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# Effect of Thermal Pre-Treatments Method on Sludge Degradation Process Prior Usage in Membrane-Less Microbial Fuel Cell for Electricity Generation

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# Effect of Thermal Pre-Treatments Method on Sludge Degradation Process Prior Usage in Membrane-Less Microbial Fuel Cell for Electricity Generation

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**Abstract**. Microbial Fuel Cell (MFC) is gaining high interest by researcher around the globe as this is a promising renewable technology that has capability in producing electrical energy. In this study, the effect of the thermal pretreatment method (60, 80, 100, 120 and 140 ºC) on dewatered sludge (DS) as substrate for electrogenic bacteria (EB) in ML-MFC was optimized using one-factor-at-a-time (OFAT) method. The treated sludge then was used in ML-MFC by exposing them to the constant incubation temperature (35 ºC) with constant parameter of pH (6), initial moisture content (30 % vol/wt) and electrodes distance (3 cm) for 7 days incubation period. The performance of ML-MFC was relied on generation of soluble chemical oxygen demand (SCOD), power generation and EB biomass. Results shows the thermal pretreatment at 80 ºC was the optimum condition as the DS degradation rate (increment 78 %) when DS was treated for 4 h time treatment prior used in the ML-MFC. The degradation of DS was further degraded in ML-MFC via bio-catalysis process (51 % of SCOD produced compared initial DS inserted in MFCs ≈ 66 % SCOD removal). It was important to have a high DS degradation as the EB in ML-MFC would obtain more 'food' to growth thus increased the voltage (239 mV) and power density (98.34 mW/m$^2$) generation. In comparison, the control ML-MFC recorded low performance of DS degradation rate (21 %), voltage (24 mV) and power density 0.88 mW/m$^2$. Thus, by combining the pretreatment method of dewatered sludge prior used in ML-MFC it could improve the performance and expanding the application of ML-MFC as future renewable technology for sustainability of power sources. The EB strains was *Bacillus subtilis sp.*

## 1. Introduction

Nowadays the world is searching for an alternative renewable energy that can replace the current reliance on fossil fuel due to finite supplies of the energy source [1]. The increasing usage of non-renewable energy such as fossil fuel as the main energy consumption in the world have led to the global energy challenge with respect to climate change and local environment pollutions, this lead for intense research in implementing renewable energy sources as replacement [2]. In this past years, Microbial Fuel cells (MFCs) technology

gained a lot of interest from researchers and emerged as one of the popular alternative sustainable energy sources in wastewater treatment technology and green energy production [3]. MFCs is a device that convert chemical energy that stored in the bonds of organics matter into electrical energy through the biocatalysts of microorganism [4]. The microbial fuel cell (MFC) is a technology that uses electrochemically active bacteria as a catalyst to oxidize organic and inorganic matter to generate current [5]. Common MFCs system composed of anode, cathode, the separator and external circuit. The MFC consists of an anode compartment where the electrons are released by electrochemically active bacteria and transferred to the electrode [6]. Upon the application, the configuration of MFCs system can be develop into variable type such as single chamber or double chamber [7].

Unlike conventional technology, MFCs technology possess ability to utilizing a wide range of organics and inorganics matters as it substrate and this technology have high-energy transformation efficiency compare than other technology as it convert the chemical energy that stored in the substrates into electrical power energy [8]. In the term of configuration of MFCs, the single chamber MFCs can lower the operational cost due to no energy input requires for aeration and this device has great sustainable alternative technology as it has widespread application in wastewater treatment, biosensor, bioremediation, hydrogen production and generation of electrical power energy [8].The productions of dewatered sludge (DS) that produce from the treatment plant possess major issues in term of maintenance cost and environmental pollution. Based on S. Oh (2014) about 60 % of capital cost of treatment plant are used in managing DS production problem due to the stringent DS disposal laws. The ability of MFC technology which can utilize the organic waste as substrates provided an opportunity in reducing the operational cost of wastewater treatment plant. Plus it is also a sustainable energy production by converting the DS into energy [4]. DS is a complex heterogeneous mixture of microorganism and characteristics of DS which contain high energy of nutrient content possess a very stringent criteria for researcher to develop a sustainable energy solution as sludge can be translated into a viable source of energy instead of waste [9]. This supported the global action for sustainable development goals (SDGs) which focusing on issue of climate change and affordable energy, thus reduce the dependency on non-renewable energy [9]. Research done by S. Oh (2014) presented that the generation of power during MFCs process influenced by the DS degradation efficiency in ML-MFC due to the high composition of biomaterial in sludge. Thus, to make this persistent biomaterial more susceptible to efficient microbial degradation, the pretreatment of DS prior usage in ML-MFC could be the solution. The DS pretreatment method might promote value-added productivity into the sludge and can increase the overall performance of ML-MFCs. The release of organic matter from the DS could be monitored by determining the amount of SCOD formation. Thermal, ultrasonication and alkaline pretreatment method were found as an efficient DS degradation method which resulted in an increment of soluble chemical oxygen demand (SCOD) thus increase the availability of simple compound acted as 'food' for EB in ML-MFC. Hence improved the power generation in ML-MFCs via the EB biocatalyst process [10]. The pretreatment of DS is necessarily in providing a simple degraded organic compound therefore EB growth could be boosted [2]. Previously, the combination of ultrasonication and alkaline pretreatment in double chamber MFC [11], high pressure homogenization [12] and alkaline pretreatment also being tested on the sewage sludge[13]. The aerobic digestion [14] also had been used to improve the performance of MFC. However, there was lack information on study and research on thermal pre-treatment of DS which the DS acted as the pseudo membrane at the same time as the substrate in the membrane-less single chamber MFC for power generation. Therefore, the effect of pretreatments method by using one-factor-at-a-time (OFAT) method was carried out to see the impact toward the production performance of soluble chemical oxygen demand (SCOD), power generation and biomass of EB.

## 2. Materials and Methods

### 2.1 Sample collection
The DS sample was collected from two different wastewater treatment plants that located at Juru and Butterworth Wastewater Treatment Plant (WTP), Pinang, Malaysia. Then the samples was stored and kept in a cold room at 4 ºC under precaution method that proposed by Muaz et al. (2019) to maintain it freshness [5] [15].

### 2.2 Characteristics of dewatered sludge
The trace element in DS such as nickel (Ni), zinc (Zn), magnesium (Mg), iron (Fe) and Manganese (Mn) was detected using atomic absorption spectrophotometry (AAS) (GBC Model 903, Australia). In analyzing the macronutrient and micronutrient of DS, elemental analyzer (PerkinElmer 2400 Series II) was used and the preliminary treatment on DS were done using microwave digester method as proposed by Muaz et al. (2019). For this preliminary treatment by using microwave digestion method, 0.5 g of DS was added into solution of 10 mL nitric acid ($HNO_3$) and a 1 mL of hydrogen peroxide ($H_2O_2$) in the digestion vessel. The vessel was heat and reflux at temperature of 175 ºC for 10-15 min without boiling. The vessel was cooled in fume chamber at room temperature. 5 ml of $HNO_3$ was added into the sample and reflux process was repeated for 30 minutes. If there are brown fume formed the step was repeated until there are no fume were formed [15].

### 2.3 Thermal treatment
In order to determine the optimum time on thermal treatment for the DS, the experiment was carried out at four different time (2, 4, 6 and 8 h) to obtain the optimum condition. The DS samples were put into oven at constant temperature 120 ºC and treated at different time. The optimum time treatment was obtained by determining the SCOD concentration and degradation rate for each treatment time. Using the optimum treatment time obtained, the thermal pretreatment was carried out at different temperature 60, 80, 100, 120 and 140 ºC to find out the best temperature for thermal treatment. The SCOD and degradation rate were analyzed in each level of temperature.

### 2.4 Construction of ML-MFC
ML-MFC was built using cylindrical glass reactors (diameter: 10 cm; height: 10 cm) (Fig. 1) with the radiuses and surface areas of the graphite felt electrodes (anode and cathode) were 0.043 m and 0.5808 $m^2$ respectively. The initial pH (6), electrode distance (3 cm), initial moisture content (30 % vol/wt) were set as constant and the configuration was setup as used by as referred to Muaz et al. (2019).
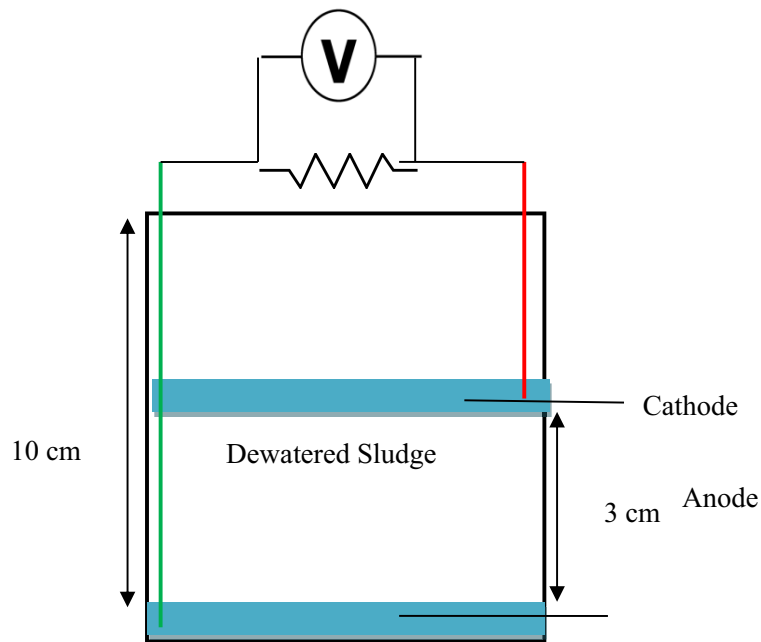
**Figure 1**. Construction diagram of ML-MFC using cylindrical glass reactors [16]

2.5 *Analytical Methods*

*2.5.1 COD analysis.* COD digester (Checkit Direct Lovibond) was used to analyze the organic compound in the DS samples. The soluble chemical oxygen demand (SCOD) is measured using Standard APHA Method [17] for SCOD, DS obtained from ML-MFC sample is centrifuge at 5000 rpm for 30 min [18] and filter using syringe filter (MF – Millipore Millex GS syringe filter with pore size 0.22 μm) and the filtrate is use as SCOD. The filtrate was put into 3 mL cod vial that consist of premixed chemicals ($K_2Cr_2O_7$, $AgNO_3$, $HgSO_4$, Potassium hydrogen phthalate, $H_2SO_4$).
The mixture of filtrate and chemical premixed were digested for 2 hours at 150 ℃. The cod value concentration was measured using UV-spectrophotometer at wavelength of 620 nm (Chemopharm-DR2800) and the blank sample was prepared by adding 2 mL of distilled water into the premixed chemical in the cod vial. From that the SCOD degradation rate was calculated based on the based on equation (1) [19]

(1)

$$SCOD\ (\%) = \frac{SCOD(final) - SCOD\ (initial)}{SCOD\ (final)} \times 100$$

*2.5.2 Determination of power using polarization curve technique.* The voltage produce by ML-MFC was measured using digital multimeter. Power output from ML-MFC was calculated according to Ohm's law equation (2) and (3) shown in Table 1 below. Polarization curve of ML-MFC was determined by varying the external resistance (47, 100, 220, 470 and 1000 Ω) and the power density is represented in equation (4). The peak of the power curve represent the maximum power of the ML-MFC [19] [20].

**Table 1**. The Ohm's law equation

| Parameter | Equations | |
| --- | --- | --- |
| Resistance | $R = \dfrac{V}{I}$ | (2) |
| Power | $P = VI$ | (3) |
| | $P_{density} = \dfrac{VI}{A}$ | (4) |

*$R$ is resistance $V$ is voltage, $I$ is current, $P$ is power and $A$ is area.

*2.5.3 Phylogenic Analysis.* The isolation method was done using serial dilution method from DS sample. A total of 1 g of DS sample was added into 10 ml sterile 0.9 % NaCl. The sample then were shake vigorously to separate the soil particle and bacterial cell. 500 µL of sample was transferred using micropipette to sterile microcentrifuge tube. Vortex the microfuge tube for 30 s to mixed the cell and saline solution. Six sterile microfuge tube that contained 900 µL of 0.9 % NaCl were labelled with dilution factor: $10^{-1}$ to $10^{-6}$. 100 µL cell from undiluted (100) were transferred to tube labelled $10^{-1}$ and mixed using vortex for 30 s. the process was continued 1:10 dilution until 10-6. Then 100 µL from each dilution were put in agar plate and incubated for 24 hours in 37 °C. The clear single strain was obtained from isolation of bacterial cell was transferred into slant agar plate [16]. The isolated samples (agar slants) were sent to Macrogen, South Korea, for microbial identification. The primers used for the polymerase chain reaction analysis were 27F 5' (AGA GTT TGA TCM TGG CTC AG) 3′ and 1492R 5′ (TAC GGY TAC CTT GTT ACG ACT T) 3'. The sequences obtained were analysed using the National Centre for Biotechnology Information (NCBI) online nucleotide BLAST tool and ribosomal database-II to identify the taxonomic hierarchy of the sequences. Taxonomically related 16S rRNA gene sequences were obtained from the NCBI nucleotide database. The sequences collected were aligned using the MUSCLE multiple sequence alignment algorithm.

The phylogenetic tree was constructed and inferred using the neighbour joining method and validated using the bootstrap method (1,000 replications). The evolutionary distances were computed using the maximum composite likelihood method and are presented as the number of base substitutions per site. All positions containing gaps and missing data were eliminated. All analyses were performed using MEGA6.

*2.6 Biochemical Test by Gram-Staining of EB*
The cell culture were aseptically transferred using inoculate loop. The culture material was then fixed on the slide by using heat. The cell culture were heat-fixed smear for 1 min with crystal violet staining reagent. Then the slide were washed using indirect tap water for 2 s. The iodine was flood into the glass slide for 1 min then gently washed using tap water for 2 s. Decolorized the cell culture with 95 % ethanol. The decolorized was stopped when the purple color has stopped leaching from the slide then washed with tap water for 2 s. The smear was covered with safranin for 30 s and gently washed the top and bottom of the glaas slide with tap water. The excess water at the glass was wiped using tissue paper. Then the slide that containing staining cell culture was observed under light microscope. The gram staining was done using method described by Tranter et al. (2016).

*2.7 Experimental Design*
Operational condition was set at constant throughout the experiment, with electrode distance, moisture content and incubation temperature were 3 cm, 30 % (vol/wt) and 35 ºC respectively. The voltage generation and SCOD removal of ML-MFC were studied. Throughout the experiment, the ML-MFCs were incubated for 7 days and the samples were collected for SCOD analysis and the voltage was recorded every 24 h using digital multimeter [15].

**3. Results and Discussion**

*3.1 Sludge Degradation Rate and SCOD Formation in Thermal Pretreatment*
The breakdown of complex carbon in the DS via thermal activity had impact the SCOD formation and it reflected to the DS degradation rate. The effect of treatment time for the DS degradation rate and SCOD concentration during thermal pretreatment were shown in Fig. 2 and 3.  Both figures showed the value of SCOD formation in pretreated sludge for both samples (Juru and Butterworth) were higher than the raw DS sample (untreated). Clearly each sample had their optimum time for formation of SCOD. Butterworth DS showed increases of SCOD concentration from 0 to 4 h and started to decrease once treated for 6 h. While, Juru DS it only increased until 2 h treatment and started to decrease at 4 h of treatment.  The increasing of SCOD concentration and rate of DS degradation (Fig.2 & Fig.3) for Juru and Butterworth DS occurred due to the disruption of chemical bonds of carbon source in DS sludge compound by thermal treatment, thus increase of DS degradation rate [19].

The disruption of complex DS structure by thermal treatment release intracellular and extracellular organic material in DS such as proteins, carbohydrate and lipid to the liquid phase thus it increase the SCOD value shown in Fig. 2 (Jayashree et al., 2014;Aboulfoth et al., 2015). Here we can conclude that as the SCOD value increase thus the degradation rate of DS also increase significantly. The decrement in SCOD after 6 h may be due to the occurrence of refractory compound catalyzed by the thermal energy over prolonged periods of treatment [19]. Juru DS showing the highest degradation rate with 85 % when the sludge treated at 2 h treatment time compared to Butterworth DS slightly low as 83 % when treated at 4 h treatment. This might be due to the DS that come from WWTP of Butterworth have higher concentration of heavy metal such as Magnesium (Mg), Zinc (Zn), Iron (Fe) and Manganese (Mn) with concentration of 0.8915, 0.2738, 0.1473 and 0.0113 mg/L respectively (Table 2).

DS that collected from Juru WWTP have higher concentration of Magnesium compare with Butterworth (1.4034 mg/L) but Juru DS have lower concentration of Zinc (Zn), iron (Fe) and Manganese (Mn) with 0.0383, 0.0536 and 0.0818 mg/L respectively (Table 2). The higher concentration of the other three element of heavy metal (Zn, Fe and Mn) in Butterworth DS was the factors that contribute into longer time treatment and slightly low degradation rate (%) compare with Juru DS.  Toxicity of heavy metal is ability of metal that cause detrimental effect on sludge degradation and it depend on the bioavailability of heavy metal and

absorbed dose. The presence of heavy metal in DS can severely inhibit the biodegradation of organic contaminant and carbon source in DS [23]. Heavy metal interfere with the physiological activity of DS by forming a complex functional side chain groups or by displacing essential metal ions in metalloproteinase and interfere with biological activity of DS thus caused a low degradation rate of Butterworth DS [24].

**Table 2**. Composition of DS for Juru DS and Butterworth DS

| Element | Butterworth (mg/L) | Juru (mg/L) |
|---|---|---|
| Mg | $0.8915 \pm 0.003$ | $1.4034 \pm 0.001$ |
| Zn | $0.2738 \pm 0.003$ | $0.0383 \pm 0.003$ |
| Fe | $0.1473 \pm 0.002$ | $0.0536 \pm 0.008$ |
| Mn | $0.0113 \pm 0.008$ | $0.0818 \pm 0.003$ |
| SCOD | 1930 | 1760 |

From the graph in Fig. 3 the degradation rate (%) profile of DS from WWTP Butterworth and Juru started to decrease significantly at 4 h treatment time, thus it clear that treatment time after 4 h not contributed to the degradation of DS and resulting the extracellular and intracellular of organic material that were contain in DS remained inside and the EB couldn't use it as source of food. Hence the treatment time of 4 h was found to be the optimum time treatment (its time friendly due to the shorter time period). Therefore, the effect of thermal pretreatment using optimum time was conducted at five different temperatures at 60, 80, 100, 120 and 140 ºC to determine the effect of different thermal temperature for SCOD performance and ML-MFC voltage output. According to Ruiz-hernando et al. (2013) the impact of temperature improves the biodegradability and dewater ability of DS by causing cell lysis due to pressure difference. Common treatment temperature usually between 60 ºC and 180 ºC which can help in destroying the cell wall and broken the bond of carbon source in DS [25]. According to Jayashree et al. (2014) and Ruiz-hernando et al. (2013), the temperature above 180 ºC leads to the production of recalcitrant soluble organics or toxic intermediates, hence this reducing the biodegradability and the alternative way to overcome this drawback was to apply temperature below 180 ºC and it was the suggested approach as a biological predigesting step. It had an incremental effect on DS degradation effect (under thermophilic condition) and significantly reduces the energy requirement [19]. DS that under high thermal temperature consumes little energy for degradation into simple organic compound and this can attributes to increase of activation potential and mass transfer electron loss lead to decrease of current production of ML-MFC [2].
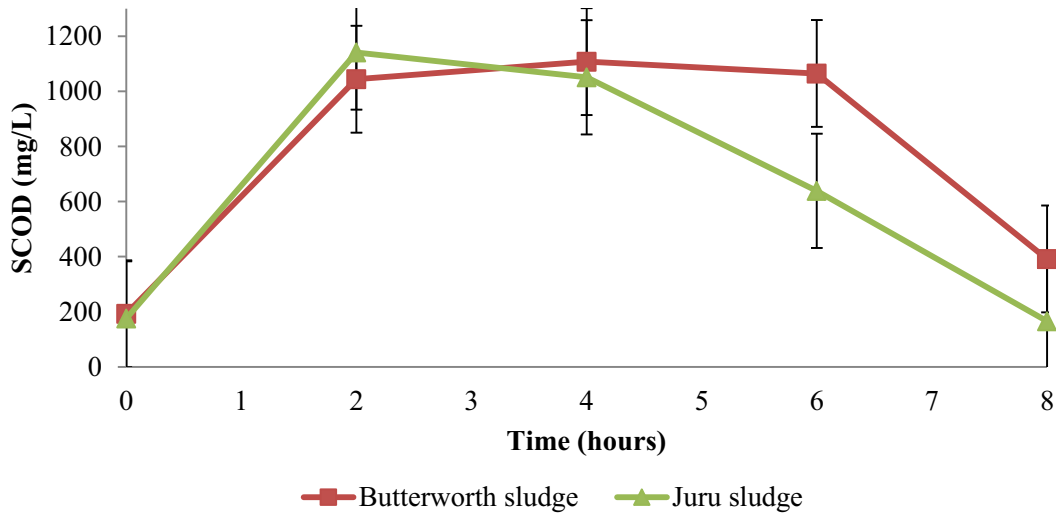
**Figure 2**. The SCOD profile under thermal pretreatment at 120 ºC
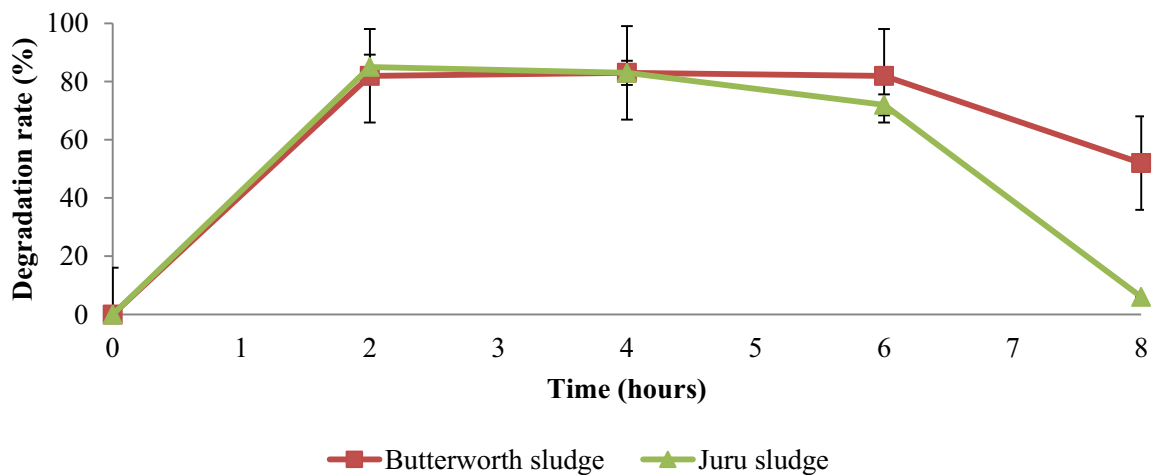


**Figure 3**. The degradation rate (%) profile of DS from WWTP Butterworth and Juru at 120 ºC.

The optimization of time treatment of DS and the effect of different temperature for SCOD formation and DS degradation rate during thermal treatment were shown in Fig. 4 and 5. The Butterworth DS sample was increased when treated at 60 to 80 ºC with the recorded highest SCOD formation (8650 mg/L) and DS degradation rate (78 %). The SCOD and DS degradation rate started to decrease once it reached treatment at 100 ºC to 140 ºC.

While the Juru DS result were contradicted results as it showed the highest SCOD (8150 mg/L) and degradation rate (78 %) when the DS were treated at 140 ºC.
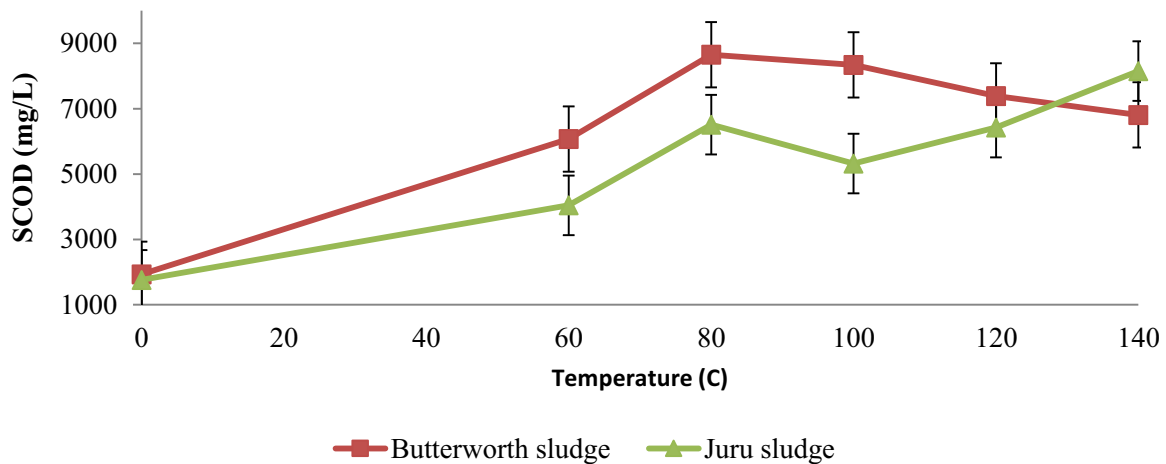


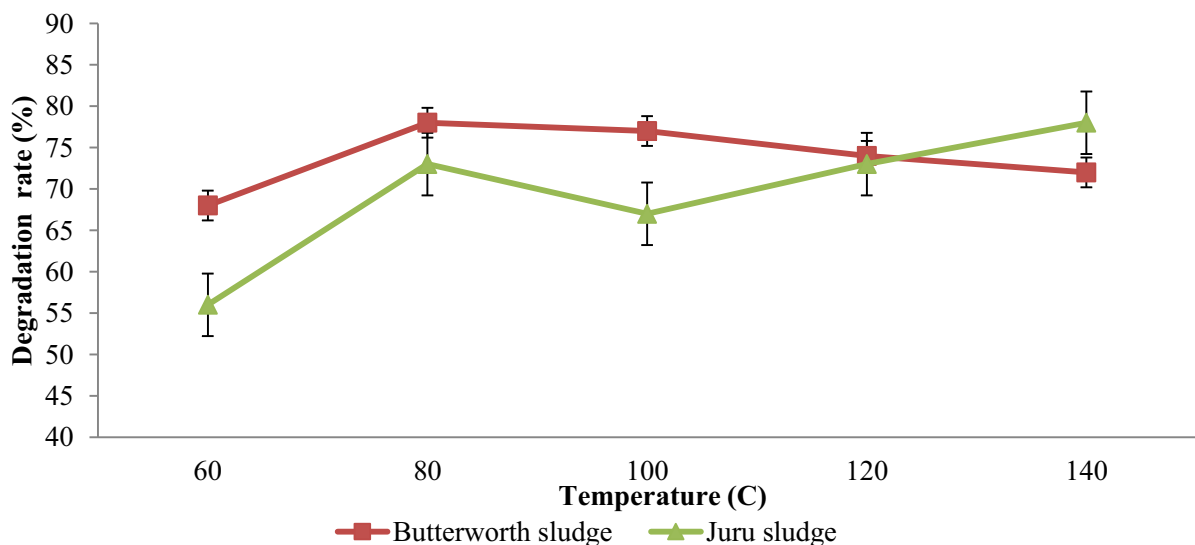**Figure 4**. Effect of temperature on SCOD concentration (mg/L) at 4 h treatment time



**Figure 5**. Effect of temperature on degradation rate (%) at 4 h treatment time

*3.2 ML-MFC Performance*

*3.2.1 Effects of thermal sludge pretreatment on voltage output generation.* After sludge was pretreated by using different temperature of thermal treatment, the pretreated DS was introduced into single chamber ML-MFC for further degradation and simultaneous power generation. In comparison ML-MFC was also operated using untreated DS samples a control. Figure 6(a), 6(b) and 7 show the pattern of voltage generation from different type of pretreated DS under external load 1000 Ω.

The maximum voltage output from ML-MFC was obtained from Juru DS (239 mV) when treated at 80 ºC when ML-MFC in day 6 under external load of 1000 Ω and second highest voltage output in Juru DS sample was 51 mV 120 ºC of thermal treatment in day 4. Voltage output from raw (control) for Juru DS yielded highest peak at day 6 with only 24 mV. For the case of Butterworth DS yielded maximum voltage output of 34 mV when the DS was treated at 120 ºC in day 6 with the second highest voltage was 31 mV treated at same thermal temperature in day 4. The voltage output from the raw Butterworth sludge (control) showed the voltage peak at day 5 with only 10 mV.
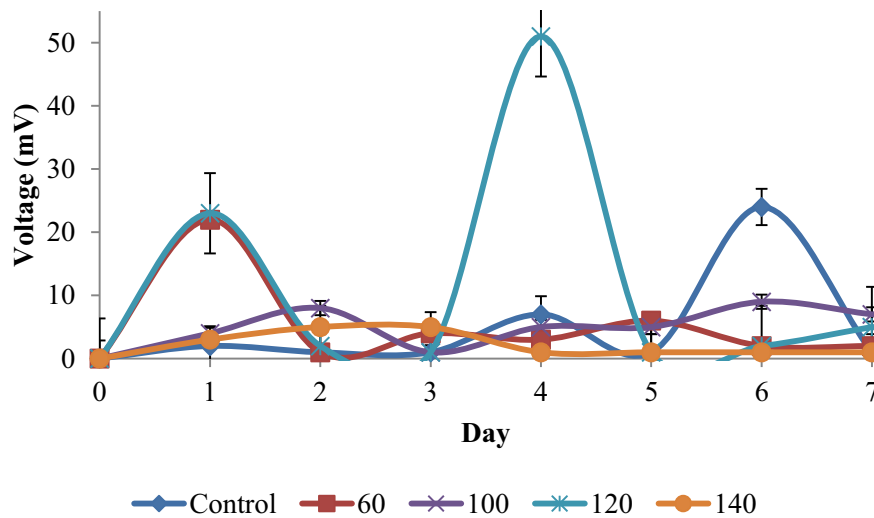


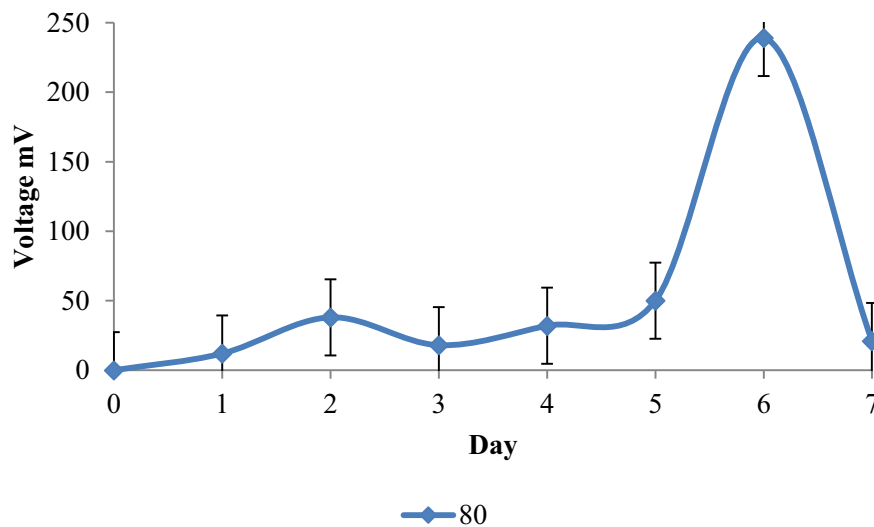**Figure 6(a)**. Voltage generation profile in Juru DS ML-MFC under external load 1000 Ω



**Figure 6(b)**. Voltage generation profile in Juru DS treated at 80 ºC under external load 1000 Ω
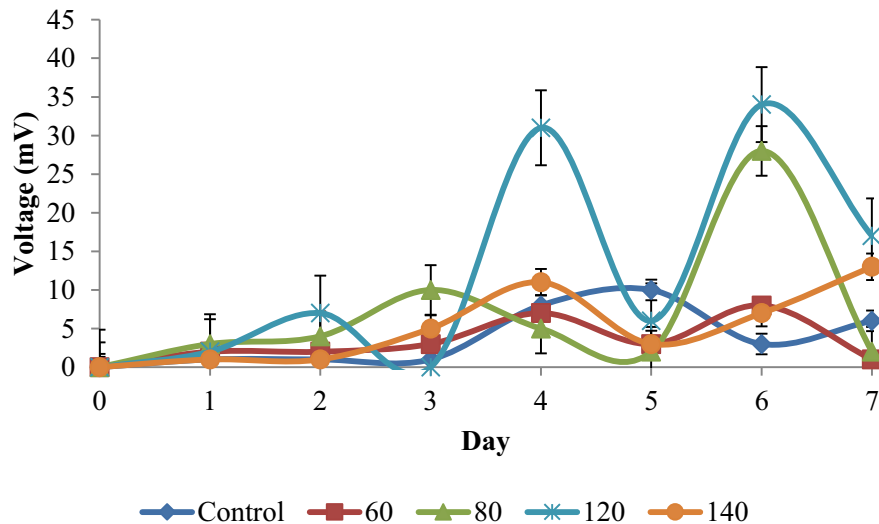
**Figure 7**. Voltage generation profile in Butterworth DS ML-MFC under external load 1000 Ω

Based on Table 3 it can be summarized that 60 ºC of Juru DS shown the highest voltage output (22 mV) in 1st day meanwhile Butterworth DS that undergo thermal temperature of 60 ºC achieved its highest voltage output in 5th day incubation time. Table 3 show that Juru DS that undergo thermal temperature of 80 ºC achieved highest voltage output (239 mV) when treated at 6th day incubation period. Juru DS that undergo thermal treatment of 140 ºC show the lowest voltage output (5 mV) compare to other temperatures, while Butterworth DS that undergo 140 ºC produce 13 mV when treated at 7th day incubation time.

**Table 3.** Summarize the optimum voltage at their different rate of thermal pretreatment

| DS | Temperature (ºC) | OCV (mV) | Optimum Voltage (mV) (Under 1000 Ω resistor) |
|---|---|---|---|
| **Juru** | 60 | 285 | 22 (1st day) |
| | 80 | 312 | 239 (6th day) |
| | 100 | 234 | 9 (6th day) |
| | 120 | 237 | 51 (4th day) |
| | 140 | 135 | 5 (3rd day) |
| **Butterworth** | 60 | 199 | 10 (5th day) |
| | 80 | 465 | 28 (6th day) |
| | 100 | 146 | 18 (6th day) |
| | 120 | 103 | 34 (6th day) |
| | 140 | 264 | 13 (7th day) |

*3.2.2 Effect of thermal temperature on power density.* Based on Fig. 8, the polarization curve was plotted using Juru DS as it produces the highest voltage output under external load of 1000 Ω (239 mV) to describe the overall performance of single chamber ML-MFC of Juru at 6th day. The open circuit voltage (OCV) of Juru DS at 6th day was 312 mV with SCOD of 5240 mg/L. The polarization curve as a function of current, potential and power measured at variable external resistance $R_{ext}$ between (47 – 1000 Ω). From the Figure, the polarization curve were divided into three region; (i) activation losses, (ii) ohmic losses and (iii) mass transport losses [26].

Based on Fig 8 (i) activation losses occurred due to energy losses for initiating the oxidation or reduction reaction and the energy lost through the transfer of an electron from the cell to the anode surface [4]. Figure 8 also demonstrated the dominance activation loss was decrease in the voltage from 312 mV to 239 mV. This loss of energy can be reduced by improving the electron transfer between EB bacteria and the anode. At region (ii), the subsequent slopes of voltage decrease linearly from 193 mV to 182 mV indicate the dominance of ohmic losses. These losses happen due to arise of ion resistance that was occurred in ML-MFC where the DS acts as substrate medium that contribute to complete the electrical circuit. The ohmic losses can be reduce by decreasing the electrode distance between anode and cathode.

Fig. 8 (iii) shown the mass transport losses as the voltage dropped significantly at 182 to 115 mV. These due to the flux of reactant to the electrode insufficient and limiting the rate of reaction. The polarization experiment showed that ML – MFC was able to supply a maximum power 281 mW for 115 mV under load of 47 Ω (Figure 8). The curve depicts a maximum power density of 570.32 Watt/$m^2$ at current 1.82 mA and at resistance 100 Ω.
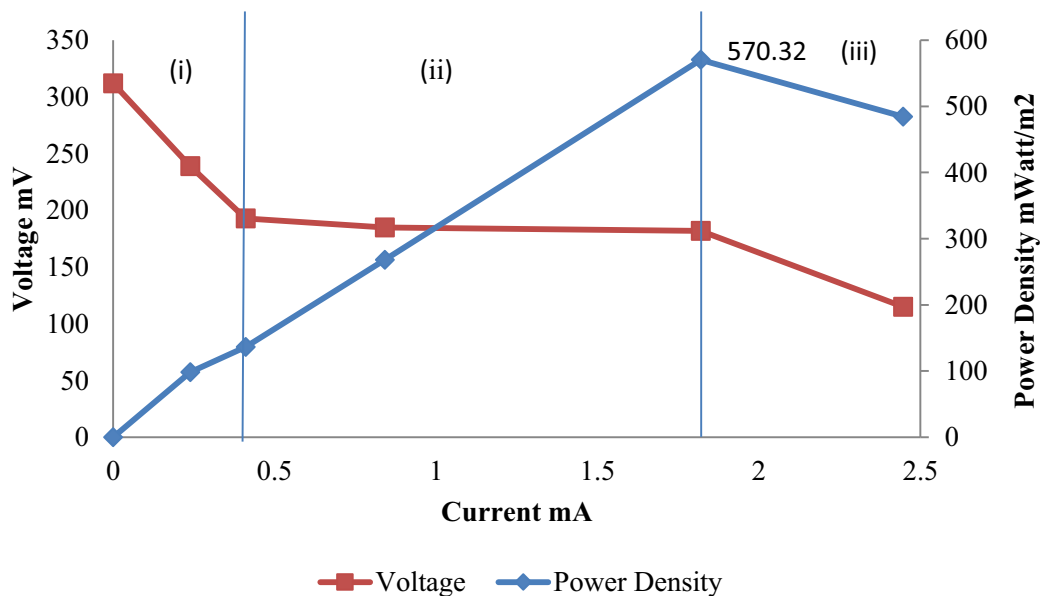


**Figure 8.** polarization curve of ML-MFC at 6th day

*3.3 Gram Staining Analysis*
The EB was observed under a light microscope and it was recorded as gram positive microorganism because of the distinctive purple appearance of the cell (due to thick peptidoglycan layer of the cell wall). The cell wall of gram-positive bacteria is host to wide variety of molecule and can serves multitude of functions that can be an advantage in ML-MFC such it has thick peptidoglycan. Gram-positive bacteria have simpler chemical composition compare to gram negative with 90 % peptidoglycan and 10 % teichoic acid and the covalent bonding between peptidoglycan and teichoic acid of gram positive bacteria may cause higher negative surface change [27]. Contrary on that gram negative bacteria have only 10 % peptidoglycan composition compare to gram positive thus, these gram-negative bacteria have low negative surface changes this makes the cell walls of the gram positive microorganism more rigid compare to gram negative microorganism [27].

*3.4 Phylogenic Analysis*

The EBs that was colonized at the anode electrode were isolated and were sent to the microbiology company Macrogen, Korea. The analysis revealed the presence of genus *Bacillus subtilis*. The presence of *B. subtilis* gives an impact in the MFC as it has good biocatalyst ability in ML-MFC, and it can generate energy stably. It also secretes several enzymes that play a significant role in hydrolyzing the DS's component.

The enzymes secreted are protease, glycolipid, α-amylase, endo-β-glucanase penicillin acylase, 50-inosine monophosphate, and riboflavin and will be used to strengthen the microbial biochemical pathway. This finding shows why a stable energy production was able to be obtained via this study because presence *B. subtilis* enhances the oxidation processes around anodes.

## 4. Conclusion

Based on the study of effect of thermal pretreatment of DS had increased the degradation rate significantly (Juru: 1760 mg/L to 8150 mg/L (78 % increment) when it been treated at optimum treatment time (4 h). The optimized treatment had led improvement of the power density output under external load of 1000 Ω from 0.88 mW/m$^2$ to 98.34 mW/m$^2$ (98 % increment) with highest voltage output was 239 mV (OCV was at 312 mV) for Juru DS. The EB was identified as gram positive via the gram staining test and result from the phylogenic analysis exhibited the electrogenic bacteria used was from *Bacillus subtilis* (BS) group. The thermal pretreatment on DS could enhance organic matter degradation as the SCOD of DS has increased and significantly improved the performance of ML-MFC which thermal pretreatment DS showed the best performance in voltage generation. Thus, the thermal pretreatment on DS prior usage in ML-MFC showed it importance as it could improve the overall performance of ML-MFC.

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