

BIOELECTRICITY GENERATION BY *SACCHAROMYCES CEREVISIAE* USING METHYLENE BLUE MEDIATOR IN VARIOUS MICROBIAL FUEL CELL DESIGNS

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Abstract

The discovery of electroactive microorganisms and their electron transfer mechanisms has opened new possibilities for bioelectrochemical wastewater treatment. Microbial fuel cells (MFCs) generate electricity by converting organic substrates through microbial metabolism. In this study, Saccharomyces cerevisiae was utilized as a biocatalyst to assess the performance of various MFC designs using domestic wastewater as the anode substrate. The effects of reactor configuration and the addition of methylene blue as a redox mediator on voltage generation, power density, and chemical oxygen demand (COD) removal were investigated. Two batch-mode reactors were tested: a nested cylindrical dual-chamber MFC (NDMFC) and a cylindrical H-type MFC (CHMFC). The maximum power densities achieved were 3.22 mW/m² for the NDMFC and 3.35 mW/m² for the CHMFC, while COD removal efficiencies were 90.33% and 91.44%, respectively. As all operational conditions were maintained constant, the observed performance variations were attributed to differences in reactor design. Overall, this study aimed to emphasize the importance of mediators that facilitate electron transfer in microbial fuel cells. The effect of methylene blue on power generation was effectively investigated using different reactor configurations.

Keywords: *Saccharomyces cerevisiae*, Mediator, Power density, Microbial fuel cell, Biotechnology

ÇEŞİTLİ MİKROBİYAL YAKIT HÜCRESİ TASARIMLARINDA METİLEN MAVİSİ ARACISI KULLANILARAK *SACCHAROMYCES CEREVISIAE* TARAFINDAN BİYOELEKTRİK ÜRETİMİ

Özet

Elektroaktif mikroorganizmaların ve elektron transfer mekanizmalarının keşfi, biyoelektrokimyasal atıksu arıtımı için yeni olanaklar açmıştır. Mikrobiyal yakıt hücreleri (MFC'ler), mikrobiyal metabolizma yoluyla organik substratları dönüştürerek elektrik üretir. Bu çalışmada, *Saccharomyces cerevisiae*, anot substratı olarak evsel atıksu kullanan çeşitli MFC tasarımlarının performansını değerlendirmek için biyokatalizör olarak kullanılmıştır. Reaktör konfigürasyonunun ve metilen mavisinin redoks aracı olarak eklenmesinin voltaj üretimi, güç yoğunluğu ve kimyasal oksijen ihtiyacı (KOİ) giderimi üzerindeki etkileri araştırılmıştır. İki kesikli mod reaktör test edilmiştir: iç içe geçmiş silindirik çift odacıklı MFC (NDMFC) ve silindirik H tipi MFC (CHMFC). Elde edilen maksimum güç yoğunlukları NDMFC için 3,22 mW/m² ve CHMFC için 3,35 mW/m² iken, KOİ giderim verimlilikleri sırasıyla %90,33 ve %91,44 olmuştur. Tüm çalışma koşulları sabit tutulduğundan, gözlemlenen performans değişimleri reaktör tasarımındaki farklılıklara bağlanmıştır. Genel olarak, bu çalışma mikrobiyal yakıt hücrelerinde elektron transferini kolaylaştıran araçların önemini vurgulamayı amaçlamaktadır. Metilen mavisinin güç üretimi üzerindeki etkisi, farklı reaktör konfigürasyonları kullanılarak etkili bir biçimde incelenmiştir.

Anahtar Kelimeler: *Saccharomyces cerevisiae*, Medyator, Güç yoğunluğu, Mikrobiyal yakıt hücresi, Biyoteknoloji

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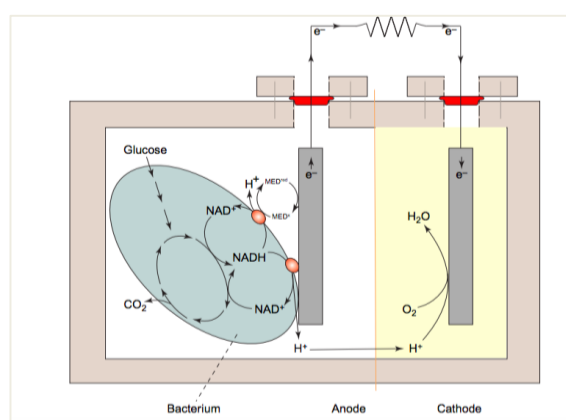
1. Introduction

Millions tons of wastewater are produced each year and often discharged untreated into agricultural lands or inland lakes. These wastewaters contain high concentrations of microbial, organic and inorganic pollutants [1]. Additionally, researchers have been

focusing on the use of alternative renewable energy sources that are environmentally friendly and economically sustainable in recent years [2]. Microbial fuel cell (MFC) is an encouraging bioenergy technology with the potential to generate sustainable and renewable electrical energy from wastewater [3]. The

preference for microbial fuel cell technology in wastewater treatment can help reduce high operating costs, such as those associated with aeration in activated sludge systems, by generating electrical energy through the oxidation of organic matter in wastewater [4]. Microbial Fuel Cells (MFCs) are defined as bioelectrochemical systems that produce electricity from organic matter using electroactive bacteria [5]. MFC is a two-electrode system that produces electrical power by oxidizing carbon source such as that found in wastewater at the anode through bacterial respiration, which is coupled with oxygen reduction at the cathode [6]. The typical dual chamber microbial fuel cell is given in Figure 1.

Figure 1. The typical dual chamber microbial fuel cell [7].



MFCs have also been used in a variety of different areas, including chemical production, desalination and use as biosensors [8]. In laboratory studies, various designs such as two-chamber H-type reactors, single or two-chamber cylindrical/cubic reactors, flat or cube-shaped reactors are generally used. In scaling studies, it is seen that flat plate or tubular reactors are mostly used [9]. Reactor and electrode modifications can affect parameters like microbial community growth, power output and removal efficiency [10]. Diverse reactor configurations [11], electrode materials [12] and different substrates [13] have been investigated and these results are given in the literature. It has been seen that the power generation of MFCs is limited by internal resistance, mass transport loss and activation loss [14]. MFC studies include the conversion of organic substances including amino acids, acetate, lactate, butyrate, glucose, cellulose, lignocellulosic substrates, ethanol, albumin, cysteine and marine sediments directly into energy by microorganisms for electricity production. Moreover, different domestic and industrial wastes have been utilized in this technology, with redox technology widely applied to facilitate electron transfer between microorganisms- substrates for the production of renewable energy from biomass [15]. In addition, the amount of electrical energy produced by MFCs is relatively low, which limits MFC

practice in broad-scale studies. Another limit is that microorganisms can only generate electricity from specific substrates, which restricts the types of waste that can be effectively used as feedstock [16].

Recent studies have shown that there has been an increase in the number of electrochemically active microorganisms that transfer electrons from substrates to the electrodes in MFC reactors without the need for external mediators. These microorganisms are referred to by various terms such as "electrochemically active bacteria", "anode-respiring bacteria", "electricigen" and "exoelectrogenic bacteria". Examples of these microorganisms include bacteria such as *Shewanella spp.*, *Rhodospseudomonas spp.* and *Geobacter spp.* [17]. MFCs are classified into fuel cells that facilitate direct electron transfer and those that rely on mediators to transfer electrons. The electron transfer efficiency of MFCs can be improved by using a suitable electron mediator. In most microbial fuel cells, mediators such as neutral red, methylene blue, methyl viologen, thionine, humic acid and ferricyanide are added to the anode chamber to improve the power output of the reactor. The use of electron mediators is essential for enhancing the performance of MFCs [18]. Literature findings have shown that the incorporation of mediators in microbial fuel cell (MFC) systems plays a crucial role in enhancing electron transport. Accordingly, this study aimed to evaluate the influence of mediator addition on MFC performance under different reactor configurations.

The remainder of this study is organized as follows: Section 2 explains the experimental parameters used in the study and gives the equations of the calculations. The effect of adding electroactive bacteria to *S. cerevisiae* in the presence of a mediator on the generation of electricity is investigated using two different MFC reactor designs, and the results are presented in Section 3. Finally, Section 4 presents the conclusion of this study.

2. Materials

This section describes reactors and chemicals used in the study, measurement devices, electrochemical calculations and other experimental details.

2.1. Fabrication of Reactors

Two different reactor types to determine the effect of reactor designs were used in this study. Reactors produced from polypropylene material in Ankara Ostim Seymen Machine (with 700 mL capacity) are nested cylindrical dual-chamber reactor (NDMFC) and cylindrical H-type reactor (CHMFC). The NDMFC, the cathode chamber is placed inside the anode chamber to reduce the internal resistance of the reactor and produce higher power. The cylindrical H-type reactor is frequently used in literature studies [19] but it is manufactured in a cylindrical shape to prevent the formation of dead space inside the reactor. The NDMFC and CHMFC reactors were shown in Figure 2.



Figure 2. The NDMFC and CHMFC reactors.

2.2. Experimental Design

In this work, all experiment and analyses were carried out in the laboratories of Gazi University Chemical Engineering Department. Graphite rod electrodes (Beijing Dongfangle Trading Co., Ltd., China) were used as anode and cathode electrodes on NDMFC and CHMFC reactors [19,20]. The anode and cathode electrodes used are 1 cm in diameter and 5 cm in height. Before use, the electrodes were kept in ethanol for 15 minutes to remove any contaminants that may be present on the electrode surface.

Nafion 117 membrane (Ion Power, 720 Governor Lea Rd, New Castle, DE 19720) was used to separate the reactor chambers [21]. PEM was activated by first boiling in 0.5 M H₂SO₄ at 80°C for 2 hours, then in deionized H₂O for 2 hours, and the final stage stored in 0.5 M H₂SO₄ before use. The Nafion 117 used in both NDMFC and CHMFC reactor has the same surface area. Dried powder *S. cerevisiae* cells were activated according to the method and yeast used in the literature and used as inoculum to produce electrical energy from domestic wastewater [22,23].

The presence of mediators such as neutral red, thionine or methylene blue is critical for *S. cerevisiae* to transfer its electrons [24]. For this purpose, as electron mediator, 300 µM methylene blue (MB) was added in the anode solution [25].

The anode chambers were filled with domestic wastewater and inoculum (1/20 ratio). The cathode chambers were filled with deionized water. Both anode and cathode electrodes were connected with a copper wire and a resistance of 100 kΩ. The experimental setup was shown in Figure 3.

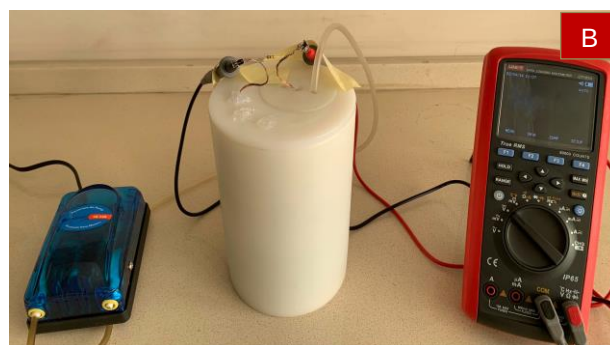
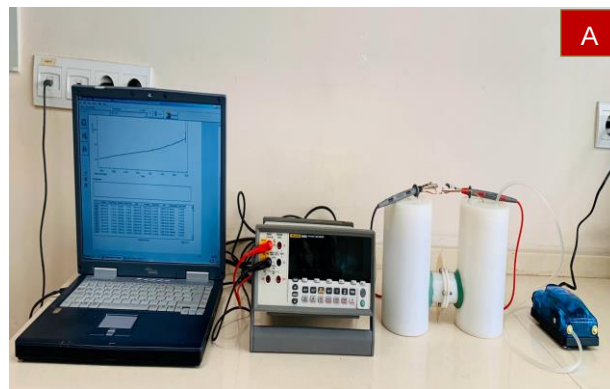


Figure 3. The CHMFC (A) and NDMFC (B) experimental setup.

2.3 Electrochemical Analysis

The reactors were operated at the same time in batch conditions and daily monitored by analysing the anodic solution in term of pH (by using pHmeter, Ezdo pHmeter PL-700PV) and chemical oxygen demand (COD) (Matriks COD test kits and Lovibond MD 600 fotometer). The percentage of removals were calculated according to from (1).

$$\text{Removal (\%)} = \frac{(C_i - C_f)100}{C_i} \quad (1)$$

where, initial concentration (C_i) (mg/l), final concentration (C_f) (after treatment; mg/l) [26].

Voltage values were measured at 10-minute intervals by multimeter (UNI-T UT181A and FLUKE 8808A). Power density was calculated used from (2);

$$\text{Power Density} = \frac{I \cdot V}{A} \quad (2)$$

where; voltage (V), anode electrode surface area (A) and current (I) [27]. Coulombic efficiency (%) is defined as the ratio of the total coulombs actually transferred from the substrate to the anode to the maximum possible coulombs that could be transferred. For a batch MFC reactor, the coulomb efficiency is calculated as follows: (3).

$$CE (\%) = \frac{8 \int_0^t I dt}{F V_{an} \Delta COD} 100 \quad (3)$$

where; Faradays constant (F), the change of substrate concentration over the time ΔCOD , the volume of the NDMFC and CHMFC anode chamber (V_{an}) [28].

3. Results and Discussion

This study examines the impact of *S. cerevisiae* electrochemical performance in different types of microbial fuel cells (MFCs) using domestic wastewater as substrate. The most effective parameter of electrical performance is the output voltage. The output voltage of NDMFC and CHMFC's during the experiment is shown in Figure 4.

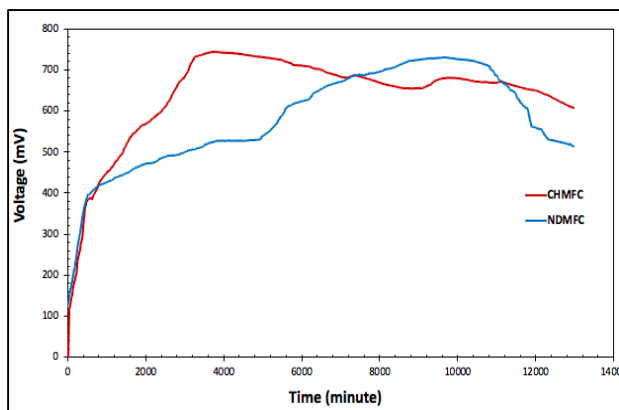


Figure 4. Comparative voltage trends of CHMFC and NDMFC operated with *S. cerevisiae*.

The voltage-time graphs express the impact of microbial fuel cells (MFCs) effect the electrochemical performance and *S. cerevisiae* behavior of MFCs. In Fig. 4, the highest voltage peaks for CHMFC and NDMFC are 744.4 mV and 729.8 mV, respectively. These findings may indicate that microorganism-substrate contact is prevented to some extent by placing the cathode chamber in the placed of the anode chamber in the NDMFC reactor.

When literature studies were examined, Permana et al. [18] investigated the performance of MFCs in generating electricity using glucose as a substrate and *S. cerevisiae* as biocatalysts. At the end of the experiment, the maximum OCV value was observed a value of 0.689 V at 36 hours with mediatorless MFC. In another study, Rossi et al. [29] studied *S. cerevisiae* as an active biocatalyst and glucose as a substrate in a two-chamber MFC. The maximum voltage value was obtained as 479 mV with the use of hydrogen peroxide as an electron acceptor in the cathode chamber. Voltage values recorded at 10-minute measurement intervals and the anode electrode surface area were applied to the Equation (2) to obtain power density curves and are given in Figure 5.

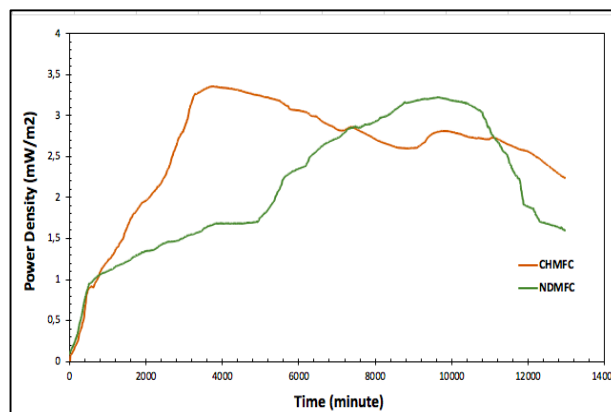


Figure 5. Comparative power density trends of CHMFC and NDMFC operated with *S. cerevisiae*.

In Figure 5, the NDMFC design shows that decreasing the distance between the anode and cathode compartments decreased diffusion resistance and increased ion transport. However, because the cathode compartment was located in the center of the anode compartment, the substrate accumulation around the cathode reactor delayed the interaction of *S. cerevisiae*. Consequently, the CHMFC exhibited a higher internal resistance than the NDMFC but performed more effectively, enabling faster microbial adaptation and earlier onset of electron transfer. The maximum power density values for CHMFC and NDMFC were measured as 3.35 mW/m² and 3.22 mW/m², respectively. In a study conducted by Rahimnejad et al. [24], MFC's were investigated for the acceleration of biofilm formation when thionine was used as an electron mediator. *S. cerevisiae* and an exoelectrogenic mixed culture were used as inoculum and the maximum power density was obtained as 3 mW/m² without a mediator. Shrivastava et al. [30] measured ethanol production and power generation by *Saccharomyces cerevisiae* and *Pichia fermentans*. In this study, 100 mL single-chambered cell was used as MFC and maximum power density value was achieved as 4.47 mW/m² on the 15th day.

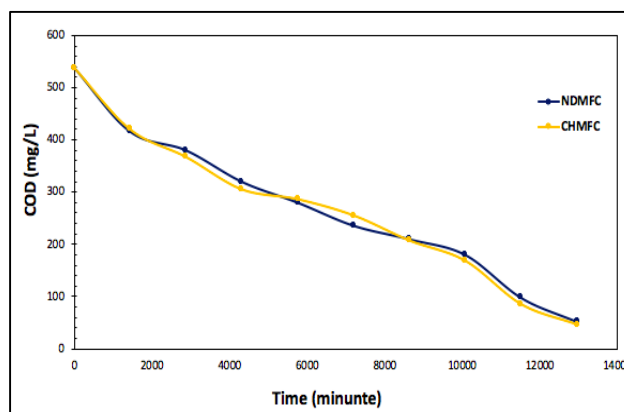


Figure 6. Comparative COD removal trends of CHMFC and NDMFC operated with *S. cerevisiae*.

Figure 6 shows that the COD removal efficiency of NDMFC and CHMFC was obtained as 90.33% and 91.44%, respectively. The higher COD removal efficiency obtained in the CHMFC reactor reveals that the substrate-microorganism contact was prevented to some extent in the NDMFC reactor. In this study, the addition of *S. cerevisiae* to the anode chamber provided high COD removal efficiency compared to the literature studies. Verma and Mishra [31] studied banana peel slurry based dual-chamber H-shaped MFC with *S. cerevisiae* inoculum and obtained maximum COD removal from BSY-MFC as 88.8%. Fang and Zhang [32] investigated a dual chamber microbial fuel cell using molasses wastewater simulated, and COD removal was found to be 79.25%.

In the study, coulombic efficiency was obtained using the area of the current graph, Faraday constant and COD removal efficiencies. The coulombic efficiency for NDMFC and CHMFC was calculated as 0.11% and 0.12%, respectively. When the coulombic efficiency values are examined, it is shown that most of the electrons obtained from organic matter are not used for current generation. The current situation may be caused by biomass formation, methanogenesis, and the presence of various electron acceptors [27]. Juang et al. [33] investigated effects of flow rate on power generation. The optimum flow rates to acquire power density and coulombic efficiency in MFC-1 as 0.85 mW/m² and 0.063%, respectively. Pişkin et al. [34] were performed of oxidation of yeast wastewater at the anode and reduction of azo dye at the cathode in MFC. With OPT 1, azo dye removal of 16.8 mg/L, maximum power density of 95.34 mW/m², COD removal of 5.8 g/L and coulombic efficiency of 0.23 % were achieved.

4. Conclusion

In this study *S. cerevisiae* and two reactor design are performed with use domestic wastewater as a substrate in order to increase the performance of MFC. Electrochemical determinations reveal a maximum voltage values of CHMFC and NDMFC are obtained as are 744.4 mV and 729.8 mV, respectively. Furthermore, the maximum power densities achieved were 3.22 mW/m² for the NDMFC and 3.35 mW/m² for the CHMFC, and domestic wastewater COD removal efficiency of NDMFC and CHMFC was obtained as 90.33% and 91.44%, respectively. The observed increases in both voltage and COD removal with CHMFC and NDMFC reactors demonstrate that methylene blue mediator is a highly suitable choice for *S. cerevisiae*. The study investigated the electricity generation performance of *S. cerevisiae* in two different reactor types using 300 µM methylene blue mediator. This analysis revealed the effectiveness of the reactor design by determining the parameters required for electron transfer. Ongoing innovations in electroactive microorganisms, reactor design and bioprocess optimization could offer higher power output in MFC

studies, potentially overcoming barriers to laboratory or large-scale MFC studies.

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