

# Potential of Electricity Generation and Wastewater Treatment of Organic Brewery Effluent Using Inoculated H-Type Microbial Fuel Cell

## ABSTRACT

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. Bio-electrochemical systems, such as microbial fuel cells (MFCs), serve as greener alternatives to conventional fuel energy. In recent years, MFCs have drawn science community interest as a method for direct bioelectricity recovery from wastewater while simultaneously treating the wastewater. Moreover; they gain a competitive advantage over other water treatment technologies due to their unique features such as huge energy benefits, less environmental impact, good operating stability, and high economic efficiency. Reports reveal that MFCs are the subject of much interest to researchers, and the number of papers on MFCs in wastewater treatment is increasing. The ever-growing demand for green waste management and renewable sources of energy has enthused research efforts all over the world. This study, therefore, investigated the effect of process variables on the bio-electrical performance of H-type microbial fuel cells fueled with brewery wastewater and inoculated with distillery plant waste. 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 35°C, 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 MFC 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

32 Nonrenewable energy sources, such as fossil fuels and nuclear power, are widely used in the world [1].  
33 When it comes to fossil fuels, this source of energy does more damage to the environment and  
34 continuous use of fossil fuels emits carbon dioxide, which becomes toxic when there is too much of it in  
35 the air. The two major global problems facing human beings are energy shortage and environmental  
36 pollution [2]. Therefore, great efforts have long been exerted in a simultaneous response to both the  
37 energy consumption and water contamination [3]. The wastewater containing pollutants must be treated  
38 before being discharged into the environment [4,5]. At present, wastewater treatment is commonly treated  
39 with a conventional aerobic activated sludge reactor, anaerobic digester, membrane filtration, ion  
40 exchange, adsorption, coagulation, electrolytic reduction and so on [6]. Nevertheless, the high  
41 expenditure of energy and the running cost are the two major restraining factors for the current  
42 wastewater treatment technologies [7]. In addition, the presence of a large amount of residual generation  
43 can lead to secondary pollution among these technologies, which can be deleterious for the environment  
44 and ineffective in catching the energy potential from wastewater [8]. Therefore, it is essential to establish  
45 a wastewater treatment technology, which is required to be reliable, sustainable, and cost-effective [9].

46 Microbial fuel cells (MFCs) are a type of bio-electrochemical fuel cell that requires the presence of active  
47 bacteria that function as biocatalyst for bioenergy generation in anodic chambers [10, 11]. They are  
48 recognized as a future technology with a unique ability to exploit metabolic activities of living  
49 microorganisms for simultaneous conversion of chemical energy into electrical energy. This technology  
50 holds the promise to offer sustained innovations and continuous development towards many different  
51 applications and value-added production that extends beyond electricity generation, such as water  
52 desalination, wastewater treatment, heavy metal removal, bio-hydrogen production, volatile fatty acid  
53 production and biosensors. Compared with other wastewater treatment technologies, MFCs have the  
54 following significant advantages: (1) direct conversion of substrates energy into electricity, (2) low  
55 activated sludge generation, (3) being robust and insensitive to environmental factors (e.g., temperature),  
56 (4) absence of gas treatment, (5) without any energy input for aeration, and (6) a widespread application  
57 in places lacking electrical infrastructures [12,13]. MFCs have proven to have great potential for industrial  
58 applications in several types of wastewater treatment [14]. To date, the number of papers on MFCs in  
59 wastewater treatment is increasing.

60 "MFCs are considered as one of the Bio Electrochemical Reactors (BERs), which essentially based on  
61 the ability of "electrogenic" or "electroactive" bacteria to exchange electrons with the anode through  
62 developing effective anodic biofilm" [15]. The addition of biological organisms responsible for catalyzing  
63 electrochemical reactions, gives these systems a level of complexity that is perhaps above that of already  
64 complex electrochemical systems (e.g. batteries, fuel cells and supercapacitors). The main differences of  
65 MFCs with the conventional low temperature fuel cells (direct methanol fuel cell or proton exchange  
66 membrane fuel cell) are: i) "the electrocatalyst is biotic (electroactive bacteria or proteins) at the anode"  
67 [16-18]; ii) "the temperature can range between 15 °C and 45 °C, with close to ambient levels as optimum  
68 [19-21]"; iii) "neutral pH working conditions" [22-25]; iv) "utilization of complex biomass (often different

69 types of waste or effluent) as anodic fuel” [26,27]; v) “a promising moderate environmental impact  
70 assessed through life cycle analysis” [28,29].

71 The typical MFCs usually consists: (i) anode—oxidation of organic matter takes place, catalyzed by  
72 electroactive bacteria; (ii) cathode—reduction of oxygen or carbon dioxide, a thermodynamically favorable  
73 reaction catalyzed in the presence or absence of catalysts; (iii) ion exchange membrane—a proton  
74 exchange membrane that favors the passage of protons from anode to cathode through simple diffusion;  
75 (iv) electroactive microorganisms—microorganisms with the ability to respire electrodes under anoxic  
76 conditions; (v) biofilm—the colonization of bacteria on the surface of the material; (vi) electric circuit—an  
77 external load where the electrons are passed through a fixed resistor to regulate the flow of electrons. In  
78 the anodic chamber, microbial decomposition (biological oxidation) of organic substrates generate  
79 electrons and protons that are transferred to the cathode through the circuit and membrane, respectively  
80 (Figure 1). By transferring electrons from the negative terminal (anode) to the positive terminal (cathode)  
81 against a load, an electric current is generated [30]. On the other hand, the generated protons drift  
82 over to the cathode through the proton exchange membrane (PEM), which prevents the  
83 movement of oxygen into the anode compartment to avoid the inhibition of electricity  
84 generation. Instead, the cathode exposed to the oxygen initiates the formation of water [31]. On  
85 the cathode surface, the electrons react with the final electron acceptor. Mostly  $O_2$  is applied as electron  
86 acceptor because of its abundance in nature. Recently, air-cathodes, based on the gas-diffusion layers,  
87 are in use, avoiding forced  $O_2$  provision at the cathode. Commonly used Catholyte include oxygen,  
88 ferricyanide, and permanganate [32].

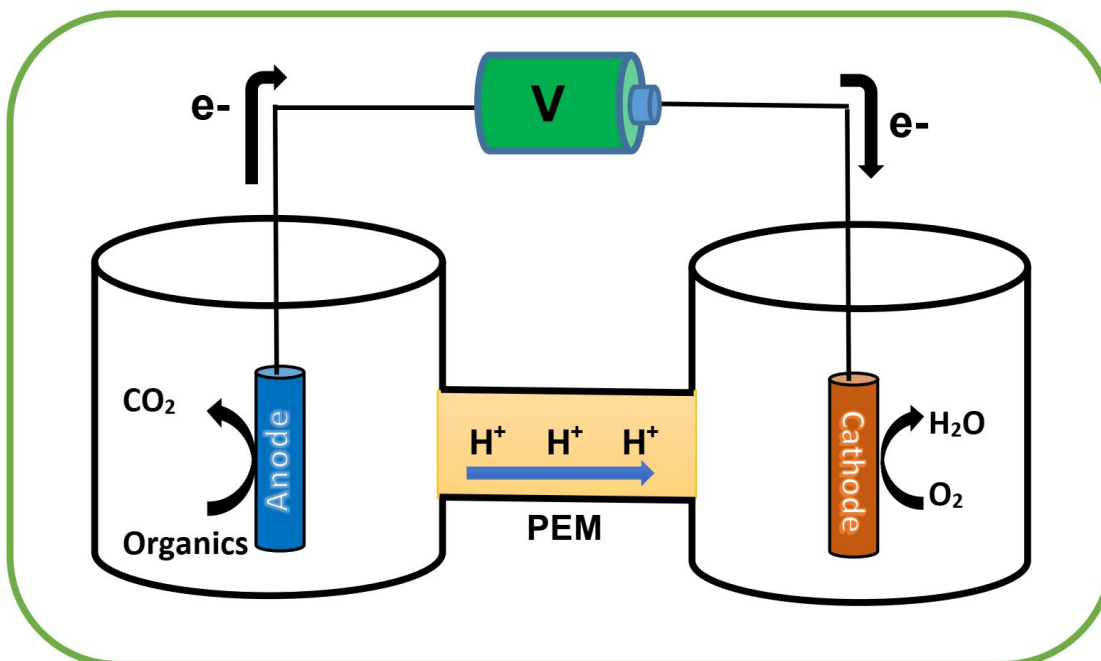
89 In this work, we report the bio-electrical performance of H-type or dual-chamber microbial fuel cell  
90 (DCMFC) fueled with brewery wastewater as an electron donor, inoculated with distillery plant waste from  
91 working biogas reactor as a source of microorganisms to run the experiment. The linear effect of process  
92 variables such as pH, time, and temperature on the responses has been investigated by keeping other  
93 variables constant. Seventeen dual-chamber microbial fuel cells (DCMFCs) were designed as adopted  
94 from [33], thirty four polyethylene (PE) cylinders of equal volume (600 ml) and height (20cm) were used.  
95 Two PE cylinders were used for one MFC set up in which one was used as a cathode and the other as an  
96 anode. A side opening of 2cm diameter at a height of 8cm from the bottom of the bottle and 2mm  
97 diameter head opening was made on each cylinder for the insertion of salt bridge and copper wire  
98 respectively. The MFCs were operated in a batch mode according to the prescribed experimental  
99 conditions for 8 days. The time constraint was fixed based on the decline in power generation (open  
100 circuit voltage) [34]. We evaluate the performance of DCMFC using Raya Brewery wastewater (RBWW)  
101 for direct bio-electricity generation while treating the wastewater. The samples were analyzed for the  
102 selected significant physicochemical characteristics such as BOD, COD, TN, and TP. The concentration  
103 of BOD, COD, TN, and TP in the wastewater was determined according to the standard method [35].

104 It has been estimated that 4-10 liter of brewery wastewater is generated per liter of beer. "This  
105 wastewater is rich in organic content (3000-5000 mg/l of COD), which is approximately nine times  
106 concentrated than the domestic wastewater" [36-38]. "Consequently, the wastewater can pose hazard to  
107 human beings and the environment if not treated before discharge" [39-41]. However, "most of brewery  
108 wastewater treatment technologies are not sustainable to meet the ever-growing waste sanitation needs,  
109 basically because they are energy-intensive processes without any return which discourage the investors"  
110 [42]. Raya Brewery is one of the largest beer producers in country with an annual production capacity of  
111 600,000 hectoliters of beer. The factory generates a large volume of wastewater, which is about  
112 1250m<sup>3</sup>/day. The existing wastewater treatment plant is an up-flow anaerobic sludge blanket reactor  
113 (UASBR) with a treatment capacity of 1500 m<sup>3</sup>/day and the outlet effluent from this plant is released into  
114 the nearby river. "The plant consumes 660 KWh of electricity per 1250m<sup>3</sup> of wastewater, about 50% of the  
115 electricity is consumed to supply air for the aeration basins" [43, 44].

## 116 2. EXPERIMENTAL DETAILS

### 117 2.1. MFC assembly and operation

118 Seventeen dual-chamber microbial fuel cells (DCMFCs) were designed as adopted from [36] thirty four  
119 PE cylinders of equal volume (600 ml) and height (20cm) were used. The MFCs consists of an anode and  
120 cathode, connected by an external circuit and separated in different compartments by a proton exchange  
121 membrane (PEM). The MFCs were operated in a batch mode according to the prescribed experimental  
122 conditions for about 8 days. The time constraint was fixed based on the decline in power generation  
123 (open circuit voltage) [37]. "In the anode section of the microbial fuel cell, the microbes (mixed consortia)  
124 oxidize the organic substance in the wastewater as a fuel for growth, consequently producing electrons  
125 and protons via redox reactions, by this means a bio-potential difference (biological mediated voltage)  
126 enabling power generation" [45]. Protons passed through the salt bridge to the cathode section consisting  
127 of a solution of potassium ferricyanide (electron acceptor) and the electrons produced in the anode  
128 section flow over the carbon rod electrodes which were linked with the copper wire to complete the circuit.  
129 After 24h incubation period, the copper wires were connected to a digital multimeter using alligator clips.  
130 The voltage output was measured and recorded as an open circuit voltage.



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**Fig. 1. PEM mediated Dual-Chamber MFC**

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## 2.2. Preparation of Anolyte and Catholyte

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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 reactor of Desta Alcohols Distillery plant. "Since, the inoculum contains highly varied bacterial consortia consisting of electrochemically active bacterial strains" [46], it was served as a source of a microorganism to run the experiments. Samples were adjusted at different pH (4, 7, and 10) 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 460 ml of the solution to serve as a Catholyte (electron acceptor).

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## 2.3. Performance analysis of the MFC

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The wastewater treatment performances of the MFC were measured by the BOD, COD, TN, and TP according to the standard methods [47], 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).

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## 2.4. Analysis and calculation

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In every electrical or electronic system, the notion of current density is crucial. The current density determines the power output and efficiency of any circuit. The power density that an MFC can typically generate is low. Therefore, the MFC output voltage and power must be increased for practical uses. So

152 far, several MFCs were simply connected in series or in parallel to overcome the low voltage or power  
 153 issue. Columbic efficiency is also the efficiency with which electrons are transferred in a system to carry  
 154 out an electrochemical reaction. This is an important measure of the microbial fuel cell efficiency as it  
 155 measures the number of coulombs recovered as electrical current.

156 The MFC potential was recorded four times a day with the multimeter. The current and the harvested  
 157 power were calculated from the following formula [48, 49].

158 
$$I = \frac{V_{MFC}}{R_{ext}} \quad \dots\dots\dots (1)$$

159 Where  $V_{MFC}$  is the measured voltage,  $R_{ext}$  is the external load applied. Current density ( $\text{mA}/\text{m}^2$ ) was  
 160 calculated from the followed equation [50, 51]:

161 
$$CD = \frac{I}{A} \quad \dots\dots\dots (2)$$

162 Where  $I$  is the current per mA and  $A$  is the projected area of the anode ( $\text{m}^2$ ). The Power density (PD,  
 163  $\text{mW}/\text{m}^2$ ) was calculated from the followed equation [52]:

164 
$$PD = V_{MFC} \times CD \quad \dots\dots\dots (3)$$

165 The Columbic efficiency (CE), describes the efficiency of the MFC in facilitating the electrochemical  
 166 reactions for charge (electrons) transmission, i.e. the current represented in the recovered fraction  
 167 electrons versus the complete of oxidation of the substrate. The CE was calculated by the followed  
 168 equations [53-55]:

169 
$$CE = \frac{C_P}{C_T} \times 100\% \quad \dots\dots\dots (4)$$

170 
$$C_T = \frac{Fn\Delta cV}{M} \quad \dots\dots\dots (5)$$

171 Where the  $C_P$  is the actual current production collected by the anode during one batch cycle integrated as  
 172 ( $C_P = xt$ ) and the  $C_T$  is the theoretically available amount of produced coulombs depending on the COD  
 173 removed in the MFC from the fully oxidation of substrate organic content into  $\text{CO}_2$  and water. It was  
 174 estimated as in formula no.5, where  $F$  = faraday's constant (96485 C/mol),  $n$  = number of electrons per  
 175 mole of substrate (4 electrons),  $\Delta c$  is the daily COD removed,  $V$  is the inner reactor volume per liter,  $M$  =  
 176 molecular weight of  $\text{O}_2$  (32 g/mole).

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

178 
$$\text{Removal efficiency (\%)} = \frac{\text{COD}_{\text{influent}} - \text{COD}_{\text{effluent}}}{\text{COD}_{\text{influent}}} \quad \dots\dots\dots (6)$$

179 Where,  $\text{COD}_{\text{influent}}$  is initial COD concentration (mg/l) and  $\text{COD}_{\text{effluent}}$  is final COD concentration (mg/l) in  
 180 the reactor.

### 181 3. RESULT AND DISCUSSION

#### 182 3.1. Performance of the laboratory scale DCMFC

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

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

Run	<u>Factors</u>			<u>Responses</u>				
	Temp (°C)	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
<b>3</b>	<b>35</b>	<b>7</b>	<b>5</b>	<b>1150</b>	<b>92.85</b>	<b>91.40</b>	<b>63.20</b>	<b>70.10</b>
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
<b>6</b>	<b>35</b>	<b>7</b>	<b>5</b>	<b>1079</b>	<b>90.92</b>	<b>89.52</b>	<b>60.80</b>	<b>67.30</b>
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
<b>11</b>	<b>35</b>	<b>7</b>	<b>5</b>	<b>1110</b>	<b>92.01</b>	<b>90.70</b>	<b>62.20</b>	<b>69.12</b>
<b>12</b>	<b>35</b>	<b>7</b>	<b>5</b>	<b>1106</b>	<b>91.12</b>	<b>89.70</b>	<b>68.87</b>	<b>67.40</b>
13	35	10	8	900	88.10	86.70	57.80	65.10
<b>14</b>	<b>35</b>	<b>4</b>	<b>2</b>	450	<b>34.79</b>	<b>33.41</b>	<b>26.00</b>	<b>30.12</b>
15	35	4	8	<b>302</b>	59.00	57.70	40.10	42.60
<b>16</b>	<b>35</b>	<b>7</b>	<b>5</b>	<b>1109</b>	<b>91.60</b>	<b>90.10</b>	<b>62.04</b>	<b>68.80</b>
17	45	10	5	814	56.96	55.56	39.10	42.10

187

188 As shown in Table 1, the observed voltage outputs were varied noticeably within the range of 302 mV to  
189 1150 mV. The lowest voltage output (302 mV) was obtained on the 15<sup>th</sup> run where the experimental  
190 conditions were held at an average temperature, lowest pH and longest residence time (35°C, pH 4 and 8  
191 days). On the other hand, the maximum voltage output of (1150 mV) was obtained on the 3<sup>rd</sup> run, which is  
192 the replicate at the experimental conditions at 35°C, pH 7 and in 5 days. This value is higher than what  
193 was reported as a maximum voltage output of 750 mV by [56]. Another study by [57] also reported a  
194 maximum voltage output of 950 mV, which is still lower than the maximum value obtained in this study.  
195 This difference can be result of the type of substrate used in this study: brewery wastewater was used as  
196 a substrate that contained higher organic content than soak liquor from the tannery industry and hostel  
197 sewage, which were used in the stated studies respectively. On the other hand, the observed value in this  
198 study is lower than maximum voltage output of 1480 mV reported by [58]. This difference might be due to  
199 reasons such as the type of substrate used, concentration, ionic strength, electrode materials and the  
200 difference in the factors and levels used in the process.

201 The observed removal efficiencies for COD, BOD, TN, and TP were varied within the range of (34.79% to  
202 92.85%), (33.41% to 91.40%), (26% to 68.87%), and (30.12% to 70.10%) respectively. The lowest  
203 removal efficiencies for COD, BOD, TN, and TP were 34.79%, 33.41%, 26.00% and 30.12% respectively  
204 where the experimental conditions were held at an average temperature, acidic pH and short residence  
205 time at 35°C and pH of 4 for 2 days. On the other hand, the maximum COD, BOD, TN, and TP removal

206 efficiencies were 92.85%, 91.40%, 68.87% and 70.10% respectively, where the experimental conditions  
 207 were held at an average value of all the factors considered at 35°C and pH of 7 for 5 days as shown in  
 208 Table 1.

209 These results are comparable to other results reported by [59] where brewery wastewater treatment using  
 210 microalgae had given removal efficiency of 83.1%, 91.4%, 76 %, and 66.1% of COD, BOD, TN and TP  
 211 respectively. These values confirm that the effectiveness of wastewater treatment using MFC apart from  
 212 direct bioelectricity generation. Besides, the organic matter reduction was good enough showing that  
 213 there was biodegradation which in return indicating high voltage output [60, 61]. This concept is confirmed  
 214 in this study as a significant amount of voltage was obtained in parallel with organic load reduction.

### 215 3.2. Statistical Analysis of the Experimental Results

216 The statistical software program used to develop a model equation that describes the significance of the  
 217 independent variables, interaction effects of the independent variables, and surface plots using the fitted  
 218 equation obtained from the regression analysis. The suggested model that fits the data for this analysis  
 219 was a quadratic model. Analysis of variance is a vital tool to check the adequacy of the quadratic model.  
 220 Checking the adequacy of the fitted model is necessary to confirm that it provides an adequate  
 221 approximation to the true system and supports the least square regression assumptions. Thus, the  
 222 adequacy of the fitted model was evaluated from the coefficients of correlation as summarized in Table 2.

223 **Table 2: Model fit summary statistics**

Parameters	Responses				
	Voltage (V)	COD removal (%)	BOD removal (%)	TN removal (%)	TP removal (%)
Std. Dev.	0.0164	1.01	0.9864	2.62	2.10
Mean	0.8106	68.71	67.33	47.08	51.76
C.V. %	2.02	1.47	1.47	5.56	4.06
R <sup>2</sup>	0.9985	0.9991	0.9992	0.9871	0.9926
Adjusted R <sup>2</sup>	0.9965	0.9980	0.9981	0.9705	0.9830
Predicted R <sup>2</sup>	0.9851	0.9901	0.9909	0.9491	0.9009
Adeq, Precision	63.7724	74.7833	76.4545	19.3369	25.3826

224 The coefficient of variance (CV), which is the ratio of the standard error of the estimate to the mean value  
 225 of the observed response is a measure of reproducibility of the model. As a rule, a model can be  
 226 considered a reasonably adequate model as its CV is less than 10% [62]. In this case, the CV is 2.02%,  
 227 1.47%, 1.47 %, 5.56%, and 4.06% for voltage output, COD removal, BOD removal, TN removal, and TP  
 228 removal, respectively which indicates that the developed model is adequate.

229 The regression coefficient (R<sup>2</sup>) shows how much of the difference in the outcome is explained by the  
 230 model which is useful for checking the adequacy of a model. The regression coefficient value is in a

231 range between 0 and 1, and as it approaches 1.0 it fits well with the experimental data otherwise it  
232 indicates the inadequacy of model approximation. So, the model was found to be a highly significant  
233 model since the  $R^2$  (0.9985, 0.9991, 0.9992, 0.9871, and 0.9926) value of all the responses is closer to  
234 1.0. This means, 99.85% of the total variation in the voltage output is attributed to the experimental  
235 variables studied, or in another term, only 0.15 % of the variation was left unexplained by the model in the  
236 case of voltage output. Similarly, the  $R^2$  for other responses such as COD removal, BOD removal, TN  
237 removal, and TP removal is 99.91 %, 99.92 %, 98.71 %, and 99.26 % respectively.

238 The predicted  $R^2$  is the measure of the extent to which this developed model can be used to predict  
239 ranges of data this study has not considered, which, therefore, the difference between the predicted  $R^2$   
240 and adjusted  $R^2$  should be less than 0.2 [62]. Accordingly, the obtained predicted  $R^2$  (0.9851, 0.9901,  
241 0.9909, 0.9491, and 0.9009) were in reasonable agreement with their adjusted  $R^2$  (0.9965, 0.9980,  
242 0.9981, 0.99705, and 0.9830) for voltage output, COD removal, BOD removal, TN removal, and TP  
243 removal, respectively, because the difference was found less than 0.2. So, the model is adequate to  
244 predict the ranges of data this study has not considered since the model had 98.51%, 99.01%, 99.09%,  
245 94.91%, and 90.09% precision in fitting to all ranges of data. Besides, the adequacy of precision  
246 measures the signal to disturbance ratio due to random error. A ratio greater than 4 is desirable [62]. In  
247 this case, the ratio of all the parameters was found to be greater than 4 which indicates an adequate  
248 signal.

### 249 **3.3. Factors affecting MFCS performances**

250 So far, performances of laboratory MFCs are still much lower than the ideal performance. "There may be  
251 several possible reasons like Microbe type, fuel biomass type and concentration, ionic strength, pH,  
252 temperature, time, electrode materials, proton exchange membrane or salt bridge and operation  
253 conditions of anode and cathode that have important effect on MFCs" [63].

254 Linear effect of process variables such as pH, time, and temperature on the responses has been  
255 investigated by keeping other variables constant. The interaction effect of process variables on the  
256 responses has been also investigated by two interactive process variables at a fixed third variable.  
257 Interaction implies that the effect produced by changing the one-factor levels dependable on the level of  
258 the other factor. For the graphical interpretation, the use of three dimensional (3D) response surface plots  
259 affected by two interactive variables at a fixed third suggested variable. Thus, in this study, 3D plots were  
260 used to show the interactive effect of the variables on the responses and the optimum levels of each  
261 variable. Below is the discussion with possible reasons behind the single and interaction effect of process  
262 variables on the responses.

#### 263 **3.3.1. Effect of Time**

264 Figure 2 shows the effect of time on the voltage output keeping the temperature and pH at the center  
265 point. As shown in the figure the voltage output is slightly affected by time, as time increases from 2 to 5  
266 days the voltage output slightly increases, whereas operating beyond 5 days resulted in a slight decline in

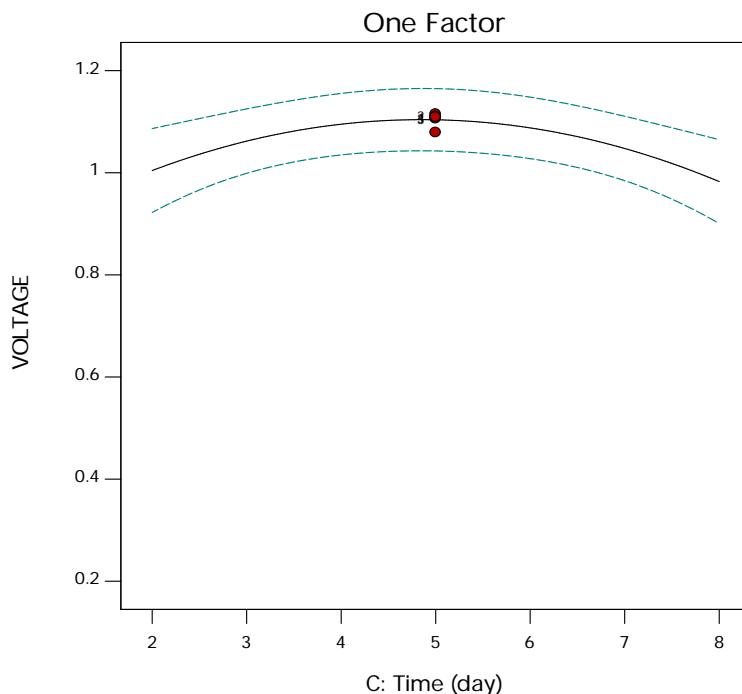
267 voltage output. The best voltage output has been observed on the 5<sup>th</sup> day. This could be for the reason  
 268 that the substrate and microbes were in contact for an optimal amount of time which might have favored  
 269 the system to have an accelerated organic substance degradation by the microbes, so do a release of  
 270 proton and electrons in the anodic section. “It was reported that the formation of most conductive biofilm  
 271 over the electrode appears after 3 to 5 days” [64-66]. These studies demonstrated that the maximum  
 272 voltage output is due to the formation of conductive biofilm which stimulates the oxidation of the organic  
 273 matter. It is fairly in agreement with the finding of this study.

Design-Expert® Software  
 Factor Coding: Actual

**VOLTAGE**  
 ● Design Points  
 --- 95% CI Bands

X1 = C: Time

**Actual Factors**  
 A: temp = 35  
 B: pH = 7



274  
 275 **Fig. 2. Effect of time on the voltage output**

276  
 277 **3.3.2. Effect of pH**

278 “The pH value had a strong effect on MFCs microbial activity which was reflected in the overall MFC  
 279 performance” [67]. Changes in the pH value also affected the metabolism and absorption of nutrients  
 280 through influencing the solubility of nutrients, thus affecting the growth rate of microorganisms. “This is  
 281 because the fact that any microorganism should live in optimal pH value for its proper/optimal microbial  
 282 growth that can also be inhibited when the pH environment is below or above the appropriate pH value”  
 283 [68]. Thus, in this study, to investigate the effect of pH on voltage output, the MFC setups were operated  
 284 under different anodic pH, ranging from 4 to 10. At all pH, the MFC setups started voltage output soon  
 285 after the incubation period.

286 Figure 3 shows the effect of pH on the voltage output holding time and temperature Constant. As shown  
 287 in figure 2, voltage output is sensitive to the changes in pH. Hence, it was observed that a sharp  
 288 increment in voltage output (302 -1150 mV) was recorded when running from acidic to neutral pH and

289 then decreased gradually to about 850 mV in the basic pH. The best performance was observed when  
290 operating at pH 7 as the peak including the highest voltage output (1150 mV). The reason could possibly  
291 be the existence of a favorable pH for the microbial metabolic activities which generates proton and  
292 electrons. Thus, as the production of electrons increased, so does the voltage output. This is in  
293 agreement with the different studies which showed that optimum condition for microbial activity is set at  
294 neutral pH. Likewise, “changes in pH tend the microbes to respond accordingly which can pointedly  
295 influence the voltage output” [69-72].

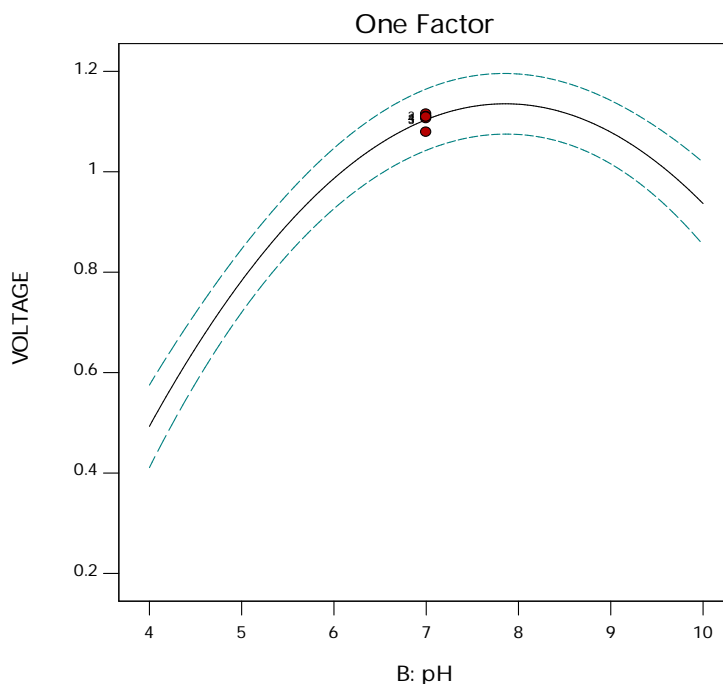
296 The voltage outputs were lower at pH 4 and pH 10, this indicates that the microbial catalytic activity is  
297 lower at these pH ranges. Comparatively, the lowest voltage outputs were observed in the acidic pH than  
298 in the basic pH. “This is because operating at lower pH inhibiting the metabolic activity resulted from the  
299 accumulation of excessive protons and therefore drops the voltage output as reported by” [73, 74].  
300 Therefore, it can be concluded that the performance of MFCs towards the voltage output is extremely  
301 dependent on pH, and neutral pH exhibit better performance.

Design-Expert® Software  
Factor Coding: Actual

**VOLTAGE**  
● Design Points  
--- 95% CI Bands

X1 = B: pH

**Actual Factors**  
A: temp = 35  
C: Time = 5



302

303

**Fig. 3. Effect of pH on the voltage output**

### 304 **3.3.3. Effect of Temperature**

305 Figure 4 shows the effect of temperature on the voltage output keeping the time and pH constant. As  
306 shown in the figure the voltage output is slightly affected by temperature, as the temperature increases  
307 from 25°C to 35°C, the voltage output slightly increases, whereas operating beyond 35°C resulted in a  
308 slight decline in voltage output. The best performance was observed at 35°C where the maximum voltage  
309 outputs of 1150 mV was recorded. This can be due to the existence of a favorable temperature for the  
310 catalytic activity of the microbes. “A report by [75] showed that operating microbial fuel cells at a

311 temperature between 30°C and 45°C is optimum to obtain higher voltage outputs”, which agrees with the  
312 finding in this study. Therefore, it can be concluded that the temperature has insignificant effect on the  
313 voltage output but operating at a temperature of 35°C gives a better result than the other temperature  
314 ranges.

Design-Expert® Software

Factor Coding: Actual

VOLTAGE

● Design Points

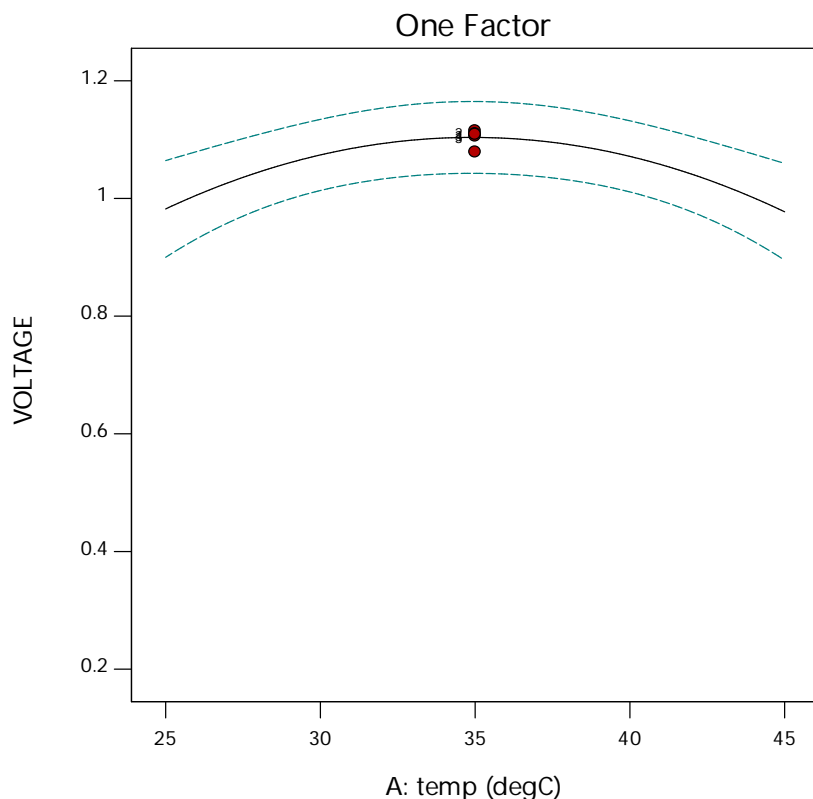
--- 95% CI Bands

X1 = A: temp

Actual Factors

B: pH = 7

C: Time = 5



315

316

Fig. 4. Effect of temperature on the voltage output

#### 317 4. CONCLUSION

318 This study investigated the bio-electrical performance of DCMFC fueled with brewery wastewater as an  
319 electron donor and inoculated with distillery plant waste from working biogas reactor as a source of  
320 microorganisms to run the experiment. From the experimental results, 1150mV maximum voltage output,  
321 92.85%, 91.40%, 68.87%, and 70.10% removal efficiencies of COD, BOD, TN and TP respectively were  
322 obtained at 35°C, pH 7, and 5 days. These results confirmed that the wastewater has been effectively  
323 treated and significant amount of direct bio-electricity is generated. This shows findings supported the  
324 hypothesis that bacterial heterogeneity of the anode surface is the main responsible factor for MFCs  
325 efficiency. The obtained results were compared with the previous literatures and the current study  
326 demonstrated that the potential of well-prepared MFCs to remove organic matter and other pollutants of  
327 interest, as well as to produce electricity. Results revealed that DCMFC provides an alternative insight  
328 into an effective treatment of wastewater that can simultaneously generate a direct bio-electricity. In this  
329 study, the inoculum was used as a source of microorganism. This might influence the voltage output.  
330 Thus, the type of microorganism involved in MFCs should be isolated and identified for further

331 investigation. Moreover; further research into novel and economically feasible electrode and membrane  
332 materials, the improvement of electrogenicity of the microbes used, and the potential of hybrid MFCs will  
333 provide opportunities to launch MFCs from the laboratory to the commercial-scale as a bid to improve the  
334 global energy security in an eco-friendly way.

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### 339 **AUTHORS' CONTRIBUTIONS**

340 All authors took part in the evaluation of the results, read and approved the final manuscript.

### 341 **COMPETING INTEREST**

342 The authors declare that they have no competing interest!

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