Study the relationship between bacterial growth, Congo red dye removal and voltage production using single chamber microbial fuel cell

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Abstract. In this work single chamber microbial fuel cell (SCMFC) incorporating agar salt bridge pipe were used to investigate the interaction mechanisms between bacterial growth, Congo red decolorization, and bioelectricity generation. After 20 days of SCMFC operation in a batch test using a mixture of Congo red (CR) and sucrose as fuel, graphite plates electrodes, temperature 32ºC and under anaerobic working condition results showed that 98% decolorization at dye concentration 300 mg/L demonstrated at UV-Visible Spectrophotometer (wavelength 500 nm) and maximum voltage output of approximately 263.9 mv. Microbial community analysis showed the high species of bacteria Shigella dysenteria and Salmonella sp. (23×10⁹ and 19×10⁹ Count CFU/mL respectively). The bacterial growth activates start to decrease due to substrate reduction for metabolic process and pH automatically dropping from 8.2 to 6.9 as a result of the reaction. This study offered a feasible option for the dye wastewater treatment and electricity generation by using single chamber microbial fuel cells.

Key Word: SCMFC, Congo Red, Decolorization, Bacterial Growth, Voltage.

1. Background

Production of energy, storing and utilizing are subjects which are raising prevalent within modern study fields and are of world interest and significance [1, and 2]. Study into alternate renewable power production sources are raising rapidly, with vast research, in an abundance of areas including: wind energy [3], solar energy [4], geothermal energy [5], tidal energy [6] and bioenergy generation [7, and 8]. Recently no single renewable energy source has the potential to overcome with and replace the conventional energy sources including fossil-fuel based energy production approach. However, mixing renewable energy sources such as, solar-hydrogen fuel cells and solar-wind hybrids may be promising alternatives [9, and 10]. MFC “Microbial fuel cell” is an option source for long term energy recovery and pollution control reduction system. MFCs are a biochemical system in which power is resulting by oxidizing the organic contaminants exist in the wastewater [8]. Microbial fuel cell (MFC) is a promising microbial electrochemical techniques (METs) that could elicit chemical energy and realize energy recovery from organic contaminants through bio-catalytic processes. It has the advantages of
biodegradation technique and electrochemical technology, which can bio-oxidize easily biodegradable contaminants at anode to produce protons and electrons that will be accepted by electron accepter at cathode. In current years, the utilizing of MFC increased to treat wastewater along with electricity production, such as azo dye, triclosan, and landfill leachate [9, 10, and 11]. Furthermore, it has a big ability to treat heavy metals in MFC [12]. Currently, MFCs have been evaluated for their potential to treat azo dyes, recalcitrant pollutants exist in the discharge from manufacturing of dye and consuming industry, and to produce electrical energy. Azo dyes, estimated by the presence of a nitrogen-nitrogen double bond (N=N) in their chemical structures, determined to be more than 50% of all commercial dyes (about 1000, 000 tons per year) [13], and are extensively utilized in different industries such as food, textiles, pharmaceutical, printing, and cosmetics [14, and 15]. Among the dyeing process, about 10-15% of the dyes utilized are discharged into the wastewater [13, 16, and 17]. The effluent of azo dye containing wastewater not just impacts the aesthetic appearance and transparency of surface water bodies, however also causes a big expected toxic risks to the ecosystem, posing serious environmental problems [3, 18, and 19]. Thus, the removal of azo dye contaminated wastewater has been a serious environmental problem for a long time [14]. The metabolic abilities of its microbial population widely estimate the performance of an MFC. For example, the production of energy is significantly impacted by the substrate specificities of the organisms present in an MFC. In the current research, the relationship between Congo red degradation and microbial growth in a single-chambered MFC incorporating agar salt bridge pipe was investigated. The voltage production in (mV), pH variation and electrical conductivity were studied as well.

2. Material and Methods

2.1 Studied dye

Congo red is sodium salt of 3, 3’-((1, 1’biphenyl)-4, 4’-diyl) bis (4-aminonaphthalene-1- sulfonic acid). In water and ethanol Congo red is soluble, in contrast it is very slightly soluble in acetone. Table 1 shows the characteristics of Congo red dye.

2.2 SCMFC construction

SCMFC fabricate from a plastic container has a total volume of 6 liters (working volume of approximately 5 liters plus a 1-liter headspace) which is used as an anaerobic anode chamber. This chamber connected to salt bridge PVC pipe with a length and diameter of 5 and 1.5 inches, respectively at 1 cm high from the bottom, on the other side the sample port was located at 4 cm above the bottom to take a sample for a checkup. Two graphite plates of (11cm×3.5 cm× 1cm), one is placed inside the container and other electrodes are placed at the end of the open salt bridge pipe that acts as a cathode. The electrodes are washed with distilled water before being employed in the SCMFC experiment to facilitate attachment of microbes and electron transfer. These electrodes are linked together with copper wire. The experiment was carried out at a temperature of 32 °C using heater with thermostat. SCMFC photo and schematic diagram are shown in Figure 1.
### Table 1. Characteristics of Congo red dye

| Item                      | Congo red (CR) |
|---------------------------|----------------|
| Molecular formula         | C_{32}H_{22}N_{6}Na_{2}O_{6}S_{2} |
| Wave length (nm)          | 500            |
| Molecular weight g/mol    | 696.66         |
| Packing                   | Solid / powder |
| pH range                  | Blue (3.0) to red (5.0) |
| Class                     | Diazo          |
| Structure                 |                |

![Figure 1. Schematic diagram and photo of SCMFC.](image)

#### 2.3 Sludge samples

The thick activated sludge was collected from thickener from Rustamiyah WWTP- the old project. The activated sludge samples are autoclaved for 20 min, 121 °C and 1.5 bar to exclude the effect of other
microorganisms. To diagnosis species of bacteria exist in thickener sludge, a sample of (0.1 mL) of sludge is spread on various nutrients agar and cultivated in incubator (Memmert, ICP 500, Germany) at 66 °C for 3-7 days. The bacteria found in the sludge is heterogeneous as shown in Table 2 and includes mainly of facultative anaerobic bacteria, according to the biochemical tests and using smart technology (Api 32 system) to diagnose various species of bacteria in relatively short time utilizing a stripe involved of 32 chambers as shown in Figures below.

![Figure 2. Petri dish of different nutrients agar microorganisms cultivate.](image)

![Figure 3. Microorganism appearance where in light microscope.](image)

![Figure 4. Strake of Mini Api system bacteria cultivate.](image)

![Figure 5. Mini Api system to diagnostic where bacteria, Biomereux, France.](image)

2.4 Prepare salt bridge pipe

Agar was used to prepare the salt bridge used solid media. The chemical formula and molecular weight of agar used is C\textsubscript{14}H\textsubscript{24}O\textsubscript{9} and 336.337 g/moL, respectively. The bridge was prepared using 15% of agar in a solution that contains a salt concentration of 1M KCl. The prepared solution (salt+ agar) was boiled and cast in the PVC pipes (5 inch in length and 1.5 inch in diam.). The salt bridge stored in the refrigerator for solidification to be ready for connected to the SCMFC. Figures below show the preparation of agar salt bridge.
2.5 Preparation of simulated wastewater

The medium was prepared by dissolving the dye at a concentration of 300 mg/L mixed with sucrose $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ 468 COD/L and adding 500 mL of sludge after filtered through filter paper to remove impurities, 7.75 g of NH$_4$CL, and 3.25 g of KCL and 650 ml of phosphate buffer solution (pH 7.2) to the anode chamber as nutrient. Figures below show steps of simulated dye wastewater. 300 mg/L was taken since many of dyes effluents discharge not more than this concentration [3, 7].
2.6 Analysis

The voltage measured every 12 hours using a digital multi-meter (UNI-T model), pH using digital pH meter and EC (WTW, Cond 3110, Germany). The absorbance of Congo red dye sample was measured using UV–visible scanning spectrophotometer. Maximum absorbance for the sample of Congo red was found at 500 nm. All the absorbance measurements were carried out as the respective $\lambda_{\text{max}}$ values to attain maximum sensitivity of the instrument and recording of UV–visible absorption spectra over a wavelength range from 400 to 800 nm. Initially, before analysis, dyes samples were filtered through filter paper (Whatman® Grade 542, Hardened Ashless Filter Paper, England) to remove the particulate matter. Figure 8 show prepared of samples for analysis and the spectrophotometer apparatus are shown in Figure 8.

Decolorization activity was calculating following the equation below:

\[
\text{Efficiency of decolorization} \% = \frac{A - B}{A} \times 100
\]

A: is the initial absorbance and B: is the observed absorbance

![Figure 8. Collection samples (A), sample filtered (B) and (C) Spectrophotometer (Thermo-genesys 10 UV, USA) ](image)

3. Result and Discussion

3.1 Voltage production of SCMFC

The voltage production of the SCMFC with Congo red over 20 days of batch operation obtained in open-circuit voltage (OCV) is shown in Figure 9. The maximum voltage had been obtained on the 10th day to be 263.9 and 236.7 mV at 5 AM and 5 PM, respectively. While minimum concentration were obtained on the 19th day to be 103.6 and 101.2 mV, respectively at the same times. This differences in voltage generation may be attributed to the complexity of the heterogeneous bacterial culture and the sharply changing dynamics through the bacterial species till an equilibrium is reached out. Also, bacteria have an effective role in MFC voltage generation, the total count of bacteria and species of bacteria was measured in SCMFC shown in Table 2, and it was concluded that voltage increases with an increase in the rate of growth of bacteria and started to decrease when bacteria activities decrease. The same explanations were given by other researchers [5, and 8].
Table 2. Bacterial species identified in sludge CFU/mL

| Species of Bacteria | Pseudomonas aeruginosa | Escherichia coli | Bacillus subtilis | Proteus mirabilis | Entrobacter cloacae |
|---------------------|------------------------|------------------|------------------|-------------------|-------------------|
| Count CFU/mL         | 3.1 × 10⁸               | 3.9 × 10⁸        | 2.2 × 10⁶        | 5.3 × 10⁵         | 3.1 × 10⁵         |
| Species of Bacteria  | Salmonella sp.          | Shigella dysenteria | Staphylococcus xylosus | Aeromonas caviae | Klebsiella pneumoniae |
| Count CFU/mL         | 19 × 10⁹               | 23 × 10⁹         | 1.5 × 10⁹        | 2.24 × 10⁴       | 4.23 × 10⁴       |

Figure 9. SCMFC voltage production.

3.2 Effect of pH

Figure 10 shows the recordings of pH through the removal process. The bigger value in SCMFC of pH was 8.2. However, at the end of the experiment, the smallest value was measured to be 6.9 at the end of 20 days of operation. This pH level in the natural range level of allowable concentration to throw in the river (6.5-9.5) [20]. This decreasing in pH values may be due to the effect of the anaerobic treatment which produces CO2 that combines with water and forms H₂CO₃. The bacterial growth needs could change the pH in the anode chamber which could further cause some differences in primary physiological elements, such as membrane potential, the ions concentration, proton-motive force and biofilm formation [21].
3.4 Bacterial growth

Bacterial metabolism is a major in MFC operation which witnessed some limitations due to the decreasing in the biological process taking place, specifically around the anode surface. This can be enhanced by maintaining an optimum temperature for biological activity. Therefore, the temperature of the current SCMFC was maintained at 32 °C. The bacterial growth started to increase gradually at first (Lag phase, 420CFU/100 mL), then the increase became rapidly until reach maximally 1000 420CFU/100 mL (Log phase), then the increase become steadily due to substrate decreasing for metabolic processes. The dye concentration and voltage production are high on the Log phase and then became steadily at stationary phase which prove the relationship between dye (substrate) concentration and bacterial activity and subsequently on the voltage production, same behaviors were noted by others [8, and 10].

**Figure 10.** Variation of pH.
3.4 *Decolorization of dye wastewater*

The reduction in Congo red concentration and removal efficiency are shown in Figures 12 and 13, respectively. The maximum removal efficiency was obtained on the 10th day to be 98%, where the concentration reached to 4 mg/L. The initial concentration was 300 mg/L then it gradually decreased with time until reach steady concentration of 4 mg/L where the reduction was 296 mg/L which is considerably high. The impact of maximum voltage was significant when the dye concentration decreased. The metabolism process of bacteria species is main reason for the decolorization of Congo red in the SCMFC system. Many researchers had studied the performance of different MFC modes including single chamber (SCMFC) and dual chamber (DMFC) to treat dyes contaminated wastewater and for voltage production at different circumstances. Table 3 show some of these studies. The results of the current study are considerably high which improve the successfully of SCMFC design.
Figure 12. Reduction of concentration.

Figure 13. Removal rate changes of Congo red.
Table 3. Previous studies for Congo red (CR) dye removal efficiency through MFCs.

| Type of MFC system | Target dyes based pollutant | Type of electrode | Inoculum / Microbes | Initial concentration (mg/L) | Removal efficiency Percentage | Reference |
|-------------------|-----------------------------|-------------------|---------------------|------------------------------|-------------------------------|------------|
| SCMFC             | CR                          | Carbon papers     | Mixture of aerobic and sludge | 300                          | 90                            | [22]       |
|                   |                             | (non-wet proofed porous) |                           |                              |                               |            |
| SMFC              | CR                          | Graphite felt     | Anaerobic sludge      | 300                          | 70                            | [23]       |
| DMFC              | CR                          | Plain carbon felts | Anaerobic sludge      | 100                          | 86.4                          | [24]       |
| SMFC              | CR                          | Graphite fibre brush | Anaerobic sludge      | 200                          | ≥88                           | [25], [26] |
| SCFMC             | CR                          | Graphite plate    | Anaerobic sludge      | 300                          | 98                            | This study |

3.3 Effect of EC

The electrical conductivity (EC) were measured during the operation of the SCMFC. As common, there is a big relationship between EC and voltage production. The initial EC was measured to be 18.3 mS/cm then it decreased gradually to reach 12.2 mS/cm on the 20th days. This decreased in in EC is caused by bacteria which need to consume salts during growth. Figure 14 show the EC behavior during the time period. The researchers suggest to have a good minerals salts to sustain high production of voltage [10, 11]. High value EC accelerates proton convey and subsequently decreases the internal resistance of the system. Lefebvre et al. 2012 [26] proved that adding up to 20 g/L of NaCl improved the overall performing of SCMFC by lowering the internal resistance by 33% and increasing the maximum voltage generation by 30%.
Figure 14. EC variation during SCMFC process.

4. Conclusion

Single chamber Microbial fuel cell (SCMFC) technology shows considerable promise for power generation and a good ability to remove azo Congo red dye (maximum 98%) under anaerobic conditions. The constructed SCMFC showed good performance under normal conditions of pH and temperature. This could be enhance through studying of different parameters including temperature, electrodes surface area, electrode configuration, etc. All these will be studied in next paper. Using of sludge for bacteria source seem to be feasibly economic and technologically easier than isolate and cultivate specific species of bacteria. Salt increases power production by increasing conductivity, however this need to be studied in further details.

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