Electricity generation using Glucose as substrate in microbial fuel cell

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Article Information	Abstract
	Microbial fuel cells (MFCs) provide new opportunities for energy generation through conversion of organic matter to electricity by electricity-generating bacteria. The cell voltage and degradation rate of Glucose were monitored in a mediator less single chamber air cathode microbial fuel cell (ML-SCMFCs). Glucose was used as substrate by the MFC that was inoculated with aerobic sludge. Degradation metabolites in the anode solution of MFC were analyzed according to standard methods by closed reflux titrimetric Method. The morphology of the anode has been analyzed using Scanning Electron Microscopy. The results showed that electricity output increased with the Glucose concentration increased until at definite limit 5.0 g/l the current decreased because inhibition effect of glucose in relation to cyclic voltammetry, The MFC displayed a maximum power density of 52 mW/m2 at stable current density 275 mA/m2 and a maximum glucose degradation rate 94.4%. SEM show an intensive of bacterial biofilm communities on anodic electrodes which responsible for the electron shuttling to the electrode to generate electricity. These results demonstrate that Gulcose can be used for electricity generation in MFC for practical applications.

1. Introduction

Continued use of petroleum fuel is considered as unsustainable because of depleting their supplies and the accumulation of carbon dioxide in the environment [1] . Increasing demands for energy lead to strong motivation for clean alternative energy resources (renewable energy). a direct results, renewable energy is an increasing demand in our society [2–5]. Microbial fuel cell (MFC) is a new source of renewable energy, which has the ability to use the tiniest microorganism (microbial community) and grab the electricity from wide range of organic waste. It is a galvanic cell that converts chemical energy stored in chemical bond of organic substrate into electricity through biocatalytic reaction of

microorganism [6–14] under anaerobic conditions .In a membrane less single chamber MFC, organic substrate (organic waste and renewable biomass), is oxidized in the anode producing carbon dioxide, protons and electrons [15] . The electrons and the protons 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 [16] .

In the cathode, an oxidant (normally oxygen) is being reduced forming treated water. Oxygen has been widely used as an electron acceptor in most of the cathodic reaction of MFC research because of its unlimited availability and high standard redox potential [12, 26], so the bacterial energy converted to electrical energy [11,15] . Therefore, MFC had been become a major type of bio electrochemical system (BES) [16–19]. They provide a combination between the recovery of energy and the treatment of waste simultaneously [22–25] . Electron transfer to the anode can be achieved indirectly by adding artificial electron mediators (shuttling agents). Or, directly by the cell cytochromes or by reduction of electron transport chain to transfer electrons to the electrode by means of physical contact to the electrode surface. Or , by means of nanowires and cell appendages (pili) of various electrogenic species [21–24]. These pili attached to the electrode form (biofilm) [23–26] to generate electricity from organic matter. 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.

Biodegradable substrate (or electron donor) plays an important role in electricity generation in the MFC [35]. Various organic and inorganic substrates, commonly in the form of liquid or particulate solid have been added to MFCs, and these have been reported in the literature [21] . These substrates ranging from a simple compounds such as acetate and glucose to complex organic compounds [36] . The power produced by MFCs may vary, depending on the availability of the substrate and the capability of microorganisms to metabolize the substrate [37] .Glucose is commonly used as a substrate in MFCs. Several studies have been done using glucose as carbon source in MFCs. Kim et al [38] reported that the performance of a MFC containing Proteus vulgaris depended on the glucose initiated cells in MFC run for a short time period compared with galactose . Rabaey et al [18] obtained a maximum power density of 216 Wm-3 from glucose fed-batch MFC using 100 mM ferric cyanide as cathode oxidant . Moreover, Hu [39] evaluated the feasibility of anaerobic sludge as fuel the feasibility of anaerobic sludge as fuel for electricity generation in MFC and compared it with glucose.

The main purpose of this work was to improve the performance of the mediator less single chamber microbial fuel cell (MSCMFC) and to generate electrical energy from glucose as a carbon source with a different concentration of glucose under aerobic sludge community as a source of biocatalysis. The charge-discharge cycling performances for the MFC using glucose are also 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 and implemented using transparent Perspex as a material of construction with an electrode active area of 25 cm^2 . It consists of an anode and cathode, they anode were

made from carbon paper (Laydel). The cathode electrode was treated with Poly tetrafluoroethylene (PTFE) (60wt% dispersion in water) diffusion layers on the air-exposed side. Then, loaded with 0.3 mg cm^{-2} of 30 % 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 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 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 were fed with the synthetic wastewater. The media growth was prepared using the following constituents (in grams per liter of deionized water) : glucose as a source of organic load (electron donors) ,1; NaHCO3, 2.5; NH4Cl , 0.2 ; KH2PO4, 0.42; KCL , 0.33; NaCl , 0.3 ; K2HPO4, 1.26 ; CaCl2.2H2O, 0.15 ; MgCl2, 3.15 ; yeast extract 1. 10 mL of mineral media were added. The mineral media had the following composition in (in grams per liter of deionized water): EDTA, 0.5; CoCl2.6H2O, 0.082; CaCl2.2H2O, 0.114; H3BO3, 0.01; Na2MoO4.2H2O , 0.02 ; Na2SeO3, 0.001 ; Na2WO4 2H2O, 0.01 ; NiCl2.6H2O, 0.02 ; MgCl2, 1.16 ;MnCl2.4H2O, 0.59 ; ZnCl2, 0.05 ; CuSO4.5H2O, 0.01 ; AlK(SO4)2, 0.01, and 2 ml/l from vitamins (B12 2500 μg- B6 4 mg - B1 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. PH was adjusted by NaOH solution to 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 is connected to a three electrode system comprising a carbon paste working electrode, (CPE) a Pt 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) 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 50mV S^{-1} 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 (OD$_{600}$nm).

2.4. Microbial fuel cell operation:

The MFC was inoculated with the adapted aerobic culture (activated sludge). It was operated under fed batch mode of operation. The aerobic sludge was first pre-processed by filtration to remove un dissolved material. Then, 15 ml sludge 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. Anode was set to maintain anaerobic conditions. After steady state of power generation and electricity generation, polarization curves were obtained by varying external resistance (Rex) from 100 to 125000 Ω. The chemical oxygen demands (COD chromate) of the anodic influent and the effluent were also analyzed according to standard

methods by 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) indicates 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 using a multimeter and recorded every 5 minutes with a multimeter and data acquisition system (Lab jack U6 - PRO).The potentials are related to the current flowing between the electrodes by ohms law

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

Current density (mA m^{-2})

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

Where I is the current per mA, A is the projected area of the anode (m^2).

Power density (mW m^{-2}) output calculated from voltage across the current

As:

$$P.D = V_{MFC} \times C.D$$ [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:

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

Where Ms is the molecular weight of substrate, tb time duration for the cycle, F is faraday; (96,485 c mol-1 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-1) [29].

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

Where COD inlet represents the initial COD concentration (mg/l) in the feed and COD outlet denotes COD concentration (mg l^{-1}) in the reactor outlet.

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2.6. Scanning electron microscopy

The surface of the anode electrode was visualized by scanning electron microscopy to determine microbial attachment and formation of a biofilm on the anode electrode surface. The morphology of the anode was observed using SEM (JEOL, JXA-840A). It was removed after 46 days incubation period and SEM was performed. The electrode fixed with 2.5% glutaraldehyde (Sigma-Aldrich) for 4hrs at 40° 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 % , absolute ethanol) for 10 min. Then the specimens were oven dried, mounted onto specimen stubs using graphite paste, and]zacoated with gold.

3.   Results and discussion

3.1. Microbial Fuel Cell performance

Successive experiments were initially conducted using glucose as carbon source in order to reach a reproducible state of the microbial fuel cell and to achieve enrichment of the anodic electrode with electrochemically active bacteria. In these experiments, 15 mL of aerobic sludge were inoculated in the membrane-less single chamber microbial fuel cell (MSCMFC) through the sampling port at the top, where about 35 ml of glucose was added. A rapid increase in the open circuit cell voltage (OCV) to a value of approximately 0.531V was observed at approximately 8 days after inoculation, implying that electricity can be generated using mixed aerobic culture as microbial community. The open circuit cell potential stabilized to a value of approximately 0.63 V after nearly 14 days, once the glucose in the MFC was consumed, the cell voltage decreased sharply to value about 0.05 V.

Then, the addition of glucose solution in the MFC was repeated along with the addition of a new batch of sludge in order to continue the enrichment of the anodic electrode with electrochemically active bacteria. After repeating the step of glucose and inoculum addition in three successive cycles, a stable reproducible operation of the MFC was achieved while the microorganisms were tightly attached on the surface of anodic electrode, forming anodic bio film to oxidize glucose to produce electrons, protons and CO_2 [30]. Anodic bio-film transfers the electrons outside the cell and the proton transferred to final hydrogen acceptor (O_2). The highest voltage (720 V) produced within 32 days. Then, the voltage dropped quickly to less than 0.050 V. Fig .(2) indicates the MFC performance over three cycles of fed batch operation at infinite resistance and zero current, open circuit voltage, OCV. The main objective of this OCV is to maximize power output and thus obtain the highest current density.
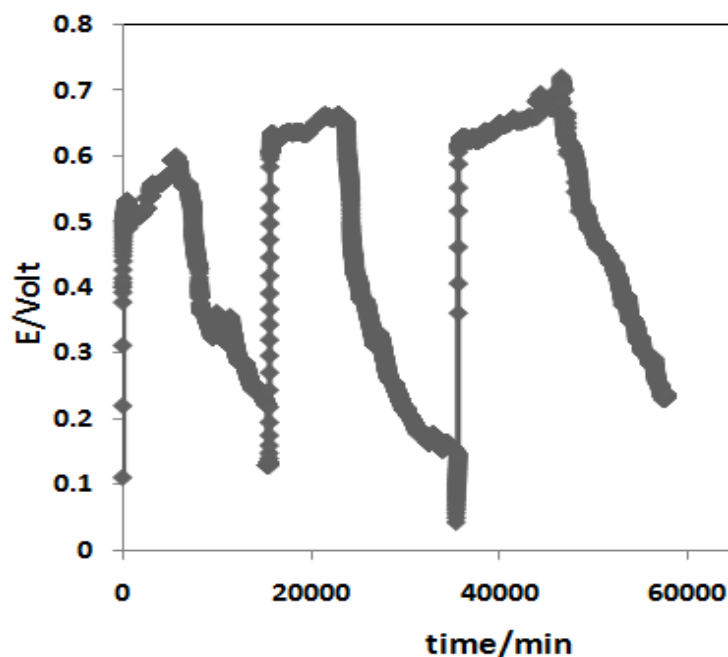
Figure 2: MFC voltage versus time (VT) curve over three cycles of fed batch operation using glucose with a Nafion as binder and a Pt catalyst.

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 2157.32 mg l^{-1}. Fig.(3) Indicates that the current rises gradually at the beginning of the cycles about 0.152 mA after 6 hrs. 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 supplied with another fresh growth media. Once the cell voltage reached to its maximum value (0.189 V) and maximum current peak (343 mA) after 7 days sharply decreased.

Variation in current and voltage is dependent on the concentration of glucose in MFC. The effect of different concentrations of glucose was studied in order to select the effective concentration of glucose on electrochemical behavior of Microbial fuel cell, Which influence on stimulation of the electron transfer pathways of the microbial community by using cyclic voltammetry at different concentration of glucose 1, 5, 10 and 20 g/l. Fig. (4) Shows the peak current output of the different glucose concentration using cyclic voltammetry technique after 3 hrs and overnight incubation period of sludge. The results were showed that the current density increased with increasing of glucose at concentration 5g/l then the current density began to decrease. 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^{-1}, 5 g l^{-1}, 10g l^{-1} and 20g l^{-1}, respectively. This may be due to its inhibitory effects as formation of byproduct as organic acids 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 increasing 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].
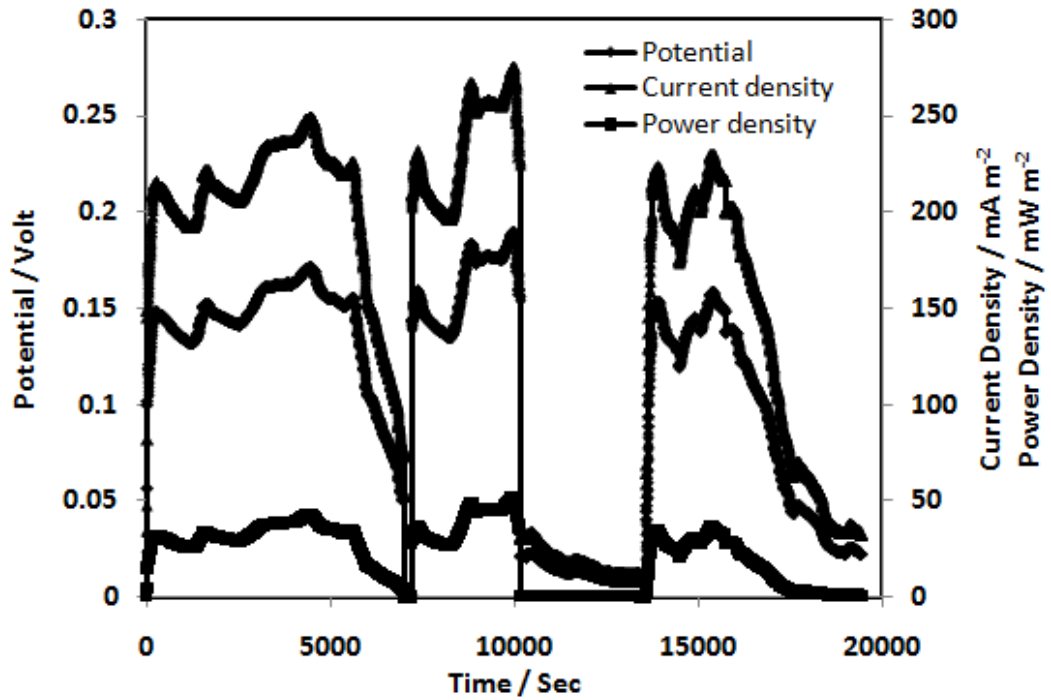
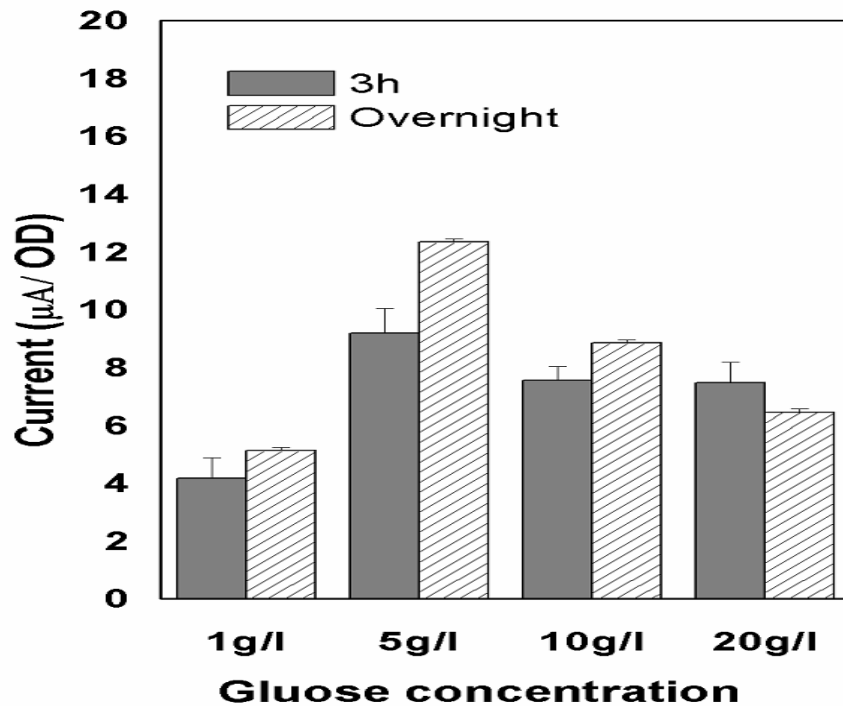Figure 3: Charge-discharge performances of the mediator less single chamber microbial fuel cell at 550Ω

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 degradation of glucose concentration and the electricity generation could be measured by chemical oxygen demand (COD). This measurement occurred by measure the sample of glucose taken from the cell at different time intervals during the period of initial electron production to the removed substrate from the cell. From the result, COD removal efficiency can be calculated. Fig. (5) Illustrates the degradation of glucose concentration versus time. The value

of removal efficiency about 94.4 % this indication to totally consumption of glucose at time duration 8 days estimated in the basis of completion conversion of glucose, also mixed communities had high capacities for removal of the glucose present in the media. The time of degradation increase due to reduction of O_2 amount, which flow to the anode where bacteria use O_2 directly as an electron acceptor instead of using O_2 indirectly via the anode bacteria for growth the biomass. 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 mWm^{-2}) at stable current density (275 mAm^{-2}). 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 of 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].
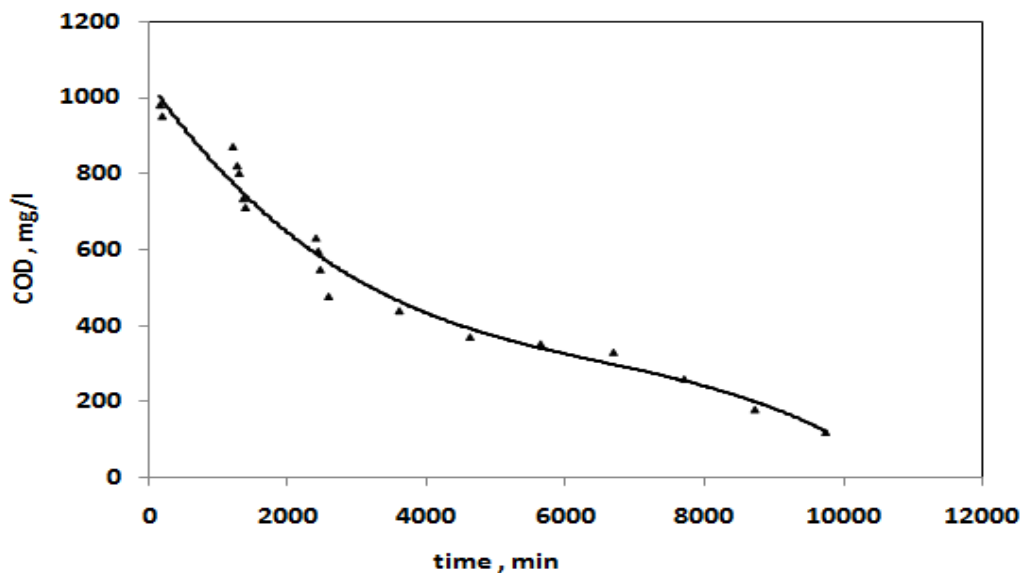

Figure 5: COD vs. time behavior when using glucose as energy source. External load: R = 550 Ω

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].
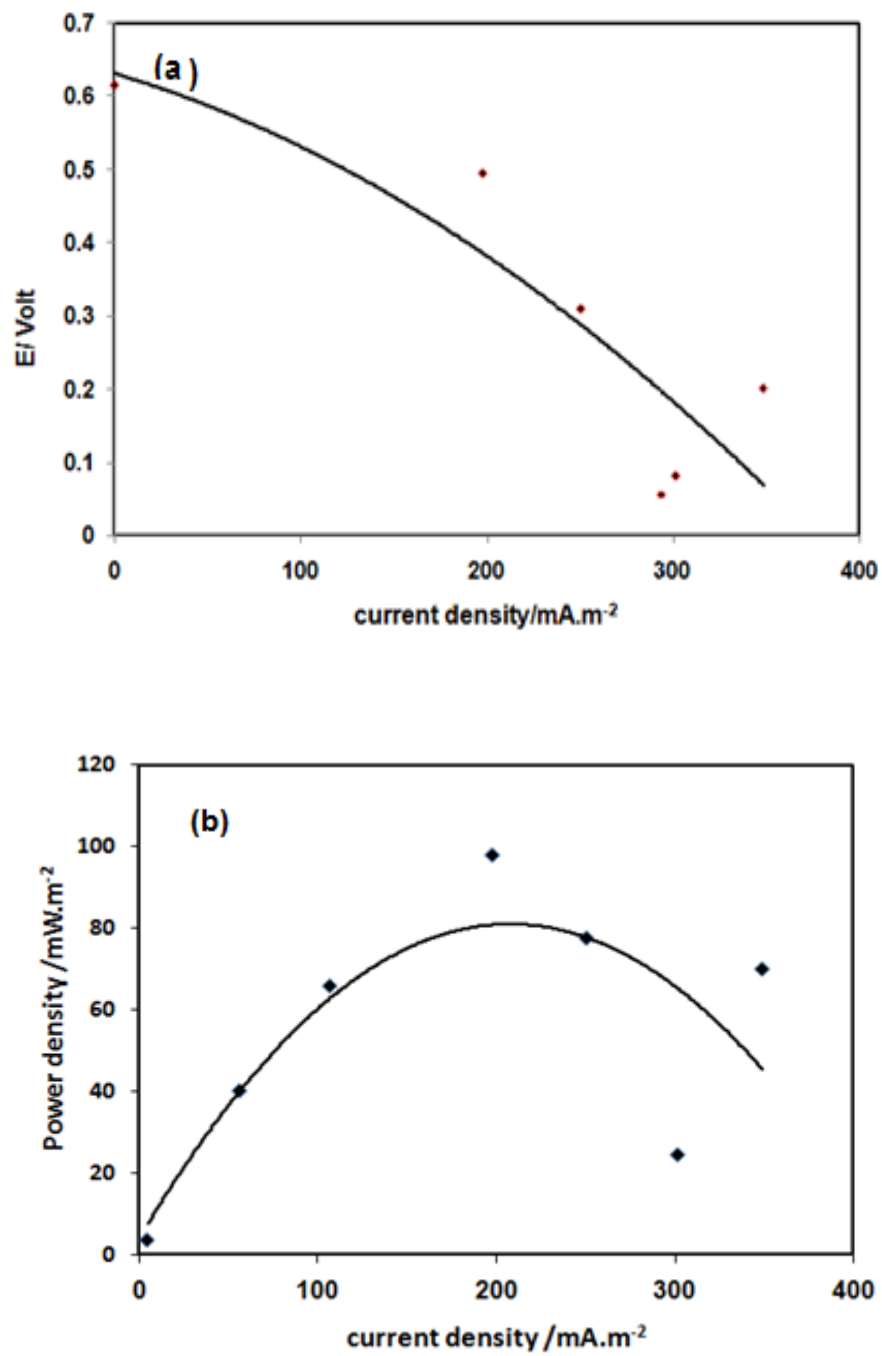

Figure 6 (a, b): Polarization curve and power curve output for glucose

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.
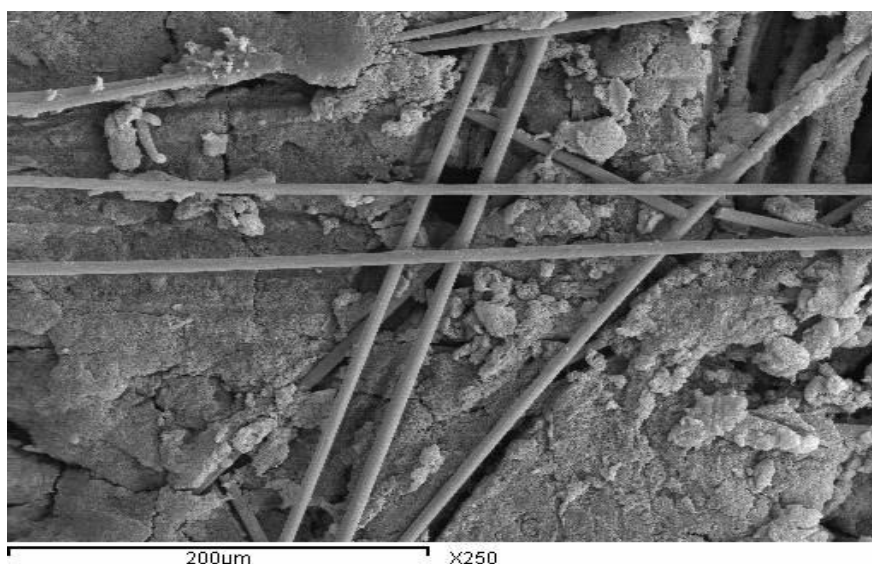

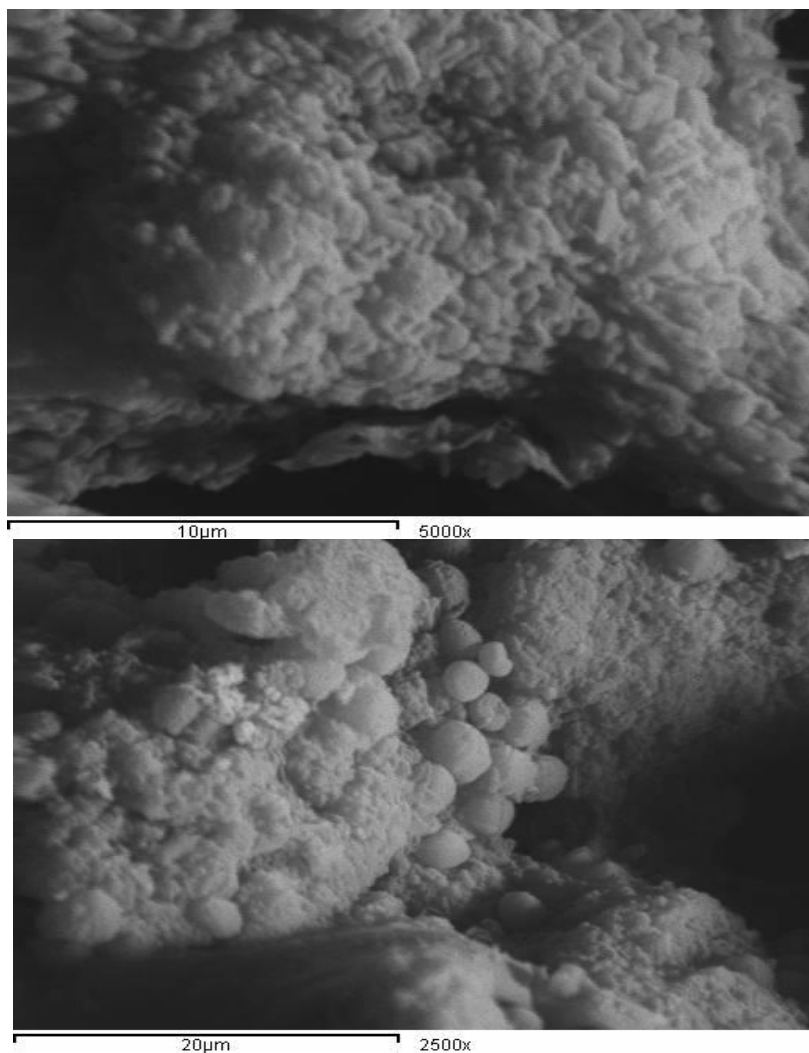Figure 7: Scanning electron microscope of anode free microbial community

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

4. Conclusions

Mediator-less single chamber microbial fuel cell (MLSCMFC) is advantageous due to their 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 the substrate concentration causing 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 best glucose concentration 5 g/l suitable for current output and bacterial growth due to an increase in glucose concentration up to 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 a (MSCMFC) not only can generate electricity but also treat wastewater simultaneously.

5. Acknowledgments

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