrogenic consortium.

HTL-WW was separated from mixed HTL products using vacuum-filtration method, which came from a 1.8 L reactor (Model 4593, Parr Instrument Company, USA) at these set values of initial TS of 20%, the top heating temperature of 312°C, and the stable stir speed of 300 r/min during the HTL experiments. The HTL-WW wastewater had a lower rate of biochemical oxygen demand (BOD) to COD (0.376), so it was determined as one of bio-recalcitrant wastewaters. In addition, other detailed characteristics of this wastewater, like volatile fatty acids (VFAs), pH, suspended solid (SS), total organic carbon (TOC), total carbon (TC), inorganic carbon (IC), ammonia nitrogen (NH_3-N), total nitrogen (TN), total phosphorus (TP) and conductivity were described in Table 1.

The reactor started up in MFC mode initially at the OLR of 2 g COD/L·d. After the enrichment of anaerobic electroactive bacteria for half a year, HTL-WW originated from corn stover was used as substrate of the anode, at the same time the running mode was changed to MEC mode under the applied voltage of 1.0 V. Then one month later, when the MEC system was in stable stage, the initial OLRs of the influent increase in a stepwise way from 2 g COD/L·d to 10 g COD/L·d, which were diluted to every certain concentration in advance with ultrapure water (Water Purification Systems, Integral-3, Millipore, Germany). Under every initial OLR, after at least 3 HRTs, the effluents were sampled to monitor different parameters, such as pH, COD removal rate, VFAs removal efficiency, the degradation of organic compounds and electrochemical properties. And the gases both anode and cathode were collected by a gas-tight balloon.

Table 1 Detailed composition of the hydrothermal liquefied wastewater from corn stover

Parameter	Recalcitrant wastewater
pH	3.24±0.01
BOD_5/mg·L^{-1}	16300.00±12.73
COD/mg·L^{-1}	43408.33±787.82
SCOD/mg·L^{-1}	36303.75±8.84
BOD_5/COD	0.376
TOC/mg·L^{-1}	11525.00
TC/mg·L^{-1}	11531.25±15.03
IC/mg·L^{-1}	5.62±0.02
NH_3-N/mg·L^{-1}	2.11±0.33
TN/mg·L^{-1}	1919.74±15.09
TP/mg·L^{-1}	76.18±2.59
SS/mg·L^{-1}	30.00±7.07
Conductivity/ms·cm^{-1}	5.45±0.01
Total volatile fatty acid/g·L^{-1}	61.66
Formic acid/g·L^{-1}	21.32
Acetic acid/g·L^{-1}	10.34
Propionic acid/g·L^{-1}	2.30
Lactic acid/g·L^{-1}	12.89
Butyric acid/g·L^{-1}	5.03
Succinic acid/g·L^{-1}	9.78
Furfural/g·L^{-1}	0.65
5-hydroxymethylfurfural/g·L^{-1}	0.63

2.2 Reaction configuration

A two-chamber fixed-bed MEC reactor, made in poly-methyl-methacrylate, was used in this experiment with continuous operation mode. Carbon nanotubes were utilized as the conductor material and simultaneously the microbial carrier material of anode chamber. In the cathode, the carbon fiber felt was served as electrode material. In order to cut the cost of MEC system, there was not any biotic or abiotic catalyst in cathode. The working volume of both the anode and the cathode was 290 mL. The proton exchange membrane (PEM) was used to separate the two chambers. A more detailed description about this reactor was in the previous study[23]. Real time voltages were monitored regularly (one time per minute) via a data acquisition system in order to analyze the electrochemical characteristics like current density, power density and the internal resistance (R_{in}).

2.3 Analysis methods

The pH and conductivity of the wastewater were

measured with a pH meter (FE20, Mettler Toledo, Germany) and a conductivity meter (FE30, Mettler Toledo, Germany), respectively. In order to measure the SS, the wastewater samples were filtered via a vacuum pump (SHD-III, Yangguang Kejiao, China) using filter papers (Pore size: 15-20 *u*m; paper diameter: 45-60 mm). After the filtration, the filter paper was put in the drying oven (Shanghai Fuma Company, China) for 2 h at 103-105°C. Finally, the filter paper was weighed by the balance (AL204, Mettler Company, Switzerland).

COD was analyzed by the HACHI analyzer (DR 2800, HACHI, USA). BOD_5 was determined by the BOD analyzer (Trak^{TM}, HACHI, USA). The TOC, TC and IC were measured by a Torch Combustion TOC analyzer (TOC-VCPN, Shimadzu Co., Japan). The TN, TP and NH_3-N were determined following the standard methods[26].

VFAs were detected using the high performance liquid chromatography (HPLC, LC-10 AVP, Shimadzu Company, Japan). The sample was filtered with a 0.45 μm membrane filter prior to testing. The mobile phase was 5 mmol/L H_2SO_4 with a flow rate of 1 mL/min. Organic compounds in the HTL-WW were identified by a gas chromatography-mass spectrometry (GC-MS) (Model QP2010, Shimadzu Company, Japan). The samples were extracted with the ethyl ether in a volume ratio of 1:3. The injection volume of the sample was 1 *u*L and the top 30 peaks in the spectrogram were selected. The detailed process of test was the same as the previous paper[23].

Gases components like hydrogen, methane and carbon dioxide were measured and analyzed by a gas chromatograph (SP-6890, Shandong Lunan Company, China). In addition, the total volume of gases was obtained using a 50 mL or 100 mL injector not a gas flow indicator because of the small amounts production per day.

R_{in} was measured using the electrochemical impedance spectroscopy (EIS) method through an electrochemical workstation (CHI660E, Shanghai Chenghua Company, China), whose three electrodes system (the working electrode, the counter electrode and the reference electrode) were linked to the two electrodes of the MEC, and the other port was output to the computer. Further EIS was tested at the frequency (10^{-2}-10^5 Hz) and amplitude (0.01 V).

2.4 Calculation

In general, COD removal rate (%) stands for the situation of total organic matters removal of wastewater, so we chose this parameter to investigate in the study, the calculation formula as follows:

$$\text{COD removal rate} = \frac{\text{COD}_{\text{influent}} - \text{COD}_{\text{effluent}}}{\text{COD}_{\text{influent}}} \times 100\% \quad (1)$$

The current can be calculated according to the voltage across the external resistance (R_{ex}). The power density (mW/m^3) was calculated by the formula $P=U^2/(R_{ex}\cdot V)$, where U (V: volt) was the voltage of two ends of the MEC, and V (m^3) was the anode volume of MEC system. The calculation formula of the coulombic efficiency (C_E) was the same as the previous study[23].

3 Results and discussion

3.1 Efficiency of HTL-WW wastewater treatment via MEC

The influent characteristics of the MEC are listed in Table 1. The COD removal rate, effluent pH and C_E are listed in Table 2. In spite of the recalcitrant property of HTL-WW, up to 83.8% of COD removal rate in the influent was achieved at the initial OLR of 2 g COD/L·d, and on the whole of the removal efficiency (71.74%-83.84%) was more than 71.74% under different initial OLRs. Even at higher initial OLR of 10 g COD/L·d, the COD removal rate was close to 80.00%. A previous study has demonstrated that 85%-90% COD removal rate could be achieved at lower OLRs (0.31-0.93 g COD/L·d) in electrochemical system fed with industrial wastewaters (vegetable oil industry, chemical industry and glass industry)[27], while our study showed that even at higher OLRs (2-10 g COD/L·d) the similar COD removal rate (71.74%-83.84%) could also be obtained fed with HTL-WW in MEC. The maximum C_E was 6.01% at 8 g COD/L·d. One study reported that C_E decreased with the increase of OLRs ranging from 1.92 g COD/L·d to 4.80 g COD/L·d in single-chamber MFCs fed with fermented wastewater[28], but this phenomena was not found in this study. As shown in Table 2, C_E measured at each OLR

ranged from 6.01% to a value as low as 1.42%. In general, C_E values were lower, indicating that much of the substrate available was consumed by non-exoelectrogens and only a little of them was recovered as current intensity. Likewise, a similar situation occurred in a MEC fed with beer wastewater, in which the content of CH_4 was up to 90.6%, and most of organic matters were utilized by methanogen and fermentative bacterium rather than electro-active bacteria[11]. So it is necessary to limit the growth of methanogens in MEC anode in order to enhance the hydrogen production rate. Moreover, it was proved that if methanogenesis was inhibited, the C_E would improve significantly[29]. The factors of affected C_E were very complicated, and the lower C_E was probably due to the catalyst-free in MEC cathode. Note that it was cost-efficient and environment-friendly to achieve a high COD removal rate (79.47%) at a relatively high OLR (10 g COD/L·d) via MEC in our study. Increasing the anode size of the reactor was beneficial to increase the C_E. However, the methanogens in anode biofilm may be a negative factor for C_E[30]. The lower C_E in this study could be the result of interactions of different substrate, different operation mode (MFC or MEC), the activity of the fermentative bacteria and exoelectrogens, and R_{in} of MEC system.

Abundant carbohydrates existing in corn stover were converted into organic acids through the depolymerization and re-polymerization. So there were higher VFAs concentrations in the influent, which led to the slight acidity of the HTL-WW with a pH of 3.24. But the pH of wastewater was adjusted to 7.00 prior to use in order to meet the growth of electrochemical active bacteria. Various large-molecule organic matters were degraded to intermediate products via MEC, and finally converted into small-molecule VFAs. These VFAs contributed to a decline of pH. The exoelectrogenic consortium would convert VFAs to CO_2 and H_2O, making the pH recover in the end. Table 2 shows that the pH of effluent approached to neutral (7.36-7.70). In addition, VFAs with longer carbon (Figure 1), especially the propionic acid, could inhibit the activity of methanogens in anaerobic fermentation[31]. It would decrease the performance of wastewater treatment, but this phenomenon did not exist in MEC system. Even, there was nearly no VFAs in the effluent. As previously reported[32], respiring bacteria in dark-fermentation could not directly utilize kinds of VFAs except for acetic acid, while firstly VFAs were degraded into acetic acid, at the same time hydrogen was produced during this process. And then hydrogen and acetic acid were used to produce methane by methanogens further. The yield of hydrogen production via dark-fermentation could be 12 mol H_2/mol glucose theoretically, but in practice the value was only 2-3 mol H_2/mol glucose[33]. The reason for this is due to accumulation of VFAs during the dark-fermentation process[34]. But hydrogen production via MEC technology has not this kind of barrier, even many researches have demonstrated that acetic acid could be the ideal substrate for MECs[35,36]. In MEC anode, electroactive bacteria can oxidize acetic acid to produce gases like hydrogen or methane. Thus, from above explanation, it was a high efficiency way to treat wastewater of high VFA concentrations via MEC system.

Table 2 Effects of OLRs on coulombic efficiency, COD removal rate and pH of effluent in MEC

Feedstock	OLRs/g COD·L^{-1}·d^{-1}	Coulombic efficiency/C_E, %	COD removal rate/%	Effluent pH	References
Artificial beer wastewater	1.50-2.00	15	85	5.90-7.30	[11]
Pharmaceutical wastewater	1.98-7.98	-	75-85	-	[37]
Sucrose wastewater	0.04-0.41	4-49	51-82	6.90	[38]
Fermented wastewater	3.84	<1.0	93	-	[28]
Leachate	4.30	1.2	58.5	-	[39]
Industrial wastewater	0.31-0.93	-	85-90	7.3-11.8	[27]
Synthetic wastewater	0.62	1.4	99	-	[40]
Hydrothermal liquefied water	2	2.52	83.84	7.70±0.17	This study
	4	5.69	72.66	7.46±0.11	
	6	1.42	71.74	7.36±0.08	
	8	6.01	78.23	7.60±0.05	
	10	3.08	79.47	7.55±0.03	

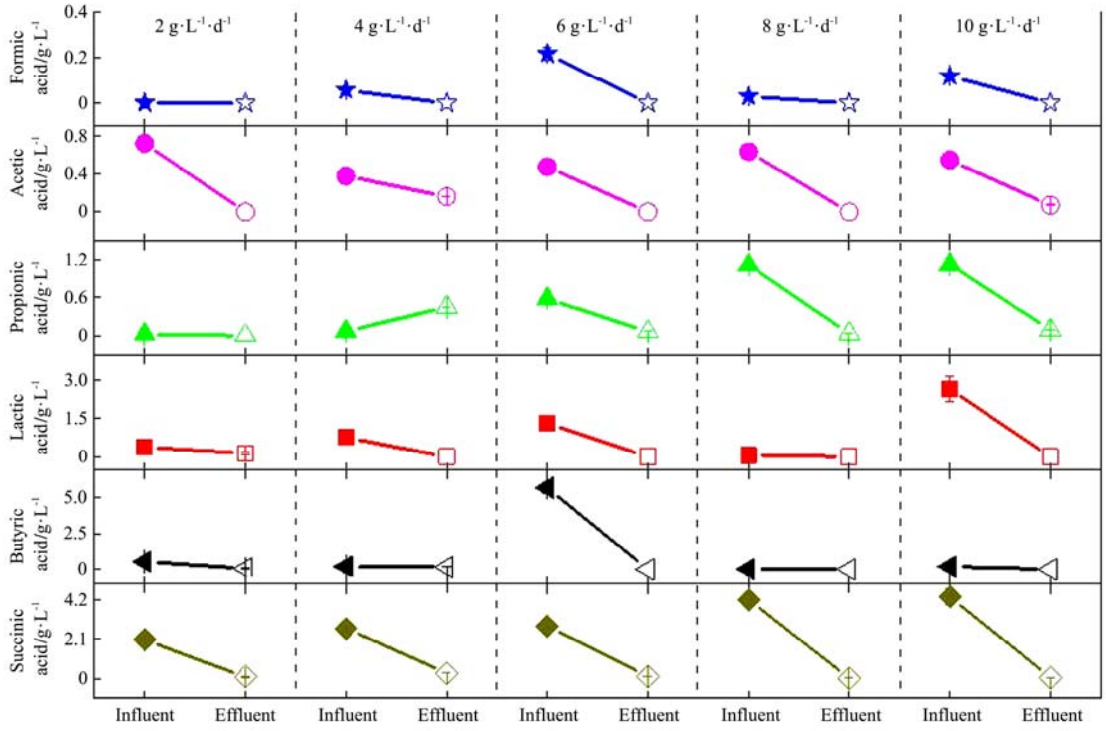

Figure 1 Analysis of VFAs before and after MEC treatment at different OLRs

GC-MS analysis was further carried out to identify the degradation of organic compounds. The higher MEC performance supported that recalcitrant compounds removal was feasible in HTL-WW. Table 3 lists the analysis results of main organic compounds by GC-MS. Major organic compounds in the influent include dibutyl phthalate, 1,4-dimethyl-7-(1-methylethyl)-azulene, glyceraldehyde, 2,2'-methylenebis-phenol, toluene, 2,4-bis(1-phenylethyl)-phenol, 1-bromo-triacontane, 1-Acetoxynonadecane, nitro-methane, etc. Most compounds belonged to phenols and heterocyclic compounds. Many organic compounds were fully degraded after MEC. As shown in Table 3, glyceraldehyde, 1,4-dimethyl-7-(1-methylethyl)-azulene, 2,4-bis(1-phenylethyl)-phenol, 1-acetoxynonadecane, 1-bromo-triacontane, as some typical recalcitrant organic compounds, were almost not detected at the initial OLR of 8 g COD/L·d in the effluent. Thus, there were phenols and heterocyclic compounds-degrading bacteria existing in the MEC anode, which played an important role during degradation. As the previous report, *Desulfovibrio* genus in anode biofilm was related to the degradation of phenolic compounds[41,42]. Thus in later research, the structure of microbial community needs to be analyzed further. Similarly, it was affirmed that other recalcitrant compounds were efficiently removed through microbial electrochemical system, such as landfill leachate[43], palm oil mill effluent[44], *p*-nitrophenol[45], phenol[46], *p*-chloronitrobenzene[47] and 2-chlorophenol[48]. Thus, it may be a promising way to degrade recalcitrant compounds via microbial electrochemical processes.

3.2 Gases production at two electrodes of MEC

Hydrogen generation under different OLRs for the continuous MEC is shown in Figure 2. The maximum hydrogen production rate was 3.92 mL/L·d and hydrogen content was 7.10% in MEC cathode at 2 g COD/L·d. The reason for the lower hydrogen production rate may be explained from the following respects. The complex HTL-WW as one of real wastewaters, had a lower conductivity (5.45 ms/cm), which affect the electron transfer to some extent. In addition, there was no PBS to add in the anode of MEC. It has been reported that PBS played a more important role than conductivity[49]. Even, previous research has demonstrated non-PBS MEC did not work in two-chamber MEC because of the higher pH drop in the anodic chamber[49]. Moreover, some works have showed how to prevent the pH changes for two-chamber MEC, like the use of bipolar membranes[50] and periodic polarity reversal[51]. Interestingly, in this

study there was no the situation of the pH drop according to the pH change between the anodic influent and effluent. So it was inferred that the lower conductivity of the HTL-WW and weak catalysis characteristics of cathode resulted in the lower hydrogen production rate.

Table 3 Analysis results of main organic compounds in MEC influent and effluent by GC-MS at 8 g COD/L·d

Number	Name of compounds	Absolute peak area of compounds	
		Influent	Effluent
1	Methane, nitro-	2 832 066	3 840 789
2	Glyceraldehyde	443 746	ND
3	Butoxyacetic acid	ND	367 374
4	Toluene	6 870 852	8 592 769
5	Phenol, 2-methoxy-	142 258	ND
6	1,3,5,7-Cyclooctatetraene	155 237	152 903
7	Ethylbenzene	155 722	184 370
8	1-Heptanol, 2,4-diethyl-	ND	134 192
9	Tetradecane, 1-chloro-	181 741	ND
10	Cycloheptasiloxane, tetradecamethyl-	205 639	193 340
11	Azulene, 1,4-dimethyl-7-(1-methylethyl)-	226 745	ND
12	Dibutyl phthalate	1 849 544	908 866
13	1-Cyclohexene-1-carboxylic acid	169 406	ND
14	Cyclooctasiloxane, hexadecamethyl-	264 660	272 721
15	Cyclononasiloxane, octadecamethyl-	160 484	262 289
16	Glutaric acid, isobutyl undecyl ester	145 685	142 769
17	Mandelic acid di(tert-butyldimethylsilyl)-	182 290	ND
18	1-Acetoxynonadecane	285 422	ND
19	Phenol, 2,4-bis(1-phenylethyl)-	1 641 175	ND
20	Phenol, 2,2'-methylenebis-	2 958 551	ND
21	15-Isobutyl-(13.alpha.H)-isocopalane	161 108	ND
22	Triacontane, 1-bromo-	278 363	ND

Note: ND stands for "not detected".

In addition, there was a small amount of methane (0.12%-0.34%) appeared in the cathode of MEC, which might derive from the anode through the PEM. According to the MEC cathodic reaction, the only product is hydrogen theoretically, but in practice some other gases can pass through the PEM and diffuse into the cathode. Similar previous research has demonstrated that PEM can allow the permeation of oxygen[52]. Another research confirmed that the gas in cathode was more than biohydrogen of 99.5% with trace amounts of carbon dioxide and methane via electrohydrogenesis, when two chambers were separated using an anion exchange membrane[34]. So here, in cathode of MEC, main gas component was hydrogen with little methane.

Then, with the continuous increase of OLRs, hydrogen production stopped in cathode. But it has been recovered to a certain extent at the initial OLR of 10 g COD/L·d which was 1.49 mL/L·d with a hydrogen content of 0.35%. Hydrogen production rate did not appear to be affected exactly by the different OLRs (Figure 2). It was inferred that some toxic compounds in the HTL-WW affected the activity of exoelectrogenic microorganism with the increase of initial OLRs. According to previous research, there were hundreds of compounds in the HTL-WW[53]. Once the activity of exoelectrogenic microorganism was restrained, methanogens would become the dominant microorganism in anode. Some electrons produced in anode were consumed directly by methanogens[54], so the production of methane was obvious in anode. While electrons cannot arrived the cathode to produce hydrogen in time, and finally hydrogen was not detected. Another reason for this may be trace amount of hydrogen produced in cathode may differ into the anode via PEM, next it was used by methanogens, which was similar to the previous study[52]. With the continuous increase of OLRs, after the acclimatization for long time, the activity of exoelectrogenic microorganism were recovered. When the initial OLR was 10 g COD/L·d, the hydrogen production rate has returned to 1.49 mL/L·d. So it was the combination result of the microbial activity, competitive inhibition of electrons, and hydrogen diffusion through PEM from cathode to anode.

In the anode of MEC, methane, as the dominant gas, showed an increasing trend with the increase of OLRs ranging from 2 g COD/L·d to 10 g COD/L·d, similar to the previous study[55]. The maximum methane production rate was 826.87 mL/L·d and its content was 54.75% at the initial OLR of 10 g COD/L·d. Clearly methane production rate in anode was much better than that of hydrogen in cathode, which hinted a significant activity of methanogenic archaea proliferation in anode. As previously reported, hydrogen production would be negatively affected in a single-chamber MEC if the content of methane was more than 1.50%[56]. But Dhar et al.[32] found there was no methane in the MEC anode fed with sugar beet juice. This was attributed to the differences of substrates, exchange membranes, initial pH of influent, etc. In addition, other microorganisms in

anode could consume hydrogen which leaked through the PEM. And according to previous research, shortening the retention time may be a tentative strategy of enhancing hydrogen production in the MEC system[57].

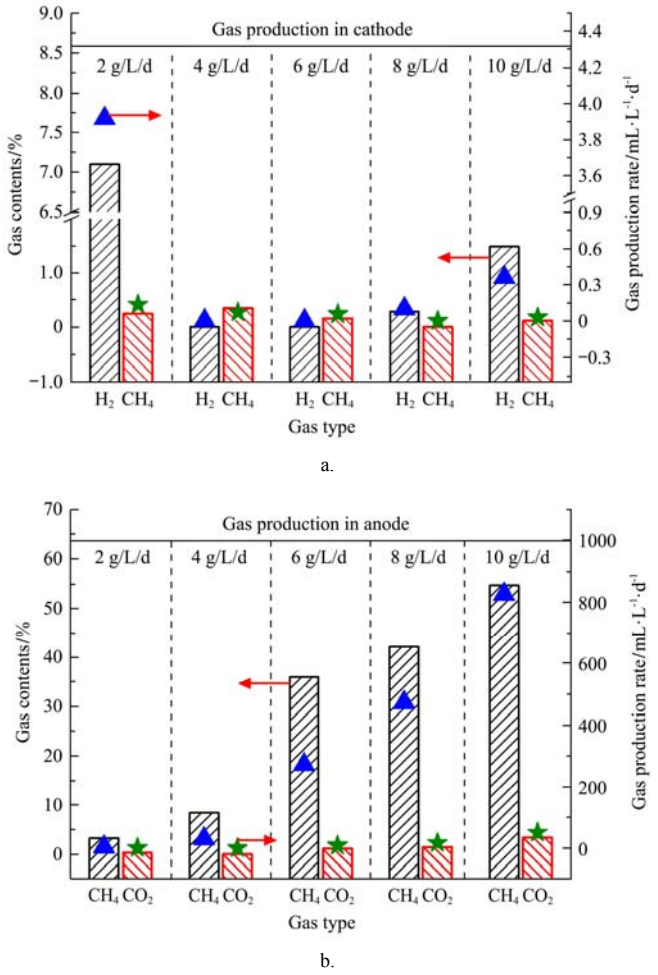

Figure 2 Effect of OLRs on gas production at the cathode (a) and the anode (b)

3.3 Electrochemical characteristics of MEC at different OLRs

The electrochemical properties of MEC at different OLRs were illustrated in Figure 3. The maximum open circuit voltage was 0.48 V at 10 g COD/L·d, and the maximum power density was 1546.22 mW/m^3 at 8 g COD/L·d. The maximum power density increased with the increase of current density from 2 g COD/L·d to 8 g COD/L·d. However, the power density suddenly decreased when the OLR was up to 10 g COD/L·d. This result was similar to Juang et al.[58]. There was a good linear relationship between the maximum power density and current density at different OLRs (from 0.96 g COD/L·d to 4.94 g COD/L·d) with the R_{ex} of 1000 Ω, but different anodic inoculum had different relationships. For example, the coefficients of determination were 0.95 if *Pseudomonas putida* was used as the inoculum, but the value was only 0.76 when *Arthrobacter polychromogenes* was used. This was due to the high or low surface charges on microbial cell surfaces[59], which resulted in different electron transport capabilities. Moreover, previous results have reported that different substrate natures could affect MEC electrochemical properties and its metabolism, such as the hydrolysis of complex organic matters[28].

The Nyquist plots of MEC at different OLRs (Figure 3c) showed that the R_{in} reached the maximum at the initial OLR of 6 g COD/L·d, while the corresponding C_E was the minimum (1.42%). Thus it hinted that the R_{in} was one of important factors affecting the C_E of MEC. The highest R_{in} was the combination results of polarization R_{in}, ohmic R_{in} and diffusion R_{in}. Supposing that polarization R_{in} was changeless, and the ohmic R_{in} should be decreased with the increase of OLRs, so we ascribed the highest R_{in} at the initial OLR of 6 g COD/L·d to the diffusion R_{in}, which resulted from mass transfer resistance of anodic products in MEC system. At the same time, methane production in anode increased sharply compared with the situation at the initial OLR of 4 g COD/L·d, so it suggested that limitation of mass transfer accelerated the process of methanogenesis. In addition, the R_{in} was not only related to surface areas, distance between two electrodes, and material characteristics of electrodes, but also the nature and the composition of wastewater. As we known, C_E indicated the situation of recovered electrons in cathode from total available electrons of anodic substrate (HTL-WW), and low C_E has been a general problem in the electrochemical systems when fed with real, complex or recalcitrant wastewaters[54]. Here, lower C_E may be related to some factors including substrate consumption via competing metabolic processes like fermentation and methanogenesis, the growth of non-exoelectrogenic microorganism, low electron transfer efficiency associated with electrodes, and biocatalyst grazing, etc.[54] In addition, it was reported that reducing the tunable R_{ex} could heighten C_E[57]. Further studies could focus on increasing the R_{ex}, using potential electrode materials,

and adding some cheap catalyst into the MEC system to accelerate the hydrogen evolution reaction.

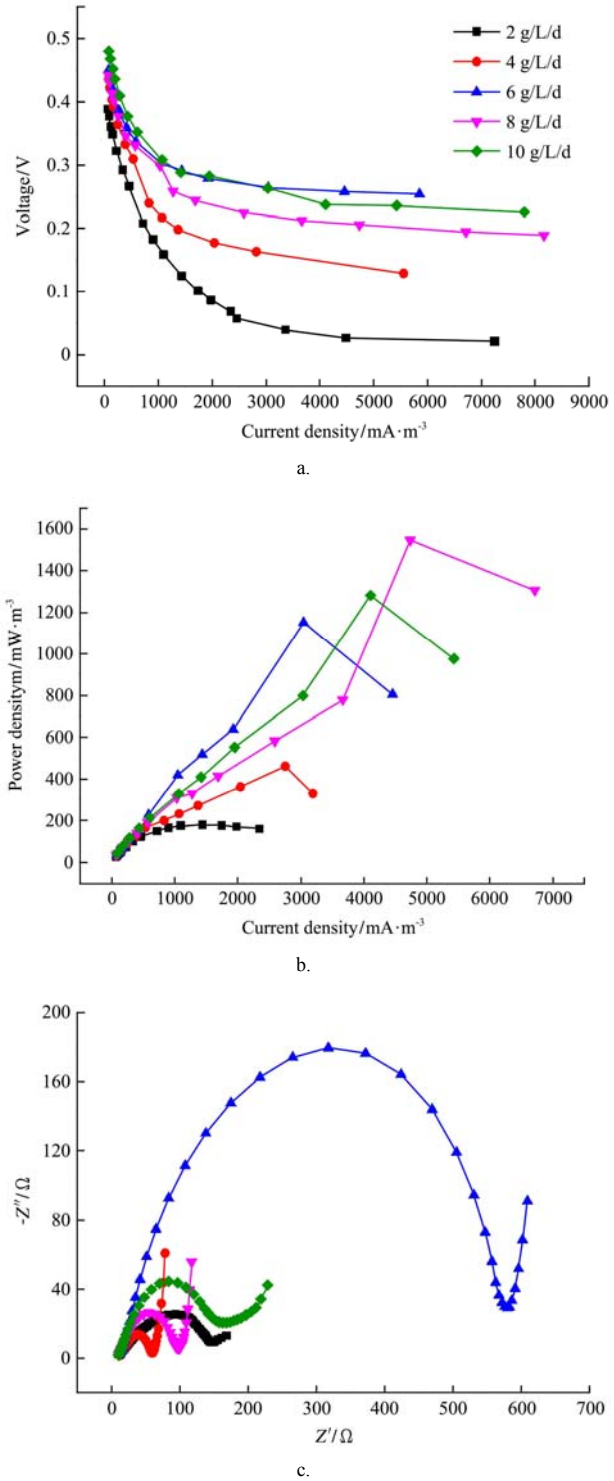

Figure 3 Electrochemical properties of MEC at different OLRs

4 Conclusions

OLRs remarkably affected the MEC performance including gases production, wastewater treatment efficiency, electrochemical properties, recalcitrant compounds removal and C_E. On the whole, the COD removal rate was more than 71.74% under different initial OLRs. Even, up to 83.84% of the COD was achieved at the initial OLR of 2 g COD/L·d. In the cathode of MEC, the maximum hydrogen production rate was 3.92 mL/L·d with a hydrogen content of 7.10% at 2 g COD/L·d. And in the anode, the maximum methane production rate was 826.87 mL/L·d with the content of 54.75% at 10 g COD/L·d. There was nearly no VFAs in the effluent. Further in view of the electrochemical properties, the maximum open circuit voltage was 0.48 V at 10 g COD/L·d, and the maximum power density was 1546.22 mW/m^3 at 8 g COD/L·d. The maximum C_E was 6.01% at 8 g COD/L·d. GC-MS analysis evidenced the existing and degradation of HTL-WW with recalcitrant compounds, such as 2,4-bis(1-phenylethyl)-phenol, 1-acetoxynonadecane, etc. Further work is needed to enhance hydrogen production through improving cathode reactions, optimizing MEC configuration, and better understanding of some microbial community.

Acknowledgements

This work was supported through National Science Foundation of China (21106080, 51561145013) and Beijing Youth Top-notch Talents Program (2015000026833ZK10).

[References]

[1] Croese E, Pereira M A, Euverink G W, Stams A J M, Geelhoed J S. Analysis of the microbial community of the biocathode of a hydrogen-producing microbial electrolysis cell. Appl. Microbiol. Biotechnol., 2011; 92(5): 1083–1093.

[2] Zhang Q G, Wang Y K, Hu J J, Guo J, Zhang Z P, Jing Y Y, et al. Temperature variation of reaction liquid of ultrafine corn stover in photosynthetic hydrogen production. Int J Agric Biol Eng, 2014; 7(5): 65–71.

[3] Chookaew T, Prasertsan P, Ren Z J. Two-stage conversion of crude glycerol to energy using dark fermentation linked with microbial fuel cell or microbial electrolysis cell. New Biotechnol., 2014; 31(2): 179–184.

[4] Rodrigo M A, Cañizares P, Lobato J, Paz R, Sáez C, Linares J J. Production of electricity from the treatment of urban waste water using a microbial fuel cell. J. Power Sources, 2007; 169(1): 198–204.

[5] Pilli S, Ghangrekar M M, Tyagi R D, Surampalli R Y. Effect of cathode biofilm and non-feeding condition on the

performance of membrane-less microbial fuel cell operated under different organic loading rates. Int. J. Environ. Prot., 2013; 2(4): 8–14.

[6] Lu L, Xing D F, Ren N Q. Pyrosequencing reveals highly diverse microbial communities in microbial electrolysis cells involved in enhanced H_2 production from waste activated sludge. Water Res., 2012; 46(7): 2425–2434.

[7] Wang H, Luo H, Fallgren P H, Jin S, Ren Z J. Bioelectrochemical system platform for sustainable environmental remediation and energy generation. Biotechnol. Adv., 2015; 33(3-4): 317–334.

[8] Wang C F, Shao X H, Xu H L, Chang T T, Wang W N. Effects of compound microbial inoculant treated wastewater irrigation on soil nutrients and enzyme activities. Int J Agric Biol Eng, 2016; 9(6): 100–108.

[9] Ma J Q, Zhu H G, Fan M. Distribution of heavy metals in pig farm biogas residues and the safety and feasibility assessment of biogas fertilizer. Int J Agric Biol Eng, 2013; 6(4): 35-43.

[10] Escapa A, Mateos R, Martínez E J, Blanes J. Microbial electrolysis cells: An emerging technology for wastewater treatment and energy recovery. From laboratory to pilot plant and beyond. Renew. Sust. Energ. Rev., 2016; 55: 942–956.

[11] Sangeetha T, Guo Z, Liu W, Cui M H, Yang C X, 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. Hydrogen Energ., 2016; 41(4): 2189–2196.

[12] Fang Z, Song H, Cang N, Li X N. Electricity production from Azo dye wastewater using a microbial fuel cell coupled constructed wetland operating under different operating conditions. Biosens. Bioelectron., 2015; 68: 135–141.

[13] Lewis A J, Ren S, Ye X, Kim P, Labbe N, Borole A P. Hydrogen production from switchgrass via an integrated pyrolysis–microbial electrolysis process. Bioresour. Technol., 2015; 195: 231–241.

[14] Tenca A, Cusick R D, Schievano A, Oberti R, Logan B E. Evaluation of low cost cathode materials for treatment of industrial and food processing wastewater using microbial electrolysis cells. Int. J. Hydrogen Energ., 2013; 38(4): 1859–1865.

[15] Biller P, Sharma B K, Kunwar B, Ross A B. Hydroprocessing of bio-crude from continuous hydrothermal liquefaction of microalgae. Fuel, 2015; 159: 197–205.

[16] Gai C, Zhang Y, Chen W, Zhou Y, Schideman L, Zhang P, et al. Characterization of aqueous phase from the hydrothermal liquefaction of *Chlorella pyrenoidosa*. Bioresour. Technol., 2015; 184: 328–335.

[17] Wang M, Wang X F, Zhu Z B, Lu J W, Zhang Y H, Li B M, et al. Biocrude oil production from Chlorella sp. cultivated in anaerobic digestate after UF membrane treatment. Int J Agric Biol Eng, 2017; 10(1): 148–153.

[18] Zhang L, Lu H F, Zhang Y H, Zhang Y H, Ma S S, Li B M, et al. Effects of strain, nutrients concentration and inoculum size on microalgae culture for bioenergy from post hydrothermal liquefaction wastewater. Int J Agric Biol Eng, 2017; 10(2): 194–204.

[19] Li R R, Ran X, Duan N, Zhang Y H, Liu Z D, Lu H F. Application of zeolite adsorption and biological anaerobic digestion technology on hydrothermal liquefaction wastewater. Int J Agric Biol Eng, 2017; 10(1): 163–168.

[20] Tommaso G, Chen W T, Li P, Schideman L, Zhang Y H. Chemical characterization and anaerobic biodegradability of hydrothermal liquefaction aqueous products from mixed-culture wastewater algae. Bioresour. Technol., 2015; 178: 139–146.

[21] Chen H H, Wan J J, Chen K F, Luo G, Fan J J, Clark J, et al. Biogas production from hydrothermal liquefaction wastewater (HTLWW): Focusing on the microbial communities as revealed by high-throughput sequencing of full-length 16S rRNA genes. Water Res., 2016; 106: 98–107.

[22] Yang X, Lyu H, Chen K F, Zhu X D, Zhang S C, Chen J M. Selective extraction of bio-oil from hydrothermal liquefaction of salix psammophila by organic solvents with different polarities through multistep extraction separation. BioRes., 2014; 9(3): 5219–5233.

[23] Shen R, Liu Z, He Y, Zhang Y H, Lu J W, Zhu Z B, 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. Hydrogen Energ., 2016; 41(7): 4132–4142.

[24] Liu Z D, Liu J, Zhang S P, Su Z G. Study of operational performance and electrical response on mediator-less microbial fuel cells fed with carbon- and protein-rich substrates. Biochem. Eng. J., 2009; 45(3): 185–191.

[25] He Y H, Liu Z D, Xing X H, Li B M, Zhang Y H, Shen R X, et al. Carbon nanotubes simultaneously as the anode and microbial carrier for up-flow fixed-bed microbial fuel cell. Biochem. Eng. J., 2015; 94: 39–44.

[26] Zhang L, Lu H F, Zhang Y H, Li B M, Liu Z D, Duan N, et al. Nutrient recovery and biomass production by cultivating *Chlorella vulgaris* 1067 from four types of post-hydrothermal liquefaction wastewater. J. Appl. Phycol., 2016; 28(2): 1031–1039.

[27] Abbasi U, Jin W, Pervez A, Bhatti Z A , Tariq M, Shaheen S, et al. Anaerobic microbial fuel cell treating combined industrial wastewater: Correlation of electricity generation

with pollutants. Bioresour. Technol., 2016; 200: 1–7.

[28] Nam J, Kim H, Lim K, Shin H S. Effects of organic loading rates on the continuous electricity generation from fermented wastewater using a single-chamber microbial fuel cell. Bioresour. Technol., 2010; 101(1): 33–37.

[29] Kim J R, Min B, Logan B E. Evaluation of procedures to acclimate a microbial fuel cell for electricity production. Appl. Microbiol. Biot., 2005; 68(1): 23–30.

[30] Van Eerten-Jansen M C A A, Heijne A T, Buisman C J N, Hamelers H V M. Microbial electrolysis cells for production of methane from CO_2: long-term performance and perspectives. Int. J. Energ. Res., 2012; 36(6): 809–819.

[31] Cerrillo M, Viñas M, Bonmatí A. Overcoming organic and nitrogen overload in thermophilic anaerobic digestion of pig slurry by coupling a microbial electrolysis cell. Bioresour. Technol., 2016; 216: 362–372.

[32] Liu Z, Zhang C, Lu Y, Wu X, Wang L, Wang L J, et al. States and challenges for high-value biohythane production from waste biomass by dark fermentation technology. Bioresour. Technol., 2013; 135: 292–303.

[33] Logan B E, Oh S E, Kim I S, Van Ginkel S. Biological hydrogen production measured in batch anaerobic respirometers. Environ. Sci. Technol., 2002; 36(11): 2530–2535.

[34] Cheng S, Logan B E. Sustainable and efficient biohydrogen production via electrohydrogenesis. PNAS, 2007; 104(47): 18871–18873.

[35] Venkata Mohan S, Prathima Devi M. Fatty acid rich effluent from acidogenic biohydrogen reactor as substrate for lipid accumulation in heterotrophic microalgae with simultaneous treatment. Bioresour. Technol., 2012; 123: 627–635.

[36] Lindermeir A, Horst C, Hoffmann U. Ultrasound assisted electrochemical oxidation of substituted toluenes. Ultrason. Sonochem., 2003; 10(4-5): 223–229.

[37] Velvizhi G, Mohan S V. Electrogenic activity and electron losses under increasing organic load of recalcitrant pharmaceutical wastewater. Int. J. Hydrogen Energ., 2012; 37(7): 5969–5978.

[38] Kim J R, Premier G C, Hawkes F R, Rodríguez J, Dinsdale R M, Guwy A J. Modular tubular microbial fuel cells for energy recovery during sucrose wastewater treatment at low organic loading rate. Bioresour. Technol., 2010; 101(4): 1190–1198.

[39] Zhang J N, Zhao Q L, You S J, Jiang J Q, Ren N Q. Continuous electricity production from leachate in a novel upflow air-cathode membrane-free microbial fuel cell. Water Sci. Technol., 2008; 57(7): 1017–1021.

[40] Oon Y, Ong S, Ho L, Wong Y S, Dahalan F A, Oon Y S, et al. Synergistic effect of up-flow constructed wetland and microbial fuel cell for simultaneous wastewater treatment and energy recovery. Bioresour. Technol., 2016; 203: 190–197.

[41] Wierckx N, Koopman F, Ruijssenaars H J, de Winde J H. Microbial degradation of furanic compounds: biochemistry, genetics, and impact. Appl. Microbiol. Biot., 2011; 92(6): 1095–1105.

[42] Zeng X F, Borole A P, Pavlostathis S G. Biotransformation of furanic and phenolic compounds with hydrogen gas production in a microbial electrolysis cell. Environ. Sci. Technol., 2015; 49(22): 13667– 13675.

[43] Mahmoud M, Parameswaran P, Torres C I, Rittmann B E. Relieving the fermentation inhibition enables high electron recovery from landfill leachate in a microbial electrolysis cell. RSC Adv., 2016; 6(8): 6658–6664.

[44] Cheng J, Zhu X, Ni J, Borthwick A. Palm oil mill effluent treatment using a two-stage microbial fuel cells system integrated with immobilized biological aerated filters. Bioresour. Technol., 2010; 101: 2729–2734.

[45] Shen J, Feng C, Zhang Y, Jia F, Sun X Y, Li J S, et al. Bioelectrochemical system for recalcitrant *p*-nitrophenol removal. J. Hazard. Mater., 2012; 209: 516–519.

[46] Luo H, Liu G, Zhang R, Jin S. Phenol degradation in microbial fuel cells. Chem. Eng. J., 2009; 147(2-3): 259–264.

[47] Xu X, Shao J, Li M, Gao K T, Jin J, Zhu L. Reductive Transformation of *p*-chloronitrobenzene in the upflow anaerobic sludge blanket reactor coupled with microbial electrolysis cell: performance and microbial community. Bioresour. Technol., 2016; 218: 1037–1045.

[48] Strycharz S M, Gannon S M, Boles A R, Franks A E, Nevin K P, Lovley D R. Reductive dechlorination of 2-chlorophenol by *Anaeromyxobacter dehalogenans* with an electrode serving as the electron donor. Env. Microbiol. Rep., 2010; 2(2): 289–294.

[49] Ruiz Y, Baeza J A, Guisasola A. Microbial electrolysis cell performance using non-buffered and low conductivity wastewaters. Chem. Eng. J., 2016; 289: 341–348.

[50] Rozendal R A, Sleutels T H J A, Hamelers H V M, Buisman C J N. Effect of the type of ion exchange membrane on performance, ion transport, and pH in biocatalyzed electrolysis of wastewater. Water Sci. Technol., 2008; 57(11): 1757–1762.

[51] Jiang Y, Liang P, Zhang C Y, Bian Y H, Sun X L, Zhang H L, et al. Periodic polarity reversal for stabilizing the pH in two-chamber microbial electrolysis cells. Appl. Energ., 2016; 165: 670–675.

[52] Kim J R, Cheng S A, Oh S E, Logan B E. Power generation using different cation, anion, and ultrafiltration membranes in microbial fuel cells. Environ. Sci. Technol., 2007; 41(3): 1004–1009.

[53] Pham M, Schideman L, Scott J, Rajagopalan N, Plewa M J. Chemical and biological characterization of wastewater generated from hydrothermal liquefaction of *Spirulina*. Environ. Sci. Technol., 2013; 47(4): 2131–2138.

[54] Pandey P, Shinde V N, Deopurkar R L, Kale S P, Patil S A, Pant D. Recent advances in the use of different substrates in microbial fuel cells toward wastewater treatment and simultaneous energy recovery. Appl. Energ., 2016; 168: 706–723.

[55] Sun R, Xing D, Jia J, Liu Q, Zhou A J, Bai S W, et al. Optimization of high-solid waste activated sludge concentration for hydrogen production in microbial electrolysis cells and microbial community diversity analysis. Int. J. Hydrogen Energ., 2014; 39(35): 19912–19920.

[56] Hu H, Fan Y, Liu H. Hydrogen production using single-chamber membrane-free microbial electrolysis cells. Water Res., 2008; 42(15): 4172–4178.

[57] Montpart N, Rago L, Baeza J A, Guisasola A. Hydrogen production in single chamber microbial electrolysis cells with different complex substrates. Water Res., 2015; 68: 601–615.

[58] Juang D F, Yang P C, Chou H Y, Chiu L J. Effects of microbial species, organic loading and substrate degradation rate on the power generation capability of microbial fuel cells. Biotechnol. Lett., 2011; 33(11): 2147–2160.

[59] Juang D F. Effects of mono- and di-valent cations on the zeta potential and settling velocity of activated sludge. J. Chin. Inst. Environ. Eng., 2001; 11(1): 11–20.