A low-cost yeast-based biofuel cell: an educational green approach

Gustavo Silveira\textsuperscript{a}, Masaharu Ikekagi\textsuperscript{a} and José Maurício Schneerdo\textsuperscript{b}

\textsuperscript{a}Department of Food and Drugs, Faculty of Pharmaceutical Sciences, Federal University of Alfenas, Alfenas, Brazil; \textsuperscript{b}Department of Biochemistry, Institute of Biomedical Sciences, Federal University of Alfenas, Alfenas, Brazil

ABSTRACT

This paper describes the construction and characterization of a biofuel cell with low-cost materials. The system uses the baker’s yeast (\textit{Saccharomyces cerevisiae}) as the microorganism responsible for the generation of voltage, whose interaction with the electrode is mediated by methylene blue. Simple experiments are proposed to evaluate the effects of different substrates, inhibitors and cell viability, improving both the knowledge of the system as well as metabolic pathway concepts to the student. The proposed device was able to generate a power density of $41 \pm 0.3 \text{ mW} \cdot \text{m}^{-2}$, similar to those obtained with yeast-based biofuel cells. The low cost and easily acquisition of materials described allow the introduction of biofuel cell theme in different teaching levels, from high school to the college level.

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Introduction

Biological fuel cells, commonly named biofuel cells, are devices similar to conventional fuel cells, but use biocatalysts for the conversion of chemical energy into electrical energy \((1)\) instead. As in any galvanic cell, the electrical work occurs whenever electrons flow from the anode to the cathode. In the case of two-chamber biofuel cell, electrons released by the oxidation of an organic substrate in the anode chamber causes a reduction of an oxidizing agent in the cathode compartment, such as oxygen or ferricyanide \((2, 3)\). The circuit is completed by migration of generated protons through the electrolyte, to ensure charge balance. Figure 1 shows a general scheme of a biofuel cell.

These devices are classified according to the biocatalyst, as microbial or enzymatic biofuel cell. In the latter group, the electrodes are modified with enzymes to catalyze the oxidation reaction of specific substrates \((4, 5)\), although the complete oxidation of a substrate can be achieved by the combination of several enzymes. For instance, Sokic-Lazic and Minteer \((6)\) modified an anode of a biofuel cell with 11 enzymes in order to mimic the citric acid cycle, and showed that there was an increased efficiency of enzyme biofuel cell whenever the enzymes involved in ethanol oxidation were immobilized in series, according to Equations (1) and (2):

\begin{align*}
\text{CH}_3\text{CH}_2\text{OH} + 2\text{NAD}^+ & \xrightarrow{\text{alcohol dehydrogenase}} \text{CH}_3\text{CHO} + 2\text{NADH}, \quad (1) \\
\text{CH}_3\text{CHO} + \text{H}_2\text{O} + 2\text{NAD}^+ & \xrightarrow{\text{aldehyde dehydrogenase}} \text{CH}_3\text{COOH} + 2\text{NADH}. \quad (2)
\end{align*}
In the microbial biofuel cell, bacteria or fungi are responsible for all the enzymatic machinery necessary for substrate oxidation. As whole biological systems, they are capable of using a wide range of enzymes in order to metabolize the substrate completely (1, 7, 8). Fairly common examples of microorganisms employed in these devices are *Shewanella* spp. (9–12), *Geobacter* spp. (13–16), *Escherichia* (14, 17), *Proteus* sp. (18, 19), *Pseudomonas* spp. (20, 21), *Rhodoferax* sp. (22, 23), *Saccharomyces* sp. (24–27), as well as mixed cultures (28–30). The interaction mechanism of the microorganisms with the electrode can be classified according to their nature: direct electron transfer – when the charge transfer occurs directly between the surface of the microorganism and the electrode; and mediated electron transfer – when the presence of a molecule is required to transport the electrons generated by metabolism to the electrode (1, 7, 31, 32).

These electron transfer mechanisms have been widely investigated in the literature. Physical contact with electrode enzymes or cytochromes present in the cell membrane is essential for direct electron transfer to occur. Inoue et al. (33) found over 200% increase in the current density of a biofuel cell inoculated with a mixed culture of bacteria, and target the occurrence of redox reaction. This finding is corroborated by Babanova et al. (32) in the evaluation of different mediators (neutral red, bro-mocresol green, methylene blue, methyl red and methyl orange) on the performance of a biofuel cell inoculated with a mixed culture of bacteria, and target to a wastewater treatment. In the case of organic mediators, the authors argued that the neutral red has a redox potential of −325 mV, similar to the oxidation reaction of NADH/NAD⁺ (−320 mV), thus favoring the interaction of neutral red and potassium ferri-cyanide mediators on the performance of a biofuel cell inoculated with a mixed culture of bacteria, and target to a wastewater treatment. In the case of organic mediators, the authors argued that the neutral red has a redox potential of −325 mV, similar to the oxidation reaction of NADH/NAD⁺ (−320 mV), thus favoring the occurrence of redox reaction. This finding is corroborated by Babanova et al. (32) in the evaluation of different mediators (neutral red, bromocresol green, methylene blue, methyl red and methyl orange) on the performance of a two-chamber biofuel cell inoculated with *Candida melibiosica*. As a result, neutral red and bromocresol green were both able to interact with NADH/ NAD⁺ system, whereas methylene blue, methyl red and methyl orange interacted preferentially with fumarate/succinate system.

In order to compare the performance of these electronic transfer mechanisms, leropoulos et al. (14) has studied three biofuel cells systems employing similar operating conditions, but with different microorganisms. The biofuel cell with external mediator employed
methylene blue and *Escherichia coli*; the second model was based on the mediators produced by *Desulfovibrio desulfuricans*, a sulfate reducing strain and a third model that evaluates the ability of direct electron transfer promoted by *G. sulfurreducens*. The highest average current and power output were given by the second biofuel cell, followed by the system that had used methylene blue mediator.

The main applications of biofuel cells are in wastewater treatment, biosensor development, implantable devices and electric power generation (1, 8, 45, 46). These applications can be related to the Twelve Principles of Green Chemistry (47), especially regarding “prevention” and “energy efficiency,” because it utilizes waste or organic matter for energy production. However, the latter application is still a challenge because energy production is generally under 50 mW m$^{-2}$ (48), requiring further studies to improve the performance of these systems. In this sense, some goals involving novel architectures (2, 8, 48–50), electrolytes (16, 40, 51, 52), organic matter sources (15, 53–55) and microbial community (30, 37, 56) had been encouraged for a better understanding and application of these systems. The construction and application of biofuel cell involves many scientific concepts, such as charge transfer, chemical equilibrium, thermodynamics, kinetics and general metabolism, among others. These concepts are difficult to understand, making the construction and application of biofuel cells attractive for Biology and Chemistry learning. However, to the knowledge of the authors, there are few biofuel cells reported for educational purposes, for example, Bennetto (57) who has dedicated an interdisciplinary approach using biofuel cells at the undergraduate level. Another initiative led by Logan (58) involves a whole web page for guidance, construction and dissemination of biofuel cells projects conducted by students throughout the world.

The objective of this work was to construct a biofuel cell based on green chemistry principles, employing low-cost materials to explore concepts inherent to equilibrium electrochemistry and cellular metabolism as teaching tools for the chemical and biochemical learning. In short, the biofuel cell employed the microorganism *Saccharomyces cerevisiae*, methylene blue as mediator, a two-chamber architecture of easy construction and a commercial multimeter for signal monitoring.

**Experimental**

**Materials and equipment**

Common graphite electrodes (HB) purchased at local stores, with dimensions of 2.0 mm × 10 cm, were used as electrodes. Digital multimeters with 1 mV resolution and ±0.5% accuracy were purchased at local stores and used for potential measurements. The difference in potential readings between the different multimeters was less than 1%.

**Solutions and reagents**

All solutions were prepared using distilled water. The anolyte consists of sodium phosphate buffer solution 0.05 mol L$^{-1}$ pH 7.0 in saline (sodium chloride 0.9%); the catholyte was potassium ferricyanide 0.025 mol L$^{-1}$ in the same buffer. For the study of enzymatic inhibitions, the anolyte was prepared in sodium citrate buffer 0.05 mol L$^{-1}$ pH 7.0 in saline. Dry yeast acquired at local market was used as a source of *S. cerevisiae*, and methylene blue (CAS #61-73-4, Vetec, Brazil) stock solution was prepared with a concentration of 0.010 mol L$^{-1}$. Glucose, fructose and sucrose (Vetec, Brazil) were used as fuels in different experiments. The electrodes were cleaned with ethyl alcohol/acetone solution (1:1 v v$^{-1}$).

**Biofuel cell assembly**

Two 25 mL beakers were employed for assembling a two-chamber biofuel cell, composed of an anode and a cathode chamber separated by a salt bridge consisting of a U-shaped glass tube (length between 10 and 1 cm × 0.4 cm diameter) filled with a 2% agar solution in potassium chloride (1.5 mol L$^{-1}$). The cathode compartment was filled with 20 mL of catholyte solution. The anode compartment was filled with 20 mL of anolyte, 2 mL of methylene blue stock solution and 1 mL of a glucose solution (0.4 g mL$^{-1}$). The electrodes were inserted into each compartment and connected to the multimeter terminals (black probe – anode; red probe – cathode; *Figure 2*), and a potential range of 2 V was selected for multimeter reading. After stabilization, 2 mL of yeast suspension (previously prepared by homogenizing 0.8 g of yeast with 2 mL of the respective anolyte for 5 min) was added following potential readings at 5 min intervals up to 70–80 min. The anolyte was maintained unaffected during the experiments to minimize the O$_2$ diffusion into the solution, except in the studies of the influence of O$_2$. For these experiments, N$_2$ flow (Praxair Inc., SP) or O$_2$ (atmospheric, employing an aquarium pump) was bubbled into the anode chamber before (30 min) and throughout each experiment. Data were normalized by subtracting the value of the potential at zero time (immediately before adding the yeast suspension) from the voltage values at each interval. The experiments were performed in
triplicate and data were presented as mean ± standard deviation, except as specified. Difference between groups was evaluated by analysis of variance (ANOVA), considering as significant a p-value <.05.

Cell fractionation

A procedure for cellular disruption by abrasion was done following adaptation from Medeiros et al. (59). Briefly, an aliquot of dry yeast was ground in a mortar and pestle for 30 min, and were suspended in 0.05 mol L\(^{-1}\) phosphate buffer solution at pH 7.0. The homogenate was centrifuged at 3600 rpm for 10 min. Then the supernatant was recovered and stored in refrigerator. The pellet was resuspended in the phosphate buffer and centrifuged at 3600 rpm for 10 min three times, and the final fractions (supernatant or debris containing membrane fragments) were used as inocula for the biofuel cell.

Polarization studies

Polarization measurements were done after the stabilization of the voltage values (30 min after the yeast preparation and its addition into the anolyte), varying the external resistance from 18 kΩ to 2.2 kΩ with resistors. Different solutions containing carbon sources at 0.4 g mL\(^{-1}\) (glucose, fructose or sucrose, as above), glucose in the citrate buffer, glucose saturated with O\(_2\) or deaerating it with N\(_2\) flow throughout the measurements were conducted to characterize the biofuel cell. For each resistor (external resistor – \(R_{\text{ext}}\)), the voltage of biofuel cell (\(E\)) was recorded after the establishment of a pseudo-steady state (2) (3–5 min). Current and power densities were calculated employing Ohm’s law (Equation (3)) and Joule’s law (Equation (4)), respectively, and the data were normalized by the geometric area of the anode:

\[
I = \frac{E}{R_{\text{ext}}},
\]

\[
P = IE.
\]

Finally, polarization curves (\(E\) vs. \(j\)) and corresponding power curves (\(P\) vs. \(j\)) were constructed and analysed.

Results and discussion

The principle of operation of a biofuel cell is based on metabolic reactions of organic matter oxidation to produce energy for the survival of microorganisms. Equations (5) and (6) represent the oxidation of glucose in aerobic and anaerobic conditions, respectively (60, 61):

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 32\text{ADP} + 32\text{P}_i + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 38\text{H}_2\text{O} + 32\text{ATP},
\]

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 2\text{ADP} + 2\text{P}_i + 2\text{H}^+ \rightarrow 2\text{C}_2\text{H}_5\text{OH} + 2\text{CO}_2 + 2\text{H}_2\text{O} + 2\text{ATP}.
\]

In order to convert chemical energy into electrical energy from the above reactions, it is necessary that both the oxidizing and reducing agents are physically separated by an electrolyte, allowing the electrons to flow through the external circuit to complete the reactions. Equations (5) and (6) show that under ideal conditions, the glucose oxidation under oxygen produces about 16 times more ATP than the anaerobic process. However, the presence of oxygen in the anodic chamber inhibits the generation of electricity (8) and reduces the efficiency of the system (16, 48) because the O\(_2\) acts as a final acceptor of the electrons. Although some investigators have shown that some biofuel cells inoculated with \(S.\) cerevisiae can transfer electrons to a load without the need of mediators (25, 62), its presence can improve the biofuel cell performance and simplify the interpretation of the results by the student. According to Babanova (32), these compounds are reduced by electrons released by the catabolism after permeating the cells; in reduced form, the mediators escape from the cell and reach the oxidized electrode promoting a charge transfer on its surface. This process generates a potential difference observed in these devices.

Although the formal potential of methylene blue is influenced by the pH of the solution (63, 64), their changes observed in this work cannot be attributed to this mechanism. The medium acidification caused by
the microbial metabolism shifts the average potential to less negative values (65), which would reduce the cell potential measured by the multimeter ($E_{\text{cell}} = E_{\text{cathode}} - E_{\text{anode}}$). Tests conducted with the biofuel cell in which the anolyte has been acidified with HCl (final pH range between 7 and 2) support this fact (data not shown).

In recent years, some papers have reported the role of methylene blue as an exogenous electron shuttle in biofuel cells using S. cerevisiae (66). In this respect, Figure 3 shows the results obtained with a biofuel cell to demonstrate the effect of a mediator presence in the reaction medium. In the absence of methylene blue, the measured potential did not exceed 25 mV, while the potential rises continuously up to 400 mV for 50 min with the dye.

**Suggestions to illustrate the use of the biofuel cell in chemical/biochemical learning**

Given the simplicity to assembly and to implement the biofuel cell described in this work, different experimental conditions can be easily tested in the period of an experimental class, to evaluate chemical and biochemical changes under the influence of inoculants, substrates, inhibitory agents and cell viability.

**Effect of inoculum concentration and different substrates**

The baker’s yeast S. cerevisiae is a very attractive microorganism for educational purposes, since it is harmless to the human body, has a low cost, is easy to find, cultivate and store and presents a fast growth. Furthermore, S. cerevisiae is able to oxidize different substrates (25, 27) and, at least for the period of a common classroom experiment (50–60 min), there is no need for aseptic conditions of the materials and reagents.

To test the effect of the inoculum concentration, appropriate amounts of yeast were hydrated with anolyte in order to obtain the microorganism at 8, 20, 32 and 42 mg mL$^{-1}$ in the anodic chamber. Figure 4 shows the potential changes obtained after 80 min of experiment using glucose as the carbon source.

The data shown in Figure 4 suggest a value of 32 mg mL$^{-1}$ as the most suitable yeast concentration to the experiment. Furthermore, this amount was able to discolor methylene blue in the same manner as the highest concentration evaluated in the experiment. The potential values observed in Figure 4 was similar to those obtained in another studies (3, 26, 27) in spite of a larger range of inoculum concentration reported (1–150 mg mL$^{-1}$).

Although S. cerevisiae is able to metabolize different carbohydrates, glucose is certainly the most used (3, 24–27, 44, 67). In order to compare the performance of the system that utilizes glucose to systems that uses other carbon sources, fructose and sucrose were evaluated as alternatives. For these experiments, the mass of fructose and sucrose were calculated to ensure the same carbon amount. The results showed a potential generated at 80 min of 380 ± 16 mV for fructose, 354 ± 6 mV for glucose and 342 ± 20 mV for sucrose (ANOVA, $p < .05$), suggesting the possibility to use any of these carbon sources with biofuel cells.

The high potential changes recorded for fructose indicated that the metabolism favored the electronic transfer in the biofuel cell when compared to glucose or sucrose. However, it is well known that S. cerevisiae preferentially metabolizes glucose, mainly in mixtures containing both the substrates (68, 69), due to some known mechanisms, such as the Crabtree effect and phosphate sequestration. During glycolysis, hexose phosphate accumulates in the cell and inhibits the mitochondrial metabolism for energy production, changing the rate of consumption of mitochondrial O$_2$. This event leads to an increase in α-D-glucose-6-phosphate and a decrease in β-D-fructose 1,6-bisphosphate (70, 71). However, according to Rosas-Lemus et al. (70), the inhibitory effect of β-D-fructose 1,6-bisphosphate seems to prevail. This compound inhibits cell respiration acting directly on two complexes in the mitochondrial respiratory chain (71), and shifting
the metabolism to the fermentative pathway thus favoring the Crabtree effect (70–73). This upregulation of glycolysis is considered as a suppression of respiration by fermentation caused by an excess of substrate even in the presence of oxygen (72, 74). The other mechanism, the ability of a fructose excess to sequester phosphate in ATP form in the glycolytic pathway, thus reduces its inhibitory effect on phosphofructokinase-1 (60, 61).

Effect of oxygen and citrate

*S. cerevisiae* is a facultative aerobic microorganism, wherein the pyruvate derived from the fermentation process is oxidized to CO₂ and H₂O in the presence of oxygen, by the citric acid cycle and the respiratory chain. In anaerobic conditions, however, pyruvate is converted to ethanol by the action of some enzymes such as pyruvate decarboxylase, alcohol and aldehyde dehydrogenase (60, 61, 75). The presence of oxygen inhibits the generation of electricity due to its competition with the mediator for the generated electrons.

In this work, the influence of oxygen to the electricity generation was evaluated in duplicate and different conditions. The results presented mean changes of voltage value of 367 ± 11 mV for a control biofuel cell (without agitation), 287 ± 1 mV for the anaerobic biofuel cell (under N₂ flow throughout the runs) and 184 ± 2 mV for aerobic biofuel cell (under air flow). In the latter case, the methylene blue, which was reduced by microbial metabolism, was reoxidized by oxygen molecule interrupting the charge transfer to the electrode surface, and thus lowering the voltage output (27). The strictly anaerobic conditions showed an intermediate performance when compared to the other two biofuel cells. A possible explanation lies in the fact that a small amount of oxygen is essential for the growth of the *S. cerevisiae* (76). Therefore, the oxygen present in the biofuel cell used as a control may have ensured the proper balance between growth of the yeast and methylene blue reduction needed to generate the potential (7, 27). With the continuity of metabolic reactions, the medium became anaerobic due the O₂ consumption and CO₂ production, as evidenced by the mediator discoloration.

The reduction in voltage observed in biofuel cell under oxygenation suggested the possibility to evaluate it as a teaching tool for explaining inhibitory compounds in the glycolytic pathway, for example, citrate, a well-known inhibitor of phosphofructokinase-1. To study the inhibitory effect caused by citrate, phosphate buffer in the anodic chamber was replaced by citrate buffer at the same pH and concentration, as described in methodology. The results presented in Figure 5 indicate that citrate was able to decrease the voltage of the biofuel cell around 55% (ANOVA, p < .05) after 80 min with values of 148 ± 27 mV for the citrate group compared to controls (336 ± 23 mV).

These results show that the system response is dependent on regulatory enzyme activity of microorganisms, opening a possibility for other applications such as the development of biosensors (77).

The control points of the glycolytic pathway for the citrate can be seen in Figure 6. During the glycolysis pathway in eukaryotes, hexokinase, phosphofructokinase-1 and pyruvate kinase catalyze reactions which are practically irreversible, having an important regulatory role (60, 61). The activity of these enzymes can be controlled primarily by substrate supply, hormonal action, allosteric regulation or covalent modification (60, 61). For example, high levels of ATP in the cell allosterically inhibit the phosphofructokinase-1 through binding to a specific regulatory site, thus reducing its affinity for β-D-fructose 6-phosphate substrate. Citrate, being an intermediate of the citric acid cycle, is a probe for the energy needs to the cell. High citrate levels suggest a high availability of chemical

![Figure 5](image)

**Figure 5.** Inhibitory effect of citrate (●) when compared to phosphate (○) buffers on the voltage changes in the biofuel cell, using glucose as carbon source.

![Figure 6](image)

**Figure 6.** Representation of the glycolytic pathway, showing the metabolic pathways for glucose and fructose conversion to pyruvate, highlighting key regulatory enzymes and inhibition points (**) by citrate. P = phosphate; P₂ = bisphosphate (adapted from Roche Biochemical Pathways (75)).
energy as ATP molecules, inhibiting the action of phosphofructokinase-1 by increasing the inhibitor effect caused by ATP (60, 61). Hexokinase catalyzes the first reaction of glycolysis, producing α-D-glucose 6-phosphate. On the other hand, the inhibition of phosphofructokinase-1 leads to an increase in concentration of β-D-fructose 6-phosphate and α-D-glucose 6-phosphate, which decrease the enzymatic action of hexokinase. In other words, citrate promotes an indirect downregulation on the activity of hexokinase.

**Cellular integrity and cell fraction as origin for electricity generation**

Live yeast and yeast inactivated by humid heat was used to study the cell viability in voltage production of the biofuel cell. The performance of the system inoculated with living cells (control) showed a potential difference \( \Delta E = 317 \pm 49 \text{ mV} \) after 70 min, whereas the system with inactivated cells showed a \( \Delta E = 29 \pm 21 \text{ mV} \). The inactivation of the yeast suspension (0.8 g of yeast in 2 mL of anolyte) was performed by autoclaving samples at 121°C for 15 min. Similar results were found using boiling water for cell inactivation. At the end of this experiment, a complete reduction of methylene blue, characterized by the disappearance of the blue coloration in the medium, has been observed with living yeasts. In biofuel cells inoculated with inactivated yeast, no color change could be detected and a visible amount of strong stained cells was found precipitated in biofuel cell, thus indicating its cell inviability (78).

Some authors attribute the electrical response to confined compounds on the surface of the cell membrane (25, 62), while others claim that the intracellular enzymes are responsible for the mean changes in the voltage in biofuel cells (79). To locate the cellular fraction responsible for the observed potential generated in the biofuel system, membranes fragments and intracellular content were fractioned before the assays. After this procedure, the resuspended pellet revealed few viable cells and a large amount of debris, whereas the supernatant presents no cells in the sample (Supplementary Material). The final fractions (supernatant and pellet) were used as inoculum for the biofuel cell. The results presented in Figure 7 revealed very small values for potential changes obtained with the pellet (i.e. cell debris and fragmented membranes), thus discarding the presence of compounds that would lead to the reduction of methylene blue in the conditions assayed.

However, the addition of the supernatant to the anode compartment caused an increase in the voltage of the biofuel cell, confirming the catalytic effect already reported by Sayed et al. (79). This result would be also related to enzyme-catalyzed redox reactions of methylene blue, as depicted by Babanova (32). In fact, while the anodic chamber of the control presented visible bubbles and methylene blue discoloration during sugar fermentation, the supernatant inoculum presented only dye discoloration, and the pellet inoculum no discoloration or bubbles (Supplementary Material). Hence, methylene blue discoloration probably indicates the ability of these enzymes to reduce the mediator. When compared to the control group, the decreased voltage values observed for the cellular lysate can be related to the fractionation treatment of the samples, as the lysate may have lost part of its machinery for protein synthesis needed to keep the glycolytic pathway under control.

**Biofuel cell characterization**

The power generated by a biofuel cell is the most common parameter used to evaluate its performance. Changing the external resistance, a new electric potential value is obtained and electrical current is calculated by Ohm’s law (8). This classic method for polarization curves was chosen in this study, but the data can also be obtained by other methods (2, 8, 80). From the current and voltage data, a polarization curve (\( E \) vs. \( j \)) and power curve (\( P \) vs. \( j \)) can be constructed in which the current \( i \) is generally normalized by the anode area in order to allow comparisons between different devices. Figure 8 shows a further characterization of the biofuel cell through power and polarization curves in different assay conditions (carbohydrate sources, \( O_2 \) influence). In this representation, the maximum in the power curve represents the limit of the generated power, and allows an estimation of the internal resistance of the biofuel cell (8).
A sharp drop in potential at higher current densities was more pronounced for glucose (Figure 8(A)) and fructose (Figure 8(B)). This signal decrease can be attributed to a mass transfer effect, that is, a limiting in the reaction rate led by the flow of reactants and products (2, 8), and agrees with these preferential carbon sources by yeasts (68, 69). Nevertheless, minor effects due to mass transfer were found in the other conditions (sucrose, citrate, O2). In descending order, the maxima on the power curves for the carbon sources were 41 ± 0.3 mW m⁻² (fructose, Figure 8(B), 38 ± 1.0 mW m⁻² (glucose, Figure 8(A)) and 34 ± 0.5 mW m⁻² (sucrose, Figure 8(C)). The other conditions assayed strengthen the inhibitory effect already found for citrate (27 ± 0.1 mW m⁻², Figure 8(D)), deaeration with N₂ (14 ± 0.2 mW m⁻², Figure 8(F)) and saturated O₂ (3.3 ± 0.3 mW m⁻², Figure 8(E)). The peak values of polarization curves obtained in Figure 8(A–C) are in agreement with the values compiled by Hubenova and Mitov (66) for biofuel cells containing S. cerevisiae and methylene blue. When considering the low-cost materials used, the absence of metal catalysts (Pt) and the simplicity of the proposed architecture (81), the achieved value indicates satisfactory for use as a teaching tool on biofuel cell and green chemistry, and reinforce the robustness of the system.

Conclusions

The present work evaluated the construction and characterization of a yeast-based biofuel cell for chemical and biochemical teaching, using low-cost materials (two-chamber architecture and graphite electrodes). The advantages of using the biofuel cell presented include their non-toxic reagents or low toxicity (methylene blue, glucose, phosphate buffer and potassium ferricyanide), and the well-known microorganism S. cerevisiae. The effect of different substrates, inhibitors and cell viability on the generation of voltage observed in the
biofuel cell together with the mapping of cell fraction responsible for signal generation was evaluated. The experiments are simple and affordable, making the biofuel cell didactically interesting for chemistry and biochemistry teaching, while continuing to attract the interest of young students (future researchers) for new renewable sources of energy.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Notes on contributors

Gustavo Silveira was born in 1986 and graduated in Chemistry from the Federal University of Alfenas in 2008. In 2010, Silveira completed the MSc and since 2014 is a Chemistry Ph.D. student working on Microbial Fuel Cells.

Originally from Japan, Dr Masaharu Ikegaki is Professor of Fermentation Technology in the Faculty of Pharmaceutical Science at the Federal University of Alfenas, Brazil. He obtained his Bachelor’s degree in Biological Science from the Federal University of Alfenas, Brazil. He obtained his Master’s degree in Microbial Technology in the Faculty of Pharmaceutical Science at the State University of Campinas. His research interests are in the discovery of novel compounds with biological activities produced by microorganisms by fermentation processes isolated from Brazilian biodiversity.

José Maurício Schneedorf is a Biologist with D.Sc. degree in Biochemistry from the Federal University of Minas Gerais (1998, UFMG/Brazil, ligand–protein interaction), and postdoc at Federal University of Vioasa (1999, UFBV/Brazil, biothermodynamics of tumor cells). Actually, the author is Associated Professor at the Federal University of Alfenas (UNIFAL-MG/Brazil) with interest in biomolecular interactions, biothermodynamics and enzyme kinetics.

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