A microbial biofuel cell with encapsulated microorganisms and gas vent for long-term operation

Chien-Chou Chen and Yu-Chuan Su*

National Tsing Hua University, Department of Engineering and System Science
101, Section 2 Kuang Fu Road, Hsinchu, 30013, Taiwan

Abstract

We have successfully demonstrated a self-contained microbial biofuel cell for long-term operation. This fuel cell consumes glucose and oxygen to generate electricity in a reaction catalyzed by encapsulated microorganisms. The microorganisms are immobilized inside liquid-core alginate capsules, which prevent the microorganisms from depositing on device surfaces while allow substances to diffuse through. In order to discharge unwanted carbon dioxide gas, this biofuel cell is equipped with a bubble guiding and venting system, which facilitates the transport of charges as well. As such, this biofuel cell is capable of self-regulating the electricity harvesting process and therefore able to produce steady voltage and current outputs for an extended period of time.

Keywords: Microbial biofuel cell, Microorganism encapsulation, Gas vent, Micro fuel cell, Capillarity

1 - INTRODUCTION

The concept of using microorganisms as catalysts in biofuel cells, known as microbial biofuel cells, has been explored since the 1970s [1, 2]. Microbial biofuel cells are capable of converting chemical energy, which is available in bio-convertible substrates, directly into electrical energy. By replacing expensive catalysts with naturally occurring micro-organisms to promote the oxidation of electron donors, the manufacturing cost of fuel cells could be greatly reduced. Potentially, microbial biofuel cells could supply electricity for a variety of applications and help fulfill the increasing demand for energy. More importantly, they are promising in powering life-supporting implantable medical devices, which need to work inside biofuel-abundant bodies for a long period of time. Previously, researchers have demonstrated micro-machined microbial fuel cells that function for less than 15 minutes [3]. Since there is no fuel circulating or supplying component in these cells, their probable working period is severely limited. For long-term operation, an additional control system (preferably passive) is required to regulate the mass transfer of reactants and products in and out of the reactor. To address this need, this work presents a system that integrates capillary fluidic components to guide the transport inside the cell. As such, this biofuel cell is capable of self-regulating the electricity harvesting process and producing steady outputs for an extended period of time.

2 - OPERATION PRINCIPLE

2.1 - Microorganism Catalysis

Figure 1 shows the principle of our microbial biofuel cell and the details are illustrated in Figure 2. In the anode com-partment, Saccharomyces cerevisiae (yeast) is used as the bio-catalyst to oxide glucose and hydrogen protons, electrons, and carbon dioxide are generated through the reaction. The electrons are transferred to the anode and the protons are driven across a proton exchange membrane (PEM). In order to facilitate transfer of electrons, an electron transfer mediator, methylene blue, is added to the anolyte suspension. In principle, mediator molecules collect electrons from the biological electron transport chain of the microorganisms and transport them to the anode of the biofuel cell. On the other hand, the oxidizing solution (catholyte) is a solution of potassium ferricyanide, which accepts electrons and reduces to ferrocyanide, in potassium phosphate buffer. The reduced ferrocyanide is then oxidized by oxygen and returns to ferricyanide and water. Overall, the reaction consumes glucose and oxygen while produces carbon dioxide and water.
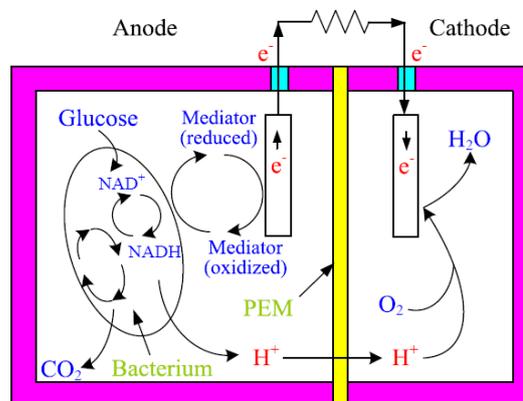

Figure 1 – Working principle of a microbial biofuel cell.

---
* Contact author: Tel. 886-3-574-2374, Fax: 886-3-572-0724, email: ycsu@ess.nthu.edu.tw

Anode
$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24e^- + 24H^+$$

$$4Fe(CN)_6^{3-} + 4e^- \rightarrow 4Fe(CN)_6^{4-}$$
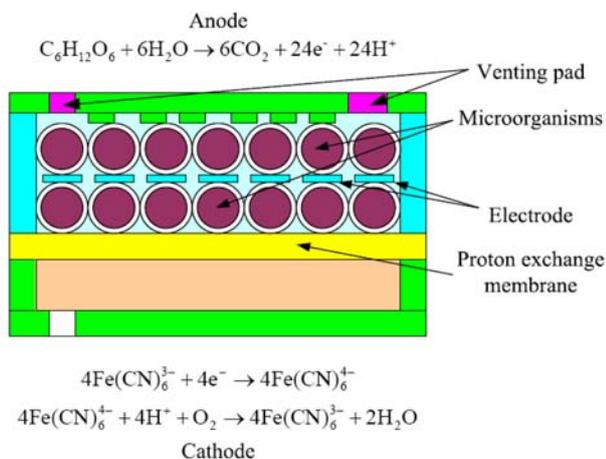$$4Fe(CN)_6^{4-} + 4H^+ + O_2 \rightarrow 4Fe(CN)_6^{3-} + 2H_2O$$
Cathode

Figure 2 – Structure of the proposed microbial biofuel cell.

2.2 - Carbon Dioxide Degassing

In order to prevent the build up of pressure that eventually degrades the system, carbon dioxide gas must be efficiently discharged from the anode compartment. Typically, bubbles can be removed by hydrophobic gas vent if the electrolyte is a simple gas/liquid mixture [4]. However, in our case the microorganisms in the electrolyte tend to stick on hydrophobic surfaces and eventually block the gas vent. In order to immobilize microorganisms inside the anode compartment and space them from the hydrophobic vent, thin alginate membranes are employed to encapsulate microorganisms as indicated in Figure 2. Carbon electrodes and microorganism capsules are arranged layer by layer to increase the effective surface for electron transport. Once carbon dioxide gas is generated, it eventually merges into tiny bubbles all around the anode compartment. While CO_2 bubbles are entrapped in the gaps between electrode and compartment surfaces, pressure gradient could be built by capillarity to passively guide the movement of these bubbles. As indicated in Figure 3, a hydrophilic channel diverging from right to left will apply a pressure gradient along the direction to an entrapped bubble. In order to facilitate the degassing of CO_2, specific patterns could be formed on the electrode and compartment surfaces to control the flow of bubbles. Capillary and buoyancy forces will drive unwanted CO_2 moving to hydrophobic venting holes and diffusing out of the compartment.
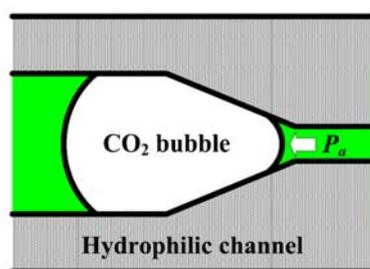

Figure 3 – Capillary pressure gradient applied on an entrapped bubble.
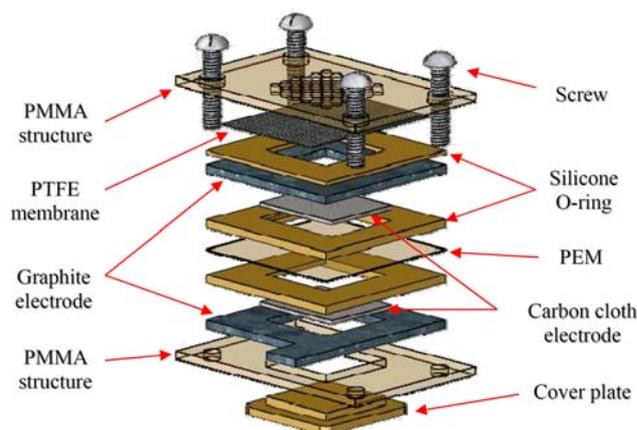

Figure 4 – Assembly diagram of the proposed microbial biofuel cell.

3 - FABRICATION

3.1 - Fuel Cell

An assembly diagram of the prototype system, which is composed of polymeric parts and carbon/graphite electrodes, is shown in Figure 4. Polymethylmethacrylate (PMMA) plates shaped by milling are used as the main structure for mechanical support and assembly. On the top PMMA structure, there is an array of holes designed for gas venting. A hydrophobic polytetrafluoroethylene (PTFE) membrane underneath these holes is employed to provide the pathways for discharging the CO_2 gas accumulated in the anode compartment. On the other hand, molded and machined graphite plates are used to construct the anode and cathode compartments. In this way, the effective area for electron transfer is further increased. Furthermore, soft polydimethylsiloxane (PDMS) o-rings are sandwiched between adjacent structural layers to achieve reliable seal, which is required in order to sustain the pressure built by CO_2 accumulation.

In this work, Dupont Nafion-117 thin film is chosen as the PEM material because of its unique semi-permeable property. Before sandwiching PEM with PDMS o-ring layers, the membrane has to be cleaned to remove the contaminations on its surfaces. On the other hand, the working electrodes for electron collection are made of either carbon cloths or graphite plates. In the case of carbon cloth being used, the effective electrode area is notably magnified because of its weaved texture. However, it will be difficult to further shape carbon cloth into specific 3-D structure, which functions to guide the flow of CO_2 bubbles. Comparatively, graphite plates can be conveniently shaped by either hot embossing or precise milling to form microscale pattern on their surfaces. Once the components are packed and aligned accordingly, the microbial biofuel cell is assembled by screws to provide the required clamping forces. For the prototype demonstration, cells with 1 cm^2 projected electrode area are fabricated and characterized. A prototype system having an overall size of 30 x 15 x 5 mm^3 is shown in Figure 5.
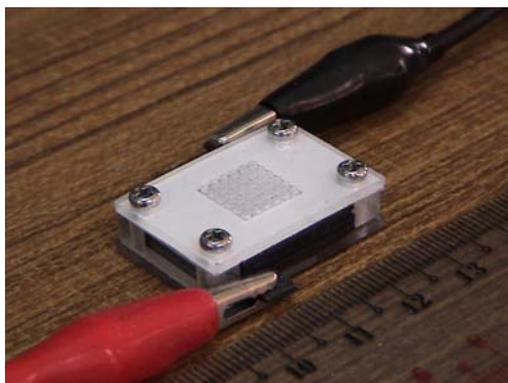
**Figure 5** – Photography of the fabricated microbial biofuel cell prototype.

3.2 - Microorganism Encapsulation

The encapsulation process is modified from a previous work, in which millimeter-scale capsules are formed by the inter-action between surface tension and gravity [5]. For the current application, smaller capsules with higher surface-to-volume ratios and catalysis performance are desired. In order to realize the spontaneous forming of micrometer-scale capsules, a hydrodynamic-focusing mechanism [6], which discretizes a continuous aqueous flow into uniform droplets, is employed. The discretization process of aqueous droplets in oil, which happens at a microscale cross junction, is illustrated in Figure 6. The transparent PMMA channels, which have dimensions in the hundreds of micrometers range, are fabricated using hot embossing and machining processes. Two oil streams from the top and bottom channels squeeze and break the Na-alginate solution flowing out of the left channel. The Na-alginate solution is a mixture of alginate, yeasts, and Tween-20 surfactant, which is used to improve the permeability of capsule membrane. Otherwise, the generation of CO_2 gas may eventually rupture capsules because of the accumulated pressure. By adjusting the flow rates of oil and Na-alginate solution, droplets of roughly 600 μm in diameter are generated at a frequency of 2.5 Hz. After that, the Na-alginate emulsion is dripped into a calcium ion solution to produce stable Ca-alginate coating on the outer surface. These liquid-core alginate capsules are then densely packed in the anode compartment as illustrated in Figure 2.

4 - EXPERIMENT RESULT

4.1 - Sample Preparation

The microorganisms used in the experiments are baker's yeasts, which are commercially available from a variety of sources. Before the experiments, the microorganisms are hydrated and re-activated. For anolyte, the microorganisms are mixed with glucose (with various concentrations) in a phosphate buffer (0.1 M and pH 7.0). The electron transfer mediator, methylene blue (0.01 ~ 0.05 M), is then added in to promote the biochemical-to-electrical reaction efficiency. On the other hand, the catholyte, in most cases a potassium ferricyanide solution (0.02 M), is prepared in a phosphate buffer (0.1 M, pH 7.0).
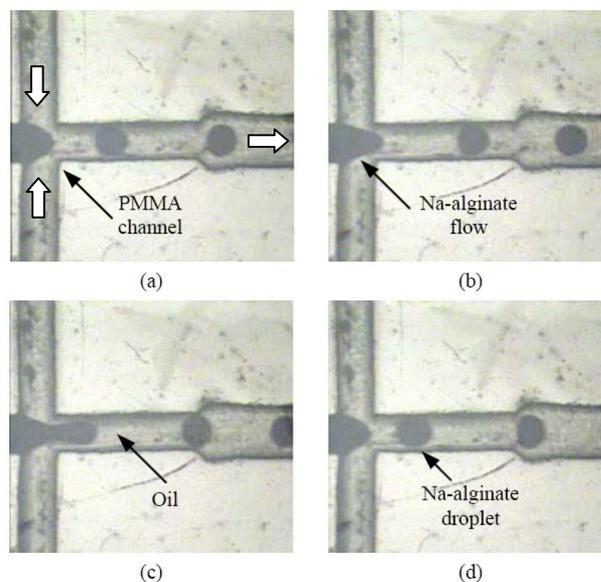
Figure 6 – The discretization process of a continuous Na-alginate solution flow into uniform micro droplets at a cross junction.
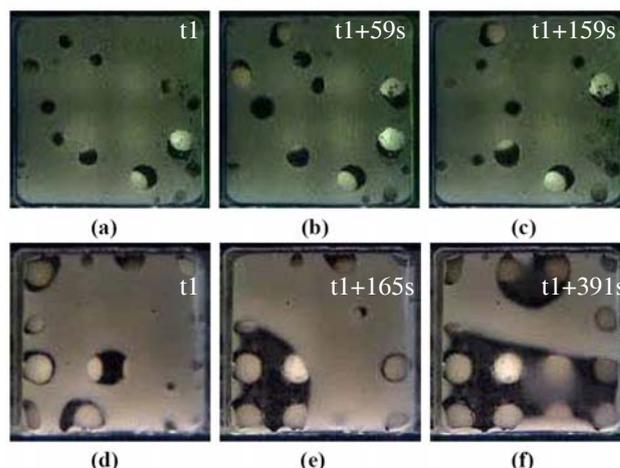
Figure 7 – From (a) to (c): with a functional venting design, bubbles are discharged once reaching a certain volume limit. From (d) to (f): without a functional venting design, anolyte is discharged and bubbles will eventually fulfill the anode compartment.

4.2 - CO₂ Degassing

The forming and discharging of CO_2 bubbles inside the anode compartment are observed and recorded under an optical microscope. Figure 7 shows the sizes and distribution of bubbles while a functional venting design is included or excluded from the system. Through the oxidation process, carbon dioxide gas is continuously generated and accumulates inside the anode compartment. Typically, CO_2 bubbles tend to stick on the surface and merge together to form larger bubbles as indicated in Figure 7 (d) to (f). At the end, CO_2 gas will drive all the anolyte out and stop the biofuel cell reaction. On the other hand, while a functional

venting system is integrated into the system, it causes the movement of CO_2 bubbles by capillary forces. In addition to gas discharging, this movement also results in the circulation of anolyte inside the compartment, which notably facilitates the transfer of charge and improves the performance of bio-fuel cells as well.

4.3 - Biofuel Cell Performance

The performance of the proposed microbial biofuel cells is determined by several key parameters, including the con-centrations of microorganisms, mediator, and glucose, the properties and geometries of electrodes, and the performance of guiding and venting sub-system. In order to investigate the characteristics and optimize the performance of the system, a series of experiments have been performed. Figure 8 shows the best performance we have ever achieved in this research. The voltage output is relatively steady at around 320 mV through a 3-hour-long period. On the other hand, the current output is up to $80\mu A/cm^2$ in the first 30 minutes and drops to around $10\mu A/cm^2$ after 2 hours. In order to achieve higher voltage and current output, sets of biofuel cells connected in parallel and in serial are built and characterized. It has been demonstrated that with six proposed cells connected in serial, the output is capable of powering a LED for up to an hour.
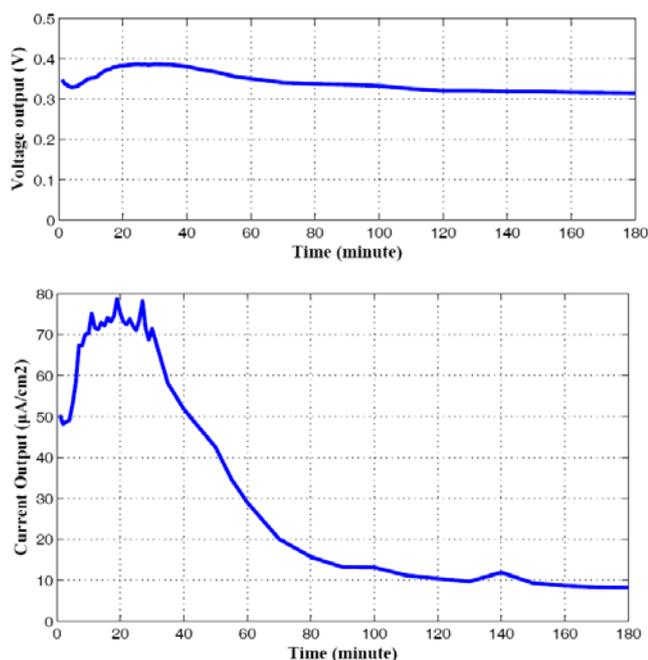

Figure 8 – Voltage and current outputs of the prototype microbial biofuel over time.

5 - CONCLUSION

In this work, a self-contained microbial biofuel cell for long-term operation has been successfully demonstrated. This fuel cell consumes glucose and oxygen to generate electricity in a reaction catalyzed by encapsulated Saccharomyces cerevisiae. The yeasts are immobilized inside microscale alginate capsules, which prevent the yeasts from depositing on device

surfaces while allow substances to diffuse through. In order to discharge unwanted carbon dioxide gas, this biofuel cell is equipped with a bubble guiding and venting system, which facilitates the transport of charges as well. In the prototype demonstration, voltage output at around 320 mV and current output up to $80\mu A/cm^2$ is achieved through a 3-hour-long period. As such, this biofuel cell is capable of self-regulating the electricity harvesting process and therefore able to power devices such as LEDs and micro-machines for an extended period of time.

ACKNOWLEDGMENTS

REFERENCES

[1] Katz, E., Shipway A.N., and Willner, I., "Biochemical Fuel Cells," in *Handbook of Fuel Cells*, Wiley, 2003.
[2] Palmore, G.T.R. and Whitesides, G.M., "Microbial and Enzymatic Biofuel Cells," in *Enzymatic Conversion of Biomass for Fuels Production*, American Chemical Society, 1994.
[3] Chiao, M., Lam K.B., and Lin, L., "Micromachined Microbial Fuel Cells," *Technical Digest of 12th Int. Conf. on Solid-State Sensors, Actuators and Micro-systems,* pp. 383-386, 2003.
[4] Meng, D.D., Kim, J., and Kim, C.-J., "A Degassing Plate with Hydrophobic Bubble Capture and Distributed Venting for Micro-fluidic Devices," *J. Micromech. Microeng.*, Vol. 16, pp. 419-424, 2006.
[5] Park J.K. and Chang H.N., "Microencapsulation of Microbial Cells," *Biotech Advances*, Vol. 18, pp. 303-319, 2000.
[6] Huang, K.S., Lai, T.H., and Lin, Y.C., "Manipulating the Generation of Ca-alginate Microspheres Using Microfluidic Channels as a Carrier of Gold Nanoparticles," *Lab on a Chip*, Vol. 6, pp. 954-957, 2006.