



**The Effect of Electrode Type on Energy Efficiency in Microbial Fuel Cells Using
vgb⁺ Recombinant *Enterobacter aerogenes***

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Abstract

In this study, microbial fuel cells were designed using *E. aerogenes*, an important proton producing bacterium, to determine energy efficiency. In the design of these fuel cells, nafion membrane is preferred as a proton permeable membrane. Potassium ferricyanide was used in the cathode section when the anode partition mediator was preferred as methylene blue. Within the scope of the study, the efficiency of the electrode route on the optimization and energy efficiency of fuel pellets prepared using *E. aerogenes* was determined. A comparative study was carried out using graphite, composite and copper electrodes as the electrode type. In the experiment with copper electrode, the highest voltage value was read as 0.23 V, 0.38 V on the composite electrode and 0.52 V on the carbon electrode. It is determined that the electrode giving the highest voltage is the carbon electrode. Furthermore, optimization of designed microbial fuel cell's nutrient, pH and microorganism incubation time has been realized.

Keywords: Fuel cell, Microbial fuel cell, *Enterobacter aerogenes*, Vitreoscilla hemoglobin.

***vgb*⁺ Rekombinant *Enterobacter aerogenes* Kullanılan Mikrobiyal Yakıt Pillerinde Enerji Verimliliği Üzerine Elektrot Türünün Etkisi**

Özet

Bu çalışma kapsamında önemli bir proton üreten bakteri türü olan *E. aerogenes* kullanılarak mikrobiyal yakıt hücreleri tasarımı gerçekleştirilerek enerji verimlilikleri belirlenmiştir. Bu yakıt hücrelerinin tasarımında proton geçirgen membrane olarak nasyon membran tercih edilmiştir. Anot bölgesi medyatörü metilen mavisi olarak tercih edilirken katot bölgesinde Potasyum ferrisiyanid kullanılmıştır. Çalışma kapsamında *E. aerogenes* kullanılarak hazırlanan yakıt pillerinin optimizasyonu ve enerji verimliliği üzerine elektrot türünün etkinliği belirlenmiştir. Elektrot türü olarak grafit, alaşım ve bakır elektrotlar kullanılarak kıyaslamalı bir çalışma gerçekleştirilmiştir. bakır elektrotla yapılan denemede en yüksek voltaj değeri 0.23 V, kompozit elektrotta 0.38 V, karbon elektrotta ise 0.52 V olarak okunmuştur. En yüksek voltaj miktarını veren elektrotun karbon elektrot olduğu saptanmıştır. Ayrıca hazırlanan mikrobiyal yakıt pilinin, besiyeri, pH ve mikroorganizma inkübasyon süre optimizasyonları gerçekleştirilmiştir.

Anahtar Kelimeler: Yakıt pili, Mikrobiyal yakıt pili, *Enterobacter aerogenes*, vitreoscilla hemoglobin.

1. Introduction

Due to their high energy efficiency and modular structure, fuel cells are important in the production of electrical energy. In addition, increasing energy demand and decreasing fossil fuels are increasing the need for fuel cells. Fuel cells have an important potential for the environmental aspects of reducing pollution caused by fossil fuels, and controlling CO₂ emissions [1]. Fuel cells reduce the emission of sulfur and nitrous oxide to values close to zero and decreases carbon dioxide emissions due to their high operating efficiency and the electrochemical transformation reaction formed much more controlled than the combustion reaction. This is a very important factor in our atmosphere where the greenhouse effect is getting more and more important [2].

Furthermore, the advantage of using fossil fuels for all types of fuel, including coal, and the possibility of using first-order hydrocarbon fuels with fuel cells increases the importance of such batteries. However, considering the rapid consumption of fossil fuels and not being renewable instead of such fuels, it is necessary to create fuel cells where wastes will be evaluated. Microbial Fuel Cells (MFC) are an important alternative in this context [3-4].

In its simplest form, MFC can be defined as the conversion of chemical energy in organic wastes into electrical energy by microorganisms [5-8]. Electrical energy can be produced in microbial fuel cells and waste treatment can be done at the same time [9-11]. In general, MFCs are a system consisting of two chambers called cathodes and anodes and separated from each other by proton exchanger membrane (selective permeable membrane). Microorganisms growing on the electrode surface in the anode cell oxidize organic matter and produce electrons and protons (hydrogen). The electrons produced in the anode chamber are transferred to the cathode chamber by a circuit from the electrode surface. Hydrogen passes through the proton exchanger membrane by diffusion to reach the cathode chamber, where it combines with oxygen (another electron acceptor can be used) to turn into water. Thanks to the presence of O_2 , a strong electron acceptor, and H^+ ions that constitutes a positive electric charge, electrons in the anode are drawn towards the cathode and generate an electric current on this line [3-12, 15].

The anode compartment is completely anaerobic and the cathode compartment is aerobic in MFC. Anodic section in the structure of MFC; is the development section that provides all necessary conditions for the growth of certain microorganisms. Anodic section of MFC consists of an electrode, microorganism, mediator (depends on the condition of being MFC with mediators and without mediators) [3] and growth medium (glucose etc.). With bacterial growth in this compartment; protons and electrons needed are produced during metabolic reactions. In the anode, usually used graphite electrodes. Some of the anode electrodes covered with elements Fe ions, Mn ions, etc. increasing electrical efficiency and reducing redox potential [16].

There are also various mediators to enhance the electron transfer efficiency. Increasing the energy efficiency of microbial fuel cells due to population growth and the increase in energy demand has become another important issue. For this reason, such

tissues the design of proton permeable membranes in MFC, the development of electrode materials, the type of microorganisms used or increasing the efficiency of the media are continuously studied.

In this study, it is aimed to improve with the type electrode used the energy efficiency of microbial fuel cells formed by *E. aerogenes* microorganism. For this purpose, three different types of electrodes were used to design the MFC with the highest energy efficiency. Within the scope of the study, the reason why *E. aerogenes* microorganism is preferred; capable of produce fermentative hydrogen in temperate environment conditions and therefore to offer the ease of production of hydrogen which will be carried by proton permeable membranes.

The first researcher to discover that *E. aerogenes* is a hydrogen producer is Tanisho, which isolates it from the soil [17]. In addition to being a species capable of producing hydrogen, as a facultative anaerobe, *E. aerogenes* has the advantage of growing in an oxygenated environment compared to those with mandatory anaerobes. Hydrogen has the ability to grow in an intense atmospheric environment and shows a high rate of hydrogen production through the nicotinamide adenine dinucleotide (NADH) pathway (10 mol H₂ / mol glucose). In our study, firstly, the effect of on the energy formation activity of the electrode type which *E. aerogenes* strains can hold microorganisms and effective eletron transfer in MFC can be determined.

2. Materials and Methods

2.1. Used Chemicals and Devices

In this study, chemicals used in the preparation of media; NaCl, agar, peptone, yeast extract, tripton, KH₂PO₄, K₂HPO₄, HCl, NaOH were of analytical purity and were obtained from Sigma company.

In this study, both *E. aerogenes* and *E. aerogenes* (pUC8) and *E. aerogenes* (pUC8:15) which this bacterium's *vgb*⁻ and *vgb*⁺ (*Vitreoscilla* hemoglobin gene) recombinants found in our laboratory were used. The *vgb*⁻ and *vgb*⁺ strains of *E. aerogenes* are recombinants obtained in our own laboratory, which are called to as *Ea* [pUC8], *Ea* [pUC8:15], respectively. *Ea* [pUC8:15] is a recombinant bacterium strain formed by cloning the genomic fragment of *Vitreoscilla* carrying the *vgb* gene together

with the 2.3-Kb promoter to the Hind III restriction site in the multi cloning region of the pUC8 plasmid having 665 base pairs. The plasmid size [pUC8: 15] is about 5 Kb. It was stored in stock with glycerol at -20 °C to ensure the viability of the this bacteria. *E. aerogenes* we used in our studies were passaged to the LB plates every 30 days with the help of a core and were allowed to grow overnight and the next day, wrapped around the plates with parafilm and stored at 4 °C. These stocks were used during the experiments.

In this study, Luria-Broth (LB), Terrific Broth (TB), Whey and Molasses were used as rich foods. The pH of the medium was adjusted to 6.0, 7.0 and 8.0 according to the working steps, and the flasks containing 20 ml of medium were autoclaved in autoclave at 120 °C and 1 atm for 25 minutes. In the study, an MFC with a total of 10 ml of anode + cathode section made specifically for this study was used. The MFC is shown in Figure 1. The data collection system (PICOTEST M3500A 61/2 Digit Multimeter) was used to measure the electrical current obtained in the MFC and the average voltage values were recorded for 24 hours and this data was transferred to the computer by a software belonging to PICOTEST.

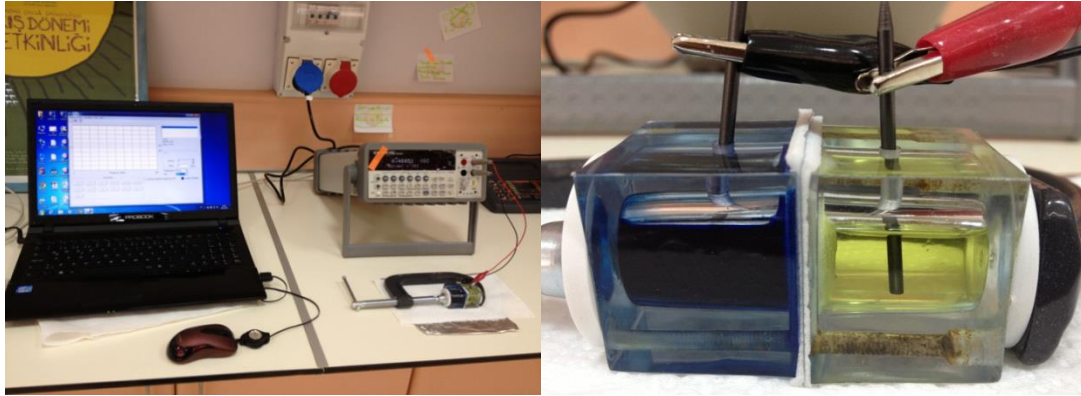


Figure 1. The MFC system used during the experiments. Yellow color: cathode section; Blue color: The anode section shows.

2.2. Electrode Optimization

The flasks containing 20 ml of LB (pH 7) medium were autoclaved at 25 °C for 25 minutes at 120 °C. Then, this medium was cultured from the stock bacteria cultures

were stored in petri dishes at 4 °C. However, for recombinant strains, 100 µg/ml solution from stock antibiotic solution (100 mg / ml) was added to the medium. Bacterial growth was achieved in a shaking incubator at 200 rpm at 37 °C during overnight. The next day, inoculation is done to new mediums by pipetting 200 µl from these mediums observed growth and were allowed growths at the incubator. Bacterial cultures were transferred to microbial fuel cell at 8th hour and electrical current obtained during 24 hours to be three replicates for each bacterium using copper, composite and graphite electrodes was measured with a multimeter in PICOTEST M3500A 61/2 brand and model and data was transferred to the computer by the same multimeter firm's software.

2.3. Optimization of Microorganism Incubation Time

The flasks containing 20 ml of LB (pH 7) medium were autoclaved at 25 ° C for 25 minutes at 120 °C. Then, this medium was cultured from the stock bacteria cultures were stored in petri dishes at 4 °C. However, for recombinant strains, 100 µg/ml solution from stock antibiotic solution (100 mg/ml) was added to the medium. Bacterial growth was achieved in a shaking incubator at 200 rpm at 37 °C during overnight. The next day, inoculation is done to new mediums by pipetting 200 µl from these mediums observed growth and were allowed growths at the incubator. Bacterial cultures were transferred to microbial fuel cell at 6. 8. 10. 12th and 14th hours and electrical current obtained during 24 hours to be three replicates for each bacterium using copper electrode was measured with the multimeter and data was transferred to the computer.

2.4. Medium Optimization

The flasks containing 20 ml of LB (pH 7), TB (pH 7), whey (pH 7) ve molasses (pH 7) mediums were autoclaved at 25 ° C for 25 minutes at 120 °C. Then, this medium was cultured from the stock bacteria cultures were stored in petri dishes at 4 °C. However, for recombinant strains, 100 µg/ml solution from stock antibiotic solution (100 mg/ml) was added to the medium. Bacterial growth was achieved in a shaking incubator at 200 rpm at 37 °C during overnight. The next day, inoculation is done to

new mediums by pipetting 200 μ l from these mediums observed growth and were allowed growths at the incubator. Bacterial cultures were transferred to microbial fuel cell at 8th hour and electrical current obtained during 24 hours to be three replicates for each bacterium using copper electrode was measured with the multimeter and data was transferred to the computer.

2.5. pH Optimization

The flasks containing 20 ml of molasses (pH 6), molasses (pH 7) ve molasses (pH 8) mediums were autoclaved at 25 $^{\circ}$ C for 25 minutes at 120 $^{\circ}$ C. Then, this medium was cultured from the stock bacteria cultures were stored in petri dishes at 4 $^{\circ}$ C. However, for recombinant strains, 100 μ g/ml solution from stock antibiotic solution (100 mg/ml) was added to the medium. Bacterial growth was achieved in a shaking incubator at 200 rpm at 37 $^{\circ}$ C during overnight. The next day, inoculation is done to new mediums by pipetting 200 μ l from these mediums observed growth and were allowed growths at the incubator. Bacterial cultures were transferred to microbial fuel cell at 8th hour and electrical current obtained during 24 hours to be three replicates for each bacterium using copper electrode was measured with the multimeter and data was transferred to the computer.

3. Results and Discussions

The experiments were done with three replicates and were written with the average of three experiments.

3.1. Effect of Electrode Type on MFC Efficiency

Type of electrode used in microbial fuel cells affects both the performance of the microbial fuel cell and the amount of voltage to be obtained from this fuel cell. In this step of our study, three different electrodes were used to determine the electrode that would give us the best microbial fuel cell performance. The results obtained from these experiments are presented graphically below (Figure 2,3,4).

During the experiments, the same type of electrodes were immersed in the microbial fuel cell with the cultures of the *E. aerogenes* and recombinant strain,

respectively, and the highest voltage was observed in the recombinant strain *Ea* [pUC8:15], which was amplified at 37 °C in LB medium. Therefore, electrode research was continued in this recombinant strain.

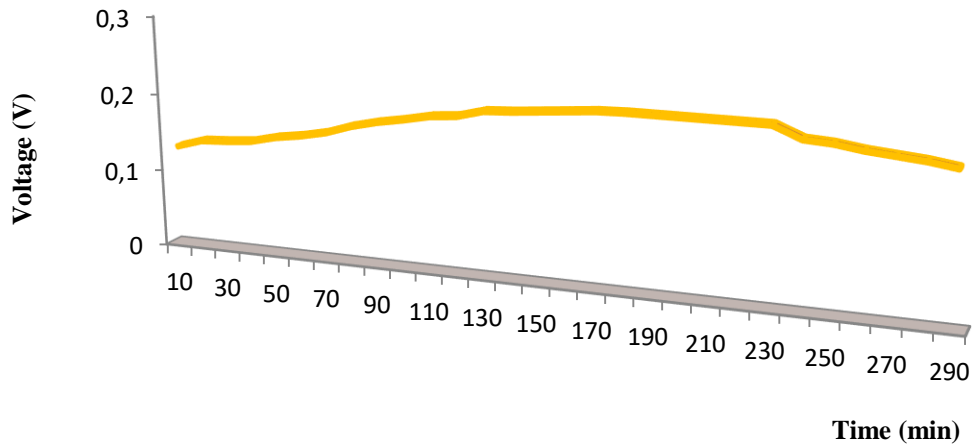


Figure 2. Experimental data graph made with copper electrode in *Ea* [pUC8:15]

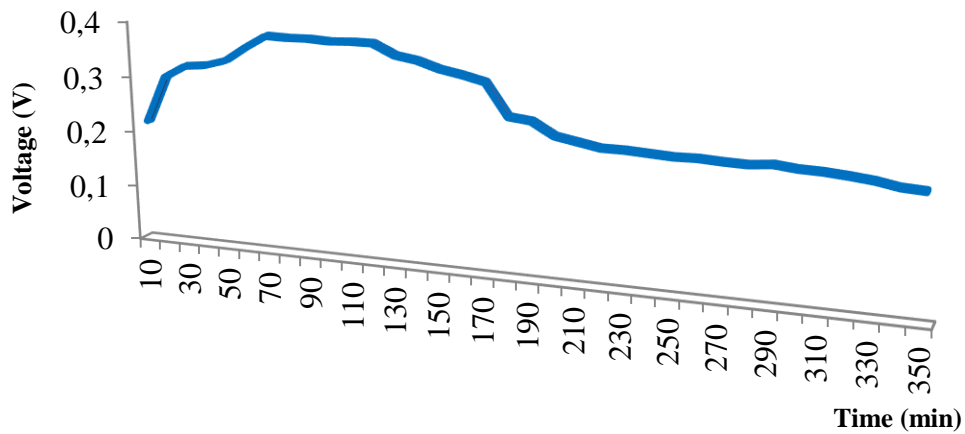


Figure 3. Experimental data graph made with composite electrode in *Ea* [pUC8:15]

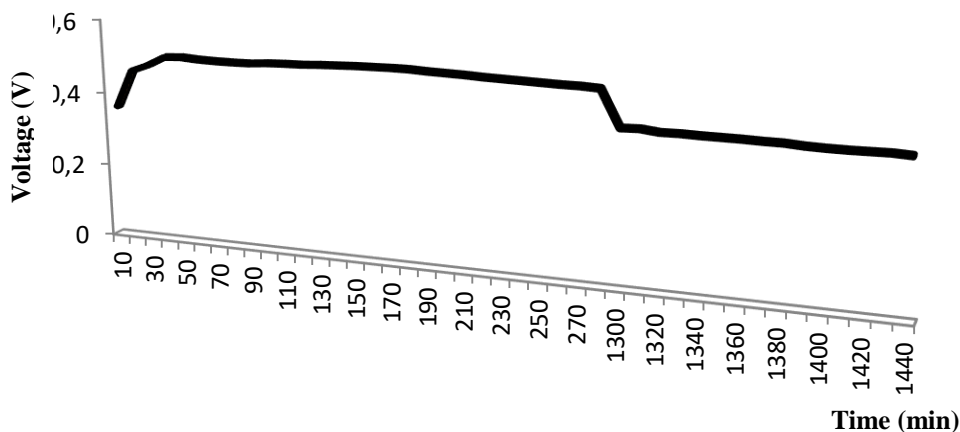


Figure 4. Experimental data graph made with graphite electrode in *Ea* [pUC8:15]

The result of the experiments; it was found to be highest voltage value in copper electrode test is 0.23 V, in composite electrode is 0.38 V, in graphite electrode is 0.52 V. Graphite electrode was found to be the electrode that gave the highest voltage amount and the following studies were continued with this electrode.

3.2. Optimization of Microorganism Incubation Time

The time period when the bacteria we use give the maximum amount of voltage is not known. In order to determine this, it has been tried to determine when the microbial fuel cell has the best performance by using various time periods. At this stage; In the previous study, the highest voltage value by using the culture at 6. 8. 10. 12th and 14th hours of *Ea* [pUC8: 15] which was grown at 37 ° C in LB medium which gave the highest voltage yield with carbon electrode was determined. The experimental data are plotted below (Figure 5).

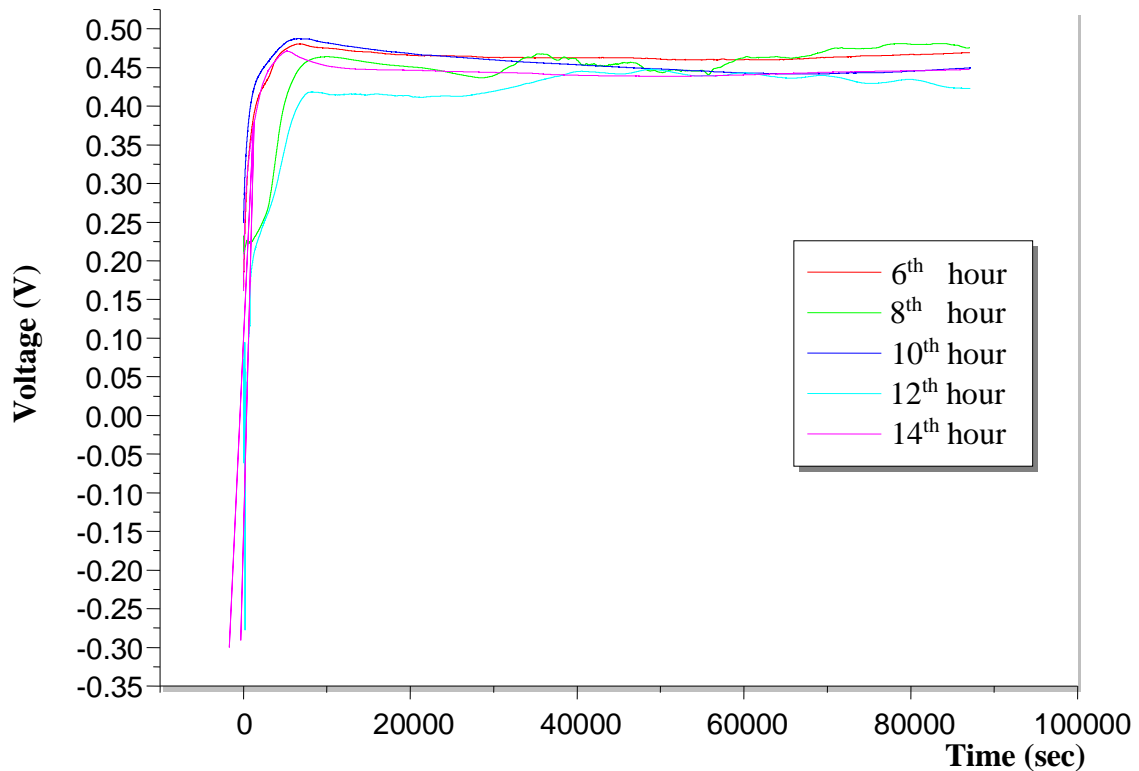


Figure 5. Experimental data graph of incubation time optimization performed using culture at 6. 8. 10. 12th and 14th hours of *Ea* [pUC8:15]

In the made measurements, the highest voltage value of *Ea* [pUC8:15] at 6.8.10.12th and 14th hours was read as 0.480 V, 0.494 V, 0.490 V, 0.453 V and 0.476 V, respectively.

Based on this data and graph, it was determined that *Ea* [pUC8: 15] had the highest voltage yield at 8th hour compared to the time periods above, and the next steps of the study were continued at this time.

3.3. Effect of Medium Type on MFC Efficiency

In order to determine in which medium the bacteria we use give the highest voltage these bacteria were allowed to multiply in 4 different medium and were measured voltage values give in these mediums. In addition to the amount of voltage obtained when determining the best and most suitable medium, it was also taken into consideration that the selected medium is economical.

Data obtained from experiments performed in molasses, TB, Whey and LB media at 37 °C for each of the recombinant strains of *E. aerogenes* and its [pUC8], [pUC8:15] are presented as a graph below (Figure 6).

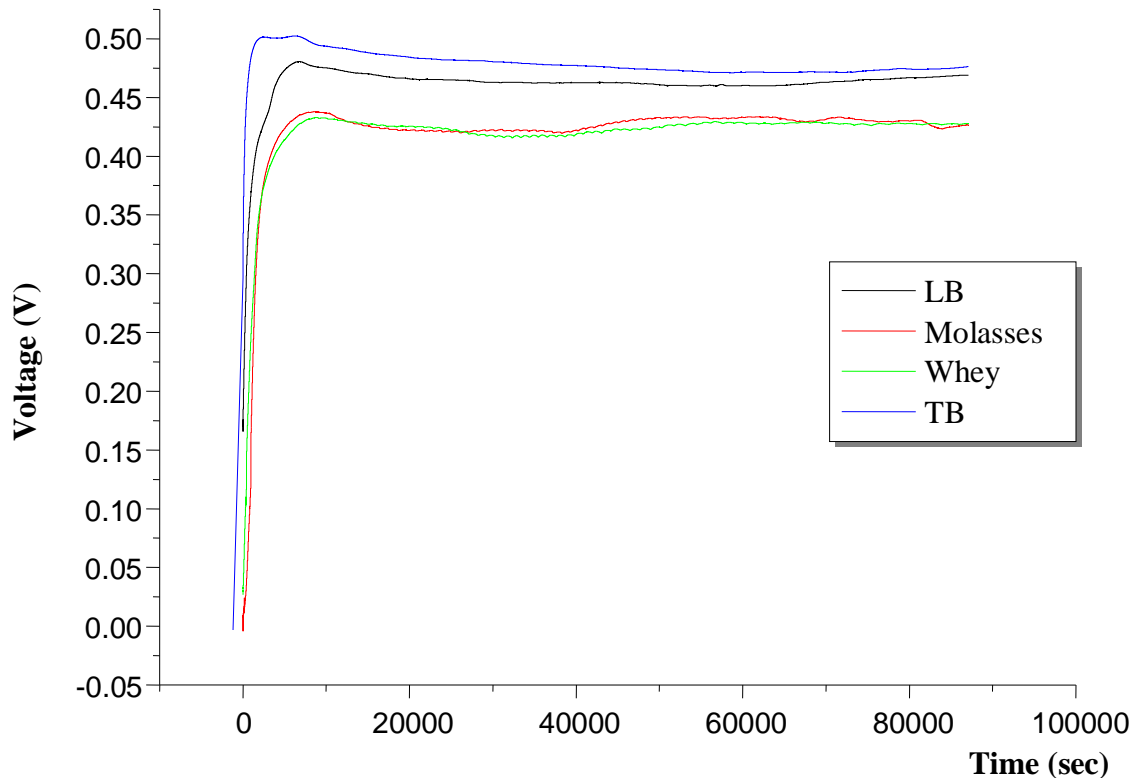


Figure 6. A graph of experimental voltage values performed in 4 different medium of *Ea* [pUC8:15]

In the made measurements at Molasses, Whey, TB and LB mediums the highest voltage value of *Ea* [pUC8:15] were read as 0.438 V, 0.436 V, 0.508 V and 0.476 V respectively. The highest voltage value for *Ea* [pUC8:15] was observed to be TB, and the lowest voltage was read in Whey.

When the study findings are examined completely; the highest voltage value for all three bacteria was observed in TB and the lowest voltage was observed in Whey. Since TB is economically cost-effective, LB and Molasses, which give the highest voltages after this medium, were evaluated. Molasses has been identified as the most suitable medium for further studies in terms of both its waste assessment and economic suitability and its ease of access.

3.4. Effect of Cell pH on MFC Efficiency

The pH effect of bacteria on voltage production was studied at 3 different pH (pH 6.0 - 8.0). As the culture medium, Molasses, which was determined as the most suitable medium in the previous study, was used and each bacterium was reproduced in 3 different pH conditions in Molasses at 37 °C and the obtained voltage values were plotted (Figure 7).

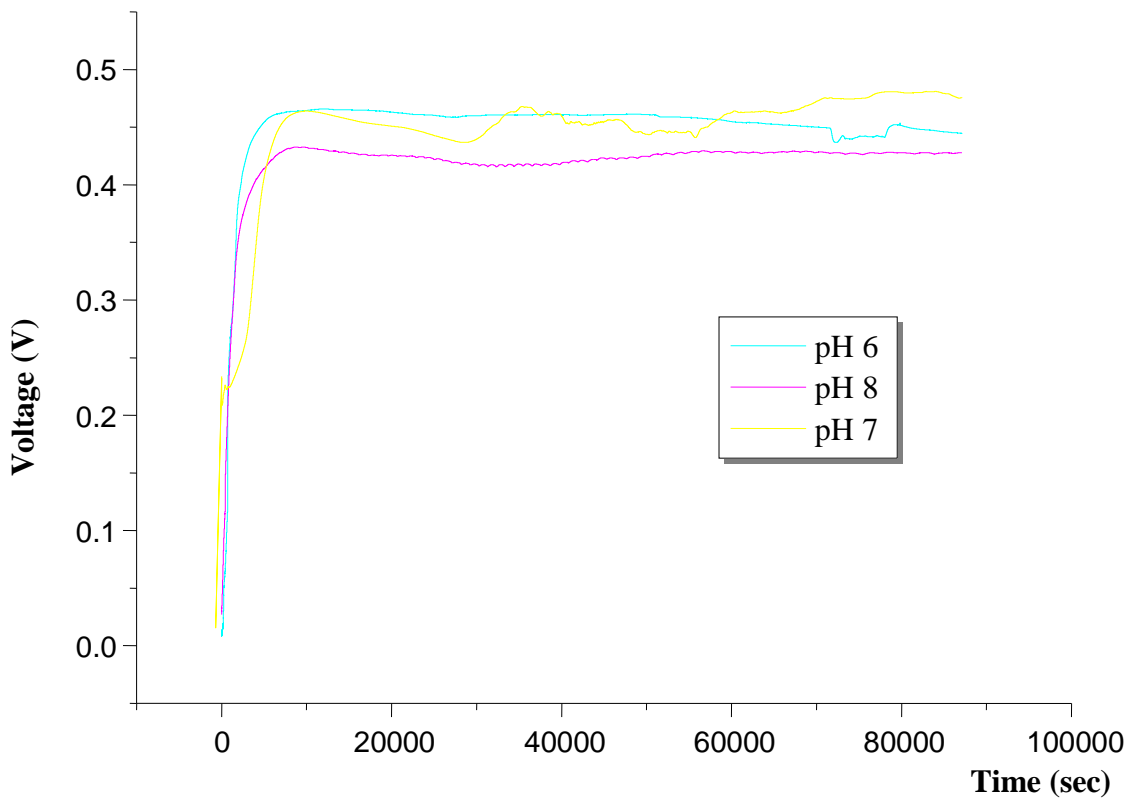


Figure 7. Graph of voltage values obtained from in 3 different pH media in Molasses for *Ea* [pUC8:15]

The result of the study; For *Ea* [pUC8:15], the highest voltage values in Molasses at pH 6.0, pH 7.0 and pH 8.0 were 0,472 V, 0,476 V, 0,438 V, respectively. For *Ea* [pUC8:15], it was determined that the pH that gave the highest voltage amount was pH 7.0. The studies were examined completely and the pH value of the three bacteria was determined as 7.0. Subsequent studies were continued at pH 7.0.

3.6. Analysis of pH and Spectrophotometric Values of *E. aerogenes* and Its Recombinant Strains in Molasses

Acid production of bacteria in molasses was determined by measuring the pH of the culture. For this, pH values of *E. aerogenes* and its recombinant cultures grown in molasses medium were measured at 8th hour and given below as table (Table 1.). At the same time, absorbances of 600 nm wavelength are measured and given in continuation of Table 1.

The pH values in the environment of microorganisms are particularly important for enzymatic activities. The pH of the environment is effective on the metabolites of bacteria and this is important for voltage production as this will affect the amount of protons and electrons.

At the end of the 24-hour incubation period in Molasses culture media that started with three different pH values; there is a general increase in cultures with pH 6.0 starting pH, while there is a general decrease in pH 7.0 and 8.0. The pH with the maximum pH change was found to be 8.0 (pH decrease of about 1 unit), while the lowest pH change was recorded in the environment with an initial pH value of 6.0 (a pH increase of about 0.5 units). The highest biomass formation was observed in pH 7.0 medium, followed by pH 6.0 medium.

Table 1. pH change and biomass formation of *E. aerogenes* and its recombinant strains in molasses culture medium. Each data is the average of three independent replicates. Standard deviations (σ_{n-1}) are indicated as \pm values.

| | pH 6.0 | pH 7.0 | pH 8.0 |
|-------------------------|---------------|---------------|---------------|
| <i>E. aerogenes</i> | 6.46±0.07 | 6.68±0.13 | 6.80±0.01 |
| <i>Ea pUC8</i> | 6.1±0.09 | 6.15±0.10 | 6.95±0.33 |
| <i>Ea pUC8:15</i> | 6.6±0.28 | 6.81±0.04 | 6.81±0.45 |
| OD₆₀₀ | | | |
| <i>E. aerogenes</i> | 0.197±0.1 | 0.146±0.3 | 0.158±0.2 |
| <i>Ea pUC8</i> | 0.205±0.2 | 0.173 ±0.2 | 0.158±0.2 |
| <i>Ea pUC8:15</i> | 0.185±0.1 | 0.140±0.3 | 0.161±0.3 |

Table 2. *E. aerogenes* and its recombinant strains at pH 7.0, at the end of 8 hours, pH changes and biomass formation in LB, TB, Whey and Molasses mediums. Each data is the average of three independent replicates. Standard deviations (σ_{n-1}) are indicated as \pm values.

| pH 7.0 | | | | |
|-------------------------|-----------------|-----------------|-----------------|-----------------|
| | LB | TB | Whey | Molasses |
| <i>E.aerogenes</i> | 7.95 \pm 0.13 | 6.85 \pm 0.22 | 4.67 \pm 0.45 | 6.68 \pm 0.13 |
| <i>Ea pUC8</i> | 7.88 \pm 0.22 | 6.80 \pm 0.07 | 4.64 \pm 0.72 | 6.15 \pm 0.10 |
| <i>Ea pUC8:15</i> | 7.93 \pm 0.05 | 6.89 \pm 0.16 | 4.93 \pm 0.86 | 6.81 \pm 0.04 |
| OD₆₀₀ | | | | |
| <i>E.aerogenes</i> | 0.629 \pm 0.2 | 0.660 \pm 0.2 | 0.544 \pm 0.1 | 0.205 \pm 0.3 |
| <i>Ea pUC8</i> | 0.574 \pm 0.2 | 0.510 \pm 0.1 | 0.518 \pm 0.1 | 0.185 \pm 0.2 |
| <i>Ea pUC8:15</i> | 0.548 \pm 0.3 | 0.747 \pm 0.3 | 0.465 \pm 0.2 | 0.197 \pm 0.3 |

In the LB medium with an initial pH of 7.0, there was a general increase in pH after culture, whereas there was a general decrease in pH values in TB, Whey and Molasses. While the highest change was seen in Whey medium (approximately 2.5 units pH decrease), the least change was recorded in LB and TB media (approximately 0.2 units pH change). The highest biomass formation is observed in TB environment, followed by LB, Whey and Molasses.

4. Discussion and Conclusion

It has been shown that bacteria are advantageous systems in electricity production with MFC systems with studies done so far. In various studies, experiments have been performed on the maximum power outputs and electrical power generation in MFC of the bacteria such as *Geobacter* [18], *Enterobacter* [19], *Shewanella* [20] and *Bacillus* [21]. In MFC studies, mixed cultures are preferred rather than pure cultures. Mixed cultures are more resistant to stress and high nutritional adaptations ensure that they are stable. However, pure cultural studies have the advantage of allowing direct observation of both mechanistic and physiological details [22]. In study made by Kiely et al. [23],

Shewanella (MR-1) has been shown to produce more power density than a mixed culture. In our study, the application of pure culture was preferred because it was aimed to observe the effect of recombinant systems on electricity production. *E. aerogenes* is one of the most widely studied species in fermentative hydrogen production [17]. Tanisho and his team found out that *E. aerogenes* is a good hydrogen producer and studied the basic fermentation properties of *E. aerogenes* in hydrogen production during the 1980s and 1990s [24, 25]. *E. aerogenes* in the MFC system produces 10 moles of hydrogen corresponding to 1 mole of glucose used as substrate. Therefore, it is a preferred type of bacteria in energy studies [26]. In our study; *E. aerogenes* and its recombinant strains were used (*Ea puc8*, *Ea puc8:15*). *Ea* [pUC8:15] is a recombinant bacterium strain formed by cloning the genomic fragment of *Vitreoscilla* carrying the *vgb* gene together with the 2.3-Kb promoter to the Hind III restriction site in the multi cloning region of the pUC8 plasmid having 665 base pairs. *Ea* [pUC 8] strain carries only pUC8 plasmid. *Vitreoscilla* Hemoglobin gene (*vgb*) consists of a total of 648 nucleotides with the promoter-operator region and the region encoding the hemoglobin. The promoter of the gene is an oxygen-responsive promoter and also demonstrates catabolite suppression. It is sensitive to oxygen through sequences specific to the FNR and ArcAB proteins it carries on the promoter. The cAMP-CRP binding site on the promoter provides catabolic suppression [27].

Due to oxygen-sensitive *vgb* promoter of VHb synthesis, the synthesis occurs at high level in low oxygen environment in its natural hosts and organisms in which it was clone and the growth and development of organisms with VHb synthesis has been recorded. In addition, various metabolites and recombinant proteins requiring a certain level of oxygen for their production have also shown significant increases in organisms carrying the VHb / *vgb* system [28, 29]. Therefore, it has been suggested that this protein can be used as an effective agent for some microaerobic fermentations in industrial use. In general, MFCs have two designs, namely single-chamber and double-section, in which the cathode electrode directly contacts the air. The MFC we use in our study is the dual-part MFC design. Such MFCs generally consist of an aerobic cathode section separated by a proton exchange membrane and an anaerobic anode section. In our experiments, we injected the bacteria into the anode section and in this section it was observed that the substrate was oxidized by bacteria and electrons were captured by

the MFC system and read as voltage. In one study; it has been reported to oxidize the substrate by bacteria that produce electrons and protons in anode section [30]. It has been observed that the ratio between the electrode and membrane surface areas used in MFC reactors and the reactor volume is significant. Although the two-section cubic-MFC reactor is small in volume, it is thought that the equalization of the electrode and membrane surface area positively affects the power generation performance. Compared to the studies using similar reactors, the findings obtained were confirmed by the literature. In particular, the membrane structure and the reactor model have been modified and it is recommended to continue research to increase the energy production efficiency with a suitable cheap chemical mediator.

The modeling of the reactor we used in our study was performed by taking into operation the principles of MFC by us. Among the various factors that affect the performance of MFC; selection of electrodes is quite important. Type of electrode used in MFCs; both the performance of the MFC and the amount of voltage to be obtained from this fuel cell. The electrode used should increase MFC performance as well as economically. For this purpose, various carbon and metal materials have been developed in recent years. Copper, graphite and carbon electrodes were used in our study and the highest voltage was measured on carbon electrodes. In our study with carbon electrode, the maximum voltage is 0.5 V. In various studies; In the study performed with platinum electrode, 0.5 V [31], 250 mV [32] with carbon electrode, 360 mV in the study with flat carbon electrode [33], in another study with platinum electrode 0.4 V [34] and in another study with porous carbon electrode A value of 0.33 to 0.57 V was obtained [34]. The voltage obtained in our study with carbon electrodes is efficient and is similar to the results obtained studies with platinum electrodes with high cost [31, 35]. In addition, the value we obtained are higher than other studies made with carbon electrodes [32, 33, 35]. This shows that the performance of our MFC system is high depend on the high hydrogen production efficiency of the bacteria we use. The time period when the bacteria we use give the maximum amount of voltage is not known. In order to determine this, it has been tried to determine when the microbial fuel cell has the best performance by using various time periods. At this stage; In the previous study, the highest voltage value by using the culture at 6. 8. 10. 12th and 14th hours of *Ea* [pUC8: 15] which was grown at 37 ° C in LB medium which gave the

highest voltage yield with carbon electrode was determined. In the made measurements, the highest voltage value of *Ea* [pUC8:15] at 6.8.10.12th and 14th hours was read as 0.480 V, 0.494 V, 0.490 V, 0.453 V and 0.476 V respectively. Based on measurement results, it was determined that *Ea* [pUC8: 15] had the highest voltage yield at 8th hour. Earlier studies have shown that the optimum growth time for this group of bacteria is 8th-10th hour. Our measurement results are parallel with the literature in this sense [36].

In order to determine in which medium the bacteria we use give the highest voltage these bacteria were allowed to multiply in 4 different medium and were measured voltage values give in these mediums. The highest voltage for all three bacteria was observed in TB and the lowest voltage was observed in Whey.

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