Microbial fuel cells (MFC) in the treatment of dairy wastewater

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Abstract. Microbial Fuel Cells (MFC) appear to offer a feasible alternative to conventional wastewater treatment techniques; thus, constructing and testing MFCs for this purpose was accomplished in this research. The experiments were carried out in two stage; In first stage, synthetic dairy wastewater was used as substrate in an anode chamber using Saccharomyces cerevisiae at different pH values (5, 6, 7, and 8) and different operational temperatures (25, 30, 34 °C) in order to evaluate the performance of MFC in term of COD reduction and electricity generation, while in the second stage, the MFC testing was conducted using real dairy wastewater with an initial concentration of COD equal to 2,610 mg/L, inoculated with Saccharomyces cerevisiae in an anaerobic anode chamber under optimum operation conditions. The results revealed that increasing operation temperature had a significant effect on COD reduction and operation time. The optimum pH and temperature were 6 and 34 °C, respectively. After treatment of the dairy wastewater, the COD, TSS, TDS, EC, SO4, and NO3 removal efficiencies of the MFC were found to be 92%, 79.3%, 62.5%, 38.6%, and 60%, respectively. These removal ratios confirm ability of living microorganisms to digest the organic matter present in dairy wastewater to produce electrical power, with maximum values of 850 mV and 28 μA produced for voltage and current, respectively. Microbial Fuel Cell technology thus offers an efficient approach to both dairy wastewater treatment and energy recovery.

1. Introduction

Among the most essential environmental issues facing humanity currently is the problem of pollution of surface water, which is one of the most imperative environmental issues affecting human health [1]. The dairy industry is the largest source of food processing wastewater in many countries, and as awareness of the importance of improved standards for wastewater treatment has grown, process requirements have become increasingly stringent [2]. Dairy wastewaters are typically treated by means of aerobic and anaerobic biologic treatments, which may include any or all of activated sludge, trickling filters, aerobic lagoons, anaerobic lagoons, sequencing batch reactors (SBR), anaerobic sludge blankets (UASB), anaerobic filters, and constructed wetlands. Physical and chemical treatment is also applied using membrane technology or coagulation/flocculation [3,4]. However, these conventional techniques come with many disadvantages, including high cost, high energy requirements, and considerable sludge generation [5]. The high energy requirements of conventional treatment systems have also boosted demand for alternative treatment technologies that are cost effective and require less energy for efficient operation. A great deal of research has been carried out to find renewable energy sources, and among such renewable alternatives, microbial fuel cells (MFCs) have attracted interest from researchers due to the possibility of directly harvesting electricity from organic wastes and renewable biomass [6].
MFCs have emerged in recent years as a promising yet challenging technology that offers the tantalising possibility of treating a wide range of wastewaters to remove soluble organic pollutants while simultaneously generating electrical current [7]. MFC technology may thus be an efficient way to solve energy, budget, and environmental problems. These bio-electrochemical systems convert carbonaceous substances into electricity, with bacterial systems acting as catalysts to oxidise organic and inorganic matter to generate current [8]. An MFC typically consists of two chambers, the anode chamber and the cathode, which are separated by a proton exchange membrane or a salt bridge. Microorganisms are then used as catalysts to oxidize the substrate in the anode chamber under anaerobic conditions, making them the power house of MFCs. The electrons produced are transferred to the anodic (electrode) surface, and then directed to the cathode through an electrical connection. Different microbial populations can be used as biocatalysts in MFC, many of which are present in wastewater. However, multiple factors can affect these living microorganisms’ efficiency and growth that must thus be monitored and stabilised, such as temperature, pH value, and humidity [9].

Many studies have investigated the ability of MFC technology to treat different types of wastewater. Patrick and Zhen [10] used four identical MFCs to treat different stages of a cheese wastewater treatment process, with results that indicated a decline in Chemical Oxygen Demand (COD) coupled with power generation. Sunil et al. [11] reviewed the feasibility of using chocolate industry wastewater as a substrate in a double chamber MFC in batch mode, generating a significant reduction in the COD, BOD, TSS, and TDS of the wastewater. The treatment of refinery wastewater in a dual compartment MFC was studied by Fang Zhang et al. [12], whose work revealed the ability of MFC to mitigate waste effluent by the elimination of COD alongside its ability to generate power. Afaf [13] investigated COD removal and electrical generation for both synthetic and municipal wastewater by used two chambers MFC under different operation conditions. Jayashree et al. [14], studied the effect of substrate in the MFC by applying different real wastewaters (municipal wastewater, dairy wastewater, cassava wastewater) and found a superior performance by using particular options.

This study aimed to construct an MFC to study the optimum operating conditions for wastewater treatment and generating electricity in order to then treat real dairy wastewater in the anode chambers of the MFC to investigate its performance in terms of actual wastewater treatment, and bioelectricity generation.

2. Materials and Methods

2.1. MFC Configuration

The constructed microbial fuel cell (MFC) consisted of two glass containers of the same volume (3,000 cm³). One container was used as the anode chamber and the other as the cathode chamber. The dimensions of these chambers were 15 x 10 x 20 cm. The anode was linked to the cathode chamber using an agar salt bridge with a diameter and length of 1.5 and 5 inches, respectively. Graphite plates were used as both anode and cathode materials, with electrodes attached via an electrical conductor (copper wire) that extended outside the microbial fuel cell to connect it to an external electrical circuit through which electrons were transferred. The anode compartment had three openings at the top, one that allowed the wastewater to enter, another for nitrogen pumping, and the final one that allowed the oxygen to exit; a single port in bottom of anode chamber was used for wastewater sampling. The cathode chamber had two openings at the top, one for inserting the catholyte (waste water in this research) and the other for pumping the air or oxygen. Leak-proof sealing was employed to maintain an anaerobic environment in anode chamber. In order to enhance yeast attachment on the anode, the graphite plates were abraded using sand paper. Wastewater samples were fed into the anode chamber, while the cathode chamber was filled with water, used as catholyte. Figure 1 shows the schematic diagram of the MFC while Figure 2 shows the microbial fuel cell during operation.
2.2 Salt Bridge Preparation

The salt bridge was prepared by dissolving 5% agar with a 1 M concentration of potassium chloride (KCl) in 100 ml distilled water. Agar was used to create a solid media. The prepared solution (salt+agar) was boiled for two minutes and cast in a U-shaped glass tube of 70 cm length and 1.8 cm diameter, as shown in Figure 2.
2.3 Substrate

Two types of substrate were used in this study:

2.3.1. Synthetic Dairy Wastewater Preparation.

In order to simulate real wastewater 0.5g of glucose, 100mg of MgSO4.7H2O, 6 mg of FeCl3.6H2O, 1 mg of CaCl2.H2O, 120mg of KCL, and 5 g of milk powder, NH4Cl and (NH4)2PO4 were dissolved in distilled water to prepare 2,000 ml of anode solution [15]. The initial COD concentration for synthetic wastewater was 2,300 mg/l.

2.3.2. Actual Dairy Wastewater

Real dairy wastewater was utilized as a substrate in the MFC in the second stage. This was collected from the Abu Ghraya dairy factory located west of Baghdad, Iraq. The sample of real wastewater was taken from the equalisation tank in the dairy plant and then stored in the refrigerator at 4 °C in order to inactivate any microbial activity. The initial COD concentration for dairy wastewater was 2,600 mg/l.

2.4. Inoculum

In the anode chamber of the MFC, Saccharomyces cerevisiae were used as pure microorganisms, with 4 grams of dry yeast dissolved in 40 mL of warm water, then transferred to a 250 mL closed bottle containing wastewater at room temperature for 24 hours to acclimate it to the experimental environment. At that point, 80 mL of this solution was transferred to the anode chamber in the MFC to allow the experiment to begin [16].

2.5. MFC Operation

In this study, the MFC was operated in a batch mode under anaerobic conditions for each experiment. The experimental work was done in two stages: in the first stage, 2,300mg/l of COD synthetic dairy wastewater was used under a variety of operational conditions (temperatures and pH levels) in order to evaluate MFC performance in terms of reducing COD and generating current and voltage in order to determine the optimum operational conditions; then, in the second stage, real dairy wastewater was treated under these optimum operational conditions. To start the MFC for each experiment, 2 L of wastewater and 80 ml of Saccharomyces cerevisiae that had previously undergone the adaptation process were placed in the anode chamber, with nitrogen gas pumped in for 10 minutes to ensure an anaerobic environment; in the cathode chamber, 2 L of distilled water was added as a catholyte and the chamber was connected to air pump that provided air bubbles to the cathodic solution. Each experiment was continued until the measured COD and electrical current values reached a steady state.

3. Results and Discussion

Chemical oxygen demand (COD), current and voltage were assessed daily by the researchers, while other tests such as TDS, TSS, EC, SO4, and NO3 measurements were carried out before and after treatment via MFC according to the procedures outlined in the standard methods, per APHA 2005. Several different instruments and measuring devices were thus used, such as a COD reactor (model: Dr Lange Thermostat LT IW, Germany), a COD photometer (model: multi direct, Lovibond, Germany), a pH meter (model: portable HI- 83141, HANNA, Romania), and a digital multimeter (model: Pros Kit MT-1232) air pump (model Big boy BB-600).
3.1 Assessment of MFC with synthetic dairy wastewater

3.1.1. Effect of pH on chemical oxygen demand removal and electricity generation

To find the impact of pH on COD removal and power production within the MFC, a 2,300mg/l concentration of synthetic dairy wastewater was prepared. The pH was then amended to various different values (5, 6, 7, and 8) under constant operation temperature of 25 °C. The results of these experiments are shown in Figures 3, 4 and 5, which illustrate that the pH value of 6 offered the best degradation of COD by yeast in the MFC. The maximum removal efficiency at a H of 6 was 82%, and this clearly provides the best medium for increasing yeast activity with regard to digesting organic matter. These results were supported by those of a previous study [13]. No removal of COD was seen at a pH of 8 due to optimum yeast growth ranges spanning only the pH 4 to 6 range [17], as shown in Figure 6.

Figure 3. COD concentration and removal efficiency of MFC at pH 5

Figure 4. COD concentration and removal efficiency of MFC at pH 6
Figure 5. COD concentration and removal efficiency of MFC at pH 7

Figure 6. Maximum COD removal efficiency by MFC at different values of pH

The measured values for voltage and electrical current for each experiment is given in Figures 7 and 8. The MFC showed zero current and voltage generation during the first few days of MFC operation, as the biomass required an acclimation period; this was followed by a rapid increase in the value of current and voltage after 4, 5, and 13 days at pH values of 5, 6, and 7, respectively. The maximum current and voltage generated were 14.3 µA and 590 mV, respectively, at pH 6, as shown in Figure 8 and 9. The anodic pH plays important role on MFC performance by improving the metabolic rate of the microorganisms, allowing them to digest the organic matter more effectively and hence to increase the concentration of protons and electrons in the anodic chamber [18]. However, there was no electricity generation at pH 8, as the yeast only thrives within an acidic medium of pH 4 to 6.
3.1.2. Effect of Temperature on COD removal and electricity generation

To investigate the effect of MFC operational temperature, experiments were conducted at 25, 30, and 34 °C with the pH kept constant at 6, for an initial COD of 2,300 mg/l synthetic dairy wastewater. The results are presented in Figures 11, 12, and 13. These figures show that operation time was shortened from 21 to 10 days as the temperature increased from 25 to 30 °C, and then further reduced to 7 days at 34 °C, with the removal efficiency of COD increased to 90.2%, as shown in Figure 14. As the temperature increased, the biochemical reaction rate increased, causing enhancement of microbial kinetic rates [19]. Similar findings were reported by [20], who found that when the temperature increased from 20 to 40 °C, COD removal efficiency increased from 62 to 84%. Figure 13 shows the maximum efficiency obtained with regard to removal of COD at different operational temperatures in the microbial fuel cell.
Figure 11. COD Concentration and removal efficiency in the MFC at 25 °C

Figure 12. COD Concentration and removal efficiency in the MFC at 30 °C
Figure 13. COD Concentration and removal efficiency in the MFC at 34 °C

Figure 14. Maximum COD removal efficiency at different operational temperatures in the MFC

The voltage and current generated between the anode and cathode in the microbial fuel cell are illustrated in Figures 15 and 16. It is clear that the generation of voltages and current were recorded from the second day at 30 and 34 °C, but that at 25 °C, such generation did not begin until the fifth day, indicating that the acclimation period decreased with the increase in temperature. From the figures, as the temperature increased, more electrons passed through circuit and more current and voltage was generated; the maximum values for voltage and current were 780 mV and 23 µA, respectively at 34 °C as, shown in Figures 17, and 18. This was due to increases in the substrate utilisation rate, with higher growth rates promoting faster microbial attachment to the electrode. All of these factors have positive effects on system performance in terms of reducing operation time. The researchers in [21] reported same behaviours for MFC in the range 10 to 60 °C, though they also noted that the optimum operation temperature was under 40 °C.

It was clear from these figures that a long operation time required to produce significant power at relatively low temperatures such as 25 °C due to the lower activity of microbial metabolism at such temperatures; in such cases, the degradation of organic matter is decreased, leading to less generation and transfer of electrons and a decrease in power and energy generation. Similar behaviours at low
and moderate operational temperatures were also reported by [22].

**Figure 15.** Effect of different operation temperatures on the current in the MFC

**Figure 16.** Effect of different operation temperatures on the voltage in the MFC

**Figure 17.** Maximum current at different operation temperatures in the MFC

**Figure 18.** Maximum voltage at different operation temperatures in the MFC
3.2. Application of MFC to real dairy wastewater

In this study, MFC performance was evaluated with regard to treating real dairy wastewater and electric power generation. Chemical oxygen demand (COD), current, and voltage tests were performed on samples taken from the MFC every day, with the temperature and pH values continuously monitored and controlled in the optimum range. This was around 6 for pH, achieved by adjusting the acidity with 1 N HCl and 1 N NaOH solutions, and around 34 °C for temperature. Figure 19 shows the COD concentration and removal rate with time, which illustrates that the COD concentration profile decreased as treatment proceeded; the COD removal continued, increasing until it reached a steady state condition due to the depletion of all organic matter present in the anode chamber. The maximum removal efficiency of COD was 92% with real dairy waste water, higher better than the results reported by [23], which used an MFC with zinc electrodes for dairy wastewater treatment and energy generation, in which the COD removal rate was 77.58%.

The characteristics of the influent and effluent dairy wastewater used in the MFC are listed in Table 1.

![Figure 19. COD concentration and removal efficiency for dairy wastewater](image-url)
### Table 1. Characteristics of dairy wastewater before and after treatment via MFC.

| Test                          | Dairy wastewater | Removal (%) |
|-------------------------------|------------------|-------------|
|                              | influent con.    | effluent con. |       |
| Chemical oxygen demand (COD) mg/l | 2610            | 210         | 92%   |
| Electrical conductivity(EC) µS/cm | 2210            | 1030        | 53.4% |
| Total suspended solids (TSS) mg/l | 373             | 77          | 79.3% |
| Total dissolved solids (TDS) mg/l | 1315            | 866         | 62.5% |
| Sulphate(SO₄²⁻)mg/l          | 75              | 49          | 38.6% |
| Nitrate (NO₃⁻) mg/l          | 55              | 22          | 60%   |

Figure 20 illustrates the values of current and voltage over time for the treatment of the real dairy wastewater. From the figure, on the second day of operation, there was a rapid generation of voltage and current, which reached its highest values of 850 mV and 28 µA at the end of 11th day. This was followed by a decrease in voltage and current due to death of the yeast on depletion of the nutrients in the anodic chamber.

![Figure 20. Current and voltage values generated from dairy wastewater treatment](image-url)
4- Conclusion

In this work, a dual chamber MFC was manufactured and studied. The effect of operational temperature and pH were investigated in first stage by using synthetic wastewater, then in the second stage, the treatment of real dairy wastewater to produce power generation using a microbial fuel cell (MFC) was successfully demonstrated. The following points were noted:

• The results showed a pH of 6 in anode chamber offered optimum value in terms of COD reduction and electricity production, with the latter expressed in both current and voltage.
• The results revealed that increasing the temperature from 25 to 34 °C positively affected COD reduction and the production of voltage and electrical current, as well as reducing the operation time.
• The decrease in COD, with a removal percentage of 92% after treatment, highlighted the feasibility of actual dairy wastewater treatment by MFC; reductions in TDS, TSS, EC, SO₃, and NO₃ were also observed. The maximum current and voltage generated during dairy wastewater treatment were found to be 28 µA and 850 mV, respectively, offering significant evidence that such cells can be used for real wastewater treatment and be scaled for use in more economical forms.
• The reduction in COD for both synthetic and dairy wastewater revealed the ability of the *Saccharomyces cerevisiae* to digest organic matter in dairy wastewater and similar contaminated solutions.
• This method offers good sustainability because both treats wastewater and produces electricity simultaneously in an environmentally-friendly manner.

5- References

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