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Characterization of Electron Mediated Microbial Fuel Cell by *Saccharomyces Cerevisiae*

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Microbial Fuel Cells (MFCs) is an innovative technology able to both clean organic waste of domestic or industrial origin and produce renewable energy converting directly chemical energy stored in chemical bonds to electrical energy. Our findings showed that the rate of substrate consumption (such as glucose) by Saccharomyces Cerevisiae (a common baker's yeast) in the anaerobic compartment of a dual chambered MFC presented a great potential to generate electrons in a microbial fuel cell. Methylene blue was used as the electronophore in the anode compartment, while oxygen and hydrogen peroxide were tested as electron acceptors in the cathode compartment. The experiment was performed in closed circuit configurations under different loads ranging for evaluating the performance of the MFC with different concentration of methylene blue and electron acceptor. The fuel cell mediated by methylene blue carried out a maximum power generation of 110 mWm^{-2} and generated a maximum open circuit voltage of 479 mV with a short circuit current of 1.17 Am^{-2} with hydrogen peroxide as electron acceptor. The different contribute on the cell output of the mediator and electron acceptor concentration in anode and cathode compartment have been studied.

1. Introduction

The demand for energy is increasing at an exponential rate due to the exponential growth of world population. The combined effect of the widespread depletion of fossil fuels and the gradually emerging consciousness about environmental degradation has given priority to the use of conventional and renewable alternative energy sources. In the World Energy Report (2013) was suggested that use of fossil fuels cause damage to climate, environment and human health, also create economic problems to many nations and produce unbearable inequalities and political tensions at international levels which have already given rise to wars.
Biomasses represent the most used renewable vectors for producing electrical and thermal energy through traditional combustion technologies (World Energy Report, 2013).
Domestic or industrial wastewater, hold high energy density, theoretically higher than necessary for disposal (Logan and Rabaey, 2012); the development of techniques for capturing the energy contained in this biomass would provide a new source of electrical power that would also avoid the consumption of energy for wastewater treatment (Logan and Rabaey, 2012). This energy can be partly exploited through transformations of the organic fraction in liquid biofuels like bioethanol (Bjerre et al., 1996) or gaseous biofuels, for examples biomethane (Liu et al., 2002) or hydrogen (Logan, 2004). In a microbial fuel cell, the biochemical energy contained in the organic matter is directly converted into electricity, this implies that the overall conversion efficiencies that can be reached are potentially higher compared to other biofuel processes (Rabaey et al., 2005a). Microbial fuel cell is based on a biochemical oxidation process which generates electrical energy to be transferred to the cathode chamber. Most biological fuel cells use mediator molecule in presence of biocatalysts either fermentative to speed up produced electrons to electrode surface (Bullen et al., 2006).
Bioelectrochemical devices can transform wastewater from a problem for their disposal to renewable resources from chemical to electrical energy production (Rabaey and Verstraeten, 2005b).
Microbial Fuel Cells are usually classified according of electron transfer between reaction site and electrode: electrons can be transferred through the diffusion of a secondary fuel, via a mediator molecule that repeatedly

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cycles (MET) or via a direct electron transfer (DET) (Villano et al., 2010). In devices with mediated electron transfer, an intermediate molecule, for example a dye, will shuttle electrons between the microbe and the electrode (Schröder, 2007). The staining ability of a dye helps it to stick to the cellular membrane and helps transfer of electrons and protons (Gunawardena et al., 2008). Most recent studies on the conversion of organic wastes direct to electricity have focused on DET, from this researches emerged that DET usually allows very low current and power densities, so it is required large electrode for high power output, also few microbial species can achieve this particular kind of transfer (Schröder, 2007). However, in baker's yeast-based fuel cells, exogenous mediators are a necessity since Saccharomyces Cerevisiae is not known to produce such mediators indigenously.

During past years, papers on bioelectrochemical devices increased exponentially. The scientific communities worked on develop new MFC with different features, it resulted in hundreds of papers about as many different cells. However, only little part of the scientific groups focused on the identification of the limiting factor affecting MFC performances.

For this reason we decided to construct the simplest laboratory scale MFC and characterize all of the electrochemical step, with the aim of identifying the limiting one. Once all of the steps are clear, we can concentrate our attention on those that permit to obtain the best improvement in power output.

As schematized in Figure 1, we constructed a Microbial Fuel Cell, operating in batch condition, with two chamber connected with salt bridge. We choose common baker's yeast, Saccharomyces Cerevisiae, as biocatalyst, the electron transfer from the yeast cells to the anode was accomplished by methylene blue as electronophore (electron shuttle). An electron mediator to wire the microbial metabolism to the fuel cell anode was necessary in our configuration, this should form a reversible redox couple at the electrode, cross the microbial membrane and catch electrons from the microbial metabolism. The mediator polarity should be such that the mediator is soluble in aqueous systems (near pH 7) and can pass through or be absorbed by the microbial cytoplasmic membrane.

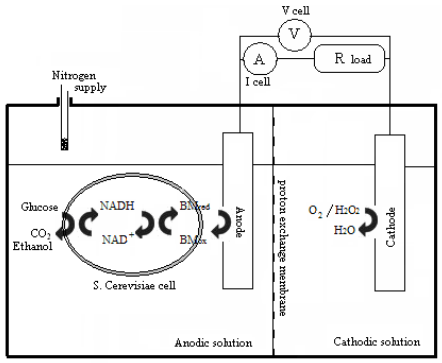

Figure 1: Schematic diagram of the microbial fuel cell where Saccharomyces Cerevisiae is the biocatalyst and methylene blue the mediator responsible for the electron transfer.

The conversion efficiency itself is inadequate to determine the fuel cell behaviour 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 fulfil this knowledge gap using Saccharomyces Cerevisiae as the model microorganism

In our MFC, methylene blue can be used as an electron acceptor for "hijacking" the electrons, accumulated in chemically reduced nicotinamide adenine dinucleotide (NADH) into the cell, from the microbial metabolism to an external electrode (Justin et al., 2004).

2. Materials and Method

2.1 Substrate consumption, ethanol determination and cell growth

Substrate consumption, ethanol determination and cell growth were measured on separated batch at the same operative conditions of the electrochemical cell. In presence of mediator we worked with a 5.0 mM concentration. Glucose consumption was calculated by the determination of the remaining sugars in the culture. ADNS reagent was employed to detect and measure substrate consumption using a modified colorimetric method by Bailey et al. (1992). Ethanol determination was accomplished by a first oxidation of

ethanol to formaldehyde (further oxidation was prevented by adding Fe(II)) followed by a reaction between aldehyde, MBTH and ferric chloride resulted in a blue complex spectrophotometrically dosable. Cell growth was also monitored by optical density using spectrophotometer (Uvikon 923, Bio-teck Instrument s.r.l. Milano Italy) at wavelength of 750 nm in presence or absence of methylene blue.

2.2 Construction of the Fuel Cell

The fuel cell was constructed with two separate glass chambers, the volume of each one was 0.1 L, with working volume of 0.05 L (50% of total). Each chamber had three different entrances for electrode, salt bridge and gas supply or thermometer.
The salt bridge was a solution of KCl 10 gL^{-1} in phosphate buffer 0.1 M at pH 7.8, heated and stirred 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 electrodes were made of carbon and it was extracted from a 4.5 V battery (Duracell). The current density was calculated based on the open area, 7.8 cm^2, of the rod used for the anode current collector.

2.3 Operation of the fuel cell

Cell growth measurements during electrochemical 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 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)).

2.4 Yeast and anolyte preparation

Saccharomyces Cerevisiae (baker's yeast, CLECA S.p.a. Mantova Italy) was used as a biocatalyst. A 0.5 g (5 gL^{-1}) sample of the dried yeast in 0.1 L of phosphate buffer solution 0.1 M pH 7.8 was centrifuged twice at 3000 rpm for 3 min. at 25 °C in ALC 4239R centrifuge, each times 0.08 L were replaced with fresh phosphate buffer solution.
A 0.05 L aliquot of sample mixed with 0.25 g (5 gL^{-1}) glucose as the substrate and methylene blue, in order to obtain the desired concentration of the mediator, was used as anolyte.

2.5 Cell operation and electrochemical measurements

The anolyte, 0.05 L, was injected into the anode chamber and purging (14 Lh^{-1}) with gaseous nitrogen during all the experiment, firstly gas was purged for 10 minutes in the solution then the supply was lift up to maintain a nitrogen atmosphere in the anode chamber.
Before every measurement the yeast was cultivated for at least 22 h in the anode compartment, in open circuit configuration, with electrode left disconnected.
Anodic and cathodic solution were stirred continuously during all the experiment and the temperature of each chamber was 27.5 ± 2.5 °C.
The open circuit voltage (OCV) was recorded first, the cell circuit was then closed and the current-voltage, i-V, characteristics 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.)
All chemicals and reagents used for the experiments were analytical grades and supplied by Sigma-Aldrich. The pHmeter, Crison micropH2001 with Hamilton glass electrode was employed for measuring pH of the working solution.

3. Results and Discussion

During the first phase of the experiment spectrophotometric studies were conducted to understand the nature of methylene blue and its influence in the anode compartment. Cell growth was observed by looking at the number of cells based on the technique of OD (optical density) against incubation time.
The pattern obtained from the growth curve of Saccharomyces Cerevisiae represented in Figure 2A in medium showed that the microbial population was stable until 46 h or did not provide a sufficient number of cells increased significantly. This is due to the fact that the cell growth was limited by lack of nitrogen source (Salmon, 1989); even if, the yeast cells activity was demonstrated by the glucose consumption curve. The results obtained in presence of mediator demonstrates the non-toxicity of the mediator.

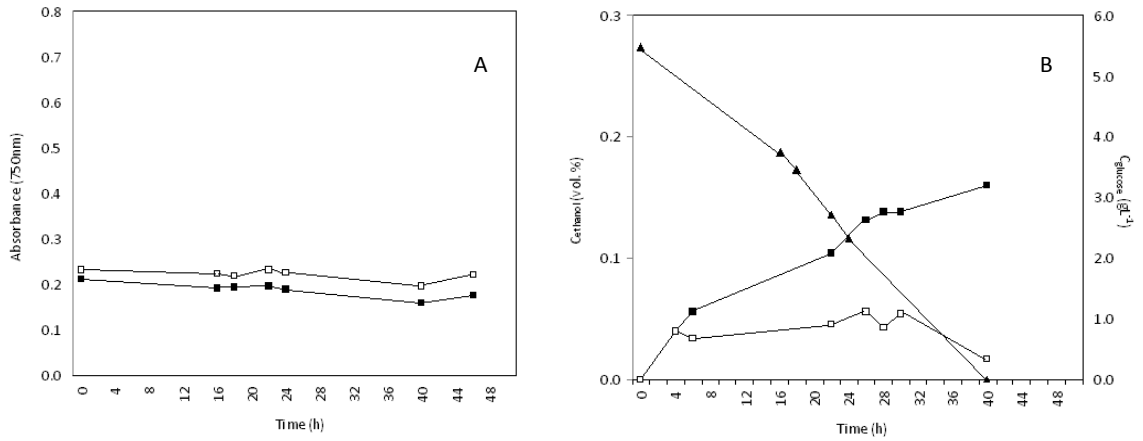

Figure 2: (A) Growth of Saccharomyces Cerevisiae in an anaerobic condition in presence (open squares) or absence (closed squares) of methylene blue (5.0 mM); (B) glucose consumption (closed triangles) and ethanol production in presence (open squares) or absence (closed squares) of mediator over time.

Anaerobic conditions could favour the fermentation of glucose to ethanol as was evidenced in the absence of mediator (Figure 2B). In presence of methylene blue decreases the production of ethanol, showing its capacity to be used as an electron acceptor for the regeneration of the yeast's co-enzymes according to Babanova et al. (2011).

Metabolism of Saccharomyces Cerevisiae was then active to consume glucose as carbon source in the anode chamber and generating electrons and protons for the reduction of methylene blue as terminal electron acceptor. We had previously investigated (unpublished data) the reaction between methylene blue and NADH, observing that methylene blue, chemically reduced by NADH, could leave on the electrode surface the electrons accumulated when the presence of an electron acceptor, such as oxygen or hydrogen peroxide, was in the cathode chamber. The electrochemical experiments were carried out after a lag phase of 22 h in which the yeast had operated for the reduction of the methylene blue. This reaction could be followed by the discoloration of the aqueous solution, which is blue when the mediator is oxidized and colorless when reduced.

Figure 3 shows i-V characteristics of a microbial fuel cell using oxygen as electron acceptor in acidified aqueous solution at the cathode chamber, with and without methylene blue as electron mediator at the anode chamber. We also report a polarization curve with hydrogen peroxide as electron acceptor. The experiment was performed in both open and closed circuit configurations under different loads ranging.

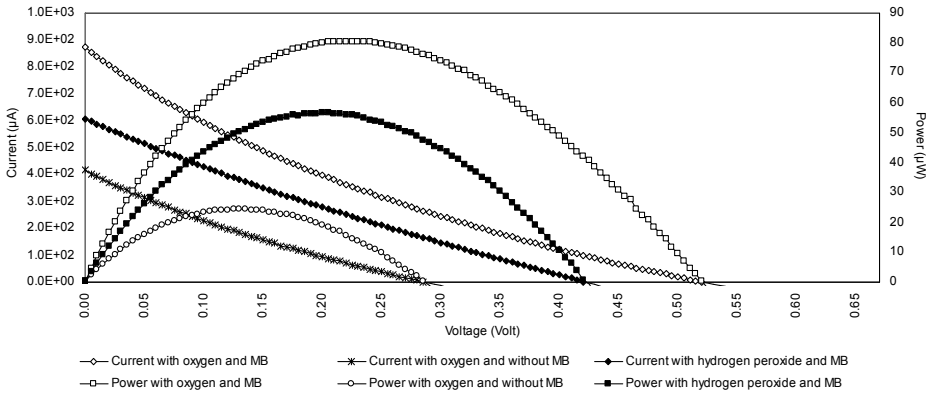

Figure 3: i-V polarization curve and MFC power with oxygen as electron acceptor in presence (5.0 mM) (open rhombus and open squares) or absence (asterisk and open circle) of methylene blue; and with hydrogen peroxide (4.41 molL^{-1})as electron acceptor in presence of methylene blue (5.0 mM)(closed rhombus and closed squares).

Due to presence of resistance, the power and voltage were considered as operational electricity. Maximum power and current generated with addition of methylene blue in anaerobic conditions in the anode

compartment were respectively 81 μW and 870 μA, in absence of mediator power and current resulted in 24 μW and 416 μA. These results confirmed that the exogenous mediators such as methylene blue are a necessity since Saccharomyces Cerevisiae is not known to produce such mediators indigenously

The oxygen as electron acceptor was then substituted with hydrogen peroxide because of the more stability in terms of OCV, power and current.

Also the influence of mediator concentration and electron acceptor concentration on battery output was investigated. Several concentrations of hydrogen peroxide (0; 0.18; 0.88 and 4.41 M) in phosphate buffer 25 mM pH 6) were experimented at fixed methylene blue concentration (5.0 mM). The results of i-V curves, registered after 22 h of incubations are shown in Figure 4 in terms of OCV (Open Circuit Voltage) and SCC (Short Circuit Current).

The value of OCV and SCC increased increasing the hydrogen peroxide concentration, even if the maximum values were lower than that obtained in the presence of oxygen as electron acceptor. The current output from the point without hydrogen peroxide was caused by the reduction of hydrogen ions in the cathode chamber because the chamber was purged with gaseous nitrogen for 22h prior to the analysis in order to avoid the oxygen contamination.

The current curve followed a hyperbolic model, and it is demonstrated in the panel in Figure 4 B, evidencing a saturation of the cell response at the hydrogen peroxide concentration close to 0.88M.

In order to determine if the saturation was due to the anode or cathode chamber, the concentration of the electro-active species in the anode compartment was increased increasing the lag phase until the complete discoloration of the aqueous solution, waiting for the methylene blue in reduced form. The results of polarization curves showed higher values of OCV and SCC when the lag phase was 46 h. Our findings demonstrated that the electrochemical reaction was limited by the anode chamber.

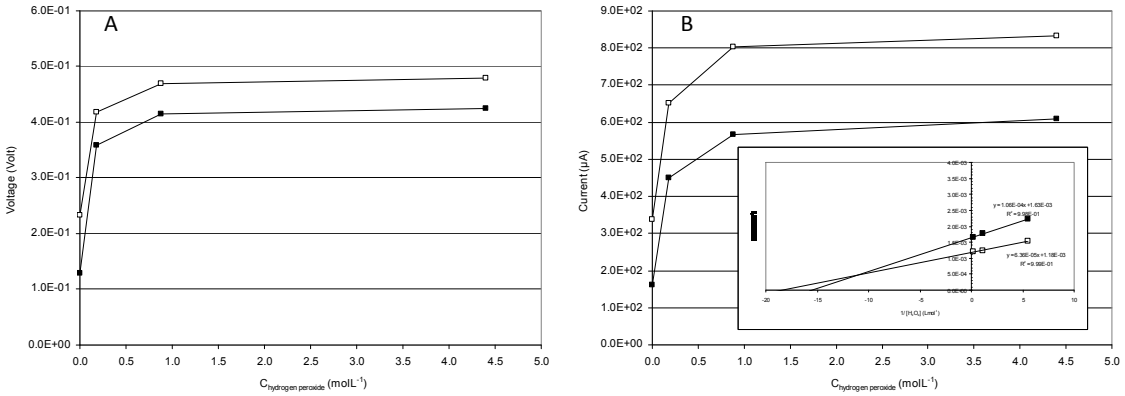

Figure 4: Effect of hydrogen peroxide concentration on OCV (A) and SCC (B) of the fuel cell after 22 h (closed squares) and 46 h (open squares) of incubation.

However, this model was not confirmed by the values of OCV and SCC at different concentration of mediator. Experiments were carried out at different concentrations of MB (0.01, 0.05, 0.5 and 5 mM) and the results of polarization curves are reported in Figure 5.

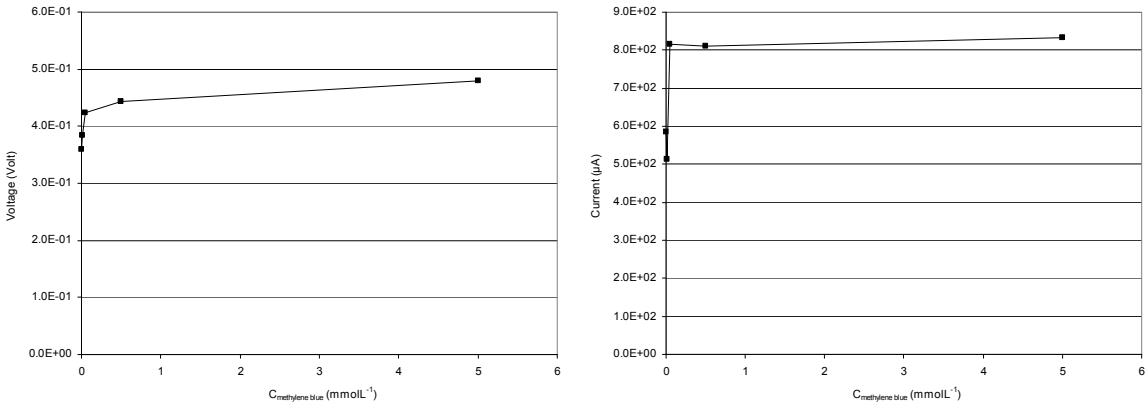

Figure 5: Effect of methylene blue concentration on OCV and SCC of the fuel cell.

The electrochemical experiments were started after the complete reduction of the methylene blue in anode chamber between 22 h and 46 h of lag phase.
The electrochemical reaction would be independent to the mediator concentration into the bulk of the aqueous solution in the range 0.05-5.00 mM. This result could be only explained by a stable micro-environmental conditions reached on the surface of the anode, probably due to the yeast adsorption.
Accordingly the limiting step in the anode chamber must be searched in the capacity of the biocatalyst to address the mediator in the reduced form to the electrode surface rather than on the ability of the last one to interact with the electrode and discharge.

4. Conclusions

Electric current was generated by Saccharomyces Cerevisiae as active biocatalyst in a dual-chamber MFC. Glucose and methylene blue were used as substrate for the bioelectricity generation and electron mediator in the anode chamber, respectively. The effect of concentration on MFC performance of both electron acceptor and electron donor was investigated. We proved the importance of working with high concentrations of electron acceptor, the increased hydrogen peroxide concentration, in the range 0.18 - 0.88 $molL^{-1}$ resulted in an increment of current generated of 25 %.
The biocatalysts in the media with concentration of 5 gL^{-1} of glucose, 0.05 mM methylene blue and oxygen at 27.5 °C as electron acceptor demonstrated the maximum power and optimum current density of 115 mW/m^{-2} and 1.14 A/m^{-2}. The obtained averaged power and current density using hydrogen peroxide as electron acceptor were 110 mW/m^{-2} and 1.17 A/m^{-2}.

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