Bioelectrical evaluation of microbial fuel cell fed with outlet wastewater of the sugar industry in Egypt

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May 5, 2020

Abstract

Background: Evaluation of the electricity generated by the single-chamber mediator–less microbial fuel cell (MFC) fed with sugar industry wastewater (SIW), besides the characterization of bacterial diversity of anodic biofilm. Results: The maximum MFC voltage in open circuit (OCV) mode was 911 mV after 24 operational days, while the closed-circuit voltage (CCV) was 360 mV when 550 Ω as the external load applied. From the polarization curve, the maximum power density of 189.16 mW/m2 as power output was observed at a current density of 370.9 mA/m2. The efficiency of the MFC was measured based on coulombic efficiency and chemical oxygen demand (COD) removal rate. While the CE was 51%, the COD removal efficiency reached 90.4%. The anodic biofilm bacterial diversity was observed through several identifying morphologically, microscopy, and molecularly. The anodic biofilm phylogenetic bacterial consortia based on the molecular analysis of 16S rRNA sequences was indicated seven dominant strains: Pseudomonas aeruginosa, Lactobacillus sp., Enterococcus sp., Aeromonas hydrophila, Bacillus methanolicus, Geothrix fermentans, and Bacillus thermocloacae with similarity value 100% for each strain. Conclusion: These results proposed that SIW bacterial communities in the anodic biofilm have balanced symbiotic behavior, which has been translated into the bioelectricity production in parallel with the SIW substrate treatment.

Introduction

Sugar cane is one of the strategic crops worldly, as well as the Egyptian sugar cane crops, enjoys with a comparative advantage among countries, where Egypt ranks at first grade in the world with average productivity of 13.49 tons/acre in 2016 with annual productivity reached 931.3 million tons (Eladawy, 2017). Meanwhile, Egypt's production of white sugar has been reached about 2.2 tons in the same year. Water enters the sugar industrial stages such as Cane washing, juice clearing, evaporator cleaning, boiler and heaters filters, cooling units and sanitary systems (Delden, 2015). The sugar industry from sugar cane generates a large amount of wastewater, this wastewater re-used again through previous processing stages; however, the residual wastewater has to be treated in wastewater treatment plants before drained into surface water. The drained sugar industry wastewater (SIW) is dominated by high organic content, and the treatment of the SIW consumes a large amount of energy(Ramjeawon, 2000). One of the most promising technologies in recent years is the microbial fuel cells (MFCs) technology due to its significant ability to produce electricity combined with substrate biodegradation among the microbial activity (Pant, Van Bogaert, Diels, & Vanbroekhoven, 2010). The operation of MFCs as bioreactors based on the ability of electroactive microorganisms to exports electrons to the anode through building effective anodic biofilm(Logan et al., 2006; Lovley, 2011). The transmission of electrons forming potential between the electrodes depends on the physiology of the electroactive bacteria forming biofilm in the anode zone and to the terminal electron acceptors in the cathode zone (Pinto, Coradin, & Laberty-Robert, 2018). In the MFCs, the microbes are biocatalysts for electrical power generation from a variety of biodegradable substrates. Many organic substrates act as the MFCs including wastewater, industrial wastewater, and food industrial wastes (Gude,

2016; Rahimnejad, Ghoreyshi, Najafpour, & Jafary, 2011a).The MFCs Classified according to electrons transmission mode from the microbial catalyst to the anode into two types, the first one is the mediated MFC, which external mediators required for electrons transmission (Cao et al., 2019; Lin, Wu, Chiu, & Tsai, 2014; Rahimnejad et al., 2011a). While, the second type is a mediator-less MFC, where the electrons transmit directly from the microbial cell wall by nanowire to the surface of the anode (Roh & Kim, 2012). The MFCs operational key feature is the electroactive microbial content, due to its ability for recovering and releasing electrons from organic substrates (Huarachi-Olivera et al., 2018; Sydow, Krieg, Mayer, Schrader, & Holtmann, 2014). The electrically active bacteria stimulation required an electrons donor, which represented in a carbon source (substrate). The SIW conceder's idealistic carbon source for microbial growth Depending on its chemical properties and its high content of organic compounds(Ansari, 2006). The most significant issues in sugar manufactories industrial wastewater management are the finding of eco-secure treatment systems so that the MFCs can play an important role in the treatment process as well as producing clean electrical energy. The high organic content in SIW represented in high concentrations of the chemical oxygen demand (COD) and the biochemical oxygen demand (BOD), which the BOD5 concentration may reach to 7,000 mg L^{-1}, while COD concentration may be records 10·000 mg L^{-1}(Zhou, Chi, Luo, He, & Jin, 2011). The electrically active microbial content is the main driver that affects the MFCs performance; therefore, the anodic microbial diversity needs to characterized precisely. 16S rRNA gene sequencing is the most famous molecular technique used to analyses the bacterial content involved in MFCs bioelectricity generation (Clarridge, 2004; Drancourt et al., 2000; Pepè Sciarria, Arioli, Gargari, Mora, & Adani, 2019). These molecular techniques include clone library analysis and Denaturing Gradient Gel Electrophoresis (DGGE), which used for analyzing microbial diversity structure (Shimano, Sambe, & Kasahara, 2012). The advantages of DGGE over other techniques are that it is rapid, affordable, and valuable as a first-line bacterial community's investigation (Burr, Clark, Spear, & Camper, 2006).These techniques were used to define the microbial content of the anodic biofilm to know the dynamics of the coexistence of bacterial groups, which involved the bioelectricity production using MFCs.

The current research aimed at the assessment of the single-chamber mediator-less MFC performance and characterizing of the anodic biofilm bacterial structure when SIW was utilized as a bacterial source and electrons donor.

Experimental Methods

MFC configuration and operation

A mediator-less air-cathode single-chamber MFC was used as a bioreactor for bioelectricity production as well as bio-incubator of SIW substrate. Fig. 1 illustrate the diagrammatic design of fabricated MFC. The MFC made-up from Perspex material (Homemade, Assiut University, Assiut, Egypt) with a total inner volume of 60 ml. The MFC design allows the cathode external surface directly contacted with air through small holes while keeping the anode zone within the anaerobic condition. The MFC electrodes have the same size, but the anode made from carbon cloth, while the cathode electrode made from a Microporous Wet Proofed Layer (MWPL) carbon cloth to avoid water leakage (www.fuel cell store.com). The intra-distance between the electrodes is 6 cm without separating membrane and the electrodes surface-active area is 25 cm^2. All tests completed at room temperature of about 30°C, which considered the optimum temperature for the bacteria to be active (Khater, El-Khatib, & Hassan, 2017). The MFC was operated in both, the open circuit mode (without external load) and the closed mode using external resistance (550 Ω) as mentioned in previous literature (Katuri, Scott, Head, Picioreanu, & Curtis, 2011; F. Liu et al., 2019). The performance analysis of MFCs conducted according to the mathematical calculation of the system bioelectricity outputs records as mentioned in latrature (Logan et al., 2006; Oh, Min, & Logan, 2004; Wang, Zheng, Jia, & Zhang, 2014).

Bacterial growth stimulation

The MFC was configured, sterilized, and fueled with SIW as mixed bacterial and carbon source. SIW samples obtained from the effluent of Abu Qurqas Sugar Factory (Minyaw Egypt). The chemical properties

of SIW samples was determined according to the association of official analytical chemists (AOAC) (Horwitz, Chichilo, & Reynolds, 1970) (Table.1). The MFC was operated and the outputs data was recorded continuously during three consecutive cycles of fed-batch operational mode.

MFC operational Procedures

The MFCs was operated under both OCV mode and CCV mode (550 Ω as external resistance). The MFC voltage was recorded momentously among three consecutive operational cycles using a multimeter (FLUKE 289 – TRUE RMS). After the OCV steady maximum voltage was achieved, the polarization curves was plotted by applying external resistances (R_{ext}) from 100 to 10000 Ω (Horwitz et al., 1970). The chemical oxygen demands (COD chromate) were estimated according to the standard methods at the end of one single operational cycle (Association, Association, Federation, & Federation, 1920).

Analysis and calculation

The MFC current (ampere) and the MFC power were calculated from the following equations (Rabaey, Lissens, Siciliano, & Verstraete, 2003; Rahimnejad, Ghoreyshi, Najafpour, & Jafary, 2011b).

$$I = \frac{V_{\mathrm{MFC}}}{R_{\mathrm{ext}}} \tag{1}$$

Where V_{MFC} is MFC voltage, $R_{ext}$is the external resistance, The Current density (mA m^{-2}) was calculated from the followed equation (H. Liu & Logan, 2004; Min, Cheng, & Logan, 2005):

$$C.D = \frac{I}{A} \tag{2}$$

Where I is the current per mA and A is the active area of the anode (m^2). The Power density (PD, mW/ m^2) calculated from the followed equation (Catal, Li, Bermek, & Liu, 2008):

$$P.D = \mathrm{V}_{\mathrm{MFC}}\ x\ C.D \tag{3}$$

The Columbic efficiency (CE), describes the efficiency of the MFC in facilitating the electrochemical reactions for charge (electrons) transmission, i.e. the Current represented in the recovered fraction electrons versus the complete of oxidation of the substrate. The CE was calculated by the followed equations (Catal et al., 2008; Heilmann & Logan, 2006; Ieropoulos, Greenman, Melhuish, & Hart, 2005; Ringeisen, Ray, & Little, 2007):

$$CE = \frac{C_P}{C_T}\ x\ 100\% \tag{4}$$

$$C_T = \frac{F\ n\ \Delta c\ V}{M} \tag{5}$$

Where the C_P is actual current production collected by the anode during one batch cycle was integrated as (C_P = x t) and the C_T is the theoretically available amount of produced coulombs depending on the COD removed in the MFC from the fully oxidation of substrate organic content into CO_2 and water. It was estimated as in formula no.5, where F = faraday's constant (96485 C/mol), n = no. of electrons per mole of substrate (= 4 electrons), Δc is the daily COD removed, V is the inner reactor volume per liter, M = molecular weight of O_2 (= 32 g/mole).

COD removal efficiency calculated as the following equation (Freguia, Rabaey, Yuan, & Keller, 2007; Rodrigo et al., 2007):

$$COD\ removal\ efficiency = \frac{\mathrm{COD_{inlet}} - \mathrm{COD_{outlet}}}{\mathrm{COD_{inlet}}}\ x\ 100 \quad (6)$$

While COD_{inlet} refers to the initial COD concentration (mg/l), COD_{outlet} represents COD concentration (mg/ l) at the end of full operational cycle.

Anodic Bacterial diversity analyses

Under aseptic conditions, 1.0 mL from anolytic bacterial mixed culture sampled from the anodic zone and the suspension centrifuged for 5 min at 4°C and 20,000×g then filtered to obtain the bacterial cells(Mortimer, Petersen, Buchholz, & Holden, 2016). The extracted Bacterial isolates screened on Nutrient Agar medium (NA) plates by the pour plate method. The NA Plates was incubated at 37°C/24h-48h and the characterized colonies were subjected for further purification analyses. The isolated bacterial strains was exposed to the systematic identification lines such as morphological characterization, gram staining, and biochemical tests as described in previous literature (Bernhardt, Pennell, Almer, & Schell, 1991; Sanders, 2012)

2.7. Phylogenetic characterization

The bacterial Isolates was sent to SolGent laboratories (Solgent Company, Daejeon city, South Korea) for 16S rRNA gene sequencing. The Bacterial isolates subjected to solGent lab identification procedures initially SolGent purification bead was used to extract the bacterial strains DNA. Prior to 16S rRNA gene sequencing, the ribosomal rRNA gene was amplified using the polymerase chain reaction (PCR) technique in which Two universal primers were used for amplification: forward primer 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer 492r (5'-TACG GYTACCTTGTTACGACTT-3'). The PCR products purified and sequenced using a PCR purifying kits (Cosmo Genetech, Republic of Korea) the purified PCR products reconfirmed (using size marker) by electrophoreses on 1% agarose gel. Then these clusters sequenced and eluted with the integration of dideoxy nucleotides (dd NTPs) in the mixture of reactions. Further analysis conducted with BLAST of the website of the National Biotechnology Information Center (NCBI). Phylogenetic analysis of sequences conducted using software version 5.05 MegAlign (DNA Star).

The partial 16s rRNA sequences of the selected bacterial strains have been aligned with BLAST (Basic Local Alignment Search Tool) which is a web-based program able to align unknown sequences to thousands of dissimilar and similar sequences in a gigantic Database and show the list of top matches Instantly. BLAST platform performs its alignment by matching up each position of search sequence to each position of the sequences in the database (Robinson, Bohannan, & Young, 2010). BLAST provide accurate positive score for matched nucleotides, as well as it demonstrate the gaps when performing the alignment. The inserted gaps have a negative effect on the degree of the alignment, but in the case of the alignment of a sufficient number of nucleotides as a results of the gap, is to overcome this negative impact and the gap is accepted in the alignment (Bayat, Gaëta, Ignjatovic, & Parameswaran, 2019). These results then used for constructing the phylogenetic tree through determine the alignment score, in "bits" which is translated to the statistical E- value, which estimates the degree of sequences similarly with the database matched sequences (Altschul et al., 1997; McGinnis & Madden, 2004).

Results & discussion

Microbial fuel cell performance

The MFC was operated in a fed-batch mode to encourage the growth of electro-active bacteria to reach the maximum performance. Figure 2 shows the MFC performance over three consecutive cycles under fed-batch manner at OCV mode. It observed that the maximum voltage output may contributed to decreasing cell life, so the voltage generated from the MFC expected to decrease overtime. Through the OCV mode, there are Consecutive increase in voltage, which was reached to about 878 mV after 100 hours of the first operational

cycle, after that, the voltage was stapled for 80 hours before decreased sequentially. Due to the microbial activity and an increase in substrate consumption, the voltage dropped to less than 100 mV at the end of the 10th day of operation. Based on fed batch operational mode the reactor was re-fed with SIW substrate to start the second operational cycle, as a result, the voltage values increased again and the highest value was 900 mV at 13^{th} to 17^{th} day of the MFCoperation then began to decrease again. The Voltage value reached to maximum steady value 910 mV within 24 days under OCV mode of the third operational cycle (Fig. 2). The MFC operated in OCV mode for three consecutive rounds, and at the end of each cycle, SIW was added to reactivate the anodic electrode with active microorganisms and anodic biofilm development [53].

In this study, the MFC was inoculated with SIW as a bacterial cultures source and showed an increase in the bioreactor output voltage over the time. The voltage values are not reported at the start of bioreactor operation on OCV mode because no biofilm has been formed when the bacterial consumption of the substrate started the voltage values was increased in the first OCV operating cycle After that, the cell potential dropped gradually with time . On the other hand, the MFC was operated in closed-circuit voltage mode (CCV) through applying 550 Ω as external resistance. The reactor was operated for three consecutive cycles in fed-batch mechanism as the previous OCV operation. The highest CCV voltage value in the third cycle of operation reached 360 mV (Fig. 3). During all three operational cycles in both OCV and CCV operational modes, the recorded voltage results may be attributed to the anodic biofilm formation, furthermore the ability of bacterial content to release and transfer the electrons to the anode surface directly. The current study results showed that the ability of SIW bacterial content to catalyzing bioelectricity production through the MFCs. In a previous research by Kumar et al. (Kumar, Singh, & Zularisam, 2016), they used the aerobic mixed bacterial culture from sugar mill effluent and their the reactor maximum voltage was 318 mV (1000) with power density140 mW/m^2(1000,50 mA/m^2).

Polarization characteristics

Polarization curves were constructed for examining and analysing the changes in MFCs potential from balance condition based on a streaming current (Walter, Santoro, Greenman, & Ieropoulos, 2019). The polarization curve properties were determined when the voltage reached a stable state in OCV mode and various external loads have been applied. Fig. 4 shows the polarization curve of current and power density against MFC voltage. The maximum obtained MFC power density and current density 189.16 mW/m^2 and 370.9 mA/m^2 respectively. The MFC eternal resistance was obtained from the slope of the plotted voltage versus current as mentioned elsewhere [57] and it was 225 Ω. Parasad et al.(Prasad, Sivaram, Berchmans, & Yegnaraman, 2006) reported that the maximum power density obtained from polarization curves of MFC fed with sugar industry effluents was of 5.62 W m^{-3} at current density reached 6.8 W m^{-3}. The variability in behavior of polarization curves and results may be due to many factors such as electrode composition, bacterial activity as well as internal resistance generated (Prasad et al., 2006). The polarization curve indicated the power harvested by the reactor, which was considered the principal objective of MFCs. In addition, the polarization curves demonstrates the relation between SIW as a carbon source for the bacteria activity and produced power.

Substrate degradation and COD removal

The COD removal rate of the operated MFC was increased continuously overtime. Fig. 5 clarifies the degradation of SIW substrate (COD values) over time. The operated SIW fed-batch MFC was started with initial COD 2300 $mg.L^{-1}$. The graph cleared that there is a correlation between the substrate consumption and the bioelectricity production, where the voltage value on the CCV MFC operational fifth day was 360 mV and COD efficiency percentage was about 70 %. While the maximum removal efficiency was reached by the end of the cycle duty and recorded 90.4%. These results are in line with previously recorded results of Patil et al. (Patil et al., 2009), they revealed that the percentage of COD removal of sugar industrial effluent was more than96 % at the end of the MFC operation time. The coulombic efficiency (CE) referred to the full charge proportion transmitted into the anode over the highest extractible charge after complete oxidation of the substrate (Ishii et al., 2012). The calculated coulombic efficiency was 51%; this result verified the inverse relationship between coulombic efficiencies and substrate concentration. The CE ratio recorded in this study

falls within the CE measurements range of Kumar et al. (Kumar et al., 2016) which reached 72%.

Molecular analysis of the anodic biofilm

The molecular investigation demonstrated that the anodic biofilm were enriched with aconsertium of phylogenetic bacterial strains with no single dominant bacterial species. The anode microbial community was dominated by Firmicutes phylum (60%), accompanied by phyla g-proteobacteria (40%) with six bacterial strains: *Pseudomonas aeruginosa, Lactobacillus sp., Enterococcus sp., Aeromonas hydrophila, Bacillus methanolicus, and Bacillus thermocloacae* (Table. 2) with alignment similarity was more than 99% for each strain. Most of these species were facultative anaerobes, this may be in line with the fact that the species, which dominates the electrons releasing, are limited to the biofilm strains (Chae, Choi, Lee, Kim, & Kim, 2009). The phylogenetic tree has been created based on bacterial partial 16S rRNA gene sequences of MFC anodic biofilm (Fig.6). The phylogenetic profile detected a bacterial verity in the anodic biofilm confined in phylum g-proteobacteria Proteobacteria and Firmicutes phylum. All identified strians was assigned in the genbank and received an accession numbers and the strains was named as:*Pseudomonas aeruginosa* MAR strain*, Lactobacillus sp* MAR strain*., Enterococcus sp* MAR strain*., Aeromonas hydrophila*MAR strain*, Bacillus methanolicus* MAR strain*, and Bacillus thermocloacae* MAR strain.

Conclusion

The bioelectrical performance of mediator-less single-chamber MFC fed with SIW as a microbial source and substrate was determined after three consecutive operational cycles during 33 days. The molecular Analysis of anodic biofilm demostrated that the Bacterial heterogeneity on the anode surface is the main responsible factor of MFC efficiency. According to the MFC COD removal efficiency analysis, the study was indicated that the SIW was oxidized completely, which translated into a direct transmission of the electrons to the anode. The COD removal efficiency of SIW at maximum voltage yield was 91.6% with a coulombic efficiency of 55% was achieved within the operational cycle. The maximum reported open-circuit voltage (OCV) is 910 mV in addition to the system successfully revealed a maximum power density of 189.16 mW/m^2 at a stable current density of 370.9 mA/m^2. Based on 16s rRNA sequences analysis, the anode bacterial comunity was dominated by g-Proteobacteria phylum (57%) followed with phyla Firmicutes (43%) with sex bacterial strains which identified and registered in GenBank as: *Pseudomonas aeruginosa*MAR strain*, Lactobacillus sp* MAR strain*., Enterococcus sp*MAR strain*., Aeromonas hydrophila* MAR strain*, Bacillus methanolicus* MAR strain*, and Bacillus thermocloacae* MAR strain.

ACKNOWLEDGEMENTS

This work is financially supported by Al Azhar University, Assiut, Egypt (www.Azhar.edu.eg). The authors would like to thank Prof. Dr. Kamel El-Khatib (Chemical Engineering at National Research Center) for critical reading, helpful comments, constructive suggestions, and careful corrections for further improvement of this manuscript.

DECLARATION OF CONFLICTING INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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