The Effect of External Resistance on the Performance of an Annular Single Chamber Microbial Fuel Cell (ASCMFC) Using Chocolate Industry Wastewater

Abstract

The following goals were followed by the study to treat wastewater and produce power and electricity simultaneously using a single chamber microbial fuel cell (SCMFC). Firstly, the stainless-steel mesh with graphite coating as the anode brought about a porous surface for the proper growth and attachment of the biofilm. Secondly, using the anode in the spiral geometry, the surface of the anode electrode increased and the time for the substrate to reach the microorganisms decreased. Wastewater of the chocolate industry was used as substrate. In this study, two ASCMFCs with a volume of 90 cm^3, with a completely identical configuration and the only difference in the electrode distance, were examined discontinuously. The maximum voltage values in the open circuit state and the power density for the first system with an electrode distance of 1.3 cm were obtained as 742 mV and 7.98 W/m^3, respectively. The second fuel cell was built to optimize the electrode distance and started at three electrode distances of 1, 0.7 and 0.4 cm, and its performance was examined and compared with the results obtained from the first fuel cell at these three distances. The maximum voltage in the open circuit mode was obtained at the optimal electrode distance of 0.7 cm and 856 mV. The experiments were repeated to obtain the maximum current and power density. The maximum power density at the optimal distance was 22.898 W/m^3. The performance of the microbial fuel cell as an electricity generator is given according to the polarization behavior and the cell potential. In the next stages of the experiments, the input substrate concentration and chemical oxygen demand were analyzed. A significant decrease in turbidity and chemical oxygen demand was seen after 96 hours, respectively, at 79.66% and 91.2%.

Keywords: *Microbial fuel cell, annular structure, external resistance, wastewater of chocolate industry*

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Introduction

The theory of using microbial cells was presented to produce electricity. MC Potter - a professor of botany at Durham University - was the first to propose this theory in 1912 [1]. Potter managed electricity production from E.coli microbe but could not reach the desired results [2]. Electricity is produced based on the fermentation of cellulose to hydrogen in the microbial fuel cell. This process is carried out by a hydrogen reaction with a platinum catalyst [3].

The microbial fuel cell is a new technology that deals with the latest methods of obtaining electricity and producing bio-electricity from biomass using bacteria. In other words, the microbial fuel cell is a type of technology to convert the energy stored in chemical bonds in organic compounds into electric energy by reactions catalyzed by microorganisms, receiving much attention in recent years in academic studies [4]. Microbial fuel cell technology is a promising and, at the same time, completely different method for wastewater treatment. Besides purification, this technology can be used to extract energy in the form of electricity or hydrogen gas. In late 1990, Kim et al. found that using bacteria in microbial fuel cells can be used to determine the concentration of lactic acid in water [5]. Following that, Kim et al. showed the production of electricity from starch in industrial wastewater in a microbial fuel cell [6].

Nonetheless, the low production power made the effect of this technology to be overlooked in wastewater treatment. When it was proven that domestic wastewater could be treated to a useful level while simultaneously generating electricity, the link between electricity use and wastewater treatment was strengthened [7]. Riemers et al. proved that the organic and inorganic materials in marine sediments could be used in a new type of microbial fuel cells, and in these cells, a wide range of diverse substrates, materials and structures can be used to harness electricity from organic materials with bacteria [8].

Biofuel cells are the general term for cells using organic substrates as fuel and microorganism activities as catalysts [9]. Leo et al. (2004) presented a structure for SCMFCs that was used for the simultaneous production of electric current and wastewater treatment and could be used continuously. This structure is composed of two concentric acrylic cylinders. A SCMFC with a volume of 520 mL for power generation was examined by Leo et al. [10]. They designed this fuel cell according to the information of previous scholars. The maximum power of this large-scale battery using sodium acetate as a substrate was 20 W/m^3. Moreover, the study used more than 90% of sodium acetate.

In their study on a SCMFC, Leo et al. indicated that the power generated using acetate (506 mW/m^2, 800 mg/L) was 66% higher compared to when butyrate was used (305 mW/m^2, 1000 mg/L) [11].

Forming a suitable biofilm structure on the surface of the microbial fuel cell electrode is very important to achieve maximum power and stable current. Laboratory studies have revealed that the biofilm tends to form a uniform, compact and less active structure at higher external resistances compared to lower resistances. The first test of the SCMFC system was carried out by measuring the potential difference in the open circuit state to find the optimal distance between the anode and the cathode, and the microbial enrichment was performed simultaneously with the open circuit voltage measurement. One factor that reduces ohmic losses and increases power is reducing the distance between electrodes. Moreover, the very close distance of the electrodes will result in a decrease in the production power despite the decrease in ohmic resistance [12]. Accordingly, the study aims to examine the effect of external resistance on the performance of the ASCMFC using chocolate industry wastewater.

Materials and methods

The study used two air SCMFCs with a volume of 90 ml. stainless steel mesh with graphite coating was used as anode in spiral geometry to reduce the manufacturing cost. Graphite coating reduces the resistance and increases the conductivity of the anode at the same time. Chocolate industry wastewater with a high oxygen demand load containing large values of industrial detergents with high stability and hardness was selected as the SCMFC feed to prove the system's ability to produce electricity and purify wastewater. The activated sludge of the Ghaem Shahr treatment plant, with different useful microorganisms such as bacteria, protozoa, rotifers, and so on, was used as a mixed culture. Then 20 ml of a solution with a 1:1 ratio of wastewater and nutrient solution was added to the mixed culture every 48 hours, and this work was repeated for 90 days to adapt the microorganisms in the mixed culture to the wastewater. It must be noted that the nutritional solution contains a combination of minerals, salts and vitamin substances necessary for the continued growth and activity of microorganisms.

Design, manufacture, and operation of biological fuel cell

SCMFC body is a 3 mm thick transparent plexiglass plate (for top and bottom caps), 3 cm thick transparent plexiglass plate (for cell wall) and 6 mm thick plexiglass plate for cathode holder in the cell center.

In the upper and lower cover of the SCMFC, the other two pieces that make up the cell body are cut on a 3 mm thick plexiglass plate, and a circle with a diameter of 3.5 cm was cut on each of these covers for air passage. Four holes with a diameter of 4 mm were made, which screw together to connect the covers to the main body. The overall design of the body of the microbial fuel cell with a volume of 90 cm^3 and its aspects is given in Figure (1).

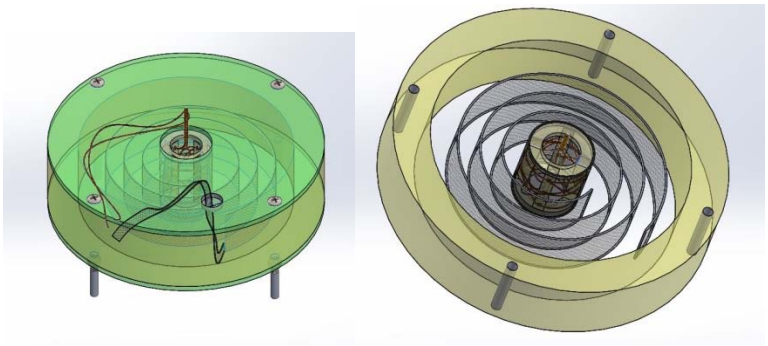

Figure 1. Schematic of the SCMFC with a helical anode

Fabric carbon produced by the company (E-TEK, USA) was used to manufacture the cathode electrode. It is better to use porous surfaces to make the anode electrode; therefore, stainless steel with 300 mesh was used for this purpose. 57 x 2 cm dimensions were separated from stainless steel and then washed using deionized water. This net was immersed in acetone solution to remove the organic pollutants in the next step. Subsequently, this net was boiled in 0.1 M hydrochloric acid solution and again boiled in deionized water to remove the bad smell of hydrochloric acid.

Results

1. Measuring open circuit voltage

The initial distance between the inner wall of the cathode and the starting point of the anode electrode was selected as 1.3 cm in the initial experiment. To this end, 3 ml of activated sludge, 45 ml of chocolate wastewater, and 42 ml of the feeding solution, a combination of salts, minerals, soluble vitamins, and microorganisms need them for their metabolic activities and making their protoplasm; we entered the microbial fuel cell. We reached the chemical oxygen demand concentration of 1400 mg/liter after 3 dilutions.

The delayed or dormant stage starts with the entry of microorganisms into the fuel cell. Then in the continuation of this stage, their growth limit and reproduction speed gradually increase until it reaches the maximum at the end of this stage.

At this stage, there are practically no environmental metabolites and waste gases. This stage lasts almost two hours, and the bacteria adapt to the stated wastewater for metabolism. As seen in Figure (2), the open circuit voltage of the fuel cell was examined for 257 hours. The initial potential difference of the microbial fuel cell with an electrode distance of 1.3 cm is 118 mV. Such a potential difference is due to the difference in biological and chemical conditions in the cathode and anode electrodes. This potential difference decreased during 4 hours and reached 99 mV because of the stated reasons and the lack of adaptation and familiarity of microorganisms with their surrounding environment and nutrient solutions.

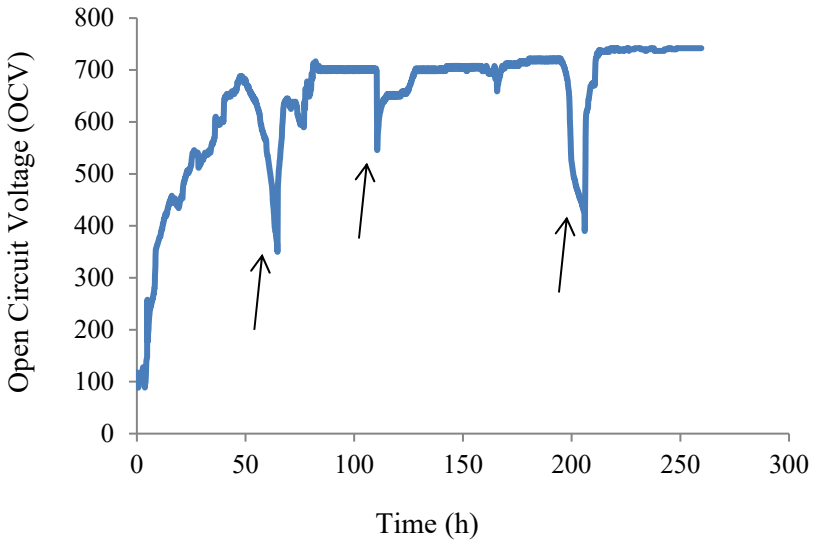

Figure 2. Measuring the open circuit potential difference of the first microbial fuel cell with an electrode distance of 1.3 cm

Log growth starts with the completion of the lag phase. Bacteria carry out metabolic reactions at their maximum speed and reproduce freely without any obvious obstacles during log growth.

Ultimately, the stationary stage or slow reproduction begins after passing this stage and maximizing the number of bacteria. After this stage, the growth rate gradually decreases, and the increase in the number of bacteria is not exponential anymore. As the figure shows, the fuel cell voltage reached 673 mV after 46 hours from the start. The stage of stability in the number of bacteria or the stability of the voltage lasted about 7 hours, and the voltage of the microbial fuel cell showed a value of 678 mV. With the start of bacterial death, they enter an incremental death and very soon enter a log death. Due to the severe lack of food and required gases and the increase of metabolites, some of the bacteria are autolyzed, and the potential difference of the fuel cell decreases until the potential difference reaches 350 mV during the 64 hours that passed after entering the wastewater. The wastewater used by bacteria during this period was 23 ml. By injecting fresh wastewater with a 1:1 ratio of diluted wastewater and feed solution, the potential difference starts to increase rapidly so that after 3 hours of the second injection, the potential difference reaches 630 mV. This voltage showed decreasing trend within 8 hours until it reached 595 mV and then started to increase again.

Seventeen hours after the second injection, the voltage increased to 708 mV. As is seen, the value of voltage that arose from the degradation of hard degradable compounds was more than that of easily degradable compounds. Then it remained constant for 27 hours, and the voltage decreased again because of the reduced available substrate for microorganisms. By adding 17 milliliters of fresh wastewater with the ratio stated, the voltage increased rapidly inside the fuel cell and reached 644 mV in the first stage and 708 mV in the second. It is important to note that after the second injection of the fresh substrate into the fuel cell, the voltage could have decreased instead of increasing. This decrease in the maximum voltage compared to the previous state is linked to the decrease in the pH of the anolyte solution. This is because by reducing the pH and its reaching below 7, the electric charge accumulated on the surface of the microorganisms becomes positive, and the microorganisms will not be able to break down the metals they need from the activity of the active part because of the positive charge of the metal cations in the nutrient solution. Enzymes are then reduced and unable to metabolize hard degradable organic compounds [12]. This problem was predicted earlier and prevented by adding a buffer solution. After the third injection, the voltage did not exceed 742 mV. Then the tests were completed, and results were obtained by applying various resistances to the first fuel cell system, which will be discussed in the next sections. In the experiment with the first fuel cell with an electrode distance of 1.3 cm, the results were much lower than the values previously reported by the same system with dairy wastewaters in line with the expectations of the system.

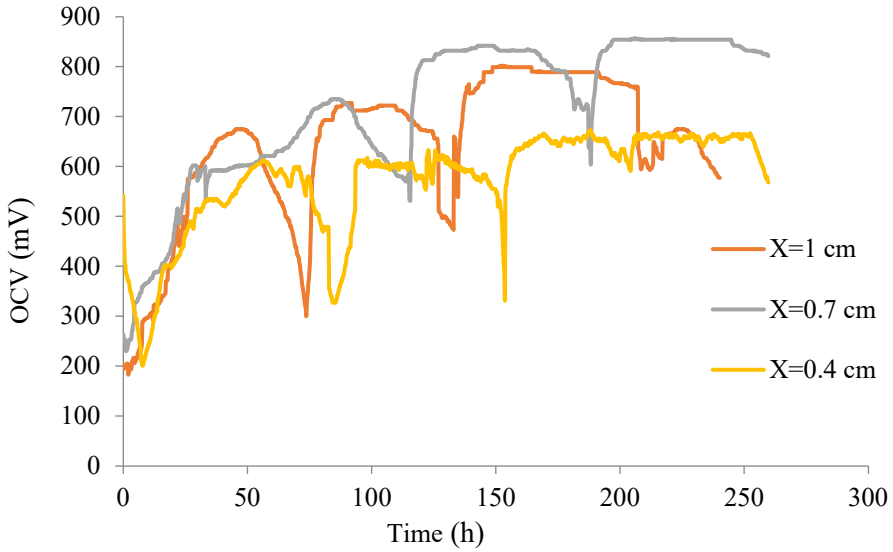

Figure 3. Measuring open circuit potential difference for the second cell at three electrode distances

A re-examination was conducted on all the effective factors to find the cause of this difference. This examination showed that the only parameter not addressed and optimized in the previous study was the initial distance between the cathode and anode electrodes. Hence, the second fuel cell was started this time, and the potential difference was measured at three electrode distances of 0.4, 0.7 and 1 cm (Figure 3). Concerning the subsequent experiments with various electrode distances, the instructions were carefully followed in line with what was done in the case of the first cell. Concerning Figure (3), the system became stable after 143, 192, and 205 hours for electrode distances of 1, 0.7, and 0.4 cm, respectively. The maximum current was 800, 856, and 660 mV for distances of 1, 0.7 and 0.4, respectively. These values were obtained after 3 times of fresh feed injections; the potential difference dropped each time. As is seen, the maximum voltage was obtained at 0.7 cm; thus, 0.7 cm between the two electrodes was selected as the optimal distance, and the performance of the system at this distance compared to the first battery was compared in the polarization and power density curves.

2. External resistance to the performance of SCMFC

After measuring the open circuit voltage at the optimal distance and reaching the sufficient thickness of the biolayer, measuring the power and intensity of the current in variable external resistances and during different time intervals were evaluated, and the microbial fuel cells applied different current densities based on the external resistances. In the first cell, with an electrode distance of 1.3 cm, resistances of 50, 100, 150, 200, 300, and 500 ohms were applied to the system. We applied a wider range of resistances, including 50, 100, 150, 200, 300, 500, 600, 800, and 1000 ohms, to the system to examine the performance of the second system with an optimal distance of 0.7 cm.

Microbial fuel cell with an electrode distance of 1.3 cm

The initial resistance of 500 Ohms was selected as the consumer of the electric charge resulting from the oxidation of the substrate, and the changes in the potential difference were examined over 265 hours in the case of the first cell with an electrode distance of 1.3 cm based on the Multiple Cycle method (Figure 4).

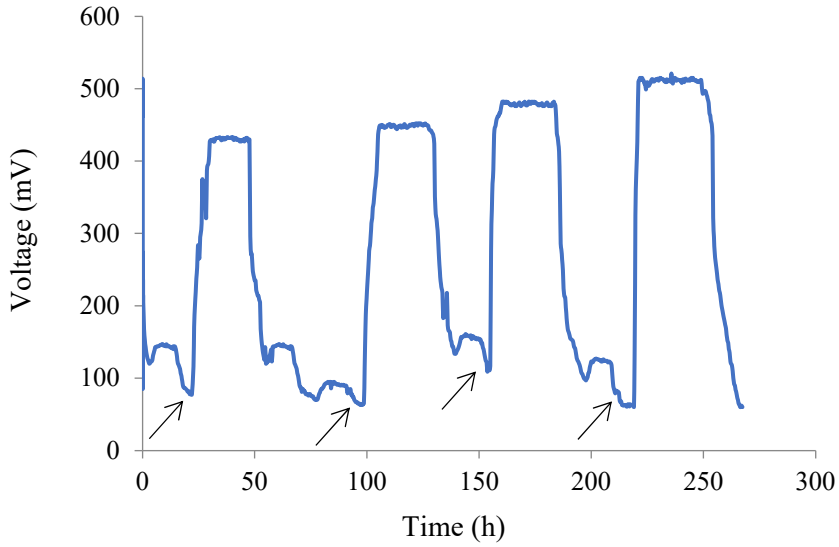

Figure 4. The potential difference of the single-compartment microbial fuel cell with an electrode distance of 1.3 cm at an external voltage of 500 ohms

By applying an electrical resistance of 500 ohms to the first system, the electrical circuit is out of the open circuit state, and the potential difference drops from 742 mV to 130 mV. Over time, the potential difference decreased again and reached 86 mV after 19 hours. To compensate for the voltage drop like the open circuit state, 8 ml of fresh feed was injected into the microbial fuel cell with a ratio of 1:1 from the feed solution. An arrow in the figure indicates this action. As Figure (4) shows, adding fresh wastewater results in an increase in the potential difference. Such an increase is caused by the decomposition of wastewater and the release of electrons resulting from oxidation-reduction. Hydrolytic enzymes on the surface of bacteria convert suspended organic particles into soluble organic compounds that bacteria can consume, resulting in an increase in voltage. The fresh feed has been injected at various voltages to reach the lowest voltage attainable in the 500 Ohm resistance (Figure 4). The maximum voltage obtained in this resistor was 514 mV, obtained after 265 hours after the start of measuring the voltage in the 500 Ohm resistor.

The wastewater consumed by microorganisms in this resistance was about 0.131, 0.133, 0.142, and 0.154 ml/h in the first to fourth injections. The wastewater consumed by microorganisms has increased from the first injection to the fourth injection. With time, as already stated, the organic compounds that had been dissolved and turned into simpler compounds increased, and the microorganisms had more available nutrients; therefore, the wastewater was consumed by them during the hours that passed since the beginning of the experiment. Increased. Furthermore, the average value of wastewater consumed during this resistance was about 0.140 ml/hour. Figure (5) shows the current intensity and electric power in 500 Ohm resistance.

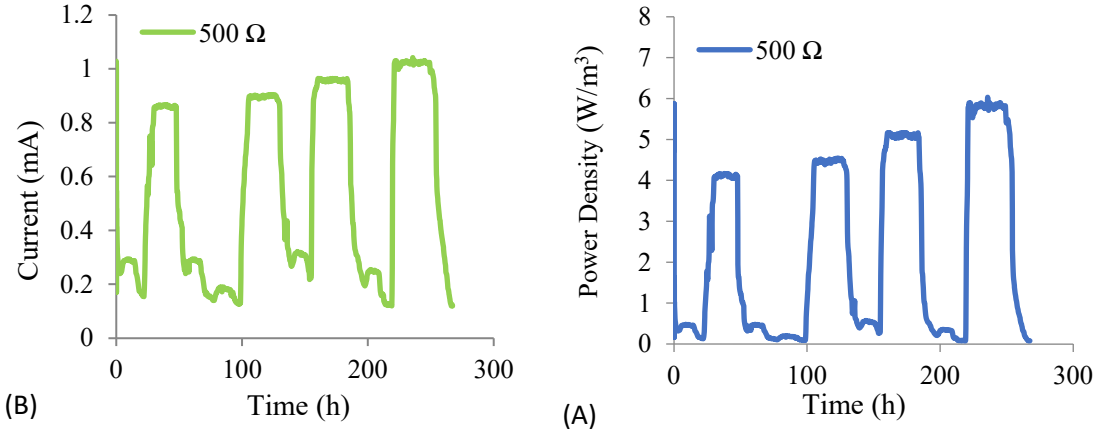

Figure 5. (a) power diagram, (b) current intensity of microbial fuel cell with 1.3 cm electrode distance, in hours at 500-ohm resistance

1. Applying lower external resistances and comparing system performance

Figures (6) and (7), respectively, indicate the changes in electric current intensity and output power in 300 Ohm resistance and its comparison with 500 Ohm resistance. In higher resistance, the system response was longer as the high external electrical resistance prevented the passage of more electrons. Thus, the waste consumption rate in the anode compartment decreased and caused the wastewater to produce electrons for a longer time in the anode. If the resistance decreased from 500 to 300 ohms, the time to reach stable voltage decreased from 265 hours to 211 hours. At 500-ohm resistance, the value of the injectable substrate increased from the first to the fourth injection.

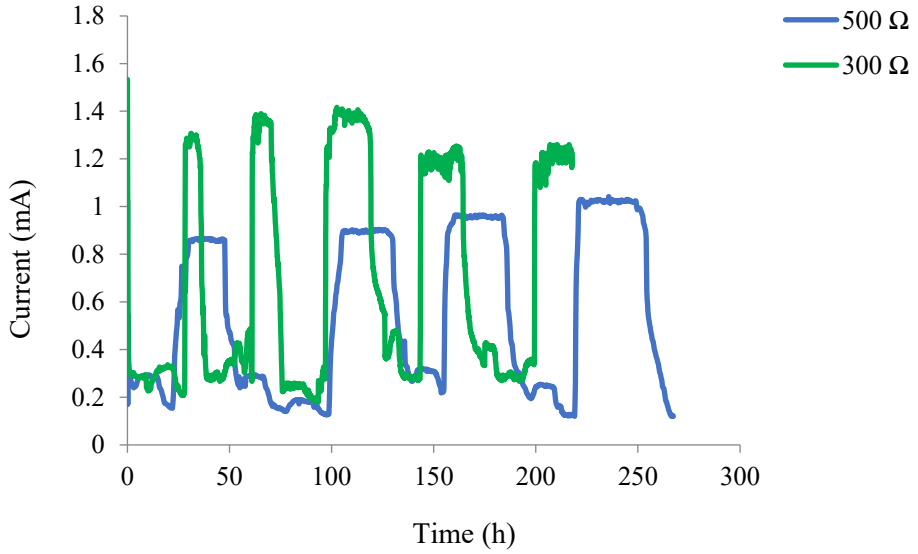

Figure 6. The electric current intensity in 500- and 300-ohm resistances

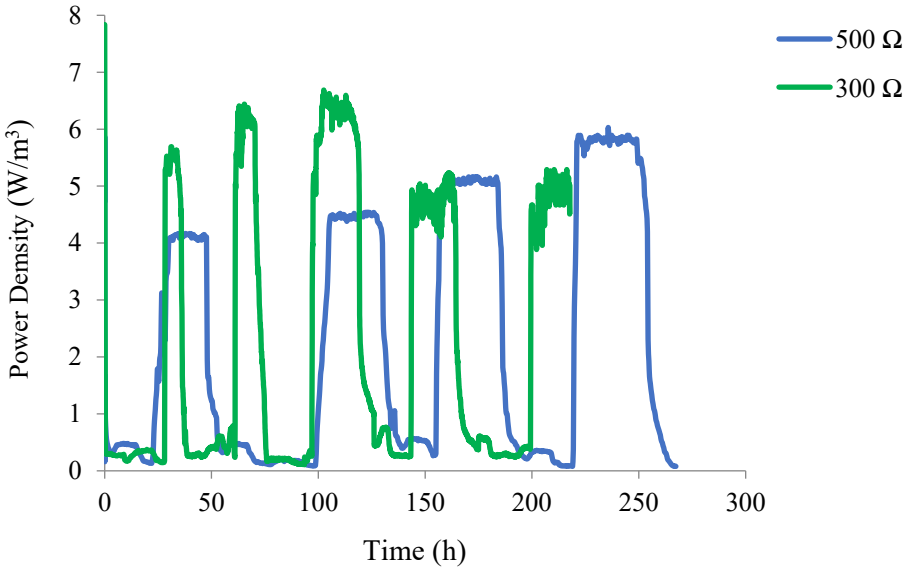

Figure 7. Power density in 500- and 300-ohm resistances

Table (1) shows that the substrate consumption has increased in the 300-ohm resistance compared to the 500-ohm resistance.

$CH_3COO^- + 2\ H_2O_2 \rightarrow CO_2 + 7\ H^+ + 8\ e^-$

$O_2 + 4\ e^- + 4\ H^+ \rightarrow 2\ H_2O$

Table 1. Comparison of substrate consumption in 300- and 500-ohm resistances

R_{ex} Ω	First)mL/ h(	Second)mL/ h(	Third)mL/ h(	Fourth)mL/ h(
500	0.131	0.131	0.142	0.154
300	0.157	0.171	0.186	0.204

Table (2) summarizes the comparison of the duration of the three increasing, static, and decreasing phases in each resistance. This phenomenon is associated with the increase in the thickness of the active biofilm on the surface of the electrodes. The substrate decomposes in less time with an increase in the thickness of the active biofilm.

Table 2. Comparison of the duration of increasing, static, and decreasing phases in 500- and 300-ohm resistances

	Cycle 1		Cycle 2		Cycle 3		Cycle 4	
Rex Ω	500	300	500	300	500	300	500	300
(h) Increasing phase	0±10.3	3.5	8.0±3.3	1.9	7	1.0±9.1	4	1.6
(h) Stationary phase	0±16.3	2.65	20.0±6.6	7.45	22	17	25.0±6.6	20
(h) Decreasing phase	8	4.4	10	6	14	10	16	12

2. Application of external resistance of 100 and 50 ohms

Examining the output current intensity in 100- and 50-Ohm resistances. For instance, Figures (8) (b) and (9) (b) are shown for a SCMFC with an electrode distance of 1.3 cm. The current intensity was measured in 100 Ohm resistance for 78 hours and 50 Ohm resistance for 168 hours, and the maximum current intensity for 100 Ohm resistance was obtained at 2.68 mA, and for 50 Ohm resistance, 2.48 mA. The mean wastewater consumed in 100 Ohm resistance is about 0.167 ml/h and 0.367 ml/h in 50 Ohm resistance. As is seen, wastewater consumed by microorganisms increases with a decrease in resistance.

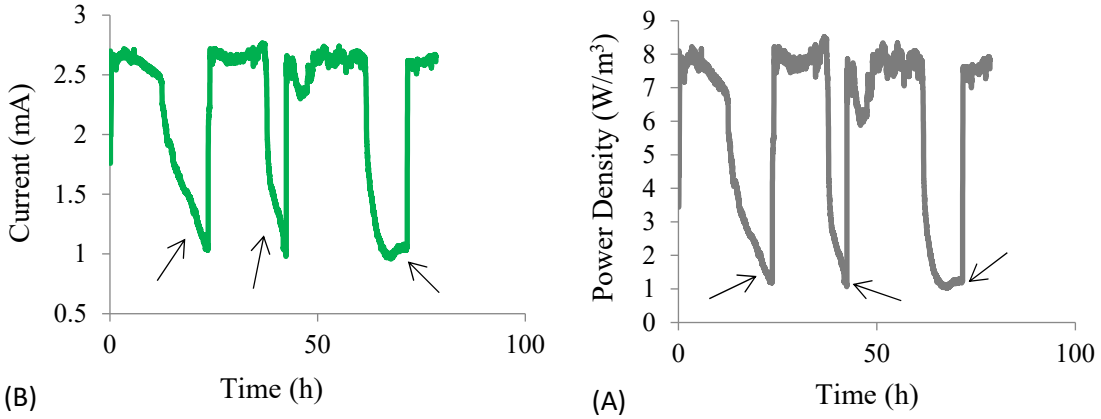

Figure 8. (a) power density, (b) current intensity in 100-ohm electrical resistances

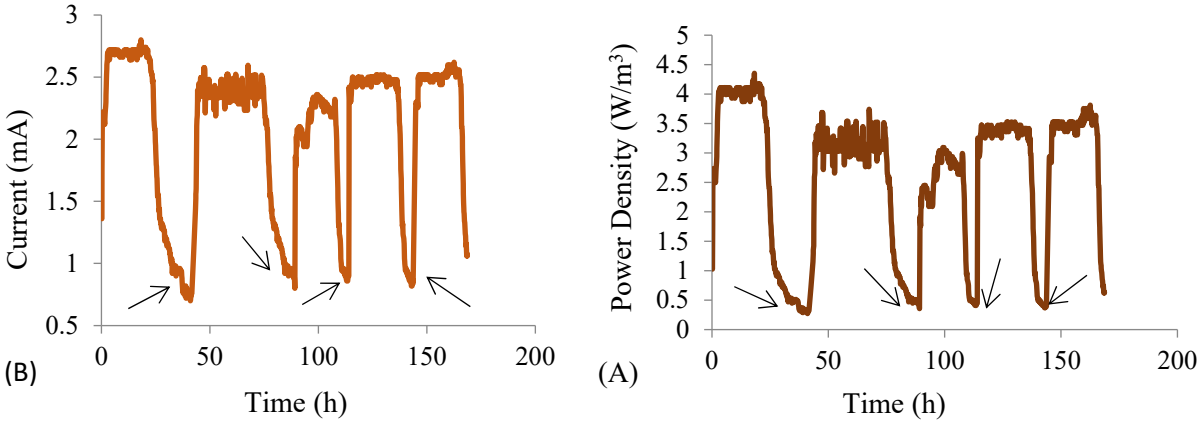

Figure 9. (a) power density, (b) current intensity in electrical resistance of 50 ohms

As the comparison of Figures (8) (b) and (9) (b) shows, in the first 21 hours, the current intensity for 50 Ohm resistance is higher than the stable current intensity in 100 Ohm resistance. In the resistance of 50 ohms, in the first 21 hours, the rate of electron passage is in balance with the rate of electron production by microorganisms; however, with time, the rate of electron passage exceeds their production rate, and microorganisms cannot provide the electrons that can be passed or are unable to provide electrons anyway. Therefore, the intensity of the current after 21 hours in the 50 Ohm resistance is lower than the 100 Ohm resistance. Figures (8) (a) and (9) (a) show the power density for the microbial fuel cell at an electrode distance of 1.3 cm, in resistances of 50 and 100 ohms. The maximum power density in 100 Ohm resistance was 7.98 $W/m^{3,}$ and in 50 Ohm resistance, 2.48 W/m^3. The maximum power density and intensity of the output current were obtained in the resistance of 100 ohms.

3. Microbial fuel cell with an optimal electrode distance of 0.7 cm

Figure (10) indicates the changes of potential difference measured during 257 hours. The maximum potential difference was measured to be 777 mV with a drop of approximately 10% from the open circuit value. Figure (11) shows the power and output current changes for this system in 1000 Ohm resistance. As is seen in the figure, the maximum power density and current were measured as 6.7081 W/m^3 and 0.777 mA, respectively. The overall behavior of the system in this electrode distance was almost the same as the first system, and the only difference was evident in the findings.

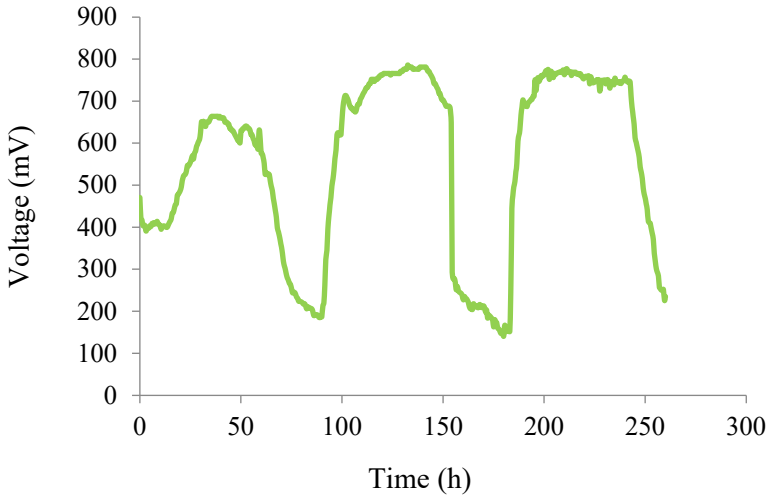

Figure 10. The potential difference of SCMFC with an electrode distance of 0.7 cm at an external voltage of 1000 ohms

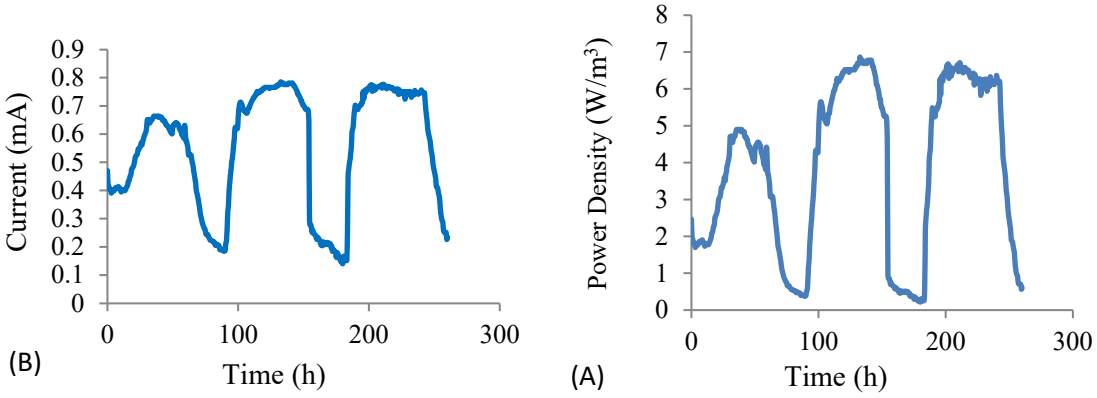

Figure 11. (a) power density diagram, (b) current intensity of microbial fuel cell with 0.7 cm electrode distance, in hours at 1000 Ohm resistance

4. Polarization diagram and power density

To obtain the polarization diagram, it is necessary to calculate the intensity of the output current in each resistance using Ohm's law and the relationship I=V/R. The polarization diagram was obtained by plotting the voltage changes in current.

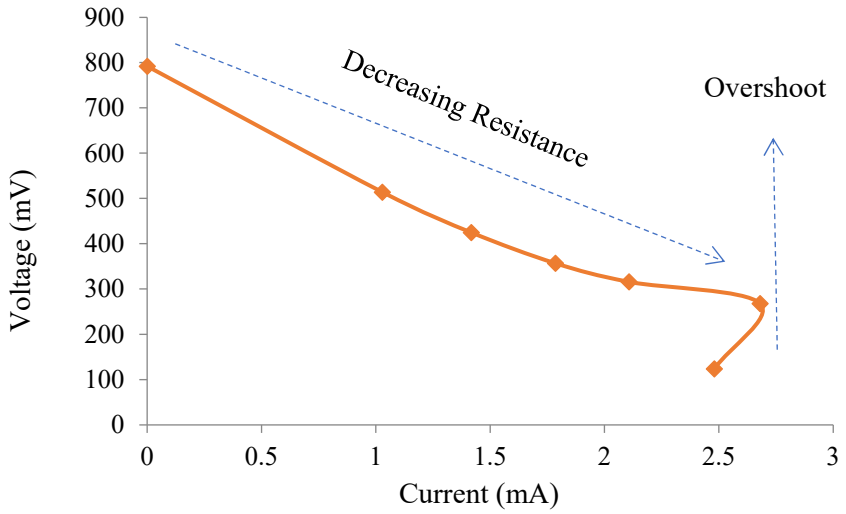

Figure 12. Polarization diagram of SCMFC with 1.3 cm electrode distance

As Figure (12) shows, the potential difference of the microbial fuel cell decreases and the current intensity increases with the decrease of electrical resistance (the direction shown in the figure). This reduction starts from the open circuit voltage (742 mV). The open circuit voltage is the starting point of the polarization diagram, which has infinite resistance and zero current. Ultimately, as the figure shows, further reduction of resistance from a certain value did not increase the intensity of the current, and the intensity of the current resulted in a sharp drop; this phenomenon is known as overshoot.

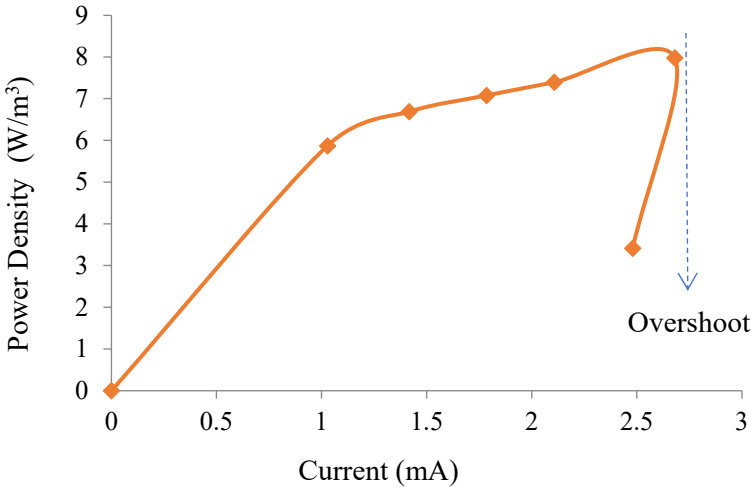

Figure 13. Power density diagram of a single-chamber microbial fuel cell with an electrode distance of 1.3 cm

As is seen in the power density diagram in Figure (13), the power of the microbial fuel cell increased until it reached its maximum value at 100-ohm resistance with the reduction of electrical resistance. The maximum power obtained from this system is 7.98 Wm^3. The power density diagram shows that the decrease in electrical resistance resulted in a sharp drop in power, and an overshoot phenomenon appeared. The dashed line shows this phenomenon in Figures (12) and (13).

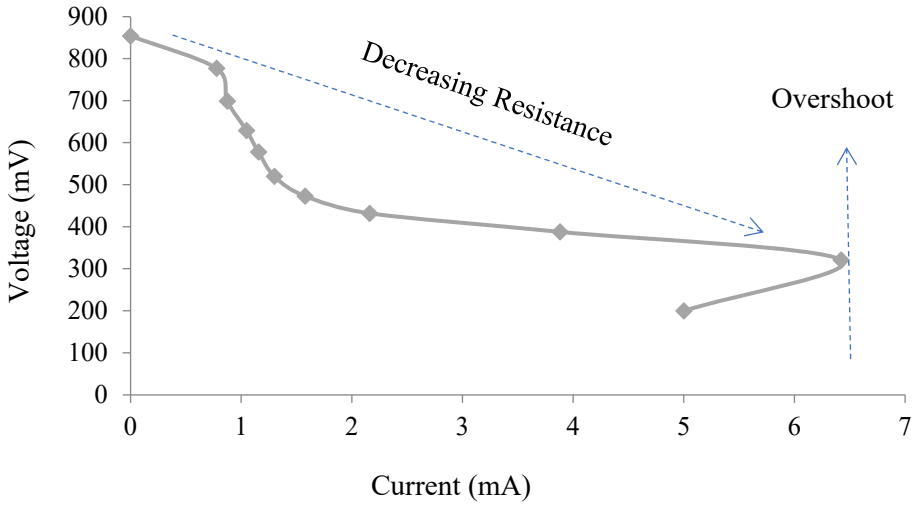

Figure 14. Polarization diagram of SCMFC with an optimal electrode distance of 0.7 cm

Figure (14) indicates the polarization diagram for the second system at the electrode distance of 0.7 cm. As is seen, the maximum current value of 6.42 mA was obtained in the resistance of 50 ohms in the second system.

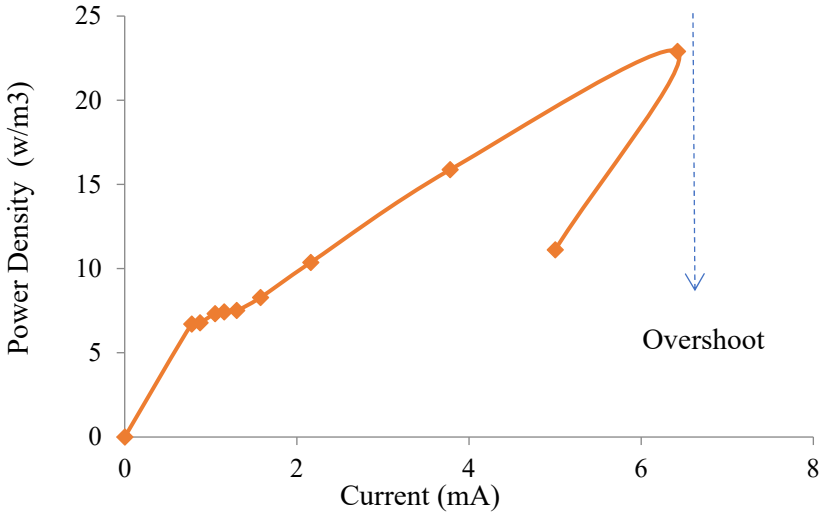

Figure 15. Power density diagram of SCMFC with an optimal electrode distance of 0.7 cm

As Figure (15) shows, the maximum power of the microbial fuel cell with an electrode distance of 0.7 cm is 22.898 W/m^3 at an external resistance of 50 ohms. When we reduced the resistance to 40 ohms, the power was reduced to 11.11 Wm^3. As stated, at the point where the power becomes maximum, the internal resistance of the microbial fuel cell is the external resistance, so the internal resistance of the microbial fuel cell with an electrode distance of 0.7 cm was calculated to be about 50 ohms. This resistance was lower compared to the internal resistance of the first system.

5. Examining the reduction of wastewater turbidity

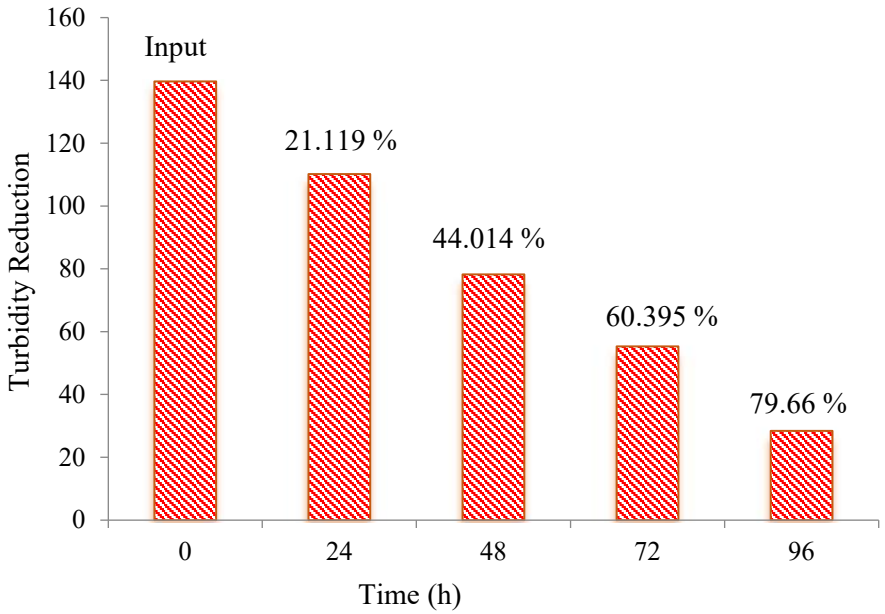

Figure 16. Turbidity reduction of the chocolate industry wastewater using a SCMFC

Figure (16) indicates the reduction of turbidity by the SCMFC system. The wastewater turbidity reduction is a function of the residence time of the wastewater in the microbial fuel cell, as well as the amount of initial turbidity. The initial turbidity of the wastewater was measured as 139.68 NTU. Turbidity was measured using a turbidity meter. As the figure shows, the turbidity has decreased to 44.01% after 48 hours and to 80% at the end of 96 hours. Such a decrease shows the success of the system in wastewater treatment and the decomposition of complex organic and colloidal substances in it during biological processes by microorganisms.

6. Examining the reduction of chemical oxygen demand

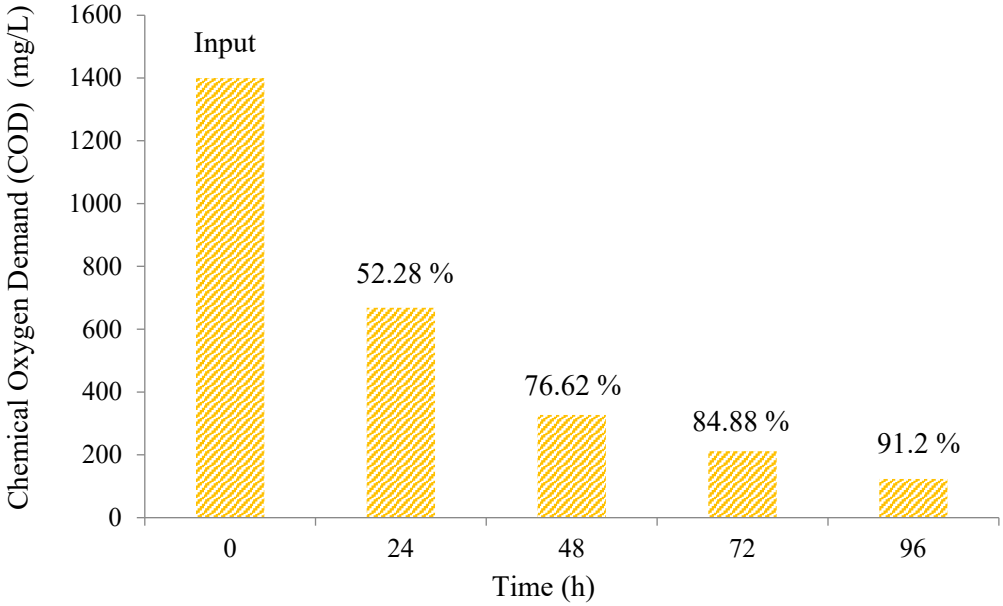

Figure 17. The reduction of chemical oxygen demand in terms of time in 100 Ohm resistance

Figure (17) indicates the oxygen demand reduction needed for a SCMFC. The incoming wastewater with a COD of about 1400 mg/liter was fed into the microbial fuel cell. The electrical resistance of 100 ohms was applied to the system as a consumer of electrons resulting from the decomposition of organic substances. The chemical oxygen demand of the wastewater was measured for 96 hours. As Figure (17) indicates, COD has decreased by 76.62% after 48 hours and by

91.2% after 96 hours. Such a reduction in chemical oxygen demand indicates the success of the experiments in wastewater treatment in the minimum time.

Conclusion

The study used two air SCMFCs with a volume of 90 ml. stainless steel mesh with graphite coating was used as anode in spiral geometry to reduce the manufacturing cost. Graphite coating reduces the resistance and, at the same time, increases the conductivity of the anode. Chocolate industry wastewater with a high oxygen demand load and containing large amounts of industrial detergents with high stability and hardness was selected as the SCMFC feed to prove the system ability to produce electricity and purify wastewater.

The first fuel cell system with an electrode distance of 1.3 cm succeeded in producing a voltage of 742 mV in the open circuit mode, and the maximum power and current for this system, respectively, 7.98 W/m^3 and 2.68 mA, were obtained during the resistance range of 100, 150, 200, 300, 500 and 50 ohms. A re-examination was made on all the factors affecting the power increase to find the cause of this difference, as the power obtained for this system was a large difference outside the expected limit and lower than the values previously reported by the same structure. The study results showed that the only parameter not discussed in the previous study was the initial distance between the cathode and anode electrodes. After finding the optimal distance between the electrodes, the open circuit potential difference was re-measured at three distances of 1, 0.7 and 0.4 cm in the second fuel cell system. Ultimately, the best result and the largest potential difference were obtained at X=0.7 cm distance, and this distance was reported as the optimal distance between the cathode and anode electrodes, and the polarization and power density tests were like the first fuel cell system. This time it was repeated in a wider range of resistances, including 1000, 800, 600, 500, 400, 300, 200, 100, 50 and 40 ohms.

It was the first test examined in both microbial enrichment systems and open circuit voltage measurement. To increase the biolayer thickness, fresh wastewater was introduced into the microbial fuel cell to stimulate the system at each stage when the potential difference started to decrease. In both systems, the speed of voltage increase was higher than the speed of its increase in the previous stage, happened because of the increase in the thickness of the active biolayer on the surface of the electrodes, and the substrate decomposes in a shorter period with the injection of fresh wastewater in each stage. As already stated, the maximum voltage obtained for the first system in the open circuit mode was 742 mV, which increased to 856 mV after optimizing the electrode distance.

Then 500 Ohm electric resistance was selected as the first consumer of electric current in the first system. Recording the potential difference over time and injecting fresh wastewater at each stage resulted in a noticeable increase in the potential difference. Such an increase shows that the microbial ecology of the system is affected due to applying external resistance. Replacing fresh wastewater in the system ended in an increase in its potential difference. Such an increase occurred due to the decomposition of wastewater and the release of electrons from the oxidation-reduction reaction. Reducing the wastewater concentration available for microorganisms results in a decrease in the flow intensity of the microbial fuel cell. Indeed, during this reduction, the electrons passing through the electrical resistance are more than their production through waste decomposition. Considering three phases for the potential difference of the system, during the injection of wastewater to it until the voltage decrease, the phase of increasing current intensity was faster in each phase than the previous phase, the static phase was longer, and the phase of reducing the potential difference was slower. Increasing the increasing phase in each stage relative to the previous stage is associated with microbial enrichment.

The maximum power for the system with an electrode distance of 1.3 cm in resistance of 100 ohms was obtained with a value of 7.98 Wm^3. Moreover, the maximum current intensity and power density for the second system with the optimal electrode distance of 0.7 cm in the resistance of 50 ohms were 6.42 mA and 22.898 W/m^3. The internal resistance for the first system with an electrode distance of 1.3 cm was calculated to be 100 ohms. On the contrary, using the same annular structure and the wastewater of the chocolate industry, but with an optimal electrode distance of 0.7 cm, the internal resistance is reduced to 50 ohms. Hence, optimizing the electrode distance and reducing it by 46.15% has significantly reduced the internal resistance and greatly increased the output power.

The maximum current intensity decreased from 3.77 mA to 2.76 mA by reducing the oxygen demand of wastewater from 1400 mg/L to 700 mg/L. Furthermore, the duration of the decreasing phase of the flow was also reduced.

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Conflict of interest

None.

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Ethics statement

None

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