Effect of different yeast extract concentration in membrane-less microbial fuel cell (ML-MFC) for electricity generation using food waste as carbon sources.

Nurul Najwa Adam Malik¹, Mohammad Mirza Mohammad Faizal¹, Husnul Azan Tajarudin¹, Noor Fazliani Shoparwe² and Muaz Mohd Zaini Makhtar¹

¹School of Industrial Technology, Bioprocess Technology Division, Universiti Sains Malaysia, 11800 Penang, Malaysia
²Faculty of Bioengineering and Technology, Universiti Malaysia Kelantan, 17600 Jeli, Kelantan, Malaysia.
³Fellow of Centre for Global Sustainability Studies, Universiti Sains Malaysia, 11800 Penang, Malaysia

Email: muazzaini@usm.my

Abstract. Electricity constitutes one of the basic energies of our everyday life and approximately 14 % of the global population does not have the access to electricity. An abundance of waste is generated daily wherein food waste constitutes 45 % of the composition. A mediator-less and membrane-less single-chambered microbial fuel cell (ML-MFC) has the potential to serve as a cost-effective solution for food waste treatment and electricity power generation at no additional cost for the substrate. Food waste from E-Idaman Sdn. Bhd. Kedah was utilised in this study to generate electrical energy while focusing on the effect of different yeast extract concentrations on the performance of the ML-MFC. Electrogenic bacterial (EB) culture employed in this study acted as a catalyst for the power generation and was isolated from a previous ML-MFC study. The proximate analysis of food waste observed carbon constituting the greatest composition at 30.02 %. From the conducted preliminary study which compared three different strains of EB to be introduced in the ML-MFC, Bacillus subtilis sp. exhibited the highest specific growth rate, $\mu$ (0.117 g. L$^{-1}$/h) and shortest doubling time, $T_d$ (5.93 h). One-factor-at-a-time (OFAT) method was utilised to evaluate the performance of the ML-MFC. 15 g/L yeast extract concentration obtained the greatest power density ($628.05 \times 10^6$ mW/m²), substrate degradation efficiency (12.3 %), COD removal (99 mg/L) and biomass (44.32 mg/L). This showcased that the addition of extra yeast extract concentration into the food waste had boosted the efficiency of EB’s growth resulting in greater consumption of carbon source (removed COD value; bioremediation) in the food waste.

1.0 Introduction

Approximately more than one billion people around the globe still do not have the access to a stable source of electricity [1] and Malaysia has not been excluded. Approximately 809 schools in two of its states; Sabah and Sarawak, still lack the access to a constant 24-hour electrical supply due to its rural location [2]. Withal, the world is also currently facing a rapid upsurge in electrical consumption and demand as a result of wide industrialisation [3] signifying the importance of energy security. The main sources of energy can generally be divided into three main groups which are renewable sources, fossil
fuel sources and nuclear sources [3], of which, both of the latter has also been categorised as non-renewable energy [4]. The extensive commercial use of fossil fuel sources has negatively impacted the environment through its high amount of carbon dioxide emission. This largely contributed towards the world temperature’s rapid rise, ultimately, also contributing to the global warming and atmospheric pollution [5]. Nevertheless, various countries around the globe have made remarkable efforts to find alternative solutions against the energy issues and to achieve energy security by venturing into renewable energy sources such as water, wind, wave and solar energy. Malaysia has also been facing the issue of rising solid waste production whereby an estimated 15,000 tonnes/day of food waste has been produced in 2016, of which, 3000 tonnes are still good for consumption. From the amount of food waste produced, it showed that the source of food waste was sufficient for renewable energy generation via microbial fuel cells (MFCs) in Malaysia. In the same time, the utilization of food waste for energy generation via MFC also can counter the problem that come from the landfilling. For example, landfilling needs large area for landfill which causes land scarcity and the management varies depends on the type of landfill causing contamination to occur if not managed properly.

Currently, the usage of MFCs as an alternative energy source is being extensively studied. MFC is a device that converts the chemical energy to electrical energy by catalytic reaction of microorganisms. Researchers using various substrates to increase MFC efficiency have been reported [6]. However, as of today, the optimization of food waste as substrate in membrane-less MFC (ML-MFC) and yeast extract concentration for electricity generation is still lacking. The optimization of the condition in ML-MFC will increase potential of ML-MFC to produce a higher yield of electricity generation. Therefore, there is a need to carry out the proposed work and it would be referred data with respect to electricity generation, yeast extract concentration and food waste.[7].

2.0 Methodology

2.1 Sample collection
Extracted food waste was collected from E-Idaman Sdn. Bhd.’s landfill and was then kept at cold temperature of 4 °C. All the preservation steps and testing for the food waste characterisation follow the methods employed by a group research lead by Muaz [8].

2.2 Analytical methods
2.2.1 AAS analysis
Trace elements such as nickel (Ni), zinc (Zn), manganese (Mn) and cadmium (Cd) were determined by atomic absorption spectrometry (AAS) (GBC model 903, Australia). The food waste sample needed to undergo preliminary treatment prior to the analysis of the trace elements using filtration to obtain pure samples. A total volume of 40 mL of food waste sample was transferred into a 50 mL falcon tube and centrifuged at 6000 rpm for 5 min. The supernatant was then collected and filtered using syringe-filter (MF-Millipore Millex GS syringe filter with a pore size of 0.22 μm). Concentrated HNO3 was then added into the obtained filtrate until pH 2 is achieved. The solution was utilised for AAS analysis (APHA Method 3110). Standards that were used for the elements were 1000 ppm.

2.2.2 ICP-OES analysis
Micronutrients such as phosphorus (P), potassium (K) and ferum (Fe) were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES). The food waste sample needed to undergo preliminary treatment prior to the analysis of the trace elements using filtration and acid digestion to obtain pure samples. A total volume of 40 mL of food waste sample was transferred into a 50 mL falcon tube and centrifuged at 6000 rpm for 5 min. The supernatant was then collected and filtered using syringe-filter (MF-Millipore Millex GS syringe filter with a pore size of 0.22 μm). Concentrated HNO3 was then added into the obtained filtrate until pH 2 is achieved. A total volume of 10 mL of the acidified filtrate was transferred into a tube and added with 0.5 mL of HNO3. The tube was then placed
in a block heater at 105 °C for 2 h to undergo acid digestion. Concentrated nitric acid with pH 2 was added until digestion was completed (sample is in a form of clear solution). The tube was then removed and cooled at room temperature. The sample was diluted with deionised water to its original volume of 10 mL. The sample was used for ICP-OES analysis (APHA method 3110). Standards that were used for the elements were 1000 ppm.

### 2.2.3 Elemental analysis
The composition of carbon (C), hydrogen (H) and nitrogen (N) present in the sludge were determined using an elemental analyser (PerkinElmer 2400 series II). Purified helium, oxygen and compressed air were purged into the equipment at 20, 15, and 60 psi, respectively. Combustion and reduction furnace were operated at 9758 °C and 5008 °C. The food waste sample needed to undergo pre-treatment before it could be analysed as the elemental analyser require small amount of solid sample. A total volume of 40 mL of food waste sample was transferred into a 50 mL falcon tube and centrifuged at 6000 rpm for 5 min. This step was repeated to increase the volume of sample required. The supernatant was discarded, and the sample was then dried in an oven at 90 °C for 24 hours. The dried sample was utilised for the elemental analyser.

### 2.2.4 COD analysis
Substrate degradation efficiency (SDE) of the food waste was determined using COD analysis. A total mass of 1 g dewatered sludge supplemented with food waste sample was diluted in 9 mL of deionised water in a 30 mL falcon tube. The sample was then filtered using syringe filter (MF-Millipore Millex GS syringe filter with pore size 0.22 μm). Next, a volume of 2 mL of the filtrate was added into a COD vial that contains a solution of premixed chemicals (K2Cr2O7, AgNO3, HgSO4, potassium hydrogen phthalate, H2SO4). A blank sample was prepared by adding 2 mL of deionised water into a COD vial. The COD vials were then digested at 150 °C for 2 h. After digestion, the digested samples were cooled to room temperature. The COD kit was used to measure the COD values of the samples.

### 2.2.5 Biomass of Bacillus Subtilis species
It is important to acquire data on samples’ biomass in order to know the availability of bacteria in the ML-MFC. The data on the samples’ biomass was obtained at designated hours for a period of seven days. To determine the biomass in ML-MFC a standard curve is required. A total mass of 1 g of sludge was diluted with a volume of 1 L of deionised water forming a sample stock solution. A straight-line standard curve was then plotted utilising the sample stock solution using UV-Vis spectrometer (turbidity of the sample) at 580 nm single light ray. In this study, the EB biomass was determined through the utilisation of UV-Vis. A mass of 1 g of the sample from the ML-MFC was diluted with 9 mL of deionised water in a 30 mL falcon tube. The optical density (OD) of the diluted sample was then obtained using a UV-Vis spectrometer with deionised water acting as the blank. The OD values were then translated into biomass data through the utilisation of the plotted standard curve.

### 2.2.6 Specific growth rate of electrogenic bacteria
Specific growth rate, \( \mu \), is the rate of population increase with each consecutive time period. Specific growth rate was calculated at the log phase of the microbial growth. The rate of biomass growth was then correlated with the specific growth rate, \( \mu \), and the cell number or biomass, \( X \). The specific growth rate of the bacteria in ML-MFC was calculated using the equation below:

\[
\frac{\ln X - \ln X_0}{t} = \mu
\]

where, \( X \) is number or mass of cells, \( X_0 \) is initial number or mass of cells, \( t \) is time and \( \mu \) is specific growth rate.
2.2.7 Doubling time of electrogenic bacteria

Doubling time, \( Td \), is the time or period that is required by the electrogenic bacteria to double its population during a specified time also known as generation time. Doubling time varies with different organisms. The doubling time of the bacteria in the ML-MFC was calculated by using the equation below:

\[
Td = \frac{\ln 2}{\mu}
\]  

where, \( Td \) is Doubling time and \( \mu \) is specific growth rate.

2.3 Construction of ML-MFC

A ML-MFC device was built using cylindrical PVC reactors (diameter: 10 cm, height: 10 cm). The anode electrode was placed at the bottom of the PVC reactor and food waste was placed on top of the anode with a depth of 5 cm. The cathode electrode was placed on top of the food waste (substrate) with its upper surface exposed to air. The graphite felt electrodes (anode and cathode) had radiuses, thicknesses, and surface areas of 4.6 cm, 0.65 cm, and 0.0066 m\(^2\), respectively. The total loading of substrate in the ML-MFC device was 100 g of dewatered sludge which also acted as a pseudo-membrane with 30 % of moisture content consisting of 27 mL of food waste and 3 mL of inoculum. The cylindrical PVC reactor was then capped with a lid and is left at temperature 35 °C. Generation of electricity was measured using a digital multimeter (UT33D, UNI-T, Hong Kong) that had probes connected to the anode and cathode wires in the ML-MFC.

2.4 Determination of power using polarization curve technique.

The electricity generation was determined by using the polarisation curve. The polarisation curve is a conventional method utilised to evaluate the performance of a MFC. The ML-MFC device was connected to a multimeter to record its cell voltage values at different external resistances (47, 100, 220, 470 and 1000 \( \Omega \)) and its electrical powers were then determined using Ohm’s law \( R = \frac{V}{I}, P = IV \). A polarisation curve was then plotted throughout the voltage and current measurements. The peak of the power curve of the plot is considered to be the maximum power generated by the ML-MFC.

2.5 Experimental Design

2.5.1 Operation of ML-MFC

In the preliminary study, three types of bacteria strains, Geobacter sp., Bacillus subtillis sp., and mixed culture of both strains were to be chosen for the ML-MFC. The strains were compared on their growth rates and doubling times to find the best strain with the highest growth rate and shortest doubling time. Bacillus subtilis sp. was chosen as it complied with both the requirements. To evaluate the effect of yeast extract concentration, the yeast concentration of YPG media was varied from 5 to 15 by adjusting the yeast concentrations in the YPG media formulation. In the study, the ML-MFCs were set at 30 % moisture content, 5 cm electrode distance, and incubation period of 7 days same setup as Muaz and Vel (2019) [9]. Samples were collected and recorded for COD analysis, biomass data, and voltage readings at every 6 h interval for the first 24 hr and an interval of every 24h after the first day until the seventh day of incubation period using a multimeter.

3.0 Result and Discussion

3.1 Proximate analysis of food waste.

The composition of the food waste was determined in order to check the food waste’s usefulness in acting as the source of substrate (Table 1). In this study the food waste was utilised was in its slurry
form. It is necessary to know the food waste’s basic chemical properties such as its organic contents, trace element compositions and inorganic compounds characteristics.

Figure 1 exhibited the food waste collection’s path before it is collected for its analysis in this project. In regard to macronutrients, elemental analysis of food waste by using a CHN analyser obtained compositions of carbon, hydrogen and nitrogen in the food waste at 30.02 %, 6.7 % and 3.7 % respectively. An AAS analysis of the food waste observed concentrations of micronutrients P, K and Fe at 43.4 mg/L, 2.5 mg/L and 1.4 mg/L respectively. Trace elements in the food waste were detected using ICP-OES showcasing 4.3 mg/L of Zn, 0.1 mg/L of Cd, 1.8 mg/L of Mn and 15.6 mg/L of Ni. These elements in food waste are essential for EB growth, an example being that manganese (Mn) is essential in acting as co-factors for the enzymatic functions and most importantly for proton energy generations in the bacterial cells [10].

Table 1. The composition of elements in food waste.

| Elements       | Type of nutrients | Concentration (mg/L)/Percentage (%) | Value |
|----------------|-------------------|-------------------------------------|-------|
| Macronutrients | Carbon            | %                                   | 30.02 |
|                | Nitrogen          |                                     | 6.70  |
|                | Hydrogen          |                                     | 3.70  |
| Micronutrients | Phosphorus        | mg/L                                | 43.40 |
|                | Potassium         |                                     | 2.50  |
|                | Ferum             |                                     | 1.40  |
| Trace elements | Zinc              | mg/L                                | 4.30  |
|                | Cadmium           |                                     | 0.10  |
|                | Manganese         |                                     | 1.80  |
|                | Nickel            |                                     | 15.60 |

Figure 1. Food waste collection’s path.
3.2 Preliminary study on growth kinetic of different electrogenic bacteria cultures in batch culture.

The preliminary study was carried out to screen the best bacterial culture among two different bacterial cultures; *Geobacter sp.*, and *Bacillus subtilis sp.*, and one culture being a mixture of both prior to the ML-MFC operation. The selected culture was chosen by comparing its specific growth rate, \( \mu \), and doubling time, \( T_d \). The comparison of the three cultures is shown in Table 2. The cultures were cultured in conical flasks under batch condition for 24 hours utilising Yeast-Peptone-Glucose (YPG) as the media for the electrogenic bacteria growth. Batch culture is a closed system which do not have any inlet or outlet in comparison to a continuous culture. EB have certain condition requirements to grow and catalyse the process in the ML-MFC, wherein, yeast extracts provide vitamin and mineral, peptones supply the needed nitrogen and glucose acts as the energy source for the EB consumption. Once these are met, the EB would degrade the carbon sources in the ML-MFC resulting in an increase of EB growth.

There are five different phases in a typical EB batch growth which are lag phase, log phase, deceleration phase, stationary phase and death phase (Figure 2). As observed, the growth profile of *Bacillus* has a lag phase period of \( t = 0 \) h to \( t = 4 \) h during which its biomass had increased from 0.8 g/L to 1.1 g/L. During this phase, the cells had started to adapt in its new environment by reorganising their molecular constituents based on the composition of nutrients in the media [11]. Next is the log phase with time intervals of \( t = 4 \) h to \( t = 12 \) h during which its biomass had increased from 1.1 g/L to 2.8 g/L. In this phase, the biomass had increased exponentially due to the cells already been adjusted to the new environment and started multiplying rapidly. This phase is also an important phase to calculate and obtain the specific growth rate, \( \mu \). Then the EB entered its deceleration phase where the essential nutrients had started to deplete. The
deceleration phase occurs in a very short of period of time [11]. Hence, was not visible in

Figure 2 but it can be predicted that deceleration phase had occurred after $t = 12$ h. Next, the EB entered it stationary phase at time $t = 12$ h to $t = 18$ h where the biomass increased from 2.8 g/L to 3.3 g/L. During the stationary phase, the cells’ growth rate and its death rate are equal. The biocatalytic activities of the EB may also gradually decrease as the cells age and die. The EB then entered its death phase with a time interval of $t = 18$ h to $t = 24$ h during which the biomass had decreased from 3.3 g/L to 3.2 g/L. During this phase, the cells’ growth had stopped, and the death rate increased due to exhaustion of essential nutrients and increase in waste accumulation.

![Figure 2](image-url)

**Figure 2.** Electrogenic bacteria growth profile in batch culture.

The strain with the best specific growth rate, $\mu$, and doubling time, $T_d$, was chosen prior to ML-MFC operation. Specific growth rate, $\mu$, is one of basic tools in microbiology that is used to study microbial growth. It also has a significant relationship with the concentration of the substrate utilised in a fermentation. According to Kova & Englí (1998), the specific growth rate decreases with the depletion of the substrate. Doubling time is the period or time that is required by the cells to start multiplying itself more actively, resulting an exponential increase of the cell mass and cell number density with time (Equation 1 & Equation 2). From Table 2. The comparison of the specific growth
rate and doubling time for each strain, it can be observed that *Bacillus subtilis* sp. had the greatest specific growth rate and shortest doubling time at 0.117 gL⁻¹/h and 5.93 h respectively while *Geobacter* sp. had the slowest specific growth rate and longest doubling time at 0.101 gL⁻¹/h and 6.86 h respectively. The greatest specific growth rate and the earliest doubling time were chosen based on the ability of the cells to achieve the maximum biomass, $X_m$, in a shorter period. The specific growth rate is determined during the logarithmic growth phase based on cell number or cell mass.

Table 2. The comparison of the specific growth rate and doubling time for each strain.

| Strain           | Specific growth rate, $\mu$ (gL⁻¹/h) | Doubling time, $T_d$ (h) |
|------------------|--------------------------------------|--------------------------|
| Geobacter sp.    | 0.101                                | 6.86                     |
| Bacillus subtilis sp. | 0.117                               | 5.93                     |

Table 3. The absorbance and biomass readings for each strain with time.

| Time (hour) | 0  | 2  | 4  | 6  | 12 | 18 | 24 |
|-------------|----|----|----|----|----|----|----|
| Absorbance  |    |    |    |    |    |    |    |
| Geobacter sp.| 0.041 | 0.069 | 0.064 | 0.144 | 2.096 | 2.211 | 2.128 |
| Bacillus subtilis sp. | 0.035 | 0.077 | 0.085 | 0.425 | 2.143 | 2.236 | 2.102 |
| Biomass (g/L) |    |    |    |    |    |    |    |
| Geobacter sp. | 0.2  | 0.6 | 0.8 | 1.2 | 2.2 | 3.3 | 3.1 |
| Bacillus subtilis sp. | 0.8  | 1.3 | 1.1 | 1.8 | 2.8 | 3.3 | 3.2 |

### 3.3 Effect of yeast extract concentrations on EB growth in the membrane-less microbial fuel cell (ML-MFC)

Based on the preliminary study, *Bacillus subtilis* sp., was observed to be the best strain to be implemented in the ML-MFC due to its specific growth rate, $\mu$, and doubling time, $T_d$. Hence, *Bacillus* was used throughout the experimental tests to find the optimum yeast extract concentration for EB growth and electricity generation. The EB growth was measured through utilising its biomass value calculated from the standard curve’s equation ($y = 0.0451x - 0.025$) which correlated the OD values with its biomass profile.

Yeast extract was essential in providing the necessary components for the propagation of the cells, including biosynthetic building blocks, and it is also frequently employed in initial stages of a fermentation that requires large inoculum [12]. According to Zhang et al (2003), yeast extract also contains a number of other compounds, which strongly influence fermentation performance [13]. A study on the influence of Mg²⁺ concentration on the growth of *E. aerogenes* culture was also conducted by increasing the concentration from 5 mg/L to 22 mg/L. In this study however, the yeast extract concentration was increased from 5 g/L to 15 g/L instead. The study showed the trend of increased Mg²⁺ concentration can reduce the lag phase period in the cell growth. Hence, by increasing the yeast extract concentration a shorter lag phase of the EB’s growth could be achieved in order enter its exponential phase at a quicker time. This results in a rapid increase of EB biomass and degradation of carbon source; thus, electricity generation is increased. The ML-MFC with 15 g/L of yeast extract concentration showed the highest biomass (44.32 mg/L) and fastest time taken ($t = 120$ h) to achieve maximum biomass, compared to ML-MFC with 5 g/L of yeast extract concentration. The lowest yeast extract concentration ML-MFC, (5 g/L), had the slowest time ($t = 144$ h equivalent to 6 days) to achieve its maximum growth (43.19 mg/L).

There were also double lag phases observed in the ML-MFCs (Figure 3). The phenomenon observed can also be known as diauxic growth which takes place as a result of a shift in metabolic pathways in the middle of a growth cycle [11]. This phenomenon had taken place in this study due to the EB having more than one carbon sources to consume which are from the YPG media and food.
waste. After one carbon source is exhausted, e.g.: glucose in YPG media, the EB would then adapt their metabolic activities to utilise the second carbon source, which, can be the product from the occurred glucose degradation or carbon sources from the food waste.

3.4 Optimisation using OFAT method.

The operating conditions for EB in the ML-MFC were optimised through the classical OFAT method. The effect of yeast extract concentration ratio factor was evaluated by varying one factor at a time, while keeping the peptone and glucose concentrations fixed. The results were recorded in Table 4.

3.4.1 Effect of yeast extract concentration on voltage generation and substrate degradation efficiency in the ML-MFC.

This study observed the effect of yeast extract concentration towards voltage generation and COD removal by using OFAT method. The voltage generation was observed to be low during the initial stages. However, the voltage generation started to increase with the increase in time (Figure 4). This low initial voltage was as a result of the EB still being in its lag phase during which it is adapting to its new environment within the ML-MFC. The COD value was observed to generally decrease with the increase in time despite a few fluctuations occurred during the COD removal (Figure 5). The generation of voltage observed also indicated that the EB had degraded the organic compounds in the ML-MFC. The recorded maximum power density generation, COD removal and substrate degradation efficiency can be observed in Table 4 through utilising OFAT method on the effect of different yeast extract concentrations. In this study, the ML-MFC with 15 g/L yeast extract concentration had obtained the greatest power density ($628.05 \times 10^6$ mW/m$^2$), best COD removal (99 mg/L) and the highest substrate degradation efficiency (12.3 %).

![Figure 3. Electrogenic bacteria growth profiling in membrane-less microbial fuel cell.](image)
Figure 4. Trend of voltage production against time (low and high concentration of yeast extract in (ML-MFC)

From Table 4, it can be concluded that COD removal by the bacteria had significant relationship with the voltage generation. In ML-MFC, the EB at anode electrode oxidises (consumes) the organic matters (food waste) [14]. The organic matters were oxidised into simpler products through subjection from the metabolic pathways in the cells: glycolysis, citric acid cycle and oxidative phosphorylation [8]. During its metabolism the EB generate protons which were then passed through the sludge to the cathode, and the electrons which were also passed to the cathode from the anode results in a flow of electrons generate the current. Hence, a greater oxidization of organic matters by the EB results in a higher substrate degradation efficiency via COD removal consequently resulting voltage generations in ML-MFC. Table 4 observed 15 g/L YE ML-MFC obtaining the highest substrate degradation efficiency (12.3 % = 99 mg/L COD removed) and 5 g/L YE ML-MFC having the lowest substrate degradation efficiency (8.0 % = 56 mg/L COD removed). The EB performed well to reduce the substrate in 15 g/L YE ML-MFC since the EB had a greater yeast extract concentration for its rapid growth producing high amount of biomass, thus, more EB is available to degrade a greater amount of substrate ultimately increasing the COD removal in ML-MFC.

Table 4. Voltage generation and COD removal using one-factor-at-a-time (OFAT).

| Parameters                     | Varying parameter (g/L) | Power Density (mW/m²) (10⁶) | COD removal (mg/L) | Substrate degradation efficiency (%) |
|--------------------------------|-------------------------|-----------------------------|--------------------|--------------------------------------|
| Varying:                       |                         |                             |                    |                                      |
| Yeast extract (g/L)            | 5                       | 507.54                      | 56                 | 8.0                                  |
| Fixed:                         |                         |                             |                    |                                      |
| Peptone: 20 g/L, Glucose: 20 g/L | 15                      | 628.05                      | 99                 | 12.3                                 |

The voltage generation in the ML-MFC was determined by the difference in the redox potential between anode and cathode. In theory, the ML-MFC was noted to achieve maximum voltage generation at 1.2 V because the reduced biomolecules had a minimum of -0.4 V at anode and the redox.
potential of oxygen at cathode was at 0.8 V. As previously mentioned, Table 4 observed the greatest power density at 628.05 x 10^6 mW/m^2. Due to the ML-MFC technology also being aimed for bioremediation (treatment of food waste) the study had also focused on the ML-MFC which had a higher COD removal to further investigate the effectiveness of treatment and electricity generated.

![COD profile for three ML-MFCs (5g/L and 15 g/L).](image)

**Figure 5.** COD profile for three ML-MFCs (5g/L and 15 g/L).

### 3.4.2 Voltage generation and biomass

Figure 6 showed the bacterial growth and voltage generated by the 15 g/L yeast extract ML-MFC. The EB biomass profile is in its log phase at early stage, from $t = 0$ to $t = 6$ h, due to the EB already adapting into its new environment and entering its log phase at earlier stages in the ML-MFC. The biomass constantly increases until 120 h, at which the voltage generated, and the biomass were 257 mV and 40.19 mg/L respectively. The biomass then reached its maximum value and entered its deceleration phase at $t = 144$ h. The general trends in Fig.6 showcased the same trends of increase in biomass, from 19.78 mg/L at $t = 0$ h to 28.96 mg/L at $t = 168$ h, and increase in voltage generated, from 360 mV at $t = 0$ to 653 mV at $t = 168$ h, proving the EB biomass and voltage generation were associated with growth. In theory, the biomass and voltage would obtain the highest value at $t = 120$ h, however, a voltage drop at $t = 120$ h was observed in this study despite its biomass having the maximum value. This is due to occurrence of mass transfer loss in which the rate of oxidation at anode was not equal to the reduction rate at the cathode. The oxidation of organic compounds at the anode by EB had released a greater number of electrons, while the reduction of oxygen at the cathode was low and had generated a lower redox potential resulting in a voltage drop. The process of voltage generation is initiated when the donated compound (organic matters in food waste) is being subjected into a series of metabolic pathways, e.g.: glycolysis, to be broken down generating nicotinamide adenine dinucleotide (NADH), which, is a highly reducing biomolecules that act as electron carriers [14]. The substrate degradation took place at the anode at which the electrons NADH was generated and passed. The electrons reacted with oxygen and protons at the cathode and electrical energy is generated.
3.5 ML-MFC performance.

In this study, the ML-MFCs performances were evaluated using polarisation curve technique. A MFC was tested through the polarisation curve technique using five different types of resistors (47, 100, 220, 470 and 1000 ohm). The current and power generation were calculated by using equations $V = IR$ and $P = IV$. The power density was calculated by dividing the power produced with the area of the electrodes in the ML-MFC. Figure 8 showcased the trends of power density produced by each ML-MFC at different resistances. ML-MFC with 15 g/L yeast extract concentration obtained the highest power density at 628.05 mW/m$^2$ because it had a better and sufficient nutrient for its EB growth to produce high yield of biomass in the ML-MFC yielding the highest power output. It can be observed from the polarisation curve that the performance of the ML-MFC could be further improved in the future by overcoming the voltage losses. A common phenomenon that often takes place in the ML-MFC is the mass transfer lost [15]. Mass transfer loss takes place due to the rate of oxidation at anode.

**Figure 6.** Biomass and voltage profile in 15 g/L yeast extract ML-MFC.

**Figure 7.** Biomass and COD profile in 15 g/L yeast extract ML-MFC.
and reduction rate at cathode not being equal [15, 16]. Due to a greater number of EB at the anode the system’s oxidation rate is greater than that of its reduction rate at the cathode resulting in a lower power density in the ML-MFC. In a MFC that utilises a membrane, the mass transfer loss can be reduced by increasing the mass transfer area. By using a larger mass transfer area or membrane in the MFC, the system is allowed for a greater generation of hydrogen ions in the anode to be transferred to the cathode for reduction [16]. Due to this study employing a membrane-less MFC system, mass transfer loss is effectively reduced as the proton (H⁺) are more easily transmitted from its anode to the cathode without having any resistant, thus, lowering the mass transfer loss.

In the ML-MFC, there were three characteristic regions of voltage decrease: 1) a rapid voltage dropped as current flows through the circuit; 2) a nearly linear decrease in voltage; 3) a second rapid voltage decrease at high current densities [17]. This MFC comply with all the characteristics mentioned. The activation potential for the ML-MFC took place when the current increases from 0 to 0.66 mA and the voltage had dropped from 0.656 V to 0.655 V. The activation potential took place due to the energy being lost as heat for initiating the oxidation and reduction reactions by the EB, and the energy lost through the transfer of an electron from EB to the anode surface [18]. Activation loss can be overcome by increasing the temperature in the ML-MFC system for a better electron transfer [8].

As the current increases from 1.4 mA to 6.47 mA, there was a constant voltage drop was observed from 0.652 V to 0.647 V. This region is also known as the ohmic loss or ohmic over potential that had occurred due to electrical resistance of the electrode. According to Muaz et al. ohmic losses can be reduced by shortening the distance between the anode and cathode to decrease the loss of energy during the transmission of protons to cathode [8]. Subsequently, as the current increases from 6.5 mA to 13.1 mA, the voltage experienced a rapid drop from 0.647 V to 0.615 V due to an occurrence of large oxidative forces at the anode. This event or region is also known as the mass transfer losses and it takes place when the oxidation rate at the anode is non-equivalent to the reduction rate at the cathode [15]. In order to achieve better performing MFCs with greater power generations, all of the mentioned polarisation losses should be further investigated and tackled upon in the future.

![Figure 8. Polarization curve of 15 g/L ML-MFC.](image)

**4.0 Conclusions**

The concentration of yeast extract in YPG media displayed its effect on EB growth, voltage generation and substrate degradation efficiency (SDE) in the ML-MFC. The proximate analysis conducted on the
food waste observed carbon constituting the greatest composition at 30.02%. Based on this study it was found that the ML-MFC device and system could be utilised in generating electrical energy by employing food waste as its substrate. The strain Bacillus subtilis obtained the greatest specific growth rate (0.117 g. L\(^{-1}/h\)) and fastest doubling time (5.93 h) compared to Geobacter sp. (0.101 g. L\(^{-1}/h\) and 6.86 h respectively). Thus, Bacillus subtilis was selected in this study to further investigate the optimum yeast concentration in ML-MFC. Further tests on Bacillus subtilis profiles were carried out by manipulating the different concentrations of yeast extracts (5g/L and 15 g/L). ML-MFC with 15 g/L of yeast extract concentration obtained the highest amount of biomass (44.32 mg/L), and fastest time taken (\(t = 120\) h) to achieve its maximum biomass, compared with ML-MFC with 5 g/L of yeast extract concentration (43.19 mg/L for 144 h). Through the optimisation using OFAT, it was observed that the greatest power density of 628.05 x 10\(^6\) mW/m\(^2\) and COD removal (99 mg/L) were obtained from the optimum ML-MFC of 15 g/L of yeast extract concentration.

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