Effects of Methyl-blue Addition in Sewage Substrate on the Performance of Microbial Fuel Cell

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ABSTRACT

Experimental study was undertaken to evaluate performance of microbial fuel cell. Without addition of methyl blue, the cell generated 1.265 V, 0.403 mA and 56.12 mW/m^2 after three hours of operation. With 300 µM methyl blue in sewage anolyte, 6.7% increase in voltage, 20.5% increase in current and 28.6% increase in power density was observed. By using ash-water catholyte, addition of methyl blue in anolyte led to increase of 9.0% in voltage, 38.5% in current and 50.9% for power density. Between three and twenty four hours of continuous operation of the cell with phosphate buffer catholyte, the average voltage was 1.305 V and the average current was 0.321 mA before addition of methyl blue. Methyl blue addition led to 5.1% increase in voltage and 55.2% increase in current. For the case of ash-water catholyte with methyl blue in anolyte, led to 6.2% increase in voltage and 59.1% increase in current.

Keywords: Methyl-blue, Microbial fuel cell, Phosphate buffer, Sewage anolyte.

INTRODUCTION

Microbial Fuel Cell (MFC) technologies represent a new approach for generating electricity from biomass by using bacteria. MFC is a bio-electrochemical device that through microbial-catalyzed redox reactions convert the energy stored within bio-convertible substrates to electricity (Permana *et al.*, 2015). It works similar to many other types of fuel cells. As opposed to the other types of fuel cells, MFC uses bacteria to generate electricity from the breakdown of organic substrates. The fundamental physical components of a typical dual-chamber MFC includes the electrolytes, an anode and a cathode partitioned by a Proton Exchange Membrane (PEM) also known as Cation Exchange Membrane (CEM). In the anode chamber, the bacteria oxidizes these substances to generate protons and electrons (Rabaey *et al.*, 2005). The electrons are attracted to the electron accepting molecules in the cathode, and are transferred through the external circuit and are further used to produce electrical energy. Protons are exchanged from the anode to the cathode through the CEM, thus balancing the charge of the overall system. Bacteria gain energy for metabolism and reproduction by transferring electrons and protons from a reduced substrate at a lower potential to an electron acceptor at a higher potential (Li *et al.*, 2014).

Anode should be available with a higher positive potential than other possible substrates in the waste stream, such as

sulphate or iron, so that the energetic gain will be much higher for bacteria that can deliver to the anode (Logan and Regan, 2006). However, if the anode potential is too low, electricity production will cease and fermentation processes will start. Several methods have been shown to increase anode performance beyond the standard graphite electrode: from bioengineering a reconstituted glucose oxidase monolayer (Katz *et al.*, 2003) to bound electron mediators including Mn^{4+} - graphite and neutral red covalently linked woven graphite anodes (Park and Zeikus, 2000). Cathode plays important factor in the performance of a MFC due to the poor kinetics of oxygen reduction reaction in a neutral pH medium (Cheng *et al.*, 2004). Other physical and chemical environmental effects also influence the thermodynamics and the kinetics of the electro catalytic oxygen reduction (Zhao *et al.*, 2006). There are two general options for a cathode, either a chamber filled with some form of dissolved electron acceptor or a chamber with less cathode that is exposed directly to oxygen in the air. Proton or cation exchange membrane (PEM/CEM) can be used to separate the cathode and anode liquids into different chambers, or just to act as a barrier that keeps materials other than protons from reaching the cathode (Logan *et al.*, 2006).

The first observation of electrical current generated by bacteria is generally credited to Potter's elementary work (Ieropoulos *et al.*, 2005). This didn't generate much interest until when it was discovered that current density and the power output could be greatly enhanced by the addition of electron mediators. Electron mediators accelerate the transfer from inside the cell to exogenous electrodes (Davis and Higson, 2007). Studies of MFCs indicate that the efficiency of operation of MFCs depend much on rate of electron transfer from microorganism (You *et al.*, 2006). The major drawback is that the rate of electron transfer from bacteria is too small

for high electricity generation. The electron transfer from microbial cells to the electrode can be facilitated by mediators such as thionine, methyl viologen, methyl blue, humic acid, ferric cyanide, and neutral red (Permana *et al.*, 2015). Some of mediators are too expensive and some are highly poisonous like thionine. Dissolved artificial electron mediators assist the electron transfer from bacterial cells to the electrode and further routed through an external circuit to the cathode to deliver electrical energy. Mediators in an oxidized state can easily be reduced by capturing the electrons from within the membrane. The mediators then move across the membrane and release the electrons to the anode and become oxidized again in the bulk solution in the anodic chamber (Parkash *et al.*, 2015). This cyclic process accelerates the electron transfer rate and thus increases the power output.

The use of sewage and ash for electricity generation paves new potential for complementing decentralized resources for off-grid solutions. Along with decentralized availability of sewage and ash from domestic cooking stoves, MFC provides relatively cheaper electricity generation technology. Other common alternatives resources for off-grid electricity generation include solar PV, wind turbines and microhydro (Kihedu and Mhilu, 2012). However, rural electrification through use of these resources, shows slow penetration among the target rural population in developing countries. Among other factors hindering the expected success in rural electrification by using these resources, include higher investment costs for relevant technologies (Kimambo and Kihedu, 2008). Therefore MFC technology need to be developed and explored. This study analyzed effects of methyl blue mediator as electrons prompter in microbial fuel cell for power generation. Charcoal anode is used instead of graphite, copper, platinum or $Ti-TiO_2$

used by other researchers (Rabaey *et al.*, 2004; Ghangrekar and Shinde, 2006; Davis and Higson, 2007; Permana *et al.*, 2015; Taskan *et al*., 2014).

MATERIALS AND METHODS

Laboratory scale microbial fuel cell

Microbial fuel cell (MFC) used featured two chambers built in H shape, consisting of two heavy duty rectangular polyethylene containers, each with 2,200 cm^3 volume and with sealable lids (Figure 1A). Containers were connected by using 10 cm PVC pipe containing a separator that served as Cation Exchange Membrane (CEM). CEM was made from solution of NaCl 0.04 g/cm^3 distilled water heated to 85 °C, then agar powder was added and the hot mixture was stirred. The mixture was poured to the 10 cm PVC pipe and allowed to solidify to form salt bridge between the polyethylene containers. Charcoal anode of 3.5 cm internal diameter and 6.5 cm long was used (Figure 1B). Aluminium cathode used was 10 cm long, 8 cm width and 0.2 cm thick. Surface area for charcoal anode and aluminium cathode was 90.71 cm^2 and 167.2 cm^2, respectively.

Preparations of anolyte and catholyte solutions

Anolyte solution or substrate used was wastewater collected as fresh incoming sewage from University of Dar es Salaam wastewater stabilization ponds. Nzabuheraheza *et al*. (2012), reported that inflow sewage to University of Dar es Salaam constructed wetland has total dissolved solids (TDS) of 790.0 ± 160.9 mg/l and total suspended solids (TSS) of about 416.2 ± 61.1 mg/l while chemical oxygen demand (COD) is 450.6 ± 73.9 mg/l. Biological oxygen demand (BOD) is 347.2 ± 23.5 mg/l.

Methylene blue ($C_{37}H_{27}N_3Na_2O_9S_3$) was selected for use as a mediator (Taskan *et al.,* 2015). The two catholytes used are phosphate buffer solution and ash water solution. Phosphate buffer solution of 10 pH and 1 M was prepared by adding 0.7 L of potassium dihydrogen phosphate (KH_2PO_4) solution to 1.3 L of 1 M sodium hydroxide ($NaOH$). Ash-water solution of pH 10 and concentration of 15 g/L was prepared by dissolving 30 g of ashes in 2 L of distilled water. Then, two polyethylene containers connected by the salt bridge, were filled with 2 L each of anolyte and catholyte solutions, respectively. Anode charcoal and cathode aluminium were totally immersed and hence connected by using 0.15 cm copper cables (Figure 1A).

Equipment used

The following equipment were used;
a) Fluke 289/FVF multi-meter with data logging capacity for voltage and current measurements, with IEC/EN 61010-1 complience.
b) Electrical heater for NaCl solution and agar heating.
c) Digital pH meter.

**Figure 1: Experimental set-up (A) and charcoal anode and Al-cathode (B)**

Experimental methods

Six sets of anolyte with methyl blue concentration of 0, 100, 200, 300, 400 and 500 μM were used. Experiments were conducted for both catholyte solutions, phosphate buffer solution and ash-water solution. Results for six levels of methylene blue concentration were compared on basis of the two catholyte solutions. Optimum concentration of methylene blue in anolyte used as anolyte solution was determined with respect to resulting voltage, current and anodic power density, after three hours of operation of the MFC. Hence, methyl blue concentration that yielded highest voltage, current and anodic power density was selected was selected as optimum and considered for further performance tests.

Determination of performance of methylene blue in MFC was carried out for anolyte solution with methyl blue addition.

Duration for this set of experiments was 24 hours and covered both catholytes, phosphate buffer solution and ash water solution. Variation of voltage and current with methyl blue addition in anolyte solution were compared with results obtained from mediatorless set-up for both catholyte solutions, phosphate buffer solution and ash-water solution. Lohar *et al.* (2014) also investigated performance of MFC on 24 hours basis, but Permana *et al.* (2015) opted for 36 hours period.

Each set of experiment was conducted three times, among these some were affected by noises caused by unintended interference such as physical interference of measurement cables, loss of data owing to low voltage battery cut-off points during 24 hours period of experiments. Therefore, data presented in this paper represent single valued data selected from three sets of replicates.

$$C_{12}H_{22}O_{11} + 13H_2O \rightarrow 12H_2O + 48H^+ + 48e^- \dots\dots\dots (1)$$
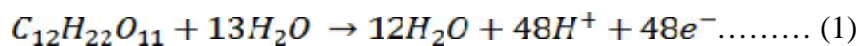
Cell reactions and anodic power density

Cathodic reaction for MFC using sewage as substrate, can be represented as sugar consumed by the microorganisms under anaerobic condition leading to production of carbon dioxide, protons and electrons

(Chonde, 2014). Anodic reaction involves oxygen reduction to form hydroxide ions with protons available from water.

$$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O \dots\dots\dots (2)$$
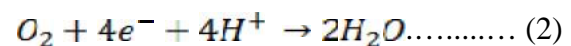
Cation transfer from the anolyte to the catholyte ensures electro-neutrality. It can be noted that, as for the case of equation (1), similar reactions can be deduced from reaction of sucrose, glucose and acetate (Ghangrekar and Shinde, 2006).

Power was calculated from equation (3) in which voltage and current represent measured values.

$$Power = Voltage \times Current \ldots \ldots (3)$$

Anodic power density, P_d was determined by using equation (4) as suggested by Permana *et al*. (2015). The power was derived from measured values of voltage and current.

$$P_d = \frac{P}{A} \ldots \ldots \ldots (4)$$

Where P is power and A is the surface area of the anode.

RESULTS AND DISCUSSION

Optimum concentration of Methyl blue

Figure 2 shows voltage variation with methyl blue concentration for phosphate buffer and ash-water catholytes. For the case of phosphate buffer catholyte, it was observed that voltage output increased from 1.265 V to 1.349 V with increase in methyl blue concentration in anolyte solution from 0 µM to 300 µM. However, further increase in methyl blue concentration to 400 µM and 500 µM resulted to slight decrease in voltage output to 1.347 V and 1.343, respectively. For ash-water catholyte, voltage output increased from 1.221 V to 1.331 V before decreasing to 1.317 V when methyl blue concentration was increased from 0 µM, to 300 µM and 500 µM, respectively.
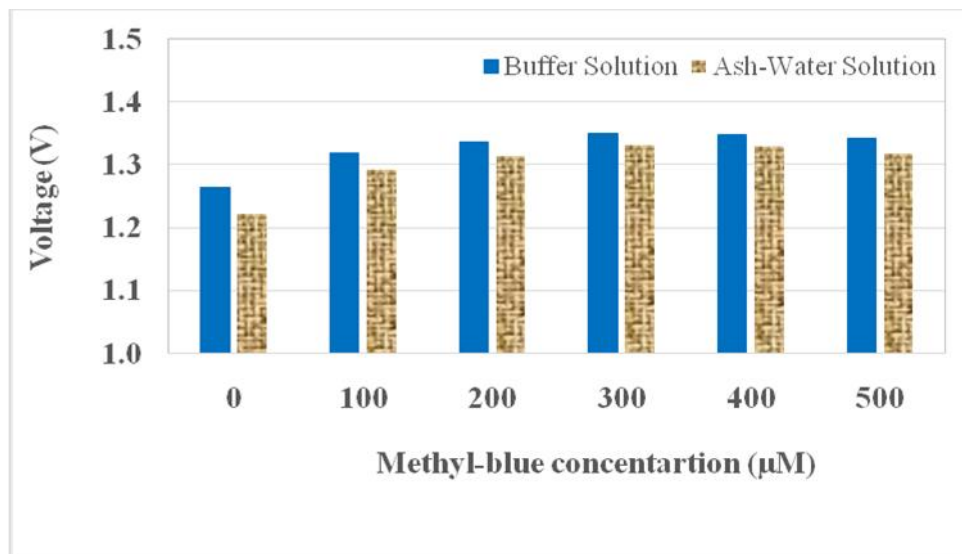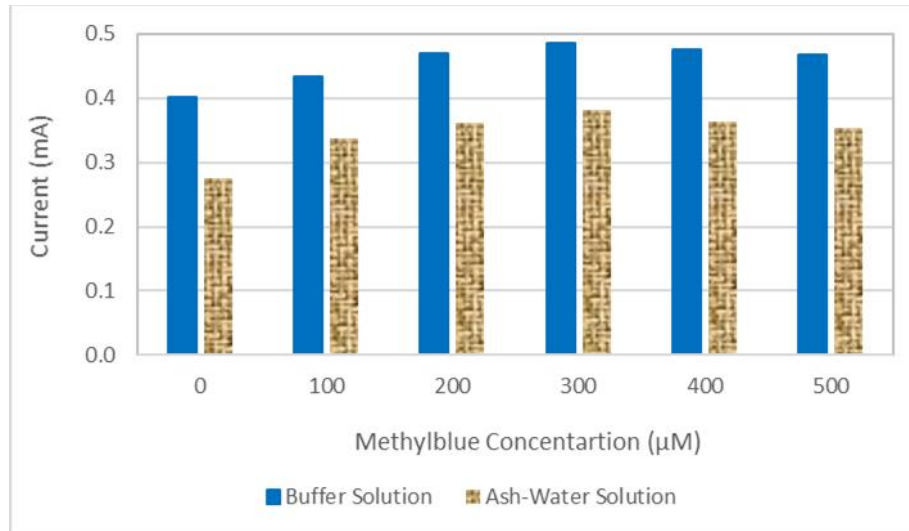

Figure 2: Voltage variation with methyl-blue concentration for phosphate buffer and ash-water catholytes

Figure 3 shows current variation with methyl blue concentration for phosphate buffer and ash-water catholytes. It was observed that for phosphate buffer catholyte current output increased from 0.403 mA to 0.485 mA with increase in methyl blue concentration in anolyte solution up to 300 µM. Nevertheless, current reading decreased to 0.477 mA and to 0.468 mA with increase in methyl blue concentration from 400 µM to 500 µM, respectively. For ash-water catholyte, current output increased from 0.275 mA to 0.381 mA and then decreased 0.354 mA when methyl blue concentration was increased from 0 µM to 300 µM and hence to 500 µM, correspondingly.
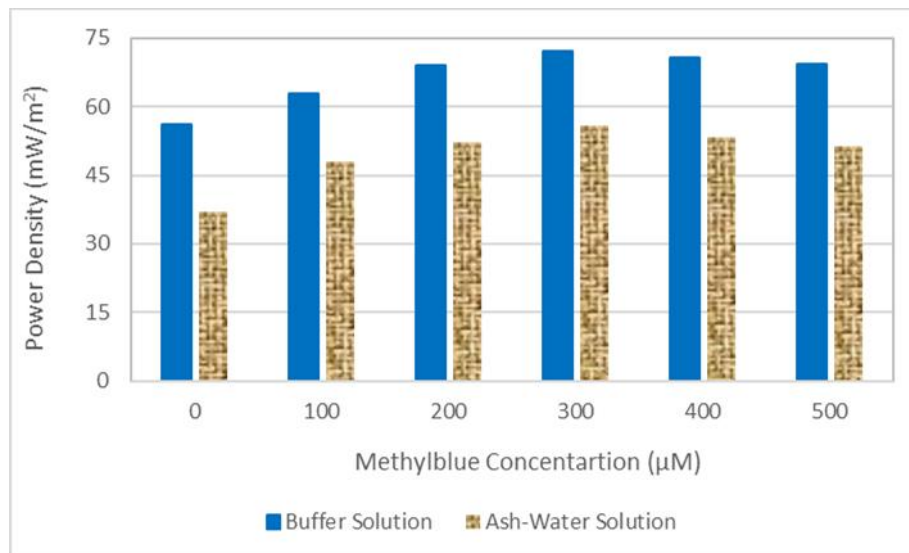
Figure 3: Current variation with methyl blue concentration for phosphate buffer and ash water catholytes

Figure 4 shows anodic power density variation with methyl blue concentration for phosphate buffer and ash-water catholytes. For phosphate buffer catholyte, anodic power density increased from 56.12 mW/m^2 to 72.15 mW/m^2 with increase in methyl blue concentration in anolyte solution up to 300 µM. Nevertheless, power density decreased to 69.31 mW/m^2

with increase in methyl blue concentration to 500 µM, correspondingly. For ash-water catholyte, power density increased from 37.07 mW/m^2 to 55.95 mW/m^2 and then decreased 51.43 mW/m^2 when methyl blue concentration was increased from 0 µM to 300 µM and hence to 500 µM, correspondingly.

Figure 4: Power density variation with methyl-blue concentration for phosphate buffer and ash-water catholytes

Figures 2 to 4, shows that microbial fuel cell generated 1.265 V, 0.403 mA and

56.12 mW/m^2 after three hours of operation without addition of methyl blue

into sewage anolyte. Correspondingly, with 300 µM methyl blue in sewage anolyte, fuel cell produced 1.349 V, 0.485 mA and 72.15 mW/m^2 after three hours. This is equivalent to 6.7% increase in voltage, 20.5% increase in current and 28.6% increase in power density. However, 500 µM methyl blue in anolyte resulted in slight decrease in cell output to 1.343 V, 0.468 mA and 69.31 mW/m^2. Similarly, microbial fuel cell with ash-water anolyte generated 1.221 V, 0.275 mA and 37.07 mW/m^2 after three hours of operation (Figures 2 to 4). However, with 300 µM methyl blue in sewage anolyte, the cell produced 1.331 V, 0.381 mA and 55.95 mW/m^2 after three hours. This increase is equivalent 9.0% for voltage, 38.5% for current and 50.9% for power density. In addition, slight decrease in cell output to 1.317 V, 0.354 mA and 51.43 mW/m^2 was observed with 500 µM methyl blue in anolyte. It can be noted that with reference to any specific methyl blue concentration in anolyte, the difference in current is larger than the difference in voltage. This phenomena indicates that methyl blue acts as electron mediator that accelerate electron transfer leading to improved current flow (Davis and Higson, 2007; Permana *et al.*, 2015).

Compared to 100 µM, 200 µM, 400 µM and 500 µM concentration of methyl blue in anolyte solution, 300 µM yielded highest voltage, current and anodic power density for both phosphate buffer and ash-water catholytes. Hence, 300 µM methyl blue in anolyte was selected as optimum concentration. Similarly, Taskan *et al.* (2014) investigated the effect of 0 µM 50, 50 µM, 100 µM, 200 µM, 300 µM, 400 µM and 500 µM concentration of methyl blue in anolyte. As a result, 300 µM concentration of methyl blue was selected as optimum concentration after observation of 101% increase in average

power density from 17.9 mW/m^2 without methyl blue to 36 mW/m^2.

Performance under phosphate buffer catholyte

Figure 5A shows voltage variation in 24 hours of operation of MFC with phosphate buffer catholyte. Without methyl blue addition, the voltage was recorded at 0.829 V at commencement of the experiment when electrodes were dipped into anolyte and catholyte. Then, the voltage increased to 1.167 V and 1.260 V corresponding to one and two hours of continuous operation. Thereafter, the voltage increased gradually to 1.327 V after 24 hours of operation. With 300 µM concentration of methyl blue in anolyte solution, the voltage was 1.094 V at the beginning of the experiment and increased to 1.346 V after two hours of operation. Even after 24 hours of operation, the voltage remained almost stable with slight increased to 1.381 V.

Figure 5B shows current variation in 24 hours of operation of MFC with phosphate buffer catholyte. Without methyl blue addition, current was recorded at 0.796 mA during the initiation of the experiment, and then dropped abruptly to 0.456 mA in two hours of operation. Afterwards, the current decreased steadily to 0.261 mA after 24 hours. With 300 µM concentration of methyl blue in anolyte solution, the current was 1.152 mA at the start of the experiment and decreased to 538 mA in two hours. However, it was observed that current slightly increased 0.521 mA five hours later. Subsequently, the current remained almost stable for other eight hours before decreasing back to 0.453 mA after 24 hours of operation.
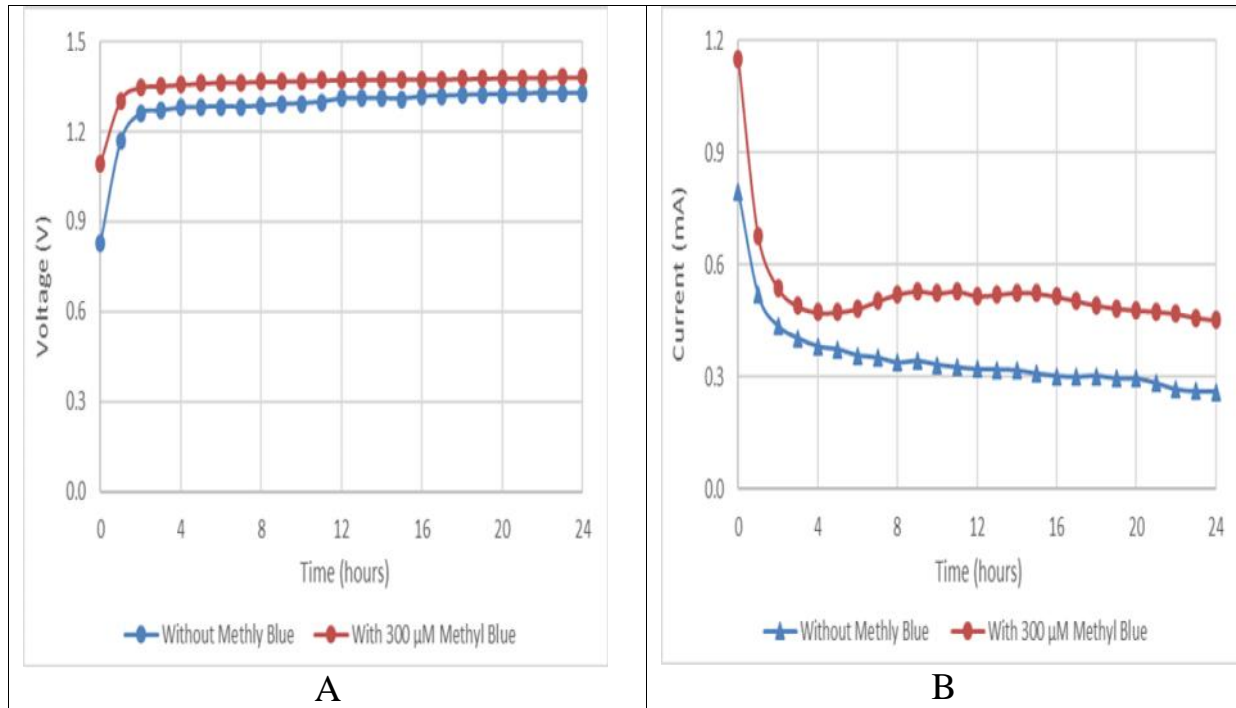
Figure 5: Variation of voltage (A) and current (B) in 24 hours of operation of MFC with phosphate buffer catholyte

Between three and twenty four hours of continuous operation of the microbial fuel cell, the average voltage was 1.305 V with phosphate buffer catholyte and without addition of methyl blue in anolyte (Figure 5A). With 300 µM concentration of methyl blue in anolyte, the voltage remained almost stable at average of 1.371 V during the similar span of period. With phosphate buffer catholyte, the average current was 0.321 mA without addition of methyl blue in anolyte (Figure 5B). With 300 µM concentration of methyl blue, the average current increased to 0.498 mA. In comparison to fuel cell operation without methyl blue addition in anolyte, 300 µM concentration of methyl blue lead to 5.1% increase in voltage, 55.2% increase in current and consequently 63.3% increase in power density. Permana *et al*. (2015) referring to 36 hours of MFC operation, observed 28.7% increase in average voltage from 0.689 V without methyl blue to 0.887 V with methyl blue in anolyte. Similarly, Lohar *et al*. (2014) reported 11.8% increase in average voltage from

0.68 V without methyl blue and 0.76 V with methyl blue equivalent.

Performance under ash-water catholyte

Figure 6A shows voltage variation in 24 hours of operation of MFC with ash-water catholyte. Without methyl blue addition, the voltage was recorded at 1.013 V at start of the experiment. The voltage increased to 1.197 V and 1.222 V after one and two hours of operation, respectively. Subsequently, the voltage decreased to 1.209 V after 24 hours of operation. With 300 µM concentration of methyl blue in anolyte solution, the voltage was 1.231 V at the beginning of the experiment before increasing to 1.331 V after two hours of operation. The voltage dropped to 1.272 V after 24 hours of operation.

Figure 6B shows current variation in 24 hours of operation of MFC with ash-water catholyte. Without methyl blue addition, current was recorded at 0.541 mA during the beginning of the experiment, and then

dropped to 0.275 mA in two hours of operation. Thereafter, the current decreased steadily to 0.219 mA after 24 hours. With 300 µM concentration of methyl blue in anolyte solution, current was 0.845 mA at the start of the experiment and decreased sharply to 0.370 mA in two hours. However, it was observed that current slightly increased to 0.430 mA after three hours later. Subsequently, the current remained almost stable for other six hours before decreasing further to 0.310 mA after 24 hours of operation.

By using ash-water as catholyte, microbial fuel cell provided the average voltage of 1.222 V between three and twenty four hours of operation with phosphate buffer catholyte and without addition of methyl blue in anolyte (Figure 6A). With 300 µM

concentration of methyl blue in anolyte, the average voltage was 1.298 V during the similar period of operation. With phosphate buffer catholyte, the average current was 0.244 mA without addition of methyl blue in anolyte (Figure 6B). With 300 µM concentration of methyl blue, the average current 0.388 mA. In comparison to fuel cell operation without methyl blue addition in anolyte, 300 µM concentration of methyl blue lead to 6.2% increase in voltage, 59.1% increase in current and hence 69.1% increase in power density. Similarly, Permana *et al.* (2015) observed over 111% increase in average power density from 2.12 mW/m^2 without methyl blue to 4.48 mW/m^2 with methyl blue. Correspondingly, Lohar *et al.* (2014) observed 25.0% increase in average power density from 8.99 mW/m^2 without methyl blue to 11.24 mW/m^2 with methyl blue.
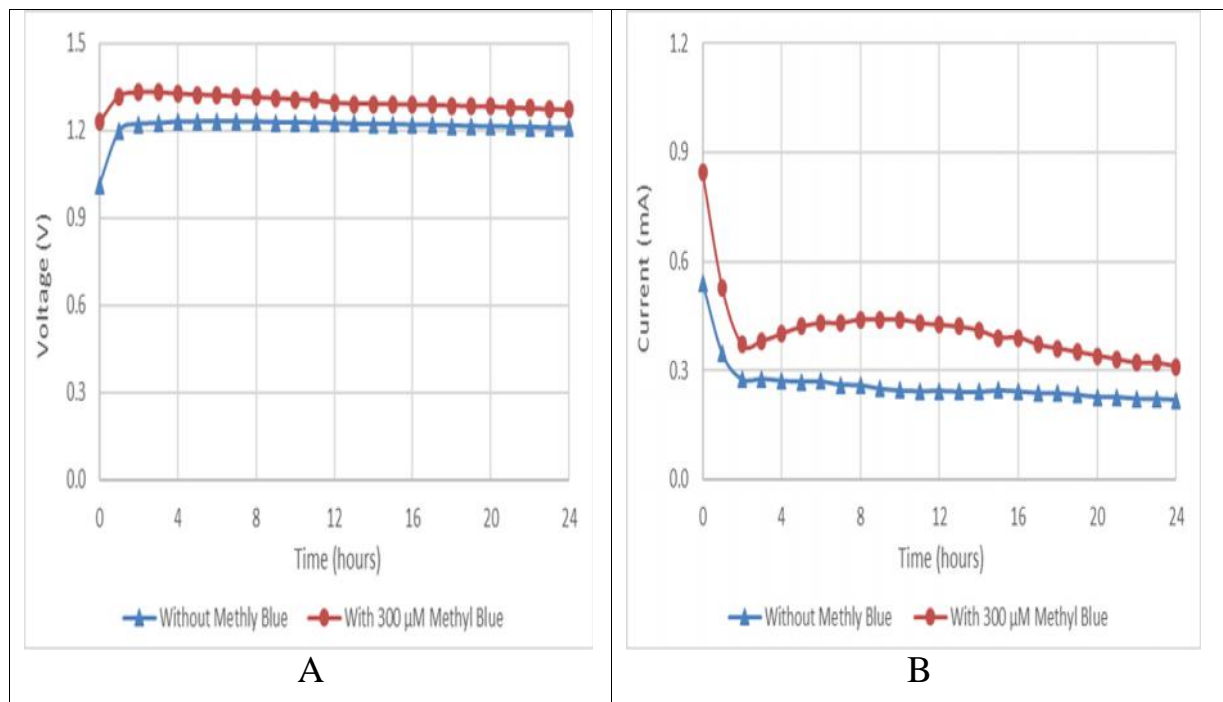
Figure 6: Variation of voltage (A) and current (B) in 24 hours of operation of MFC with ash-water catholyte

Without addition of methyl blue to anolyte, between three and twenty four hours of operation of the microbial fuel cell using phosphate buffer catholyte produced higher average voltage and current of about 1.305 V and 0.321 mA (Figure 5) as compared to ash-water catholyte which attained 1.222 V and 0.244 mA (Figure 6). Therefore, without addition of methyl blue to anolyte, use of ash-water catholyte reduced voltage, current and subsequently power density to by 6.4%, 23.8% and 28.5% as compared to when phosphate buffer catholyte was used. With 300 µM concentration methyl blue in anolyte, higher average voltage and current of about 1.371 V and 0.498 mA was also produced by using phosphate buffer catholyte (Figure 5) as compared to ash-water catholyte which attained 1.298 V and 0.388 mA (Figure 6). Therefore, with 300 µM concentration methyl blue of methyl blue in anolyte, use of ash-water catholyte reduced voltage, current and subsequently power density to by 5.3%, 21.9% and 26.0% as compared to when phosphate buffer catholyte was used.

CONCLUSIONS

After three hours of operation microbial fuel cell without addition of methyl blue into sewage anolyte, its output was 1.265 V, 0.403 mA and 56.12 mW/m^2. With 300 µM methyl blue in sewage anolyte, 6.7% increase in voltage, 20.5% increase in current and 28.6% increase in power density equivalent to 1.349 V, 0.485 mA and 72.15 mW/m^2, respectively. By using ash-water anolyte, the cell generated 1.221 V, 0.275 mA and 37.07 mW/m^2 after three hours of operation. With 300 µM methyl blue in sewage anolyte, the cell generated 1.331 V, 0.381 mA and 55.95 mW/m^2, which is equivalent to increase of 9.0% in voltage, 38.5% in current and 50.9% for power density. With reference to specific methyl blue concentration in anolyte, the difference in current is larger than the difference in voltage. Compared to 100

µM, 200 µM, 400 µM and 500 µM concentration of methyl blue in anolyte solution, 300 µM yielded the highest voltage, current and anodic power density for both phosphate buffer and ash-water catholytes. Hence, 300 µM methyl blue in anolyte was selected as optimum concentration.

Between three and twenty four hours of continuous operation of the microbial fuel cell with phosphate buffer catholyte, the average voltage was 1.305 V without addition of methyl blue in anolyte and 1.371 V when 300 µM concentration of methyl blue was used in anolyte. Similarly, the average current was 0.321 mA before addition of methyl blue and increased to 0.498 mA when anolyte had 300 µM concentration of methyl blue. Therefore methyl blue addition with phosphate buffer catholyte, led to 5.1% increase in voltage, 55.2% increase in current and consequently 63.3% increase in power density. By using ash-water as catholyte, average voltage of 1.222 V while average current was 0.244 mA without addition of methyl blue in anolyte. With 300 µM concentration of methyl blue in anolyte, the average voltage and average current increased to 1.298 V and 0.388 mA, respectively. This is equivalent to 6.2% increase in voltage, 59.1% increase in current and hence 69.1% increase in power density for ash-water as catholyte.

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