***Original Research Article***

# Bio-Electrochemical Reactor: Inoculated H-type Microbial Fuel Cell for Wastewater Treatment and Energy Recovery


## ABSTRACT

Microbial fuel cells (MFCs) are bio-electrochemical devices that are capable of transforming chemical energy stored in waste organic matter into direct electrical energy through catalytic activity of microorganisms under anaerobic conditions. This study investigated that the bio-electrical performance of H-type or dual-chamber microbial fuel cell (DCMFC) fueled with brewery wastewater served as an electron donor inoculated with distillery plant waste from working biogas reactor as a source of microorganisms to run the experiment. From the experimental results, 1150mV maximum voltage output, 92.85%, 91.40%, 68.87%, and 70.10% removal efficiencies of COD, BOD, TN and TP respectively were obtained at 35ºC, pH 7, and 5 days. These results confirmed that brewery wastewater effectively treated would generate a considerable amount of direct bio-electricity. Results also revealed that the DCMFC provides an alternative insight into an effective treatment of wastewater that can simultaneously generate a direct bio-electricity in a sustainable and eco-friendly manner.



*Keywords:Biofilm, Electrogenic bacteria, Energy recovery, Inoculation, Microbial fuel cell, Renewable energy*

## 1. INTRODUCTION

The depletion of global fossil fuel reserve makes it necessary to develop alternative technologies for energy production, not just employing traditional renewable sources (solar, wind, etc.), but also tapping in nonconventional ones, such as wastes of different origin and nature. “Renewable bioenergy from wastes, presenting a neutral or even negative carbon footprint, is also viewed as one of the ways to alleviate the current global warming crisis” [1].

“Microbial fuel cells (MFCs) represent one of the most promising technologies of recovering electrical energy from wastewater which are not exploited and dumped in landfills while simultaneously treating the wastewater” [2]. “The MFCs are considered as one of the Bio Electrochemical Reactors (BERs), which essentially based on the ability of “electrogenic” or “electroactive” bacteria to exchange electrons with the anode through developing effective anodic biofilm” [3]. The addition of biological organisms responsible for catalyzing electrochemical reactions, gives these systems a level of complexity that is perhaps above that of already complex electrochemical systems (e.g. batteries, fuel cells and supercapacitors). The main differences of MFCs with the conventional low temperature fuel cells (direct methanol fuel cell or proton exchange membrane fuel cell) are: i) “the electrocatalyst is biotic (electroactive bacteria or proteins) at the anode”[4-6]; ii) “the temperature can range between 15 ℃ and 45 ℃, with close to ambient levels as optimum [7-9]”; iii) “neutral pH working conditions”[10-13]; iv) “utilization of complex biomass (often different types of waste or effluent) as anodic fuel”[14,15]; v) “a promising moderate environmental impact assessed through life cycle analysis”[16,17].

This study investigated that the bio-electrical performance of H-type or dual-chamber microbial fuel cell (DCMFC) fueled with brewery wastewater as an electron donor, inoculated with distillery plant waste from working biogas reactor as a source of microorganisms to run the experiment. The linear effect of process variables such as pH, time, and temperature on the responses has been investigated by keeping other variables constant. Seventeen dual-chamber microbial fuel cells (DCMFCs) were designed as adopted from [18], thirty four polyethylene (PE)cylinders of equal volume (600 ml) and height (20cm) were used. Two PE cylinders were used for one MFC set up in which one was used as a cathode and the other as an anode. A side opening of 2cm diameter at a height of 8cm from the bottom of the bottle and 2mm diameter head opening was made on each cylinder for the insertion of salt bridge and copper wire respectively.The MFCs were operated in a batch mode according to the prescribed experimental conditions for 8 days. The time constraint was fixed based on the decline in power generation (open circuit voltage) [19].

In this study, we evaluated the performance of DCMFC in utilizing/using/ Raya Brewery wastewater (RBWW) for a direct bio-electricity generation while simultaneously treating the wastewater. It has been estimated that approximately 4-10 liter of brewery wastewater is generated per liter of beer being produced. “This wastewater is rich in organic content concentrated up to 3000-5000 mg/l ofCOD, which is approximately nine times concentrated than the domestic wastewater” [20-22]. “Consequently, the

wastewater from breweries can pose hazard to human beings and the environment if not well treated before discharge" [23-25]. However, "most of the wastewater treatment technologies employed in the breweries are not sustainable to meet the ever-growing waste sanitation needs, basically because they are energy-intensive processes without any return which discourage the investors" [26]. These problems were also observed in RBWW.

Raya Brewery is one of the largest beer producers in country with an annual productioncapacity of 600,000 hectoliters of beer. The factory generates a large volume of wastewater, which is about 1250$m^3$/day. The existing wastewater treatment plant is an up-flow anaerobic sludge blanket reactor (UASBR) with a treatment capacity of 1500 $m^3$/day and theoutlet effluent from this plant is released into the nearby river. "The plant consumes 660 KWhof electricity per 1250$m^3$ of wastewater, about 50% of the electricity is consumed tosupply air for the aeration basins" [27, 28].

The present article aims to investigate the performance of dual chambered microbial fuel cells using BWW as an electron donor for a direct bio-electricity generation while simultaneously treating the wastewater. The obtained results were compared with the previous studies. The current study clearly demonstrated that the potential of well-prepared MFCs in removing organic matter and other pollutants of interest, as well as producing electricity. Results revealed that DCMFC provides an alternative insight into an effective treatment of wastewater that can simultaneously generate a direct bio-electricity in a sustainable and eco-friendly manner.

## 2. EXPERIMENTALDETAILS

### 2.1. MFC assembly and operation

Seventeen dual-chamber microbial fuel cells(DCMFCs) were designed as adopted from [18] thirtyfour PE cylinders of equal volume (600 ml) and height (20cm) were used. Two cylinders were used for oneMFC set up in which one was used as a cathode and the other as an anode. Apart from opening of2cm diameter at a height of 8cm from the bottom of the cylinder and 2mm diameter head opening was made on each cylinder for the insertion of salt bridge and copper wire respectively.The MFCs were operated in a batch mode according to the prescribed experimental conditions for about 8 days. The time constraint was fixed based on the decline in power generation (open circuit voltage) [19]. "In the anode section of the microbial fuel cell,the microbes (mixed consortia) oxidize the organic substance in the wastewater as a fuel forgrowth, consequently producing electrons and protons via redox reactions, by this means a bio-potential difference (biological mediated voltage) enabling power generation" [29]. Protons passed through the salt bridge to the cathode section consisting of asolution of potassium ferricyanide (electron acceptor) and the electrons produced in the anodesection flow over the carbon rod electrodes which were linked with the copper wire to completethe circuit. After 24h incubation period, the copper wires were connected to a digitalmultimeter using alligator clips. The voltage output was measured and recorded as anopen circuit voltage.

### 2.2. Preparation of Anolyte and Catholyte

Raya Brewery wastewater which contains organic matter accessible for the microorganisms was used as a substrate in the anodic chamber. Besides,50ml of inoculum per anode was taken from a working biogas reactorofDesta Alcohols Distillery plant. “Since, the inoculum contains highly varied bacterial consortia consisting of electrochemically active bacterial strains” [30], it was served as a source of a microorganism to run the experiments. Samples were adjusted at different pH (4, 7, and10) using the prepared standard solution (0.1M HCl, 0.1M NaOH). The anodic chambers of the microbial fuel cells were filled with 410 ml adjusted sample. For the cathode chamber of the microbial fuel cells, 0.1M potassium ferricyanide solution was prepared and the chambers were filled with 460mlof the solutionto serve as a Catholyte (electron acceptor).

### 2.3. Performance analysis of the MFC

The wastewater treatment performances of the MCF weremeasured by the BOD, COD, TN, and TP according to the standard methods [31], before and after each parameter goes through the MFC. The direct bio-electricity generation performance of the MFC was evaluated by measuring the voltage output using advanced digital multimeter (UNI-T UT61B).

### 2.4. Analysis and calculation

The MFC potential was recorded four times a daywith the multimeter. The current and the harvested power were calculated from the following formula [32, 33].

$$I = \frac{V_{MFC}}{R_{ext}} \quad \text{…….(1)}$$

Where $V_{MFC}$ is the measured voltage, $R_{ext}$ is the external load applied. Current density (mA/m$^2$) was calculated from the followed equation [34, 35]:

$$CD = \frac{I}{A} \quad \text{……. (2)}$$

Where I is the current per mA and A is the projected area of the anode (m$^2$). The Power density (PD, mW/m$^2$) was calculated from the followed equation [36]:

$$PD = V_{MFC} \times CD \quad \text{……. (3)}$$

The Columbic efficiency (CE), describes the efficiency of the MFC in facilitating the electrochemical reactions for charge (electrons) transmission, i.e. the current represented in the recovered fraction electrons versus the complete of oxidation of the substrate. The CE was calculated by the followed equations [36-39]:

$$CE = \frac{C_P}{C_T} x100\% \quad \text{..... (4)}$$

$$C_T = \frac{FnΔcV}{M} \quad \text{…… (5)}$$

Where the $C_P$ is the actual current production collected by the anodeduring one batch cycle integrated as ($C_P$ = xt) and the $C_T$is the theoretically available amount of produced coulombs depending on the COD removed in the MFC from the fully oxidationof substrate organic content into $CO_2$ and water. It was estimatedas in formula no.5, where F = faraday’s constant (96485 C/mol),n = number of electrons per

mole of substrate (4 electrons), Δcis the daily COD removed, V is the inner reactor volume per liter,M = molecular weight of $O_2$(32 g/mole).

The COD removal efficiency of the microbial fuel cell was calculated using:

$$\text{Removal ef iciency } (\%) = \frac{COD_{influent} - COD_{effluent}}{COD_{influent}} \ldots\ldots\ldots (6)$$

Where, $COD_{influent}$ isinitial COD concentration (mg/l) and $COD_{effluent}$ is final COD concentration (mg/ l) in the reactor.

## 3. RESULT AND DISCUSSION

### 3.1. Performance of the laboratory scale DCMFC

Table 1 shows the experimentally investigated results of the 17 experimental runs. The results depict that performance of the DCMFC in terms of the voltage output and removal efficiencies for COD, BOD, TN, and TP at each experimental run.

**Table 1.Three-variable with five responses for the process performance of DCMFC**

| | Factors | | | Responses | | | | |
|---|---|---|---|---|---|---|---|---|
| Run | Temp (ºC) | pH | Time (Day) | Voltage Output (mV) | COD Removal (%) | BOD Removal (%) | TN Removal (%) | TP Removal (%) |
| 1 | 45 | 7 | 8 | 794 | 91.81 | 90.41 | 62.10 | 69.00 |
| 2 | 35 | 10 | 2 | 767 | 37.10 | 35.90 | 27.00 | 31.10 |
| **3** | **35** | **7** | **5** | **1150** | **92.85** | **91.40** | **63.20** | **70.10** |
| 4 | 25 | 4 | 5 | 401 | 45.10 | 43.70 | 31.50 | 35.20 |
| 5 | 25 | 7 | 8 | 909 | 90.80 | 89.40 | 60.50 | 67.20 |
| **6** | **35** | **7** | **5** | **1079** | **90.92** | **89.52** | **60.80** | **67.30** |
| 7 | 25 | 10 | 5 | 798 | 53.99 | 52.59 | 36.10 | 40.10 |
| 8 | 25 | 7 | 2 | 823 | 52.82 | 51.41 | 35.10 | 39.20 |
| 9 | 45 | 4 | 5 | 351 | 45.21 | 43.81 | 31.70 | 35.28 |
| 10 | 45 | 7 | 2 | 952 | 54.00 | 52.60 | 36.19 | 40.19 |
| **11** | **35** | **7** | **5** | **1110** | **92.01** | **90.70** | **62.20** | **69.12** |
| **12** | **35** | **7** | **5** | **1106** | **91.12** | **89.70** | **68.87** | **67.40** |
| 13 | 35 | 10 | 8 | 900 | 88.10 | 86.70 | 57.80 | 65.10 |
| **14** | **35** | **4** | **2** | 450 | **34.79** | **33.41** | **26.00** | **30.12** |
| 15 | 35 | 4 | 8 | **302** | 59.00 | 57.70 | 40.10 | 42.60 |
| **16** | **35** | **7** | **5** | **1109** | **91.60** | **90.10** | **62.04** | **68.80** |
| 17 | 45 | 10 | 5 | 814 | 56.96 | 55.56 | 39.10 | 42.10 |

As shownin Table 1, the observed voltage outputs were varied noticeably within the range of 302 mV to 1150mV. The lowest voltage output (302 mV) was obtained on the 15$^{th}$run where the experimental conditions were held at an average temperature, lowest pH and longest residence time (35°C, pH 4 and 8 days). On the other hand, the maximum voltage output of (1150 mV) was obtained on the 3$^{rd}$run, which is the replicate at the experimental conditions at 35°C, pH 7 and in 5 days. This value is higher than what

was reported as a maximum voltage output of 750 mV by [40]. Another study by [41] also reported a maximum voltage output of 950mV, which is still lower than the maximum value obtained in this study. This difference can be result of the type of substrate used in this study: brewery wastewater was used as a substrate that contained higher organic content than soak liquor from the tannery industry and hostel sewage, which were used in the stated studies respectively. On the other hand, the observed value in this study is lower than maximum voltage output of 1480 mV reported by [42]. This difference might be due to reasons such as the type of substrate used, concentration, ionic strength, electrode materialsand the difference in the factors and levels used in the process.

The observed removal efficiencies for COD, BOD, TN, and TP were varied within the range of (34.79% to 92.85%), (33.41% to 91.40%), (26% to 68.87%), and (30.12% to 70.10%) respectively. The lowest removal efficiencies for COD, BOD, TN, and TP were 34.79%, 33.41%, 26.00% and 30.12% respectively where the experimental conditions wereheld at an average temperature, acidic pH and short residence time at35ºC andpH of 4for 2 days. On the other hand, the maximum COD, BOD, TN, and TP removal efficiencieswere 92.85%, 91.40%, 68.87% and 70.10% respectively, where the experimental conditions were held at anaverage value of all the factors considered at35ºC andpH of 7for 5 days as shown in Table 1.

These resultsare comparable to other results reported by [43] where brewery wastewater treatment using microalgae had given removal efficiency of 83.1%, 91.4%, 76 %, and 66.1% of COD, BOD, TN and TP respectively. These valuesconfirm that the effectiveness of wastewater treatment using MFC apart from direct bioelectricity generation. Besides, the organic matter reduction was good enough showing that there was biodegradation which in return indicating high voltage output [44, 45]. This concept is confirmed in this study as a significant amount of voltage was obtained in parallel with organic load reduction.

### 3.2. Factors affecting MFCS performances

So far, performances of laboratory MFCs are still much lower than the ideal performance. “There may be several possible reasons like Microbe type, fuel biomass type and concentration, ionic strength, pH, temperature, time, electrode materials, proton exchange membrane or salt bridge and operation conditions of anode and cathode that have important effect on MFCs”[46].

Linear effect of process variables such as pH, time, and temperature on the responses hasbeen investigated by keeping other variables constant. The interaction effect of processvariables on the responses has been also investigated by two interactive process variables at afixed third variable. Interaction implies that the effect produced by changing the one-factorlevels dependable on the level of the other factor. For the graphical interpretation, the use of three dimensional (3D) response surface plots affected by two interactive variables at a fixed thirdsuggested variable. Thus, in this study, 3D plots were used to show the interactive effectof the variables on the responses and the optimum levels of each variable. Below is thediscussion with possible reasons behind the single and interaction effect of process variableson the responses.

### 3.2.1. Effect of Time

Figure 1 shows the effect of time on the voltage output keeping the temperature and pH atthe center point. As shown in the figure the voltage output is slightly affected by time,as time increases from 2 to 5 days the voltage output slightly increases, whereas operatingbeyond 5 days resulted in a slight decline in voltage output. The best voltage output has beenobserved on the 5$^{th}$ day. This could be for the reason that the substrate and microbes were incontact for an optimal amount of time which might have favored the system to have an acceleratedorganic substance degradation by the microbes, so do a release of proton and electrons in theanodic section. “It was reported that the formation of most conductive biofilm over theelectrode appears after 3 to 5 days”[47-49]. These studies demonstrated that the maximum voltage output is due to the formation of conductivebiofilm which stimulates the oxidation of the organic matter. It is fairly in agreement with thefinding of this study.

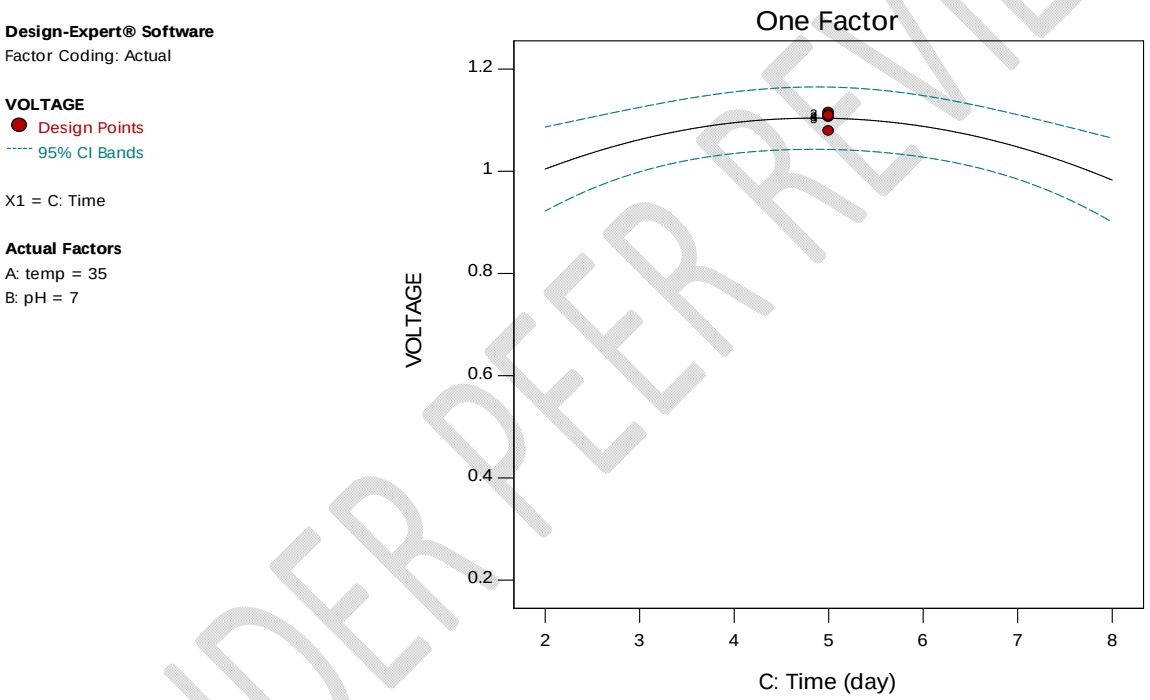


**Figure 1. Effect of time on the voltage output**

### 3.2.2. Effect of pH

“The pH value had a strong effect on MFCs microbial activitywhich was reflected in the overall MFC performance” [50]. Changesin the pH value also affected the metabolism and absorption ofnutrients through influencing the solubility of nutrients, thusaffecting the growth rate of microorganisms. “This is because the fact that any microorganism should live in optimal pH value for its proper/optimal microbial growththat can also be inhibited when the pH environment is below or abovethe appropriate pH value” [51]. Thus,in this study, to investigate the effect of pH on voltage output, the MFC setups were operatedunder different anodic pH, ranging from 4 to 10. At all pH, the MFC setups started voltageoutput soon after the incubation period.

Figure 2 shows the effect of pH on the voltage output holding time and temperature Constant. As shown infigure 1, voltage outputis sensitive to the changes in pH. Hence, it was observed that a sharp increment in voltage output (302 -1150 mV)was recorded when running from acidic to neutral pH and thendecreased gradually to about 850mV in the basic pH. The best performance was observed when operatingat pH 7 as the peak including the highest voltage output (1150mV). The reason could possibly be the existence of a favorable pH for themicrobial metabolic activities which generates proton and electrons. Thus, as the production ofelectrons increased, so does the voltage output. This is in agreement with the different studieswhich showed that optimum condition for microbial activity is set at neutral pH. Likewise,"changes in pH tend the microbes to respond accordingly which can pointedly influence thevoltage output"[52-55].

The voltage outputs were lower at pH 4 and pH 10, this indicates that the microbial catalytic activity is lower at these pH ranges. Comparatively,the lowest voltage outputs were observed in the acidic pH than in the basic pH. "This is becauseoperating at lower pH inhibiting the metabolic activityresulted from the accumulation of excessive protons and therefore drops the voltage output asreported by"[56, 57]. Therefore, it can be concluded that theperformance of MFCs towards the voltage output is extremely dependent on pH, and neutral pH exhibit better performance.

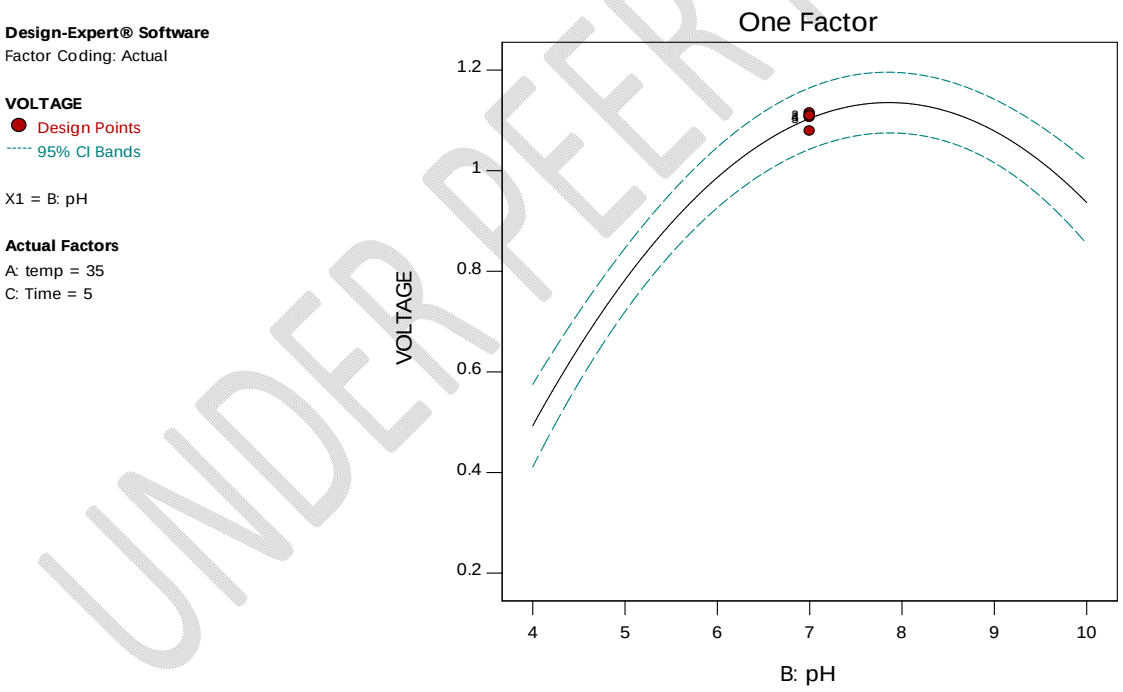


**Figure 2. Effect of pH on the voltage output**

### 3.2.3. Effect of Temperature

Figure 3 shows the effect of temperature on the voltage output keeping the time and pH constant. As shown in the figure the voltage output is slightly affected bytemperature, as the temperature increases from 25ºC to 35ºC, the voltage output slightlyincreases, whereas operating beyond 35ºC resulted in a

slight decline in voltage output. The best performance was observed at 35ºCwhere the maximum voltage outputs of 1150mV wasrecorded. This can be due to the existence of a favorable temperature for the catalytic activityof the microbes. “A report by [58] showed that operating microbial fuel cells ata temperature between 30ºC and 45ºC is optimum to obtain higher voltage outputs”, whichagrees with the finding in this study. Therefore, it can be concluded that the temperature has insignificant effect on thevoltage output but operating at a temperature of 35ºC gives a better result than theother temperature ranges.

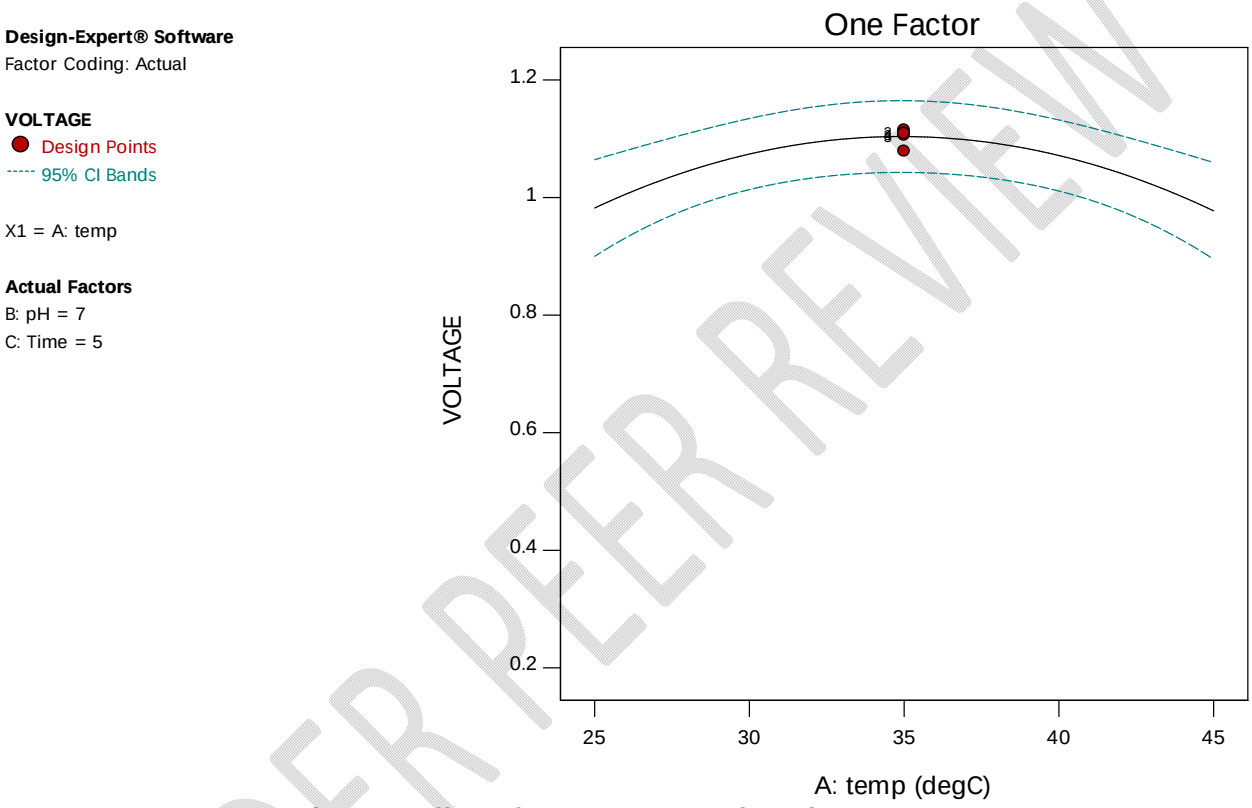


**Figure 3. Effect of temperature on the voltage output**

## 4. CONCLUSION

This study investigated the bio-electrical performance of DCMFC fueled with brewery wastewater as an electron donor and inoculated with distillery plant waste from working biogas reactor as a source of microorganisms to run the experiment. From the experimental results, 1150mV maximum voltage output, 92.85%, 91.40%, 68.87%, and 70.10% removal efficiencies of COD, BOD, TN and TP respectively were obtained at 35ºC, pH 7, and 5 days. These results confirmed that the wastewater has been effectively treated andsignificant amount of direct bio-electricity is generated.This shows findings supported the hypothesis that bacterial heterogeneity of the anode surface is the main responsible factor for MFCsefficiency. The obtained results were compared with the previous literatures and the currentstudydemonstrated that the potential of well-prepared MFCsto remove organic matter and other pollutants of interest, as well as toproduce electricity.Results revealed that DCMFC provides an

alternative insight into an effective treatment of wastewater that can simultaneously generate a direct bio-electricity in a sustainable and eco-friendly manner.

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