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Activated Sludge-based Microbial Fuel Cell for Bio-electricity Generation

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Abstract

Based on the catalytic properties of electrochemically active organisms, activated-sludge based-microbial fuel cell (MFC) system was designed for the electricity generation. As a microbial energy source, glucose has been exploited as electron provider. During the incubation time of bacterial culture in a mediated-less MFC, the cell voltage and degradation rates of glucose were determined. The results showed that electricity output was increasing due to the increase of glucose concentration. The MFC displayed a maximum power density of 52 mW/m^2 at stable current density 275 mA/m^2 and a maximum glucose degradation rate 94.4%. However, the electrical current was dropped when the glucose level in the bacterial culture was higher than 5.0 g/l. This fact was confirmed by studying the glucose concentrations using cyclic voltammetry.Concerning to the extracellular electron transfer mechanism(s), the biofilm formation on the anode was visible by Scanning electron Microscope (SEM). SEM showed the intensive adherence of microbes on anodic electrodes.On the other hand, rates of glucose consumption (degradation) were analyzed, the degradation rate was in accordance with the electrical current.Therefore, the current study demonstrates the applicable use of activated sludge-based microbial fuel cells for bioelectricity generation.

1. Introduction

Continued use of petroleum fuel is unsustainable energy source because of depleting their supplies and the accumulation of carbon dioxide in the environment [1]. Increasing demands for energy in addition to the crisis of global warming need a clean, cheap and sustainable energy resources (renewable energy). Renewable energy is the best to keep our life safe and maintain the environmental problems [2–5]. Among several renewable energy sources, Microbial fuel cell (MFC) is a very promising one, which has the ability to use microorganisms (microbial community) and grab the electricity from wide range of organic substances. Basically, it is a galvanic cell that converts energy that stored in chemical bonds of organic substrate into electricity through biocatalytic reaction of microorganism [6–10]. In the anodic compartment of the MFC, the microbe-anode interaction is taking place. Whereas the organic substrate (microbial carbon sources) is fully oxidized into electrons which will be produced in the anode chamber ends up at the cathode, via the external electrical circuit that carries the electrons from the anode to the cathode, completing the reaction and sustaining the electric current [14] .

In the cathode, an oxidant (normally oxygen) is being reduced forming water molecules. Therefore, MFC had been become a major type of bio electrochemical system (BES) [16–19]. There are two common ways of transferring (delivering) the electrons to the anode : Mediated Electron Transfer (MET), the use of exogenous electron mediators to wire the microbe-electrode interaction(s). In this case, the mobility feature of the used artificial redox mediators will

enable extracellular electron transfer [19] . However, the use of addition chemicals is not recommended in the MFC. Consequently, Direct Electron Transfer (DET) was discovered [20] . In this regards, a physical contact between the microbe and the anode surfaces have to be found. Therefore, the outer layer of the microbe has to have a conducive surface (e.g. cytochromes or by forming nano-wire (pili) [21–24]. Which form (biofilm) [23–26] to generate electricity from organic matter. Better understanding of electron transfer is necessary to construct a sufficient MFC system. However, the most important thing is to build up the MFC on good operating conations. Therefore, the selection of microbial community that provide are able to liberate electrons from degradable carbon sources in addition to utilize an easily oxidizable organic substances are very important factors that have to be considered.

Biodegradable substrate (electron donor) is the source of electrons in the MFC [29]. These substrates ranging from a simple compounds such as acetate and glucose to complex organic compounds [30]. Thus, the power produced by MFCs may vary, depending on the availability of the substrate and the capability of microorganisms to metabolize the substrate [31]. Glucose is commonly used as a substrate in MFCs. Several studies have been done using glucose as carbon source in MFCs. It has been reported by Kim et al [32] that the performance of a MFC was dependent on the glucose initiated cells in MFC run for a short time period compared with galactose. On the other hand, Rabaey et al [33] has obtained a maximum power density of 216 Wm-3 from glucose fed-batch MFC using 100 mM ferric cyanide as cathode oxidant. However, the development of high performance microbial fuel cell still needs more improvements and better understanding of reaction mechanism. In recent studies, activated sludge bacteria were shown to produce electricity in MFCs from domestic wastewater with no mediators but with, electrochemically active special bacteria. Thus, the main concern of the current work is to study the performance of activated sludge as a microbial source in a mediator-less single chamber microbial fuel cell (MSCMFC). Based on the utilization of glucose as a degradable carbon source, the charge-discharge cycling performances for the MFC will be tested. The morphology and the heterogenicity of the microbial coverage generated on the anode forming biofilm have been investigated.

2. Material and Methods

2.1. Microbial Fuel Cell Architecture

All MFC tests were carried out using an air-cathode single-chamber mediator-less microbial fuel cell (ACSCMFC) with inner volume of 50 ml. The MFC was designed (Homemade, NRC, Egypt) and implemented using transparent Perspex as a material of construction with an electrode active area of 25cm2. It consists of an anode and cathode. anode was 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. Then, loaded with 0.3 mg cm-2 of 30 % Platinum (Pt) supported on carbon Vulcan XC-72R. The catalyst loaded on only one side (the water facing side) [35, 36] to reduce water loss and oxygen diffusion into the MFCs, causing an increase in both Coulombic efficiency (CE) and power output [26, 27]. The anode and cathode electrode were placed on opposite sides and were connected through an external circuit across different external resistance (open circuit, 550 Ω).

2.2. Preparation of synthetic media solution

MFCs reactors were seeded with aerobic activated sludge (mixed culture) from the municipal wastewater treatment plant (Benha Municipal sanitation unit), which acts as a biocatalyst role in oxidation process of organic matter. The microbial fuel cell was fed with the synthetic wastewater. The media growth was prepared using the following constituents (in grams per liter of deionized water): glucose as the degradable organic substrate (electron donors) $NaHCO_3$, 2.5; NH_4Cl , 0.2 ; KH_2PO_4, 0.42; KCL , 0.33; NaCL , 0.3 ; K_2HPO_4, 1.26 ; $CaCl_2.2H_2O$, 0.15 ; $MgCl_2$, 3.15 ; yeast extract 1. 10 mL of mineral media were added to the previous constituents. The mineral media consists of (gram/ /L liter of deionized water): EDTA, 0.5; $CoCl_2.6H_2O$, 0.082; $CaCl_2.2H_2O$, 0.114, H_3BO_3, 0.01, $Na_2MoO_4.2H_2O$, 0.02 ; Na_2SeO_3, 0.001 ; Na_2WO_4 .$2H_2O$, 0.01; $NiCl_2.6H_2O$, 0.02 ; $MgCl_2$, 1.16 ;$MnCl_2.4H_2O$, 0.59; $ZnCl_2$, 0.05; $CuSO_4.5H_2O$, 0.01 ; $AlK(SO_4)_2$, 0.01, and 2 ml/l from vitamins (B_{12} 2500 µg- B_6 4 mg - B_1 5 mg- Folic acid1000 µg – Nicotinamide 20 mg - D-pantheno l6 mg - Orotic acid 10 mg) All chemicals were in analytical grade and purchased from sigma Aldrich. The value of pH was value was adjusted by NaOH solution to pH 7 using (HANNA pH211). The anode solution was refreshed when the cell voltage decreased below 50 mV.

2.3. Electrochemical procedure

Direct electrode reaction of bacterial cells was examined by cyclic voltammetry. All electrochemical measurements were performed using a computer controlled Gamry Potentiostat/Galvanostat/ZRA G750, which was connected to a three electrode system comprising a carbon paste working electrode, (CPE) a Platinum disc auxiliary electrode and an Ag/AgCl/3M KCl reference electrode. The carbon paste electrode was prepared by thoroughly mixing 1 g of synthetic carbon powder 1-2 micron with 0.4 ml paraffin oil in a small hand mortar. The hollow electrode (5mm) was filled with the carbon paste. The working electrode was electrochemically activated prior to measurements by applying ten cyclic scans from -0.3 to 1.0 V with a sweep rate of 50m V/S in phosphate buffer (pH 7) as a supporting electrolyte. All electrochemical experiments were carried out at room temperature. Shimadzu spectrophotometer

(Shimadzu UV-240, Japan), was used for measurement of the optical density of microbial community at 600 nm (OD600).

2.4. 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. Then, 15 ml sludge were added to 35 ml synthetic media. The cathode was facing to air on one side and the Pt loaded side of cathode was faced the solution, while the node was set to maintain anaerobic conditions. After steady state of power and electricity generation, polarization curves were obtained by varying external resistance (Rext) from 100 to 12.5×10^4 Ω .The chemical oxygen demands (COD chromate) of the anodic influent and the effluent were also analyzed according to the standard method (closed reflux titrimetric method using chromate as the oxidant) [28]. The MFC was operated for three cycles until the anode-biofilm was formed, while the electricity generations of MFC were recorded. Fig.1 is showing the final operation of microbial fuel cell (MFC).

Figure 1: Membrane-less air cathode single chamber microbial fuel cell composed of anode and cathode electrodes connected with wire to close the circuit.

2.5. Analysis and calculation

Cell potential between anode and cathode was recorded every 5 minutes with a multimeter and data acquisition system (Lab jack U6 - PRO).The potentials were related to the current flowing between the electrodes by ohms law

$$I = \frac{V_{MFC}}{R_{ext}} \quad [1]$$

Where V is the recorded potential, R ext is the used external resistance

Current density (mA m-2)

$$C.D = \frac{I}{A} \quad [2]\ 1$$

Where I is the current per mA, A is the projected area of the anode (m2). Power density (PD, mW m-2) output was calculated from voltage across the current as:

$$P.D = V_{MFC} \times C.D \quad [3]$$

The Columbic efficiency, defined as the ratio of total charge actually transferred to the anode from the substrate to the maximum charge if all the substrate removal produced current, i.e. the fraction recovery of electrons recovered as current versus the starting of organic matter if all the substrate oxidized produces current. Columbic efficiency (CE) was determined by:

$$C_E = \frac{Ms\ I\ t_b}{F\ b_{es}\ V_{an}\ \Delta c} \quad [4]2$$

Where Ms is the molecular weight of substrate, tb is time duration for the cycle, F is faraday; (96,485 c/ mol of electrons); Δc is the substrate concentration change over the batch cycle.; bes is the number of mol of electrons produced per mol of substrate (glucose) (b = 24 electrons per mole of glucose),Van (0.05l). The molecular weight for glucose (180 g /mol) [29].

$$COD_{\text{removal efficiency}} = \frac{COD_{inlet} - COD_{outlet}}{COD_{inlet}} \times 100 \quad [5]$$

COD inlet represents the initial COD concentration (mg/l) in the feed and COD outlet denotes COD concentration (mg/ l) in the reactor outlet.

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2.6. Scanning electron microscopy

The surface of the anode electrode was characterized (after 46-days from the incubation with the microbial culture in the MFC system) using scanning electron microscope SEM (JEOL, JXA-840A) to determine the microbial-electrode attachment, possibility of biofilm formation on the anode electrode surface. Technically, the electrode was fixed with 2.5% glutaraldehyde (Sigma-Aldrich) for 4hrs at 40^{o} C .The samples were then washed three times with water and dehydrated by successive immersion in a series of ethanol solutions of increasing concentration (30%, 50%, 70%, 80%, 90 % , and absolute ethanol) for 10 min. Then the specimens were dried, mounted onto specimen stubs using graphite paste, and then the specimens were coated with gold.

3. Results and discussion

3.1. Evaluation of the microbial fuel cell performance

Testing the performance of a new MFC design was carefully done. In the reactor of a membrane-less single chamber microbial fuel cell (MSCMFC), the activated sludge (metabolically active microbial cells) was incubated in the culture medium containing glucose as the microbial energy source. As a result, a gradual increase in the open circuit cell voltage (OCV) was obtained. At 8 days, the voltage value reached to 0.531V, then, the open circuit cell potential was constant when the voltage reach to 0.63 V.On the other hand, a serious drop was found (0.05 V) after 14 days, due to the depletion of glucose in the culture medium. It's an indication that direct interaction can take place between the metabolically active cells and the electrode surface.

Regeneration of Microbial viability and recovery of the cell potential

Addition of fresh medium containing glucose to activate the microbial viability and to stimulate the metabolic pathway led to a recovery of cell potential and the same previous values were obtained. Indicating the reproducibility and the high efficiency of the proposed MFC systems.We proposed that the direct microbial-electrode interaction was due to the biofilm formation on the anode surface. Therefore, after several days the maximum potential (0.72 V) was achieved after 32-days. The main objective of this OCV is to maximize power output and thus obtain the highest current density.

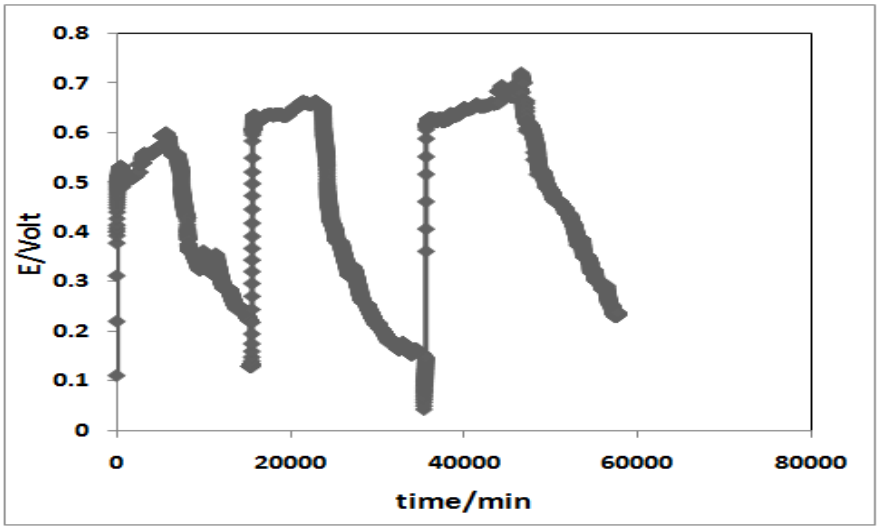

Figure 2: MFC voltage versus time (VT) curve over three cycles of fed batch operation using glucose with a Nafion as binder and a Pt catalyst.

3.2. Electrode characterization

3.2.1. Effect of external resistance

Successive three cycles of open circuit and the potential reached to its maximum value, a fixed external resistance was applied 550 Ω at the anode to the cathode and the cell was operated using glucose with nutrients in fed-batch mode at a final concentration of 2157mg/ l. Fig.(3) Indicates that the current raised gradually at the beginning of the cycles about 0.152 mA after 6 h. then, reach to a steady state at value approximately peak equal to 277 mA after one day then sharply decreased as the same trend for voltage due to consumption rate of glucose in the media. After that, the growth

media were removed and the cell was supplied with another fresh growth media. Once the cell voltage reached to its maximum value (0.189 V), maximum current peak (343 mA) after 7 days sharply decreased. The increasing of current and voltages were dependent on the concentration of glucose. In this regards, different concentrations of glucose (1, 5, 10 and 20 g/l) were incubated in the MFC reactor and the responses were measured. As a result, stimulation of the metabolic pathway resulting from the biodegradation of glucose led to generation of electrical current increasing with the increase of glucose concentration. However, a drop in the current output was occurred when the glucose concretion in the microbial culture was higher than 5g/l. As shown in Fig 4, the peak current output values were 9.6 µA/OD, 15.5µA/OD, 9.4 µA/OD and 6.7 µA/OD at glucose concentration of 1g/l, 5 g/l, 10 g/l and 20 g/l, respectively. This may be due to its inhibitory effects as formation of byproduct such as acetic acid, lactic acid, and formic acid at high concentration of glucose, which inhibit growth of microorganisms, also show deteriorating effect on the metabolic activities. Another reason might be that high concentrations of glucose limit the bacterial growth by inhibiting proteinaceous enzymes; by reducing a cell's ability to breakdown and catabolism of proteinacious resources [31-33].

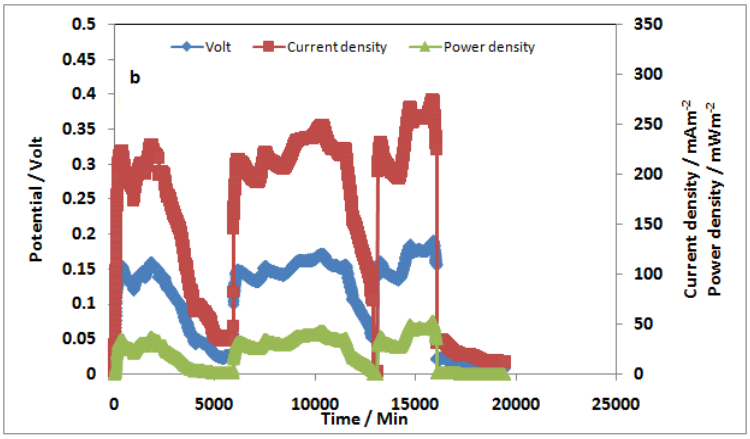

Figure 3: Charge-discharge performances of the mediator less single chamber microbial fuel cell at 550Ω.

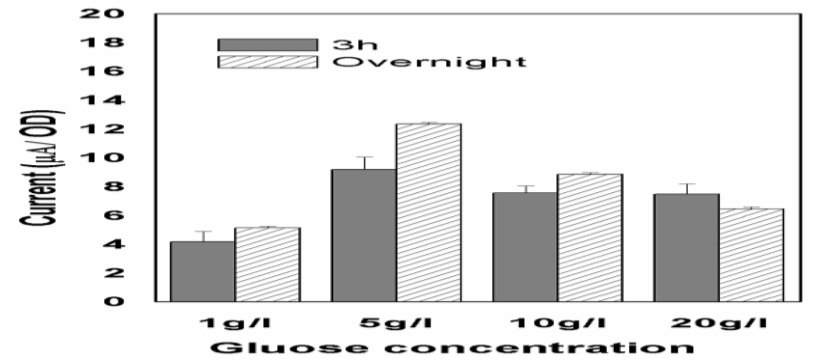

Figure 4: Effect of glucose concentration on peak current after 3 hrs and overnight incubation.

3.2.2. Effect of glucose concentration on Coulombic efficiency

The microbial activity and electrical current generation were correlated with each other. Thus, the glucose consumption rate by microorganisms is regulating the MFC performance.From this aspect, measuring the rate of glucose uptake was done to figure out the relevant relationship. The remained glucose concentration in the microbial culture was measured over the incubation time of the activated sludge in the MFC. The data of Chemical Oxygen Demand (COD) test shown in Fig 5 indicated the strong correlation between the glucose concentration and the cultivation time. By the end of the run (after 8-days), almost the total amount of glucose was consumed by the end of the electrical cycle which led to the serious drop in the cell potential. an increase in COD lead to increase in current production and decrease in an internal resistance but high substrate concentrations have been found to inhibit power generation in MFCs [34, 35] .

The glucose fed batch MFC generated the maximum power density (52 mW/m2) at stable current density (275 mA/m2). These maximum power densities demonstrate that glucose has an effect on the performance of microbial fuel cell. The recovery of electrons from glucose and extraction the electron stored in the glucose as current energy referred as coulombic efficiencies (CE), CE value (55%) . the results COD and CE can be concluded that , there is inversely

relationship between coulombic efficiencies and concentration of substrate. As a result of , high concentration of substrates inhibited the bacteria activities and thus decreased the CE [35].

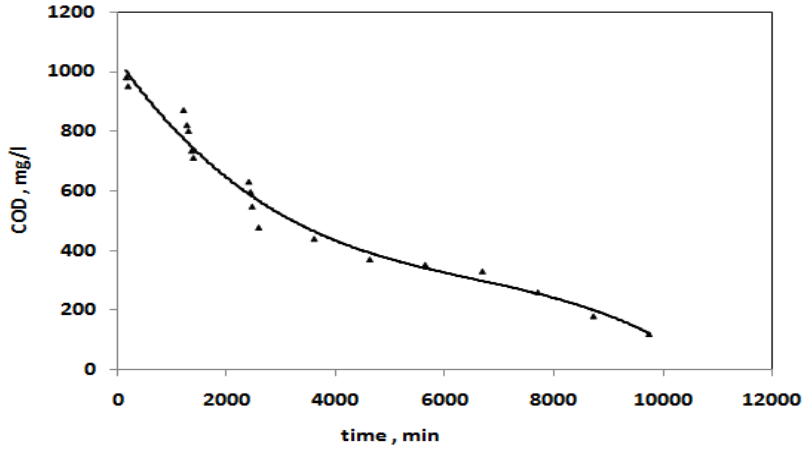

Figure 5: COD vs. time behavior when using glucose as energy source. External load: R = 550 Ω.

3.2.3. Polarization curve & power curve

The microbial fuel cell voltage vs. current density and the power density vs. current density curves for glucose were presented. The experimental data were obtained by varying the external resistance from 100 to 125000 Ω after the addition of fresh glucose a steady state of operation. Polarization curve which represent a powerful tool for the analysis and characterization of fuel cells was plotted with the function of current density against potential [37] as shown in Fig.(6a) . A power curve that describes the power (or power density) as the function of the current (or current density) is calculated from the polarization curve show the useful power produced by the system, which considered as the main goal of MFCs production as presented in Fig.(6b). Internal resistance calculated from the polarization curve from the slope line from the plot of voltage versus current [36]. Calculation of data shows that the internal resistance equals to 99 ohms this indication to lower of internal resistance than the external resistance. The lower the internal resistance the higher the power density as the high internal resistance consumes amount of power output inside MFCs and low electrochemical activity causing decrease of power generation [50, 54].

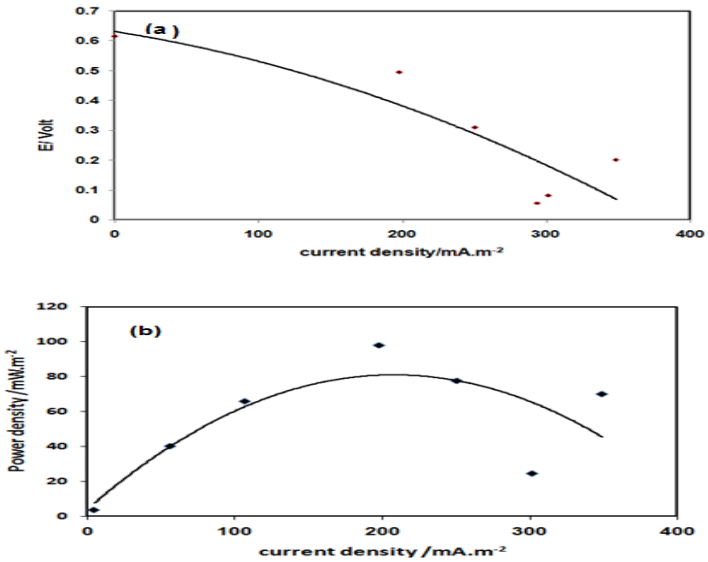

Figure 6 (a, b): Polarization curve and power curve output for glucose.

3.2.4. Bacterial diversity on both types of acetate and glucose anode

The surface of the anode electrode was visualized by scanning electron microscope SEM (JEOL, JXA-840A) to determine microbial attachment and formation of a biofilm on the anode electrode surface. It was removed after 46 days

incubation period. We observed that the anode surface covered completely with bacterial cells, then SEM was applied on anodophilic electrode in order to analyse the presence of microbial attachment and biofilm formation on anode electrodes. Fig. (7) shows the SEM of bare electrode surface in the beginning of experiments before inoculation of MFC with aerobic activated sludge, while Fig. (8) shows the SEM images of inoculated cell, which revealed that presence of abundant microbial attachment on the carbon paper as compared to the control image of plain carbon paper of the glucose fed MFC. The microbial coverage of the anode was partial and heterogeneous. The anode respiring bacteria attach themselves and colonize to the surface of anode, forming a living matrix of protein and sugar. Bacterial cell attach and electrode forming biofilm which able to degrade the substrate. The sticky accumulation (biofilm) was mainly rod shaped, (10µm) and coccied shape (20 µm). The difference in the anodophillic morphology is thought to be induced by the different electro active bacteria, which have the ability to acclimate to the anode electrode and colonize by secreting matrix materials to degrade glucose.

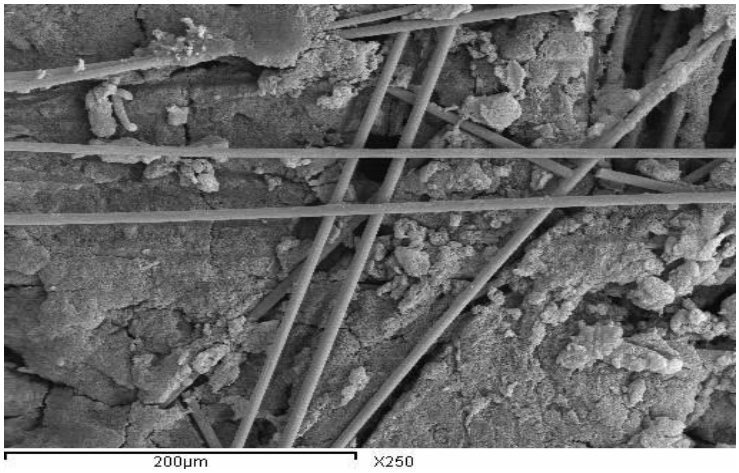

Figure 7: Scanning electron microscope of anode free microbial community.

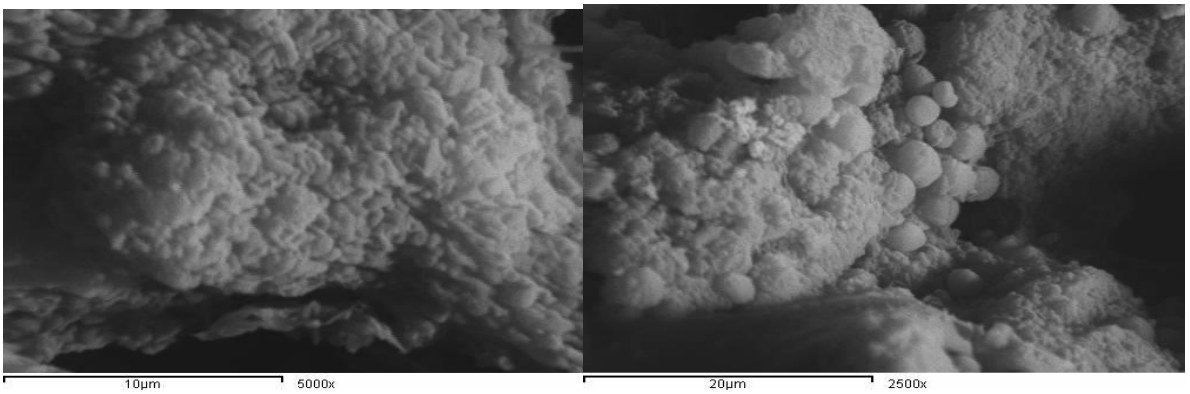

Figure 8 : Scanning electron microscopy (SEM) imaging showing morphological characters of glucose oxidizing bacteria.

4. Conclusions

Mediator-less single chamber microbial fuel cell (MLSCMFC) is advantageous due to its high power output and simplified reactor configuration. When it was enriched with electro active bacteria with glucose as carbon source, the voltage reached to its maximum peak value approximately (720 mV) within 32 days, after colonization of electroactive bacterial cell to form anodic bio film, which have the ability to oxidize the glucose to produce electricity. The higher substrate concentration resulted in an increase in power output. Moreover, it can be observed that an increase in COD value with increase in current production and decrease in internal resistance. The glucose concentration of 5 g/l was recorded as a suitable concentration for current output and bacterial growth, however, an increase in glucose concentration over this value lead to a decrease in current output, as a result of inhibition effect of glucose on the bacterial growth. These studies demonstrate that MSCMFC not only can generate electricity but also treat wastewater simultaneously.

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