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Effects of methylene blue as a mediator on pharmaceutical wastewater treatment and bioelectricity generation in a microbial fuel cell

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ABSTRACT

Pharmaceutical wastewater (PW), a type of industrial wastewater, poses a significant hazard to natural water systems due to its high organic content, which is toxic to various life forms. PW is currently a major environmental and public health concern, impacting not only aquatic ecosystems but also human health and the broader environment. In this study, Methylene Blue (MB) was utilized as a mediator in the anode chamber of Microbial Fuel Cells (MFCs), a novel technology for simultaneous wastewater treatment and electricity generation. This mediator serves as a transporter for electrons and provides a platform for the microbes to generate and reduce electrochemically active products. A double-chamber MFC (DCMFC) with graphite-based electrodes was designed and evaluated. The results demonstrated that PW could generate bioelectricity, producing 958 mV with MB and 665 mV without MB at a resistance of 100 Ω. The chemical oxygen demand (COD) removal efficiency ranged from 79.86% to 81.95%, with a power density of 1142.14 mW/m² and 248 mW/m², and a current density of 2965 mA/m² and 1745 mA/m², corresponding to open circuit voltages (OCV) of 699 mV and 521 mV, respectively, with and without MB at a design resistance of 1000 Ω. This study highlights the effectiveness of MB as a mediator for enhancing bioelectricity generation and the bioremediation of PW.

INTRODUCTION

The rapid growth of the global population, coupled with industrialization and urbanization, has significantly increased the demand for a higher quality of life. While numerous industries have been established to meet human needs, they also present various challenges, notably the generation of wastewater (Tazien, 2021). Industrial wastewater, including fertilizer production, food and dairy processing, petrochemical industries, textiles, and pharmaceuticals, poses serious environmental risks such as cancer, reproductive disorders, and organ failure (Kusui et al., 2018). Pharmaceutical wastewater, in particular, stands out due to its unique composition, which includes organic pollutants and drug residues such as antibiotics, antiepileptics, vitamins, and cosmetic ingredients. The environmental impact of these contaminants is not well understood, making it a significant issue for environmental management and public health (Singal and Kaur, 2018).

Traditional treatment methods, including chemical coagulation, osmosis, membrane filtration, advanced oxidation, and electronic coagulation, have been employed but often fall short due to their limitations and high costs (Padmaja et al., 2020). To address these challenges, microbial fuel cells (MFCs) are emerging as a promising alternative.

MFCs leverage microbial metabolic activities to generate electricity during wastewater treatment, offering an eco-friendly approach compared to conventional methods that consume energy (Rahil et al., 2019).

A dual-chamber MFC consists of an anode chamber, a cathode chamber, and a cation exchange membrane (CEM) or salt bridge, which separates the two chambers (Rahil et al., 2019). Recent studies have shown that MFCs are highly effective in treating polluted water and generating power. This study highlights the enhanced performance of Methylene Blue as a mediator in boosting power generation and improving the treatment of pharmaceutical wastewater.

MATERIALS AND METHODS

Collection and preparation of wastewater

The pharmaceutical wastewater (PW) was collected from a pharmaceutical industry located along Ogbomoso, Southwestern part of Nigeria. The wastewater with COD 1346.17 mg/LL and pH 5.4 was taken to the laboratory and left undisturbed for 24 hours at room temperature (25 ^{o}C) under anaerobic conditions for the solid particulate contents to settle (Akinwumi *et al*., 2020). The aim of storing was to preserve the nature of the wastewater and to avoid any biological change. A novel H-H-shaped graphite-based MFC (DCMFC) was constructed for the treatment of PW and simultaneously generation of electricity.

Electrodes and Preparation of Salt Bridge

Two equal-sized uncoated rods of graphite were used as electrodes for each chamber. Graphite rods with an effective length of 14.5 cm and a diameter of 0.21 cm, resulting in a surface area of 0.005 m^2, were used as electrodes in both the anode and cathode chambers. The anode was provided with uniform holes of 0.1 cm diameter. Prior to use, the graphite electrodes were soaked in de-ionized water for a period of 24 hr to enhance bacteria attachment. A copper wire was connected to each electrode and stretched outside each chamber to simply develop an electrical circuit for electron transport (Zia and Zeshan, 2019). Salt bridge which is to be used as a proton exchange membrane (PEM) was prepared according to Akinwumi *et al.* (2022).

Design of DCMFCs

Two identical laboratory-scale DCMFCs were constructed as previously described by Akinwumi *et al.* (2022). The anode chamber of the MFCs was loaded with PW while the cathode chamber was filled with the buffer solution as shown in Figure 1. The working volume capacity of the MFC was kept at 5 L on both sides. The H-shaped design with both chambers separated by a cation exchange membrane (CEM) or salt bridge is shown in Figure 1. The whole set-up was left for at least 30 minutes for stabilization and for the deflection to occur.

The process was carried out at a pH of 7.5 and a constant temperature of 35 ^{o}C using a full-strength PW. This pH value was selected based on a literature report that bacteria are active in the pH range of 4.5 to 7.5 (Tanveer *et al.,* 2022). Sulphuric acid and sodium hydroxide were used respectively by drop-wise addition for the pH (i.e., acidic and alkaline) adjustment as previously described (Akinwumi *et al.,* 2022). The inoculums for the bio-electrochemical remediation were the indigenous microorganisms in the PW (Qusheng and Mathew, 2018). After the setting up of the experiment, the MFCs were continuously monitored for 168 days.

The PW in the anodic chamber was periodically analysed at every 72 h for COD reduction. Polarization tests were carried out, employing the method of multi-cycle in which various external resistances (load) that varied from 33000 to 33 Ω in decreasing order were utilized for a complete successive batch cycle (Roveroto *et al.,* 2021).

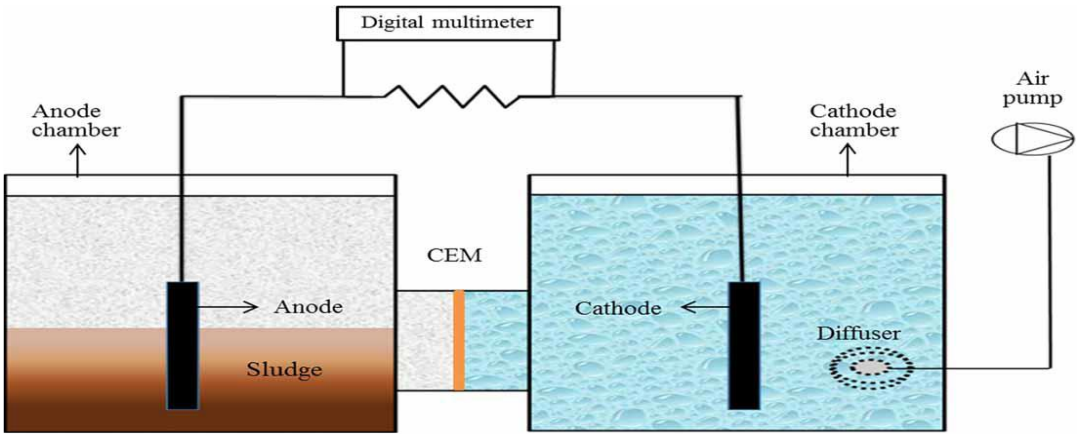

Figure 1: Schematic diagram of MFC (Modified from Zia and Zeshan, 2019)

All the MFCs were left open circuit for a cycle. Stable voltage output was achieved for more than one cycle, after which the MFC was operated under a single constant external resistance (R = 1000 Ω). A digital Multimeter (Kusam, model DT-830D) connected to the external load was used to simultaneously measure the voltage potential and current across the external load in the MFC circuit at intervals of 8 h. The voltage attained when the current is zero is referred to as open circuit voltage (OCV). As MFCs were loaded with PW samples, there was a lag phase for some hours. The optimum external resistance was 1000 Ω for full-strength PW

Electrochemical and chemical measurements

A digital multimeter (Kusam, model DT-830D) was used to continuously monitor the voltage (V) across the external load of 100 Ω in the MFC circuit at 8-hour intervals. COD was determined using the closed reflux titrimetric method (APHA et al., 2005) and pH was measured with Hach multimeter (Jenwey 3510). Power generation of the MFCs at different external loads was determined using polarization. Polarization curves were generated by decreasing the external resistance across the cell from 33 kΩ to 33 Ω, and these curves were used to evaluate the performance of MFC. (Tanvee *et al.*, 2022). All the MFCs were left open circuit for a cycle. Stable voltage output was achieved for more than one cycle, then the MFC was operated under a single constant external resistance (R= 1000 Ω). The voltage attained when the current is zero is referred to as open circuit voltage (OCV). There was a lag phase for some hours as the MFCs were loaded with PW. Also, the optimum external resistance was 1000 Ω for PW without the mediator while it was 10,000 Ω with the presence of a mediator. The calculation for current density was performed using Eq. (1):

$$Current\ density\ I_D = \frac{current}{electrode\ surface\ area} = \frac{I}{A} \quad (1)$$

where I is the current (=V/R), A is the electrode projected surface area (0.0871 m^2), V is the voltage (V), and R is the external load or resistance (Ω)

Power density was calculated according to Eq. (2)

$$PowerDensity(P_D) = \frac{Current \times Voltage}{ElctrodeSurfaceArea} \quad \frac{IV}{A} \quad (2)$$

COD removal efficiency was calculated based on concentration before and after treatment. Eq. (3) was employed for the COD estimation.

$$COD\left(\frac{mg}{L}\right) = \frac{(V_1 - V_2) \times N \times 8000}{PWVolume(mL)} \quad (3)$$

Where, V_1 = volume of titrant utilized for PW sample (mL); V_2 = volume of titrant utilized for blank (mL); and N = $FeNH_4SO_4$ normality.

The COD removal efficiency was calculated using Eq. (4).

$$E_{COD} = \frac{COD_{In} - COD_{Out}}{COD_{In}} \times 100\% \quad (4)$$

Where, E_{COD} is the COD removal efficiency, COD_{in} is the influent COD, and COD_{out} is the effluent COD. Columbic efficiency (CE) was determined by employing equation (6)

$$CE = \frac{\int Idt}{\frac{\Delta COD or BOD}{32 \times 1000} \times 4 \times V \times 96480} \times 100 \quad (3)$$

RESULTS AND DISCUSSION

Effect of Mediator on Bioelectricity Generation

Mediator aids in the transfer of electrons to the anodic electrode. Methylene Blue (MB) was utilized as a promising anode electron carrier with redox properties and being capable of efficiently working under both acidic and alkaline conditions. For bioelectricity generation to be more effective, mediators must be able to penetrate MFC. Sumaya *et al*. (2020) reported that MB has been used as an electron mediator to increase power production in MFC. The voltage generated by the addition of MB aided the transfer of electrons into the anode compartment of PW at the range of 10 mg/l to 50 mg/l as shown in Figure 2. The study showed that there was an increase in voltage output by the addition of MB to the PW in comparison with the voltage output obtained from PW without MB. Therefore, the addition of MB gradually enhanced the voltage generation from PW. Similar observations were reported by Yu *et al.* (2020). This voltage enhancement may be due to the cells' adhesion on the electrode surface because the activity of MB is known to mediate the electron transfer.

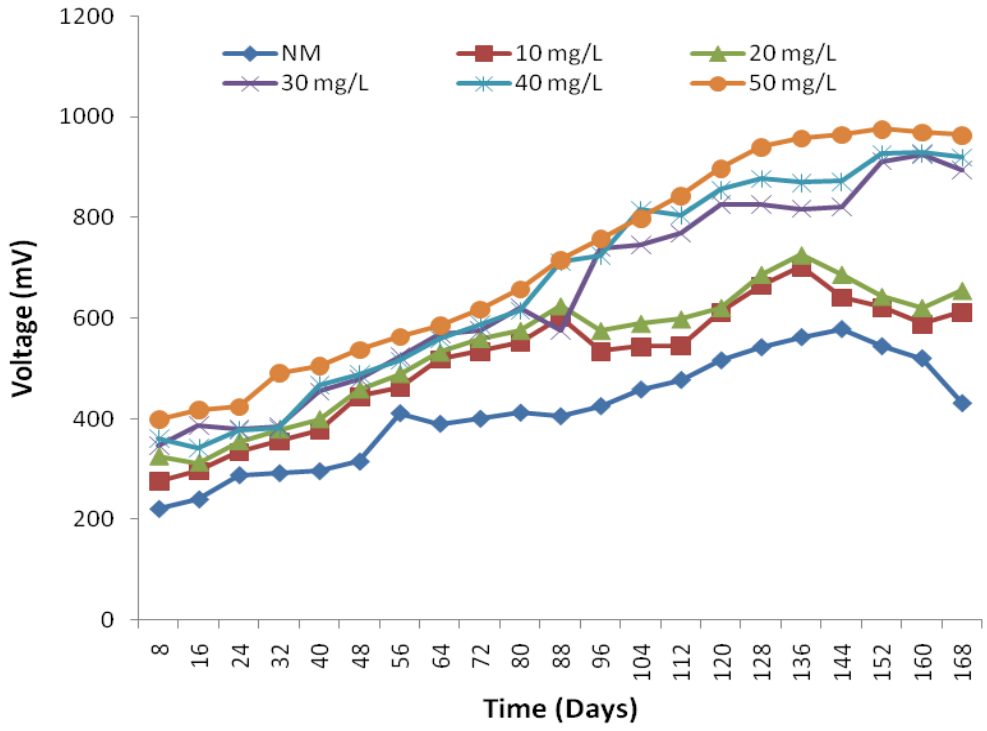

Figure 2: Variation of Voltage generation with MFC operating time using mediator of 10 - 50 mg/L and without mediator.

COD removal and Columbic efficiency

The COD removals and columbic efficiency (CE) are some of the important parameters used in the evaluation of MFC performance. The COD in an MFC is performed to determine the availability of converting wastewater to electricity or forming competitive reactions with other electron acceptors. The CE was obtained based on the utilization of total substrate into current. Figure 3 shows the percentage of COD reduction and columbic efficiency (CE) as a function of COD concentration. From the figure it is seen that at an initial concentration of 1346.17 mg/L, the percentage of COD removal increased as the COD concentration decreased, while CE proportionally increased.

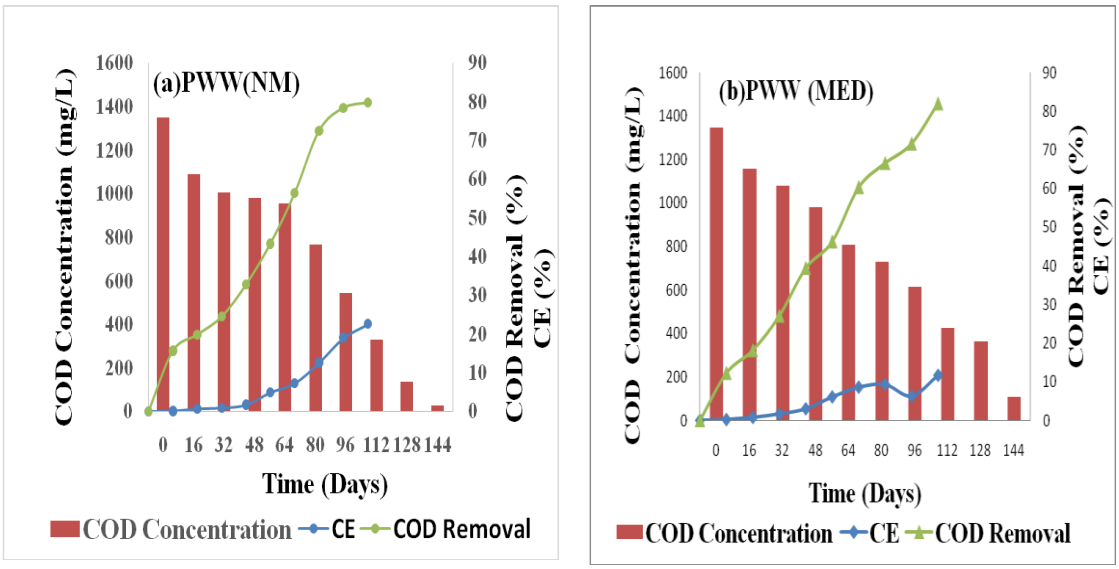

Figure 3: Columbic efficiency and COD removal from PW as function of COD absence of mediator and presence of mediator (50 mg/L)

The percentage of COD removal in the absence of mediator was 79.86%, with a corresponding CE of 22.5% attained as shown in Figure 3, while the final percentage of COD removal with the addition of MB as mediator was 81.95% with a corresponding final CE of 11.61%. The addition of MB gave higher values for PW treatment than those without MB (Gadkari and Sadhukhan, 2019).

Polarization Curve and Power Density

The polarization curve was plotted as a factor of electrode potential against the current density of MFCs. The yielding data obtained by varying the external resistance from 33 to 33,000 Ω in decreasing order after a steady state operation is shown in Figure 4. These results show polarization as a factor of current density against, voltage and power density at different resistances. It was shown from the graph that voltage decreases while current density increases as external resistance increases.

Also, the maximum power density achieved from PW using MFC in the absence of mediator was 248 mW/m^2, at a current density of 1745 mA/m^2 and cell design point of 1000 Ω while the maximum power obtained with the addition of 50 mg/L of mediator was 1142.599 mW/m^2, at a corresponding current density of 2965 mA/m^2 and cell design point of 1000 Ω. This is the point where the external resistance at a particular potential in which the maximum power point is obtained (Sumaya *et al.*, 2020).

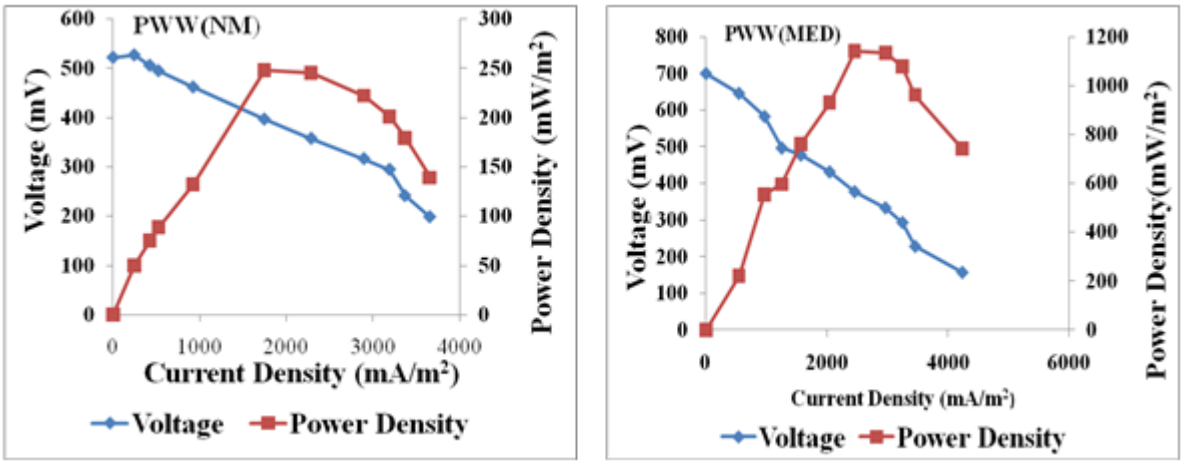

Figure 4: Polarization and power curves at lowest and highest density of MFC for PWW with mediator and without mediator

Thus addition of methylene blue enhanced bioelectricity generation, the methylene blue served as an electron promoter and liberated electrons to the anode surface.

CONCLUSION

It could be concluded that electricity was generated from pharmaceutical wastewater in a novel dual chamber. Methylene blue was used as an electron promoter in the microbial fuel cell. The addition of methylene blue played an important role in the enhancement of bioelectricity generation. Chemical Oxygen Demand removal of 81.95% was achieved with a corresponding power density of 1142.599 mW/m² and a current density of 2,965 mA/m² when 50 mg/L of methylene blue was added. In contrast, a COD removal of 79.86% was observed with a power density of 248 mW/m² and a current density of 1,745 mA/m² using full-strength PW without MB. This demonstrates that the addition of MB as a mediator in the process enabled simultaneous bioelectricity generation and treatment of PW using the MFC.

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