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Bio-electrochemical Treatment of Dye Contaminated Wastewater for Electricity Generation

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ABSTRACT

The microbial fuel cell (MFC) is a promising technology for efficient wastewater treatment and recovering energy as direct electricity for onsite applications. For treatment of biodegradable organic matters in MFCs, removal efficiencies comparable with established treatment method can be obtained. Microbial fuel cells are a powerful platform for extracting energy from various sources and converting it to electricity. As no intermediate steps are required to harness the electricity from the organic substrates stored chemical energy, MFC technology offers a sustainable alternative source of energy production. The generation of electricity from the organic substances contained in waste using MFC technology could provide a cost-effective solution to the issue of environmental pollution and energy shortages in the near future. Thus, technical advancement in bioelectricity productions is becoming commercially viable. Due to practical limitations, and although promising prospects have been reported in recent investigations, MFCs are incapable of up scaling and of high energy production.

Keywords: MFCs, wastewater, Dye, Exoelectrogens, Gram staining

1.0 **Introduction**

Bio electrochemical treatment of dye contaminated wastewater for electricity generation is interesting research because of its ability to address the challenges of energy shortage and concurrently treat wastewater.

Globally, energy shortage and access to clean/treated water has been a major challenge and problem faced by humans. To provide clean and fresh water to the modern world, which is responsible to cover the basic needs of life is also a big task in the 21st century. Water is a major part of substance for all living organisms on earth (Yaqoob et al., 2020). Water is also called the universal solvent because of its ability to dissolve many substances. The United Nations estimates the earth population at 7.2 billion inhabitants, with a projected population reaching 9.6 billion inhabitants by 2050 (UN News center, 2013). Traditional methods of wastewater treatment are energy intensive, often consuming between 950 and 2850 kj/m^3 of water treated. Polluted water has unwanted minerals on chemicals that have adverse effects on human health and cannot be used for any direct purpose (Chuo et al., 2015) also pollutants in the wastewater containing carbon, nitrogen, metals or phosphorus, can be degraded in the anode chambers of MFCs. Microbial fuel cell is one of the wastewater treatment methods in bio electrochemical system. MFC technology is a type of bio electrochemical system (BES) that has recently gain popularity due to its low cost and low level of harmfulness to the environment. Additionally, MFCs are associated with energy production, heavy metal removal, and bio-removal of toxic waste.

Microorganisms (specifically Exoelectrogens) hold a necessary part in these technologies, since they form electron rich metabolites, produces redox mediator, preserve a redox gradient, and transport electrons to an electrode through direct electron transfer or through a soluble electron transfer mediator, while producing electricity as the main product (Ardakani et al., 2020).

With this knowledge, the use of MFC in wastewater treatments shows potential as candidate for growing sustainable wastewater treatment and energy generation.

Potter introduced the first MFC in the year 1910 in which Escherichia coli bacteria and Saccharomyces bacteria were used to produce electrical energy while using platinum electrodes (Potter 1911). Simultaneously, the chemical energy trapped in these compounds are converted into electricity. The report of the International Energy Agency (IEA) shows that the expected

energy requirement was 18 billion tone oil in 2035, as compared to the current situation which is near 12 billion tone oil (Kumar et al., 2015). Currently, the world obtains its energy by utilizing fossil fuel resources, but their working efficiency, security and other environmental issues (Global warming) make them unsuitable for long term use (Ahmad et al., 2015). So, there is an urgent need to solve this major issue of the modern world related to energy demand.

Current energy production relies on fossil fuel consumption, which limits energy generation and dramatically contributes to climate change and environmental deterioration (Levin et al., 2004). Given this scenario, the development of technologies to obtain cleaner and renewable energy is highly desirable. Water pollution has been a major challenge to human existence from origin that's why it is important we treat wastewater to a reasonable percentage before disposing it.

There are different energy sources but the use of biological systems for energy production has attracted the attention of researchers worldwide.

Microbial Fuel Cells (MFCs) are among the most prominent biological systems for energy production. The possibility of using micro-organisms to generate electricity in fuel cells is based on the biochemical processes of energy production (Fabiano et al., 2015).

Fuel cells are devices which are capable of converting fuel from chemical energy to electrical energy (Emily R. Cooksey 2018).

Microbial fuel cells (MFCs) follow the same principle as all other fuel cells in terms of oxidizing fuel from a fuel source and converting them into electrical power (Cooksey, 2018). However; unlike other fuel cell systems, MFC systems use a live bacterial culture as the catalyst on the electrode surface. The bacteria used are solely responsible for facilitating the generation of ions and electrons. By forcing bacteria to respire anaerobically (without the presence of oxygen) they naturally turn to the fuel to provide them with a source of energy for survival. Digestion of the fuel by the bacteria results in the formation of ions and electrons, which are then transported around the cell in the same manner as in traditional fuel cells.

Wastewater is water whose physical, chemical and biological properties have been changed as a result of certain substances which render it unsafe for some purposes such as drinking (Amoatey et al., 2011). Wastewater is extensively generated on a daily basis from domestic and industrial

sources across the globe: posing several challenges such as water crisis and environmental deterioration.

1.1 **Microbial Fuel Cell**

Microbial fuel cell is a bio electrochemical device or system in which bacterial oxidizes organic matter and transfer the electrons through electron transport chains onto an electrode surface producing electricity.

1.2 **Microbial Fuel Cell as a method for wastewater treatment**

MFC has ability to remove different types of pollutant from wastewater and make it suitable for human use. There are many heavy metals, organic and inorganic toxic compounds are found in wastewater. The microbe's properties to accept electrons from (anode and cathode) electrodes are known as electrographs. This gives a new direction for the treatment of heavy metals through reduction and oxidation process. There were many types of bacteria with the ability to gain electrons directly from electrodes (Benetton et al., 2015). In the previous reported study, electrons are used to transfer by using different types of artificial electron shuttles, but there were many disadvantages reported of artificial shuttle electrons. Moreover, there are many types of microbes which can serve as electron shuttles to get electrons from electrodes of MFC. It can empower bacteria to enhance reduction of fermentation and different inorganic substrates. These bacteria are Staphylococcus carnosus, Clostridium ljungdahlii, Shigella flexneri, Streptococcus mutans, and Acinetobacter calcoaceticus, etc. and they also carrying active redox molecules (Rotaru et al., 2014.). A research group already extensively studied the mechanism of microbes feeding and movement of electrons from electrode to microbes (Umar et al., 2020.). The protons released by microbes are being reduced to hydrogen gas that lowers the potential of electrodes. Hydrogen is not soluble because the gas needs a high amount of energy or a catalyst at the electrode surface to overcome the reduction of protons that can reduce its applications. It is therefore essential to induce a high transfer rate of electrons by empowering the microbes with high current density. The hydrogen gas and redox molecules did not excite the cell which was attached to electrodes. The attached cell remains linked and separated from end products. Thrash and Coate's discussed first time the power concept of microbes by studying Geobacter species as electrodes. The reported studies show that Geobacter species can transfer electron directly to electrodes (Tizaoui et al., 2019). There were many toxic heavy metals such as chromium ion, nickel, zinc, lead,

mercury, copper, and vanadium, etc. that can be removed by different microbes through same mechanism (Tizaoui et al., 2019.). For example, G. sulfurreducens accept electrons directly from electrodes and reduce the U (VI) into U (IV) form (soluble to insoluble). The U (VI) is insoluble form and it was adsorbed on electrodes. G. sulfurreducens also has the capacity to reduce Cr (VI) to Cr (III), it means able to convert highly toxic nature to less toxic nature. The reduce Cr (VI) depends on the oxidation of the substrate (acetate) at anode electrode to transfer the microbes and reduction of chromium occur at the cathode. (Butler et al., 2010) stated the Enterobacter, Macellibacteroides, and Lactococcus microbes can remove vanadium with 93.6% removal efficiency and high current density of 543.4 mW/m2. In the whole studies, there is gap that no proper molecular mechanism is known to accept electrons from electrodes.

1.3 **Microbial fuel cell as a means of electricity generation**

Microbial fuel cell uses chemical energy to generate electricity just like an ordinary battery, MFCs has two electrodes held in separate chambers (the anode chamber and cathode chamber). The anode chamber that contains the bacteria is anaerobic; this means that it doesn't contain oxygen. The cathode chamber is aerobic; this means it does contain oxygen. The oxidation process occurs inside the bacteria living in the anode chamber. This takes advantage of the oxidation that bacteria carry out naturally during cellular respiration. Electron bonds hold together the molecules in the food that bacteria eat; the bacteria break these bonds to release the electrons. The set-up of MFCs helps us to coordinate the electrons to generate electricity. Exoelectrogens are bacteria used in microbial fuel cells. They are electrochemically active and can transfer electrons outside their cells. Some of the bacterial includes *Geobacter sulferreducens, Shewanella putrefaciens*, etc.

1.4 **Mechanism for wastewater degradation and electricity generation in an MFC**

Generally, MFCs has two chambers consisting of a cathode and anode, respectively. The anode chamber is enclosed into wastewater solutions (heavy metal or organic solutions) and other (cathode) in surface water (Nitisoravut et al., 2017). There are many types of microbes which can degrade different type of organic compounds and heavy metals from wastewater solutions and produce electrons and protons. The electrons travel from anode chamber to the cathode part by using an external circuit while protons move directly to the cathode and react with oxygen to

make a water molecule. MFC depends upon electro-active microbes, usually called exoelectrogens, to remove toxic organic waste along with the generation of renewable clean energy in the form of electricity (Kumar et al., 2018.). In simple words, MFCs is a tool used to degrade organic waste to convert organic energy into electric form by oxidation of substrates, using microbes that serve as a biocatalyst in the whole process, that is, it is modified type of an electrochemical fuel cell. Electrode material is considered as a significant to make MFC more reliable and commercially attractive because MFC performance depends upon the conductivity and compatibility of electrodes. In MFC electrotrophs microbes accept electrons from electrodes and convert toxic compounds into less toxic components (Tao et al., 2017). To generate power in MFCs, different type of exoelectrogens can transfer electrons from electrodes through four mechanisms such as short-range electron transfer through redox-active proteins, soluble electron shuttling molecules, and long-range electron transport by conductive pili, direct interspecies electron transfer. The powerful and efficient mechanism is long-range electron transfer through conductive pili. The pili have similar characteristics like metal, that is, conductivity (Akunna et al., 2017). However, MFC is a technique to provide safe, clean, low emission of carbon dioxide, highly efficient energy generation along with wastewater treatment to the modern world.

2.0 Materials and Method

2.1 Isolation and culture of microorganism (bacteria and fungi)

2.1.1 Synthetic dye wastewater preparation

Reactive yellow azo dyes were used for this study. 1 gram of the reactive yellow dye was diluted into 1 liter of distilled water to obtain a concentration of 1000ppm (1g/L).

2.1.2 Soil sampling and contamination of the soil sample with dye solution

Loamy soil sample was collected from a farm using a soil corer. The soil corer was inserted 30cm below the earth crust which was used to obtain 4.5kg of loamy soil. One (1) liter of the prepared

reactive yellow dye solution was mixed with 4.5kg of the loamy soil sample to contaminate the soil samples.

2.1.3 Serial dilution of contaminated Soil Sample

1g of soil sample was dissolved in beaker (A) containing 99ml of distilled water. 1ml of sample (A) was collected and added to beaker (B) containing 99ml of distilled water to obtain serial dilution of 10^{-1}. Again, 1m of sample was collected from beaker (B) and added to 99ml of distilled water contained in beaker (C) to obtain serial dilution of 10^{-2}. Next, 1 ml of sample contained in beaker B was collected and added to 99ml of distilled water contained in beaker (D) to obtain serial dilution of 10^{-3}. This process was followed to obtain serial dilution up to 10^{-9}.

2.1.4 Preparation of Microbial Culture Media

Three separate culture media consisting of MAcCONKEY Agar, Sabouraud Dextrose Agar, and Nutrient Agar were prepared. The purpose of preparing the culture media was to inoculate the microorganism present in the serial dill after which the microbes were prepared

Nutrient Agar: with concentration of 28g/L was prepared. Composition of the nutrient Agar includes: peptone - 5,000gms/ltr, sodium chloride - 5,000gms/ltr, beef extract - 1,500gms/ltr, yeast extract - 1,500gms/ltr, Agar - 15,000gms/ltr, pH - 7.4 at 25^{o}C

*MAcCONKEY Agar:*with concentration of 52g/L was prepared. Formulation of the MAcCONKEY Agar: peptone - 20g/L, Lactose - 10g/L, bile salt - 5g/L, Sodium chloride 5g/L, Agar N0. 2 - 12g/L, Neutral red - 0.05g/L, pH - 7.4

Sabouraud Dextrose Agar: with concentration of 65g/L was prepared. Ingredients of the Sabouraud Dextrose Agar includes: Dextrose - 40,000g/L, Agar - 15,000g/L, Mycological, Peptone - 10,000g/L, pH - 5.6, temp 25^{o}C

2.1.5 Introduction of serial dilution into the culture media

After the serial dilution up to 10^{-9}, serial dilutions 10^{-5},10^{-7}, and 10^{-9} were separately streaked on the three (3) different culture media; Nutrient Agar, MAcCONKEY Agar, and Sabouraud

Dextrose Agar. This was done to introduce the microbes present in the 10^{-5},10^{-7}, and 10^{-9} serial dilution to the nutrient medium, so that the microbes can grow. The separately streaked 10^{-5},10^{-7}, and 10^{-9} serial dilutions were allowed to stand in the three different media for 1 day to achieve microbial growth. After which resulting colonies of both bacteria and fungi were obtained from the three-culture media.

2.1.6 Isolation of Bacteria Strain

Bacteria strains were taken from the resulting bacterial colonies, and each strain was introduced via streaking and grown on a new plate containing mineral salt medium, so that the bacteria can be identified and used as a pure culture for dye contaminated water degradation.

2.1.7 Preparation of Mineral salt medium used for Isolation of Bacteria Strain

The media composition used for this study is a mineral salt medium (broth) with the following composition in g/l: NH_4NO_3, 1; $MnSO_4.7H_2O$, 0.2; K_2HPO_4, 1; $CaCl_2.2H_2O$, 0.1; KCl, 0.2; $FeSO_4.6H_2O$, 1; $ZnSO_4.7H_2O$, 1; $MnSO_4$, 1; and 1 yeast extract which was dissolved I 1L of sterile water and autoclaved at 121^oC for 15 minutes.

2.2 Bacteria Identification

Bacteria isolate was done using gram staining, catalase and oxidase.

2.3 Construction of Microbial Fuel Cell

Carbon electrodes with 16cm^2 surface area in a combination of graphite-graphite sheet, carbon felt-carbon felt and carbon paper-carbon paper was used as the electrodes. The electrodes were perforated by providing 2 uniform size holes of 0.1cm diameter to increase the surface area. The electrodes were soaked in 0.5M H_2SO_4 and deionized water for 20mins and 24 hrs respectively to remove metallic contaminant and improve conductivity.

The anode and cathode chambers

The anode chamber will consist of 25mls of 50 mg/L of reactive dye, contaminated wastewater and 50mls bacterial broth culture. The anode chamber was tightly sealed to accommodate an

anaerobic condition. While the cathode chamber was filled with 75 mls of 0.1M potassium permanganate ($KMnO_4$) as the electron acceptor. The cathode chamber was opened to allow for proper aeration. About 5mls of 0.1 M of NaCl will also be added to the catholyte and anolyte to increase conductivity. The external circuit completed using 1.5 mm copper wires of length 0.4 m. The setups were allowed to stand for 24 hours to stabilize and an open circuit voltage (OCV) was recorded as well as the voltage generated on connection across 330Ω, 100Ω, and 52Ω resistors in parallel to the digital multimeters. This was repeated on 12hr intervals for 25 days. Copper wire was used for contact with electrodes after sealing the contact area with epoxy resin material. All openings were carefully sealed to prevent leakages.

Nafion 117 was used as the proton exchange membrane (PEM) which was in between the H-junction of the MFCs to prevent mixture of solution and also allow passage of protons. Nafion117 sheet was arched to 25cm^2 size and pretreated by boiling sequentially in 30% H_2O_2, deionized water, 0.5M H_2SO_4 and deionized water each for one hour to increase the porosity prior to use.

3.0 Results and Discussion

3.1 Isolation, screening, and identification of microbial species

Firstly, the experiment was focused on isolating, identifying, and characterizing microbial strains that would be capable of effectively degrading reactive yellow dye and simultaneously generating electricity. The microbial strains that seemed to thrive in azo-dye-contaminated soil were subjected to biochemical tests: oxidase, catalase, and gram staining. Seventeen (17) microbial strains with different morphological and cultural characteristics were identified and assigned the numbers MO 1 to MO6, and CR 1 to CR 11. The isolate identified were both bacteria and fungi strains as shown in Table1.

Table 1: Microbial Isolates from azo dye contaminated soil

Isolate Code	Isolate Name
CR1	**Bacillus Megatrium (bacteria)**
CR2	**Lactobacillus delbrueckii (bacteria)**

CR3	**Bacillus sphaericus (bacteria)**
CR4	**Pseudomonas sp. (bacteria)**
CR5	**Bacillus lentus (bacteria)**
CR6	**Erwinia sp (bacteria)**
CR7	**Bacillus pumilus (bacteria)**
CR8	**Aspergillus flavus (fungi)**
CR9	**Aspergillus Miger (fungi)**
CR10	**Rhizopus stonolifer (fungi)**
CR11	**Fusarium sp. (fungi)**
MO1	**Kurthia species (bacteria)**
MO2	**Bacillus sp (bacteria)**
MO3	**Serratia spp (bacteria)**
MO4	**Bacillus pumilus (bacteria)**
MO5	**Aspergillus Fumigatus (fungi)**
MO6	**Fusarium sp. (fungi)**

3.2 Ultraviolet-Visible Studies

In order to investigate the dye degradation and decolorization as well as visualize the changes in the absorption spectra, and the peaks and valleys corresponding to the chromophores contained in the reactive yellow dye compound, a full wavelength scan utilizing an ultraviolet-visible spectrophotometer is crucial. Due to their capacity to absorb light, chromophore groups and

conjugate bonds can be found using the ultraviolet-visible studies approach. The spectrophotometer used is called shimadzu uv-3600 manual.

Fig.1. Shows the UV Vis absorption spectra for reactive yellow dye before and after the microbial oxidation in the Microbial Fuel Cell.

The result obtained from the UV Vis absorption spectra graph shows that the initial dye concentration of one gram per liter (1 g/L) which has an absorbance peak of 2.9 at the wavelength (λmax) of 410nm experienced a decrease in peak to 0.4after the 7 days of microbial oxidation. The change in peak from2.9 to 0.4at the wavelength of 410 nm is indicative of the loss of conjugation near the azo bond (–N=N–), and the absence of the chromophore groups that were initially present in the reactive yellow dye compound before the microbial oxidation commenced.

It was also observed in Ultraviolet-Visible Studies that there was a bathochromic shift in the chromophore's absorption maxima and that the absorption band shifted from the visible region (500–400 nm) to the ultraviolet region (400–300 nm) where a new peak of 1.1 was observed at a wavelength of 325.5 nm

The decrease in the intensity of the band from 2.9 to 0.4 at a wavelength of 410, and the new peak of 1.1 formed at a wavelength of 325.5 nm are suggestive that the large dye structure consisting of the azo bonds (–N=N–) and the multiple aromatic ring compounds present in the reactive yellow dye had fragmented into to smaller and simpler molecules during the microbial oxidation in the anode chamber of the microbial fuel cell. A similar result has been reported by Lee *et al*., 2016.

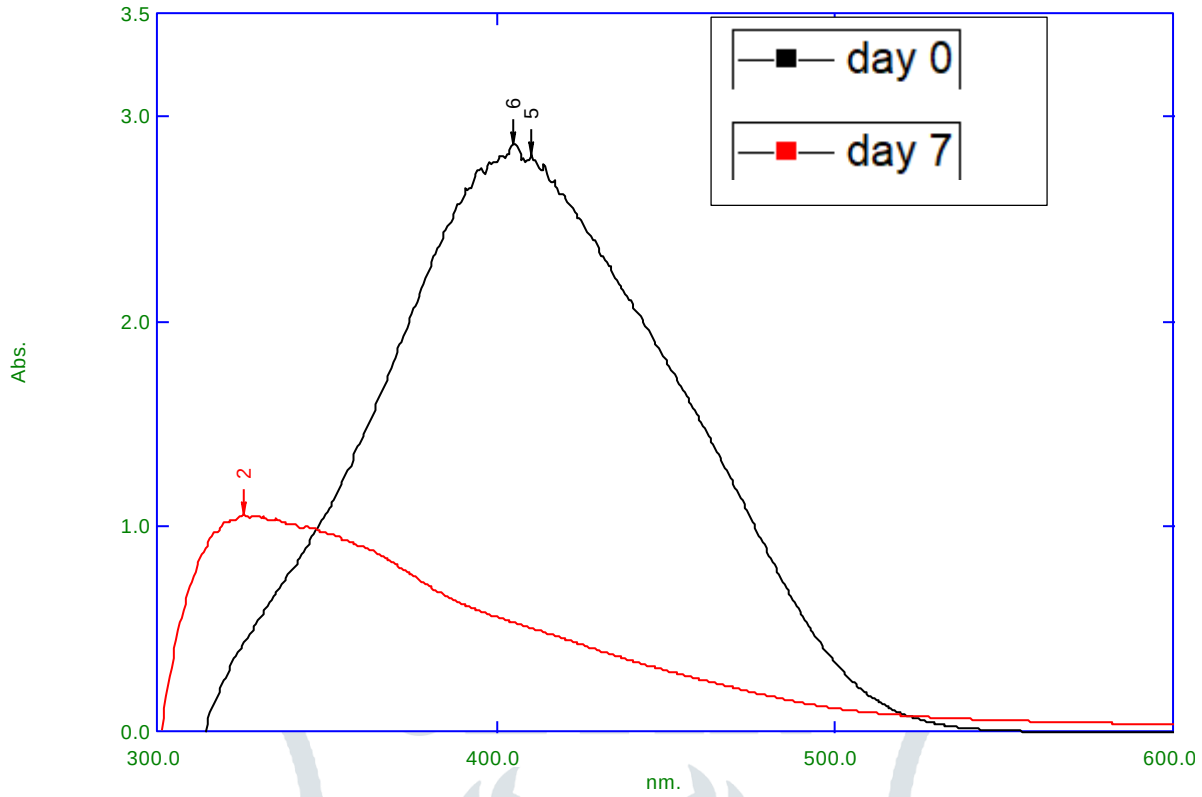

Figure 1: UV-Vis spectra analysis of Reactive Yellow Dye.

3.3 Electricity generation from Microbial Fuel Cell

In the design of MFC Fig.2. sodium chloride (NaCl) was used to increase the ionic activity of the electrolytes. The production of electricity in MFCs depends on several physicochemical and biological factors. To enhance the mass transfer of charged particles and boost solution conductivity, NaCl is typically utilized as the electrolyte (Gil et al., 2003). Additionally, one restriction on the production of electricity is the availability of protons at the cathode. NaCl may have increased the conductivity of both the anolyte and the catholyte by increasing the ionic strength in MFCs, which in turn boosted the power output (Gil et al., 2003; Jang et al., 2004).

Broth containing mixed cultures MO 1 to MO6, and CR 1 to CR 9 together with the reactive yellow dye wastewater was fed to the anode chamber of the microbial fuel cell in a batch-fed operation and used as a source of fuel for electricity generation. The time course of both the voltage and current were recorded at the open circuit for a period of 8 days.

The values of current and voltage generated (Fig.5.) throughout the course of the entire operation period were taken into consideration for MFC evaluation because the bio electrochemical processes exhibit significant oscillations over the operation cycle. It was noted that the voltage and current were low at the start of the experiment and were measured as 0.11 mV (Fig. 4.) and 0.13 mA (Fig3.) respectively, and then experienced a protracted period of severe drop after that. According to Logan (2007), "In an MFC, the bacterial colonization of the electrode and production of the necessary enzymes or structures for transferring electrons outside the cell takes time". On the sixth day of the microbial oxidation in the fuel cell, the current and voltage was found to increase because the microbes employed in the fuel cell had acclimatized to the carbon cloth used as the anode. This exemplifies the exponential development phase during which the organisms (electrogens) aggressively use the organic materials in the wastewater as fuel. The maximum output generated by the fuel cell is 0.20 mV and 0.16 mA. Later, as the 8-day period concluded, the voltage started to fluctuate once more. There was a brief period of sharp voltage decline after that. These were linked to the fact that these organisms were gradually transitioning into the decline phase of growth because of using up the organic matter in the wastewater and accumulating harmful compounds created to support inter- and intra-species competition (Peleg, 2006; McKellar, 2004).

Figure 2: Microbial Fuel Cell Configuration

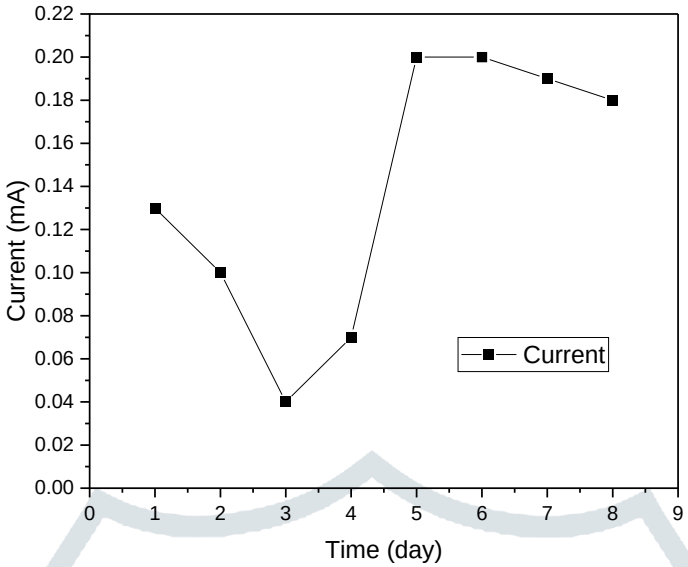

Figure 3: Current output

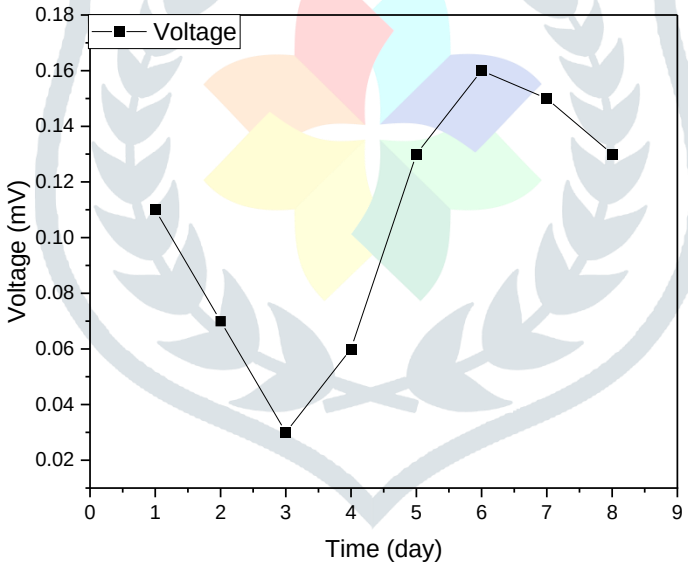

Figure 4: Voltage output

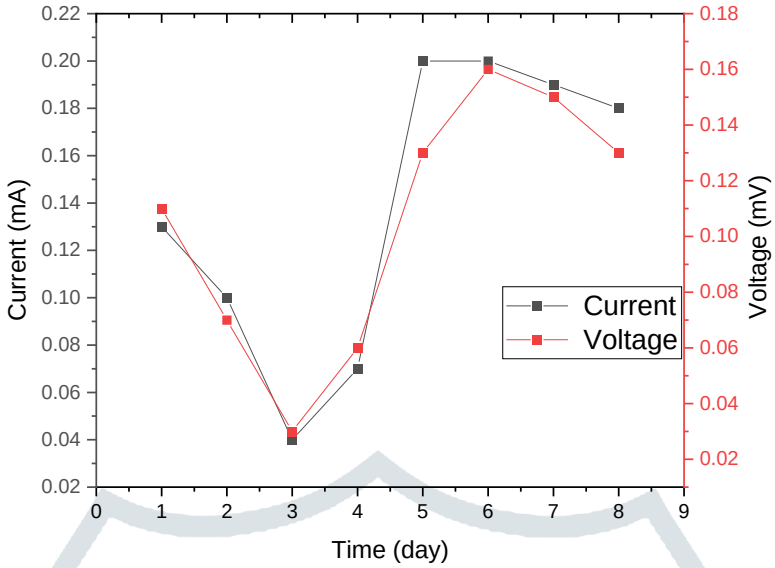

Figure 5: Current and Voltage output

Conclusion

Microbial-catalyzed fuel cells may be one of the feasible alternatives to combat climate change and the energy issue, because it simultaneously solves the issue of wastewater pollution and energy deficit. Any wastewater that contains a sizable amount of organic matter, such as carbohydrates, proteins, and lipids can be utilized to power microbial fuel cells. Use organic material and generate electrons that could be artificially collected using an organic electron acceptor like oxygen. Microbial fuel cell is a renewable energy source that generates small amount of voltage and current, but if various individual fuel cells are stacked in series, more voltage can be generated. Given the advanced study in this area, special attention is urgently necessary for terms of the practical application of this project in areas where wastewater is generated.

The findings have amply validated the hypothesis that microorganisms can generate a voltage by oxidizing the organic materials in the wastewater. This study identified opportunities for improving and increasing output by connecting the microbial fuel cells in series. Additionally, it was noted that when the setup is disturbed throughout the experiment, an effect typically occurs that is manifested as an increase in voltage. Therefore, more research is required to determine

these consequences. Despite not being published, temperature parameters showed a higher voltage during the day than at night.

In conclusion, the double-chamber microbial fuel cell under study was affected by various operating conditions, such as the presence of broth in the dye media, aeration in the cathode chamber, NaCl supporting electrolytes, and the initial dye concentration. The presence of dye enhanced electricity generation, while the aeration and conducting electrolyte significantly increased the degradation of dye in the Microbial Fuel Cell. It is interesting and encouraging that MFCs are not only effective in treatment of organic contaminants but they are also effective for the treatment of some bio-refractory pollutants, which cannot be treated in established biological treatment methods. The MFC technology holds promise towards wastewater treatment and sustainable energy generation with possible applications within a board range of life sciences in the near future.

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