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Characterization of Electron Transfer Mechanism in Mediated Microbial Fuel Cell by Entrapped Electron Mediator in Saccharomyces Cerevisiae

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Attributed to exponentially growing global energy demand in current scenario, microbial fuel cells (MFCs) are an attempt aimed towards achieving electric energy sustainability, using renewable resources such as the organic substrates in domestic or industrial wastewater.
In our findings growing cells of common baker's yeast such as *Saccharomyces cerevisiae* was immobilized by inclusion techniques in cellulose acetate membrane on the surface of a graphite electrode.
Immobilized cells were grown using nutrient broth while the substrate, like electron donor in MFC, was glucose. The rate of substrate consumption of the anode electrode indicated that *Saccharomyces cerevisiae*, also immobilised, had a huge potential to generate electrons.
Our results showed that the current and voltage output of a *Saccharomyces cerevisiae* based MFC are directly correlated to the cells on the micro-environment of the electrode and to the presence of an electron mediator such as methylene blue (MB).
Our findings suggested that reduced methylene blue was entrapped into the cells, enhancing the electron transfer on the graphite electrode as an internal mediator of the cellular metabolism, probably linked to the oxidative electron cascade.

1. Introduction

A clean energy revolution is taking place across the world, underscored by the steady expansion of the renewable energy sector.
The clean energy industry can generates hundreds of billions in economic activity, and is expected to continue to grow rapidly in the coming years. There is tremendous economic opportunity for the countries that invent, manufacture and export clean energy technologies (Armaroli and Balzani, 2006).
From waste materials of organic origin, from plants and animals, we can get a source of clean energy immediately usable. The term "biomass" is understood "the biodegradable fraction of products, waste and residues from biological origin from agriculture, forestry and related industries, including fisheries and aquaculture, as well as the biodegradable fraction of industrial and municipal waste" (Perlack et al., 2005).
Nowadays the usage of domestic or industrial wastewater is mainly developed through two steps: first the aerobic treatment of the wastes and then the anaerobic digestion in which the organic content of the wastes is converted into methane gas (Rajeshwari et al., 2000). The main disadvantage of this technology is that we can't completely recover the energy contained in the dissolved organic fraction because a lot is removed during the aerobic phase before the anaerobic treatment in which methane is produced.
The result is that the energy consumed is more than the energy gained through the entire process (McCarty et al., 2011), in consequence, a big goal is to recovery in a more efficient way the energy contained in this wastes. On this basis a lot of attention was given by the scientific community to the microbial fuel cells (MFCs), able to efficiently exploit the energy contained in the wastes (Logan and Rabaey, 2012).
The microbial fuel cells were born about one hundred years ago with the first experiment of Potter (1911), followed by those of Allen and Bennetto (1993) that answered a lot of question on how MFCs work.

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Until now great strides were done; however, the attention was concentrated mainly on microbial fuel cells based on different types of bacteria (Kim et al., 2002, Bond and Lovley, 2002, Chaudhuri and Lovley, 2003). It were investigated and identified different mechanisms of electrons transfer to the anode of microbial fuel cells (Lovley, 2008).
Recently has attracted attention the development of yeast based fuel cells, in fact there are several advantages on the use of this microorganism such as the non-pathogenic nature and the strongness, the growth capacity and the not substrate specificity (Schaetzle et al., 2008).
What it lack in this new field of research on yeast electrochemical technologies is a complete and exhaustive study on the electron transfer mechanism from the microbe to the electrode like it was done for prokaryotic bacteria (Mao and Verwoerd, 2013).
From the first experiment using eukaryotic cells, it was discovered, as could be expected, that the direct electron transfer, without adding exogenous mediators, was poor and not comparable with prokaryotic based fuel cells (Schaetzle et al., 2008). It can be due to the more complexity of the cell, linked with the presence of a thick membrane that can limit the electrical direct contact (Cohen, 1931). A mediator was added to facilitate the transfer of electrons from cells to the anode. In common view, the mediator enters the cell membrane and be reduced, and then leaves in the reduced state (Bennetto, 1990). The reduced mediator transfer the captured electrons to an electrode, producing an electric current and re-oxidizing the mediator in its original status (Walker and Walker, 2006).
The greatest problem on the use of exogenous redox mediators is the necessity of a regular addition, which is technologically unfeasible and environmentally unacceptable (Schröder, 2007).
Our paper described a new immobilization technique of a common baker's yeast on a graphite electrode by inclusion in cellulose acetate membrane (Figure 1) in which methylene blue has the function of an electron mediator inside the yeast cell.

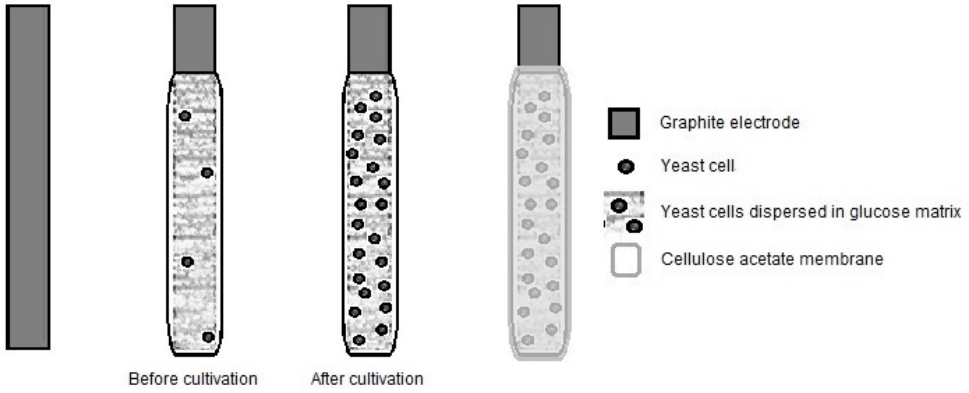

Figure 1: Illustrations of the different phases of functionalization and immobilization of the yeast cells on the electrode. The proportions are not exact and number of cells is arbitrary.

2. Materials and Method

All chemicals and reagents used for the experiments were analytical grades and supplied by Sigma-Aldrich.

2.1 Functionalized electrode development

The electrode was a graphite rod electrode purchased by Sigma-Aldrich. The current density was calculated based on the open area, 7.8 cm^2, of the rod used for the anode current collector.
On each operation, the electrodes were sonicated for 5 min. in 15 wt.% HNO_3 aqueous solution and then for another 5 min. in distilled water with Elmasonic S30H ("Elma" Hans Schmidbauer GmbH (Singen, Germany)).
All of the equipment were sterilized in autoclave for 15 min. at 121°C.
A 0.5 g sample of the dried yeast (*Saccharomyces cerevisiae* baker's yeast, CLECA S.p.a. Mantova, Italy) was dissolved in 4.0 mL of phosphate buffer solution 0.1 M pH 6.0 then was added 5.0 g of glucose. The paste formed was then transferred in a 15 mL falcon and the electrode was covered for four-fifths of the height by a dip-coating techniques (immersion speed 1.5 cms^{-1}). The glucose-yeast paste it is left to dry for 2 h before every use.

2.2 Immobilization of the yeast cells on the functionalized electrode

The functionalized electrode was covered by cellulose acetate membrane realized by a dip-coating technique through immersion in a solution of 5 % w/v (Mr ≈ 61000, 40% acetyl groups, Fluka) cellulose acetate in acetone:THF 60:40.
The coating were done with a dip coater (immersion speed 1.5 cms^{-1}) and the electrode was left to dry at room temperature for 18 h.
After dryness the electrode was transferred in a sterile YPD solution (10 gL^{-1} yeast extract, 20 gL^{-1} peptone, 2% glucose) for growing the immobilized cells.
The cells growth was determined by turbidity measurement and the substrate consumption by a modified colorimetric method by Bailey et al. (1992).

2.3 Construction of the Fuel Cell and electrochemical measurements

The fuel cell presented two glass chambers (0.10 L), the working volume was 0.05 L (50% of total). The chambers was connected by a salt bridge.
The salt bridge was a solution of KCl 10 gL^{-1} in phosphate buffer 0.1 M at pH 7.8, stirred and heated until reached 85 °C, then was added Agar until got to 2.5 gL^{-1}. The solution was poured in a silicon tube long 29 cm, 8 cm internal diameter.
The anodic chamber was purged with gaseous nitrogen (14 Lh^{-1}) during all the experiment.
Anodic and cathodic solutions were stirred continuously at the same rotational speed for all the experiments and the temperature of each chamber was 27.5 ± 2.5 °C.
Cathodic solution was hydrogen peroxide 4.41 $molL^{-1}$ in phosphate buffer 25 mM pH 6.0.
Anodic solution was glucose 5 gL^{-1} in phosphate buffer 0.1 M pH 7.8 in which methylene blue was added until reaching a concentration of 0.5 mM.
The anolyte was purged with nitrogen gas for 2 h and then the electrode was inserted into the solution.
After 46 h the methylene blue was completely reduced and the anodic solution resulted completely discoloured. The cell circuit was then closed and i-V curves were recorded. For the experiment in absence of methylene blue it was waited the same time before collecting cell characteristics.
The current-voltage, i-V, characteristics (SCC – Short Circuit Current and OCV – Open Circuit Voltage) were measured from -0.1 V to round up OCV at the scan rate of 10 mVs^{-1} by an electrochemical measurement system (Keithley series 2400 Multimeter, Keithley Instrument Inc.).

3. Results and Discussion

The growth of *Saccharomyces cerevisiae* was tested using a functionalized electrode suspended in YPD solution.
The growth curve of free yeast showed a logarithmic trend in the first 18 h reaching a stationary phase after 42 h (Figure 2).

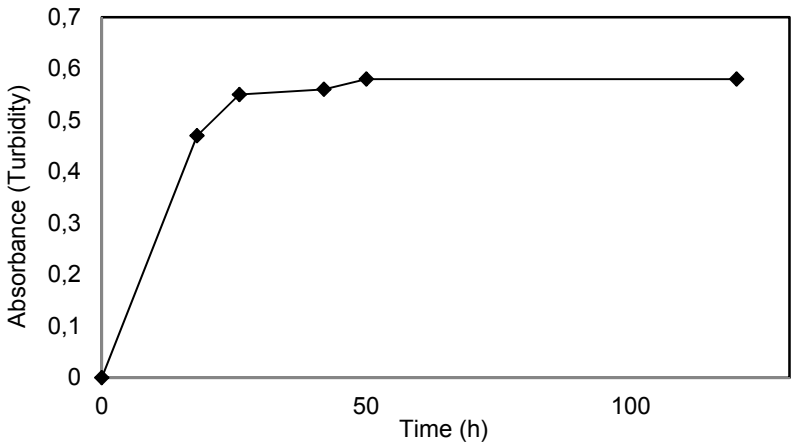

Figure 2: Growth curve of yeast cells in YPD medium after deposition of the inoculum onto the electrode surface. The solution were diluted 1:10.

The experiment demonstrated that the glucose paste was not inhibiting the yeast's growth.
The growth of the yeast immobilized on the functionalized electrode by the cellulose acetate membrane was evaluated by the consumption of the glucose in phosphate buffer solution.

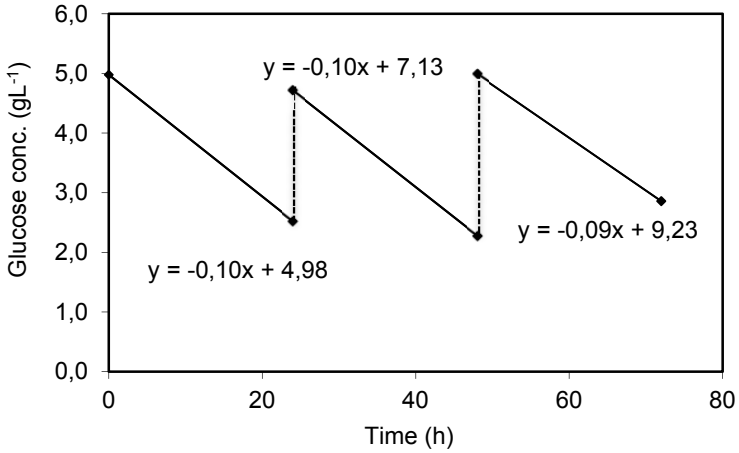

Figure 3: Glucose consumption by the yeast cells immobilized on the electrode in glucose solution changed each 24 hours.

The electrode was tested in three different glucose solutions, one after the other. Results showed that the glucose consumption was the same for all the immersions (Figure 3) demonstrating that the cellulose acetate membrane was permeable to the glucose as well as the immobilization technique did not inhibit the yeast. Furthermore, the largest amount of the cells was immobilized on the electrode as it was showed by the scarce release of free yeast after the extraction of the electrode from the glucose solution.
Figure 4 showed i-V characteristics of a microbial fuel cell using hydrogen peroxide ($4.41 molL^{-1}$) as electron acceptor in acidified aqueous solution at the cathode chamber, with and without methylene blue as electron mediator in the anode chamber. The experiment was performed in both open and closed circuit configurations under different loads ranging.

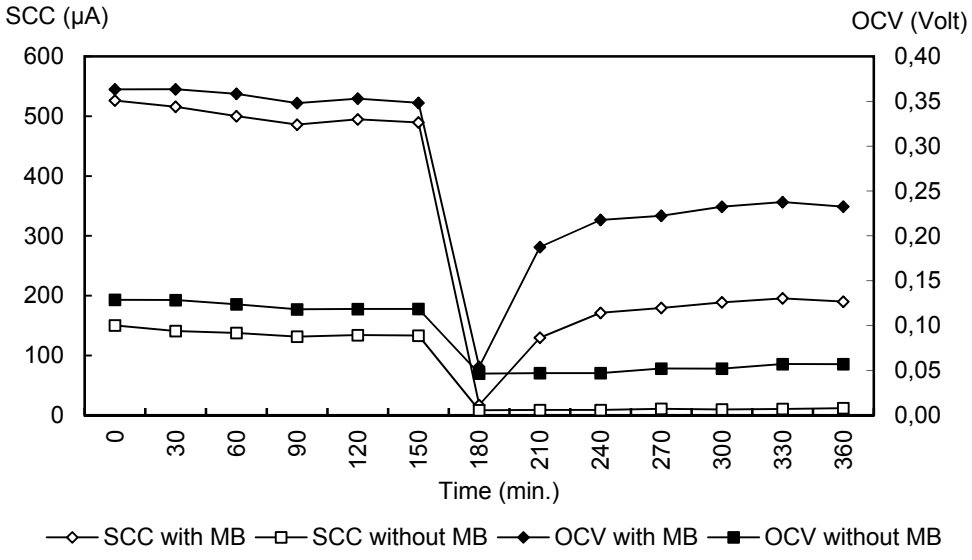

Figure 4: Values of OCV and SCC recorded by a functionalized and immobilized electrode in presence (rhombus) or absence (squares) of the electron mediator methylene blue. After 180 min. the electrode was extracted and substituted with a clean one.

At the end of the experiment in presence of the mediator it was determined spectrophotometrically the concentration of methylene blue in the anode chamber and it was 12.5 $\mu molL^{-1}$ although the initial concentration was 0.5 $mmolL^{-1}$. Our finding demonstrated that the largest part of methylene blue was then enclosed between membrane and electrode.

Open circuit voltage and short circuit current determined in the presence of methylene blue in anaerobic conditions in the anode compartment were respectively 363 mV and 526 µA while, in the absence of mediator, OCV and SCC resulted in 129 mV and 150 µA. These results confirmed that an exogenous mediator, enhanced the electron transfer on the electrode surface (Rossi et al., 2015). After 180 min. the functionalized and immobilized electrode was removed and substituted with a cleaned graphite electrode. We observed a different behavior with and without methylene blue. On the one side the short circuit current and the open circuit voltage without methylene blue collapsed to 8.53 µA and 46.5 mV, respectively, maintaining constant values until the end of the experiment. On the other side, in presence of the mediator, after the initial decrease, OCV and SCC began to rise up to 233 mV and 190 µA (Figure 4). This different behavior can only be due to the presence of the mediator (Roller et al., 1984), even if a residual community of yeast, free in solution, was moved themselves to the clean electrode surface (Figure 5).

Figure 5: Photograph showing cells on the surface of the clean electrode at the end of the experiment.

On this basis, a similar experiment was tried again replacing the functionalized and immobilized electrode (WE) with a clean graphite electrode covered with cellulose acetate membrane in absence of yeast cells. Our findings showed the values of both SCC and OCV closed to zero for all the duration of the experiment (Figure 6).

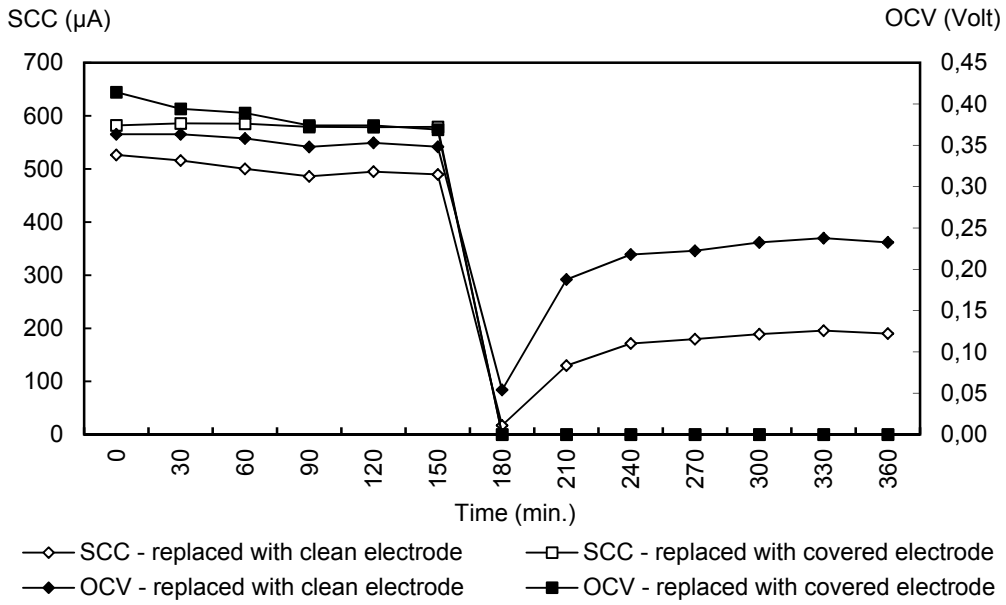

Figure 6: Values of OCV and SCC recorded by a functionalized and immobilized working electrode (WE) in presence of the electron mediator methylene blue. After 180 min. the working electrode was extracted and substituted with a clean one (rhombus) or with a clean graphite electrode covered with cellulose acetate membrane without yeast cells (squares).

The cellulose acetate membrane was impermeable to the yeast cells but permeable to methylene blue and prevent the direct electron transfer to the graphite electrode. Thus the lack of electron transfer after the electrode replacement was clearly due to the lack of the yeast cells adsorption on the graphite electrode. This result demonstrated that methylene blue expressed its electron transfer capacity inside the cells.

4. Conclusions

We demonstrated that our electron transfer mediator, methylene blue, does not act as a redox shuttle, but, once reduced, remain in the yeast cell. We also tested the functionalized electrode in an MFC. This new discovery gives the opportunity of develop yeast based fuel cells with the characteristic of a mediated MFCs but without his own disadvantages.

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