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The Analysis of Electron Transfer Mechanism Within Fuel Cell Systems: Electrochemical and Microbial Approaches

Işılay BİLGİÇ[1*]

[1]*Istanbul Okan University, Vocational School, Department of Electric and Energy, Istanbul, Turkey*

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Anahtar Kelimeler

Mikrobiyal yakıt hücreleri
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Elektron transfer mekanizması

Graphical/Tabular Abstract (Grafik Özet)

System in this study generates electricity by utilizing Thiobacillus ferrooxidans, an oxidation bacterium, at the cathode, and a mixed culture of bacteria at the anode within the MFC. The research delves into the fuel cell system's performance through electrochemical measurements. Furthermore, the study investigates the porphyrin structure of Thiobacillus ferrooxidans and unveils the electron transfer mechanism occurring at the cathode. / Bu çalışmadaki sistem, MFC içindeki katotta bir oksidasyon bakterisi olan Thiobacillus ferrooxidans'ı ve anotta karışık bir bakteri kültürünü kullanarak elektrik üretimini sağlamaktadır. Araştırmada elektrokimyasal ölçümler yoluyla yakıt hücresi sisteminin performansı ölçülmüştür. Ayrıca bu çalışmada, Thiobacillus ferrooxidans'ın porfirin yapısı ve katotta meydana gelen elektron transfer mekanizması incelenmiştir.

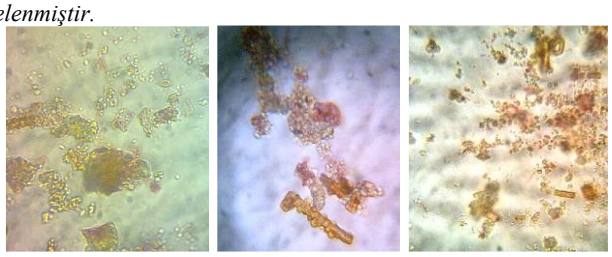

***Figure A**: Microscope images of the cathode bacteria Thiobacillus ferrooxidans / **Şekil A:** Katot bakterisi Thiobacillus ferrooxidans'ın mikroskop görüntüleri*

Highlights (Önemli noktalar)

- *Microbial fuel cells (MFCs) have gained attention as a promising avenue for alternative energy systems, offering a straightforward design and the ability to treat wastewater during energy production, thus sidestepping the use of fossil fuels. / Mikrobiyal yakıt hücreleri (MFC'ler), basit bir tasarım ve enerji üretimi sırasında atık suyu arıtma yeteneği sunarak alternatif enerji sistemleri için umut verici bir yol olarak dikkat çekmiş ve böylece fosil yakıtların kullanımını azaltmaya aday olmuştur.*
- *This study capitalizes the benefits of MFCs by utilizing Thiobacillus ferrooxidans, an oxidation bacterium, at the cathode, and a mixed culture of bacteria at the anode within the MFC. / Bu çalışma, MFC içindeki katotta bir oksidasyon bakterisi olan Thiobacillus ferrooxidans ve anotta karışık bir bakteri kültürü kullanarak MFC sisteminin avantajlarından yararlanmaktadır.*
- *In this study, the effects of different pH values and nutrients on system performance were investigated. / Bu çalışmada farklı ph değerleri ve besin maddelerinin sistem performansına etkileri araştırılımıştır.*

***Aim (Amaç):** In this study, the electron transfer mechanism and system performance of the MFC system using Thiobacillus ferrooxidans as the cathode bacteria were examined. / Bu çalışmada katot bakterisi olarak Thiobacillus ferrooxidans kullanılan MFC sisteminin elektron transfer mekanizması ve sistem performansı incelenmiştir.*

***Originality (Özgünlük):** The effects of the use of the iron oxidation bacterium Thiobacillus ferrooxidans in MFCs have been added to the literature./ Demir oksidasyon bakterisi olan Thiobacillus ferrooxidans'ın MFC'lerde kullanımının etkileri literatüre eklenmiştir.*

***Results (Bulgular):** node and cathode pH values, bacteria used in the cathode and anode nutrients are important in terms of the power values to be obtained from the system. / Anot ve katot pH değerleri, katotta kullanılan bakteriler ve anot besin maddesi sistemden elde edilecek güç değerleri açısından önemlidir.*

***Conclusion (Sonuç):** Conditions where the system reaches 0.8 V open circuit potential. Acetate was seen as the most suitable nutrient. / Sistem 0,8 V açık devre potansiyeline ulaşmıştır. En uygun besin maddesinin asetat olduğu görülmüştür.*

*Corresponding author, e-mail: isilayulusoy@gmail.com
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The Analysis of Electron Transfer Mechanism Within Fuel Cell Systems: Electrochemical and Microbial Approaches

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Abstract

Fuel cells are recognized for generating energy through electrochemical reactions while being environmentally friendly, as their only waste product is water. However, for these systems to achieve widespread commercial adoption, it's crucial to reduce the costs associated with catalysts used in the core of the system, known as the membrane-electrode assembly. Microbial fuel cells (MFCs) have gained attention as a promising avenue for alternative energy systems, offering a straightforward design and the ability to treat wastewater during energy production, thus sidestepping the use of fossil fuels. MFCs offer several advantages by employing inorganic molecules instead of traditional catalysts and microorganisms in lieu of enzymes. This study capitalizes on these benefits by utilizing Thiobacillus ferrooxidans, an oxidation bacteria, at the cathode, and a mixed culture of bacteria at the anode within the MFC. This approach leads to improved conductivity and overall system performance. The research delves into the fuel cell system's performance through electrochemical measurements. Furthermore, the study investigates the porphyrin structure of Thiobacillus ferrooxidans and unveils the electron transfer mechanism occurring at the cathode.

Yakıt Hücresi Sistemlerinde Elektron Transfer Mekanizmasının Analizi: Elektrokimyasal ve Mikrobiyal Yaklaşım

Makale Bilgisi

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Öz

Yakıt hücreleri, tek atık ürünü su olduğundan, çevre dostu olmasının yanı sıra elektrokimyasal reaksiyonlar yoluyla enerji üretmesiyle de tanınmaktadır. Bununla birlikte, bu sistemlerin ticari olarak yaygın şekilde benimsenmesi için, sistemin merkezinde kullanılan, membran-elektrot düzeneği olarak bilinen sistemin katalizör içeriği maliyetlerinin azaltılması çok önemlidir. Mikrobiyal yakıt hücreleri (MFC'ler), basit bir tasarım ve enerji üretimi sırasında atık suyu arıtma yeteneği sunarak alternatif enerji sistemleri için umut verici bir yol olarak dikkat çekmiştir ve böylece fosil yakıtların kullanımını azaltmaya adaydır. MFC'ler, geleneksel katalizörler yerine inorganik moleküller ve enzimler yerine mikroorganizmalar kullanarak çeşitli avantajlar sunar. Bu çalışma, MFC içindeki katotta bir oksidasyon bakterisi olan *Thiobacillus ferrooxidans'ı* ve anotta karışık bir bakteri kültürünü kullanarak bu faydalardan yararlanır. Bu yaklaşım iletkenliğin ve genel sistem performansının iyileştirilmesine yol açar. Araştırma, elektrokimyasal ölçümler yoluyla yakıt hücresi sisteminin performansını araştırmıştır. Ayrıca bu çalışmada, *Thiobacillus ferrooxidans'*ın porfirin yapısı ve katotta meydana gelen elektron transfer mekanizması incelenmiştir.

1. INTRODUCTION (GİRİŞ)

Fuel cells are capable of transforming chemical energy into electrical energy as long as a constant supply of fuel and oxidants is maintained. The efficiency of fuel cell systems is notably higher, nearly twice, compared to internal combustion engines, due to the direct occurrence of oxidation and reduction reactions on the electrode surface. Microbiological fuel cells exploit the microbial catalytic properties of organic compounds present in carbohydrate-rich waste materials. These systems harness the significant energy stored in carbohydrates, which are abundantly found in various sources including agricultural waste biomass, urban refuse, and crops like corn. The

*Corresponding author, e-mail: isilayulusoy@gmail.com
DOI: 10.29109/gujsc.1334651

conversion of carbohydrate energy into usable forms can involve processes like ethanol and hydrogen conversion. However, these methods tend to be complex and involve multiple steps, often entailing technical and economic challenges.

There is also an alternative method for directly converting sugar into electrical energy. Fuel cells using transition metals as catalysts cannot be used to generate electricity from carbohydrates. The most appropriate approach is to use microbiological fuel cells catalyzed by sugar oxidation during the life processes of microorganisms in the system or during fermentation to generate electricity directly from carbohydrates. Bacteria oxidize the organic matter at the anode and a current is obtained while electrons are sent to the cathode with the help of an external circuit. Interestingly, MFCs are seen as promising systems because they provide both the wastewater treatment process and electricity generation.

They can also be used as biosensors in the measurement of lactate, fructose and the analysis and determination of BOD (Biochemical Oxygen Demand). MFCs are also an alternative for use in remote areas such as the ocean floor where batteries cannot be used. Intelligent machines, called gastrobots, that can generate their own working power using organic materials have also been developed.

Microbial fuel cells (MFCs) are categorized into two groups based on their electron transport mechanism from bacteria to the electrode. In MFC systems employing mediators, electron carriers or mediators are introduced to facilitate electron transfer. However, most mediators are toxic substances and need to be used in high concentrations, rendering their application challenging for large-scale systems. The utilization of mediator-free MFCs holds promise for generating electricity from organic wastewater.

Metal-reducing bacteria, such as Shewanella, Rhodoferax, Geobacteraceae, and Clostridium butyricum, offer an alternative approach. These fermentative bacterial species can function without mediators. Metal-reducing bacteria are thought to directly transfer electrons to the anode, utilizing electrochemically active redox enzymes within their outer membrane.

The electrical output from MFCs typically varies based on factors like the initial bacterial inoculation, the type of substrate used, and the reactor design. The power generated ranges between 1 and 3600 mW/m^2. In the microbiological cathode chamber, ferrous sulfate present in the nutrient water acts as an electron acceptor. This chamber's solution remains clean due to constant renewal by microorganisms.

One of the metal oxidation bacteria used in these systems is *Thiobacillus ferrooxidans*. In a study in which graphite was used as an electrode and *Thiobacillus ferrooxidans* as redox bacteria at the cathode, it was observed that the open circuit potential increased up to 0.74 V. At a current density of 23 A/m^3, the power density increased up to 6.61 W/m^3 [1]. In another study by Heijne et.al. (2007) in which *Thiobacillus ferrooxidans* was used in the MFC system, a power density of 1.2 W/m^2 was obtained while the current density was 4.4 A/m^2 [2]. This result is 38% higher than the power value obtained by Heijne et.al. in another study in which they did not use the iron oxidation mechanism [3].

2. MATERIALS AND METHODS (MATERYAL VE METOD)

2.1 *Thiobacillus Ferrooxidans* Bacteria (*Thiobacillus Ferrooxidans* Bakterisi)

Thiobacillus ferrooxidans is a type of bacteria characterized by its gram-negative nature and rod-shaped morphology. It thrives in inorganic environments typical of mines and has the unique ability to utilize carbon dioxide from the air, making it autotrophic. While some research suggests the presence of heterotrophic growth, such findings are considered unreliable. Instances of Acidiphillium species, which are heterotrophic, appearing in *Thiobacillus ferrooxidans* cultures are attributed to cultivation errors. A study managed to cultivate a mixotrophic strain in an iron and glucose medium, although organic compounds generally impede its growth. In studies involving *Thiobacillus ferrooxidans* strains, carbon dioxide, the primary carbon source, can occasionally be substituted with formic acid. Yet, it's important to maintain low formate concentrations when utilizing chemostats. Notably, one strain, ATCC 21834, exhibited enhanced performance when selectively consuming formic acid [4].

Thiobacillus ferrooxidans possesses five distinctive attributes. Initially, it exhibits chemolithotrophy, where, in the presence of atmospheric oxygen and carbon dioxide, it harnesses energy and sustains its cellular growth through the utilization of reduced inorganic sulfur and Fe^{2+}. This mechanism can be represented as follows:

$$4FeSO_4 + 2H_2SO_4 + O_2 \rightarrow 2Fe_2(SO_4)_3 + 2H_2O \quad (1)$$

Another characteristic feature is that it is autotrophic. *Thiobacillus ferrooxidans* use CO_2 as a cellular carbon source. Nutrients such as N and P and trace elements such as K, Mg, Na, Ca, and CO are the elements needed for cell development and synthesis. *Thiobacillus ferrooxidans* is an aerobic bacterium. It uses oxygen as an electron acceptor. Since they live at temperatures between 20-40ºC, they have a mesophilic structure. It also has an acidophilic nature. They live at pH between 1-4.5. But the optimum pH is between 1.5-2.5. They cannot live below pH 1.0 and above 4.5 [5].

Thiobacillus ferrooxidans is ubiquitous. It can be easily isolated from soil samples collected around ore deposits or from acid drainage sites, often made for coal tailings or mine heap [6]. *Thiobacillu*s are colorless microorganisms. It enables these microorganisms to metabolize metal ions such as iron oxidase, Fe^{2+}:

$$Fe^{2+} + 1/2\ O_2 + 2H^+ \rightarrow Fe^{3+} + H_2O \quad (2)$$

Apart from its autotrophic nature, this bacterium possesses the capability to capture atmospheric nitrogen. Nitrogen fixation genes were identified in all 15 examined species of Thiobacillus ferrooxidans, indicating a probable presence of diazotrophy as a common trait among these bacteria [4]. However, the existing information regarding Thiobacillus ferrooxidans' nitrogen needs is contradictory. While one strain of this bacterium has been observed to sense free atmospheric nitrogen, other research has consistently demonstrated that ammonium salts are necessary for its growth. On the other hand, it is obvious that the pH of the environment being too acidic will cause the ammonia in the air to dissolve in the environment, and this may be due to the fact that almost every strain can grow in nitrogen-free media for at least a few generations. Tsuchiya showed that when a strain of *Thiobacillus ferrooxidans* produced nitrogen-fixing, acidophilic, heterotrophic, Beijerinckia lacticogenes together, the nitrogen requirement of *Thiobacillus ferrooxidans* could be met by Beijerinckia lacticogenes symbiotically with nitrogen in the air [5].

Energy is generated through the process of oxidizing iron or iron ions, as well as converting reduced sulfur compounds into sulfuric acid. These bacteria thrive in acidic conditions, with an optimal pH range of 1.5-2.5. They flourish best in aerobic environments, utilizing oxygen as an electron acceptor. In cases where oxygen is absent and either formate or reduced sulfur compounds act as electron donors, iron ions serve as electron acceptors. Thiobacillus ferrooxidans has the capacity to oxidize various other compounds apart from iron and sulfur. Within the species, there are three strains, including strain ATCC 23270, which are cultivated in a basic salt medium, in the presence of both CO_2 and O_2, and under conditions where H_2 is the sole energy source. Additionally, scientific literature highlights instances where Thiobacillus ferrooxidans grows by directly oxidizing UO_2 in an iron medium, a process facilitated by a molybdenum oxidation enzyme.

A detailed representation of rusticyanin in *Thiobacillus ferrooxidans* is given in Figure 1. As can be seen in the color scale in the figure, the parts of the strip shown in blue are the amino ends of the proteins and the 5' ends of the nucleic acids. The carboxy ends of proteins and the 3' ends of nucleic acids are red. The extremes between these two values are colored according to their degrees. The round structure in the figure shows the copper in the rusticyanin. In the study Barrett et al., they modeled the crystal structure of rusticyanin and blue copper in the structure of *Thiobacillus ferrooxidans*, which has a high redox potential. As a result of their quantum chemical modeling and calculations, they found the redox potential of Cu(II) to be 400 mV. This value is higher than the redox potential of rusticyanin [7].

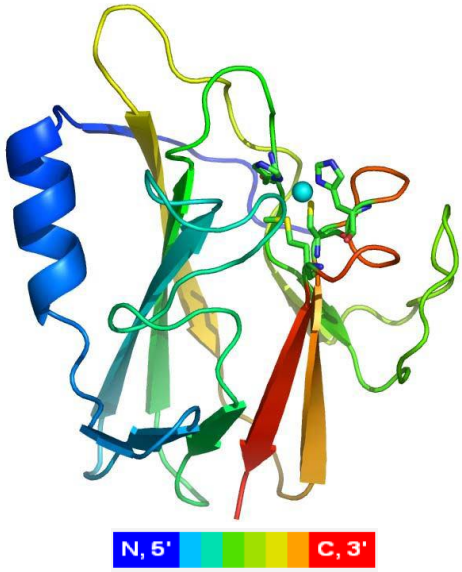

Figure 1. Structure of rusticyanin in Thiobacillus ferrooxidans *(Thiobacillus ferrooxidans*'taki rustisiyanin yapısı) [8]

The majority of *Thiobacillus ferrooxidans* isolates exhibit minimal nutritional requirements. Adequate aeration is enough to facilitate the growth of iron pyrite in acidic water. Pyrite serves as an energy source and supplies crucial trace elements, while air contributes carbon, nitrogen, and oxygen. The acidic solution forms the growth medium. The biological structure modeling of *rusticyanin* in *Thiobacillus ferrooxidans* is depicted in Figure 1. In the figure, atoms are shown in the form of sticks and in color. Carbon atoms are shown in gray, oxygen atoms in red, nitrogen atoms in blue, and sulfur atoms in yellow.

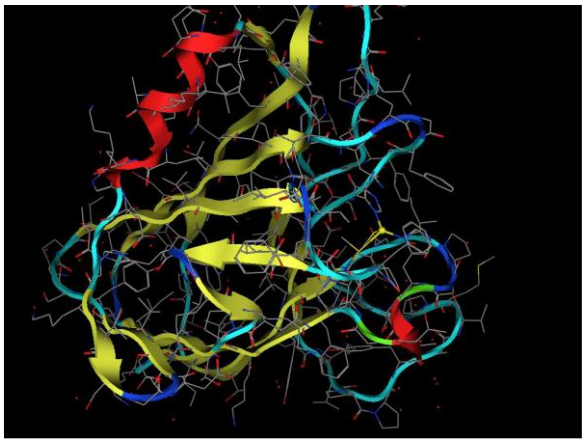

Figure 2. Biological structure modeling of rusticyanin in Thiobacillus ferrooxidans (Thiobacillus ferrooxidans'taki rustisiyanin biyolojik yapı modellemesi) [7]

In addition to its distinctive physiological traits, *Thiobacillus ferrooxidans* boasts other attributes that make it well-suited for application in bio-mining. Notably, it exhibits inherent resilience to elevated concentrations of metallic and other ions. For instance, research indicates its ability to thrive in environments rich in Zn^{2+} (120 g/l), Ni^{2+} (72 g/l), Co^{2+} (30 g/l), Cu^{2+} (55 g/l), U308 (12 g/l), and Fe^{2+} (160 g/l). Another remarkable feature is its exceptional adaptability when confronted with unfavorable growth conditions. Previously susceptible strains showcased the organism's capacity to adapt to environments with high levels of arsenic and copper, even below the optimal pH

value. Abergel et al. (2003) explored the electron transfer mechanism between rusticyanin and cytochrome-c in their study, as illustrated in Figure 2 [9].

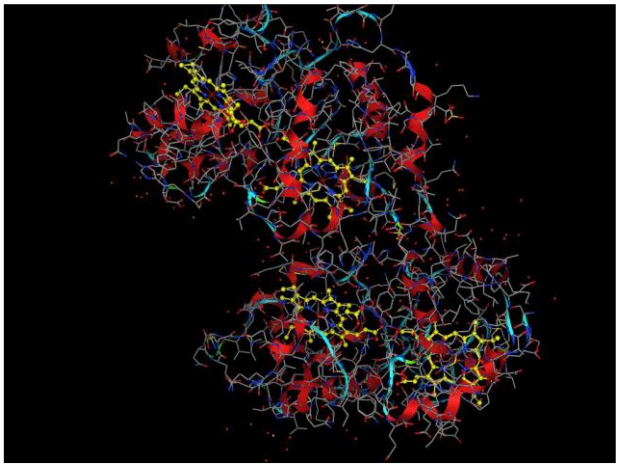

Figure 3. Thiobacillus ferrooxidans c(4)-cytochrome structure (a) (Thiobacillus ferrooxidans c(4)-sitokrom yapısı)

The adaptation mechanism is probably by the occurrence of advantageous mutations under selected conditions. Schrader and Holmes (1988) suggested that there may be other mechanisms that contribute to the rapid adaptation of bacteria in harsh environments [10]. The c(4)-cytochrome structure of *Thiobacillus ferrooxidans* is given in Figure 3 and Figure 4. There is an iron atom (dark red) in the center of this structure called the porphyrin ring. Nitrogen atoms can be seen bound to the iron atom (blue). The nitrogen atoms at the ends combined with the carbon atoms (yellow) to form the c(4)-cytochrome structure. We see that oxygen atoms (red) are attached to the carbon atoms at the ends.

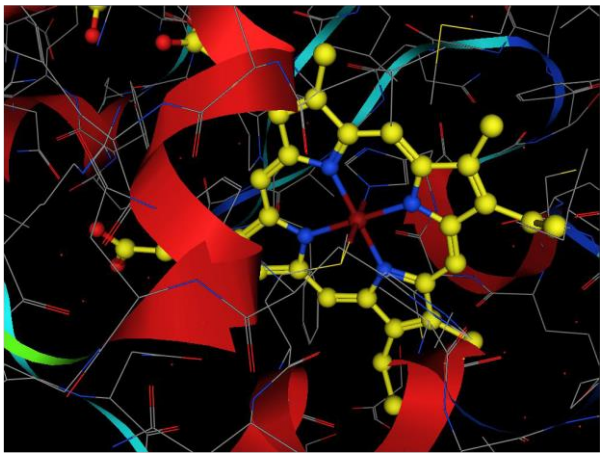

Figure 4. Thiobacillus ferrooxidans c(4)-cytochrome structure (b) (Thiobacillus ferrooxidans c(4)-sitokrom yapısı)

All *Thiobacillus ferrooxidans* isolates are genetically similar [4]. Harrison, (1984) conducted a systematic study on a large number of *Thiobacillus ferrooxidans* strains and found that the G+C content of their DNA was in the range of 55-65 mol% [11].

2.2 MEA (Membrane Electrode Assembly) Preparation (MEA Hazırlama)

The prepared catalysts were turned into a membrane electrode assembly (MEA) to test their performance in the fuel cell test system. Gas diffusion layers coated with thin film catalyst and used as anode and cathode were cut into squares of 5 cm^2 and 25 cm^2.

The selected proton exchange membrane, Nafion 115, was activated by immersing it in a 0.1 M H_2SO_4 solution for 12 hours. Subsequently, it was rinsed with deionized water and dried. The anode and cathode were positioned on opposite sides of the Nafion 115 membrane. The resulting membrane-electrode layers were then transformed into a membrane-electrode assembly (MEA) through a hot pressing process. Figure 5 illustrates the produced MEA.

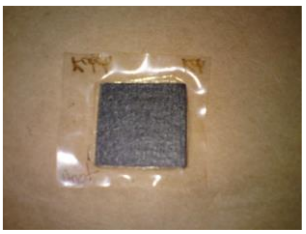

Figure 5. Membrane Electrode Assembly (Membran Elektrot Düzeneği)

A 5% nafion solution was applied as binder to the cut catalyst layers. The amount of binder used varies according to the thickness of the prepared catalyst films. This value was found as 3 µl of 5% nafion solution for the thickness used. If the binder is not optimized, the amount of binder affects the stability of the catalyst and the limited current density.

The membrane-electrode assembly (MEA) was subjected to a temperature of 117°C within the hot press. Subsequently, a pressure of 20 bars was applied for a duration of 5 minutes. Afterward, the temperature was discontinued, and the MEA was allowed to cool while maintaining the applied pressure. The hot press employed for pressing the MEA is depicted in Figure 6.

Figure 6. Hot Press Device (Sıcak Pres Cihazı)

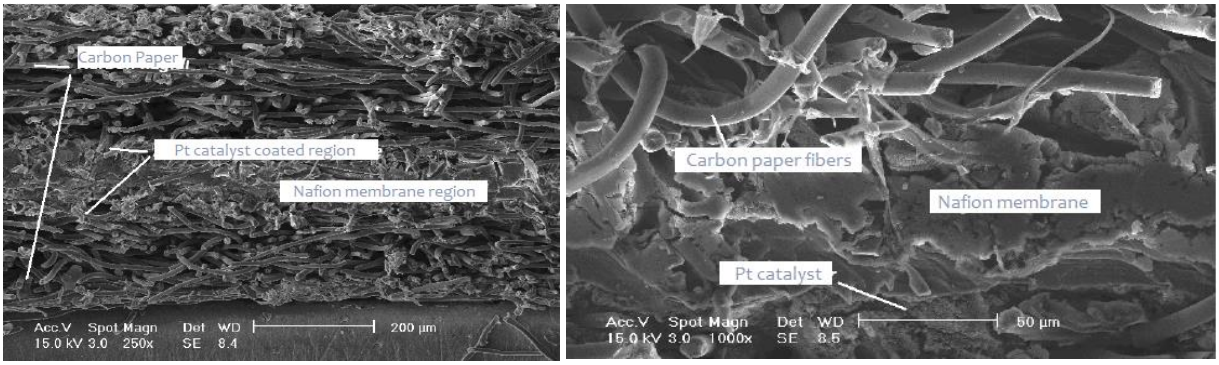

Figure 7. SEM cross-section images of the membrane electrode assembly (a), (b) (Membran elektrot düzeneğinin SEM kesit görüntüleri)

The cross-sectional images of the obtained membrane electrode assembly using SEM are given in Figure 7. The regions formed by the membrane, catalyst, and carbon paper are clearly visible in the images.

2.3. Iron Analysis (Demir Analizi)

The reaction between 5-sulfosalicylic acid (SSA) and Fe^{2+} ions is recognized to yield a red-colored compound, while the interaction with iron ions in an acidic environment results in a yellow-colored compound. Lurie (1984) introduced a relatively straightforward technique using SSA to determine Fe^{3+} and total iron concentrations [12].

This approach involves distinct steps for analyzing each form of iron. The procedure outlined by Lurie (1984) is specifically tailored for the analysis of iron in standard wastewater samples [12].

$Fe_2SO_4.7H_2O$ was used for calibration. Calibration was performed for the concentration range of 1-20 mg/L Fe^{+2}. 0.25 from stock $Fe_2SO_4.7H_2O$ solution containing 200 mg/L Fe^{+2}; 0.5; 2; 4; 6; 9 mL was taken and placed in balloon jugs. Each vessel was filled with 97 mL of distilled water.

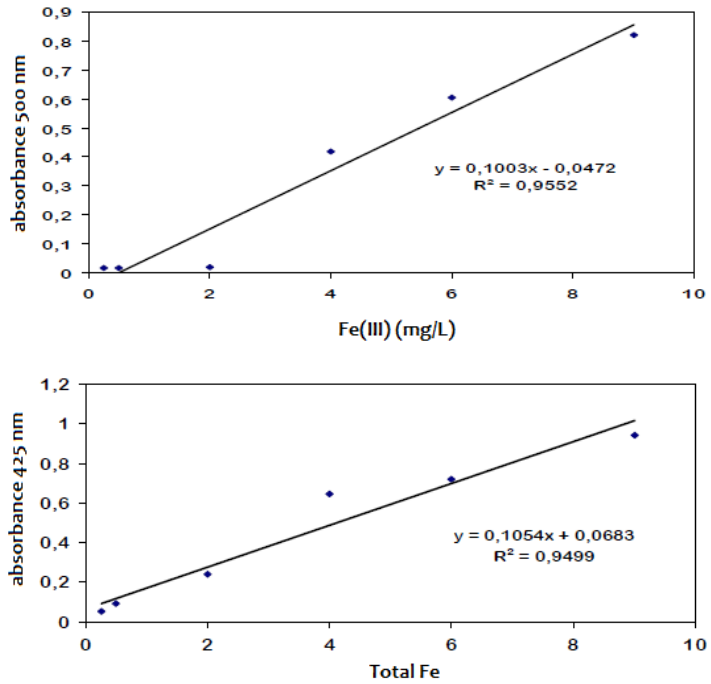

Figure 8. Fe^{+3} and Total Iron Calibration Curves (Fe^{+3} ve Toplam Demir Kalibrasyon Eğrileri)

Then, 3 mL of 10% SSA was added to these containers and mixed. It was read against the blank prepared by adding distilled water and all reagents

in a GBC brand UV-Spectrophotometer set to 500 nm.

To determine the total iron content, 3 mL of ammonia was introduced into the solutions containing added SSA, and the measurement was taken. The readings were then compared against a blank sample created by combining distilled water and all the necessary reagents. A UV-Spectrophotometer set at 425 nm was used for these measurements. The collected data points were plotted, and calibration curves were generated. The calibration curves for Fe^{+3} and total iron are visually presented in Figure 8.

2.4. Preparation of Polyurethane Foam (Poliüretan Köpük Hazırlama)

For the preparation of polyurethane foam, 83.3 g of Hypol 2002 was taken and 100 mL of distilled water was added and mixed rapidly until foam formed. It was then left to dry for 24 hours at 60ºC. The foam was divided into small pieces, kept in H_2SO_4, and after being sulfonated, it was made ready to be added to the iron oxidation.

2.5. MFC Application (MFC Uygulaması)

The aim of this study is to measure the usage performance of the redox couple Fe^{3+}/Fe^{2+} as a cathodic mediator. In addition, system performance was measured by using Pt coated with sputter technique in much lower amounts (22 μg/cm^2) as the anode and cathode catalyst material. Three main reasons for using Fe^{+3}/Fe^{+2} as a redox couple are: it can react quickly at carbon electrodes, its standard potential is high (at low pH, equal concentrations of Fe^{+3} and Fe^{+2}, +0.77V, etc.). NHE) and (+850)-(+950mV) Fe^{+2} can be biologically oxidized to Fe^{+3} by the use of oxygen as an electron acceptor.

In the system, carbon paper coated with Pt at 22μg/cm^2 charge was used as electrodes on both sides. Mixed culture anaerobic bacteria were used as anode bacteria at the anode. *Thiobacillus ferrooxidans* was used as the iron-oxidizing bacteria at the cathode. The anode and cathode are separated from each other by the ion exchanger Nafion membrane. During the experiments, Fe^{+3} reduction will take place in the MFC cathode, while Fe^{+2} oxidation will take place in the cathode reactor at the same time.

The exterior enclosure of the microbial fuel cell is constructed from plexiglass material. The fuel cell itself comprises an internal volume of 100.54 cm^3, which is evenly partitioned into two sections – the anode and the cathode. Upon inserting the membrane electrode assembly (MEA) between these anode and cathode sections, Teflon gaskets were employed for sealing, and the two segments were securely joined using bolts.

In the iron oxidation reactor of the applied design, the conversion of Fe^{+2} ions to Fe^{+3} takes place. Continuous circulation takes place in the cathode chamber. The oxidized iron ions in the reactor come to the cathode compartment of the fuel cell, where they are converted back to Fe^{+2}. This reaction takes place via electrons that pass from the anode to the cathode via the external circuit. A reactor was used for the cathode bacteria in the system. The iron oxidation reaction will take place in this chamber by forming a biofilm on the sulfonated polymer particles inside the reactor.

The electrochemical measurements were carried out using a CHI Model 800B potentiostat device. Throughout the experimentation, the pH of the system, open circuit potential, and cyclic voltammetry plots were regularly monitored. The anode compartment of the setup contained 15 mL of thoroughly mixed culture anaerobic microorganisms. To examine the impact of various nutrients on the cell's electrochemical performance, sodium acetate, glucose, and sucrose were employed as anode feed sources. A nutritional supplement was introduced into the anode compartment at a rate of 1 g/L per day. Additionally, every 4 days, 1 mL of the nutrient solution was added to the anode (4.31 g/L NH_4Cl, 5.39 g/L $CaCl_2.2H_2O$, 4.31 g/L $MgSO_4.7H_2O$, and 54 mg/L $FeCl_3$) [3].

Thiobacillus ferrooxidans was used at the cathode. The cathode's first solution was prepared with iron (II) sulfate. Afterward, feeding was carried out at regular intervals. *Thiobacillus ferrooxidans* bacteria used were isolated from Murgul Copper Enterprises drainage water. 9K medium was chosen as the medium for bacteria. The composition of the 9K medium; 3 g/L $(NH_4)SO_4$, 0.5 g/L $MgSO_4.7H_2O$, 0.5 g/L KH_2PO_4, 0.1 g/L KCl, 0.01 g/L $Ca(NO_3)_2$. Drainage water was seeded in 9K medium at a rate of 10% and its pH was adjusted to 2.

The solution prepared for activation was left to incubate in a shaker with an incubator at 200 rpm. Fe^{2+} concentration and color change were monitored daily. The samples changed from yellow to red-brown after 4 days.

Thiobacillus ferroxidans bacteria standard nutrients (0.4 g/L $(NH_4)_2SO_4$, 0.4 g/L KH_2PO_4, and 0.4 g/L $MgSO_4$, H_2SO_4 for pH 2 in 33 g/L $Fe_2SO_4.7H_2O$ medium before adding to the MFC system grown by adding [2]. The solution containing the active bacteria was added to the reactor containing polyurethane foam to form a biofilm surface. The solution was circulated continuously in the reactor by giving air to the system. Meanwhile, the conversion of Fe^{+2} ions to Fe^{+3} ions was controlled by making iron analyses. After the conversion of iron ions was completed, the solution containing Fe^{+3} ions in the reactor was transferred to the cathode compartment of MFC. The reduction reaction at the cathode was again controlled by the iron analysis. At this time, the air was supplied to the system continuously. Again, every four days, 1 mL of nutrient was added to the cathode compartment.

3. ANALYSIS (ANALİZ)

As seen in the MFC diagram presented in Figure 9.; While acetate decomposes into CO_2 and H^+ in the anode compartment, the resulting electrons are transferred to the cathode with the help of an external circuit. At the cathode, iron ions that are oxidized in the reactor from Fe^{+2} to Fe^{+3} are reduced back to Fe^{+2} by taking electrons from the anode on the cathode electrode. In the MFC system, where *Thiobacillus ferrooxidans* is used as the cathode bacteria, the anode and cathode reactions take place as follows:

Anode:

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^- \text{ (biological)} \quad (3)$$

Cathode:

$$8Fe^{3+} + 8e^- \rightarrow 8Fe^{2+} \text{ (chemical)} \quad (4)$$

Reactor:

$$8Fe^{2+} + 8H^+ + 2O_2 \rightarrow 8Fe^{3+} + 4H_2O \text{ (biological)} \quad (5)$$

Net reaction:

$$CH_3COO^- + 2O_2 \rightarrow 2HCO_3^- + H^+ \quad (6)$$

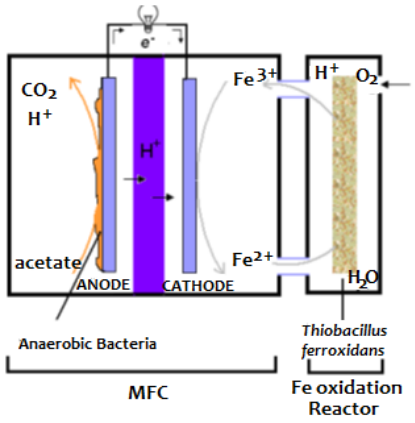

Figure 9. Schematic representation of the MFC system experimental setup (MFC sistemi deney kurulumunun şematik gösterimi)

First, biofilm formation of *Thiobacillus ferrooxidans* bacteria was performed on sulfonated polyurethane foam pieces in the cathode reactor. For this, bacterial culture and standard nutrients were added into 500 mL of purified water containing 6g/L $Fe_2SO_4.7H_2O$. A mechanism was set up to circulate the obtained solution in the cathode reactor. Circulation was provided by a peristaltic pump. Meanwhile, the air was sent to the system by using a T hose at the reactor inlet. This setup was run for one week.

In Figure 10. light microscope images of *Thiobacillus ferrooxidans* bacteria used in the cathode compartment of MFC are presented. The bacteria sample was taken from the biofilm layers formed in the reactor and on the cathode electrode.

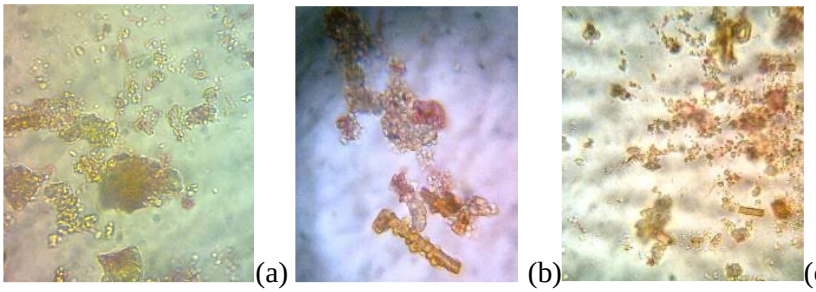

Figure 10. Microscope images of the cathode bacteria Thiobacillus ferrooxidans (a, b, c)

(Katot bakterisi Thiobacillus ferrooxidans'ın mikroskop görüntüleri)

In these images, the capsule structure of the bacterium is clearly seen. In the photo coded (a), there are images of the samples taken from the reactor. In addition, in the photo coded (b) and (c), there are capsules attached to the carbon paper fiber used as the electrode material. The fibrous structure of the carbon paper also increased the surface area where the bacterial biofilm was formed.

SEM images of mixed culture anaerobic bacteria used in the anode chamber of MFC, taken from different distances, are presented in Figure 11.

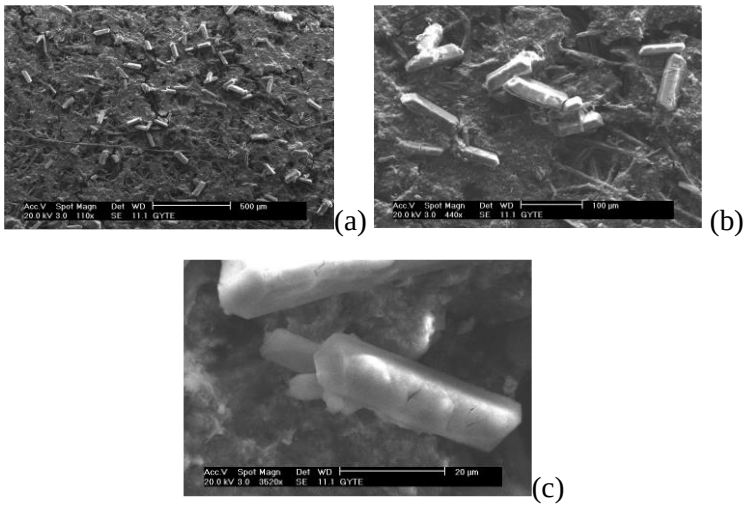

Figure 11. SEM images of mixed culture anaerobic bacteria used as anode bacteria (a (110x), b (440x), c (3520x)) (Anot bakterisi olarak kullanılan karışık kültürlü anaerobik bakterilerin SEM görüntüleri)

The bacteria sample was taken from the biofilm formed on the anode electrode. When we look at the images, it is seen that the microorganism is homogeneously distributed on the anode electrode. The capsule size of the microorganisms is 30 µm on average. After the biofilm formation was achieved, the reactor was connected to the cathode part of the fuel cell, and circulation between the cathode-reactor was ensured. Air continued to be supplied to the system. Meanwhile, biofilm formation has already taken place in the anode chamber under anaerobic conditions.

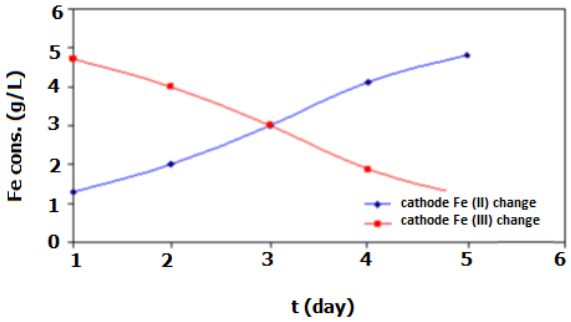

Figure 12. Fe^{+3} and Fe^{+2} concentration (g/L) change over time in the cathode chamber

(Katot bölmesinde zamanla Fe+3 ve Fe+2 konsantrasyonu (g/L) değişimi)

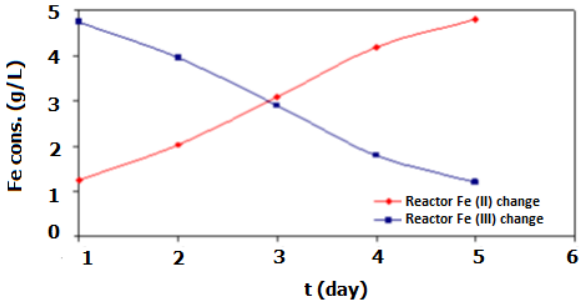

Figure 13. Variation of Fe^{+2} and Fe^{+3} concentration (g/L) in the reactor over time (Reaktördeki Fe+2 ve Fe+3 konsantrasyonunun (g/L) zaman içindeki değişimi)

The graphs are given in Figure 12. and Figure 13. show the variation of Fe^{+2} and Fe^{+3} concentrations in the reactor and cathode. As can be seen in Figure 6.7, Fe^{+2} concentration increased while Fe^{+3} concentration decreased at the cathode. Our initial Fe^{+3} concentration decreased from 4.8 g/L to 1.2 g/L within 6 days. Figure 6.7 Fe^{+3} and Fe^{+2} concentration (g/L) change over time in the cathode chamber As can be seen from the graph, Fe^{+2}, and Fe^{+3} concentrations reached the same values as a result of the reduction reaction within 86 hours. This value is 3 g/L. At the end of the 5th day, the reduction process was completely realized and the Fe^{+3} concentration was reduced to a minimum. Fe^{+2} concentration has taken its maximum value and this value is 4 g/L.

Fe^{+2} and Fe^{+3} concentration distribution in the cathode and reactor compartment is seen similarly. However, in the reactor part of the system, the oxidation process takes place. As seen in Figure 13., while the Fe^{+3} concentration increased with the oxidation process, the Fe^{+2} concentration decreased over time. The results obtained according to the calibration curve prepared give the oxidation efficiency of *Thiobacillus ferrooxidans* bacteria.

Change of Fe^{+2} and Fe^{+3} concentration (g/L) in the reactor with respect to time Nemati and Webb, (1999) stated in a study on biological and chemical oxidation of iron that the oxidation efficiency was maximum at a concentration of 20 kg/m^3[13].

The open circuit potential increases in direct proportion to the activity of bacteria. Therefore, before starting the experiments, the open circuit potential was expected to reach a certain value within a certain period of time, according to pH. These measurements are shown in the graphs below.

During the electrochemical measurements made in the fuel cell, different pH values were investigated. In Figure 14., the time graph of the open circuit potentials measured during the pH change experiments in the system is given. For the cathode, open circuit potential values and CV graphs were taken according to the pH values of 1.5, 2, and 2.5, and the results were compared. This value, which was 0.14 V at the beginning, increased to 0.8 V.

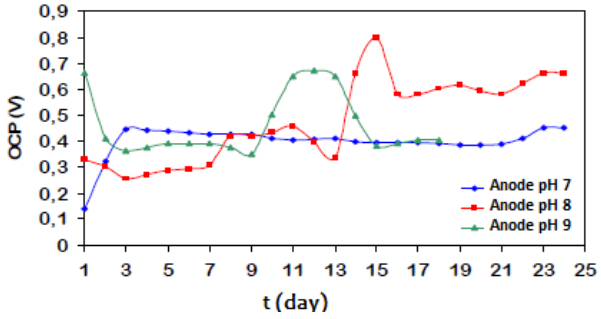

Figure 14. Cell open-circuit potential curve with respect to time (Zamana göre hücre açık devre potansiyeli eğrisi)

As seen in the system open circuit potentials at different pH values for the anode and cathode presented in Table 1., the system reached the highest open circuit potential (0.8 V) at anode pH 8 and cathode pH 2. For this reason, the next performance measurements were made under these conditions. The graphical representation of the table values is presented in Figure 15.

Table 1. System open circuit potentials at different pH values for anode and cathode (Anot ve katot için farklı pH değerlerinde sistem açık devre potansiyelleri)

	Cathode pH	OCP(V)
Anode pH 7	1.5	0.444
	2	0.409
	2.5	0.451
Anode pH 8	1.5	0.256
	2	0.800
	2.5	0.615
Anode pH 9	1.5	0.664
	2	0.391
	2.5	0.652

In addition to this, three different nutrients were used at the anode in the system. These nutrients are sucrose, glucose, and acetate. In Figure 15., open circuit potential measurement values are given by using sucrose, glucose and acetate as nutrients at the anode, under conditions of cathode pH of 1.5.

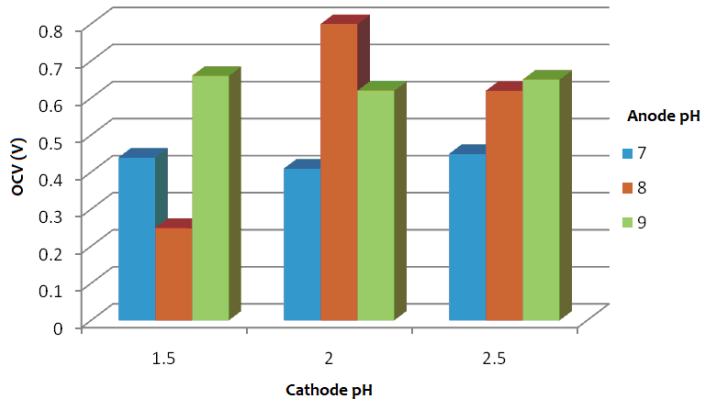

Figure 15. System open circuit potentials at different pH values for anode and cathode (Anot ve katot için farklı pH değerlerinde sistem açık devre potansiyelleri)

As seen in Figure 16, when sucrose was used as the anode nutrient, the open circuit potential, which was initially 0.14 V, increased to 0.45 V at the end of the 3rd day. At the end of the 8th day, it was fixed at 0.42 V. In the experiments using glucose, the open circuit potential, which started at 0.42 V, remained the same. In the experiments using acetate, the initial open circuit potential, which was 0.4 V, started to increase on the 6th day and was fixed at 0.45 V at the end of the 8th day. When sucrose is used as the anode nutrient, the reactions are thought to occur as follows:

Anode:

$C_{12}H_{22}O_{11} + 13H_2O \rightarrow 12CO_2 + 48H^+ + 48e^-$ (biological) (7)

Cathode:

$48Fe^{3+} + 48e^- \rightarrow 48Fe^{2+}$ (chemical) (8)

Reactor:

$48Fe^{2+} + 48H^+ + 12O_2 \rightarrow 48Fe^{3+} + 24H_2O$ (biological) (9)

Net reaction:

$C_{12}H_{22}O_{11} + 12O_2 \rightarrow 12CO_2 + 11H_2O$ (10)

When glucose is used as the anode nutrient, the reactions are:

Anode:

$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24e^- + 24\ H^+$ (biological) (11)

Cathode:

$24Fe^{3+} + 24e^- \rightarrow 24Fe^{2+}$ (chemical) (12)

Reactor:

$24Fe^{2+} + 24H^+ + 6O_2 \rightarrow 24Fe^{3+} + 12H_2O$ (biological) (13)

Net reaction:

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ (14)

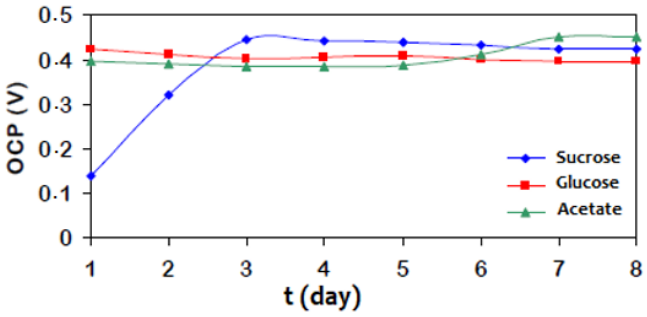

Figure 16. Change in open circuit potential in the use of different nutrients for anode bacteria when the cathode pH value is 1.5 (Katot pH değeri 1,5 olduğunda anot bakterileri için farklı besin maddelerinin kullanımında açık devre potansiyelindeki değişim)

In conditions where the cathode pH value is 2, the open circuit potential variation for different nutrients is given in Figure 16. When sucrose was used as a nutrient, the open circuit potential, which had an initial value of 0.32 V, first decreased and became 0.3 V. Then it increased and reached 0.42 V at the end of the 8th day. When glucose was used as a nutrient, the initial value of 0.42 V decreased to 0.35 V at the end of the 5th day.

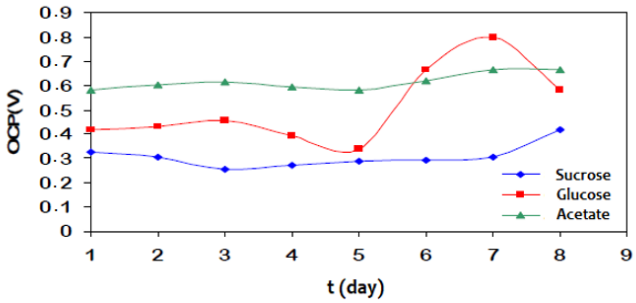

Figure 17. Open circuit potential change in the use of different nutrients for the anode bacteria when the cathode pH value is 2 (Katot pH değeri 2 olduğunda anot bakterileri için farklı besin maddelerinin kullanımında açık devre potansiyeli değişimi)

However, although it increased to 0.8 V at the end of the 7th day, it decreased again and reached 0.6 V at the end of the 8th day. In experiments using acetate, the open circuit potential, which was 0.59 V, reached 0.6 V at the end of the 5th day and 0.7 V at the end of the 8th day. When glucose was used as a nutrient, the system reached its maximum open circuit potential, but could not stay at this value for long. The curve with the most stable and highest value was obtained during the use of acetate.

In Figure 17., open circuit potential measurements made using different nutrients when the cathode pH value is 2.5 are given. The initial open circuit potential, which was 0.65 V under the conditions of using acetate as the anode nutrient, decreased to 0.4 V at the end of the 3rd day. This value was fixed at 0.42 V at the end of the 8th day.

In the use of glucose; The initial open circuit potential, which was 0.4 V, decreased to 0.38 V at the end of the 3rd day, then increased to 0.67 V at the end of the 5th day and stabilized at this value. When sucrose was used as the anode nutrient, the initial open circuit potential value, which was 0.67

V, decreased to 0.43 V until the 2nd day and stabilized at 0.4 V at the end of the 6th day.

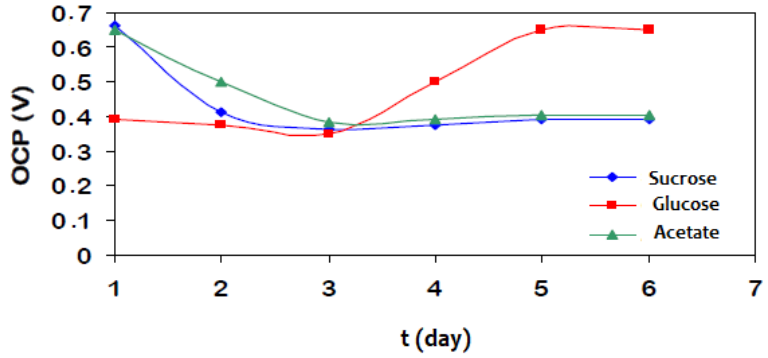

Figure 18. Change of open circuit potential in the use of different nutrients for anode bacteria when the cathode pH value is 2.5 (Katot pH değeri 2,5 olduğunda anot bakterileri için farklı besin maddelerinin kullanımında açık devre potansiyelinin değişimi)

The cyclic voltammetry graph in Figure 18. gives the electrochemical activity of the MFC in the initial state. As can be understood from here, no reaction took place in the system in the first case. The current density value observed during these experiments reached up to 1.3 mA/cm^2. In the graphs obtained after one week, the peaks of the reactions can be clearly seen.

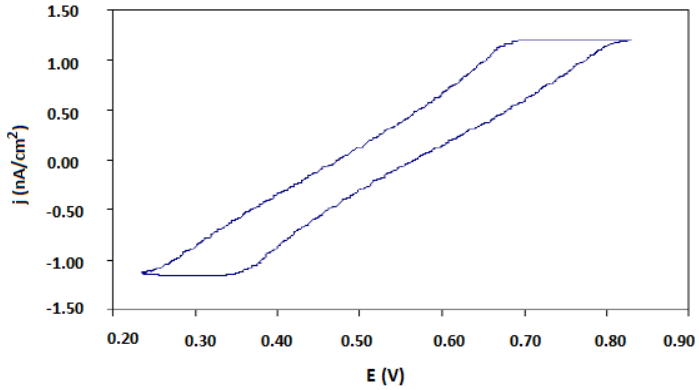

Figure 19. Initial state cyclic voltammetry graph of MFC (MFC'nin başlangıç durumu döngüsel voltametri grafiği)

When the cyclic voltammetry graphs in Figures 20. and 21. are examined, it is possible to have information about the reactions occurring in the system. Both figures show a peak of 0.35 V. This peak indicates the potential occurrence value of the reactions at the anode.

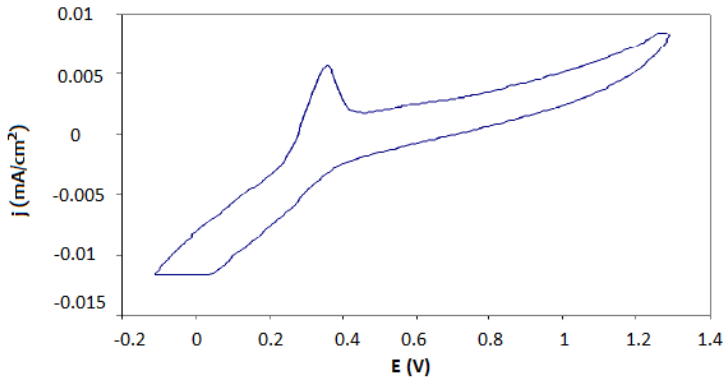

Figure 20. Cyclic voltammetry graph obtained in the system under conditions of Anode: pH 8, nutrient acetate, Cathode: pH 2 (Sistemde Anot: pH 8, besin asetat, Katot: pH 2 koşullarında elde edilen döngüsel voltametri grafiği)

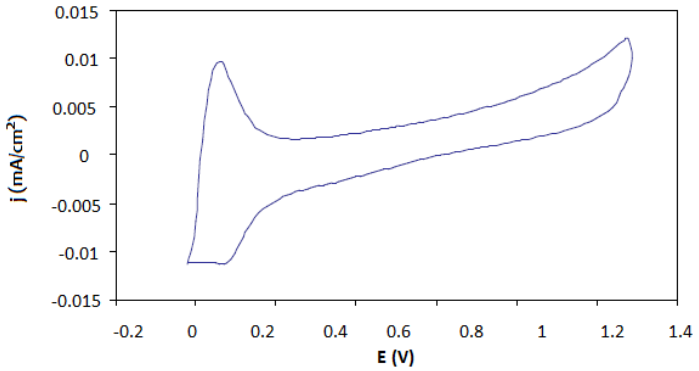

Figure 21. Anode: pH 8, nutrient acetate, Cathode: pH 2, the cyclic voltammetry graph obtained in the system (after 30 days) (Anot: pH 8, besin asetatı, Katot: pH 2, sistemde elde edilen döngüsel voltametri grafiği (30 gün sonra))

In Figure 22., two different cyclic voltammetry graphs using acetate and glucose as anode nutrients are combined. Here, an adsorption peak occurred at the same potential value (0.4 V) in both nutrients.

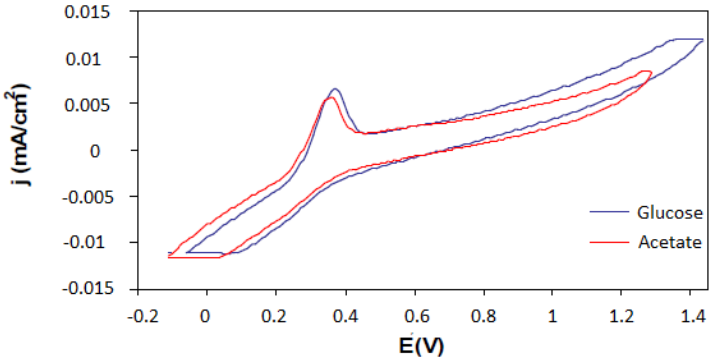

Figure 22. Cyclic voltammetry curves obtained in the system under conditions of Anode: pH 8, nutrient acetate and glucose, Cathode: pH 2 (Sistemde Anot: pH 8, besin asetat ve glikoz, Katot: pH 2 koşulları altında elde edilen döngüsel voltametri eğrileri)

According to the cyclic voltammetry graph in Figure 22., the adsorption peaks are between 0.2 and 0.5 V. The reduction peaks range from 0.2 to 0.7 V. It can be seen from the graph that the double layer region is in the range of 0.05 to 0.3 V. This graph is in agreement with the standard fuel cell cyclic voltammetry graph using a Pt catalyst.

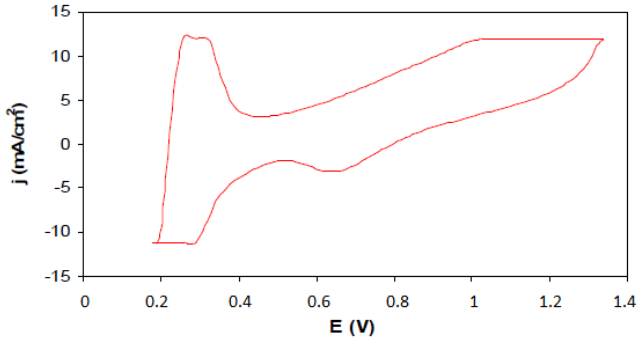

Figure 23. Cyclic voltammetry plot of MFC at system standard conditions (after 40 days) (Sistem standart koşullarında MFC'nin döngüsel voltametri grafiği (40 gün sonra))

If we compare Figures 21. and 23., it is seen that the current density obtained under the system standard conditions is considerably higher than the initial current density value. The current density value of 15 mA/cm^2 was reached under the conditions of cell anode pH 8 and nutrient acetate.

The graph in Figure 21 was obtained at the end of the 30th day. The graph in Figure 23. is the cyclic voltammetry graph obtained at the end of the 40th day. These experiments, which were carried out with an interval of 10 days, showed that the system reached high performances at the end of the 40th day. No peak is seen in the initial cyclic voltammetry graph given in Figure 19. On the other hand, a peak with a potential value of 0.7 V is seen in the graph in Figure 23. This is the potential value at which the iron oxidation reaction takes place.

In Figure 24., polarization curves obtained at different cathode pH values are given in the case where the anode pH value is 8 and acetate is used as the nutrient in the system. In this graph, the activation polarization region affected by activation losses, the ohmic polarization region where resistance losses occur, and the concentration polarization region affected by mass transfer losses are clearly seen in a typical fuel cell polarization curve.

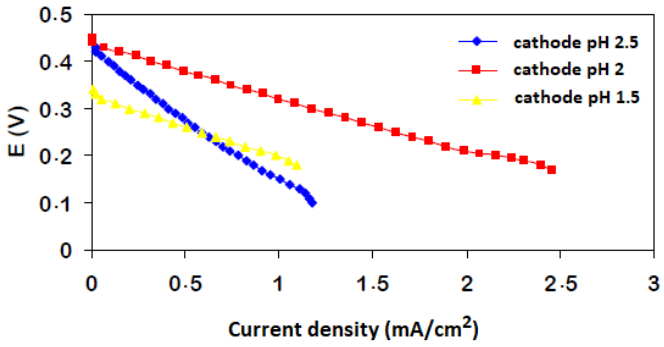

Figure 24. Anode in the system: pH 8, Tafel curves at different cathode pH values in conditions where the nutrient is acetate (Sistemdeki anot: pH 8, Besin maddesinin asetat olduğu koşullarda farklı katot pH değerlerinde Tafel eğrileri)

According to this graph, the cathode pH value is 1.5 while the initial potential is 0.34 V. This value is 0.46 V at cathode pH 2 and 0.44 V at cathode pH 2.5. Activation losses are less at cathode pH 2 compared to other pH values. Again, in the case of cathode pH 2, the system reached a higher current density value than in other cases. This value has reached 2.5mA/cm^2.

In Figure 24, the power-current density curves obtained for the same conditions are given. Again, the highest power value of 0.45 mW was reached when the cathode pH was 2. At other pHs, the obtained powers remained small as 0.15 and 0.2 mW. When these results are combined with the open circuit potential measurement results, the most suitable conditions for the fuel cell system are at the anode:

acetate fed, pH 8, and cathode: pH 2 was determined.

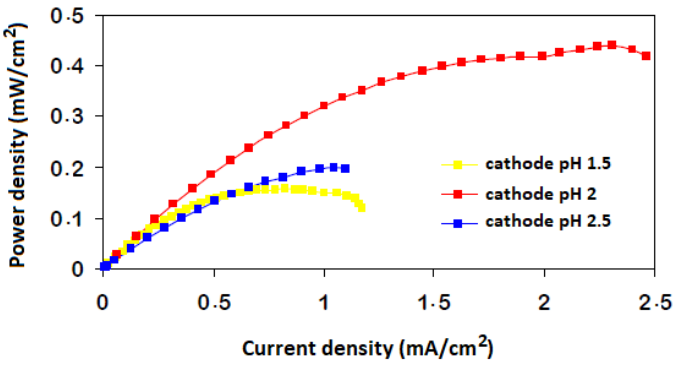

Figure 25. Current density-power curves obtained at different cathode pH values under conditions where the anode: is pH 8, and the nutrient medium is acetate (Anodun pH 8 olduğu ve besin ortamının asetat olduğu koşullar altında farklı katot pH değerlerinde elde edilen akım yoğunluğu-güç eğrileri)

The graph given in Figure 25 shows the differential pulse voltammetry (DPV) result of the MFC measured under the conditions of anode: pH 8, nutrient acetate.

$$Fe^{3+} + e^- \rightarrow Fe^{2+}\ E=+0.77V \quad (15)$$

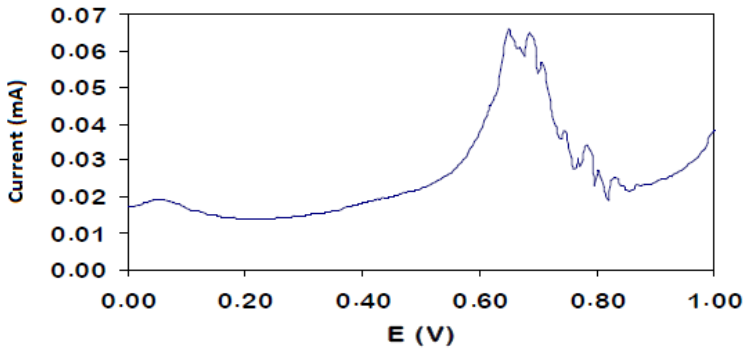

Figure 26. Anode in the system: pH 8, differential pulse voltammetry (DPV) graph obtained under nutrient acetate conditions (Sistemdeki anot: pH 8, besin asetat koşullarında elde edilen diferansiyel pulse voltammetri (DPV) grafiği)

In Figure 26., a peak with a potential value of +0.77V has occurred. This potential is the reduction potential of Fe^{+3} to Fe^{+2} realized at the cathode. This reduction reaction takes place on the cathode surface.

4. CONCLUSION (SONUÇ)

MFC systems' performance is affected by many biological and electrochemical factors. The important factors are pH, substrate, and the electrode material used. In this study performance measurements of the MFC system using *Thiobacillus ferrooxidans* as the cathode bacteria were carried out. For the MFC system using *Thiobacillus ferrooxidans* in cathode and mixed culture anaerobic bacteria in anode, the system open circuit potential values were measured at different anode and cathode pH values, and when different anode nutrient was used.

Conditions where the system reaches 0.8 V open circuit potential; the anode pH value was determined as 8, cathode pH value was determined as 2. In cases where sucrose, glucose, and acetate are used as anode nutrients, acetate with an open circuit potential of 0.7 V was seen as the most suitable nutrient.

According to cyclic voltammetry and differential pulse voltammetry measurements, the anode: pH 8 in the system is optimized for Tafel curves and power density values at different cathode pH values under conditions where the nutrient is acetate. According to the results, the system's current

density value is 2.5 mA/cm^2 and the highest power value is 0.45 mW.

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DECLARATION OF ETHICAL STANDARDS (ETİK STANDARTLARIN BEYANI)

The author of this article declares that the materials and methods they use in their work do not require ethical committee approval and/or legal-specific permission.

Bu makalenin yazarı çalışmalarında kullandıkları materyal ve yöntemlerin etik kurul izni ve/veya yasal-özel bir izin gerektirmediğini beyan ederler.

AUTHORS' CONTRIBUTIONS (YAZARLARIN KATKILARI)

***Işılay BİLGİÇ*:** She conducted the research, analyzed the results and performed the writing process.

Araştırmayı yapmış, sonuçlarını analiz etmiş ve maklenin yazım işlemini gerçekleştirmiştir.

CONFLICT OF INTEREST (ÇIKAR ÇATIŞMASI)

There is no conflict of interest in this study.

Bu çalışmada herhangi bir çıkar çatışması yoktur.

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