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Biological fuel cell and an application as a reserve power source

Alyssa L. Walker, Charles W. Walker Jr *

US Army Research Laboratory, Adelphi, Electrochemistry Branch, 2800 Powder Mill Road, AMRSD-ARL-SE-DC, Adelphi, MD 20783, USA

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Abstract

A biological fuel cell was constructed in which the metabolism of glucose by yeast provided the electrons. A two compartment plexiglass fuel cell was constructed with platinum electrodes separated by a proton conducting membrane. One half of the cell contained yeast, *Saccharomyces cerevisiae*, glucose, and an electron mediator, methylene blue. The other half contained an electron acceptor, potassium ferricyanide. Electrons produced during the metabolism of glucose by the yeast were captured by the methylene blue and transferred to the platinum electrode, traveled through the external circuit to the cathode compartment, and were finally accepted by the potassium ferricyanide. When yeast was added to the fuel cell the open circuit potential was ~0.5 V. Electrical current was measured while holding the cell voltage at 0.3 and 0.1 V. Current output was optimized for the initial concentration of yeast, temperature, glucose concentration, agitation and oxygen content. The optimum conditions for producing current within the scope of this study were obtained with agitated, partially oxygenated electrolyte at 45 °C containing 0.1 M glucose.

Keywords: Biological fuel cell; *Saccharomyces cerevisiae*; Yeast; Power; Reserve cell

1. Introduction

Although the concept of fuel cells was developed by Sir William Grove in 1839, the current search for renewable energy sources has stimulated interest in developing practical commercial systems. There is considerable effort to develop devices to provide power for applications ranging from small consumer electronics such as cell phones to automobiles and stationary power plants (e.g. to power a building). Fuel cell technologies are also being considered for other very low power applications such as body-implanted drug pumps [1,2], unattended ground sensors for military purposes [1,3], and on-chip power for microelectronics [1,4–6].

Although a hydrogen/air fuel cell is preferred, the infrastructure to handle, store and distribute hydrogen gas does not currently exist. However, other readily obtainable fuels can be utilized, such as methanol, natural gas, gasoline and diesel fuel. However, these fuels generally require complex reforming processes to extract and purify hydrogen before being fed into the fuel cell. A variation of this technology is the biological fuel cell, which can use live microbes or immobilized enzymes to produce hydrogen [7,8], or more commonly, directly provide electrons derived from the metabolic pathways that produce energy. Electron mediators are often needed to transfer electrons from the microbe to an electrode [9–11] or enzyme to the electrode [4,12–16], but in some cases electrons are transferred directly [17–22].

In this study we demonstrate a biological fuel cell "powered" by ordinary dry Baker's yeast, *Saccharomyces cerevisiae*, without the need for sterilization procedures or the maintenance of live cultures or labile enzymes. Yeasts grow rapidly under both aerobic and anaerobic conditions, have simple nutritional requirements, and can utilize a wide variety of substrates which make them ideal for a biological fuel cell. An off-the-shelf commercial yeast preparation is preferable to immobilized enzymes which by comparison are expensive, difficult to isolate, substrate specific, and much more sensitive to environmental parameters such as temperature and pH. A two compartment fuel cell similar to one designed by Bennetto [9] was constructed with platinum electrodes separated by a proton conducting membrane. One half of the cell contained yeast, *Saccharomyces cerevisiae*, glucose, and an electron mediator, methylene blue. The oxidation-reduction mediator enters the outer cell membrane and becomes reduced, then leaves in the reduced state. The reduced mediator transfers the captured electrons to an electrode, producing an electric current and re-oxidizing the methylene blue. The

* Corresponding author. Tel.: +1 301 394 0306; fax: 1 301 394 2073.
E-mail address: cwalker@arl.army.mil (C.W. Walker Jr).

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other half of the cell contained an electron acceptor, potassium ferricyanide, which accepts the electrons from the circuit and becomes reduced. Oxygen in the catholyte will oxidize Fe^{2+} back to Fe^{3+}. Ferricyanide was preferred as the electron acceptor over an oxygen-saturated solution because we did not want significant oxygen diffusion through the membrane into the anode compartment where it would oxidize the methylene blue before the methylene blue could transfer electrons to the electrode.

The optimization of several independent variables to maximize production of electricity was determined. We show here the effect of temperature, substrate concentration, quiescent versus agitated solutions, oxygen-rich versus anoxic solutions, and the influence of yeast inoculum concentration on electrical output.

2. Experimental

2.1. Fuel cell construction

Two halves of the fuel cell were constructed from 2.4 cm thick plexiglass with a 6.5 cm × 6.5 cm chamber machined into one face (1.5 cm deep) and two holes drilled from one edge (Fig. 1). One hole was for the electrode lead wire and the other for addition of solution. Both electrodes were made of platinum mesh (4 cm × 5 cm) with platinum wire leads to enable indefinite use without corrosion or fouling. Electrically insulating but ionically conducting Nafion 117 proton exchange membrane was treated to remove impurities and prepare it in the H^+ form by boiling the film for 1 h each step in 3% H_2O_2, deionized water, 0.5 M H_2SO_4, and deionized water. The Nafion membrane was sandwiched between two rubber gaskets and bolted between the two halves of the fuel cell to create two compartments. Each compartment was filled with deionized water when not in use to maintain hydration of the Nafion which is essential for good proton conductivity. A schematic of the system is shown in Fig. 2. The light bulb illustrates a simulated load and not the actual set-up. The open circuit voltage (OCV) of the cell was ~0.5 V.

2.2. Stock solutions

A 0.1 M phosphate buffer (Na_2HPO_4 and NaH_2PO_4), 10 mM methylene blue in phosphate buffer, 0.1 M potassium ferricyanide in phosphate buffer, and 1.0 M and 0.2 M D-glucose in phosphate buffer (all Aldrich Chemical) were prepared with demineralized water. Fleischmann's Dry Rapid Rise Yeast which contains nutrients to promote rapid growth was the source of *Saccharomyces cerevisiae*.

2.3. General procedure

In one half of the cell (anode) a 50:50 mixture of glucose and methylene blue solutions produced final concentrations of

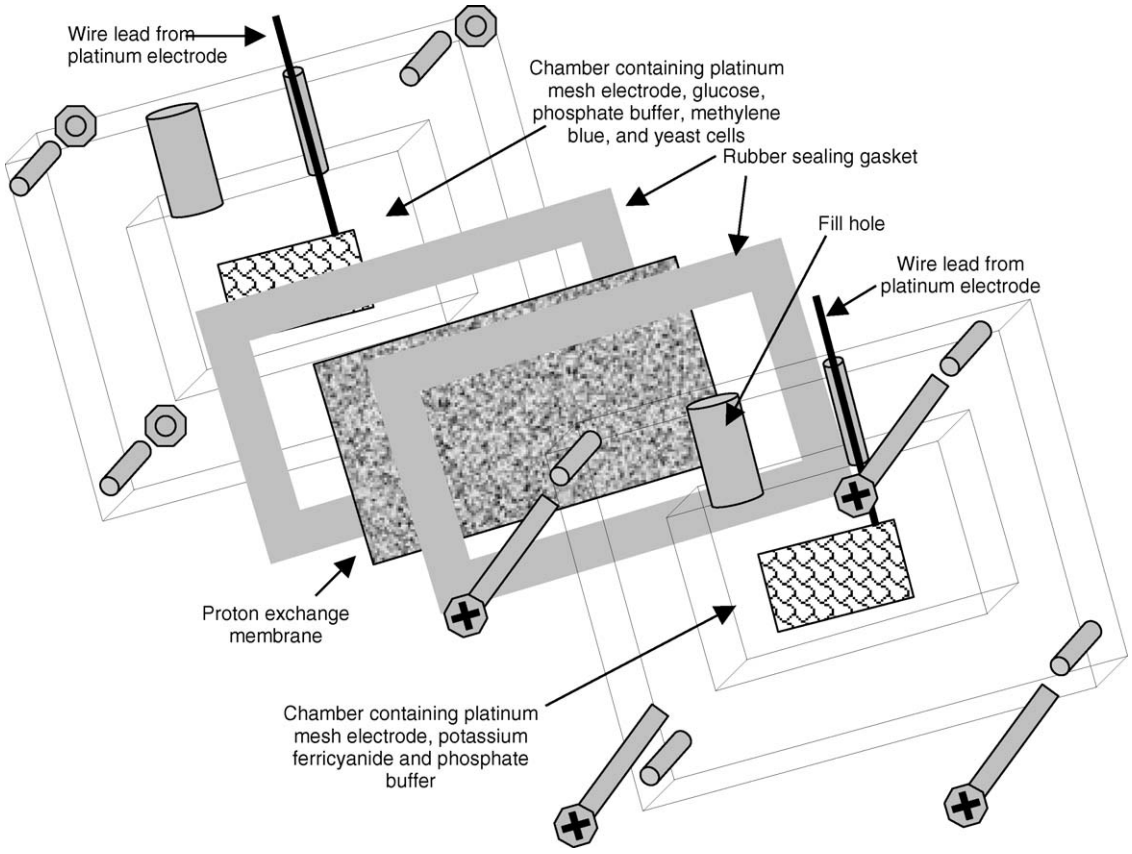

Fig. 1. Construction diagram of the plexiglass fuel cell.

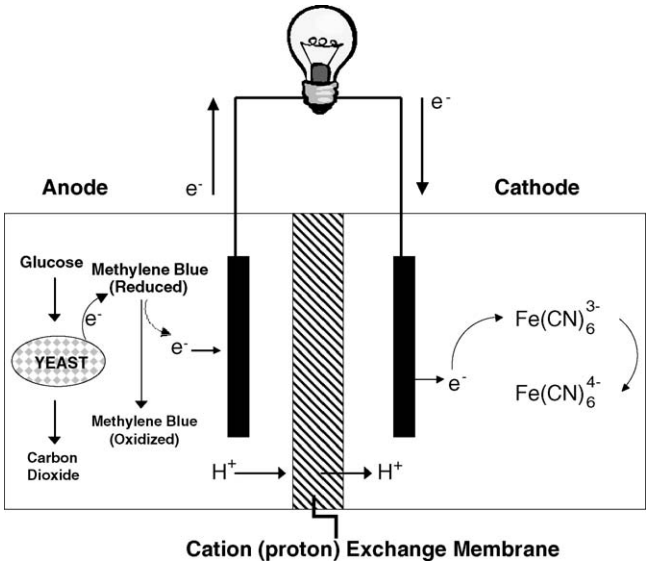

Fig. 2. Schematic of the biological fuel cell system.

0.1 and 0.005 M, respectively. The other half (cathode) contained an equal volume of 0.1 M potassium ferricyanide. The cell was placed in a Tenney Environmental Chamber to control and equilibrate temperature prior to each experiment. The electrical leads of the platinum electrodes were attached to an EG&G/PARC Mdl 273 potentiostat/galvanostat with M270 software under computer control. Chronoamperometry (current measured over time) experiments were performed by stepping cell potential from OCV to 0.3 V and measuring current output each 0.5 s for 5 min, followed by stepping voltage to 0.1 V for 5 min while measuring current. When applicable, oxygen or argon was bubbled into the anode compartment via a glass Pasteur pipette connected to a flowmeter to deliver 100 ml min^{-1} of gas to agitate the solution or to establish oxygen-rich and anoxic conditions. It is not particularly important to know the exact number of yeast cells being used if the same quality-controlled commercial source of yeast is used, so inoculum size is given by mass instead. To weigh out a mass simplifies having to determine and deliver a certain cell number from solution. Yeast was prepared as a slurry by mixing with a few ml of phosphate buffer immediately before being added to the anode compartment with a disposable polyethylene transfer pipet. The cells were briefly mixed by drawing up solution and dispensing it back into the anode compartment. A period of 30 min was allowed for yeast to grow before beginning each experiment. The two controls were: (a) a complete cell without addition of yeast (b) a complete cell without addition of glucose. Independent variables were amount of yeast inoculum, temperature, glucose concentration, ±stirring, ±oxygen. The dependent variable was the current output harnessed from the yeast.

2.4. Optimization tests

The optimum amount of yeast inoculum was determined for the conditions of 30 °C with 0.1 M glucose. Enough yeast was weighed out to produce final concentrations ranging from 1 to 35 mg ml^{-1}. A 5 min test period was arbitrarily chosen for each voltage step. The current measured at the end of 5 min was reported since this value approaches equilibrium and most clearly demonstrates differences. The effect of temperature from 10 to 55 °C was performed using 20 mg ml^{-1} yeast inoculated after equilibrating cell temperature in a Tenney environmental chamber. Glucose concentration tests were done at 30 °C with 20 mg ml^{-1} yeast for glucose concentrations of 0, 0.1 and 0.5 M. Stirred solutions were created by bubbling argon at 100 ml min^{-1} through a Pasteur pipette inserted into the anode compartment following a period of quiescent conditions. Oxygen-rich and anoxic solutions were prepared by bubbling of oxygen and argon, respectively, for 30 min prior to inoculation of yeast. Experiments were performed at least three times and results averaged.

3. Results and discussion

A control experiment without any yeast gave an OCV of about 0.175 V and baseline current of 2 μA. When potential was driven to 0.1 V the current increased about 3.5 μA. Short circuit current was only 18.5 μA. With the addition of yeast the OCV quickly reaches about 0.5 V and baseline currents exceed 100 μA.

In all of the optimization experiments high current spikes were observed when cell potential was stepped from OCV to 0.3 V. This occurs because methylene blue has accepted electrons with few being transferred to the electrode. Once the load is applied a significant transfer of electrons occurs, similar to the discharging of a capacitor. A steady state equilibrium is subsequently established, determined by the rate of glucose metabolism, electron transfer to the mediator, and diffusion of the mediator to the electrode.

3.1. Cell inoculum concentration

Inoculation of a low number of cells (1 mg ml^{-1}) results in a low current response due to the low number of electron donors, but once a sufficient number of cells were present, about 20 mg ml^{-1}, the current response (and therefore power) was maximized (Fig. 3). Although there is some uncertainty in this value shown by the error bar for the cell held at 0.3 V, this seems a reasonable choice and the data for the cell held at 0.1 V tends to support this conclusion. Addition of an excessive number of cells did not improve response and is an inefficient use of cells.

3.2. Temperature

The effect of temperature on current response is shown in Fig. 4 where typical chronoamperometric curves are shown for five temperatures. Optimal temperature for the cell conditions outlined in Section 2.4 at 0.3 V was 45 °C, although at 0.1 V 50 °C was optimal (Fig. 5). Differences may be attributed to normal distribution and the low number of replicates used to calculate the average. The power delivered by the fuel cell held

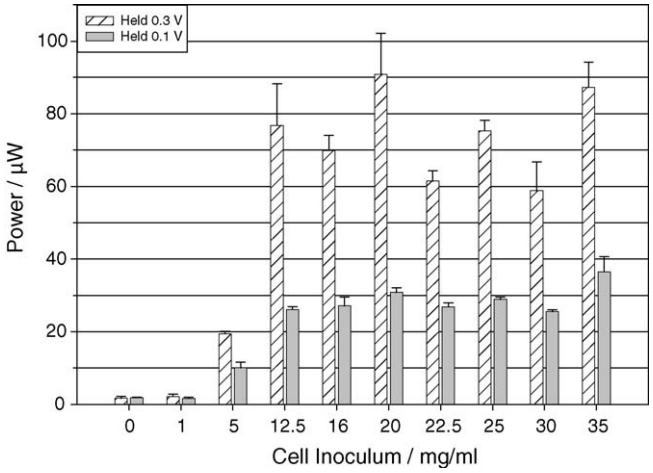

Fig. 3. Electrical power generated as a function of concentration of yeast inoculum at 30 °C and 0.1 M glucose. Cell voltage was stepped from OCV to 0.3 V for 5 min, and then stepped to 0.1 V for 5 min. Average current at the end of 5 min was used to calculate power.

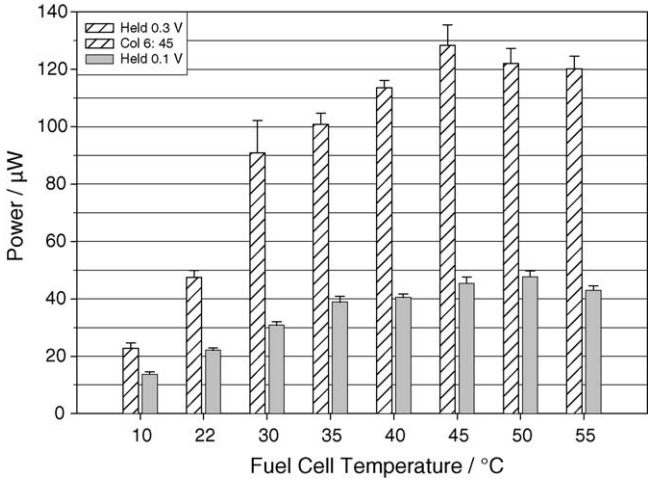

Fig. 5. Power generated as a function of cell temperature.

at 0.3 and 0.1 V is shown in Fig. 5. Although current is greater at 0.1 V less power is attained. Since the higher power was obtained at 45 °C this was taken as the optimal value. Average power increased from over 20 μW at 10 °C to about 90 μW at 30 °C and nearly 130 μW at 45 °C.

3.3. Glucose concentration

Because yeast contains endogenous stored energy and 'rapid rise' yeast are processed to contain additional nutrients to promote rapid growth there is significant cell growth even without the addition of any glucose. Without an external load a current of about 155 μA is produced. When cell potential is driven to 0.3 V an additional current of about 70 μA (average value) is realized. At 0.1 V, current increases about 135 μA to around 290 μA. Apparently, the endogenous and added energy sources are sufficient to allow nearly maximal growth over the short time periods used in these experiments since addition of glucose at a concentration of 0.1 M shows only a modest increase in current response. Current response is converted to power and shown in Fig. 6. A high glucose concentration of 0.5 M is of no additional benefit. This result was not unexpected, because even under aerobic conditions *S. cerevisiae* can simultaneously grow via respiration or fermentation under a condition of high glucose concentration. Termed the Crabtree effect [23,24], genes used for respiration are repressed and fermentation predominates. Although growth rate remains high, fermentation yields much less energy than oxidative respiration of glucose. Exceeding this limiting threshold of glucose concentration is illustrated in Fig. 6, where the power is lowest at 0.5 M glucose concentration. Any benefit of excessive glucose would be expected to become evident over long-term operation of the fuel cell. These experiments are yet to be conducted.

3.4. Stagnant versus agitated solution

Current response is diffusion limited since it is a function of the proximity of the yeast to the platinum electrode and

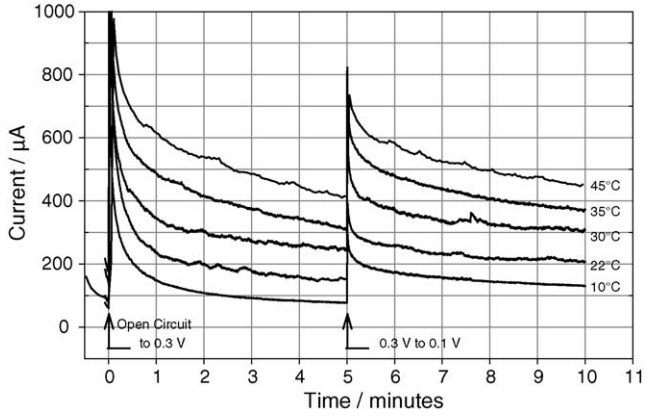

Fig. 4. Electrical current produced as a function of temperature with 20 mg ml^{-1} of yeast inoculum and 0.1 M glucose. Cell voltage was stepped from OCV to 0.3 V for 5 min, and then stepped to 0.1 V for 5 min.

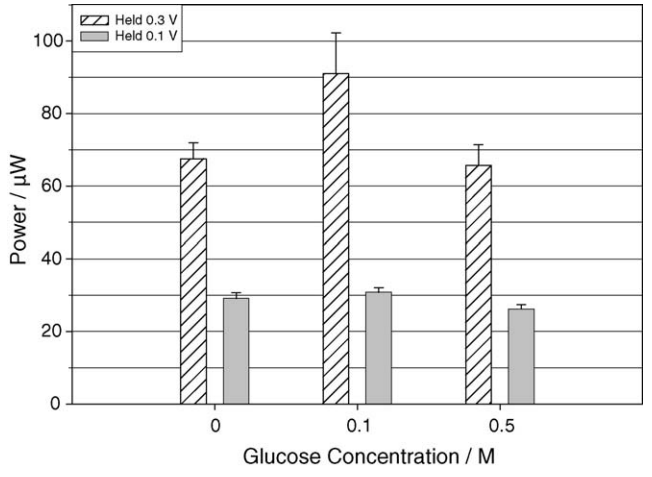

Fig. 6. Power generated as a function of glucose concentration for 20 mg ml^{-1} yeast inoculum at 30 °C.

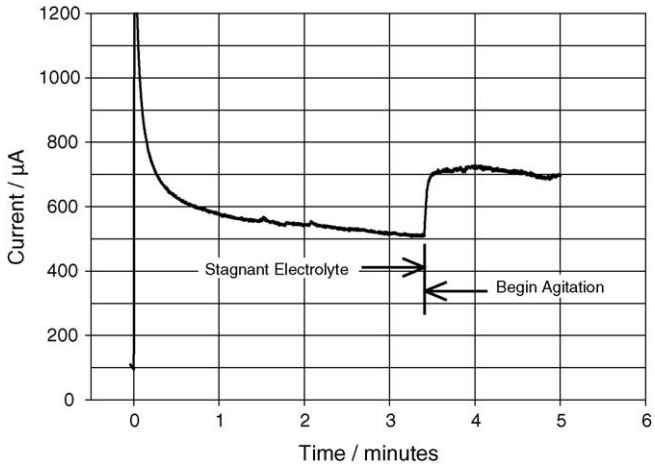

Fig. 7. Effect of solution agitation at 45 °C and 0.1 M glucose with 20 mg ml^{-1} yeast inoculum held at 0.3 V.

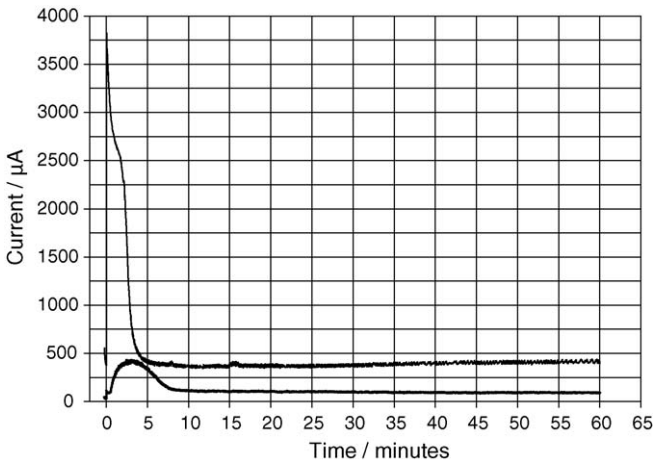

Fig. 8. Comparison of the effect of oxygen-saturated (lower trace) and anoxic (upper trace) solutions on current at 45 °C and 0.1 M glucose with 20 mg ml^{-1} yeast inoculum held at 0.3 V for 1 h.

ultimately controlled by the diffusion of methylene blue to the platinum electrode. Even under stagnant conditions, some self mixing occurs due to CO_2 evolution by yeast. Agitation or stirring of the solution eliminates this diffusion-limited condition. Fig. 7 shows an increase of about 40% in current response when a stagnant solution is agitated by bubbling argon.

3.5. Aerobic versus anaerobic conditions

Yeasts are facultative aerobes, able to utilize glucose both aerobically and anaerobically. In addition to storing energy in the cell in the form of ATP, the end products of aerobic respiration are CO_2 and H_2O (Eq. (1)) and the end products of fermentation are ethanol and CO_2 (Eq. (2)).

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 36ATP\,(net) \quad (1)$$

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + 2ATP\,(net) \quad (2)$$

Aerobic respiration more efficiently utilizes glucose and produces more energy (i.e. electrons) than fermentation. Three ATP are produced per 2 electrons. If a mediator accepts the electrons instead of oxygen, one molecule of glucose can provide up to 24 electrons that may contribute to cell current (Eq. (3)). Fermentation yields only 4/3 electrons per molecule of glucose.

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^- \quad (3)$$

Fig. 8 shows the current delivered when cells were inoculated into oxygen-rich and anoxic solutions and current response followed for 60 min. Once equilibrium is reached current response is fairly stable. A higher current response would be expected with the oxygenated electrolyte but this is not observed. The lower response in the oxygenated solution can be explained by the fact that oxygen will oxidize methylene blue, so in this oxygen-rich environment many of the electrons from the reduced form of methylene blue are captured by oxygen before they can be transferred to the electrode. The higher initial currents during the first few minutes in the anaerobic environment are most likely explained by this mechanism.

The optimal condition seems to be when a moderate amount of oxygen is available. In comparing Fig. 7 where some oxygen is present to Fig. 8 with oxygen-saturated or deoxygenated solutions, it is evident that the presence of some oxygen (ambient air contains ~21% O_2) results in obtaining higher currents by allowing maximum cell metabolism with a minimum of competition by oxygen to oxidize methylene blue.

3.6. Active and reserve configurations

Active fuel cell operation should be long lived. The Pt screen electrodes will provide unlimited electrode life enabling the cell to be used, stored, and reused indefinitely without electrode replacement. Occasional replacement of the Nafion membrane (as yet undetermined) may be the only maintenance required. Under continuous use, supplying fuel is a simple matter of withdrawing some of the anode solution containing the spent yeast, glucose, methylene blue, phosphate buffer mixture and replacing it with fresh glucose, methylene blue, phosphate buffer solution. Addition of fresh yeast cells is unnecessary since sufficient numbers of active cells will sustain the process. When not in use, a stored cell can be quickly activated by pouring out the water and adding the appropriate prepared solutions to the cell, or alternatively one can add ampoules of the dry components that will quickly be dissolved in water.

Because no active live organisms are used, this fuel cell is also amenable for use as a reserve cell. All components maybe stored in the dry state and the cell activated by hydrating with water. A very compact, inexpensive reserve cell could be produced using “pouch cell” technology. Here, the Pt electrodes could be replaced by inexpensive high surface area carbon electrodes since this is a single use application intended for a finite operating life. The surface area of carbon electrodes ranges from 60 to 1500 m^2 g^{-1}. A high surface area enhances electron transfer

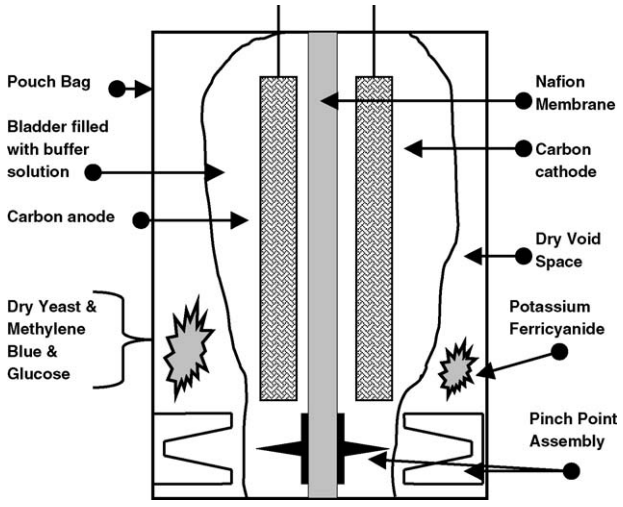

Fig. 9. Reserve pouch cell configuration.

from the methylene blue mediator thereby increasing current. One possible design is shown in Fig. 9. The dry components would be stored on the outside of a bladder filled with water or phosphate buffer that also contains the Nafion membrane and carbon electrodes. A simple squeezing of the pinch points punctures the bladder and releases its contents. A few gentle squeezes of the pouch would facilitate mixing of the components to complete cell activation.

4. Conclusion

A biological fuel cell powered by the metabolism of glucose by yeast was optimized for concentration of yeast inoculum, temperature, and glucose concentration. The effect of agitation and oxygen content were also studied. The advantage of using Baker's yeast is that it is inexpensive and readily available, does not require sterile conditions, is not substrate specific, and tolerates a wide range of environmental conditions. As long as a commercial off-the-shelf source of yeast is used, an appropriate mass of yeast can be used as inoculum. The ability to quickly weigh out the yeast is a huge benefit which eliminates the time and labor of preparing and maintaining a purified culture and determining cell concentration. Another advantage is that all of the fuel cell components could be stored or transported in the dry state and then simply activated by hydrating with water.

The optimum conditions within the scope of those used in this study for producing current were obtained with a yeast inoculum concentration of 20 mg ml^{-1} in agitated, partially oxygenated electrolyte maintained at 45 °C and containing 0.1 M glucose. Low power (>20 μW) can be delivered even at a cool temperature of 10 °C, but power peaks (∼130 μW) at about 45 °C. This suggests that devices held near the body, used in desert environments, or mounted in close proximity to heat releasing electronic devices would provide ideal conditions.

Over the short time period used in these experiments glucose concentration had a modest effect on current output up to 0.1 M. At a high concentration of 0.5 M energy output was slightly suppressed resulting from the Crabtree effect. Electrolyte agitation increased current because electron transfer was no longer limited by diffusion of methylene blue. Although the presence of oxygen will permit the greatest net number of electrons per glucose molecule, it will compete to oxidize methylene blue before electron transfer to the electrode. Under anaerobic conditions, fermentation provides fewer electrons per unit of glucose yet seems to support higher power since all of the electrons are transferred from the mediator to the electrode instead of oxygen. The preferred conditions may be different if fuel cell operation continues for longer periods of time, and will also likely show the influence of accumulated end products, high cell concentration and cell death.

Applications for the biological fuel cell might include devices only needing μW power, LEDs, trickle charging capacitors, powering retrievable unattended ground sensors, and microelectromechanical machines. Reserve pouch cells are an inexpensive alternative amenable to emergency low power such as to charge a capacitor to permit transmission of an emergency beacon or other data, disposable unattended ground sensors, and other single use miniature devices.

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