Microbial fuel cell (MFC) performance with pigments bikaverin and congo red as electrochemical mediators for optmization power energy

Desempenho de célula a combustível microbiana (CCM) com os pigmentos bicaverina e vermelho do congo como mediadores eletroquímicos para otimização de energia elétrica

Rendimiento de la celda de combustible microbiana (CCM) con los pigmentos bikaverina y congo rojo como mediadores electroquímicos para la optimización de la energía eléctrica

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Abstract

For decades, non-renewable energy resources have been used indiscriminately, but their slow depletion and extremely harmful impacts on the environment have shifted the focus to sustainable and renewable energy sources. Among the renewable energy sources, biofuel cells are defined as devices that convert chemical energy present in chemical bonds into electrical energy. Biocells are classified into two broad categories of enzymatic fuel cells, which employ enzymes as biocatalysts, and microbial fuel cells, which use microorganisms as biocatalysts. An important requirement in the functioning of a biofuel cell is the transfer of electrons from inside an active site of an enzyme to the outside, as the electrodes being solid cannot penetrate the enzymes. A wide range of molecules can be used as electrochemical mediators, some with high toxicity and many non-toxic fungal substances having an enormous potential to be used as electrochemical mediators. In this work, the fungal pigment bikaverin was compared to the synthetic dye Congo red, in order to obtain the best energy-optimizing molecule in an enzymatic fuel cell. Congo red presented a higher current density of 273 $mA.cm^{-2}$ compared to bikaverin, 230 $mA.cm^{-2}$, but because it presents a more stable chronoamperometric graph and does not have high toxicity, the fungal biopigment proved to be the best option for optimization. on the potential of energy generated in an enzymatic fuel cell.
Keywords: Biofuel cell; Enzymes; Bikaverin; Caatinga soil.

Resumo

Há decadas, recursos energéticos não renováveis estão sendo usados indiscriminadamente, mas seu lento esgotamento e impactos extremamente prejudiciais ao meio ambiente mudaram o foco para fontes de energia sustentáveis e renováveis. Dentre as fontes renováveis de energia, as biocélulas a combustível são definidas como dispositivos que convertem a energia química presente nas ligações químicas em energia elétrica. As biocélulas são classificadas em duas grandes categorias de células a combustível enzimáticas, que empregam enzimas como biocatalisadores e células a combustível microbianas, que usam micro-organismos como biocatalisadores. Um requisito importante no funcionamento de uma biocélula a combustível, é a transferência de elétrons de dentro de um sítio ativo de uma enzima para fora, pois os eletrodos sendo sólidos não podem penetrar nas enzimas. Uma vasta gama de moléculas pode ser usada como mediadores eletroquímicos, algumas com toxicidade elevada e muitas substangias fúngicas atóxicas contendo umenorme potencial para serem utilizados como mediadores eletroquímicos. Neste trabalho o pigmento fúngico bicaverina foi comparado ao corante sintético vermelho congo, a fim de obter a melhor molécula otimizadora de energia em uma célula a combustível enzimática. O vermelho congo apresentou maior densidade de corrente 273 $mA.cm^{-2}$ em relação a bicaverina, 230 $mA.cm^{-2}$, porém por apresentar um gráfico cronoamperométrico mais estável e não ter elevada toxicidade, o biopigmento fúngico se mostrou a melhor opção para otimização no potencial de energia gerada em uma célula a combustível enzimática.
Palavras-chave: Biocélulas a combustível; Enzimas; Bicaverina; Solo da Caatinga.

Resumen
Durante décadas, los recursos energéticos no renovables se han utilizado indiscriminadamente, pero su lento agotamiento y los impactos extremadamente dañinos en el medio ambiente han cambiado el enfoque hacia las fuentes de energía sostenibles y renovables. Entre las fuentes de energía renovables, las celdas de biocombustibles se definen como dispositivos que convierten la energía química presente en los enlaces químicos en energía eléctrica. Las bioceldas se clasifican en dos amplias categorías de celdas de combustible enzimáticas, que emplean enzimas como biocatalizadores, y celdas de combustible microbianas, que utilizan microorganismos como biocatalizadores. Un requisito importante en el funcionamiento de una celda de biocombustible es la transferencia de electrones desde el interior de un sitio activo de una enzima hacia el exterior, ya que los electrodos al ser sólidos no pueden penetrar las enzimas. Se puede usar una amplia gama de moléculas como mediadores electroquímicos, algunas con alta toxicidad y muchas sustancias fúngicas no tóxicas que tienen un enorme potencial para usarse como mediadores electroquímicos. En este trabajo se comparó el pigmento fúngico bicaverina con el colorante sintético rojo congo, con el fin de obtener la mejor molécula optimizadora de energía en una celda de combustible enzimática. El rojo congo presentó una mayor densidad de corriente de 273 mA.cm-2 en relación a la biverina, 230 mA.cm-2, pero por presentar un gráfico cronoamperométrico más estable y no tener alta toxicidad, el biopigmento fúngico demostró ser la mejor opción para optimización del potencial de energía generada en una pila de combustible enzimática.
Palabras clave: BioFuel Cells; Enzimas; Bicaverina; Suelo de la Caatinga.

1. Introduction

The advent of the industrial revolution and the exponential increase in urbanization marked a considerable increase in energy consumption around the globe. For decades, non-renewable energy resources have been used indiscriminately, but their slow depletion and extremely harmful impacts on the environment have shifted the focus to sustainable and renewable energy sources. Among the renewable energy sources, fuel cells are considered a relatively recent technology. Biofuel cells (BC) are defined as devices that convert chemical energy present in connections into electrical energy (Kumar *et al*. 2022).

An important subset of fuel cells are biofuel cells (BC) which employ biocatalysts to produce electricity instead of conventional costly metal catalysts. BCs are classified into two broad categories of enzymatic fuel cells (EFCs), which employ enzymes as biocatalysts and microbial fuel cells (MFCs), which use microorganisms as biocatalysts. (Nawaz *et al*. 2020).

There are many advantages in BC technology, such as bioremediation of organic waste, pollutants and simultaneous bioelectricity generation, inexhaustible environmentally friendly technology, and electrochemical parameters can be easily controlled and monitored such as internal electron transfers. An important requirement in the functioning of a biofuel cell is the transfer of electrons from inside an active site of an enzyme or microbial cell membrane to the outside, as the electrodes being solid cannot penetrate the enzymes or cell membranes. (Rahimnejad *et al*. 2020).

Despite the mechanism, the transfer of electrons to the electrode leads to reductive and oxidative species (redox) capable of establishing electrical connections between the enzyme and the electrode. This substance can be a soluble redox transport or a reduced primary metabolite and is more commonly referred to as an electrochemical mediator. A mediator must possess some significant properties, such as the ability to maintain physical contact with the electrode surface while freely transiting the compartment (Sorrentino *et al*. 2022).

A wide range of molecules can be used as electrochemical mediators, such as: azo dyes, organometallic compounds, quinone derivatives, and more. However, many of these compounds have high toxicity and high cost, which makes them very limited for application in a BC. Several substances are produced by filamentous fungi, many of them have low or no toxicity, but with enormous potential to be used as mediators. (Arkatkar *et al*. 2021). Investigations were carried out in order to expand the knowledge of the electrochemical properties of bikaverin pigments and the synthetic Congo red dye, applied in an EFC and establish in a comparative way the optimization in energy production, advantages of use and future applications.

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2. Methodology

2.1 Microorganism

For red pigment production, the filamentous fungus *Fusarium oxysporum* UCP 1137 belonging to the Culture Collection was used UCP (Uuniversidade Católica de Pernambuco), maintained at the Research Center in Environmental Sciences and Biotechnology (NPCIAMB) of the Catholic University of Pernambuco, registered in the World Federation for Culture Collection – WFCC.

2.2 Growth medium

For the maintenance of the fungus, the solid medium Agar Sabouraud was used [(g L^{-1}): Glucose, 40g; Peptone, 10g; Ágar, 15g; Distilled water, 1L] in Petri dishes, at a constant temperature of 28°C.

In the production and extraction of bikaverin, the liquid medium Batata Dextrose [Potato Dextrose (g L^{-1}): Potato infused water, 200ml; Glucose, 20g, Distilled water, 800mL], in an Erlenmyer at 25°C.

2.3 Congo red azo dye

The Congo red azo dye (C.I. No. 22120, Direct Red 28), sodium salt of benzidinediazo-bis-1-naphthylamine-4-sulfonic acid, C32H22N6Na2O6S2 and molecular weight, 696,66 g/mol) It is used abundantly in the textile and paper industry and has been reported to be an extremely carcinogenic synthetic substance, toxic to the environment and to humans. (Chung, K.-T., 2016).

2.4 Production of pigment in liquid medium

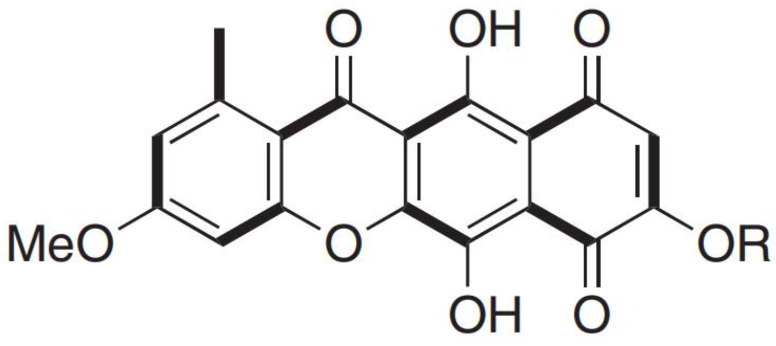
Fusarium oxysporum was inoculated in Sabouraud medium for 6 days at a temperature of 28°C to obtain young spores. After this period, the colony was drilled with a sterile well cutter, with a diameter of 12 mm, resulting in disks for liquid fermentation.

To produce the pigment in liquid broth, twenty discs of growth medium were added in a 250mL Erlenmeyer containing 100mL of Potato Dextrose medium. Fermentation continued in the dark under constant stirring at 200rpm, with pH 4.5, at a temperature of 25°C for 7 days.

2.5 Pigment extraction

Bikaverin (Figure 1) was extracted and kindly provided by Nascimento and Campos-Takaki (2021), according the methodology registered in patent BR1020210131020.

Figure 1. Bikaverin pigment structure.



Source: Cox *et al*. (2020).

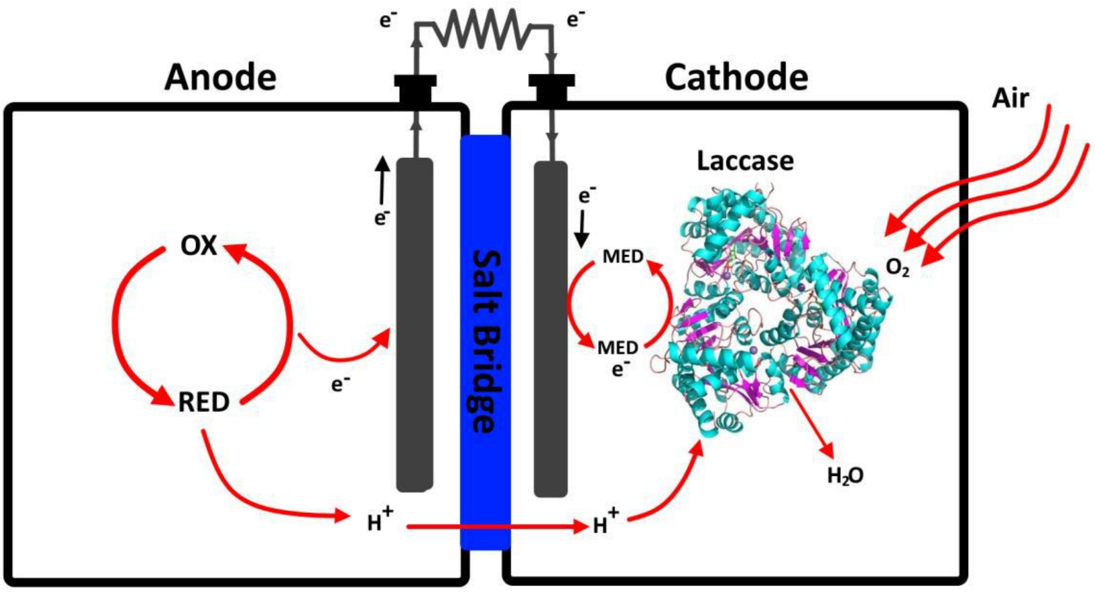
2.6 Cyclic Voltammetry

To obtain the oxidation and reduction peaks of the pigment, a cyclic voltammetry was performed. An electrolytic cell containing three electrodes was used: a glassy carbon working electrode; a platinum wire counter electrode and an Ag|$AgCl_2$ reference electrode in saturated KCl. As electrolytic support substance, H3PO4 was used at a concentration of 100mM. The readings followed a potential range of -1.0 to 1.0 V at a speed of 0.1 $V.s^{-1}$. (Di Noto, et *al*. 2022; Wang H. W., et *al.* 2021)

2.7 Enzymatic Biofuell cell

For application of the pigment with electron transport property to optimize energy generation, a Cathodic Enzymatic Biofuel Cell was used. (**Figure 2**). This cell contains two compartments, one cathodic and anodic, both with 200mL of useful volume. Each compartment contains a $9cm^2$ exploded graphite plate as electrodes for electron transport. The cationic bond between the compartments is composed of a 5 cm salt bridge composed of 3M KCl on agar. The anode was inoculated with a 20 $mmol.L^{-1}$ potassium ferrocyanide solution, while the cathode was inoculated with a commercial *Trametes versicolor* laccase solution with an enzymatic activity of 200 $U.L^{-1}$ and 10 ppm of bikaverin.

Figure 2. Structure and functioning of an enzymatic microbial fuel cell.



Source: Own authors (2022).

2.8 Chronoamperometry

To obtain the energy values generated by the BC, a chronoamperometry was performed, using a Fluke 8080 multimeter with Flukeview® data acquisition software (Fluke Corporation, USA). The data obtained in the form of potential were converted into current density (Id) using the Ohm's law equation (**Equation 1**), with an external load resistance of 1KΩ.

Equation 1. Current Density

$$Id = E \times R$$

2.8.1 Coulombic Efficiency

For an enzymatic fuel cell, an integrated model of Id vs time is used, represented by **Equation 2**. Where M = molar mass of substrate, A = the electrode area, V = the total volume of the cathode compartment, S = final substrate concentration, and F = Faraday's constant (96485 $C.mol^{-1}$) (Cao, T. N. D., *et al*. 2020 e Flores, M. M. A., *et al*. 2021).

Equation 2. Coulombic efficiency of a microbial fuel cell

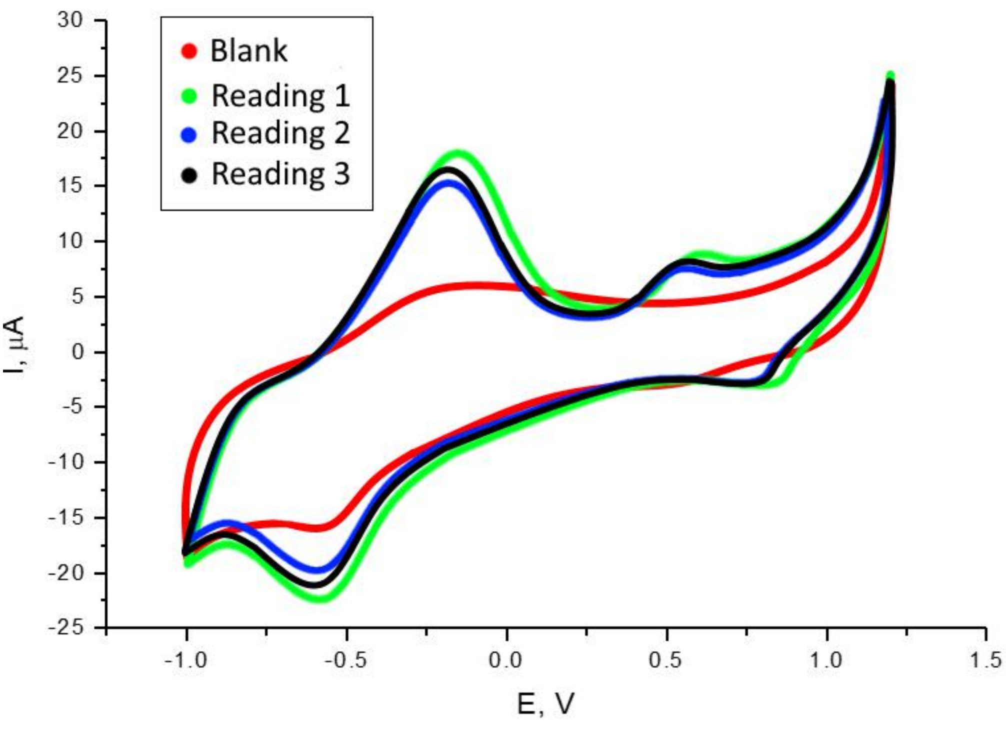
$$CE = (M\int_0^{tf} Id\ dt\ A) \times (F \times z \times V \times \Delta S)^{-1}$$

3. Results and Discussion

3.1 Capacity for electron transfer by cyclic voltammetry

Cyclic voltammetry of bikaverin demonstrated an excellent profile to be applied as an electrochemical mediator, as indicated in **Figure 3**, the amount of upper (oxidation) and lower (reduction) peaks are equal, which helps in their reversibility. The low potential for the release and reception of electrons also helps in its reversibility, since a smaller amount of energy is required for electron exchanges to occur in the cycle of a BC. According to Kuskur, C.M., *et al*. (2017), Congo red has a single redox peak, which makes it suitable for application as an electrochemical mediator. In addition, its oxidative peak is much more centered than the two peaks of bikaverin (Table 1), increasing the reversibility of the synthetic molecule in relation to the biopigment since the centralization of the oxidative peaks with their due reductive pairs increases the reversibility of the process.

Figure 3. Bikaverin Cyclic Voltammetry.



Source: Own authors (2022).

Despite the high centralization of the peaks, and presenting a redox pair, Congo red shows this behavior, when analyzed with a greater range of applied potential. (Kuskur, C. M., *et al.* 2017). With a lower energy effort, as applied to bikaverin (-0.1 to 1.0V), Congo red does not show a well-defined reductive curve, only an oxidation one (Shetti, *et al*. 2019). The need for a higher applied potential for the molecule to obtain the redox pair makes it less efficient in the mediation process, causing a longer delay in the start of its carrier activity.

Table 1. Bikaverin cyclic voltammetry redox peaks

BIKAVERIN REDOX PEAKS		
Oxidation peak	-0.25	0.5
Reduction peak	-0.6	0.8

Source: Own authors (2022).

3.2 Energy profile of electrochemical mediation in a BC

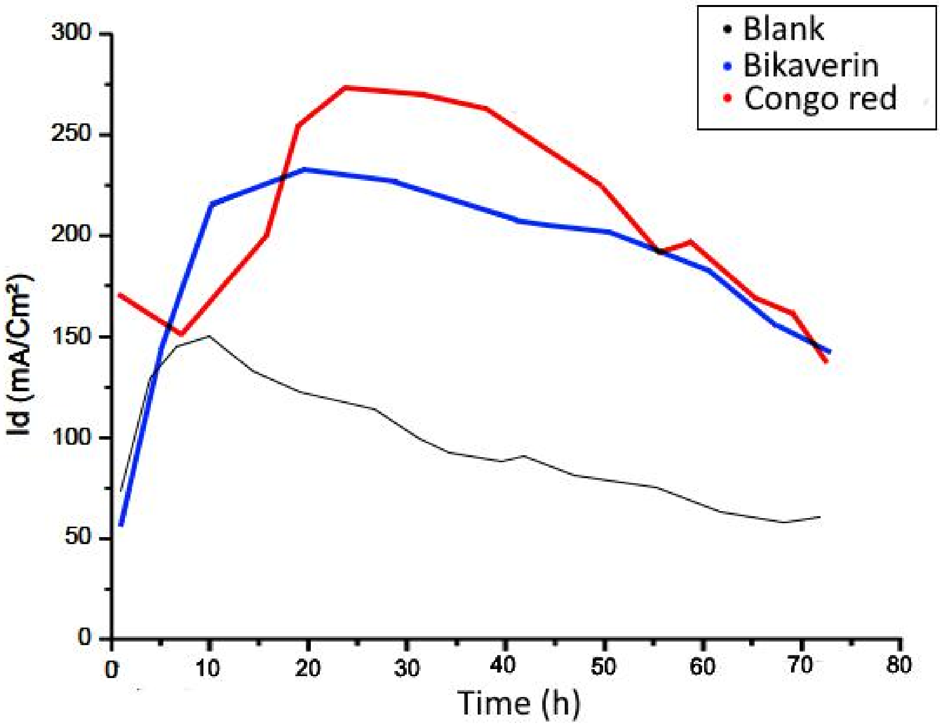
When comparing the energy profiles of the electrochemical mediating pigments, a higher current density peak can be seen in Congo red, 273 mA.cm^{-2} in relation to bikaverin, 230 mA.cm^{-2} (**Figure 4**), with a coulombic efficiency of 71% and 43% respectively. In the literature, we found higher current density results than those found in this research, as the result of Mani, P., VT, F., *et al*. (2020) which reached a maximum Id of 325 mA.cm^{-2} using a platinum catalyst on the cathode, which greatly increases the cost of a BC, but when using a bacterial consortium biocathode, it reached a maximum Id of 240 mA.cm^{-2}. However, a large amount of maintenance was required to try to maintain the stability of its system, which is a disadvantage compared to BC with bikaverin, which showed stability between 12 and 62 hours of experiment, totaling 50 hours.

Rossi, R., *et al*. (2020), used a different approach in his BC, by applying carbon brushes as electrodes, reaching a maximum current density of 420 mA.cm^{-2}. However, to reach this value, a period of 68 days was necessary, in addition to only being able to reach values above 230 mA.cm^{-2}, equal to that of bikaverin, after 20 days of experiment. Mounia, A. Y., *et al*. (2020) also took time to reach a value close to 230 mA.cm^{-2}, in this case being 200 mA.cm-^{2} in 30 days.

Stability is a property that helps to highlight the chronoamperometric profile of bikaverin in relation to Congo red. In Figure 4, it is possible to observe in the Congo red pigment the delay in reaching the maximum peak and how it presents a greater drop when compared to the fungal pigment bikaverin. It is possible that mainly degradation of the synthetic pigment by the laccase enzyme and other oxidasic enzymes occurs (Saha & Mukhopadhyay, 2020).

Another important factor that adds and confirms the value of the results obtained with bikaverin is related to the high toxicity of the Congo red pigment. Using such a harmful substance makes BC unfriendly to the environment, from contaminating soil, riverbeds, and lakes (Alsamhary, K., *et al*. 2022) to causing problems in various tissues of the human body (Frid, P., Anisimov, S. V., & Popovic, N. 2007), mainly in the central nervous system (Setti, S. E., *et al*. 2021).

Figure 4. Chronoamperometry of bikaverin and congo red



Source: Own authors (2022).

In terms of energy production optimization, the mediators proved to be quite efficient in raising a maximum blank Id, from 155 mA.cm^{-2} to 230 mA.cm^{-2} with bikaverin and 273 mA.cm^{-2}, an increase of 150% and 176% respectively. In the work of Chen, H., *et al*. (2021), a neutral red synthetic dye was used as mediator, achieving a 46% increase in its cell energy efficiency. The author Li, T., *et al*. (2021), applied several natural azo dyes with a maximum energy efficiency of 5%, a coulombic efficiency of 6.45% and associated with the decolorization of toxic dyes, like this work, but with lower efficiency in all aspects.

4. Conclusions

Compared to the Congo red synthetic dye, bikaverin proved to be an excellent option as an electrochemical mediator for application in an enzymatic fuel cell, because it has more reductive and oxidative pairs, greater stability, speed in reaching high currents and is not toxic. Thus, this biopigment has enormous potential to integrate in situ enzyme production systems in BC, increasing cell longevity and becoming a viable, cheap, and non-toxic option for energy generation.

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