Abstract
Microbial fuel cell (MFC) technologies represent the newest approach for generating electricity (bioelectricity generation) from biomass using bacteria. Bioelectricity generations by MFC have gained considerable attention due to its integration with wastewater treatment. The objectives of the work are to determine the optimisation of MFC’s bio-electrochemical process using three different factors and its interaction, and to determine the optimal pH value for acidogenic, acetogenic and methanogenic by natural mixed culture electroactive bacteria (exoelectrogens) growth in presence and absence of oxygen using MFC. The two-level factorial design is used in order to achieve the main two objectives. The current generation, power generation and maximum power have been monitored. Experimental result shows that the best interaction between these three factors is (-+-) interaction which is the interaction between tryptic soya broth (TSB), sodium hydroxide as pH controller and resistant of 200 Ω, and the interaction yield the power density of 57.44 mA m⁻². The effects between those interactions also have been analysed. The interaction of all parameters that have been used in this experiment is given out the highest significant effect which is a value of effect of 24.56 with a significant F-value of 29.51. The chemical oxygen demand (COD) reduction by MFC treatment data based on the COD effective deduction concept shows that DMP produced lower percentage of COD effective deduction efficiency compared to nDMP. nDMP was 342% to 441% more efficient to deduct COD compared to DMP. nDMP 6.8 recorded the most effective COD deduction by MFC devices at 29.17%.

Keywords: microbial fuel cell, palm oil mill effluent, 2-level factorial design, chemical oxygen demand.

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Introduction
Palm oil mill effluent (POME) is a highly-organic wastewater produced by palm oil processing mills. It is usually treated in an open pond system consisting of cooling ponds, acidification ponds, anaerobic ponds and facultative ponds. Through the system, it is able to meet the biological oxygen demand (BOD) discharge limit of 5000 mg litre⁻¹ set by the Malaysian Department of Environment...
(Malaysian Federal Subsidiary Legislation 1978) (Andrew and Manaf, 2013). POME anaerobic fermentation is a complex biochemical process where organic and inorganic matters are degraded to methane and carbon dioxide in discrete steps involving the concerted action of myriad numbers of bacteria in several different metabolite groups of microorganism. The main pathways of anaerobic digestion involve four stages, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis (Andrew and Manaf, 2013), and all these steps are essential to gain the optimal microbial fuel cell’s (MFC) performance result.

Recently, the microbial treatment of POME has gained some interest among many scientists and environmentalists, and the application of bacteria in MFC is one of the alternatives for POME treatment (Kang et al., 2017; Tan et al., 2017; Tee et al., 2017). The application of MFC technologies which generate electricity as a by-product is an alternative approach to wastewater treatment (Logan, 2008). In 2004, the relationship between generation electricity using MFC and wastewater treatment was forged when it was demonstrated that domestic wastewaters could be treated to practical levels while simultaneously generating electricity (Liu and Logan, 2004). Bio-electricity generation is a new approach for producing electricity from biomass using bacteria. Therefore, bacteria were further categorised by their ability to exogenously transfer electrons, called exoelectrogens that can produce power in an MFC (Logan, 2008). Other names for exoelectrogens are electrogens, anode-respiring bacteria (Torres et al., 2010) and electrochemically active bacteria/microorganism (Samir et al., 2010). Exogenously transfer electrons by bacteria are also known as extracellular electron transfer (EET) (Torres et al., 2010).

Design of experiment (DOE) is a well-known method in the engineering field as to determine the cause and effect relationship, which was originally designed for agriculture purposes. DOE is used to evaluate which process inputs would have a significant impact on its output, and what the target level of those inputs should be to achieve a desired output. Planning, designing and analysing are the main processes in DOE. There are three main aspects in DOE, which are factors, levels and response. Factor is the inputs of the process which can be classified either controllable or uncontrollable variables. Level is the settings of each factor in the study. Finally, the respond is the outputs of the experimental works. The purposes of DOE are comparing the alternatives, identifying the significant factors that affecting the response, achieving an optimal process output, reducing variability, improving process, balancing tradeoffs, and finally is to minimise, maximise or target an output (Anderson and Whitcomb, 2007).

Two-level factorial design is conducted for this work. It defines as factorial designs in which each factor is studied at two levels which is at high and low, or present and absent. This method is very important in factor screening experiments and many other scientific investigations. As a first approximation for the model of response controlled by these factors, it is customary to include the main effects and some specified set of two-factor interactions. It was designed to monitor all chosen parameter’s main effect and the interaction between them, by doing so we can have a better understanding on the effect of the parameters that we selected. The understanding in the interaction between parameters is a crucial information as to achieve a better performance for this experimental work. This can be achieved by implying the responses of the mains and the interactions of the model into regression model as to predict the responses of designed parameters if different combinations were used (Anderson and Whitcomb, 2007).

During hydrolysis, large polymers of carbohydrate, lipids (fat) and protein macromolecules were broken down to amino acids, long-chain fatty acids and sugars. These hydrolysates were then further fermented during acidogenesis to produce three-, four- and five-carbon volatile fatty acids, where the optimum pH range for the growth of the acidogenic bacteria was within the pH ranges between pH 5.2 to pH 6.5. These hydrolysates were consumed by the acetogenic bacteria generating acetate acids, carbon dioxide and hydrogen during acetogenesis, where the optimum pH for growth of these bacteria was between pH 6.0 to pH 7.0. Finally, during methanogenesis, where methanogenic bacteria, which grow within the range of pH 7.5 to pH 8.5, consume the acetate, hydrogen and some of the carbon dioxide to produce methane (Rappop et al. 2008; Solera, et al., 2002).

Apart from the above categorisations, bacteria may be divided into three groups according to their response to free molecular oxygen. These groups are: (1) strictly aerobes, (2) facultative anaerobes and (3) anaerobes which are inactive in the present of free molecular oxygen. Anaerobes may be divided into two subgroups; oxygen-tolerant species and oxygen-intolerant species (Gerardi, 2003). In the second part of this study, mixed POME used was a mixture of raw POME and anaerobic pond POME with the ratio of one is to one (1:1). Anaerobic bacteria present in anaerobic pond POME could be grouped on the basis of their need for oxygen to grow. Obligate anaerobic bacteria (oxygen-intolerant species) are bacteria that use an anaerobic metabolism to grow but are harmed and killed in the presence of oxygen. Meanwhile, aerotolerant anaerobic bacteria (oxygen-tolerant species) are also bacteria that use an anaerobic metabolism to grow but could tolerate the presence of oxygen. The
two categories mentioned above need an anaerobic metabolism to grow, but not for facultative anaerobic bacteria. Facultative anaerobic bacteria prefer to grow using the aerobic metabolism processes but could switch to anaerobic metabolism in the absence of oxygen.

There are two parts in this work, the first part is mixed POME substrates (1:10, raw POME: autoclaved POME) was subjected into three factors that may influence the power generation’s output. The first factor was the type of bacteria’s broth which are the brain heart infusion (BHI) and tryptic soya broth (TSB). The second factor was the type of pH controller and for this experiment were NaOH and Ca(OH)$_2$. The third factor was the external resistance which are 200 Ω and 1000 Ω. By using all these factors, its two-levels factorial was designed accordingly, similar pattern was reported by Kala et al. (2016), Samad and Zainol (2017), and Tan et al. (2016). These factors were to investigate in terms of its main and interaction effects and to determine the optimisation of MFC’s bio-electrochemical process using the MFC device. This factorial design is to provide significant justification of MFC’s power performance. The second part is to determine the optimal pH value for acidogenic, acetogenic and methanogenic by natural mixed culture electroactive bacteria (exoelectrogens) growth in original raw and anaerobic digested POME mixture (nDMP) and nitrogen deoxygenated raw and anaerobic digested POME mixture (DMP) in MFC is needed.

**METHOD**

**Part 1: DOE Procedure**

The air cathode double-chambered MFC was made locally using acrylic fibre material with 100 ml of working volume for each component. The anode chamber (anaerobic) and cathode chamber (aerobic) were separated by a salt bridge compartment filled with molten 10% agarose with 4% potassium chloride salt (Nair et al., 2013) heated in a water bath which was allowed to be cooled and solidified in room temperature. The salt bridge assists in the proton transfer mechanism during the operational of MFC. The electrode material used for anode and cathode was carbon cloth [activated carbon fibre (ACF) fabric, China] with the sizes of 3.0 x 3.0 cm (used in both chambers) with projected area of 9.0 cm$^2$. The anode chamber is sealed to maintain its anaerobic condition. The electrodes close circuit was connected externally through copper wires to provide the permanent connection to external resistance of 1000 Ω or 200 Ω resistor. The schematic diagram of MFC is shown in Figure 1.

The substrate used is mixed POME; a batch of 1.5 litres raw POME was autoclaved for 15 min at a temperature of 121°C at 100 kPa to eliminate living microorganisms entirely. The substrates were prepared in 250 ml borosilicate glass reagent bottles with a mixing ratio of 1:10 (20 ml raw POME to 180 ml of autoclaved POME) with a total of eight
bottles. The pH of the substrates were controlled by using sodium hydroxide (NaOH) or calcium hydroxide [Ca(OH)₂] maintaining a pH of 6.8 ± 0.2. Each bottle was subjected to either BHI broth’s bacteria or TSB bacteria accordingly. Then, each 250 ml bottle transferred 100 ml of substrate into 100 ml borosilicate glass reagent bottles as controlled and calibrate sample. After 24 hr of the fermentation process, the leftover substrate of 100 ml in each 250 ml bottle was used in MFC’s operation.

The electrode output voltage was recorded every 15 min with digital multimeter with data logger (DM620, 50 000 Count Logger DMM and UT803, UNI-T). From these data, power generation was calculated using power density normalised by surface area (P\text{An}, \text{Wm}^2) (Logan et al., 2006) using Equation (1):

\[ P_{\text{An}} = \frac{V^2}{A_n R} \]

where \( A = \) total area of anode electrode (m²), \( A_n = \) Anode, \( P = \) power (W), \( V = \) the potential (V) and \( R = \) external resistance (Ω).

A 2\(^3\) factorial experimental design that study the three main effects, which is type of bacteria’s broth, pH controller and resistor, is as shown in Table 1. A total of eight MFC devices were ran and each factor was carefully monitored at two different levels. High and low levels were the two types of factors that were tested as shown in Table 1. The plus symbol (+1) represents high level and the minus symbol (-1) represents the low level. In this work, as for the type of bacteria’s broth of high and low levels were the BHI broth and TSB broth, respectively. The NaOH and Ca(OH)\(_2\) that were used to represent lower and higher levels of the type of pH controller, respectively. Finally, 200 Ω represents the lower level and 1000 Ω represents the higher level of type of resistor used (Table 1). Each MFC device was ran for five days (120 hr) and all the data was recorded and analysed. The linear model that was used to predict a given responses as Equation (2).

\[ Y = \beta_0 + \sum \beta_i X_i \]

where \( Y = \) predicted response, \( \beta_0 = \) model coefficient, \( n = \) number of the variable, \( \beta_i = \) linear parameters’ coefficient, and \( X_i = \) interaction parameters’ coefficient (Samad and Zainol, 2017).

### Part 2: POME Treatment

The double-chambered MFC was designed and fabricated locally using the acrylic fibre material with each compartment working volume of 150 ml. The anode (anaerobic) and cathode (aerobic) chamber were separated by a salt bridge compartment filled with molten 10% agarose with 4% potassium chloride salt (Nair et al., 2013) heated in a water bath which was then allowed to cool down and solidified where this salt bridge assist in the proton transfer mechanism during MFC’s operation. The electrode material used for anode and cathode was carbon brush and the sizes were 2.2 x 1.6 x 0.6 cm (used in both chambers) and 1.3 x 0.7 x 0.6 cm (used in anode chamber only) with projected area of anode was 62.22 cm\(^2\) and cathode was 11.6 cm\(^2\).

The electrodes close circuit was connected by using copper wires projecting outside to provide the permanent connection to external resistance of 1000 Ω resistor. The schematic diagram of microbial fuel cell is shown in Figure 1.

Two mixed POME substrates were prepared in 2-litre beaker with mixing ratio of 1:1 of raw POME and anaerobic pond POME in each beaker. The technique by gas flushing or sparging pure N\(_2\) gas (Logan, 2008) through the mixed POME (deoxygenation pre-treatment) was a process to decreased DO (Biffinger et al., 2008; Japar et al., 2013; Logan et al., 2006) in the mixed POME was applied to the first mixed POME sample mixture, DMP, while the second, nDMP, was not. This technique (Biffinger et al., 2008) were done by a large numbers of researchers (Oh et al., 2009; Japar et al., 2013; Biffinger, 2008; Logan, 2008).

Mixed POME (DMP or nDMP), with the three groups of pH ranges prepared earlier, was used as the substrates for each separate MFC and 125 ml charged in the MFC’s anode chamber. The anode chamber was kept in an anaerobic condition by N\(_2\) purging and tightly sealed. The cathode chamber was operated in distilled water (aqueous cathode) (Rega, 2006) and constant bubbling with air into the water to provide oxygen to the cathode. The electrode output voltage was recorded against time, measured every 15 min. The anode and cathode

### Table 1. Factors and Levels in 2\(^3\) Factorial Experimental Design

<table>
<thead>
<tr>
<th>No.</th>
<th>Factors</th>
<th>Coded</th>
<th>Type of factor</th>
<th>Actual values of coded levels</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bacteria’s broth</td>
<td>A</td>
<td>Categorical</td>
<td>TSB</td>
<td>BHI</td>
</tr>
<tr>
<td>2</td>
<td>pH controller</td>
<td>B</td>
<td>Categorical</td>
<td>NaOH</td>
<td>Ca(OH)(_2)</td>
</tr>
<tr>
<td>3</td>
<td>Resistor</td>
<td>C</td>
<td>Numerical</td>
<td>200</td>
<td>1 000</td>
</tr>
</tbody>
</table>

Note: TSB - tryptic soya broth, BHI - brain heart infusion.
were connected directly to a digital multimeter with data logger (DM620, 50 000 Count Logger DMM and UT803, UNI-T). Each day, the pH value was taken by using digital pH meter (Professional Bench Top pH Meter BP3001, Trans Instruments, Singapore) and the pH values were controlled using NaOH to keep its pH ranges accordingly. These simple air bubble aqueous cathode MFC with salt bridge dual chamber were operated at ambient temperature from 25°C to 28°C. The voltage reading and its related analysis data will not be shown as this article concentrate on COD analysis.

The COD of the anode substrates was determined by using COD cell test kit Kids (20-1500 mg litre$^{-1}$ range: Hach, USA), digested by using COD reactor (Hach DRB 200, USA) and measured using COD spectrophotometer (Hach DR2800, USA) where COD reading was taken. The initial COD reading was analysed by taking small sample of mixed POME before inserting it in MFC devices. The second COD reading was taken from the MFC anode chamber and the untreated sample on the fifth day. The COD removal efficiency ($\eta$) can be calculated as the ratio between the removed and influent COD (Logan et al., 2006) using Equation (3):

$$\eta = \frac{\text{COD}_0 - \text{COD}_t}{\text{COD}_0}$$  

Equation (3)

where COD$_0$ = initial COD of the effluent in the anode chamber, mg litre$^{-1}$ and COD$_t$ = COD of the effluent in the anode chamber at measured time (Baranitharan et al., 2013).

RESULTS AND DISCUSSION

Part 1: DOE Procedure

Voltage readings were recorded, where power densities were calculated, by the MFC devices for all designed factors in $2^3$ factorial design as shown in Table 2. In Figure 2, the graph is divided into

<table>
<thead>
<tr>
<th>Standard order</th>
<th>Coded values of variables</th>
<th>Power density (mWm$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (---)</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>2 (+--)</td>
<td>+1</td>
<td>-1</td>
</tr>
<tr>
<td>3 (-+-)</td>
<td>-1</td>
<td>+1</td>
</tr>
<tr>
<td>4 (++-)</td>
<td>+1</td>
<td>+1</td>
</tr>
<tr>
<td>5 (+++)</td>
<td>+1</td>
<td>-1</td>
</tr>
<tr>
<td>6 (+---)</td>
<td>+1</td>
<td>-1</td>
</tr>
<tr>
<td>7 (+++)</td>
<td>-1</td>
<td>+1</td>
</tr>
<tr>
<td>8 (+++)</td>
<td>+1</td>
<td>+1</td>
</tr>
</tbody>
</table>

![Figure 2. Microbial fuel cell’s (MFC) electricity production of all factors in five days.](image-url)
two sections which are 1000 Ω (upper dotted-line) and 200 Ω (lower dotted-line). Since there are two external resistances involved in the experiment, the electricity generation in Figure 2 is not suitable to be used to compare the relationship between the designed factors and the generated current. The different resistors used for the experimental work make the electricity generated tend to be differ as well. As the external load is higher, the electricity that was generated also higher which apply by the formula of V=IR. The electricity generation can only be refer if the external resistor is constant, however it can be used to understand the mechanism of electricity generation and the behaviour of the electrons transferred in the system over time. A similar approach was reported by Jadhav and Ghangrekar (2009).

The power densities of all designed factors were calculated, and the comparison of all designed factors’ power generation are as shown in Figure 3. In the graph, only three peaks that have significant readings as compared to others which are factors (---), (+++) and (+++). Factor (---) recorded the highest maximum power generated as compared to (+++), that is the second highest, and lastly (+++). (---) yielded a maximum power of 57.44 mWm⁻². Factors (---) and (+++) generated maximum power of 41.48 mWm⁻² and 15.16 mWm⁻², respectively. As shown in Figure 3, the maximum powers’ peak for both factors (---) and (---) is peaked at resistance of 200 Ω, and 1000 Ω is where the maximum power of (+++) is peaked. The reason behind these results are due to correspondence of the growth of bacteria with the external resistance that were used in the experiment (Liu et al., 2008), but that is not in all cases because in this work, factor (---) shows a different result. Although, 1000 Ω was used in factor (---) but the maximum power’s peak which supposed to be 1000 Ω, but it lies on 200 Ω instead. It is proven that bacteria’s growth and external resistance play an important role in power density generation, but external resistance is not the only factor that determine its maximum power. Other factor that determine maximum power is the differences between external and internal resistance. The smaller the differences, the higher the power density will be produced (Logan, 2008). The relationship between bacteria’s growth and external resistance, that effects the maximum power generation, will be more precise, if and only if, we able to eliminate or reduce the internal resistance in a MFC system.

The ² Factorial work was carried out, in two levels of high and low. The analysis of variance (ANOVA) for MFCs’ power densities were done as to determine the significance of the designed model as shown in Table 3. The F-values is the indication whether the regression equation significant or not (Samad and Zainol, 2017). F-values can be obtained by dividing the sum of squares (SS) with degree of freedom (df) for each factor. From the Table 3, 21.63 is the F-value for this model with the p-value of 0.006 with a percentage of 99% confident that the model is significant. Any model that has percentage F-distribution of above 95%, that model is consider a significant model with the percentage of 5% to null hypothesis (Anderson and Whitcomb, 2007). The statistically significant factors for this model is

Figure 3. Microbial fuel (MCF) cell’s power densities of all parameters.
A, BC and ABC in MFC performance, and the non-significant factors in the model is B, C, AB, and AC. The non-significant factors in the model will be the residuals that be used as an optimality criterion (Anderson and Whitcomb, 2007).

From the ANOVA, the value of coefficient of determination (R²) is calculated. R² is used to study the relationship between the factors used and responses from each parameter, and also to understand the level and effect of each independent parameter (Kala et al., 2016). It is suggested by Karazhiyan et al. that a good fitting model must have a large R² and not least then 80% (Karazhiyan et al., 2011). The R² and adjusted-R² value for this model is satisfactory with the coefficient value of 0.9419 and 0.8984, respectively. The effect of the factors on responses was predicted by using the standard mathematical regression equation [Equation (4)] as follows:

\[ Y = 18.16 - 9.42A - 9.60B + 12.28ABC \]   
**Equation (4)**

where Y is response of MFC’s power density. A represents bacteria’s broth. B represents pH controller and C represents resistor as shown in Table 1. The factors A, B and C are the main effects while AB, AC, BC and ABC are the interaction effects of the model (Anderson and Whitcomb, 2007).

The Pareto chart is very helpful in showing the relative size of effects and easily determining the most significant parameters in the model. In Pareto chart, t-values are used to increase the eligibility of the chart. The t-values can be obtained by square root the F-value. There are actually two limit lines shown in Pareto chart which are the Bonferroni limit line, which is a stricter level of 0.025 by dividing p-value of 0.05 by 2, and t-value limit line with the critical t-value of 2.776 and 5.068, respectively (Anderson and Whitcomb, 2007). Figure 4, shows the Pareto chart of the model and it is clear that the factor ABC, BC and A exceed the critical t-value limit line which indicates significant effects for MFC’s power density specificallt for this model.

Among the three factors that exceed the t-value, only one factor exceeding the Bonferroni limit which is ABC with the value of 5.432, while BC is the second highest with the value of 4.246. A is the highest and only main factor that exceed the t-value limit line with the value 4.167. In this model, interaction between all factors, which are bacteria’s broth, pH controller and external resistance, is proven plays a crucial role in MFC’s performance.

### Table 3. Test of Significance for Regression Coefficient

<table>
<thead>
<tr>
<th>Source</th>
<th>Coefficient</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F-value</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>18.16</td>
<td>2 653.6</td>
<td>884.52</td>
<td>21.63</td>
<td>0.006</td>
</tr>
<tr>
<td>A</td>
<td>-9.42</td>
<td>709.9</td>
<td>709.9</td>
<td>17.36</td>
<td>0.014</td>
</tr>
<tr>
<td>BC</td>
<td>-9.60</td>
<td>737.3</td>
<td>737.3</td>
<td>18.03</td>
<td>0.013</td>
</tr>
<tr>
<td>ABC</td>
<td>12.28</td>
<td>1 206.4</td>
<td>1 206.4</td>
<td>29.51</td>
<td>0.006</td>
</tr>
<tr>
<td>Residual</td>
<td>-</td>
<td>163.5</td>
<td>40.88</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cor Total</td>
<td>-</td>
<td>2 817.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 4. Pareto chart showing a relative effect of factors on microbial fuel cell’s (MFC) performance.
MFC’s study on the type of bacteria broth used in MFC system is currently not available, but the bacteria broth that were used in this work, promote different growth of bacteria community (Sottile and Zabransky, 1977) and also reported by Logan et al. that the most researcher choose their media carefully to culture their bacteria at optimal level (Logan, 2008). The growth of a better exoelectrogens community in TSB media made bacteria’s broth a significant factor in this model. BC outcome in this model is as predicted because both factors are determining the growth of bacteria’s community in the MFC system (Jadhav and Ghangrekar, 2009). The Ca(OH)₂ is better than Na(OH) at maintaining the pH value and keeping the bacteria in its optimal pH condition (Fernandes et al., 2009). Resistance of 200 Ω given a better output than its higher level of factorial design which also aligned with Jadhav et al. study as they stated that a lower resistance produced a higher current generation (Jadhav and Ghangrekar, 2009). Although, A and BC exceed the t-value critical limit line, but ABC, that exceed both t-value and Bonferroni limit line, plays much bigger role in this model. This is proven that all factors are important in enhancing the performance of MFC’s system. From this model, we can conclude that the optimal parameters for MFC’s performance is TSB bacteria broth, Ca(OH)₂, as pH controller and 200 Ω of external resistance.

Part 2: COD Reduction

Effect of pH to COD reduction using DMP and nDMP treated by MFC devices. In Figure 5, DMP 8.0 is the highest net COD reduction treated by MFC devices in percentages and mg litre⁻¹ for DMP, that recorded 25.45% reduction that equal to 28 500 mg litre⁻¹ and it was 6.70% higher than the calibrate DMP 8.0. It was followed by treated DMP 5.5 with 16.52% reduction that equal to 18 500 mg litre⁻¹ which was 6.69% higher than the calibrate DMP 5.5 and treated DMP 6.8 with 12.05% reduction that equal to 13 500 mg litre⁻¹ and this was 4.47% higher than calibrate DMP 6.8 as shown in Table 4. From Table 5, the highest net COD reduction treated by MFC devices in percentages and mg litre⁻¹ for nDMP was with nDMP 5.5 that recorded 42.36% reduction that equal to 122 000 mg litre⁻¹ and it was 20.48% higher than the calibrate DMP 5.5. It than followed by nDMP 6.8 with 30.56% reduction that equal to 88 000 mg litre⁻¹ that was 29.17% higher than the calibrate DMP 6.8 and nDMP 8.0 with 13.19% reduction that equal to 38 000 mg litre⁻¹ and it was 11.45% higher than the calibrate DMP 8.0. Overall, the COD reduction recorded for MFC treated DMP and nDMP substrates indicate higher COD reduction as compared to the calibrate DMP and nDMP substrates. This was in accordance with previous work that MFC has the ability to treat POME by deducting the COD level as they were using natural microflora and isolated pure culture bacteria from anaerobic POME sludge in the MFC system (Md Nor et al., 2015).

The main pathways of anaerobic digestion involved four stages which usually start with hydrolysis than followed by acidogenesis, acetogenesis and methanogenesis. If considered the fermentation pathways were done in the two-stage anaerobic digestion system, where the first
stage by hydrolysis and acidogenesis stages in acidogenic reactor than followed by acetogenesis and methanogenesis stages in methanogenic reactor, therefore the total COD reduction was calculated by adding COD reduction by acidogenesis, acetogenesis and methanogenesis (MC). If considered that the stage of acetogenesis and methanogenesis cohabit (AC) and digested the same substrate, therefore the higher COD reductions between the two pathways were used in the calculation, which was reported by Cheng et al. (2010). In Figure 5, the total calculated percentages of COD reduction for DMP treated by MFC devices for pH 5.5 ± 0.2, pH 6.8 ± 0.2 and pH 8.0 ± 0.2 was between 41.97% to 54.02% and these readings were higher than DMP untreated by MFC devices which was between 30.80% to 36.16%.

On the other hand, there was an increase of 11.17% to 17.86% COD reduction for DMP between untreated and treated by MFC devices. The total calculated percentages of COD reduction for nDMP treated by MFC devices for pH 5.5 ± 0.2, pH 6.8 ± 0.2 and pH 8.0 ± 0.2 was between 42.36% to 61.10% and these readings were higher than nDMP untreated by MFC devices which was between 23.62% to 25.01%. There was an increase of 49.30% to 61.10% COD reduction for nDMP between untreated and treated by MFC devices as shown in Figure 5. The trends of COD reductions were also observed in the previous report where they used a two-stage MFC system integrated with immobilised biological aerated filters to treat POME. They found that by combining the hydrolysis and acidogenesis stages in acidogenic reactor than followed by acetogenesis and methanogenesis stages in methanogenic reactor enhanced the rate of COD reduction in MFC system (Cheng et al., 2010).

The COD effective reduction efficiency by MFC device. All the above recorded data does not reflect the true COD effective reduction by MFC devices. There were COD reduction processes done by the existing microorganism in the substrates that was not subjected to MFC devices which also contribute to the COD reduction. The percentages of MFC treated samples of nDMP and DMP were relatively higher than the calibrated sample. Therefore, the difference in these two percentages of COD reduction between the MFC devices and the calibrated sample were considered as the percentages of COD effective reduction by MFC, showing the true percentages of COD reduction by MFC devices (Table 5). This finding cannot be compared to the available reports due to the different approach of columbic efficiency method (Ghasemi et al., 2011; 2012; Hassan et al., 2014; Xing et al., 2015). Columbic efficiency is a common approach taken by other MFC’s researchers to study the effectiveness of redox reaction during electrochemical reaction. Most researchers included the columbic efficiency to determine the performance of the MFC’s device but this article only determines the MFC optimisation. The reason why the columbic efficiency was not included is due to the different approach used and it is also not included in the objectives of the experiment.

The highest percentages of COD effective reduction by MFC treated sample of nDMP were 29.17% for nDMP 6.8, while nDMP 5.5 recorded 20.48% and 11.45% for by nDMP 8.0 were relatively...

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**TABLE 4. CHEMICAL OXYGEN DEMAND (COD) EFFECTIVE REDUCTION EFFICIENCY OF MICROBIAL FUEL CELL (MFC) WITH DMP AT DIFFERENT pH VALUES**

<table>
<thead>
<tr>
<th>pH of 5.5±0.2</th>
<th></th>
<th>pH of 6.8±0.2</th>
<th></th>
<th>pH of 8.0±0.2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>MFC</td>
<td>Calibrate</td>
<td>MFC</td>
<td>Calibrate</td>
<td>MFC</td>
<td>Calibrate</td>
</tr>
<tr>
<td>Before MFC treatment (mg litre⁻¹)</td>
<td>112 000</td>
<td>112 000</td>
<td>112 000</td>
<td>112 000</td>
<td>112 000</td>
</tr>
<tr>
<td>After MFC treatment (mg litre⁻¹)</td>
<td>93 500</td>
<td>98 500</td>
<td>98 500</td>
<td>106 000</td>
<td>83 500</td>
</tr>
<tr>
<td>Net COD reduction (mg litre⁻¹)</td>
<td>18 500</td>
<td>13 500</td>
<td>13 500</td>
<td>6 000</td>
<td>28 500</td>
</tr>
<tr>
<td>% of COD reduction</td>
<td>16.52</td>
<td>12.05</td>
<td>12.05</td>
<td>5.36</td>
<td>25.45</td>
</tr>
<tr>
<td>% COD effective reduction of MFC</td>
<td>4.47</td>
<td>-</td>
<td>6.69</td>
<td>-</td>
<td>6.70</td>
</tr>
</tbody>
</table>

**TABLE 5. CHEMICAL OXYGEN DEMAND (COD) EFFECTIVE REDUCTION EFFICIENCY OF MICROBIAL FUEL CELL (MFC) WITH nDMP AT DIFFERENT pH VALUES**

<table>
<thead>
<tr>
<th>pH of 5.5±0.2</th>
<th></th>
<th>pH of 6.8±0.2</th>
<th></th>
<th>pH of 8.0±0.2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>MFC</td>
<td>Calibrate</td>
<td>MFC</td>
<td>Calibrate</td>
<td>MFC</td>
<td>Calibrate</td>
</tr>
<tr>
<td>Before MFC treatment (mg litre⁻¹)</td>
<td>288 000</td>
<td>288 000</td>
<td>288 000</td>
<td>288 000</td>
<td>288 000</td>
</tr>
<tr>
<td>After MFC treatment (mg litre⁻¹)</td>
<td>166 000</td>
<td>225 000</td>
<td>200 000</td>
<td>284 000</td>
<td>250 000</td>
</tr>
<tr>
<td>Net COD reduction (mg litre⁻¹)</td>
<td>122 000</td>
<td>63 000</td>
<td>88 000</td>
<td>4 000</td>
<td>38 000</td>
</tr>
<tr>
<td>% of COD reduction</td>
<td>42.36</td>
<td>21.88</td>
<td>30.56</td>
<td>1.39</td>
<td>13.19</td>
</tr>
<tr>
<td>% COD effective reduction of MFC</td>
<td>20.48</td>
<td>-</td>
<td>29.17</td>
<td>-</td>
<td>11.45</td>
</tr>
</tbody>
</table>

Note: nDMP – nitrogen deoxygenated POME mixture.
higher than MFC treated sample of DMP which were 6.7% and 6.69% for DMP 8.0 and DMP 6.8 respectively but only 4.46% for DMP 5.5 as shown in Figure 6.

The highest percentage of COD effective reduction by MFC sample from nDMP group is nDMP 6.8 which is 29.17%, and the percentages of nDMP 5.5’s and nDMP 8.0’s effective COD reduction were 20.48% and 11.45%, respectively. As for DMP group, the percentages of effective COD reduction done by DMP 5.5, DMP 6.8 and DMP 8.0 were 4.47%, 6.69% and 6.70%, respectively. For DMP, the percentage of COD Effective Reduction by MFC was between 4.47% to 6.70%, while for nDMP was between 11.45% to 29.17%. In this work, nDMP substrates show a better COD reduction than DMP. These were in line with the conclusions from previous study that the current generations by MFC accelerated the COD reduction (Zhang et al., 2015).

In Figure 6, if the MFC devices operation were applied as a two-stage anaerobic digestion system as mentioned earlier, the total calculated percentages of COD reduction for nDMP treated by MFC devices for pH 5.5 ± 0.2, pH6.8 ± 0.2 and pH8.0 ± 0.2 were between 49.30% to 61.10% and these reading were higher compared to DMP by MFC devices which was between 11.17% to 17.86%. Similar application was observed in (Cheng et al., 2010).

## CONCLUSION

In conclusion, the experimental result (Part 1) shows that the best interaction between these three factors is (---) interaction which is the interaction between TSB broth, Ca(OH)₂ as pH controller and resistant of 200 Ω, with the effect value of 24.56, and this interaction yields the power density of 57.44 mAm⁻². The DOE model of this work is found to be significant with the F-value of 21.63. The most significant parameter of this work is ABC with the F-value of 24.56 which exceed both Bonferroni and critical t-value limit line in the Pareto chart (Part 2). Two conclusions can be drawn in this work. Firstly,
DMP produced a relatively lower percentage of COD effective deduction efficiency compared to nDMP. Secondly, the percentage COD effective deduction efficiency treatment by MFC devices using nDMP was better than DMP. The nDMP was 342% to 441% more efficient to deduct COD compare to DMP. The nDMP 6.8 recorded the most effective COD deduction by MFC devices at 29.17% although nDMP 5.5 recorded a higher COD deduction than nDMP 6.8 but its COD effective deduction efficiency was only at 20.48%.

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