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## Performance of *Klebsiella oxytoca* to generate electricity from POME in microbial fuel cell

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**Abstract.** This study is aimed to evaluate the electricity generation from microbial fuel cell (MFC) and to analyze the microbial community structure of city wastewater and anaerobic sludge to enhance the MFC performance. MFCs, enriched with palm oil mill effluent (POME) were employed to harvest electricity by inoculating of *Klebsiella oxytoca*, collected from city wastewater and other microbes from anaerobic sludge (AS). The MFC showed maximum power density of 207.28 mW/m<sup>3</sup> with continuous feeding of POME using microbes from AS. Subsequent replacement with *Klebsiella oxytoca* resulted maximum power density of 1236 mW/m<sup>3</sup> by utilizing complex substrate POME which was six times higher as compared to MFC operated with AS. Based on Biolog gene III analysis, relatively higher abundance of *Klebsiella oxytoca* was detected in the city wastewater. Predominant microorganisms such as *Gammaproteobacteria*, *Azospiraoryzae*, *Acetobacterperoxydans* and *Solimonasvariicoloris* were isolated from palm oil anaerobic sludge as well as from biofilm of MFC. Enriched electrochemically active bacteria *Klebsiella oxytoca* showed better performance to generate electricity from complex POME substrates compare to AS. These results demonstrate that the power output of MFCs can be increased significantly using *Klebsiella oxytoca*.

### 1 Introduction

The use of fossil fuels, especially oil and gas, for all human needs in recent years has accelerated and this triggers the global energy crisis [1]. ‘How can human civilization sustain without natural petroleum reserve?’ is the prime question of the day to answer. In this regard, the central task is to formulate alternate energy sources or mechanisms or devices to combat the energy crisis [2]. During the 20th century, energy consumption increased dramatically and an unbalanced energy management exists. While there is no sign that this growth in demand will abate (particularly amongst the developing nations), there is now an awareness of the transience of nonrenewable resources and the irreversible damage caused to the environment. [3]. It is well known that fuels, such as ethanol, butanol, methane and hydrogen can be produced by microorganisms. But, the electricity production using microbes, which is known as microbial fuel cells (MFCs), is recent development in energy biology and highly attracting area [4, 5, 6].

The palm oil mill industry in Malaysia has gone through continuous and successive development as such that Malaysia has turned out to be the second largest palm oil producer in the world after Indonesia. According to the latest report, Malaysia produced approximately 80 million dry tonnes of solid biomass in 2010 and is expected to increase to about 100 million dry tonnes by 2020 [7]. The positive progress of palm oil industry

however comes with serious impact to the environment. The process of extracting crude palm oil from the fresh fruit bunch (FFB) requires an estimate of 1.5 m<sup>3</sup> of water to process one tonne of FFB where half of the amount will end up as palm oil mill effluent (POME) [8]. POME is a colloidal suspension which consists of 95–96% water, 0.6–0.7% oil and 4–5% total solids. Although POME is non-toxic, it is acidic around pH 4.0–5.0 and brownish in color with a discharge temperature between 80 and 90 °C [9]. Due to the release of POME into the environment, POME was identified as the largest pollutant discharged into the rivers of Malaysia [10, 11]. The performance of double-chambered MFC with POME as substrate was investigated by incorporating polyacrylonitrile carbon felt (PACF) as the electrode in the MFC [12]. The maximum power generated was 45 mW/m<sup>2</sup> with 45% of COD removal in 15 days. The microbial community structure from palm oil mill effluent in MFC have also been studied [13] in which electrochemically active bacterial consortium was successfully enriched from POME in the anode chamber under anaerobic condition. Analysis using 16S rDNA showed that 88.5% of the community was from the group *Deltaproteobacteria* while members of *Gammaproteobacteria*, *Epsilonproteobacteria* and *Betaproteobacteria* codominated the microbial consortium.

In this present work, the potential use of natural sources of bacteria isolated from the city waste water and

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AS for electricity generation using MFC was investigated. Firstly, a single chambered air cathode MFC was operated using AS and city wastewater as an inoculum. Next, isolation and characterization of potential electro-active bacteria from the biofilm that was formed on the anode of the MFC was conducted. Finally, the performance of MFC using *K. Oxytoca* was compared to the performance of MFC using AS. As far as the authors are concerned, no research has focused on natural source of bacteria for electricity generation in MFC especially using POME as the substrate. Previous research has only focused on POME sludge as the inoculum for the generation of electricity. In addition, it should be emphasized that in this study POME was used as the substrate in MFC for simultaneous electricity generation and treatment of POME. Since POME is rich in complex organic matter, the chemical energy within these biodegradable compounds may possibly be obtained by oxidation of these compounds and transfer of electrons by electro-active bacteria, thus generating electricity in MFC.

## 2 Methods

### 2.1 Source of microorganism and sample collection

Palm oil mill effluent (POME) and AS samples were obtained from a local palm oil mill factory in Phang, Malaysia. City waste water was collected in sterilized bottle from kuantan city in Pahang, Malaysia. All samples were stored at 40 C to minimize the degradation of samples by indigenous microbial activities, thus preserving the quality of the samples.

### 2.2 MFC configuration

The air cathode single chamber MFC was built with a cubic plexi glass which has a dimension of 5 cm x 5 cm (Shanghai, Sunny Scientific, China) and a total working volume of 20 mL. Carbon brush was directly used as anode electrode. The MEA was placed at the front opening side of the cubic chamber by facing the membrane side to the anode substrate and the PACF side was faced to the air. The whole MFC setup was tighten up with screws. Titanium wire of 5 cm was inserted through the MEA. Electric circuit consisting of resistor was connected from the anode chamber of MFC to the cathode chamber. All experiments were conducted at room temperature. For wastewater characterization, required amount of sample was withdrawn from anode chamber after two weeks of MFC operation. The COD was determined using digestive solution (0 – 1500 mg/L range; Hach, USA) and measured using a COD reactor (ACH DRB 2 00, USA).

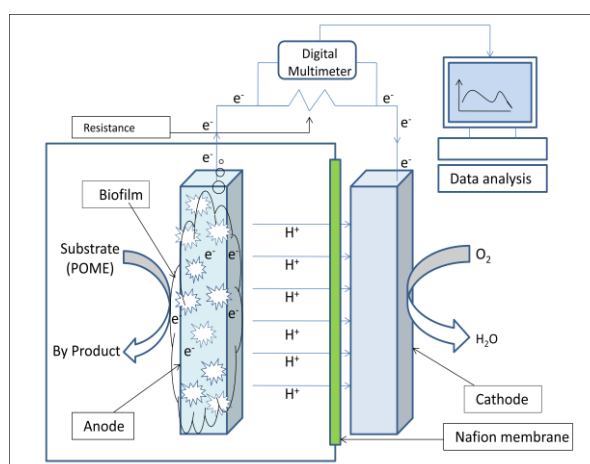


Figure 1. MFC Construction and schematic diagram.

### 2.3 Pretreatment of Nafion membrane

The Nafion 117 membrane (Dupont Co. USA) was used in this study to separate the anode and cathode compartment of MFC and acted as the proton exchange membrane (PEM). The Nafion membrane was cut with the dimension of 5 cm x 5 cm to fit the MFC. After that, Nafion membrane was heat treated with HCl solution at 100<sup>0</sup> C for an hour to remove organic impurities. The solution was leave to cool down to room temperature. The Nafion membrane was then drenched overnight in DI water followed by washing with DI water several times.

## 2.4 Isolation of potential electro-active bacteria from AS and City wastewater

In most MFC research using pure culture bacteria, the bacteria is often isolated from the waste samples containing high population of bacteria. These bacteria are selected based on their ability to grow as biofilm on the anode in the fuel cell environment. For this study, the source of microorganisms was the POME, anaerobic sludge and city waste water. Enrichment of the cultures was carried out by preparing an overnight culture in LB broth (10% v/v) incubated at 35°C with shaking at 150 rpm. The overnight culture (10% v/v) was used as inoculum in the anode compartment. After 10 days of MFC batch operation, the anode was taken out and placed in 0.1 M phosphate buffer and shaken vigorously to detach bacteria that grew as biofilm on the anode. The side of the anode was also carefully scraped and resuspended in phosphate buffer. The bacterial suspension was serially diluted and the pure culture bacteria were obtained using the spread plate technique. Biochemical tests were performed to partially identify the isolated bacteria.

## 2.5 Biolog gene III analysis

Once the pure cultures from the samples were obtained, BIOLOG GEN III test (Biolog Inc., United States) was carried out to identify the genus and species of microorganisms by performing carbon consumption tests with the isolated microorganisms under aerobic conditions. Biolog microplate consists of 96 wells out of those 71 wells for source utilization assay and 23 wells for chemical sensitivity assays. Tetrazolium redox dyes were used to colorimetrically indicate utilization of the carbon sources or resistance to inhibitory chemicals. The isolated colonies were precultivated on Tryptic soy agar plates and incubated at 33°C for 24 h. Picked up a pure culture of 3 mm diameter area of colony using cotton tipped inoculators swab from the agar plate containing pure cultures and immediately released the bacteria into the inoculating fluid with 100 µl of cell suspension and incubated at 33°C for 36 h. All of wells start out colorless when inoculated but during incubation there is increased respiration in the wells where cells can utilize a carbon source leads to increased respiration causes reduction of the tetrazolium redox dye, forming purple color. After incubation, the microplates were read using BIOLOG's microbial identification system software and based on match, species level identification of the isolate was made [14].

## 2.6 Measurement and analyses

The voltage (V) and current (I) across an external resistor (1 kΩ) in the MFC circuit was continuously monitored (15 min intervals) using a multimeter with data logger (Fluke 289 True RMS Multimeter, USA) connected to the computer through USB cable adapter. To obtain polarization data, the external resistance was varied from 50 to 20,000 Ω. Current density (I) was calculated from I

= V/R, and normalized by surface area. Power density normalized by surface area (PA, W m<sup>-2</sup>) were measured and calculated using the following equations

$$P \propto VI \quad (1)$$

$$P \propto V^2 = AR \quad (2)$$

COD was periodically checked by taking small amount of sample from the anode effluent for every 24 h.

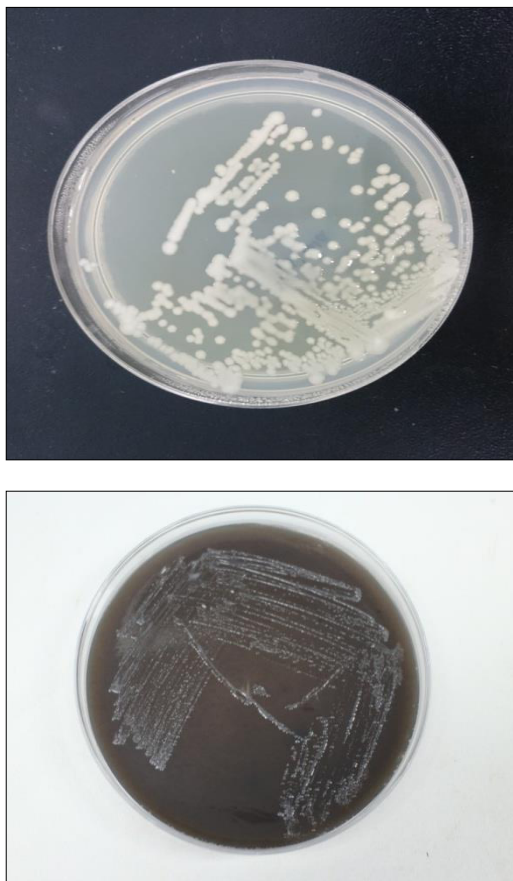
## 2.7 POME characterization

The POME characteristics such as chemical oxygen demand (COD), biological oxygen demand (BOD), total solids, total suspended solids and ammoniacal nitrogen were analyzed by standard methods [15]. The characteristics of the POME sample is shown in Table 1.

## 3 Results and discussion

### 3.1 Identification of predominant microorganism in the biofilm

The single chamber air cathode MFC was set up to allow natural microorganisms from the anaerobic POME sludge and city waste water to grow under facultative condition in the anode compartment. After the MFC was operated, the anode was taken out and it was noticed that bio-film was found to grow on the anodic electrode. Since the isolated bacteria was able to grow and survive in the MFC condition, there is a good possibility that the potential electro-active bacteria is able utilize the rich substrate in POME to generate electricity. Samples of the suspension and the biofilm (scraped from the anode) were taken and bacteria were isolated to obtain pure culture. The Biolog gene III analysis of the isolated pure culture suggested it to be *K. oxytoca* from anode after running MFC by city waste water as a source of inoculum. Most abundant microorganisms such as *Deltaproteobacteria*, *Azospiraoryzae*, *Acetobacterperoxyd* and *Solimonasvariicoloris* were isolated from palm oil anaerobic sludge as well as from biofilm of MFC.



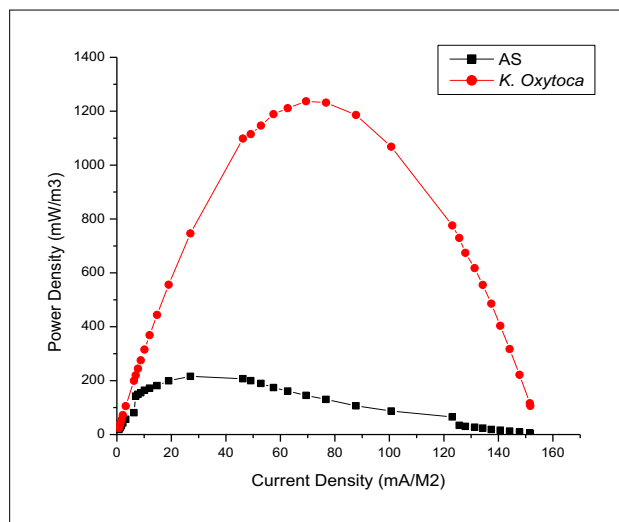
**Figure 2.** *K. Oxytoca* culture in Nutrient Agar and POME medium.

### 3.2 Identification of electrogen from anaerobic sludge and city wastewater.

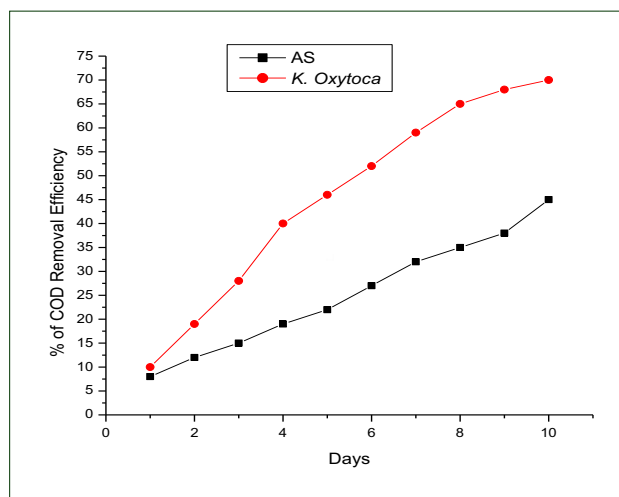
The predominant microorganisms present in the anaerobic sludge were isolated and identified as *Deltaproteobacteria*, *Actinobacillus capsulatus*, *Acetobacter peroxydans*, *Pseudomonas mendocina* and *Acinetobacter schindleri* using BIOLOG gene III analysis. Among the microorganisms identified the only known electrogen found in AS was *P. mendocina*. On the otherhand, *Klebsiella oxytoca* was detected as an electrogen from city wastewater.

### 3.3 Performance of MFC with AS

Stable open circuit potential of 500-600 mV was achieved after overnight enrichment. The MFCs were continuously fed with POME as a substrate. As early as 5 days, the MFCs started to generate electrical current. A maximum closed circuit potential of 620 mV was recorded after 7 days of enrichment. The MFCs were operated continuously for more than 10 days and recorded maximum power density of 207.28 mW/m<sup>3</sup>. The anode effluent showed a maximum COD removal percentage of 45.78%.



**Figure 3.** Polarization and power density curve of AS and *K. Oxytoca*.



**Figure 4.** COD removal efficiency of AS and *K. Oxytoca*

**Table 1.** Efficiency of MFC treatment with *K. Oxytoca*.

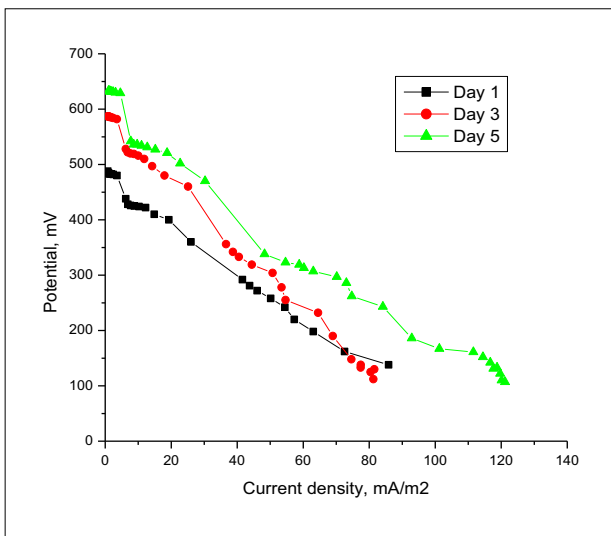
Parameters	Before MFC treatment with <i>K. Oxytoca</i> (mg/L)	After MFC treatment with <i>K. Oxytoca</i> (mg/L)	Removal % MFC with <i>K. Oxytoca</i>
COD (mg/L)	68,360	18,180	73.40
BOD (mg/L)	28,200	14,800	47.51
Total solids (mg/L)	28,108	11,226	60.06
Total suspended solids (mg/L)	9,580	3,158	65.59
Ammoniacal nitrogen (mg/L)	28	10	64.28

**Table 2.** Efficiency of MFC treatment with AS

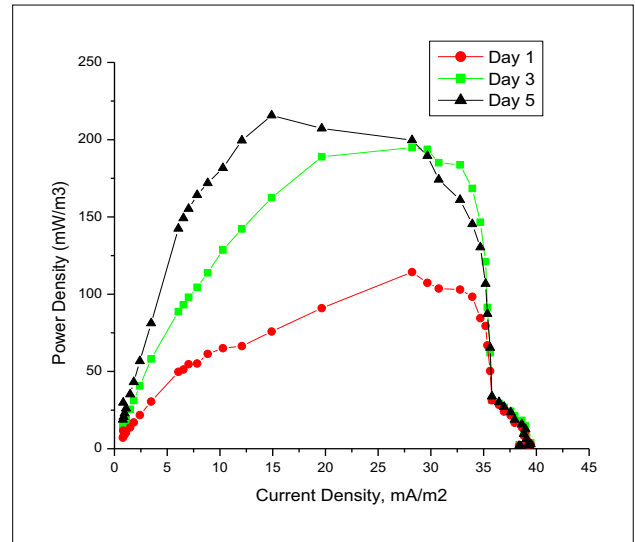
Parameters	Before MFC treatment with AS (mg/L)	After MFC treatment with AS (mg/L)	Removal % MFC with AS
COD (mg/L)	62,432	31,980	48.63
BOD (mg/L)	26,342	14,080	46.54
Total solids (mg/L)	25,687	11,654	54.63
Total suspended solids (mg/L)	11,654	2,882	75.27
Ammoniacal nitrogen (mg/L)	26	11	57.69

**3.4 Performance of MFC with city wastewater**

Stable open circuit potential of 480-520 mV was achieved after overnight enrichment. The MFCs were continuously fed with POME as a substrate. As early as 3 days, the MFCs started to generate electrical current. A maximum closed circuit potential of 648 mV was recorded after 5 days of enrichment. The MFCs were operated continuously for more than 10 days and recorded maximum power density of 1236 mW/m<sup>3</sup>. The anode effluent showed a maximum COD removal percentage of 73.40%.

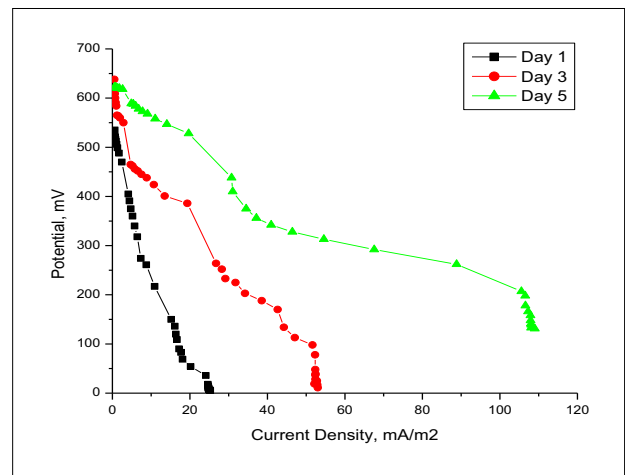


**A**

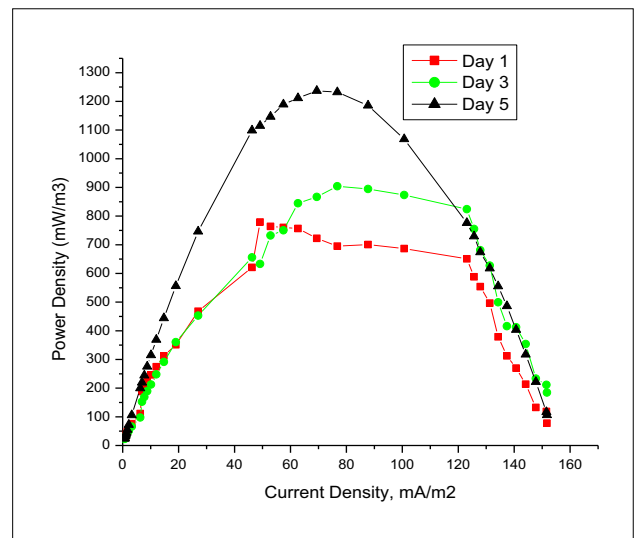


**B**

**Figure 5.** Electricity generation performance of AS, (A) Polarization curve, (B) Power density curve.



**A**



**B**

**Figure 6.** Electricity generation performance of *K. Oxytoca*, (A) Polarization curve, (B) Power density curve.

## 4 Conclusion

Final discharge POME was successfully utilized to generate electricity in a single chambered MFC without the addition of nutrient. The use of *Klebsiella oxytoca* strain, which was isolated earlier from biofilm on the anode MFC using city wastewater inoculum showed significant electricity generation in MFC compared anaerobic POME sludge. Although treatment of POME was not efficient at present, efforts to improve the overall performance of the MFC is underway including reactor design, treatment parameters and operational process in order to ensure that MFC would deliver its promise as a potential source of renewable energy and treatment of wastewater.

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