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ORIGINAL ARTICLE

Microbial diversity structure in acetate single chamber microbial fuel cell for electricity generation

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Abstract This study investigates the performance of acetate feed membrane less single chamber microbial fuel cell and physical characterization of the bio film present on the anode surface using Scanning Electron Microscope (SEM) and 16S rRNA analyzer. The performance has been investigated using Teflon treated carbon paper with 0.3 mg/cm^2 Pt/C loaded as a cathode and carbon paper as an anode. The maximum open circuit potential is noticed as 791 mV, the system successfully revealed a maximum power density of 86.1 mW m^{-2} at stable current density of 354 mA m^{-2} with high coulombic efficiency of 65% at maximum degradation rate of 96%. SEM showed the dense adherence of microorganisms on the anode. 16S rRNA sequencing results indicates phylogenetic mixture in the communities of anodic biofilm and there is no single dominant bacterial species. The dominant phyla are *Firmicutes*, *Gamma Proteobacteria*, *Alpha Proteobacteria*, *Actinobacteria*, with ten dominant microbial strains: *Bacillus firmus*, *Shewanella profunda*, *Bacillus isronensis*, *Brevundimonas bullata*, *Pseudomonas putida*, *Planococcus citreus*, *Micrococcus endophyticus*, *Acinetobacter tandoii*, *Bacillus safensis* and *Shewanella xiamenensis*.

1. Introduction

Clean environment and energy problems are two modern challenges to us, searching for permanent energy resources (renewable energy) is favorable to maintain our life safe with everlasting the environmental energy [1–5]. In a Bio electrochemical system (BES), microorganisms are interacting with electrode using electrons, which are either removed or supplied through a closed circuit. The most described type of BES is Microbial Fuel Cells (MFCs). MFCs are very promising renewable energy resources, which has the capability to employ microbial communities as the catalyst and capture the electricity from a broad range of organic matters. Microbial fuel cell (MFC) is a galvanic reactor that it can

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transfer chemical energy that stored in organic substrate into electricity during biocatalytic activity of microorganisms [5–13]. Produced electrons by microorganisms are transferred to the anode and flooded to the cathode by a resistor. The transfer of the electrons to the cathode of the MFC through a resistor, circuit completing the reaction for sustaining the electric current [14]. The power produced reflects the efficiency of the microbial respiratory chain as a result of the organic substrate degradation [15,16]. Therefore, from the environmental and economic point of view, MFCs could be exploited as a bifunctional system to facilitate simultaneous wastewater treatment and electricity generation [17,18]. There are two general techniques of transmitting the electrons to the anode: First way is Mediated Electron Transfer (MET), the use of external redox mediators to wire the electrode biofilm interaction(s) [19,20]. Second way is a physical adherence between the microorganism and the anode electrode surface surfaces (Direct Electron Transfer, DET) [21,22]. Therefore, the surface layer of the microorganism has to have a conducive surface (e.g. cytochromes or forming nanowire (pili) [23–26]. Microbial community that able to release electrons from degradable substrates in addition to consume an easily oxidizable organic substrates are very t crucial feature that have to be considered [27–29]. In the cathodic electrode, an oxidant is being reduced water molecules. Biodegradable substrate is the source of electrons donor in the MFC [30]. These substrates ranging from a simple molecules as acetate and glucose to complex organic molecules [31]. In the MFC studies, acetate has been the appropriate substrate as many types of wastewater make them more difficult to be utilized as compared to acetate [32]. Acetate is an optimum substrate, it is widely used as carbon source to stimulate electroactive microorganisms [33]. Furthermore, it is usually used as its inertness towards alternative microbial conversions at room temperature [34]. Acetate is the final product of several metabolic pathways for complex carbon source [35]. Activated sludge produce electricity in MFCs from wastewater with electrochemically active bacteria species. Using Pt in cathode materials can enhance the efficiency of a MFC because Pt and Pt black electrodes have a maximum catalytic activity with oxygen rather than graphite materials and carbon-cloth electrodes for cathode constructions [36,37]. 16S rRNA is the most common technique used to analyse microbial communities involved in electricity production [38]. These molecular methods include clone library analysis and Denaturing Gradient Gel Electrophoresis (DGGE), which commonly used for analyzing microbial community composition, diversity and dynamics [39]. The advantages of DGGE over other methods are that it is rapid and affordable for analyzing multiple unknown organisms [40]. These methods used to understand of how the microbial community dynamics is related to the functioning of a bioreactor such as wastewater treating system, the improvement of system design, treatment process, and the methods to monitor and control bioreactors [41,42]. The main objective of this paper is to generate electricity and treatment of artificial wastewater simultaneously, improve the performance of Air–Cathode Single-Chamber Mediator-Less Microbial Fuel Cell (ACSCMMFC), and Characterization of the microbial community structure that develops on the anodes, using Scanning Electron Microscope (SEM) and 16S ribosomal RNA (16S rRNA).

2. Materials and methods

2.1. Microbial fuel cell construction

Air–cathode single-chamber mediator-less microbial fuel cell (ACSCMMFCs) testing fixture was used, the cell with 50 ml volume (6 cm length and 4 cm diameter) was manufactured using transparent perspex as a material of construction (Homemade, NRC, Egypt), the cell with an electrode active area of 25 cm^2. It consists of an anode and cathode both were made from carbon paper (Laydel), The cathode electrode was treated with Poly tetrafluoroethylene (PTFE) (60 % w/v, dispersion in water) diffusion layers on the air-exposed side [43]. The catalyst layer was prepared by mixing 0.3 mg cm^{-2} of 30% Pt loading supported on carbon Vulcan xc-72R and Nafion solution (5% Nafion solution from Aldrich) to form catalyst ink, which painted on only the water facing side to reduce water loss and oxygen diffusion into the MFCs causing an increase in both Coulombic Efficiency and power output as mentioned elsewhere [27,37,43–45]. The catalyst ink composition was 13.8 mg of catalyst, 24.21 mg of carbon Vulcan XC-72R (E-TEK), and 5% Nafion solution, the mixture was ultrasonicated at 60 °C for 30 min. The first layer was obtained by spreading the ink on carbon paper electrode area, using a mask and a brush. Several successive layers were then deposited on top of each other to reach the loading of 0.3 mg cm^{-2}; the previous layers were dried before adding another layer. All electrodes were dried at room temperature for 24 h before use [44]. The anode and cathode electrode were placed on opposite sides, it was connected through an external circuit across different external resistance (open circuit, 550 Ω).The performance of MFCs was evaluated with respect to power generation and substrate degradation.

2.2. Preparation of synthetic media solution

MFC reactor was seeded with mixed culture of aerobic activated sludge obtained from the municipal wastewater treatment plant (Benha municipal sanitation unit). The microbial fuel cell was fed with the acetate synthetic media. The growth media prepared as mentioned elsewhere [27].

2.3. Microbial fuel cell operation

The MFC was inoculated with the adapted aerobic mixed culture (activated sludge); it was operated under fed batch mode of operation. The aerobic sludge was pre-processed by filtration to remove un-dissolved materials. The cathode was facing to air on one side and the Pt loaded side of cathode was faced to the solution, while the node was set to maintain anaerobic conditions. Cell Potential between anode and cathode was recorded every 5 min with a multimeter and data acquisition system (Lab jack U6 – PRO). After steady state of power and electricity generation, polarization curves were obtained by varying external resistance (R_{ext}) from 100 to 12.5×10^4 Ω. The chemical oxygen demands (COD chromate) of the anodic influent and the effluent were analyzed according to the standard method (closed reflux titrimetric method using chromate as the oxidant) at the end of three reproducible voltage cycles [46]. After electricity generation was stabilized, the MFC was operated for several cycles until the cathode-