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Full Length Research Paper

Membraneless dairy wastewater-sediment interface for bioelectricity generation employing sediment microbial fuel cell (SMFC)

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Sediment Microbial Fuel Cell (SMFC) generates electricity by microbial oxidation of organic substance in dairy wastewater sediments without using any proton exchange membrane. SMFC constructed with graphite electrodes was deployed in simulated dairy wastewater-sediment interface in laboratory conditions showed the feasibility of electricity generation. The membrane-less SMFC developed voltage gradient of 0.78 to 0.8 V with maximum power of 0.014 mW/cm^2 observed at 10 Ω. Maximum current density of 67.85 × 10^{-6} (mA/cm^{-2}) and power density of 52.92 × 10^{-6} (mW/cm^{-2}) was recorded. Native bacterial culture present in dairy waste sediment was used as catalyst for electricity generation. Bacteria present in dairy sediment showed the potential to generate electricity without any mediators (redox dyes). Production of renewable energy (bioelectricity) utilizing organic substance is an economical and sustainable process.

Key words: Bioelectricity, sediment microbial fuel cell, wastewater-sediment interface, anaerobic microenvironment; wastewater treatment.

INTRODUCTION

Energy production and supply are challenging due to the depletion of fossil fuel. Presently global energy requirements are mostly dependant on fossil fuel which eventually leads to the foreseeable depletion of limited fossil fuel resources (Aman, 1996; Das and Vetziroglu, 2001). Emission of global warming gases such as CO2 due combustion of fossil fuels is more of concerned. Concern about climate changes and increase in demand of energy resources are driving to search for alternative energy for fossil fuels (Logan, 2004).

Microbial energy technologies are an alternate to the fossil fuels that employ microbes for the conversion of chemical energy in forms of fuels (biogas, bioethanol, biohydrogen) or directly to electricity by oxidation of organic substances (Logan, 2004). Microbial energy conversion in Microbial Fuel Cell (MFC), a bioreactor in which bacteria transforms chemical energy in biomass directly to electricity is an promising technology for renewable energy production (Chaudhuri and Lovley, 2003; Rabaey and Verstraete, 2005; Venkata et al., 2007).

MFC consists of two chambers, one anaerobic (anode) and the other aerobic (cathode). In anaerobic chamber, bacteria oxidize substrate and the electrons transferred to anode or directly from the bacterial respiratory enzyme to the electrode (Siebel et al., 1984). Anaerobic chamber is separated by a proton-conducting membrane (Nafion 117 an perflurosulfonic acid membrane which separates anode and cathode that allows only diffusion of protons that creates potential diference (Electron motive force) in MFC) and by an external wire that connects cathode electrode. In the aerobic chamber electrons flow through the circuit combine with protons and oxygen to form water. MFCs have the potential to produce electricity from anaerobic sediments of marine (Bond and Lovley,

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2003; Reimers et al., 2001) and from sewage (Gil et al., 2003; Liu et al., 2004).

Sediment Microbial Fuel Cell (SMFC), is an electrochemical device that utilize the potential developed by microbial oxidation of organic substances at anode for the generation electricity (Bond and Lovley, 2003; Park and Zeikus, 2003; Rabaey et al., 2003; Reimers et al., 2001; Tender et al., 2002). Harnessing microbial power generation in seafloor by Tender et al. consist of a graphite electrode (anode) embedded in sea sediment and other graphite electrode (cathode) overlaying in seawater produced maximum power of 26.7 mW/m^2 (at 0.8V) with surface area of graphite electrode of 48.3 cm diameter. Voltage gradient developed by the sediment microbes are utilized by the fuel cells by connecting anode and cathode by an external load (resistance) capable of dissipating power at either constant voltage. Microbes at anode in anoxic conditions donates electrons (e^-) to the electrode, whereas the protons (H^+) are permeable through the sea sediment- water interface which acts as natural membrane instead of semipermeable membrane for power generation .

Bacteria identified to produce electricity in MFC are Metal Reducing Bacteria (MRB) that include *Geobacter sulfurreducens* (Bond and Lovely, 2003) *Geobacter metallireducens* (Bond et al., 2002), *Shewanella putrefaciens* (Kim et al., 2002), *Clostridium butyricum* (Park et al., 2001), *Rhodoferax ferrireducens* (Chaudhuri and Lovely, 2003). In many studies extracellular electron transfer in these bacteria which is responsible for power generation was extensively studied. The biochemistry of iron reduction of *G. sulfurreducens* was studied in detail. Cytochrome- dependent and NAD-dependent Fe (III) reductases have been purified and characterized, and evidence was provided that a porin-like protein and a special cytochrome *c* are important in the reduction of iron (Gaspard et al., 1998; Kaufmann and Lovley, 2001; Kim et al., 2005; Magnuson et al., 2000; Seeliger et al., 1998). Also soluble quinones which act as terminal electron acceptor is wide spread which mediates electron transfer (Van der Zee et al., 2000).

The present investigation is to develop SMFC for the bioelectricity production utilizing dairy wastewater – sediment interface without membrane. Evaluation of electricity generation was performed using dairy waste present in the sediment as carbon source under anaerobic microenvironment. The native bacterial culture present in the dairy waste sediment was used for the power generation.

MATERIALS AND METHODS

Collection of dairy wastewater and sediment

Dairy wastewater and its sediment were obtained from effluent pond (Aavin dairy, Madurai, India). Collection was performed in 25 L sterilized airtight plastic containers. Sediment was collected anaerobically by immersing the can to the depth of the dairy sediment and flushed along with dairy wastewater. Electrode reducing bacteria are usually present in the anaerobic layer beneath 5 cm from the surface the dairy sediment, according to which the layer was carefully collected. Collected samples were immediately transferred to laboratory for simulation of dairy wastewater - sediment interface in a fish tank for the generation of electricity. A portion of the dairy waste was used physical and chemical analysis.

Wastewater analysis

The dairy wastewater was used to study the various physical and chemical parameters like Suspended Solids (SS), Total Dissolved Solids (TDS); Total Solids (TS), COD, BOD, pH by following the methods prescribed by APHA (1998).

SMFC construction and operation

SMFC consist of two graphite electrodes obtained from Intellect Associate, Chennai were used in the experiment. Graphite electrode sized of 280 cm^2 (10 X 2 cm) rectangular blocks was held co-planner in a plastic support (Figure 1a). The distance between the two electrodes was 7 cm. Both the electrodes were connected to the digital multimeter using copper wires with all exposed metal surfaces sealed with the nonconductive epoxy. Electrodes were washed and soaked in double distilled water prior to use.

The collected dairy wastewater and sediment was transferred to the fish tank for the simulation of dairy wastewater – sediment interface. After the formation of interface, SMFC was deployed in-between the interface and allowed for settlement. Slowly the disturbed layer gets settled (Figure 1b). The digital multimeter was connected to the SMFC in open circuit in-order to observe the development of potential (Figure 2). The autoclaved dairy sediment was used as control. SMFC was operated in a constant room temperature (32 ± 2°C). The surface wastewater at cathode was sparged with air using an aquarium air pump. After the development of stable potential, power output was monitored by measuring voltage across an external resistance 10 Ω connected across the anode and cathode.

Bio-analysis

SMFC was continuously monitored according to the procedure outlined by Logan *et al*. (2004). Current (I) and potential (V) measurements were recorded at every 3 h using digital multimeter (Metravi, 901) by connecting 10 Ω as external circuit. Voltage was calculated using the formula $V = IR$ from the current. Power (W) was calculated using $P = IV$, where I is the ampere and V is the voltage in mV. Power density (mW/m^2) and current density (mA/m^2) were calculated by dividing the obtained power and current with the surface area (m^2) of anode.

The microbial colonization over the graphite electrode was visualized with Scanning Electron Microscope (SEM). After the experiment small piece of sediment graphite electrode was cut carefully without disturbing the bacterial colony. Prior to SEM imaging samples were fixed in glutaraldehyde (2%) in 0.05 M phosphate buffer (pH 7.2) for 24 h at 4°C and post fixed in aqueous osmium tetroxide (2%) in the same buffer for 2 h. After post-fixation samples were dehydrated in a series of graded alcohol and dried. Dried samples were mounted over the stubs with double-sided conductivity tape, and a thin layer of platinum metal was applied over the sample using an automated sputter coater for about 2 min and scanned in SEM.

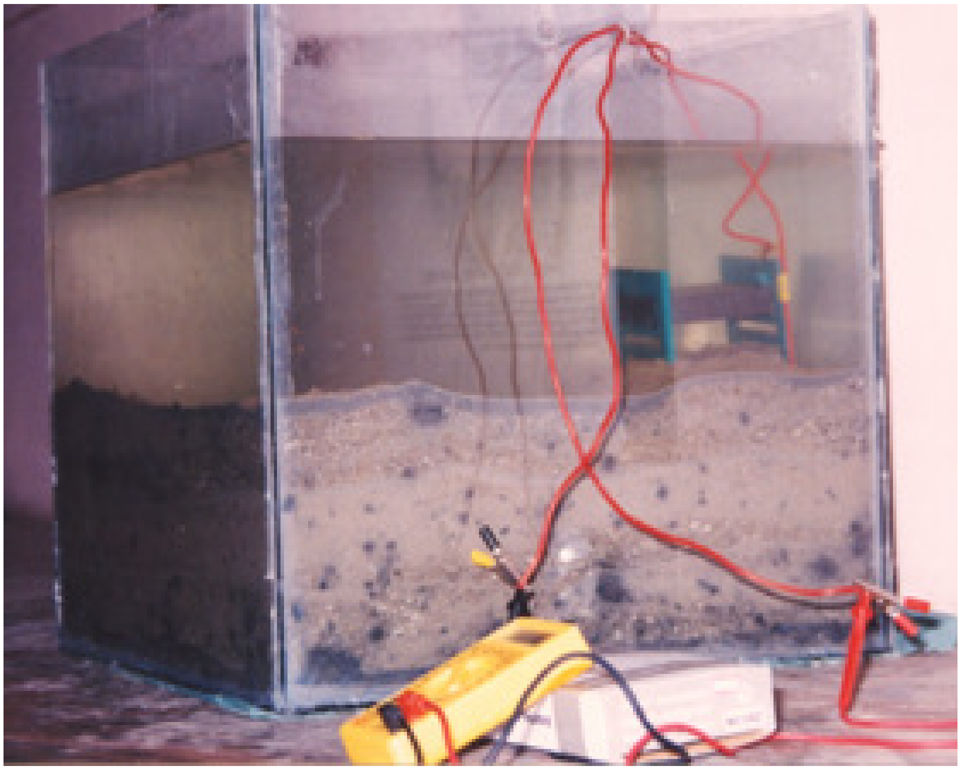

Figure 1a. Experimental setup and Voltage output, photograph of SMFC assembled in dairy wastewater-sediment interface.

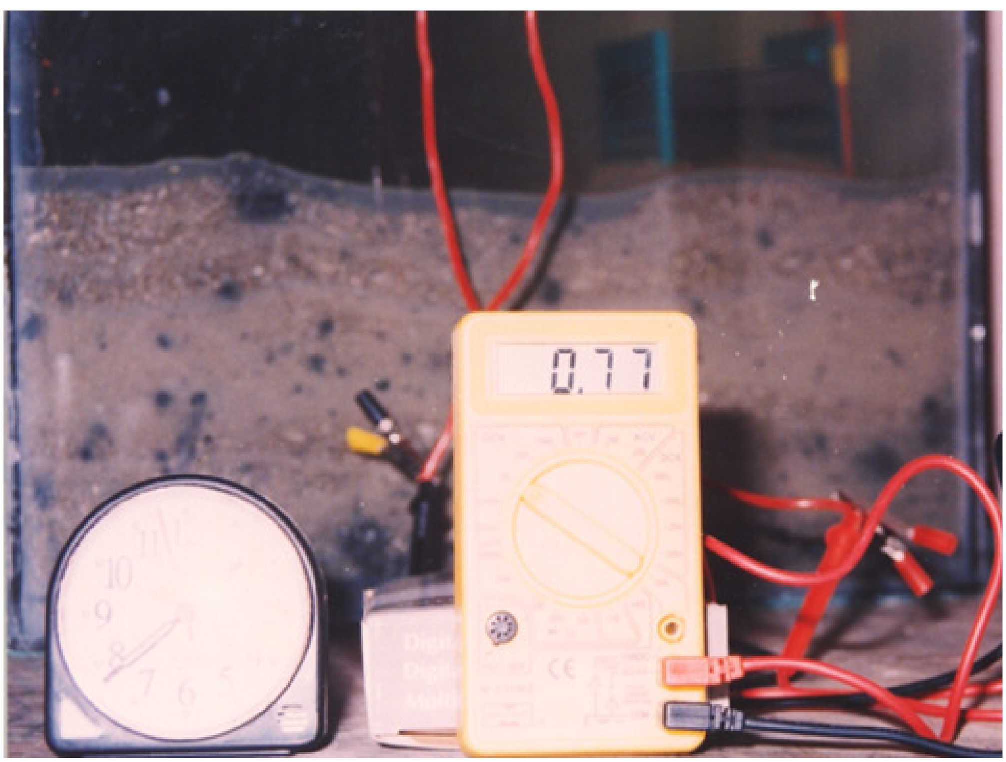

Figure 1b. Experimental setup and Voltage output, photograph showing the Voltage developed during the experiment.

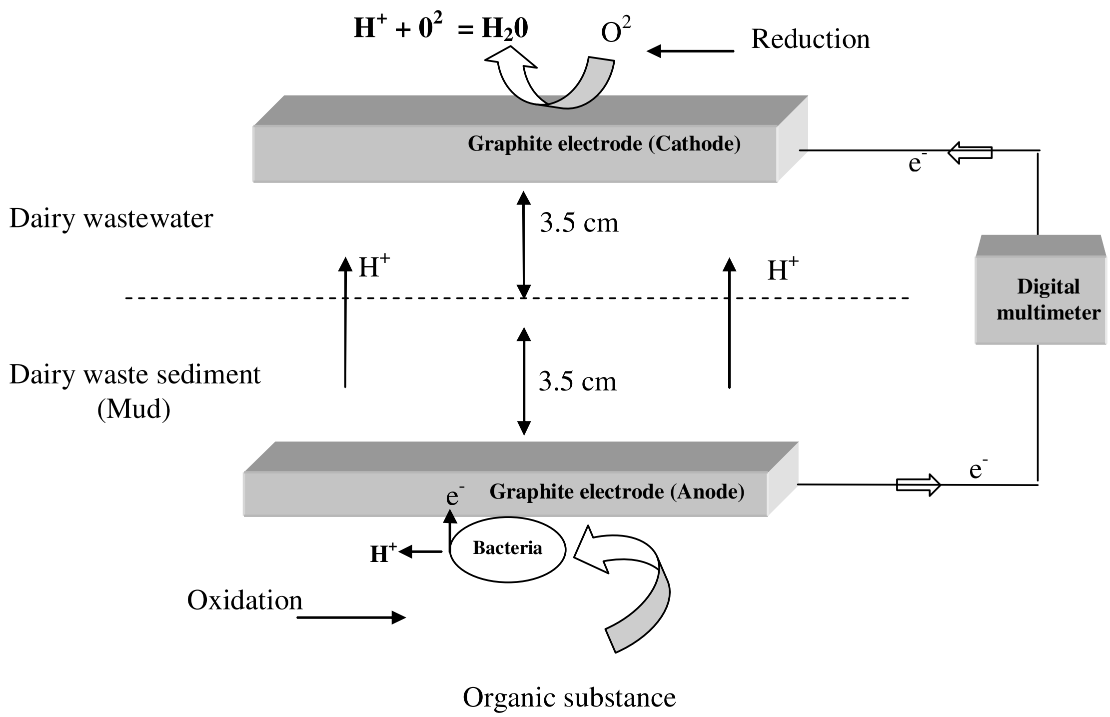

Figure 2. Schematic details of SMFC, figure shows the development of electromotive force (Voltage) due to the oxidation-reduction reaction catalyzed by bacteria attached to anode electrode. The developed voltage and current are measured in closed circuit using digital multimeter. Electrodes (sediment battery) are held co-planner, one buried in the sediment and the other in wastewater.

Table 1. Current and power generation in sediment battery using dairy wastewater sediment interface.

Time (h)	Voltage (mV)	Current (mA/cm^2)	Power (mW/cm^2)	Current density (mA/cm^2) * 10^{-6}	Power Density (mW/cm^2) * 10^{-6}
0	0	0	0	0	0
12	0.19	0.003	0.00057	10.71	2.035
24	0.34	0.012	0.004	42.85	14.57
36	0.52	0.015	0.007	53.57	72.85
48	0.78	0.019	0.014	67.85	52.92
60	0.78	0.019	0.014	67.85	52.92
72	0.68	0.014	0.009	50	34
84	0.88	0.002	0.001	7.14	2.34
96	0.28	0.001	0.0002	3.57	0.953

Readings were taken for every 3 h as given in the figure. The table value shows the readings for every 12 h from the observations.

RESULTS AND DISCUSSION

The various physical and chemical analysis results (SS, 500 mg/l; TDS, 1840 mg/l; TS, 2340 mg/l; COD, 10400 mg/l; BOD, 4700 mg/l, and pH 7.2) shown that the dairy waste water can be considered as complex in nature due to the presence of proteins, carbohydrates, and lipids content.

Table1 and Figure 3 shows the data recorded from SMFC with constant development potential and current generated using dairy wastewater – sediment interface. Stable voltage was generated (~ 24 h) indicating microbial oxidation at dairy sediment (anode) and reduction at cathode occurs. Initially placement of SMFC in interface disturbed the potential, which re-established within few hours. SMFC was continuously monitored for nearly 97 h which showed decrease at the last hours. The decrease might be due to the depletion of carbon source at the region of anode electrode. Power output was monitored by connecting 10 Ω fixed load once the

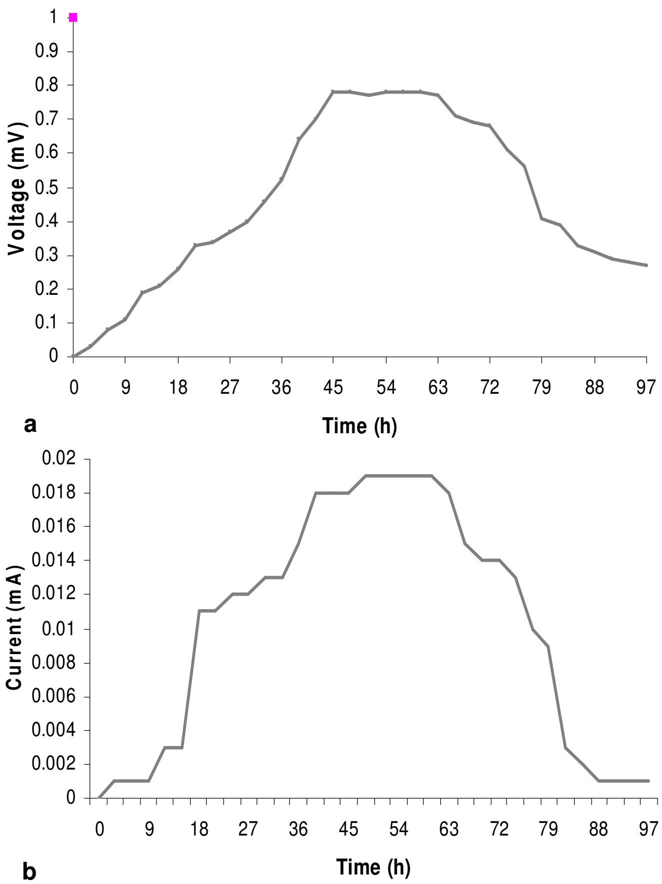

Figure 3. Voltage and current measurement during SMFC operation (measured at 10Ω), Voltage (a) and Current (b) measured at 10 Ω during sediment battery operation.

voltage stabilized. Maximum power of 0.014 mW/cm^2 was observed at ~ 60 h. This power was constant for nearly 12 h after which showed a decrease. During this maximum power output, voltage of 0.77 to 0.8 V was observed. Though the potential developed was maximum, power output was less. Several factors influence the SMFC performance are the oxidation and electron transfer to the electrode by the microbes, the resistance of the circuit, proton transport to the cathode through the membrane (interface), and oxygen supply and reduction at the cathode (Park and Zeikus, 2003).

Current density and power density with respect the surface area of graphite electrode was measured. Maximum current density of 67.85 × 10^{-6} (mA/cm^2) and power density of 52.92 × 10^{-6} (mW/cm^2) was recorded at 10 Ω resistance (Figure 4). Low current and power density might be due to the poor bacterial colonization which simultaneously affected the electron transfer to the electrode. Improved bacterial colonization (monolayer development of microbes over the anode electrode) could increase the power and current with respect to the surface area. However, the presence of additional bacteria in a biofilm capable of producing mediators could greatly increase power. The potential of large increases in power production using bacteria that produce their own mediators was demonstrated by Rabaey et al. (2003).

Control experiment with autoclaved dairy sediment did not show any improvement in voltage gradient (data not shown). This shows that the voltage gradient developed is due to the oxidation-reduction catalyzed by microbes at anode present in the dairy sediment. In an SMFC, the anode is the final electron acceptor and its potential determines the energy gain for the bacteria. Long-term enrichment and cultivation of bacteria in SMFC could lead to increased power production if the microbes possess mediators that can enhance electron transfer to the anode. Thus the contribution of exogenous mediators by bacteria plays an important role in SMFC power generation (Rabaey et al., 2004).

SEM image (figure not shown) of typical bacteria growth on the surface of sediment electrode (anode) in SMFC was observed. Examination over the electrode surface revealed two predominant bacterial morphologies. First the slightly bent, scattered and short chain rod shaped bacteria (~10 µm in length) found over the electrode resembling Fusi form Bacilli as reported by several researchers (Bond et al., 2002; Chaudhuri and Lovely, 2003; Fang et al., 2002). The other cocci-typed bacterial morphologies which are similar to bacteria found in anaerobic sludge (Wang et al., 2004).

The predominant bacterial morphology shows the similarity of *Geobacter* sp. Several researchers (Bond et al., 2002; Bond and Lovely, 2003; Seeliger et al., 1998) explained the importance of *Geobacteraceae* group in electricity generation. These electrode reducing bacteria are found mostly in anoxic sediments which can oxidize both organic and inorganic compounds. In the present study, it is evident that similar *Geobacteraceae* group bacteria found in anoxic dairy waste sediment are responsible for electricity generation in SMFC. Dairy sludge contains protein, carbohydrate and fat that is utilized as carbon for electricity generation. Continuous monitoring of SMFC reveals that it is similar to the anaerobic bioreactor. Power generation was recorded for 96 h after which the carbon source depleted. Voltage gradient was re-established once after re-locating the SMFC in fish tank.

A SMFC has a great potential since organic wastes in dairy sediments are converted to electricity. One aspect needs to improve in SMFC is power density. Based on the available anode surface and bacteria present at dairy waste, SMFC generated electricity in very short time. However, there are more factors to be addressed for the poor generation of power. There can be a several rate-limiting steps in the current generation by a SMFC. Limited proton transfer conditions, reduced microbial activity and electron transfer to anode, high internal resistance (interface resistance), and slow cathode reactions, high Dissolved Oxygen (DO) wastewater that reduces the catalytic activity of graphite reducing

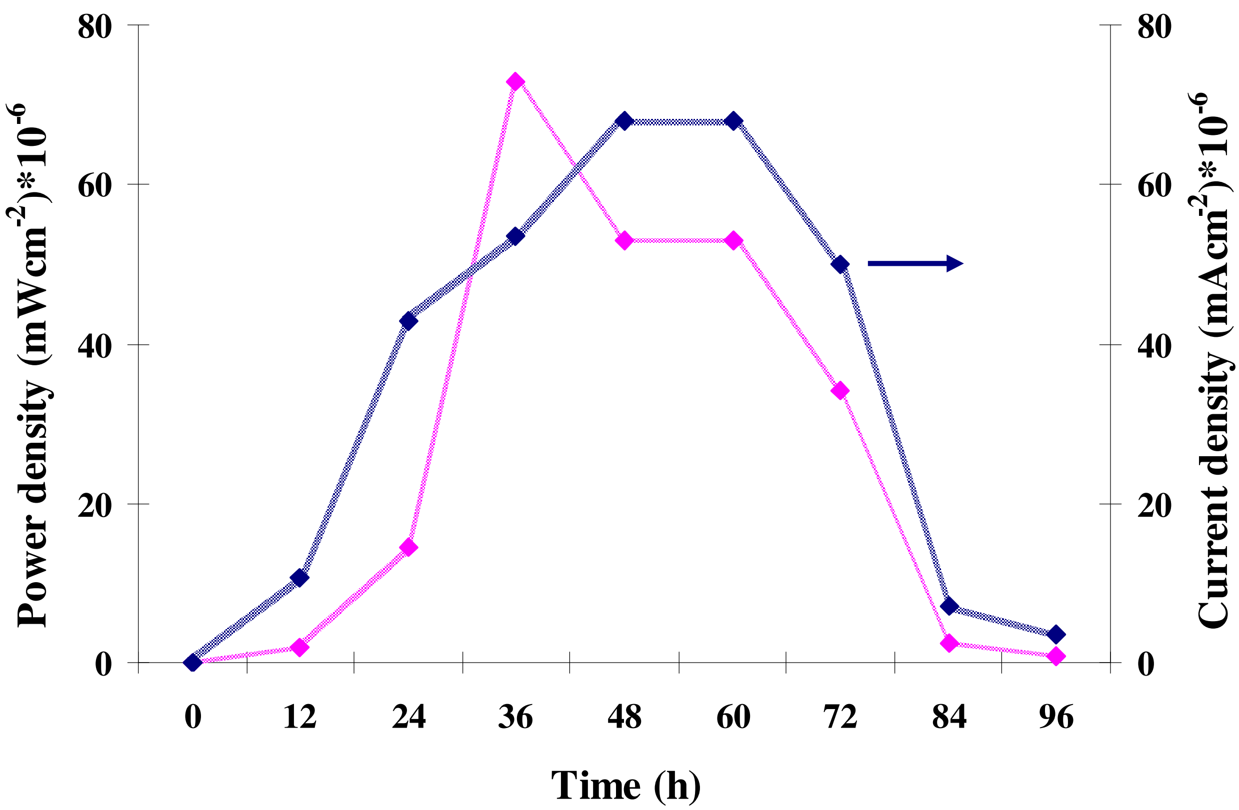

Figure 4. Power (Pink) and current density (blue) –shown here are with respect to the surface area of the graphite electrodes used measured at 10 Ω. SMFC was in operation with 10 Ω continuously. Data shown here are with respect to single batch.

dioxygen, availability of limited carbon source are the possible reasons that affects efficient functioning of SMFC. These factors should be improved for recovering maximum power.

Conclusion

In this study attempt has been made to generate electricity utilizing dairy waste sediment obtained from the effluent treatment pond. It is evident that SMFC generated electricity which is renewable, non-polluting to the environment and treating waste that will minimize the pollution. In future, several SMFC can be deployed in wastewater effluent treatment pond for the generation of more power. However, several factors must be analyzed and studied before implication of SMFC for full scale electricity generation.

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