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## Effect of different concentrations of substrate in microbial fuel cells toward bioenergy recovery and simultaneous wastewater treatment

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### ABSTRACT

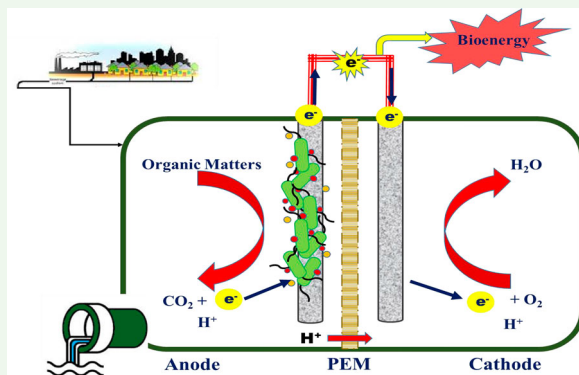
Microbial fuel cells (MFCs) is a promising and expanding technology able to eliminate various pollutants of wastewater while converting its chemical energy into power energy using biocatalysts. The potential application of double-chamber microbial fuel cell (DC-MFC) for chemical oxygen demand (COD) removal and generated power from wastewater in the different conditions is investigated. DC-MFC is operated with anaerobic sludge as an active biocatalyst in an anode section, an aerobic cathode section and a Nafion117 membrane as a separator. The performance of the bioreactor is determined with different concentrations of chemical oxygen demand (COD) loadings in the MFC process, in terms of COD removal, power generation and columbic efficiencies. The results illustrated that COD removal efficiency increased at the high concentrations of organic matter. So that at COD concentration of 2000.0 mg/L the highest COD removal efficiency (84%) was obtained. But with increasing substrate initial concentration to 10000.0 mg/L the efficiency decreased to 79%. The important outputs of the system like the highest voltage, maximum generated power, current density, and energy efficiency with the 100,000 mg/L COD are 447 mV, 50.7 mW/m<sup>2</sup>, 570.0 mA/m<sup>2</sup>, and 18.6%, respectively. The optical density levels increased due to bacterial growth while pH severely decreased in the anode chamber when using high-concentration substrates in the MFC.

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Microbial fuel cell; bioenergy production; wastewater treatment; microbial electrochemical technology; bioelectricity





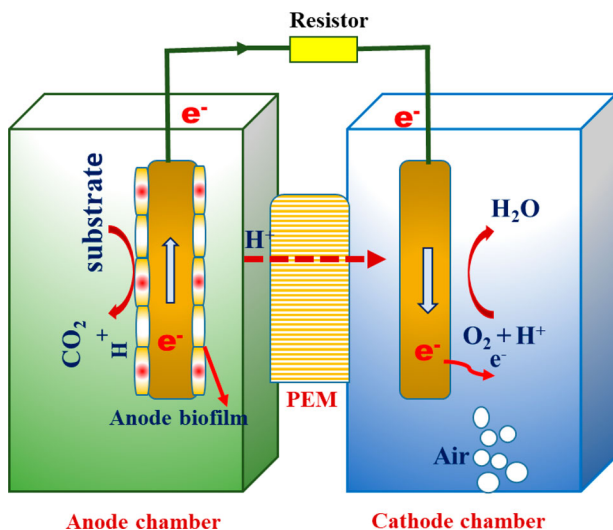
### Highlights

- MFC<sub>s</sub> as a bio-friendly technology able to eliminate pollutants of wastewater
- Performance of MFC for COD removal in different concentrations of substrates
- Discussion on the substrate for increase power production.
- Environmental parameters affecting the activity of electrogenesis bacteria.

### 1. Introduction

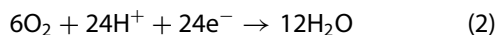
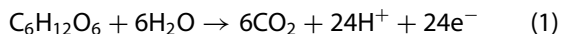
Microbial fuel cells (MFCs) can eliminate the organic matter and produce electricity and have recently been exploited as an innovative technology for wastewater treatment [1]. In general, MFCs utilise microorganisms as catalyst for production of energy at the anode chamber. Several applications are contemplate for MFCs, including wastewater treatment [2,3], power

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**Figure 1.** Schematic illustration of the double – chamber microbial fuel cell.

production, and metal recovery [4]. In addition, these fuel cells employed microorganisms as the active biocatalyst to generation of electrical energy from the chemical energy (organic substrates) [5]. The cathode and anode chambers are separated by proton exchange membranes (PEM) in a divided microbial fuel cell (Figure 1 (a)). Further, energy is generated in an anaerobic anodic chamber where the organic material is oxidised to carbon dioxide by electrochemically active microorganisms and produces electrons and protons (Equation (1)). These electrons and protons are then transferred to the cathode chamber by the external circuit and PEM, respectively, and consumed at the cathode chamber by using the electron acceptors ( $O_2$ ) [6], such relation is displayed by Equation (2).



Voltage in MFCs is production due to the potential difference of the electron acceptor and the oxidative system. The current flows from anode to cathode when the anode and the cathode compartments of MFCs are adjoined through a resistor [7]. Diverse factors affect the efficiency of MFCs, including:

- Type and concentration of organic substrate in the anode section [8];
- Supply and utilisation of the electron acceptor (oxygen) in the cathode chamber [9];
- Electron transfer from anolyte to the surface of the anode electrode [10];
- Permeability of PEM [11];
- Anode surface area and material [12].

Furthermore, columbic efficiency (CE) in MFCs is affected by multiple parameters such as external/internal resistances [13], organic matter concentration [14], microorganism community [15], various electron acceptors, and the reactor or electrode chamber design [16].

Various substrate can be employed d in MFCs for bioelectricity production for instance carbohydrates, amino acids and fatty acids [17]. Glucose, acetate and other carbohydrates as simple molecules have been tested as electron donors to power generation [18]. Researchers are using pure substrates to study the effect of different parameters on the performance of the microbial fuel cell. For instance, electricity generation from several carbohydrates in the air cathode MFCs using a mixed bacterial culture was tested and The CE ranged was obtained from 22% to 34% with over 80% COD removal [19]. Rahimnejad and etal have investigated a novel stack of MFCs in a continuous mode, 30 g/L of pure glucose, *Saccharomyces cerevisiae* as an active biocatalyst in anode resulted in 22% as CE [5].

Considering the above-mentioned explanations, this study aims to produce bioenergy from glucose as the substrate in double-chamber MFC. Moreover, the study investigated the effect of an increased COD concentration on cell potential, removal COD, and the optical density of an MFC. To this end, the current study studied the efficiency of the MFC bioreactor in removing the organic material (COD) from wastewater, which simultaneously determined the amount of the produced electrical energy. Finally, voltage, current, power density, and pH changes were measured in the anode chamber according to the substrate concentration, residence time, and its impact on the removal efficiency.

## 2. Materials and methods

### 2.1. Experimental set-up of double – chamber microbial fuel cell

All experiments were performed by using a double-chamber microbial fuel cell (DC-MFC) reactor, which functioned in a batch mode at a deterministic temperature of 30°C and the atmospheric pressure. The homemade double-chamber microbial fuel cell fabricated from plexiglass and two chambers of the same size (100 × 100 × 30 mm), which were separated by a Nafion 117 (Sigma-Aldrich, USA) with an interchange measured area of 25 cm<sup>2</sup> as shown Figure 1 [20]. In order to remove any impurities of Nafion, Several pre-treatment steps were taken. It was first boiled for 1 h in H<sub>2</sub>O<sub>2</sub> (3.0%), then was washed with deionised water and 0.5 M H<sub>2</sub>SO<sub>4</sub> and eventually, washed further with deionised water [21]. The effective volume of each anodic and

cathodic chamber was 240 mL, with the fixing of two carbon felt (30 × 50 × 5 mm) as anode and cathode electrodes with both of effective surface area of 30 cm<sup>2</sup>.

In order to purify the carbon felts, they were first soaked in acetone for 20 min and boiled in HCl (0.1 M) for 15 min before using then was washed with deionised water once more to remove all foreign substances from the surface of the electrodes [22]. Data were automatically recorded using an analogue/digital data logger (Danesh Gostar Hamgam Ba Sanat Companyand, Iran) and the external resistance of 1 KΩ were coupled to the electrodes by the copper wire with 0.5 mm diameter [23]. Figure 1 Schematic illustration of the experimental setup of the DC-MFC.

## 2.2. Microorganisms and anolyte preparation

Complex microorganisms of the anaerobic sludge of the Hamadan city refinery was applied as the inoculum and the used media included peptone, yeast extract, and glucose. Likewise, the anodic chamber was equipped with the anaerobic process tank of the wastewater treatment centre in Hamadan. Next, it was fed with 240 mL synthetic wastewater as a substrate encompassing (g/L) glucose (0.5, 1, 2, 5, & 10), KH<sub>2</sub>PO<sub>4</sub> (0.25), K<sub>2</sub>HPO<sub>4</sub> (1.4), NH<sub>4</sub>Cl (0.31), CaCl<sub>2</sub> (0.1), MgSO<sub>4</sub> (0.1), NaCl (0.1), KCl, (0.13), and trace mineral solution (0.1 mL) [24]. The anolyte was regulated for anaerobic microorganism activities at the pH of 7.4 by phosphate buffer. Similarly, the anodic chamber was purified by nitrogen gas before adding inoculums, in order to remove oxygen and provide an appropriate anaerobic condition.

## 2.3. Experimental process design

The efficiency of the MFC in the batch system was evaluated based on different chemical oxygen demand (COD) concentrations [25]. The continuous aeration was accomplished at the bottom of the cathode chamber to provide mixing and supply air bubbling with an aquarium pump. The Then, MFC performance was investigated in COD concentrations of 500.0, 1000.0, 2000.0, 5000.0, 10000.0 mg/L at different contact times. The voltage, current, and power density were calculated based on the substrate concentration, residence time, and its impact on the removal efficiency. The growth kinetics were investigated in order to estimate the required time for the bacteria to reach a steady state. Then, the optical density (OD) of the bacterial growth at different concentrations of the organic matter was measured by sampling every 2–4 h, followed by determining the percentage of the light absorption at 620 nm (photometer 6000, HACH).

Eventually, the formation of the biofilm layer onto the anode was determined by performing the scanning electron microscope (FE-SEM) technique.

## 2.4. Data processing and analysis

The computer connected to the system recorded the power, current, and voltage. The power output was constantly recorded every 10 min for 120 h. When the maximum available voltage output was approached, power density curves as a function of current density were recorded by altering the external resistance from 0.01 to 100 KΩ.

The power densities normalised relying on the projected anodic surface area [26]. The data logger provided power parameter by multiplying the current and voltage. Additionally, the provisions were supplied for the online observation of the polarisation plot, which reported the variation of voltage and DC-MFC power density with regard to the current [20].

## 2.5. Calculations methods

In a typical procedure, sampling was done from chambers (5.0 ml) in order to evaluation of pH, OD and COD removal. In this way, the samples were filtered via a Whatman filter with a pore size of 0.45 μm [6]. The calorimetric procedure was employed for the measurement of wastewater COD removal with closed reflux at 600 nm (the Palintest system, photometer 6000, England). Moreover, Equation (3) was used for calculation of the COD removal value [27]:

$$R = \frac{C_i - C_f}{C_i} \times 100\% \quad (3)$$

Where  $R$  is COD removal %,  $C_i$  is the initial concentration of COD in mg/L, and  $C_f$  is the final concentration of COD in mg/L, respectively.

A data logger connected to a computer was employed for the recording of the cell voltage of MFC reactors in real time. In the batch system the polarisation plots were drawn at a steady-state (0.1 to 100 KΩ) for assessment of power efficiency [5]. Also, in order to calculate the power density (mW/m<sup>2</sup>) and current density (mA/m<sup>2</sup>) parameters, Equations (4) and (5) were used, respectively:

$$I = \frac{V}{RA} \quad (4)$$

$$P = \frac{RI^2}{A} \quad (5)$$

Where  $P$  and  $V$  denote the power density and the

measured cell voltage, respectively. Furthermore,  $R$ ,  $I$ , and  $A$  indicate the electrical resistance, the produced current, and the total surface area of the anode electrode ( $30 \text{ cm}^2$ ), respectively. The current of MFC system was determined via dividing of the recorded voltage by the applied resistance. Then, the achieved power by the MFC reactor was calculated by multiplying the voltage in current values [28].

The achieved coulombic efficiency for MFC system was computed in terms of experimental to the theoretical ratio of coulombs according to the Equation (6) in batch mode [6,22]:

$$CE_{sys} = \frac{8It}{FV_A\Delta COD} \times 100\% \quad (6)$$

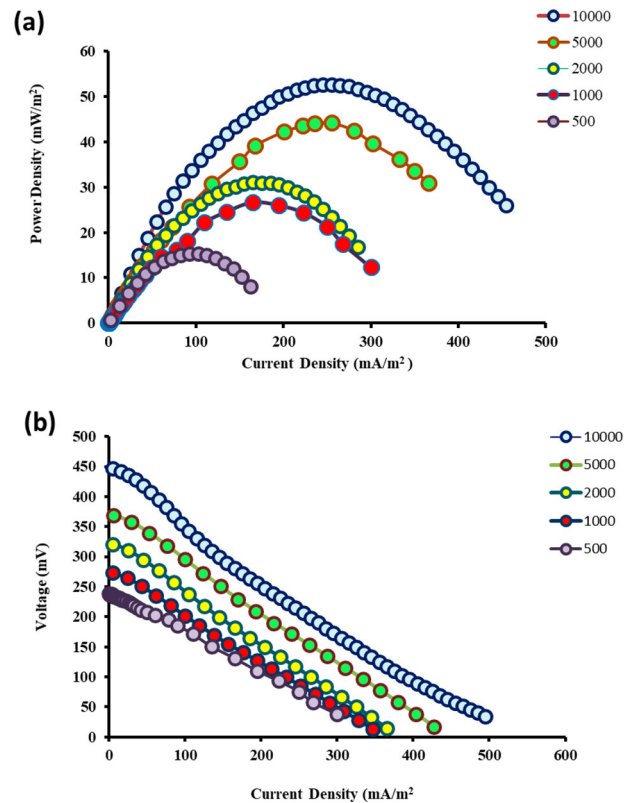
Where  $CE_{SYS}$  is percentage coulombic efficiency (%),  $t$  is the operation time (s) and  $I$  represent the achieved current of the MFC system (A), respectively. Also,  $F$  represents the Faraday's constant ( $96,485 \text{ C/mol}$ ),  $V_A$  is the volume of anolyte for batch operation (L) and  $\Delta COD$  express the organic matter removal changes (mg/L) [6].

The surface morphology of the anode was evaluated by utilising a scanning electronic microscope (FE-SEM, NANOSEM 450, USA) before and after the experiment. The graphite electrodes were aged overnight in glutaraldehyde solution (2.0% v/v) under the condition of ( $\text{pH} = 7.5$ ,  $4^\circ\text{C}$ ) to modification of electrode surface by immobilisation of bacteria. After this, the modified electrodes were immersed in the water/alcohol solution (30.0–70.0% v/v) for the hydration process. Then, the FE-SEM images of the modified electrodes (with gold coating) were recorded at a voltage of 20 kV [21].

### 3. Results and discussion

#### 3.1. Power generation of wastewater in double-chambered microbial fuel cells (DC-MFCs)

Preliminary experiments conducted by a DC-MFC represented that electricity was generated by using the synthetic wastewater and the required microorganisms were presented in the wastewater. The batch mode of functioning is considered essential for determining the best conditions regarding reaching the maximum electrical output [29]. The efficiency of the MFC reactor was evaluated by recording the polarisation plots and calculation of the maximum power generation. Also, normalised power density was achieved in terms of the anode surface area or the reactor volume. Figure 2 shows the voltage output and power density plots as a function of current density with a different initial chemical oxygen demand (COD). As shown in Figure 2(a), the maximum power and current densities are  $50.7 \text{ mW/m}^2$



**Figure 2.** Power density and voltage as a function of current density (a), (b) in batch DC-MFC with different initial COD concentrations.

and  $277 \text{ mA/m}^2$  at COD  $10000.0 \text{ mg/L}$ , respectively. In addition, the power densities are  $15.3$ ,  $26.1$ ,  $31.0$ , and  $44.2 \text{ mW/m}^2$  at the COD of  $500.0$ ,  $1000.0$ ,  $2000.0$ , and  $5000.0 \text{ mg/L}$ , respectively (Figure 2(a)). The results confirmed that COD concentration is an important factor for electricity generation. The certain resistances were used in the current path for the adjusting of Open-Circuit Voltage (OCV), in different COD concentrations at the batch system. The gradual increase of cell voltage up to steady-state (maximum amount) could be seen due to the biological activities. This pattern indicates a stable colonised microbial colony in the anode section under optimised conditions ( $447.0 \text{ mV}$  in COD  $10000.0 \text{ mg/L}$ , Table 1) (Figure 2(b)).

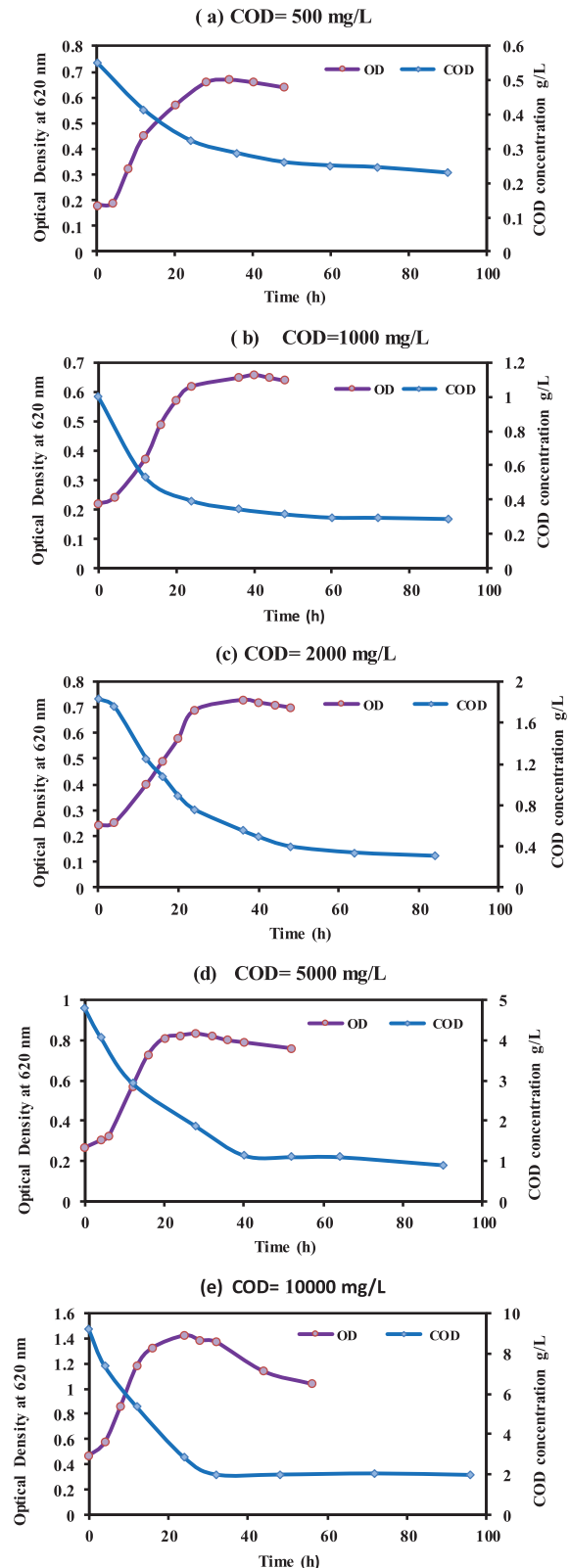
**Table 1.** Comparison of performance DC-MFC with different initial COD concentrations in power generation, current density, Coulombic efficiency.

COD concentration mg/L	Voltage mV	Current density mA /m <sup>2</sup>	power densities mW/m <sup>2</sup>	Coulombic efficiency%
500	240	103	15.3	11.5
1000	273	165	26.1	12.1
2000	321	175	31	13.7
5000	370	255	44.2	15.6
10,000	447	277	50.7	16.4

A different source of organic matters can be caused by the same increase in the initial voltage of MFC systems [26]. It is noteworthy, the increasing of COD in the anodic section can have a positive effect on the power generation. In other words, the implementation of the system with the higher COD and prolonged-time conclude at the relatively good bioelectricity production [30]. Furthermore, the substrate quality (as porosity, material and roughness) and the bacterial community that develops during adsorption can be an effect on the activity of microbial and the maximum generated power from the wastewaters.

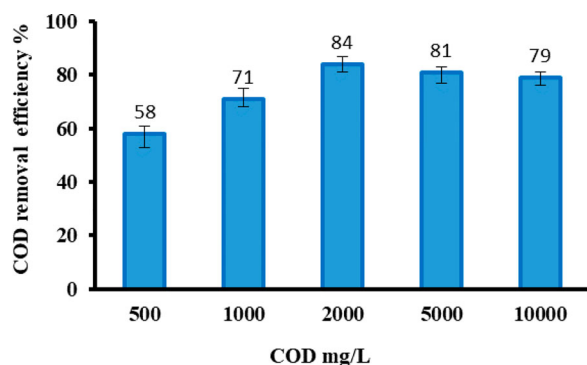
The only carbon source for the anaerobic bacteria in the anodic chamber of microbial fuel cell (MFC) reactor was different concentrations of glucose. The removal efficiency of chemical oxygen demand (COD) and the changes in OD (optical density) trend during the time was investigated over 90 h (Figure 3(a–e)). The findings indicated that COD concentration decreased in the anode section at all substrate concentrations by the activity of anaerobic microorganisms of the anode chamber, and the removal efficiency of the organic matter was constant after approximately 30 h. On the other hand, the OD resulting from the growth of the microorganisms increased over time simultaneously with the decomposition of the organic matter. Then, the growth rate of bacteria became constant after 30 h and the reactor was in a constant growth phase. In a batch MFC system, the lack of organic matter and an increase in environmental acidic and toxic substances limit the growth of microorganisms over time thus the growth curve shows a decreasing trend.

Nearly 58% of the organic matter was biodegraded and the COD removal efficiency was constant following installing the MFC at COD concentration of 500 mg/L after approximately 40 h. The bacterial growth curve was evaluated by determining the OD due to the bacterial population growth and it was revealed that OD increased from 0.2 to 0.7 overtime. The reduction in nutrition concentration and changes in the bacterial growth curve were also the same at other substrate concentrations. More specifically, the microbial growth initiated with a lag phase followed by a rapid and sharp tangent entry into the logarithmic phase. The bacteria entered the constant growth phase at different substrate concentrations after nearly 30–40 h, in which the growth rate of bacteria was equal to their mortality rate. However, OD decreased and the growth curve entered the logarithmic death phase over time and following a reduction in nutrients and the existence of inappropriate environmental conditions. The highest increase in OD was related to the COD concentration of 10,000 mg/L, which increased by 1.4 due to the



**Figure 3.** COD#concentration (g/L) and growth curve of bacteria (OD) as a function of time in DC-MFC with different initial COD concentrations.

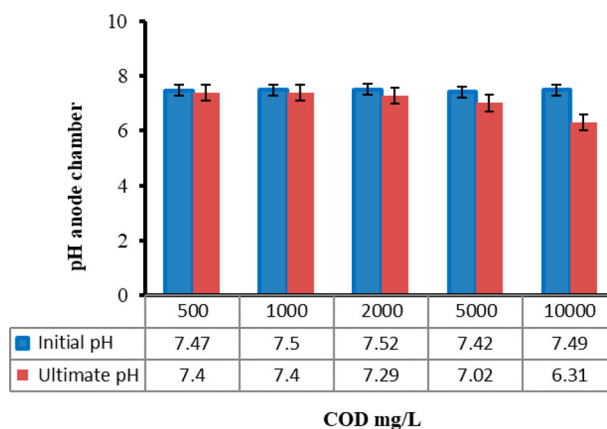
presence of nutrients with high concentration. Then, it was in a constant growth stage, and finally, decreased sharply due to increased acid content in the medium.



**Figure 4.** COD removal efficiency (%) in DC-MFC with different initial COD concentrations.

### 3.2. COD removal survey via the anodic bio-oxidation

Batch experiments were conducted to determine the required parameters for the optimum conditions including the percentage of COD concentration in the anode chamber to reach the maximum wastewater treatment. The performance of the coupled system regarding using the synthetic wastewater was evaluated based on the COD removal efficiency (Figure 4). The carbon content of wastewater as an electron donor source in the metabolic procedure causes the simultaneous decay of the substrate and power generation, too. The pH of 7–7.6 is considered as the best value for activity of microorganism in the anaerobic condition [31], thus the initial pH of the anode chamber was adjusted in the range of  $7.4 \pm 0.1$  in the MFC reactor with different COD concentrations. The pH decreased and became acidic in the anode chamber after the decomposition of organic matter by anaerobic bacteria and the production of organic acids. The produced  $H^+$  in the anaerobic reaction of organic matter decomposition was transferred to the cathode section via the PEM membrane, preventing the drastic pH reduction and inadequate environmental conditions of the anode chamber for the continuation of bacterial growth [18]. At low COD concentrations, the decrease in the pH of the anode chamber was negligible due to biochemical reactions. As shown in Figure 5, the pH decreased from 7.47 to 7.4 at a COD concentration of 500 mg/L. In addition, the accumulation of organic acids due to biodegradation in the anode section increased at the high concentration of organic matter, causing further anodic acidification. Therefore, the conditions were inadequate for microorganism activity and reduced COD removal efficiency. The final anodic pH decreased to 6.31 after 96 h in the installed MFC with the initial COD concentration of 10,000 mg/L. According to the previous reports, the highest current density and maximum COD



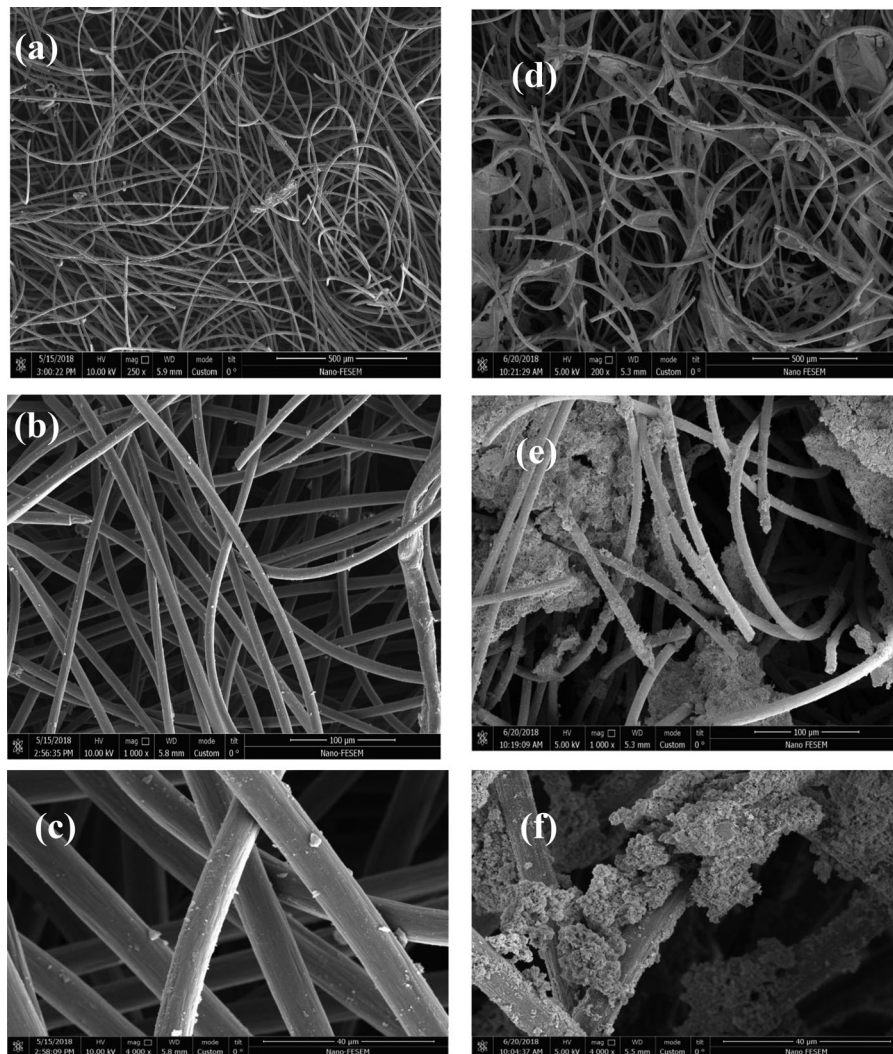
**Figure 5.** Initial and ultimate pH in anode chamber with different COD concentrations (mg/L) in batch DC-MFC.

removal percentage can be obtained at the  $pH = 7.0$ . In other words, the rate of microbial activity is slow at the acidic pHs.

Based on the COD removal percentages at different initial COD concentrations (Figure 4), biodegradation of the synthetic wastewater in the anodic chamber of DC-MFC reactor is partly low. The maximum COD removal percentage in the anodic section is 84.0% in COD 2000.0 mg/L while the removals of COD were 58.0, 71.0, 81.0, and 79.0% with CODs of 500.0, 1000.0, 5000.0, and 10000.0 mg/L, respectively, showing a slight reduction. The anodic chamber of the MFC acts as an anaerobic growth reactor, which indicates the high potential of MFC as the replacement with biological treatment processes [32–34]. The obtained results revealed that utilising the seed sludge and wastewater as the microbial source and fuel, respectively, leads to the best efficiency, and therefore there is any need to the another microbial source, which is in conformity with the results of Ahn and Logan [35].

### 3.3. Columbic efficiency and energy recovery

The aim of the present study is wastewater treatment and power generation via the anodic bio-oxidation. Meanwhile, the capacity of stored electrons in the biomass material in terms of current, and the possibility of recovering generated energy in the MFC system have investigated [36]. The recovered energy percent for the easily biodegradable materials (electron donor) such as glucose, acetate, along with amino and organic acids can be changed from 2.0% to 50.0% in the MFC systems [17]. Generally, the produced electrons in the MFC reactor can be consumed for different purposes as power output, bio-electrochemical reactions and the other electron acceptors [37].



**Figure 6.** FE-SEM Images of the (a, b & c) bare Carbon Felt; (d, e & f) attachment of microorganisms on the anode surface after inoculation (large and close view).

As shown in Table 1, the  $CE_{SYS}$  was 11.5% using COD 500.0 mg/L and increased up to 16.4% by COD 10000.0 mg/L. The  $CE_{SYS}$  was calculated with regard to the energy value of the used up COD in the anodic section. According to the previous research, the obtained  $CE_{SYS}$  for the simple substrate-fed MFC systems such as glucose or acetate is higher than the real wastewater-fed systems [6]. Rahimnejad et al. found a  $CE_{SYS}$  value of up to 13.0% by the glucose-fed MFC system [5]. The consumption of sugar by the microorganism conclude in the decreasing of  $CE_{SYS}$  because of the created intermediates [16]. Thus, the generated electricity during the synthetic wastewater treatment in the MFC reactor can be simultaneously utilised in the wastewater treatment procedure [36].

### 3.4. Morphological characterisation

The surface morphology of modified electrodes was surveyed by FE-SEM technique. Figure 6(a–c) shows the FE-

SEM image of the bare Carbon felt electrode before pre-treatment by microorganisms (large and close view). According to this image, jagged and porosity of Carbon felt surface can be used for placement and growth of bacteria. As an interesting result, Figure 6(d, e) and f illustrate the grown and immobilised microorganisms onto the anode surface exposed in the MFC systems at the end of the process. Both sides of the electrode have covered with the microorganisms after inoculation.

## 4. Conclusions

The results of this survey proved that the MFC system can be used as a new technology for the production of bioenergy from renewable raw materials and for the purification of various types of municipal and synthetic wastewater, in particular, the removal of COD from synthetic wastewater containing high organic matter. The



results show that by increasing the concentration of the substrate in the microbial fuel cell, more electricity is generated and energy efficiency rises. Accordingly, increasing the concentration of organic matter in the anode chamber led to an increase in the power generation and coulombic efficiency. The maximum power and current densities at the initial COD concentration of 10000.0 mg/L were 50.7 and 570 mA/m<sup>2</sup>, respectively. The present study showed that using a microbial fuel cell, a high percentage of COD could be removed in synthetic or municipal wastewater.

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## Disclosure statement

No potential conflict of interest was reported by the author(s).

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