Performance of air-cathode stacked microbial fuel cells systems for wastewater treatment and electricity production

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ABSTRACT

Two different air-cathode stacked microbial fuel cell (MFC) configurations were evaluated under continuous flow during the treatment of municipal wastewater and electricity production at a hydraulic retention time (HRT) of 3, 1, and 0.5 d. Stacked MFC 1 was formed by 20 individual air-cathode MFC units. The second stacked MFC (stacked MFC 2) consisted of 40 air-cathode MFC units placed in a shared reactor. The maximum voltages produced at closed circuit (1,000 Ω) were 170 mV for stacked MFC 1 and 94 mV for stacked MFC 2. Different power densities in each MFC unit were obtained due to a potential drop phenomenon and to a change in chemical oxygen demand (COD) concentrations inside reactors. The maximum power densities from individual MFC units were up to 1,107 mW/m² for stacked MFC 1 and up to 472 mW/m² for stacked MFC 2. The maximum power densities in stacked MFC 1 and MFC 2 connected in series were 79 mW/m² and 4 mW/m², respectively. Electricity generation and COD removal efficiencies were reduced when the HRT was decreased. High removal efficiencies of 84% of COD, 47% of total nitrogen, and 30% of total phosphorus were obtained during municipal wastewater treatment.

Key words | electricity, hydraulic retention time, series connection, stacked microbial fuel cells, wastewater treatment

INTRODUCTION

Nowadays, wastewater represents a source of recyclable water, despite the fact that it contains many contaminants that may affect public health and the environment. Wastewater contains large amounts of renewable energy in the form of chemical bonds. Domestic wastewater could potentially generate up to 2.2 kW/h.m³ (assuming 500 mg/L chemical oxygen demand (COD)) of energy, which corresponds to 29.3 terawatt-hours or 0.10 quadrillion kJ. If this energy is recovered correctly, it can generate electricity from wastewater (Virdis et al. 2011). Currently, microbial fuel cells (MFCs) can be utilized as a decentralized wastewater treatment, having the advantage of producing bioenergy (electricity, methane, and hydrogen) from wastewater (Logan 2012). Several studies have been carried out in single MFC units (smaller scale) to generate electricity. MFCs could produce up to 15 W/m³ using domestic wastewater (Logan 2008). However, there are only few examples of MFCs with multiple anodes and cathodes. The scaling-up of MFCs requires an intensification process, in order to reduce the size of the reactor, generate high power and COD removal, and reduce the ohmic losses, thus minimizing the voltage reversal in the reactors (Oh & Logan 2007; Kim et al. 2012). Multi-electrode MFCs are the best option so far to troubleshoot some of these problems (Jiang et al. 2010; Ahn & Logan 2012). The theoretical voltage of an individual MFC in open circuit voltage (OCV) mode is ~1.25 V, using glucose as electron donor and oxygen as the electron acceptor, according to the Nerst equation. However, in practice, the voltages produced in a single-chamber MFC are ~0.2 to 0.5 V (Oh & Logan 2007; Kim et al. 2012). In order to increase...
Production, at three different HRTs. Voltage (CCV), during wastewater treatment and electricity and nutrient removal efficiencies. The effects of HRT on voltage production, in stacked MFC systems, decreased power density has been observed (Gurung & Oh 2015). Furthermore, the scalability of MFCs is fundamental for real-world application, not only for increasing the electricity production but also (in terms of the treatment capacity) for removing the contaminants. In order to make a scaled-up MFC that is practical and sustainable, it is necessary to carry out research focused on the terms of installation of multiple anodes and cathodes (stacked MFC configurations), flow distribution, and the effect of the operational variable hydraulic retention time (HRT) on the performance of stacked MFCs. The purpose of this is to increase the capacity of organic utilization and to reduce the cathode limitations and the ionic cross-conduction. The effects of HRT on voltage profiles, organic matter, and nutrient removal efficiencies were not studied in stacked MFCs. Therefore, the optimal selection of HRT in a stacked MFC is an important factor when designing a larger-scale stacked MFC to remove contaminants and to simultaneously generate electricity. The present study has been carried out to evaluate the performance of two new air-cathode stacked MFC configurations (20 and 40 single-chamber MFCs) operated under continuous flow at OCV and closed circuit voltage (CCV), during wastewater treatment and electricity production, at three different HRTs.

**METHODS**

**Construction, start-up, and operation of air-cathode stacked MFC systems**

Two air-cathode stacked MFC systems of ~16 L volume were designed. Stacked MFC 1 module was equipped with 20 individual MFC units (un-shared reactor) (Figure 1(a)). Each individual MFC unit had a rectangular poly-acrylic plastic shape of ~800 mL volume (11.5 cm length × 11.5 cm width × 6 cm height). An anode electrode (carbon felt from Brunssen Inc., Mexico) of 6 cm length and 6 cm width (surface area of 0.0036 m²) was inserted into the rectangular anode compartment. Membrane electrode assemblies (MEAs) of 6 cm length and 6 cm height were placed between anode and cathode and were exposed to air. MEAs consisted of a cation-exchange membrane (CMI-7000, Membrane International Inc., USA) and a carbon cloth (Brunssen Inc., Mexico), which contained a PbO2 layer as catalyst (0.5 mg PbO2/cm²). The lead dioxide was utilized as an alternative catalyst to platinum with the same concentration used in MFC (Morris et al. 2007). Copper wires were utilized as electron collectors. The distance between anode and cathode was 2 cm. Stacked MFC 1 was fed in continuous cascade mode, where the wastewater flow was transported through each of the MFC compartments.

Stacked MFC 2 contained 40 units of air-cathode MFC in a shared reactor. Stacked MFC 2 module was formed by four chambers: each chamber of 70 cm height, 10 cm length, and 5.7 cm width (~4 L volume) containing 10 MFC units without separator (Figure 1(b)). An individual MFC unit was composed of three carbon felts (5 cm length, 5 cm height, and 0.65 cm thickness) and was used as anode (surface area of 0.0075 m²). The three anodes were connected externally by a single copper wire. An MEA (5 cm length, 5 cm width) was exposed to air and was utilized to separate the anodic chamber from the cathode. The distance between anode and cathode was 2 cm and the distance between each MFC unit was 3 cm. The distances between anode and cathode and surface area were selected according to Estrada-Arriaga et al. (2017), which allowed a high power density using a single air-cathode MFC to be obtained. Cheng et al. (2006) showed that a minor distance between the anode and the cathode (0.5–3 cm) in an air-cathode MFC reduces the ohmic losses and the oxygen crossover from the anode to the cathode, thus improving the MFCs’ performance.

The anode electrodes were inoculated with a mixture of 50% raw wastewater from residential housing at Jiutepec, Mexico, and 50% anaerobic granular sludge (56,000 mg volatile suspended solids per litre) from the upflow anaerobic sludge blanket reactor at a paper industry wastewater treatment plant. In order to establish a good microbial community on the anode electrodes, the anodes were immersed into the inoculum for 20 days, and then they were placed into the anodic chambers of the two stacked MFC systems. After that, the two systems were operated at HRT of 10 d at OCV until ensuring a continuous voltage in each MFC unit. This acclimation period allowed the microorganism to adapt to raw wastewater. After achieving a steady acclimation,
stacked MFC systems were operated at OCV and then the OCV was changed to CCV with an external resistor of 1,000 Ω. In each circuit mode, the stacked MFCs were operated at three different HRTs: 3, 1, and 0.5 d. Throughout all tests, stacked MFC systems were operated at shared anolyte mode under continuous flow. The anolyte is the wastewater in the anodic chamber of the stacked MFC.

OCV is voltage which is not connected to any load in a circuit in the absence of current. The electromotive force is a thermodynamic value that does not take into account internal losses. The CCV is voltage obtained when the circuit is connected to an external resistor (load); one current is generated. When the MFCs are connected in OCV or CCV the electrons flow at different rates, then the metabolism of bacteria change, and then the production of electricity and removals of organic matter are different, increasing or decreasing the MFC performance. Also the circuit mode operation in the MFC allows the voltage drop in

the fuel cell to be determined. Furthermore, it is very important to study the stacked MFC system performance in OCV and CCV.

The stacked MFC systems were fed with raw wastewater from residential housing throughout all tests. COD of raw wastewater was \(209 \pm 41 \text{ mg/L}\), and total nitrogen (TN) and total phosphorus (TP) concentrations were \(38 \pm 11 \text{ mg/L}\), and \(15 \pm 3 \text{ mg/L}\), respectively. Other parameters were analyzed in the raw wastewater: total suspended solids (\(147 \pm 20 \text{ mg/L}\)), oil and grease (\(39.5 \pm 12 \text{ mg/L}\)), heavy metal (As 0.0021 mg/L, Cd 0.030 mg/L, Cu 0.05 mg/L, Cr 0.1 mg/L, Hg 0.0010 mg/L, Ni 0.050 mg/L, Pb 0.10 mg/L, and Zn 0.1314 mg/L) and cyanides (0.02 mg/L).

### Analysis and measurements

An electronic device was developed to measure the voltages generated in each individual MFC unit and the voltages of MFC units connected in series during the operation. The voltages generated in each individual MFC unit were monitored every 5 h, and the ones generated in series were monitored every 12 h. The measurements of voltages were collected using a data acquisition system based on LabVIEW software, connected to a personal computer.

Current, \(I\) (mA), was calculated using \(I = V/R_{\text{ext}}\), where \(V\) (mV) is the voltage and \(R_{\text{ext}}\) (\(\Omega\)) is the external resistor. Power density, \(P\) (mW/m²), and current density, \(j\) (mA/m²), were calculated according to \(P = IV/(A+1,000)\) and \(j = V/(R_{\text{ext}}A)\), respectively, where \(A\) (m²) is the surface area of the anode electrode. Power density–current density \((P-j)\) curves in stacked MFCs were obtained by changing the external resistor from 68 \(\Omega\) to 10,000 \(\Omega\) (20 min intervals in each resistor) using a resistor portable box/load bank (10 \(\Omega\)–40 k\(\Omega\)) developed in-house. The polarization curves were controlled using LabVIEW software, connected to a personal computer. For \(P-j\) curves connected in series, power and current density were normalized, based on the total surface area anode electrode (0.0056 m² for stacked MFC 1; 0.0075 m² for stacked MFC 2, multiplied by the number of MFC units in each system). COD, TN, and TP were measured using Standard Methods (APHA/AWWA/WEF 2005).

### RESULTS AND DISCUSSION

#### Start-up and performance of stacked MFC systems at three HRTs

Figures 2 and 3 show the voltages obtained during the operation of stacked MFC systems at open and closed circuit modes. During the start-up (acclimatization period) of the two stacked MFC systems, the individual MFC units produced voltage at OCV, ranging from 0.7 to 567 mV for stacked MFC 1, and from 263 to 600 mV for stacked MFC 2. All individual cell units were acclimated at OCV,
showing a lag phase during the first 5 days. Similar observations have been reported by Ahn & Logan (2012), Hong et al. (2011), and Zhang et al. (2010). However, in these previous studies, MFCs were acclimated at closed circuit mode. It took from 6 to 14 days for the voltages to increase and reach steadiness. High voltage productions in individual cell units were obtained in stacked MFC 2, which was opposite to stacked MFC 1. Different voltages were obtained in each individual MFC unit, indicating that in the two systems, different oxidation-reduction reaction rates were generated and that there was a potential difference on the anode and cathode. This was due to the decreased COD concentrations in each MFC unit and due to the competition between neighboring cells for the harvest of electrons and protons. During the acclimatization period, the voltages generated from stacked MFC 2 in each MFC unit were higher, mainly due to the configuration of the anodes, which allowed a better wastewater flow through the anodes, thus allowing a better development of the biofilm on the carbon felt, increasing electron transport towards the anodes. The competition for the electron donor between electrogenic bacteria and other fermentative or anaerobic microorganisms was perhaps another factor that increased or decreased the voltage in the two stacked MFC systems during the acclimatization or colonization period on anode electrodes.

When the individual MFC units were switched to a connection in series, the maximum voltage during the acclimated period was 707 mV for stacked MFC 1 and 568 mV for stacked MFC 2. When stacked MFC 1 was switched to a different HRT, the voltages in all individual MFC units decreased. For stacked MFC 2, four individual MFC units were affected by the change of HRT. The electricity productions were generated steadily in all individual MFC units and remained positive. The electricity production for stacked MFC 1 and stacked MFC 2 at OCV were 165 ± 51 mV and 535 ± 30 mV, respectively.

Thermodynamically the maximum voltage of one MFC is ~1.1 V at open circuit (without external resistor) depending on the type of substrate being used, e.g. acetate. When the MFC is connected to an external resistance, the voltage decreases to 0.7 V if the conditions are ideal. The voltage decreases due to the internal resistance that occurs inside the MFC (ohmic losses, concentration losses and transfer mass). Hence, the size of the MFC does not increase the voltage. If the surface area of the cathode with respect to the anode is increased, the voltage will not increase to >1.1 V, but will help to better the MFC performance in terms of drop voltage; hence, the internal resistances decrease inside MFC.

The voltage obtained from stacked MFC 1 connected in series was 580 ± 65 mV, using 20 individual cells. For stacked MFC 2 connected in series, the voltage was 540 ± 35 mV, which was similar to the one generated in each individual MFC unit. According to the law of conservation of energy, when the cell units are connected in series, it is expected
that the voltages generated in each cell are the sum of all voltages generated from all cell units \(V_{\text{total}} = V_{\text{MFC1}} + V_{\text{MFC2}} + V_{\text{MFCn+1}}\). This principle was not observed in both stacked MFC systems, even when both systems were operated at open and closed circuits. This was due to a voltage drop, generated within the systems. This behavior has already been reported for other studies from stacked systems during series connections (Oh & Logan 2007; Zhuang & Zhou 2009; Kim et al. 2012). When the individual MFC units were connected in series, a voltage drop phenomenon or voltage reversal occurred due to different factors such as: substrate concentration gradient between cells, insufficient oxygen at the cathode, insufficient fuel, impedance differences, and a lack of catalyst and higher internal resistance (ohmic loss) (Oh & Logan 2007). The protons generated in the anode travel through an anolyte up to the cation-exchange membrane and to the cathode, due to electroneutrality. This oxidation-reduction reaction is faster in the anode than in the cathode. For this reason, when the individual MFC units are connected in series and share the same anolyte, or when the individual cell is in a shared reactor, the voltage drop is caused by ionic cross-conduction between units. This phenomenon was observed in the two stacked MFCs. This ionic cross-conduction was generated by the presence of an internal short current flow (parasitic current) that occurred between anodes and cathodes, due to the way the cells were connected and due to the fact that the anode kinetic loss was lower than the cathode kinetic loss, which generated voltage loss in the two systems. Another factor that caused the voltage drop between units was the architecture of the MFCs, mainly of stacked MFC 2. The individual cells that formed stacked MFC 2 were placed in a shared reactor and these cells were not divided by a separator.

When the MFC units are sharing the same reactor and the distance between each one of them is short, the force with which protons travel from anode to cathode through the anolyte is low, compared to the potential difference between the distance from anode to cathode within their respective MFC \(\text{cell}_n\). In order to increase the transportation force of a proton, which travels from the anode of the \(\text{cell}_n\) to the cathode of the \(\text{cell}_{n+1}\), it would be necessary to move \(\text{cell}_n\) away from \(\text{cell}_{n+1}\) enough to generate a potential difference between the anode of \(\text{cell}_n\) and its cathode, without generating voltage drops between \(\text{cell}_n\) and \(\text{cell}_{n+1}\). However, it would not be a practical approach, since the reactor design would be very large, impacting directly on the operating costs of the system. A practical alternative to decrease the voltage drop and increase the power production in a stacked MFC is to test different external resistors in each individual cell unit when the system is working in series or parallel circuit, until the minor voltage loss of the system is found. In this way, each individual cell will work as a single MFC without the need to change the configuration of the stacked one.

After 53 days of the stacked MFC systems operating at OCV, the HRTs were reduced to 1 and 0.5 d. In both stacked MFC systems, the reduction of the HRTs did not affect the electricity production of each individual MFC unit and of the ones connected in series. When the systems were operated in closed circuit mode (resistor of 1,000 \(\Omega\)), the maximum voltage achieved in stacked MFC 1 was \(46 \pm 28\) mV at HRT of 3 d. At HRT of 3 d, the average voltage was \(30 \pm 8\) mV, lower than in stacked MFC 1. The voltage data from two stacked cells connected in series showed that the voltage drop phenomenon was also observed in closed circuit mode. In closed circuit mode, the voltages decreased slightly due to the high internal resistance. When the HRT decreased on the stacked systems under a resistance, the voltages were affected, with values below 50 mV. The polarization curves of the individual MFC units (Figures 4 and 5) show this effect clearly. These results indicate that the selection of HRT in stacked MFCs is an important factor for scalable design of stacked systems (Ahn & Logan 2012) and it will be necessary to consider the external resistance value as a variable of the design. The overall performance of the two stacked MFC systems was slightly different when they were operated in open circuit mode. Data showed that the performances of the stacked MFC systems were affected in terms of power production at CCV, using an external resistor of 1,000 ohms; indicating that the selection of optimal external load is of vital importance to its performance. High internal resistances were observed in both systems mainly due to the type of configuration of the stacked MFC that was used and to the fact that the same anolyte was shared in all MFCs. In addition to that, the low power production generated in both systems connected in series showed a variation in metabolic activities and a different bacteria kinetic rate during substrate consumption in CCV. A low activity or weak presence of exoelectrogenic bacteria was observed, which decreased the electron transference from bacteria to anode electrode. Therefore, a lower power production was generated in the two stacked MFCs. When the MFC is operated at OCV (external resistance infinite) or at a higher external resistance, the voltage increases but no current is generated. Then, the microorganisms are unable to transfer their
electrons to the anode electrode. At low external resistance, the MFCs generate higher current due to the highest electron transfer to the cathode by the high activity of exoelectrogenic bacteria. The external resistor directly influences the anode potential, anode biofilm structure, and MFC performance. At low external resistance in an MFC, higher anode potential and current is obtained and more electrogenic microorganisms should be able to transfer electrons to the anode and gain more energy. For more sustainable low resistance to improve MFC performance, it is necessary to allow more positive potentials (the anode potential should be as low and the cathode potential as high as possible). The high external resistor decreases the anode potential, increasing the presence or activity of other microorganisms (not electrogenic) (Katuri et al. 2011; González del Campo et al. 2014).

Figure 4 | P–I curves obtained from non-stacked units 1–20 and stacked MFC 1 in series connection using 10 Ω, 68 Ω, 180 Ω, 330 Ω, 680 Ω, 1 kΩ, 3 kΩ, 6 kΩ and 10 kΩ at different HRTs (3, 1, and 0.5 d).
Polarization curves in non-stacked MFCs and series connection of MFCs at three HRTs

Figures 4 and 5 show $P-j$ curves obtained from non-stacked units and from stacked MFCs connected in series. The polarization curves were obtained after running at least three HRT values under continuous flow. Polarization curves help to understand the performance of MFCs (internal resistances: activation losses, ohmic losses, and concentration losses) and determine the maximum power generation of the MFC. The peaks indicate the maximum power density of the MFC with respect to the current density. Likewise, $P-j$ curves were obtained under continuous flow mode. The power and current densities generated by each individual cell under different HRTs were not the same due to differences in the substrate concentrations.
different microbial activities and anode potential generated in each cell and, also, due to the configuration of the stacked MFC. For the two stacked MFC systems, the maximum power densities generated in both systems were obtained with HRT of 3 days. For individual MFC units from stacked MFC 1, the maximum power density was 1,106 ± 1.2 mW/m², which corresponds to the last individual MFC unit (MFC unit 20). The individual cell that showed low power density (peak of power density) was MFC Unit 10 (10 ± 1 mW/m²). For stacked MFC 1 (connected in series), the maximum power density observed was 79 ± 0.65 mW/m² for 14 ± 2 mV, working voltage at external resistor of 680 ohms. The current density was 1.3 ± 0.4 mA/m². The polarization curves of individual cells from stacked MFC 2 showed a maximum power density of 473 ± 2.5 mW/m² for individual cell 10. Individual MFC Unit 13 was the cell that presented a low power density (maximum power 73 ± 0.5 mW/m²). Lower current densities (below 1 mA/m²) were obtained when the individual MFC units were connected in series. The maximum power density obtained from stacked MFC 2 (connected in series) was 4.2 ± 0.6 mW/m². The magnitude of the voltage drop observed in the two stacked systems was higher when the individual MFC units were connected in series. The P-j curves suggest that the ohmic and activation losses were the main action mechanisms during the voltage drop in the two stacked MFC systems connected in series (Oh & Logan 2007; Ieropoulos et al. 2008; Gurung & Oh 2012; An et al. 2015a, 2015b). Current densities were smaller in stacked MFC 2 and hence the anode potentials were lower, which altered the metabolic activities on the anode biofilm and reduced the electro-active bacteria activity as well as the power densities. When all MFCs were connected in series, the power and current density were calculated based on the surface area of the anode electrode. For this reason, the power and current densities were lower with respect to each individual MFC. Also, the lower current densities in the two MFC systems connected in series connection showed that activation losses were the principal mechanism of voltage drop. The two stacked MFC systems at high HRT showed that the power densities were increased due to an increase of microbial activities, both exoelectrogenic and anaerobic, thus obtaining a high power production and COD removal.

**COD removal at different HRTs**

COD removals of stacked MFC systems at different HRTs are shown in Figure 6. The COD of the two stacked system influents was 210 ± 42 mg/L during the tests. The results showed that the COD removal increased when the organic load was reduced from 0.45 ± 0.06 g/L-d (HRT 0.5 d) to 0.075 ± 0.008 g/L-d (HRT 3 d), at OCV and CCV. At HRT of 3 d, maximum COD removals were obtained in both systems. When the cell systems were operated in open circuit mode, COD removal for stacked MFC 1 was 81 ± 12%, and for stacked MFC 2 it was 84 ± 8%. In closed circuit mode, COD removal efficiencies in the two systems decreased by up to 55% at a HRT of 0.5 d. At a HRT of 3 d, COD removal ranged from 70 to 77%. When the organic load in the influent was low due mainly to the

![COD removal during wastewater treatment from stacked MFC systems at different HRTs (3, 1, and 0.5 d).](https://iwaponline.com/wst/article-pdf/76/3/683/451363/wst076030683.pdf)
increase of HRT, COD removal increased, allowing a high metabolic activity of the anodic biofilm community during the utilization of the organic matter. At OCV, COD removals in the two stacked systems were not assisted by an electrical current. So, the microorganisms were unable to transfer their electrons to the anode electrode, thus decreasing the electron transfer to the anode, allowing less power generation and lower growth rate for the electrogenic bacteria, and promoting the growth rate of the anaerobic microorganisms with high consumption of organic matter. This generated an increase of the COD removal, with respect to the CCV. Also, perhaps this was due to an increase of oxygen intrusion in the reactor per amount of COD removed through the membrane since the oxygen was not reduced on the cathode electrode. When the MFC was operated at the external resistor, the electrogenic activity and growth rate of the bacteria increased. A low external resistor (high anode potential) enhances the presence of the anodic electrogenic microorganisms, and a high external resistor reduces the anode potential, thus increasing the presence of different bacterial species that could generate different efficiencies of COD removal (Katuri et al. 2011; Rismani-Yazdi et al. 2011; González del Campo et al. 2014). Therefore, it is important to determine the optimal anode potential range in order to favor both the electrogenic activity and COD removal. On the other hand, TN and TP concentrations in the stacked MFC system influents ranged from 23 to 37 mg/L for TN and from 13 to 22 mg/L for TP. The nutrients were effectively removed only slightly in each cell system (<47%). Effluent concentrations of nitrate were also low in all tests (less than 4 mg/L). At different HRTs (3, 1 and 0.5 d), the removal of TN and TP in two stacked MFCs was not significant.

CONCLUSIONS

The two tested stacked MFC systems connected in series were not effective for power production at OCV and CCV (low power densities), due to higher voltage drop generated by activation and ohmic losses inside the systems. The OCV and CCV of the two systems connected in series were not equal to the sum of the voltages produced in each individual MFC unit. Voltage drops – in the two systems – occurred due to the architecture of the systems (shared reactor) and due to the use of the same anolyte in all MFC units. The maximum power density of stacked MFC 1 (connected in series) was $79 \pm 0.65 \text{ mW/m}^2$ (current density of $1.5 \pm 0.4 \text{ mA/m}^2$). For the individual MFC unit (not connected in series), the maximum power density was $1,106 \pm 1.2 \text{ mW/m}^2$ (current density of $5.5 \pm 0.6 \text{ mA/m}^2$) at a HRT of 3 days. The power production of stacked MFC 2 ($4.2 \pm 0.6 \text{ mW/m}^2$) and its current density ($0.04 \pm 0.006 \text{ mA/m}^2$) were lower compared to the power generated by stacked MFC 1. The results showed that the COD removal increased when the HRT was increased from 0.5 to 3 d.

ACKNOWLEDGEMENTS

Financial support was provided by SEP-COACYT, project CB-2013/221453.

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First received 7 November 2016; accepted in revised form 18 April 2017. Available online 4 May 2017