Study of Microbes Immobilized Monolithic Electrodes in Microbial Fuel Cell

By

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Research Article

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ABSTRACT

Microbes Immobilized Microbial Fuel Cell Anode (MIMFCA) was produced using baker’s yeast, sodium alginate, activated carbon and cement, and tested in a Microbial Fuel Cell (MFC) setup to determine its workability and to determine the most suitable ratio of carbon and alginate in the anode to enhance the MFC cell performance. Five different electrodes were prepared with varying carbon–alginate contents of 15/2, 8/4, 6/6, 4/8 and 1/10 respectively. Six dual-chambers MFC set-ups of five liters each by volumes were used for the study. Five were operated with each of the immobilized yeast electrodes as anode while the sixth which served as control was operated with the cement-carbon monolithic electrode (where the microbes are introduced in the MFC system and made to freely attach themselves to the electrode as the cell was run). Glucose solution was used as the substrate and potassium ferricyanide as the catholyte. The voltage developed in each cell as well as the corresponding current were monitored for 10 days under ambient temperature and a neutral pH of (6.6-7.0) using a digital multi-meter connected to the circuit. The cell with electrode of 6/6 composition of carbon-sodium alginate was observed to have the highest electrical output of 1.68volts and 0.75 mA with power density and current density of 332.2785 mW/m² and 197.7848 mA/m² respectively while the control cell gave the lowest electrical output of 0.38volts and 0.10mA with power density of 26.3713 mW/m² and current density of 10.0211 mA/m².

Keywords: Microbes Immobilized MFC anode, bakers yeast Immobilization, sodium alginate, cement-carbon electrode.

INTRODUCTION

The microbial fuel cell (MFC) is a new form of renewable energy technology that can generate electricity from organic matter through the catalytic activities of microorganisms (Bruce, 2008). It has been known for almost one hundred years that bacteria could generate electricity (potter, 1911) but only in the past few years has this capability become more than a laboratory novelty. The reasons for this recent interest in using bacteria to generate electricity are a combination of the need for new sources of energy, discoveries about microbial physiology related to electron transport, and advancement of fuel cell technologies. In an MFC device, chemical energy is directly converted into electricity through the catalytic activities of microorganisms. These microorganisms (electrogens, exoelectrogens, anode-respiring bacteria, electrochemically active bacteria) serve as the catalysts to oxidize organic and inorganic matter and generate current. Unlike conventional fuel cells, MFC have certain advantages like high-energy conversion efficiency, mild reaction conditions and with a fuel cell’s emissions well below environmental regulations. In addition to generation of electricity, this technology has the potential of treating wastewater using the microorganisms inherent in the wastewater (Logan and Regan, 2006). MFC consists of anode and cathode chambers separated by a proton exchange membrane. The anode chamber holds the substrate (fuel) while the cathode chamber contains solution of an oxidizing agent. Bacteria on the anode surface oxidize organic compounds to carbon-dioxide producing electrons and protons. The electrons thus produced are transferred to the anode in any of the following ways: using mediators produced by the bacteria; exogenous mediators (ones external to the cell) such as methylene blue, thionine, neutral red, etc.; or by direct transfer of electrons from electrochemically active bacteria cells (cytochromes) to the electrode (Mathuriya and Sharma, 2009). The electrons are subsequently conducted over a resistance or power user towards the cathode and thus, bacterial energy is directly converted to electrical energy (Rao et al., 1976). Protons migrate to the fully aerated cathode across a proton selective membrane and are combined with the electrons and oxygen to form water. The overall reaction is the breakdown of the substrate to carbon dioxide and Water with production of electricity as a by-product. In this research a dual chamber MFC was used to investigate the possibility and performance of microbe’s immobilized MFC anode electrode produced using baker’s yeast, sodium alginate and cement – carbon monolithic. The overall cost of the cell has been reduced and its power performance enhanced by the use of this cheaply available raw material to produce the
electrode. In addition a continuous operation of the cell is made feasible. The substrate (fuel) used was glucose solution, while potassium ferricyanide was used as the catholyte.

MATERIALS AND METHODS

The materials employed include:

Baker's yeast powder, Distilled water, Agar powder, Solid sodium chloride (NaCl), Potassium ferricyanide (KFe(CN)₆), Slow-drying Epoxy, Potassium dihydrogen phosphate (KH₂PO₄), Dipotassium hydrogen phosphate (K₂HPO₄), Calcium chloride crystalline powder (CaCl₂), Powdered Glucose (C₆H₁₂O₆), Portland Cement, Activated carbon, Sodium alginate (C₆H₇NaO₆), Masking tape and PVC tape, Twelve heavy duty plastic containers with sealable lids, Short section of plastic pipe (polyethylene) for salt bridge, Plastic flanges (polyethylene) and Flexible copper wire.

NB: All chemicals employed were bought from buhzor chemicals, rumuola, Port Harcourt Nigeria and other materials used for MFC construction bought from mile one market, Port Harcourt, Nigeria (All chemicals used were manufactured by Sigma chemical company, Germany).

APPARATUS USED

<table>
<thead>
<tr>
<th>S/N</th>
<th>Equipment</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The MFC device (Assembled)</td>
<td>This is where the electrode was used for electricity generation and it houses the anolyte and the catholyte.</td>
</tr>
<tr>
<td>2</td>
<td>Digital Multi-meter</td>
<td>This was used to measure the voltage and current output of the MFC systems.</td>
</tr>
<tr>
<td>3</td>
<td>Triple beam balance</td>
<td>It was used to weigh the materials used for the study.</td>
</tr>
<tr>
<td>4</td>
<td>Tiger Razor</td>
<td>It was used as the cutting tool during the construction of the MFC device.</td>
</tr>
<tr>
<td>5</td>
<td>Medical Syringe</td>
<td>This was used to suck the yeast-alginate slurry during electrode production and made to drop in cacl₂ solution for beats to form.</td>
</tr>
<tr>
<td>6</td>
<td>Electric Soldering Iron</td>
<td>This was used to create the necessary holes on the plastics containers used in the construction of the MFC device.</td>
</tr>
<tr>
<td>7</td>
<td>Digital pH meter</td>
<td>It was used to measure the pH of the buffer solution.</td>
</tr>
<tr>
<td>8</td>
<td>Stove top Autoclave</td>
<td>This was employed for heating of the Agar solution.</td>
</tr>
</tbody>
</table>

General laboratory glass wares were employed in measuring and holding of solutions.

Preparation of the yeast immobilized electrode

Five different electrodes with fixed grams of yeast cells and carbon-alginate compositions of 15/2, 8/4, 6/6, 4/8 and 1/10 respectively were made. The electrodes mechanical strength was enhanced with fixed grams of cement. Each was made using the data shown below in table 1. The yeast was first mixed with the sodium alginate to form a slurry mixture using a buffer solution of (k₂HPO₄ +KH₂PO₄). The slurry of the yeast–alginate mixture is gel into small beats when dropped 20cm into 0.05 CaCl₂ solutions (3.775g in 1 dm3) and washed with distilled water before monolithically mixing with carbon-cement paste. Below were the compositions of the five immobilized electrode.

<table>
<thead>
<tr>
<th>Anode Electrode type</th>
<th>Carbon (grams)</th>
<th>Cement (grams)</th>
<th>Yeast (grams)</th>
<th>Sodium Alginate (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15/2 cell</td>
<td>15</td>
<td>24</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>8/4 cell</td>
<td>8</td>
<td>24</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>6/6 cell</td>
<td>6</td>
<td>24</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>4/8 cell</td>
<td>4</td>
<td>24</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>1/10 cell</td>
<td>1</td>
<td>24</td>
<td>12</td>
<td>10</td>
</tr>
</tbody>
</table>

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Experimental Procedures for producing the yeast immobilized electrode

12 grams of yeast was weighed into five different beakers and different grams of sodium alginate ranging from 2-10 grams as shown in table 1 was weighed and each mixed with the yeast with (k2HPO4 +KH2PO4) buffer solution to form a slurry.

Each of the resulting slurry from the five beakers was sucked differently with a syringe and dropped at 20cm height into 0.05 CaCl2 solutions (3.775g in 1 dm3) for beads to form.

Five different paste of carbon- cement was prepared with fixed grams of cement and different grams of activated carbon ranging from 1-15 grams as shown in table 1.

Each of the beads formed from the five different slurries are then mixed with each of the prepared carbon-cement paste to form a monolithic mixture.

The five resulting mixtures are loaded into five different moulds with the moulds covered.
Copper wire was connected in each of the mould and the wire was centralized in the mixture inside the mould to help protect from corrosion.

The five mixtures were made to set well inside the mould by landing the bottom of the mould with a force on a flat solid surface.

The moulds with the mixtures were left on an open place in the lab for 24 hours for air to dry it.

After air drying for 24 hours the resulting electrode is removed from the mould and is set for use.

Plate 3: Yeast cells mixing with the cement-carbon monolithic paste.

Plate 4: The Immobilized electrodes exposed to air for drying. Plate 5: The produced electrode.
Preparation of the cement-carbon electrode

The electrodes were made with carbon-cement ratio of 2:1 only without cell immobilization and served as cathode in all MFC systems used for the study and as a control anode on the control cell set-up.

Experimental Procedures for producing the cement-carbon electrode

Carbon and cement was weighed with 2:1 cement-carbon ratio and mixed with distilled water to form a monolithic mixture.

The resulting mixture was loaded into seven different moulds with the moulds covered.

The mixture was made to set well inside the mould by landing the bottom of the mould with a force on a flat solid surface.

Copper wire was connected in each of the moulds and the wire was centralized in the mixture inside the mould to help protect from corrosion.

The moulds with the mixtures were left on an open place in the lab for air to dry it.

After air drying for about 48 hours the resulting electrode is removed from the mould and is set for use.

Procedure for Construction of the MFC device

Two plastic containers with lids for anode and cathode respectively were drilled from the side with about 1 inch diameter of the hole. Proton exchange membrane was fixed with one end connected to the anode chamber and the other connected to the cathode chamber. Epoxy was used to seal and firmly fixed the PEM to the chambers. These were repeated six times to obtain the six set-ups. After some hours, distilled water was used to check for leakages. Holes open enough to accommodate the electrode were made on the lids for electrodes. Each set-up has two electrodes, one on the anode and the other on the cathode chamber respectively.

Procedure for constructing the proton Exchange Membrane

Long PVC pipe of 2.54 cm diameter was cut into 12 pieces of 8 cm each after which PVC screw sockets (flanges) was fixed with epoxy at both ends of the pipe, thereby giving the pipe a new length of 10.3 cm.

Each of the pipes was sterilized with hot distilled water to free the surface of microorganisms; the open ends were sealed with masking tape to prevent microorganisms from acting on the internal surface after sterilization.

20 grams of agar was measured and mixed with 3.75 g of NaCl. 500 ml of distilled water was added to the mixture and stirred. The prepared agar was autoclaved at 121°C for 20 mins to gel, after which it was removed and poured into the sterilized pipe to solidify.
After about 30 min of solidification, the PEM was ready for use.

**Procedure for connecting the proton exchange membrane**

A hole was drilled at the side of the plastic containers with the soldering Iron. PVC Tape was used to tape the membrane's socket, and then screwed into the end cap on the container.

The end cap of the socket was fixed in the hole. Epoxy was used to gum the end caps or flanges to sides of plastic pipe, and allowed to harden.

**Preparation of the buffer solution**

20.7g of di-potassium hydrogen hoto-phosphate (base) was weighed and dissolved in 4.5 liters of distilled water.

12.2g of potassium di-hydrogen hoto-phosphate (acid) was also weighed and dissolved in 4.5liters of distilled water and shake to dissolve it completely.

The dissolved acid was poured into the dissolved base and stirred to have a homogeneous solution.

A manual pH meter was inserted into the solution to ensure a neutral solution with pH of 7.0.

**Preparation of anolyte**

The anolyte was prepared by dissolving 320g of glucose in 5 liters of distilled water and stirred very well to completely dissolve for each of the six chambers.

**Preparation of the catholyte**

The cathode chamber is the oxidant chamber which uses potassium ferricyanide as the protons acceptor. The catholyte was prepared by dissolving 100g of the potassium ferricyanide in 5.0liters of distilled water and stirred very well to completely dissolve for each of the chambers. The ratio for the preparation of the catholyte is 5.01g of potassium ferricyanide to 250ml of distilled water.

**Assembling the MFC device procedure**

The PEM was connected between the two plastic containers and epoxy was used to seal it to ensure no leakage of solution on both ends.

The inoculum (glucose solution) was added to the anode chamber.

Also the conductive solution (potassium ferricyanide) was added to the cathode chamber. Anode electrode was inserted into the anode chamber and was sealed with epoxy to ensure air tight condition in the chamber. Cathode electrode was equally inserted into the cathode chamber. The external circuit was connected and voltage readings begin with the aid of a digital multi-meter.

NB: The anode was covered and sealed to prevent oxygen from entering, since the anode chamber must be anaerobic. The cathode chamber (the oxidant) was filled with the catholyte (potassium ferricyanide) and was not sealed but partially covered to allow enough oxygen which helps in oxidation. The microbial fuel cell voltage for the set-ups was monitored daily using a digital multi-meter. Reading of voltage and current was done within few minutes of stabilization.
RESULTS AND DISCUSSION

The results of individual voltage and current obtained for 10 days operation of the cells under ambient temperature and a neutral pH of (6.6 - 7.0) are shown in figure 1 to 6 below.
Figure 2: Voltage and Current against Time(Days) for 8/4 Cell

Figure 3: Voltage and Current against Time(Days) for 6/6 Cell
Figure 4: Voltage and Current against Time (Days) for 1/10 Cell

Figure 5: Voltage and Current against Time (Days) for 4/8 Cell
Figure 7 to 12 below show the results of individual current density and power density obtained for 10 days operation of the cells under ambient temperature and a neutral pH of (6.6-7.0).

![Graph of Voltage and Current against Time (Days) for Control Cell](image1)

![Graph of Power Density and Current Density against Time for 15/2 Cell](image2)
Figure 8: Power Density and Current Density against Time for 8/4 Cell

Figure 9: Power Density and Current Density against Time for 6/6 Cell
Figure 10: Power Density and Current Density against Time for 4/8 Cell

Figure 11: Power Density and Current Density against Time for 1/10 Cell
Figure 12: Power Density and Current Density against Time for Control Cell

Figure 13 to 16 below show the results of voltage, current, current density and power density for the whole cells obtained for 10 days operation of the cells under ambient temperature and a neutral pH of (6.6-7.0).

FIGURE 13: Voltage against Time(days) for The Whole Cell
FIGURE 14: CURRENT Against TIME(DAYS) for THE WHOLE CELLS

FIGURE 15: CURRENT DENSITY against TIME(DAYS) for THE WHOLE CELLS
DISCUSSION

The graph behavior of the whole MFC systems as show in figure 1 to 6 is in corroboration with the normal pattern of a transit growth graph (Monod, 1949). But in this experiment, the microbial growth was monitored using the resultant electrical output (voltage and current) in the MFC cells. As shown in the figure 1 to 6, there was a lag phase, where no increase in cell number is evident which was evident at the inoculation phase of the MFC cells. Then the exponential growth phase, a period of rapid growth which was evident by constant increase in the electrical output values of the whole MFC cells after inoculation. The stationary growth phase is the point of no substantial growth. This was evident on the fifth day in the cells (except in the control cell in which it was on the seventh day) where the MFC cells started dropping in performance and the last phase which is the death phase where exponential decrease in the number of cells was observed. This was evident in the constant decrease in the electricity production performance of the MFC cells after the fifth day on the yeast immobilized cells and on the seventh day in the control cell.
Also in the figure 1 to 6 that gave the result of the electrical output (voltage and current) for the whole cells (15/2, 8/4, 6/6, 4/6, 1/10 and control cell). The stationary growth phase evident in the electrical output on the fifth day in yeast immobilized cells lasted just a day (24 hours) and the cells performance (except in the control cells) drops sharply and decreases as the days of running of the MFC cells increases. The short duration of the stationary growth phase evident in the electrical output obtained on the fifth day and the subsequent sharp drop in performance of the yeast immobilized MFC cells could be as a result of mass transfer restriction of the nutrients within the yeast cells inside the electrode. The microbes (the immobilized yeast cells) in the electrode depend on the nutrient (glucose solution) for survival and growth. Depletion of the nutrient as a result of mass transfer restriction could result to death of the microbes and the system decreases in production and in most critical situation ceases to produce (Brodelius et al., 1979; Williams and Mavituna, 1992). The restriction of the mass transfer of the nutrient (glucose solution) could be due to blockage of electrode pores.

The current density and power density graph behavior of the electrodes in figure 7 to 12 follows the same pattern of behavior as in that of current and voltage. This is because the two parameters have a direct relationship with current and voltage of the cells (Wang et al., 2008; Rabaey et al., 2003).

The Evaluation of the whole MFC cells parameters (current, voltage, current density and power density) in figure 13 to 16 showed that the 6/6 cell generated the highest electrical output followed by 1/10, 15/2, 4/8, 8/4 and control cell respectively. Also it was observed that the MFC cell that contains electrode cell in excess of sodium alginate (1/10) gave better result than that of excess activated carbon (15/2) but the best result was obtained in the cell that has sodium alginate and activated carbon in equal ratio. This trend justifies that in the production of the Microbes Immobilized Microbial Fuel Cell Anode (MIMFCA) electrode a composition of equal quantity of the binder (sodium alginate) and the conductor (activated carbon) is the best.

The control cell that was operated with the same quantity of the yeast cells and glucose where the yeast cells were not immobilized in the electrode but were freely made to attach themselves to the walls of the electrode for electron transfer showed the least result. Low cell density of the yeast cells on the electrode surface is the major cause of the poor result. As the yeast cells were not immobilized, they were found more in the anolyte solution than on the electrode surface. Most of the electrons produced by these floating cells need a mediator to be conducted out of the system. The cost of buying this mediators and side effect in the anode chamber is expensive (Brodelius et al., 1979). This justifies the need to immobilize the cells.

CONCLUSION

Microbes Immobilized Microbial Fuel Cell Anode (MIMFCA) could actually been produced using baker’s yeast, sodium alginate, activated carbon and cement and could practically produce electricity in MFC cell if fed with any carbon containing substrate like glucose and has potentials to serve as MFC anode electrode to enhance the whole system performance.

REFERENCES