

## The utilization of *Eschericia coli* and *Shewanella oneidensis* for microbial fuel cell

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**Abstract.** Microbial Fuel Cell (MFC) is a technology that convert chemical energy into electrical energy with catalytic reaction from microorganism. The research method using bacteria in organic waste on anode compartment and ferricyanide solution on cathode compartment. Wastewater from sugar factory was used as organic waste with bacterial concentration of 10%, 12.5%, 15%, 17.5% (v/v) and with bacteria mixture ratio 1:1, 1:2, 2:1. The result of the research showed that the best voltage of bacteria concentration was 12.5% for *Eschericia coli* and *Shewanella oneidensis* bacteria, which were 847 mV and 988 mV, and for the mixed bacteria variable was 1:2 ratio with the voltage was 1261 mV. For 12 days, the largest percentage of the decrease of BOD<sub>5</sub> was 12.5% *Eschericia coli* bacteria concentration variable reached 84.531% and 17.5% *Shewanella oneidensis* was 73.779%. The best Fe<sup>3+</sup> reduction was 53.52% for *Escherichia coli* at 10% concentration (v/v), and for *Shewanella oneidensis* bacteria reached out of 62.22% at 15% concentration (v/v). In the variable with mixed bacteria was obtained the best reduction result on the ratio of *Eschericia coli* : *Shewanella oneidensis* 1:2 was 77,44%.

### 1. Introduction

MFC is one of the ways to produce energy continuously in the form of electricity from degradable materials. MFC is a tool for converting chemical energy into electrical energy with the aid of catalytic reactions of microorganisms [1]. MFC generate electricity by oxidizing anaerobic organic matter through the aid of bacteria. The catalytic activity and proton transfer are carried out using enzymes or additional mediators [11]. The working principle of MFC is the use of microbes that perform metabolism of the medium at the anode to catalyze the converter of organic matter into electrical energy by transferring electrons from the anode through a cable and generating current to the cathode [13]. The electron transfer of the anode is received by the complex ion at the cathode which has free electrons. Protons were also delivered to the cathodic through cation exchange membrane. The protons are transported through the salt bridge. In the cathodic electrons were received by Fe(III). And the power was generated by the movement of electrons from the anodic to the cathodic. Fe(III) reduction and current production occurred because of the movement of these protons and electrons [6]. MFC has several advantages that can generate electricity from organic waste and renewable biomass [7]. Bacteria are able to catalyze and adapt well to different organic materials present in environmental

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waste to produce electrons. In the cathode compartment, there is a conductive electrolyte solution. Potassium ferricyanide ( $K_3Fe(CN)_6$ ) is very well known as an electron acceptor in MFC systems [6].  $K_3Fe(CN)_6$  is an electroactive species capable of capturing electrons well with a standard reduction potential value of +0.36V. The greatest advantage in the use of potassium ferricyanide is the resulting low overpotential when using a carbon electrode [11].

## 2. Materials And Methods

### 2.1 MFC construction

Dual chamber microbial fuel cell was constructed, with a tubular junction of 2 cm diameter, connecting the compartments contained the salt bridge [9] at a height of 4 cm above the base. The salt bridge was formed by dissolving 4% KCl salt in 10% agar [12]. The working volumes for the anode and cathode chambers were 800 mL. The graphite size 22.844 mm were used as electrodes for both the chambers. The electrodes in the two chambers were connected with a copper wire for electron ( $e^-$ ) transfer. The exposed metal surfaces were sealed with non-conductive epoxy tapes. Dry electrodes were introduced into anode and cathode chambers. The whole anaerobic set up was autoclaved before assembling [2].

### 2.2 Cathodic and anodic reagent preparation

The cathodic chamber was filled with the solution of  $K_3Fe(CN)_6$  made by mixing 400 ml a solution of 0.1 M and 400 ml of buffer phosphate 0.1 M with pH 7.0 [10]. The anodic chamber was inoculated with the bacteria (*Escherichia coli* and *Shewanella oneidensis* and binary culture of both bacteria) and sugar waste with 5% concentration [14].

### 2.3 Electrode preparation

Carbon electrodes soaked into a solution of HCl of 1 M for 1 day later flushed by using aquades. After that, the electrodes soaked again into a solution NaOH of 1 M for 1 day with aquades. Electrodes soaked in solution aquades until the electrodes will be used.

### 2.4 Procedure

A digital multimeter was connected on both the electrodes. The positive end at a cathodic chamber and the negative end at a anodic chamber. The voltage was recorded every 24 h. Then, the power was calculated by using Eq 1

$$P = (V I)/A \quad (1)$$

Where P is the power density in milli watts (mW), V is the voltage in milli volts (mV), I is the current density in milli ampere (mA) and A is the surface area of electrode. Voltage and current measurement is carried out by using a multimeter with a system OCV (Open Circuit Voltage) [8]. By getting the voltage and the current, then it can be calculated that the resulting power density.

The variable of bacteria concentration of this experiment is 10%, 12.5%, 15% and 17.5% (v/v) with volume of chamber is 800 ml. For example, to obtain the variable of bacteria concentration 10% is measured 10 ml for the volume of bacteria and 790 ml for the wastewater. In anodic chamber was record the voltage, pH and BOD, otherwise in cathodic chamber was measured the voltage, pH and the Fe reduction. The BOD value is obtain with measured the liquid of the anodic chamber with DO meter, and then the result value of DO meter was calculated by using Eq 2

$$BOD_5 = DO_s^o - DO_d^f + \text{volume of bottle}/\text{volume of sample} (DO_d^f - DO_s^f) \quad (2)$$

Where,

$DO_s^o$  = initial dissolved oxygen concentration in sample

$DO_d^f$  = final dissolved oxygen concentration in water

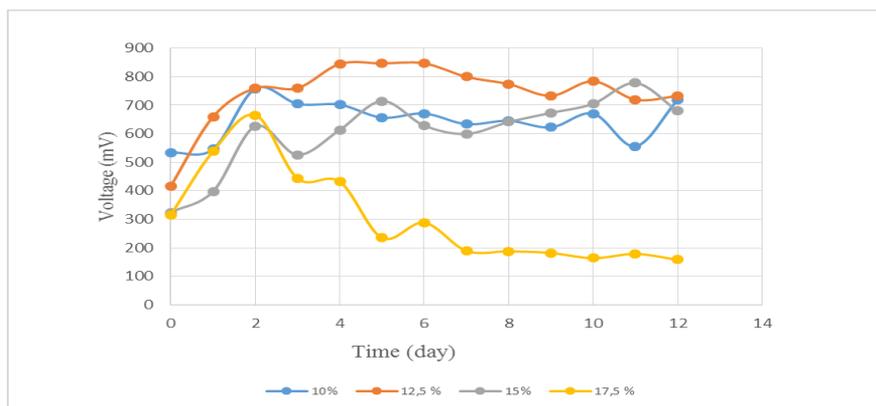
$DO_s^f$  = final dissolved oxygen concentration in sample

### 3. Result And Discussion

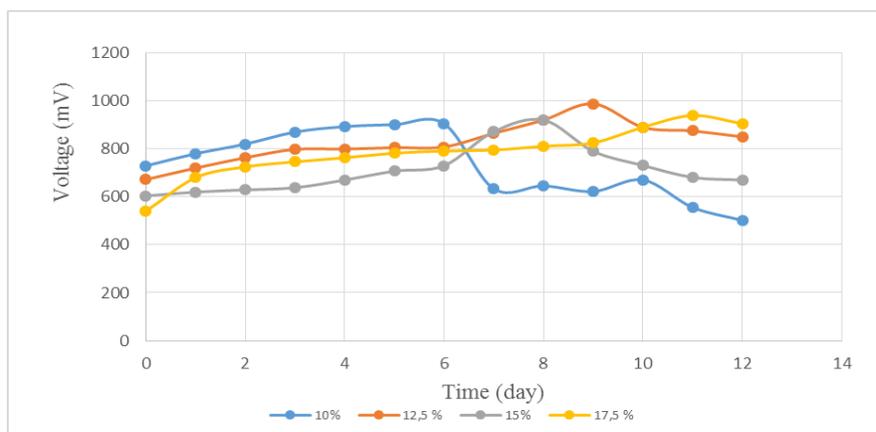
#### 3.1 Voltage and Power Density

Analysis of voltage and current are performed every day. The result of this experiment, maximum voltage of *Eschericia coli* was generated at a concentration of bacteria 12.5% (v/v) was 847 mV and maximum voltage of *Shewanella oneidensis* was generated at a concentration of bacteria 12.5% (v/v) was 988 mV. For the mixed bacterial variables, the voltage was generated in ratio 1:2 was 1261 mV. Power density is affected by voltage, electric current and the surface area of electrode. In this experiment, an electric current recorded was 0.01 mA, and for the surface area of the electrodes was 22,844 cm<sup>2</sup>. Maximum power density of *Eschericia coli* at a 12.5% (v/v) was 0.371 mW/m<sup>2</sup> and maximum power density of *Shewanella oneidensis* at a 12.5% was 0.432 to mW/m<sup>2</sup>. For the mixed bacterial variables, the maximum power density at a 1:2 was 0.552 mW/m<sup>2</sup>.

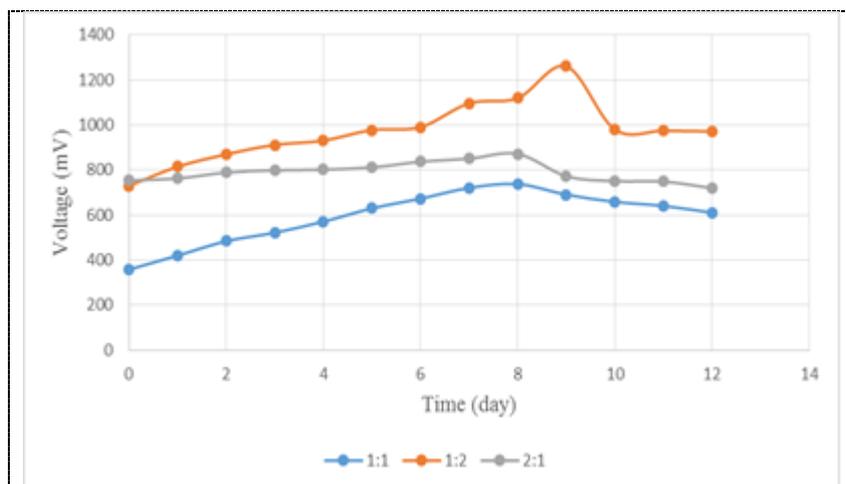
The number of microorganisms can affect in the voltage, because of the more microorganisms it will be more electrons were released. The substrate on the anode chamber become food for the microorganism and also energy to release electrons. In this experiment, there are a many bacteria but the voltage generated is small. This is due to the presence of a biofilm layer, formed by bacterial metabolic activity, where the layers can also be barriers to bacteria to release electrons and degrade organic materials in the substrate.



**Figure 1.** Voltage of *Eschericia coli* for 12 days



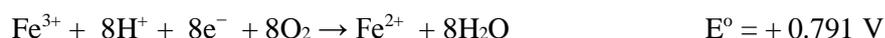
**Figure 2.** Voltage of *Shewanella oneidensis* for 12 days



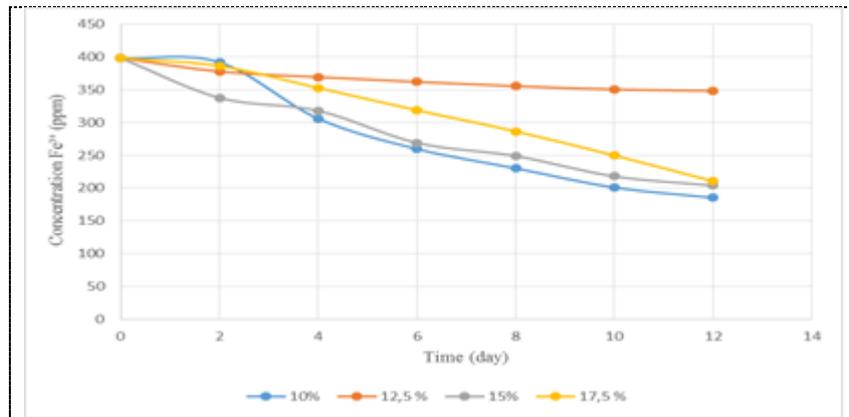
**Figure 3.** Voltage of mixture bacteria *Escherichia coli*:*Shewanella oneidensis* for 12 days

### 3.2 Reduction of Ferricyanide

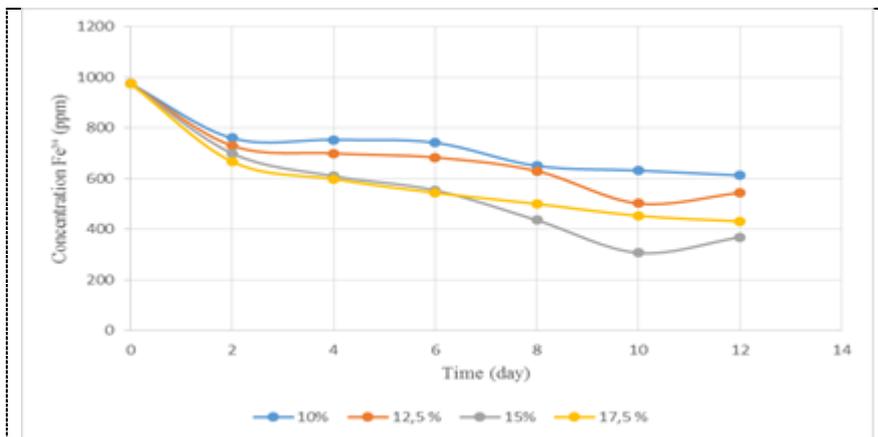
In the cathode chamber of the MFC reactors, used 400 mL ferricyanide solution 0.1 M mixed with 400 mL phosphate buffer solution. In the cathode chamber there is a reduction reaction of Fe(III) to Fe(II). The results of this experiment indicated that the Fe(III) level was decrease. In variable of *Escherichia coli*, Fe(III) was reduced 53.52 %, 12.56 %, 48.86% and 47.16 % for bacteria concentrations of 10%; 12.5%; 15% and 17.5%. In variable of *Shewanella oneidensis*, Fe(III) was reduced 37.14 %, 44.20 %, 62.22% and 55.78 % at bacteria concentrations of 10%; 12.5%; 15% and 17.5%. And then in variable of ratio of *Escherichia coli* and *Shewanella oneidensis*, Fe(III) was reduced 76.54 %, 77.45 %, and 66.74 % at bacteria ratios of 1:1 ; 1:2 and 2:1. In the cathode, reduction reaction occurs, where Fe<sup>3+</sup> will turn into Fe<sup>2+</sup> with the help of the electrons coming from the anode. Fe<sup>2+</sup> is oxidized to Fe<sup>3+</sup> by releasing the electrons and will reacted with H<sup>+</sup> coming from the anode chamber through the salt bridge to form water molecules. The Fe(III) reacted with these electrons and reduced Fe(III) to Fe(II) form as in equation below :



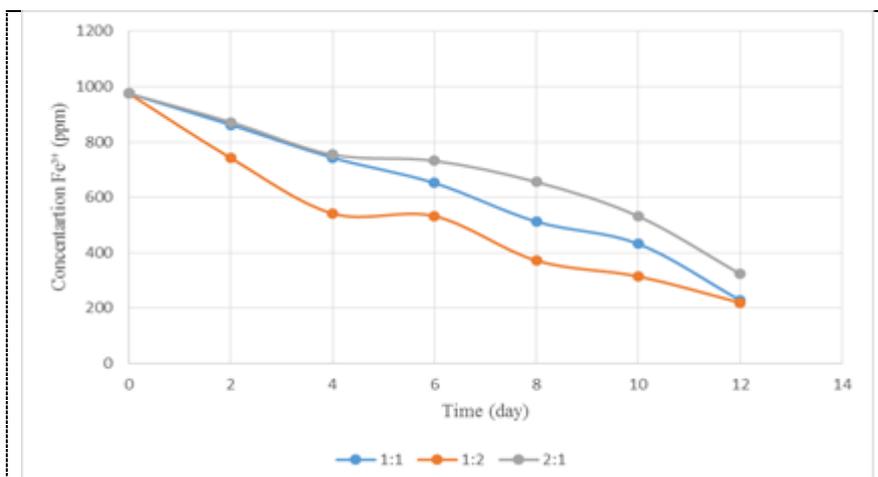
The half-cell reaction has a redox potential ( $E^\circ$ ) of 0.791 V [5]. The positive potential ( $E^\circ = + 0.791 \text{ V}$ ) indicates the greater affinity of Fe(III) for electrons and tendency to be reduced [4].



**Figure 4.** Reduction of Ferricyanide concentration of *Eschericia coli* for 12 days



**Figure 5.** Reduction of Ferricyanide concentration of *Shewanella oneidensis* for 12 days



**Figure 6.** Reduction of Ferricyanide concentration of mixture bacteria *Eschericia coli:Shewanella oneidensis* for 12 days

#### 4. Conclusion

In this experiment, it can be concluded that the higher density power generated at *Escherichia coli* concentration of 12.5% was 0.371 mW/m<sup>2</sup>, whereas *Shewanella oneidensis* concentration of 12.5% was 0.432 mW/m<sup>2</sup>, and for variable ratio of *Escherichia coli* and *Shewanella oneidensis* is in ratio 1:2 was 0.552 mW/m<sup>2</sup>. The largest decrease in the reduction of Fe of *Escherichia coli* are at concentrations of 10% was 53.52%, *Shewanella oneidensis* at concentrations of 15% was 62.22%, while for a variable mixture of bacteria on the ratio 1:2 was 77.45%.

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#### References

- [1] Allen RM and Bennetto HP. 1993. *Microbial fuel cells: electricity production from carbohydrates*. J. Appl. Biochem. Biotechnol. 39: 27-40.
- [2] A. Carmalin Sophia and Saikanth Sai. 2016. *Modified microbial fuel cell for Cr(VI) reduction and simultaneous bio-electricity production*, Journal of Environmental chemical engineering 4 2402–2409
- [3] Gralnick A. Jeffrey dan Heidi H. Hau. 2007. *Ecology and Biotechnology of the Genus Shewanella*. Annual Review of Microbiology. 61:237-58
- [4] Karthikeyan. 2016. *Effect of composites based nickel foam anode in microbial fuel cell using Acetobacter aceti and Gluconobacter roseus as a biocatalysts*, J. Bioresource Technology.
- [5] Liu H and Logan BE. 2004. *Electricity Generation Using An Air Cathode Single-Chamber Microbial Fuel Cell In The Presence and Absence of Proton Exchange Membrane*, J. Environmental Science Technology 38: 4040
- [6] Logan E. Bruce, et al. 2006. *Microbial Fuel Cells : Methodology and Technology*. Environmental Science & Technology. Vol. 40, No. 17.
- [7] Lovley, Derek R. 2008. *The microbe electric: conversion of organic matter to electricity*, Current Opinion in Biotechnology, 19:564-571
- [8] Miroliaei M.R, Abdolreza Samimi, Davod Mohebbi-Kalhari. 2015. *Competition Between E. Coli and Shewanella s. for Electricity in Air Cathode MFC in Presence of Methylene Blue as Artificial Mediator*. Environmental Progress & Sustainable Energy. Vol. 34 No. 4.
- [9] Miroliaei M.R, Abdolreza Samimi, Davod Mohebbi-Kalhari. 2014. *Kinetics Investigation of Diversity Cultures of E.coli and Shewanella sp., and their Combined Effect with Mediator on MFC Performance*. Journal of Industrial and Engineering Chemistry. Vol 36 No.4
- [10] Mohan S.V, G Mohanakrishna, B Purushotham Reddy, R Saravanan, P.N Sarma. 2007. *Bioelectricity generation from chemical wastewater treatment in mediatorless (anode) microbial fuel cell (MFC) using selectively enriched hydrogen producing mixed culture under acidophilic microenvironment*. Biochemical Engineering Journal 39 121-130
- [11] Rabaey K, Boon N, Siciliano SD, Verhaege M, dan Verstaete W. 2004. *Biofuel cell select for microbial consortia that self-mediate electron transfer*. J. Applied Environmental Microbiology 70: 5373-5382.
- [12] Ramya N, Renganathan K, S Barathi, Venkatraman. K. 2013. *Performance of Salt-Bridge Microbial Fuel Cell at Various Agarose Concentrations Using Hostel Sewage as Substrate*. International Journal of Advancements in Research & Technology, Volume 2, Issue 5, May
- [13] Rozendal, R. A., Hamelers, H. V. M. & Buisman, C. J. N. 2006. *Effects of Membrane Cation Transport on pH and Microbial Fuel Cell Performance*. Journal Environmental Science Technology, Vol. 40, No.17, 5206–5211.
- [14] Scott, Keith dan Murano, Cassandro. 2007. *Microbial Fuel Cells Utilising Carbohydrates*. Journal of Chemical Technology and Biotechnology (82):92-100