Decolorization of Methyl Orange using a Double-chamber Microbial Fuel Cell with the Use of Soil Microorganisms

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Abstract. In this study, the decolorization of methyl orange was carried out in a double chamber microbial fuel cell. Microbial fuel cells (MFC’s) have been widely studied using various materials and operating conditions, these bioelectrochemical devices that use exoelectrogenic bacteria as biocatalysts have shown great potential to oxidize a variety of substrates while simultaneously generating electricity. Soil microorganisms consisting of lactic acid bacteria, *Saccharomyces*, *Rhizobium*, *Rhodopseudomonas*, *Rhodobacter*, *Actinomyces*, and fungi were obtained from a concentrated soil microbial inoculant. Comparatively, the results of the experiments showed that using titanium mesh, the open circuit voltage and the power density of the MFC in close-circuit condition were 1.005 V and 1.223 Wm⁻² while using carbon fiber brushes, values were 0.992 V and 0.338 Wm⁻². On the other hand, results also revealed that the system with carbon fiber brush electrodes is more effective in decolorizing methyl orange at a maximum of 78 percent removal efficiency. In 10 hours of continuous operation, the concentration of methyl orange reduced from 0.10-0.022 mM.

1. Introduction
Microbial fuel cells are bioelectrochemical systems that use exoelectrogenic microbes as biocatalysts to decompose a variety of substrates and produce electricity [1-5]. The transformation of chemical energy into electrical energy by microbial action allows treatment of wastewater while simultaneously producing renewable energy [6-8]. MFCs occur in different configurations but are common in dual compartments. This configuration of the MFC allows the examination of the number of reactions and the simultaneous degradation of some substances either through oxidation by the action of aerobic or anaerobic microorganisms or reduction by electron transfer from the anode [9-12].

Currently, effluent discharge containing azo dyes is an important environmental hazard [13]. Azo dyes are organic compounds used extensively as synthetic dyes for printing, textile, and leather manufacturing among others. In textile manufacturing, 10-15 percent of these dyes are discharged with the effluent during the treating process [14]. Discharge of these persistent pollutants is adverse because of their color and their breakdown into toxic and mutagenic products [15]. When discharged into the surface water, azo dyes obstruct the penetration of light and oxygen diffusion into the water, posing negative impacts to aquatic life [16].

Electrochemical degradation is considered as a more efficient process for decolorizing dyes because of easier control and complete decomposition of compounds [17]. A special bioelectrochemical environment in the anode of the MFC creates favorable conditions for complete azo dye decolorization while producing bioelectricity [18-20]. Various reports show that other compounds like phenol [21-
22], arsenic [23], pyridine [24], 1,2-dichloroethane [25], and p-nitrophenol [26], have been effectively degraded in the MFC anode. Among microbial inoculants in MFCs, mixed cultures are generally preferred for practical applications since they can achieve higher power densities [8, 27], they are more accessible in large quantities, have higher tolerance to environmental fluctuations and more responsive to different substrates [28]. Various types of microbial inoculants are found in the market and one of this is known as effective microorganisms (EM). EM is a mix of around 80 beneficial aerobic and anaerobic bacteria in a liquid medium with pH 3.0-3.5 [29-31]. The main species of bacteria found in EM preparations include lactic acid bacteria, photosynthetic bacteria, yeasts, Actinomycetes, and fungi [32].

This paper investigated the degradation of methyl orange, an azo dye, in a dual chamber MFC using soil microorganisms derived from EM inoculant. The dye was selected as target contaminant due to its wide applications and strong toxic effect on aquatic species and humans. Furthermore, the bioenergy generation potential of the MFCs using meat processing wastewater as substrate was also investigated. While EM is locally available, meat establishments are also widespread in the Philippines and there is abundance of the meat processing wastewater discharge to the environment [33]. The bioenergy generation potential of the MFCs and the decolorization efficiency were assessed and compared using carbon fiber brush and titanium mesh electrodes. The combination of aerobic and anaerobic microorganisms in the microbial inoculant and the treatment power of the MFC systems revealed that the MFC is a promising technology for treating azo dyes in wastewaters while simultaneously generating bioenergy.

2. Materials and methods

2.1. Construction of the MFCs and pre-treatment of electrodes and the CEM

Four units of modified, 250-ml Pyrex reagent bottles were used in the double-chamber MFCs with two pairs of different electrodes. The carbon fiber brush anodes made of PANEX 35 carbon fill materials wound to twisted titanium core (purchased from Mill-Rose Company) were acid and heat-treated as described in published literature [10, 17, 34]. All brushes were first cleaned by soaking in pure acetone overnight to remove the impurities. The brushes were then acid treated by soaking in a solution of 200 gL⁻¹ (NH₄)₂S₂O₈ and 1 M H₂SO₄ for 15 min followed by heat-treatment in a forced convection oven at 200°C for 30 min. The brushes were washed repeatedly with deionized water before using in the MFC. On the other hand, the titanium electrodes were modified to form a cylindrical mesh then attached to a stainless steel filler rod by tungsten inert gas welding. A 50.8-micron thick CEM, Dupont™ Nafion 212® (purchased from Fuel Cells Etc.) was pretreated through a modified method [34]. The membrane was boiled for 1 hour in each of the following solutions: 3 percent H₂O₂; 0.5 M H₂SO₄; and deionized water. The membrane was rinsed repeatedly with deionized water before using in the MFC.

2.2. Preparation of the microbial inoculant

The soil microorganisms used as inoculant in the MFCs was derived from the activated solution of effective microorganisms. The activated solution was prepared by mixing one liter of EM1 (purchased from MC Enterprises) to 30 L of non-chlorinated water and three kilograms of molasses. The resulting solution was transferred to an air-tight carboy and stored in a dark room at room temperature to activate by fermentation for seven days.

2.3. Collection and characterization of meat processing wastewater

Meat processing wastewater was collected from the meat processing plant and cold storage facility of METS Logistics, Inc. in Carmona, Cavite. Fresh and unsettled influent was taken from the collection tank of the treatment plant and transported in ice to the laboratory. Temperature, total dissolved solids (TDS) and pH level of the wastewater were measured in situ using a COM100 digital multimeter and pH2 pen-type tester. The chemical oxygen demand (COD) of the wastewater samples was assessed by a third-party testing laboratory (JefCor Laboratories, Inc).
2.4. Operation of the MFC

The MFCs were operated in batch mode in ambient temperature and pressure in open circuit condition and in close circuit condition under a constant external load of 200Ω. The cation exchange membrane was replaced in each phase of the experiment to avoid contamination of the anode and cathode chambers. The cathodic chamber was fed with 0.01 KMNO₄ solution with pH maintained between 6-6.5 and with continuous aeration using an aquarium air pump. In contrary, the anode chamber was fed with various compositions of the anolyte.

In determining the effects of different concentrations of microbial inoculant on power generation, the anolytes were fed with different ratios of meat processing wastewater and microbial inoculant (percent by volume). The percentage of the inoculant ranged from zero up to 25 and 50 percent (1:0, 1:3, and 1:1). For the investigation of methyl orange degradation, the anolytes containing 1:0 and 1:3 ratio of wastewater to microbial inoculant were spiked with 25 mL of methyl orange to simulate an initial concentration of 0.10 mM in the anolyte.

2.5. Data collection procedure

A data logging system was developed to help in the monitoring of the MFCs. The open circuit voltage (OCV) and the voltage and current in close circuit connection of the MFC systems were observed and logged using a PuTTY desktop software. To ensure validity of the data collected, the output of the data logger was randomly checked with a digital multimeter. The power density values for each MFC setup was normalized by the estimated cross-sectional area of the anode. Power, power density, and energy values were calculated using equations (1), (2), and (3), respectively:

\[ P = I \times V \]  
\[ P_d = \frac{P}{A} \]  
\[ E = P \times t \]

where \( P \) is power (W), \( I \) is current (A), \( V \) is electrode potential (V), \( P_d \) is power density (Wm⁻²), \( A \) is the surface area of anode (m²), \( E \) is energy transferred (J) and \( t \) is fermentation time (s).

The current profile and the degradation of methyl orange were observed during the pollution experiment. In monitoring the pollutant degradation, samples were obtained from the anode chambers at an interval of one hour for 10 hours and the absorbance was measured using a 722G visible spectrophotometer at 524 nm wavelength. The final concentration of methyl orange in the anolyte was estimated using equation (4):

\[ y = 0.3185 \ln(x) + 1.8619 \]  

where \( y \) is the absorbance of the sample (AU) and \( x \) is the concentration of methyl orange, mM.

3. Results and discussion

3.1. Characteristics of the meat processing wastewater

On-site and laboratory analysis of the wastewater quality revealed the following characteristics: temperature - 30°C, TDS - 1,690 ppm, EC - 2,370 µS, pH - 4.7, and COD - 3,326 ppm.

3.2. Open circuit voltage of the MFC systems using meat processing wastewater

The MFCs fed with full strength meat processing wastewater demonstrated high open circuit voltage. Using carbon brush, the MFC achieved an average potential difference of 922.17 mV with a peak value of 1,032 mV and lowest value of 700 mV. With titanium mesh, the average potential difference was significantly higher at 1,005.50 mV with a peak value of 1,052 mV and lowest value of 620 mV. These values were comparable to published studies which achieved high OCV values using different simple and complex substrates and inocula [35-36].
3.3. Current profile

The current profile for MFC-A (with carbon brush) was highest when the volume of wastewater and microbial inoculant in the anode was equal. In close circuit condition, current peaked at 1.38 mA and dipped to 1.22 mA. Significantly, the average current for different anolytes was highest at 1.326 mA (1:1), followed by 1.112 mA (1:3), and 0.942 mA (1:0). A correlation analysis of the increasing percentage of the inoculant and current profile revealed a strong linear relationship between the two variables \((p = 0.000)\). The performance of the MFC-B was less stable, though, it exhibited increasing values in all setups.

3.4. Power and energy generation with meat processing wastewater

With MFC-A and MFC-B, power densities were computed for the anolyte containing 1:1 and 1:3 ratio of wastewater and microbial inoculant. Comparatively, MFC-B has significantly higher average power density at 1.223 Wm\(^{-2}\) \((t(24.164) = -32.610)\) probably due to the surface area of the electrodes. With similar voltage and current values, a smaller surface area will yield higher power density. The corresponding bioenergy generation profiles also revealed that with a carbon brush, the MFC achieved an average of 15.609 kJ with a peak value of 30.109 kJ while using titanium mesh, the average is 9.440 kJ and the peak value was 19.415 kJ.

3.5. Effect of methyl orange on current output profile

The average current output values before and after spiking the anolytes with methyl orange were compared for the MFCs with 1:0 and 1:3 ratio of wastewater to microbial inoculant (Figure 1). For MFC-A (1:0), methyl orange increased the average current from 0.94 mA to 1.25 mA. In contrary, the average current in MFC-B (1:0) remained constant at 1.01 mA. For both setups with 1:3 ratio of wastewater to microbial inoculant, average current decreased. Statistical findings revealed that except for MFC-B (1:0), the change in the current before and after the addition of the contaminant was highly significant.

3.6. Decolorization of methyl orange

The results demonstrated the ability of the MFC in decolorizing methyl orange. The electrochemical degradation of the contaminant may be attributed to reaction between hydroxyl radicals that are formed from water electrolysis and organic molecules that are adsorbed on the anode surface. When the conjugated structure of the methyl orange cleavages upon reaction with OH radicals, it leads to color disappearing and part of the intermediates is oxidized into a series of aromatic metabolites then finally into \(\text{CO}_2\) and \(\text{H}_2\text{O}\) [37-39].

The final concentrations of methyl orange and the removal efficiencies (Figure 2) were estimated after 10 hours of operation. The concentration of methyl orange decreased from 0.10 mM and was lowest in MFC-A where 0.038 mM (for 1:0 ratio) and 0.022 mM (1:3 ratio) final concentrations were achieved. These are equivalent to 62.28 percent and 78.09 per cent removal. On the other hand, final concentrations of the methyl orange in MFC-B were also significantly reduced to 0.041 mM (1:0 ratio) and 0.039 mM (1:3) which correspond to 59.10 and 61.08 percent removal. Generally, it was observed that higher removal efficiencies of the azo dye in the MFCs were achieved when the concentration of the microbes in the anodic chamber was higher.

The decolorization efficiencies of the MFC setups were compared with results of previous studies. Decolorization of methyl orange was investigated in dual-chamber MFC using graphite electrodes coupled with photocatalysis which achieved 73 percent removal in 24 hours [40]. An enhanced removal efficiency of 90.4 percent was observed a single-chamber MFC within six hours of operation [41]. Removal efficiency of 100 percent using methyl red was documented from an electrolysis cell coupled with MFC [42]. Using photocatalytic single-chamber MFCs, a maximum of 98 percent decolorization was observed [38]. The most recent publication reported 89 percent efficiency in degrading methyl orange using graphite polyester composite electrodes doped with metal ions which proved the capability of the MFC in eliminating azo dyes [43].
Figure 1. Comparison of the average current in MFC systems before and after contamination of the anolyte with methyl orange.

Figure 2. Percentage removal of methyl orange in the MFC setups after 10 hours of operation.

4. Conclusions and recommendations
A new approach to treating meat processing wastewater containing azo dyes while simultaneously producing bioelectricity in double-chamber microbial fuel cells inoculated with soil microorganisms was investigated in this study. Meat processing wastewater was identified as substrate due to its abundance. Methyl orange was selected as contaminant because of its wide applications and toxic effects to aquatic animals and humans. Based on the results, the MFCs fed with meat processing wastewater can be a potential source of renewable energy. Furthermore, the MFC systems can be effectively used to remove contaminants such as azo dyes from wastewater. The study confirmed that the MFCs inoculated with these soil microorganisms have the capability to degrade azo dyes while simultaneously producing electricity. Further investigation is required to confirm the pathways in which methyl orange is degraded and the rate of decolorization to determine the conditions wherein
the contaminant will be completely degraded. It is also recommended to analyse the nature in which contaminants like azo dye affects the behaviour of microorganisms in the MFC anodes.

5. References
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