

Article

## Performance of a Yeast-mediated Biological Fuel Cell

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**Abstract:** *Saccharomyces cerevisiae* present in common Baker's yeast was used in a microbial fuel cell in which glucose was the carbon source. Methylene blue was used as the electronophore in the anode compartment, while potassium ferricyanide and methylene blue were tested as electron acceptors in the cathode compartment. Microbes in a mediator-free environment were used as the control. The experiment was performed in both open and closed circuit configurations under different loads ranging from 100 k $\Omega$  to 400 $\Omega$ . The eukaryotic *S. cerevisiae*-based fuel cell showed improved performance when methylene blue and ferricyanide were used as electron mediators, rendering a maximum power generation of 146.71 $\pm$ 7.7 mW/m<sup>3</sup>. The fuel cell generated a maximum open circuit voltage of 383.6 $\pm$ 1.5 mV and recorded a maximum efficiency of 28 $\pm$ 1.8 % under 100 k $\Omega$  of external load.

**Keywords:** Fuel cell, yeast, mediators, bio-catalyst.

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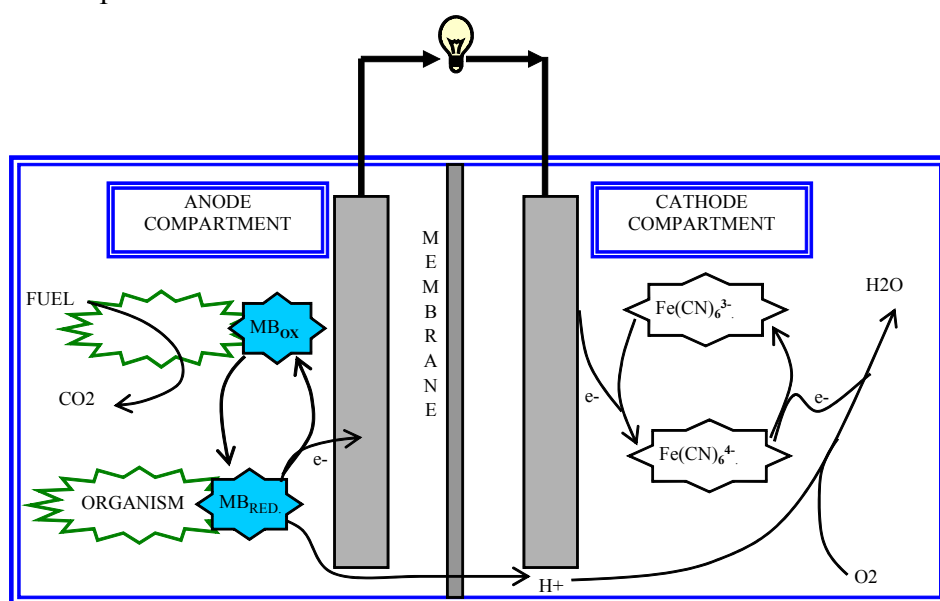
### 1. Introduction

Due to increasing energy prices and concerns on long-term energy security, there is a renewed interest in bio-renewable energy generation technologies. Consequently, direct electricity generation from glucose, a bio-renewable feedstock, has regained momentum. This study is an in-depth look at

the behavior of a fuel cell using Baker's yeast (*Saccharomyces cerevisiae*) in a proton exchange membrane (PEM) microbial fuel cell (MFC). Yeast based fuel cells are of interest since these could be easily retrofitted into ethanol plants for in situ power generation.

The function of microbes in a fuel cell is to catalyze the reaction that involves conversion of chemical energy into electrical energy [1-3]. The metabolic processes of these microorganisms produce electrons by oxidizing a carbon source, which in many applications, is a carbohydrate monomer. The electrons generated at the anode can then be passed through an external circuit to produce power. These electrons enter the cathode to combine with the protons ( $H^+$ ) that transfer through a PEM and bind with externally provided oxygen to form water (Figure 1).

**Figure 1.** The yeast fuel cell with its constituent redox cycles in the anode and the cathode compartment.



Note: MB<sub>ox</sub> – Methylene Blue (oxidized); MB<sub>red</sub>–Methylene Blue (reduced)

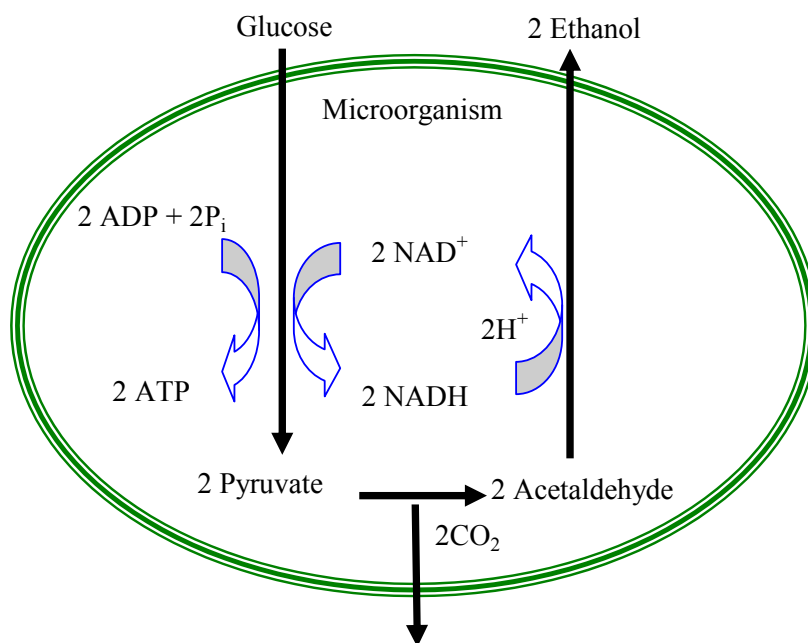
Presently, MFC research is highly focused on wastewater treatment [4-6]. The organic sludge is used as the carbon source for organisms to oxidize. To date, different organisms have been experimented in MFCs and depending on the need for presence or absence of mediators to complete the redox reactions, two main types of fuel cells have been documented: (i) Fuel cells with mediated electron transfer and (ii) Fuel cells with direct electron transfer [7].

In fuel cells with mediated electron transfer, an intermediate molecule, preferably a dye, will shuttle electrons between the microbe and the electrode. The staining ability of a dye helps it to stick to the cellular membrane and helps transfer of electrons and protons. Direct electron transfer is now a research interest where the organisms possess metal complexes on its membrane making exogenous mediators unnecessary [8-11]. Indigenous mediators reduce the risk of poisoning the medium, which is considered a serious drawback of the exogenous mediators. Commonly used organisms in direct electron transfer fuel cells are *Shewanella putrefaciens*, *Geobacter sulfurreducens*, *Geobacter metallireducens* and *Rhodospirillum rubrum* [12-14]. However, in Baker's yeast-based fuel cells,

exogenous mediators are a necessity since *S. cerevisiae* is not known to produce such mediators indigenously. Tests with organisms capable of generating mediators have proved higher conversion efficiencies compared to other MFCs with mediators which transports electrons between the species and the electrode. However, the conversion efficiency itself is inadequate to determine the fuel cell behavior as it does not sufficiently explain the energy generation mechanisms in the cell. Therefore, additional parameters such as: (i) metabolism of the bacterial species, (ii) electron transfer rate of the microorganisms, (iii) the effectiveness of the proton exchange membrane, and (iv) internal resistance have to be considered for effective performance analysis of a fuel cell. This work, in part, attempts to fulfill this knowledge gap using *S. cerevisiae* as the model microorganism.

The presence of oxygen in the anode compartment is not favorable for the overall fuel cell function. Oxygen is the terminal electron acceptor in the cathode compartment and therefore, the presence of it in the anode compartment would disrupt the electron flow through the external circuit. This obliges an oxygen free environment in the anode compartment of the MFC. *S. cerevisiae* like many other microorganisms can function in anaerobic conditions. It is an easily accessible microorganism with a well understood metabolism. The growth of *S. cerevisiae* is optimum at the ambient temperature which is around 30 °C. These factors prompted us to choose *S. cerevisiae* for this study.

**Figure 2.** Anaerobic fermentation pathway of *Saccharomyces cerevisiae* - under anaerobic conditions, yeast will transform pyruvate to ethanol. The reduction of  $\text{NAD}^+$  to  $\text{NADH}$  will generate two ATP molecules, two  $\text{H}^+$  ions and two electrons [15].



Under anaerobic conditions, as shown in the Figure 2, this organism switches to a fermentation reaction where two molecules of pyruvate are produced from one glucose molecule. Pyruvate will be further transformed to two molecules of acetaldehyde by the enzyme pyruvate decarboxylase. Acetaldehyde thereafter will be transformed to alcohol by alcohol dehydrogenase, which is an NADH dependent enzyme. In anaerobic fermentation the recycling of  $\text{NADH}$  to  $\text{NAD}^+$  is important to keep the glycolysis process continuous [15, 16]. Since this glycolysis reaction takes place in the cytosol of

the cell rather than in the mitochondria, NADH is easily accessible to a mediator molecule that is attached to the cell membrane. The MFC which operates using *S. cerevisiae* extracts the energy using NADH/NAD<sup>+</sup> redox cycle. The organism can sustain life as long as the glycolysis pathway is not obstructed. The energy extraction process in the fuel cell does not disrupt the glycolysis as NADH is oxidized back to NAD<sup>+</sup> when a mediator gets reduced.

The main objective of this study was to parameterize the performance of a *Saccharomyces cerevisiae* based fuel cell. The effect of extraneous mediators on open circuit voltage, current and power under different loads, the internal resistance and the efficiency were determined.

## 2. Materials and Method

### 2.1. Construction of the Fuel Cell

The fuel cell chamber was constructed from PVC and the internal diameter of the chambers was 5 cm in diameter and each compartment had a volume of 500 mL. The two chambers were separated by a Proton Exchange Membrane (PEM) where the membrane was held by a coupling between the chambers. A DuPont Nafion 117 PEM was purchased from the Ion Power Co. and each experiment was performed with a new membrane to avoid any interferences and/or contaminations from previous experiments. The electrodes were 45 PPI (Pores per Inch) Reticulated Vitreous (RV) carbon, with dimensions 1" × 1" × 6" purchased from ERG (Oakland, CA). Copper (Cu) wires were soldered to the electrodes by using a conductive epoxy. A digital pH transmitter (Sensorex, PHMA transmitter and pH probe) was used to measure pH variations during experimentation. The IoTech WaveBook-12 with a sampling rate of 0.1Hz was used as the automated data acquisition system.

### 2.2. Operation of the Fuel Cell

Cell growth measurements were not observed in order to minimize oxygen contamination to the fuel cell as well as to keep liquid volumes in the anode compartment static during experiments. On each operation, the fuel cell compartments were cleaned and the electrodes were autoclaved for 30 min. All the substrates, i.e., *Saccharomyces cerevisiae*, methylene blue (MB), potassium ferricyanide (PF) and D-glucose were purchased from Sigma Aldrich. The concentration of both MB and PF solutions were 50 mM. The anode solution was prepared with 2 g of yeast and 0.12M D-glucose which was prepared in either MB or de-ionized water — stirred well using a magnetic stirrer. In the experiment, the combinations of the solutions were selected according to Table 1. MB was tested as the anode mediator and on the cathode side both MB and PF were tested as both have the property of being reduced by oxygen. After a new batch was fed, both anode and cathode compartments were sparged with 300 mL/min CO<sub>2</sub> and O<sub>2</sub> respectively. The open circuit voltage (OCV) was measured continuously using the automated data acquisition system. A completely randomized design (CRD) was used in conducting the experiment and the data were analyzed using a statistical analytical software SAS. All the inferences were based at P = 0.05 significance level. The data points and the values are represented with ± standard error.

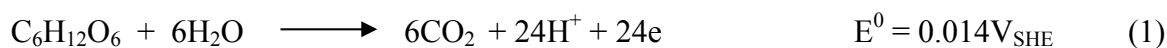
**Table 1.** The experimental design of the experiment.

Exp. Number	Organism	Substrate	Mediator- (anode)	Catholyte
EX1	Yeast	Glucose	Methylene blue	Methylene blue
EX2	Yeast	Glucose	Methylene blue	K <sub>3</sub> Fe(CN) <sub>6</sub>
EX3	Yeast	Glucose	Methylene blue	Water
EX4	Yeast	Glucose	Water	Water
EX5	Yeast	Glucose	Water	Methylene blue
EX6	Yeast	Glucose	Water	K <sub>3</sub> Fe(CN) <sub>6</sub>

### 3. Results and Discussion

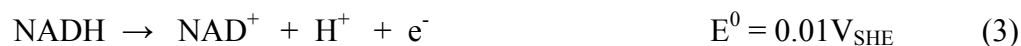
#### 3.1. Variation of Open Circuit Voltage

During the first phase of the experiment, a cyclic voltametry (CV) study was conducted to understand the reversible nature of the methylene blue (MB) and potassium ferricyanide (PF). Since MB is not toxic for the microorganism, it can be used as the mediator in the anode compartment - provided it is reversible between the reduced and the oxidized states. According to Figure 3, it can be explained that MB is reversible between its reduced and the oxidized states as the peak potential difference is very close to 59 mv. The CV study for the PF as given in Figure 4 indicates a quasi-reversible behavior between its reduced and the oxidized states.

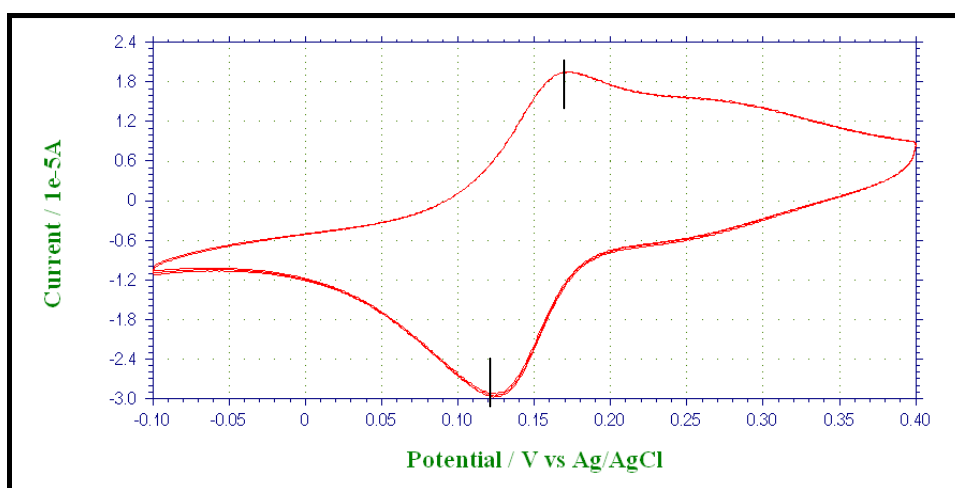


In the microbial fuel cell, glucose oxidation is the main source of energy generation. The individual potential of both the anode and the cathode depends on the redox potential of the reactions at the electrode. The oxidation half reaction of glucose, which is given in Equation 1, has a standard potential of 0.014 V, and theoretically, will determine the anode potential. However, it should be noted that the energy generated will not be available to extract directly at the electrode - since this conversion takes place in a microbe cell extraneous to the anode. On the other hand, the half reaction of the cathode, which is the reduction of molecular oxygen (Equation 2) will determine the potential of the cathode. The difference between the Equations 1 and 2 will give the fuel cell its theoretical maximum open circuit voltage of 1.216 V.

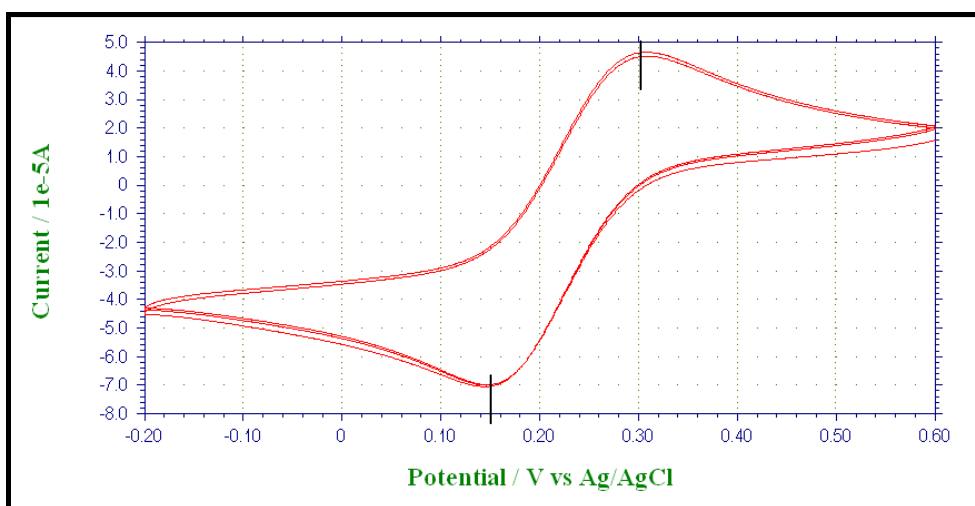
Under anaerobic conditions, *S. cerevisiae* undergoes fermentation where NADH oxidizes by alcohol dehydrogenase. However, some of the NADH will permeate outside the cell membrane. This will initiate a redox couple NADH / NAD<sup>+</sup> on the periphery of the anode where an electron and a proton will be discharged (Equation 3) [7]. Due to the cellular membrane, the NADH transfer to the outside is significantly hindered. Therefore, the presence of a mediator attached to the membrane surface will greatly enhance the electron transfer to the electrode. The redox reaction that occurs within methylene blue is given in Equation 4.



**Figure 3.** The cyclic voltammogram for 50 mM methylene blue solution with carbon electrode. Scan rate 0.05 V/s, potential range -0.1 V- 0.4 V. Reduction and oxidation peaks at 0.13 V and 0.18 V respectively.



**Figure 4.** The cyclic voltammogram for 50 mM potassium ferricyanide solution with carbon electrode. Scan rate 0.05 V/s and the potential range -0.2 V to 0.6 V. Reduction and oxidation peaks at 0.13 V and 0.20 V respectively.



The oxidation process of NADH is slow and inefficient [17]. In the case of yeast,  $\text{NAD}^+/\text{NADH}$  redox couple would determine the anode potential, if no electron shuttling mediator was available. The use of MB as an intermediate molecule will be effective in this regard as it will get reduced relatively easily and will undergo a reversible reaction [18]. In the presence of a mediator molecule, the potential of the anode will be determined by the potential of the oxidation reaction given in Equation 4. In the

six experiments conducted, the reactions at the anode and the cathode compartments are depicted in Table 2.

**Table 2.** Fuel cell reactions for different experiments: (a) the reactions in the anode compartment and (b) the reactions in the cathode compartment.

(a)

Experiment	Reactions in the anode compartment
EX1	$\text{MB}_{(\text{oxidized})} + \text{NADH}_{(\text{yeast})} \longrightarrow \text{NAD}^+_{(\text{yeast})} + \text{MB}_{(\text{reduced})}$
	$\text{MB}_{(\text{reduced})} \longrightarrow \text{MB}_{(\text{oxidized})} + 2\text{e}^- + \text{H}^+$
EX2	$\text{MB}_{(\text{oxidized})} + \text{NADH}_{(\text{yeast})} \longrightarrow \text{NAD}^+_{(\text{yeast})} + \text{MB}_{(\text{reduced})}$
	$\text{MB}_{(\text{reduced})} \longrightarrow \text{MB}_{(\text{oxidized})} + 2\text{e}^- + \text{H}^+$
EX3	$\text{MB}_{(\text{oxidized})} + \text{NADH}_{(\text{yeast})} \longrightarrow \text{NAD}^+_{(\text{yeast})} + \text{MB}_{(\text{reduced})}$
	$\text{MB}_{(\text{reduced})} \longrightarrow \text{MB}_{(\text{oxidized})} + 2\text{e}^- + \text{H}^+$
EX4	$\text{NADH}_{(\text{yeast})} \longrightarrow \text{NAD}^+_{(\text{yeast})} + \text{H}^+ + \text{e}^-$
EX5	$\text{NADH}_{(\text{yeast})} \longrightarrow \text{NAD}^+_{(\text{yeast})} + \text{H}^+ + \text{e}^-$
EX6	$\text{NADH}_{(\text{yeast})} \longrightarrow \text{NAD}^+_{(\text{yeast})} + \text{H}^+ + \text{e}^-$

(b)

Experiment	Reactions in the cathode compartment
EX1	$4\text{MB}_{(\text{oxidized})} + 4\text{H}^+ + 8\text{e}^- \longrightarrow 4\text{MB}_{(\text{reduced})}$
	$4\text{MB}_{(\text{reduced})} + \text{O}_2 \longrightarrow 2\text{H}_2\text{O} + 4\text{MB}_{(\text{oxidized})}$
EX2	$4\text{PF}_{(\text{oxidized})} + 4\text{e}^- \longrightarrow 4\text{PF}_{(\text{reduced})}$
	$4\text{PF}_{(\text{reduced})} + \text{O}_2 + 4\text{H}^+ \longrightarrow 2\text{H}_2\text{O} + 4\text{PF}_{(\text{oxidized})}$
EX3	$4\text{H}^+ + \text{O}_2 + 4\text{e}^- \longrightarrow 2\text{H}_2\text{O}$
EX4	$4\text{H}^+ + \text{O}_2 + 4\text{e}^- \longrightarrow 2\text{H}_2\text{O}$
EX5	$4\text{MB}_{(\text{oxidized})} + 4\text{H}^+ + 8\text{e}^- \longrightarrow 4\text{MB}_{(\text{reduced})}$
	$4\text{MB}_{(\text{reduced})} + \text{O}_2 \longrightarrow 2\text{H}_2\text{O} + 4\text{MB}_{(\text{oxidized})}$
EX6	$4\text{PF}_{(\text{oxidized})} + 4\text{e}^- \longrightarrow 4\text{PF}_{(\text{reduced})}$
	$4\text{PF}_{(\text{reduced})} + \text{O}_2 + 4\text{H}^+ \longrightarrow 2\text{H}_2\text{O} + 4\text{PF}_{(\text{oxidized})}$

With respect to the reactions given in the Table 2, the theoretical open circuit potentials for each experiment are given in the Table 3. Figure 5 (A) shows the results that were obtained by direct measurement of the potential difference between the electrodes. It was evident that the direct measurement results were different from the values obtained by taking the difference of the individual anode and the cathode potentials. The possible explanation for this is the existence of a potential difference between the interfacing liquids in the cathode and anode compartments.















