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Carbon-Neutral Abattoirs: Modelling Novel Self-Subsisting Wastewater Microbial Fuel Cells for Bioenergy Generation and Organic Removal

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Abstract

Environmental challenges such as climate change, emissions, indiscriminate waste disposal, and energy crisis are the focus of the Sustainable Development Goals (SDGs). There is a nexus between effluents and Bioelectrochemical Systems such as Microbial Fuel Cells (MFCs). In this study, twelve (12) locally fabricated Abattoir Wastewater Microbial Fuel Cells (AWMFCs) were developed, comprising (A, B, C, D – first stage) and (1, 2, 3, 4, 5, 6, 7, and 8 – second stage) from reusable HDPE/Plastic. The Microbial Fuel Cells (MFCs) were developed with different configurations and volumes, then fed with abattoir wastewater. The electrode used was a graphite rod, made from carbon, and was connected to a multimeter. The voltage across the circuit was monitored and recorded at an interval of twenty (20) minutes.
Reactor A and B produce a maximum density of 58.27 mWm^{-2} (0.28 Wm^{-3}, 573.53 mAm^{-2}) and 64.75 mWm^{-2} (0.31 Wm^{-3}, 604.57 mAm^{-2}) respectively. The 150 mL reactors (C and D) perform similarly to A and B. The maximum output of Reactor C was 27.42 mWm^{-2} (0.22 Wm^{-3}, 0.04 mA cm^{-2}, 3.1 A cm^{-3}), which occurred at 200 minutes, compared to 37.50 mWm^{-2} (0.3 Wm^{-3}, 0.04 mA cm^{-2}, 3.6 A cm^{-3}). Chemical Oxygen Demand (COD) removal was highest in Reactor D (32%), while efficiency ranged from 31% to 59%. Post Hoc Analysis of Variance shows a significant difference in power densities and current densities, while volumetric power shows no significant difference at an alpha value of 0.05. Moreover, COD removal in the second stage with eight (8) reactors in a two-cycle process was between 6.70% and 25.14%. The reactors reduced the organic matter of the wastewater and simultaneously generated bioenergy. Power and organic content were inversely proportional until a specific stage, after which this relationship changed.
Keywords: *Bioenergy, Biofuel, Energy, Microbial Fuel Cells, Optimization, SDG, Sustainable, Voltage, Wastewater*

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1.0 Introduction

Due to population growth and rapid industrialization, there has been a significant surge in energy demand (Apeh & Nwulu, 2024; Logan, 2004; Mohsin et al., 2019). This comes with more waste generation, especially municipal and industrial wastewater. EIB (2022) stated that around 380 billion m^3 of municipal wastewater is generated globally per year, which may surge by half before the next three decades. The two economic superpowers generated most of these. Domestic wastewater generated in the United States of America is estimated to be over 46 billion m^3 (Liu et al., 2004) while about 55.7 billion m^3 of wastewater are generated in China (U.S. International Trade Administration, 2023).

Wastewater is more environmentally problematic in developing nations of Asia and Africa. Slaughterhouse spews out substantial amounts. The abattoir industry has been reported to produce thousands of tons of organic effluents (Dentel et al., 2004; Min et al., 2005; Philipp et al., 2021), and the necessity of treating and properly managing such wastes has been asserted (Suzuki et al., 2002; Joachim et al., 2022). However, conventional wastewater treatment processes to remedy these wastes are energy-intensive. Activated sludge processes, for instance, typically require approximately 0.6 kWh per cm^3 of domestic wastewater treated (McCarty et al., 2011; Rosso et al., 2008). Aeration consumes about 0.3 kWh of the total energy required. The costs of sludge production and treatment in Activated Sludge (AS), Trickling Filter (TF), and Membrane Bioreactor (MBR) systems are relatively high, posing a serious environmental hazard if not properly conditioned (Aelterman et al., 2006). Thus, wastewater treatment plants are arguably among the most energy-intensive industrial infrastructures globally. Nearly 3% of the total electricity supply is consumed by wastewater treatment facilities, and approximately 30% of their operating budgets are dedicated to power (Rathore, 2014). This is not sustainable. Sustainable wastewater treatment targets not only water reuse but also energy recovery and nutrient management (Clauwaert et al., 2007). One of the sustainable systems is the Microbial Fuel Cell (MFC). MFC uses interactions between microorganisms as biocatalysts to generate bioenergy (Logan et al., 2006; Rabaey & Verstraete, 2005; Leung et al., 2023); thus, chemical energy is converted to direct electric current (Wang & Ren, 2013) but it is affected by many factors (González del Campo et al., 2013; Liu et al., 2005). This technology has the potential to revolutionize wastewater treatment globally by becoming a net energy producer (Hassan et al., 2024; Janicek et al., 2014; McCarty et al., 2011; Rozendal et al., 2008). It can serve not only as an energy source (Cheng et al., 2006; Mulyono et al., 2020) but also organic substance removal (Marks et al., 2020; Wang et al., 2014),

denitrification (Yao et al., 2024), and metal recovery (Chakraborty et al., 2020; Heijne et al., 2010; Lim et al., 2021).

MFC architectures can ultimately dictate performance (Logan, 2008). Configurations developed over the years include single-chamber (Daghio et al., 2015), continuous flow mode systems (Faria et al., 2017; Ismail & Jaeel, 2013; Li et al., 2020), and stacked MFCs (Gajda et al., 2020; Gurung & Oh, 2012; Oh & Logan, 2007). Others include coupled systems for organic reduction (Abdulwahab et al., 2021; Tang et al., 2019; Villaseñor et al., 2013), nitrogen removal (Ge et al., 2020; Sun et al., 2016; Zekker et al., 2020) or dye removal (Li et al., 2010; Long et al., 2017; Sonu et al., 2020) in batch and continuous flow mode (Hartl et al., 2019; Zhao et al., 2013).

Moreover, a double-chamber system with two compartments and a membrane is also prominent in many substrates (Dharmalingam et al., 2018; Fan & Gao, 2019; Ullah & Zeshan, 2020). However, power losses, internal resistance, and the cost of the membrane make MFC more challenging to scale up, which prompted a paradigm shift toward the use of single-chamber membrane-less air cathode MFC (Logan, 2010). The air-cathode MFC was first discussed over three decades ago (Sell et al., 1989), while more explicit designs of this type of bioreactor emerged later (Liu & Logan, 2004). Many studies now employ air-cathode MFCs in a single component, with the cathode exposed to oxygen (Hou et al., 2011; Logan et al., 2007).

Many compounds can be fed into the MFC as substrate. Glucose reported 3600 mWm^{-2} maximum power density (Rabaey et al., 2003a) While power has been generated from the same substrate (Kim et al., 2011; Rabaey et al., 2003b; Ray et al., 2018). Others include glucose-glutamic acid (Catal et al., 2008), municipal leachate (Haoran et al., 2014; Yuan et al., 2012), inorganic phenol was degraded to achieve a high current density of 0.1 mA cm^{-2} (Luo et al., 2009) and fermented vegetable waste (He & Angenent, 2006).

For wastewater application, the first work was arguably in the early 1990s (Habermann & Pommer, 1991; Ieropoulos, et al., 2024). The performance of NG-MFC for bioenergy recovery and simultaneous reduction of organic content from wastewaters shows auspiciousness (Liu et al., 2013). Other wastewaters substrate includes synthetic wastewater (Cheng et al., 2021; Jadhav & Ghangrekar, 2009; Rodrigo et al., 2009; Sotres et al., 2016), urban wastewater (Capodaglio et al., 2013; Colares et al., 2021; Rodrigo et al., 2007), chemical process wastewater (Venkata Mohan, Mohanakrishna, et al., 2008; Venkata Mohan, Veer Raghavulu, et al., 2008), starch processing wastewater (Lu, Zhou, Deng, et al., 2009; Lu, Zhou, Zhuang, et al., 2009; Muthukumar & Sangeetha, 2014), chocolate industry wastewater (Patil et al., 2009; Rahayuning Wulan & Notodarmojo, 2020), brewery wastewater (Feng et al., 2008; Wang et al., 2008; Wen et al., 2010). Furthermore, dairy wastewater has been reported to produce 2.7 Wm^{-3} volume power, 91% COD removal, and a CE of 17% (Elakkiya & Matheswaran, 2013). In this study, the performance of AWMFC was assessed by eliminating the membrane (arguably the most expensive part of MFC) and external inoculum, while cattle abattoir wastewater was used as substrate.

2.0 Materials and Methods

2.1 AWMFC Development and Operations

The study is in two stages. For the first stage, four reactors (A, B, C, and D) were assembled using a locally fabricated reusable cylindrical HDPE container. The two ends were covered with 70 mm diameter plastic material, with a hole approximately 5 mm in diameter bored at the center to permit the insertion of electrodes at the opposite ends. Reactors A and C were membrane-less and mediator-free MFCs developed with 250 mL and 150 mL volumes, respectively. The empty free space at the top was also 20 mL and 10 mL, respectively, as shown (Fig. 1 and Fig. 2).

Carbon paper (waterproof) was used to maintain the reactor in an anaerobic state and to ensure the covered area was watertight, while the sampling point was sealed. Electrode projected surface areas of the anode (Reactor A) were 11.81 cm^2 (4.73 $m^2\,m^{-3}$) and 6.78 cm^2 (2.72 $m^2\,m^{-3}$) for the cathode (see Table 1) and connected to a multimeter (Sooer China SD 9205A) by a thin copper cable (0.75 mm -1 mm). The abattoir wastewater samples were analyzed using standard methods to ascertain the initial organic content, electrical conductivity, pH, and temperature. A continuity test was performed at every connection point before the substrate was fed into the reactors. Reactor C has the same configuration but a 150 mL volume. A circuit board was employed to complete the connections, which were operated with a 150 Ω external resistor used for circuit connections in all four reactors.

Microorganisms from abattoir wastewater were cultured in the laboratory. The inoculum was added to 10 mL of glucose, which serves as food and activates microorganisms. The inoculated solution was incubated for 3-5 hours to achieve good bacterial growth and then inserted into the reactors. The cells were monitored by recording the voltage across the circuit at an interval of twenty (20) minutes.

Reactor B (Fig. 1a) is a 250 mL membrane-less MFC developed an electrode 11.81 cm^2 each for both the electrodes (projected surface area per volume of 4.73 $m^2\,m^{-3}$ - 250 mL reactor volume) at opposite ends, exactly with Reactor A configurations and monitored at a 20-minute interval until a decrease in voltage was noted on the digital multimeter. A 20 mL volume of space was provided at the top of the reactors. The 150 mL reactor D (Fig. 2b) has a cathode and anode area of 11.81 cm^2 each, with 10 mL of free space. The projected surface area per volume is 4.73 $m^2\,m^{-3}$. Carbon paper (waterproof) was used to make all reactors waterproof.

For the second stage of the experiment, eight reactors were set up, with four having the same configurations as reactors A, B, C, and D, while the next four were exposed to oxygen at the top (sampling point). They were inoculated like the first stage. Two readings were recorded from each reactor, and the mean value was calculated for each of the eight reactors. All reactors were observed using a multimeter at 20-minute intervals for a total of 260 minutes. The circuit was disconnected after a voltage drop was detected, and the organic content was subsequently measured.

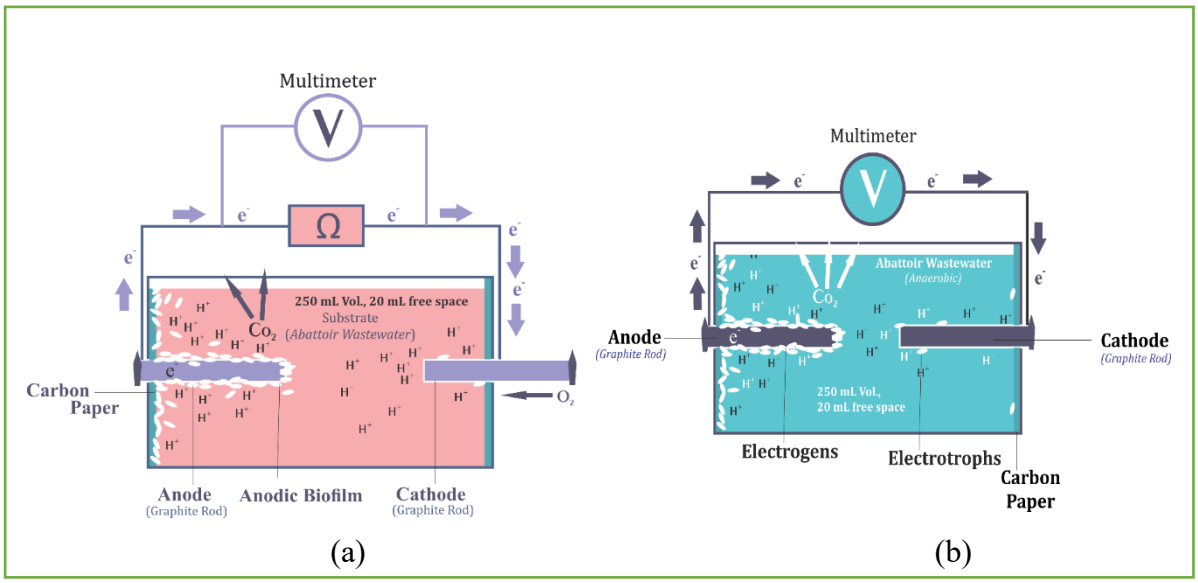


Fig. 1: Schematics of the 250 mL MFCs (a) Reactor A configuration, (b) Reactor B configuration

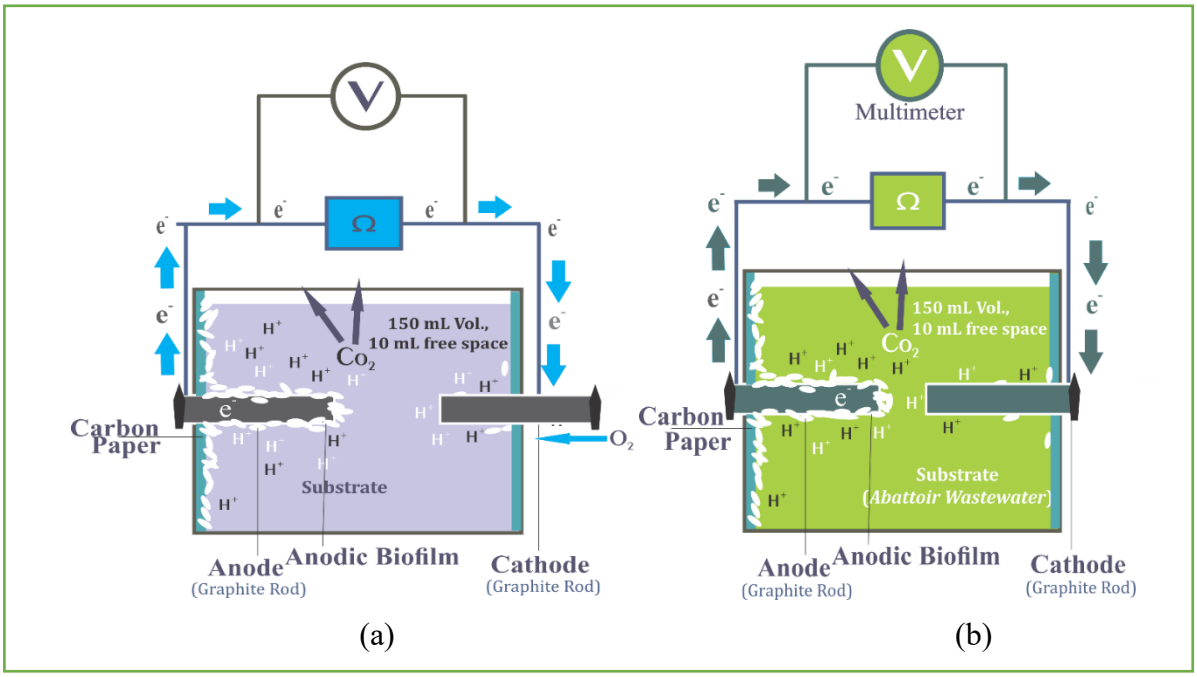
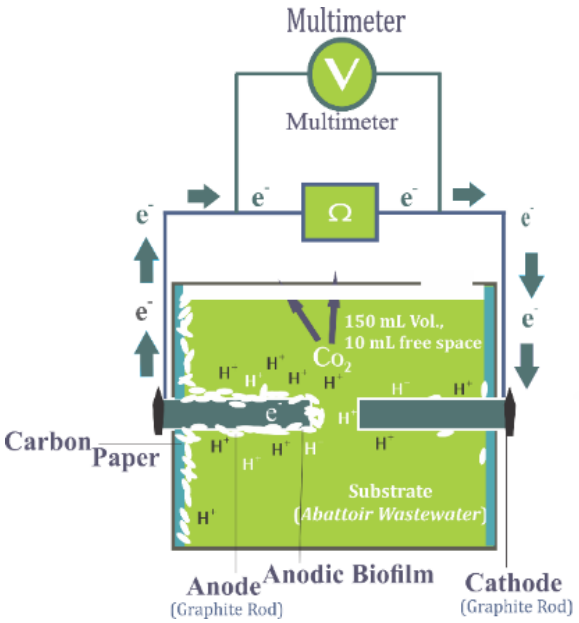
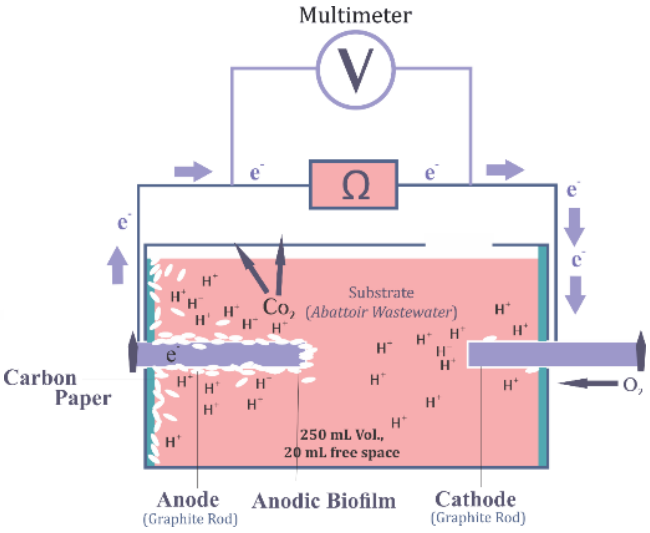


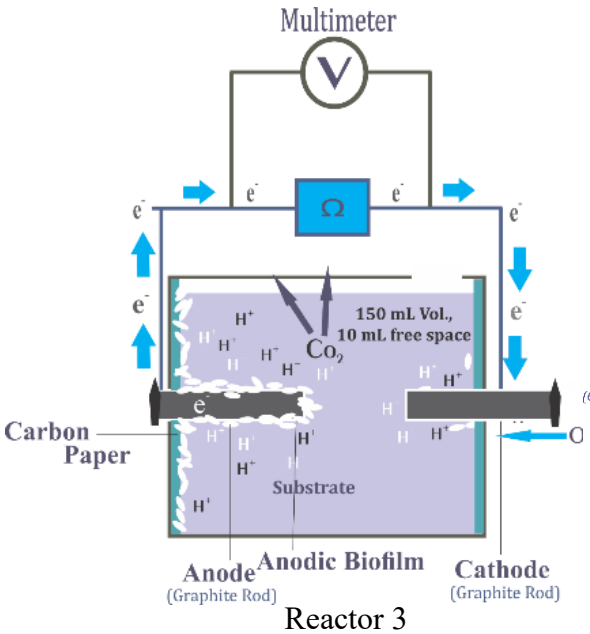
Fig. 2: Schematics of the 150 mL MFCs (a) Reactor C configuration, (b) Reactor D configuration



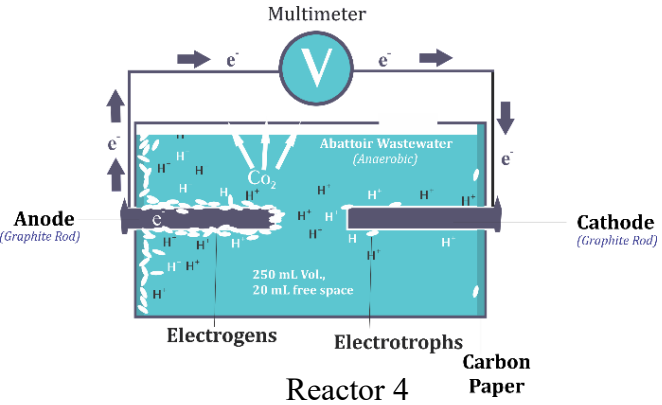
Reactor 1



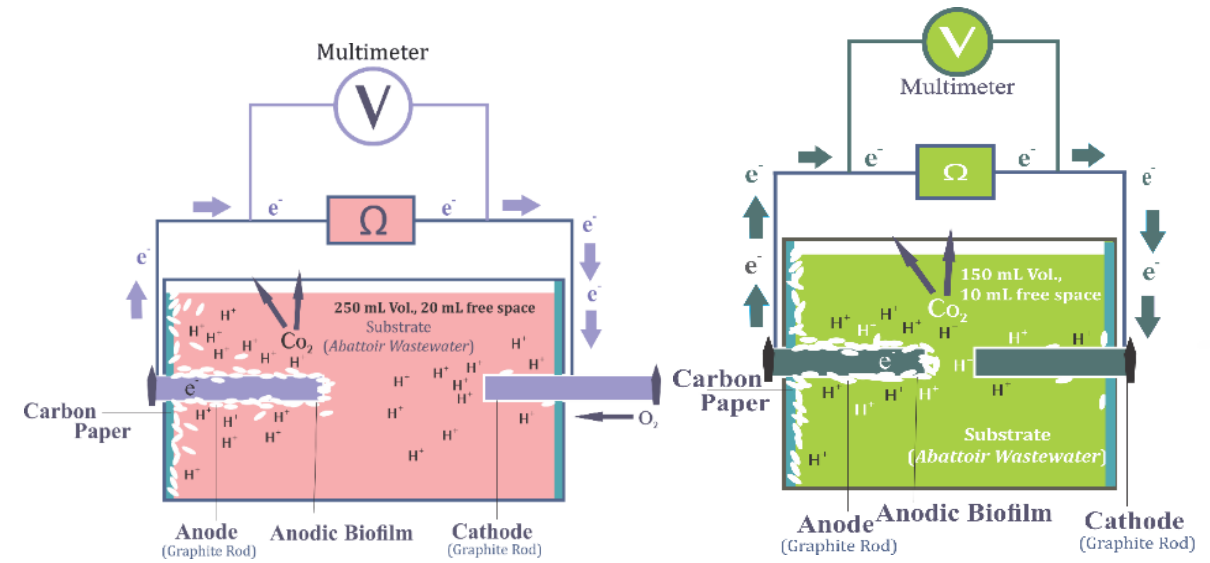
Reactor 2



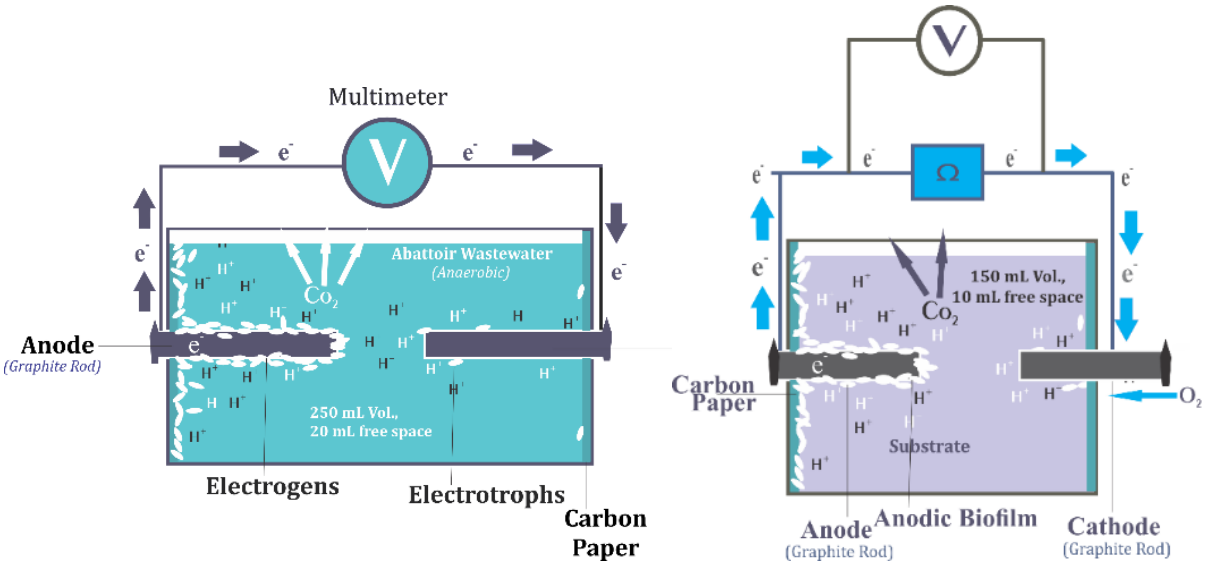
Reactor 3



Reactor 4



Reactor 5 Reactor 6



Reactor 7 Reactor 8

Fig. 3: Eight (8) reactors for optimization studies

Table 1: Summary of the reactor configuration for the first stage

	Cathode SA (cm)	Anode SA (cm)	Cathode SA (m^2m^{-3})	Anode SA (m^2m^{-3})	Volume (mL)
Reactor A	6.786	11.813	2.715	4.726	250
Reactor B	11.813	11.813	4.726	4.726	250
Reactor C	6.786	11.813	2.715	4.726	150
Reactor D	11.813	11.813	4.726	4.726	150

2.2 Optimization of Voltage Output

Design of experiment (DoE) was carried out in two cycles with eight (8) reactors, mean voltage is the response (Fig. 3). The optimal condition for voltage production was determined by using the Minitab 18 DoE application. Table 2 presents the factors considered for power generation, which are Projected Surface Area (PSA), Volume, and Category. A Full Factorial Design with three factors at two levels was employed for optimization (Table 3). The mean of voltage values from the eight (8) reactor runs was used as the response, and the values were imputed into the MiniTab software application, where a complete factorial design was performed.

2.3 Characterization of Abattoir Wastewater

Abattoir wastewaters grab samples were used as substrate for the reactors. As previously mentioned, physicochemical and biological parameters were analyzed according to standard methods (APHA, 1998). The parameters measured at the start of the study were Temperature, pH, Electrical Conductivity (EC), and Chemical Oxygen Demand (COD). To measure the COD percentage removal, the final COD was determined when a voltage drop was observed.

Table 2: Factors considered for voltage generation

Factor	Symbol	Coded factor levels	
		-1	1
Projected Surface Area (cm^2)	U	6.8	11.81
Volume (mL)	V	150	250
Category	W	*Aerobic	Anaerobic

**About half of the cathode PSA is exposed to free oxygen*

Table 3: The factorial design for optimizing the voltage generation

Standard Order	Run Order	Projected Surface Area	Volume	Category
2	1	1	-1	-1
3	2	-1	1	-1
1	3	-1	-1	-1
4	4	1	1	-1
7	5	-1	1	1
6	6	1	-1	1
8	7	1	1	1
5	8	-1	-1	1

2.4 Analysis and Calculations

The performance of the MFCs was assessed using the power density per area of the anode unit (Wm^{-2}), power density per volume of the MFC unit (Wm^{-3}), Overall Coulombic Efficiency (CE), and Treatment efficiency. From Ohm's law, Equations 1- 4 are applicable:

$$V = IR \quad (1)$$

$$P = I\,V \quad (2)$$

$$Power\ Density\ (PD) = \frac{P}{A} \quad (3)$$

$$Current\ Density\ (PD) = \frac{I}{A} \quad (4)$$

Where:

Power (P) is in mW
Current (I) in mA,
Voltage (V) in mV
External resistor R in Ω.

Others include:

Projected anode surface area (A) in m²
Current density (mAm^{-2})
Power density (mWm^{-2})

The unit for power density per volume is Watts per cubic meter (Wm^{-3}).

Coulombic Efficiency (CE), on the other hand, is the percentage of electrons recovered as current versus those in the starting organic matter of the wastewater.

$$CE = \frac{Coulombs\ recovered}{Total\ coulombs\ in\ substrate} \quad (5)$$

$$CE = \frac{8\int_{o}^{t_b} I\,dt}{FV\Delta COD} \quad (6)$$

Where:
F = Faraday's constant (96485 C.mol^{-1} of electrons)
V = the volume of liquid in the compartment (L)

Treatment efficiency is the measure of the COD removal, which can be illustrated by:

$$COD\ removal = \frac{COD_{in} - COD_{out}}{COD_{in}} \quad (7)$$

3.0 Results and Discussion

The performance of the reactors was measured with power density, current density, treatment efficiency, and CE of the cells. For the first stage, the average EC and temperature of reactors A, B, C, and D were 3.50, 3.87, 9.78, and 8.3 mScm^{-1}, and 26.7, 26.7, 27.2, and 27.2 °C, respectively. The pH ranges between 7.6 and 8.0. Conductivity, pH, and Temperature may have affected the power output of MFCs in various ways beyond the scope of this study.

3.1 Power generation

Reactors A and B produced a maximum power density of 58.27 mWm^{-2} (0.28 Wm^{-3}) and 64.75 mWm^{-2} (0.31 Wm^{-3}) respectively (Fig. 4 and Fig. 5). The maximum current density produced is 573.53 mAm^{-2} (0.05 mAcm^{-2}) and 604.57 mAm^{-2} (0.06 mAcm^{-2}) respectively. The cathode electrode area of Reactor B is greater than that of A, resulting in a higher power density in the former, which also exhibits higher conductivity (3.87 mScm^{-1} > 3.50 mScm^{-1}). One of the highest volumetric power (6.5 Wm^{-3}) was experienced using swine wastewater (Min et al., 2005). The maximum current density of 0.06 mA cm^{-2} achieved in each of A and B exceeded 0.02 mA cm^{-2} (Jang et al., 2004) using synthetic wastewater, 0.015 mA cm^{-2} with swine wastewater substrate (Min et al., 2005), and other prominent works. The temperature of 26.70 °C was the same for both reactors; likewise, the pH measured for the two reactors was 7.8, which is practically ideal for reactor performance (Bullen et al., 2006).The higher ionic conductivity of 3.87 mS cm^{-1} compared to 3.50 mS cm^{-1} recorded may also be responsible for the higher voltage generation in Reactor B than in Reactor A. The performance of anaerobic Reactor B is similar to another work with carbon electrode (graphite rod and granules) which produced 67.28 mWm^{-2} (116.00 mV 0.58 Am$^{-2;}$ 0.46 Wm^{-3}) at 200 Ω and 62.41 mWm^{-2} (79.00 mV 0.79 Am^{-2} 0.43 Wm^{-3}) at 100 Ω in an anaerobic-aerobic reactors with 220 mL volume each for the anode and cathode chamber (Li et al., 2010). The cathode can also affect power generation in MFCs (Oh et al., 2004; Pham et al., 2004; Zhang et al., 2012). The increased total projected surface area of the electrode (cathode) may have contributed to the variation in maximum power density in the 250 mL samples (A and B, 10%) or 150 mL samples (C and D, 17%).
However, Reactor D delivered higher volumetric power (power per volume) than its counterpart (Reactor B) and more COD removal (32 >27%), but lower CE overall. Power generation started at a slower pace in reactor B before the steady increase, which surpasses that of reactor A at the 200-minute mark. Reactor C, with a 150 mL volume, also produced 27.42 mWm^{-2} (0.22 Wm^{-}

^{3}; 393.45 mAm^{-2}; 3.1 Am^{-3}) at the same period, but at a slightly higher temperature (27.2 °C) and conductivity (9.78 mScm^{-1}).

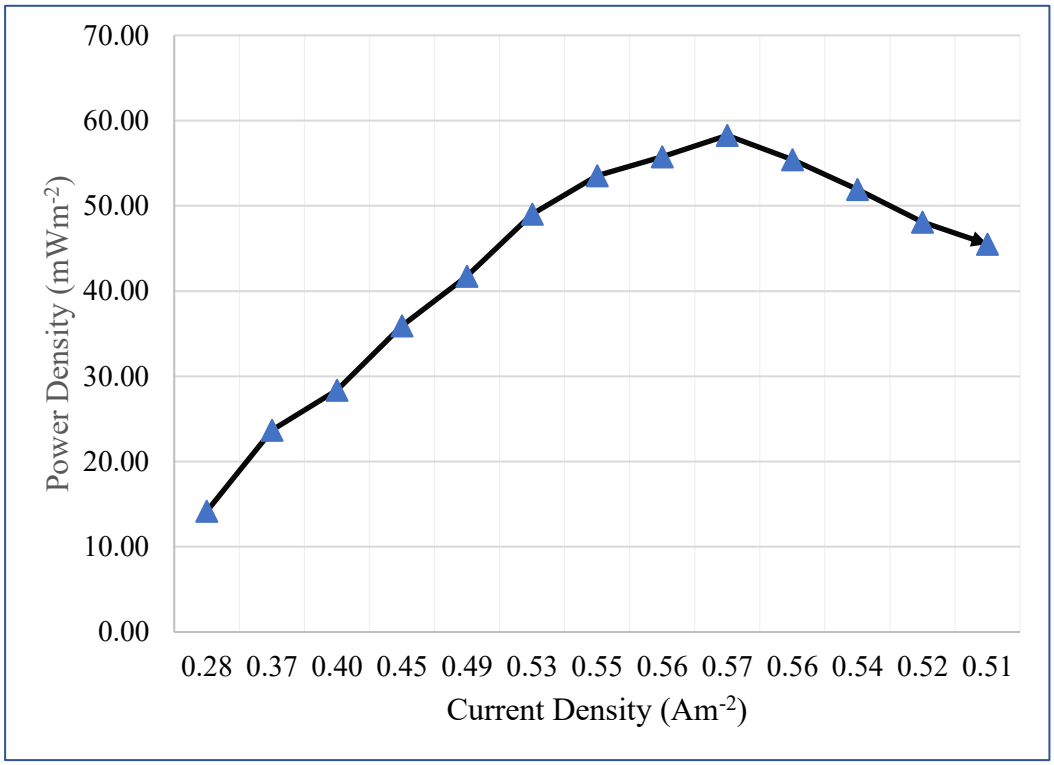


Fig. 4: The power density curve for reactor

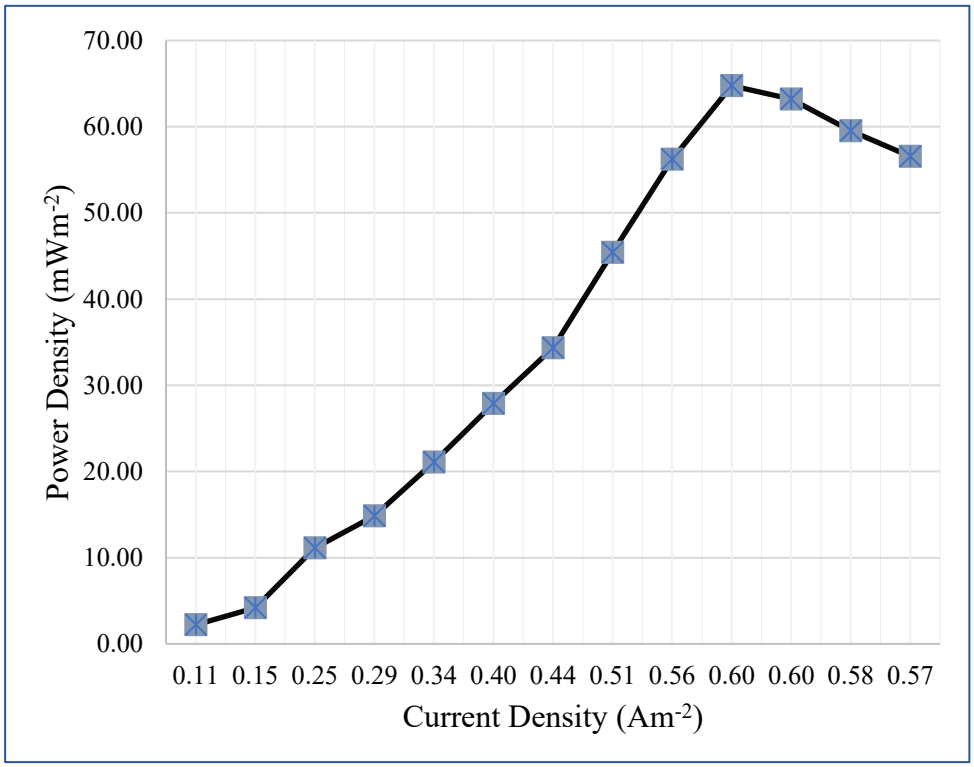


Fig. 5: The power density curve for 250 mL reactor B

Moreover, the 150 mL reactors (C and D) perform in the similitude of A and B. Maximum output of Reactor C was 27.42 mWm^{-2} (0.22 Wm^{-3}, 0.04 $mAcm^{-2}$, 3.1 Am^{-3}) which occurred at 200 minutes compared to 37.50 mWm^{-2} (0.3 Wm^{-3}, 0.04 $mAcm^{-2,}$ 3.6 Am^{-3}) which took 40 minutes more to achieve.

The maximum current density from the reactors (A - 573.53, B - 604.57 (0.06 $mAcm^{-2}$), C - 393.45, and D - 460.06 $mAcm^{-2}$) was higher than 170 $mAcm^{-2}$ (0.017 $mAcm^{-2}$) current density in a membrane-less and mediator-less synthetic wastewater MFC (Aldrovandi et al., 2009). Although the electrical conductivity of Reactor C and D is 9.78 and 3.38 $mScm^{-1,}$ respectively, at 27.2 °C, the higher maximum power density in Reactor D is primarily attributed to the larger projected electrode surface area, which enables the bacteria to transfer electrons more efficiently.

The power density curves of both reactors (C and D) performed similarly. They were almost linear until they approached their maximum power (Fig. 6 and Fig. 7). The B and D reactors, which were kept under anaerobic conditions, reached their maximum power density more slowly than reactor C. This was also the case for the two 250 mL configurations, which enabled reactor B to reach its maximum power output at 20 minutes more than its counterpart of the same practical configuration, but with a different total projected surface area. The maximum voltage achieved in B and D is more than in A and C, respectively. This may be due to the contamination of the anode with oxygen admitted through the exposed cathode.

3.2 Statistical Analysis and Comparison

3.2.1 Current density generated from the AWMFCs

Table 4 gives a summary of the maximum power generation. The current density was subjected to descriptive statistics to establish normality, which is one of the conditions for Analysis of Variance. The one-way ANOVA of the four reactors was then subjected to two comparable post hoc tests: Least Significant Difference (LSD) and Honestly Significant Difference (HSD). This is to establish the group means with a significant difference at a 95% confidence level. The skewness was -0.197. The normality and homogeneity of the data set were established statistically, and by using the boxplot (Fig. 8). For the ANOVA test, the variance ratio (F) was greater than the critical value at $\alpha = 0.05$. The calculated actual value of $F = 7.517$ is greater than the critical value 2.798 (Table 5).

Therefore, the null hypothesis is rejected because $p < 0.05$. The means of the current density of the reactors are different at a 95% confidence level. To identify the actual pairwise reactors with a significant difference, the data were subjected to post hoc LSD (critical value = 90.682) and HSD (critical value = 120.039) tests. HSD, which is more conservative, reveals that Reactor A and C (206.902), A and D (125.667), and B and C (136.121) are significantly different (Table 6). This is because the colored cells in the table have significant mean differences, which are greater than the HSD test critical value. Employing a less conservative LSD but more potent of the two tests shows that there is a pairwise not for multiple comparisons, current density of Reactor A and B (90.682 > 70.781), B and D (90.682 > 54.886), C and D (90.682 > 81.235) are not originally different from each other.

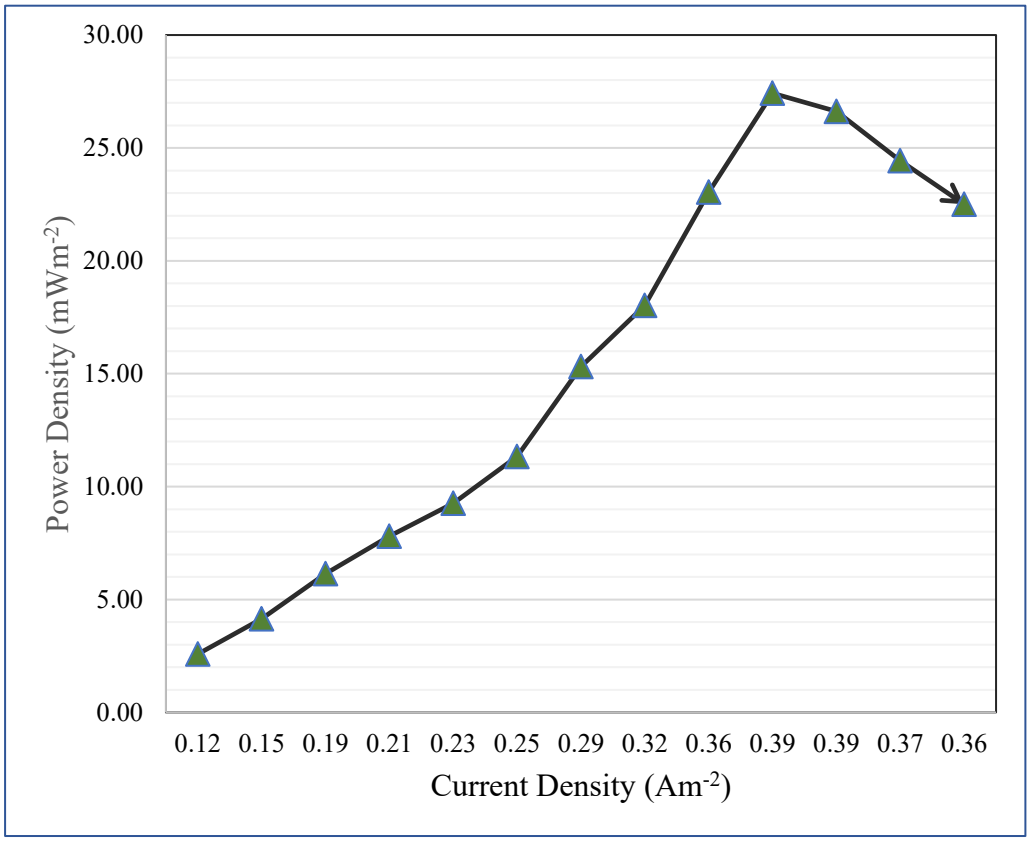


Fig. 6: The power density curve for 150 mL reactor C

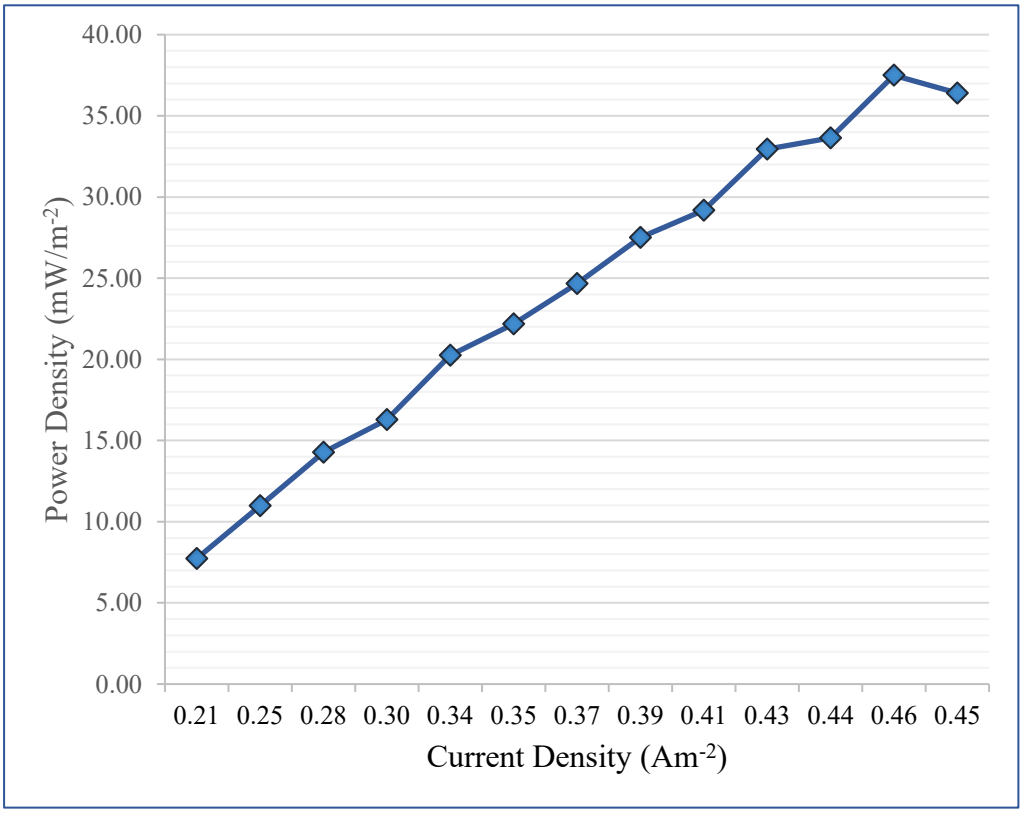


Fig. 7: The power density curve for 150 mL reactor D

Table 4: Summary of maximum power and current density of the reactors

	Power (mW)	Power density (mWm^{-2})	Vol. Power Density (Wm^{-3})	Current Density (mAm^{-2})	Vol. Current Density (Am^{-3})
Reactor A	68.82	58.27	0.28	573.53	2.71
Reactor B	76.47	64.75	0.31	604.57	2.86
Reactor C	32.39	27.42	0.22	393.45	3.10
Reactor D	44.28	37.50	0.30	460.06	3.62

Table 5: ANOVA for the current density dataset

Source of Variation	SS	Df	MS	F	P-Value	F critical
Between Groups	298190	3	99396.65	7.517636	0.001	2.798061
Within Groups	634646.3	48	13221.8			
Total	932836.2	51				

Table 6: Post hoc critical value for the dataset

Post Hoc	Reactor A	Reactor B	Reactor C
Reactor B	70.781		
Reactor C	206.902	136.121	
Reactor D	125.667	54.886	81.235

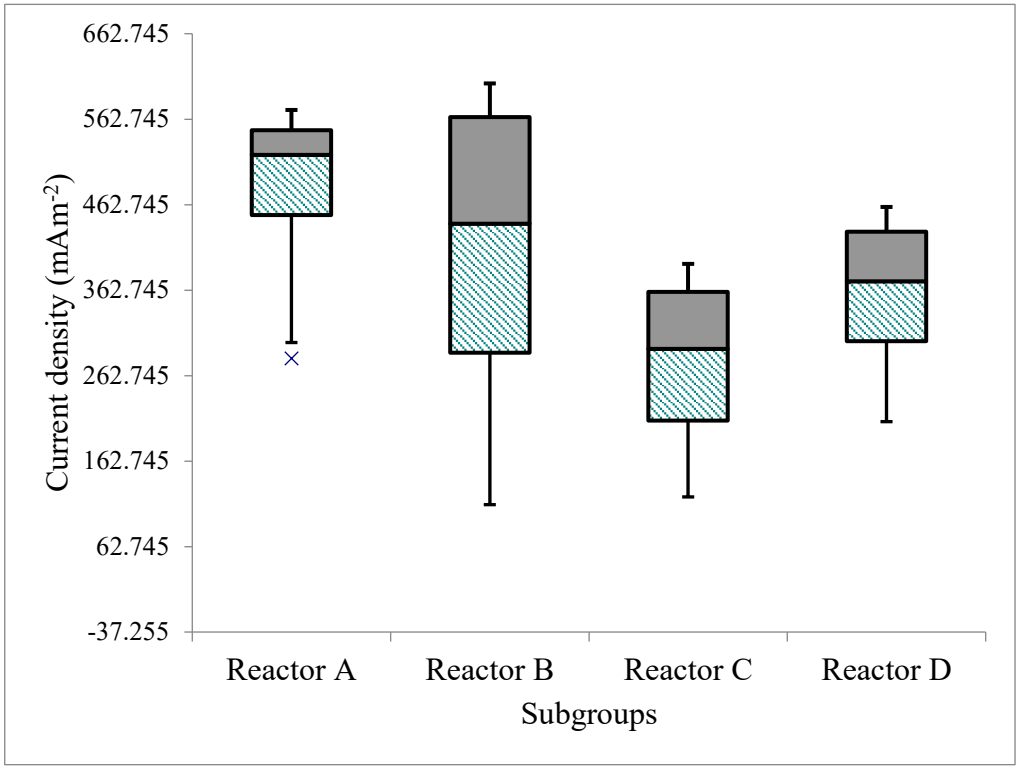


Fig. 8: Box plot for the data showing an outlier in reactor A

3.2.2 Power density of the AWMFCs

The bioreactors' power density, normalized by anode surface area, was subjected to descriptive statistics for a normality test before applying the single-factorial ANOVA analysis. Critical Value (0.787) at $\alpha = 0.05$, data tend towards normal at 0.05 due to the two outliers in Reactor A and B subgroup but is nearly normal (Fig. 9). Variance ratio 8.645 is greater than the critical value (Table 7), with 95 percent confidence level, thus significant. The rejection of the null hypothesis was because $p < 0.05$ (means are different). It is therefore concluded with a 95 percent certainty that the MFCs differ.

Using post hoc analysis, Reactor A and C, A and D, and B and C show a significant difference because all their pairwise critical values are greater than HSD (15.758 critical value). The critical value of LSD is 11.904. With the less conservative but more powerful LSD test, $F_{LSD} < F_{critical}$, (11.904 > 7.705, 11.372, 8.836), thus the null hypothesis can be accepted for A-B, B-D, and C-D pairwise groups (Table 8).

Table 7: ANOVA data for the power density

Source of Variation	SS	Df	MS	F	P-Value	F critical
Between Groups	5909.092	3	1969.697	8.645	0.001	2.798
Within Groups	10936.51	48	227.844			
Total	16845.6	51				

Table 8: Post hoc critical value for the dataset for power output

Post Hoc	Reactor A	Reactor B	Reactor C
Reactor B	7.705		
Reactor C	27.913	20.208	
Reactor D	19.077	11.372	8.836

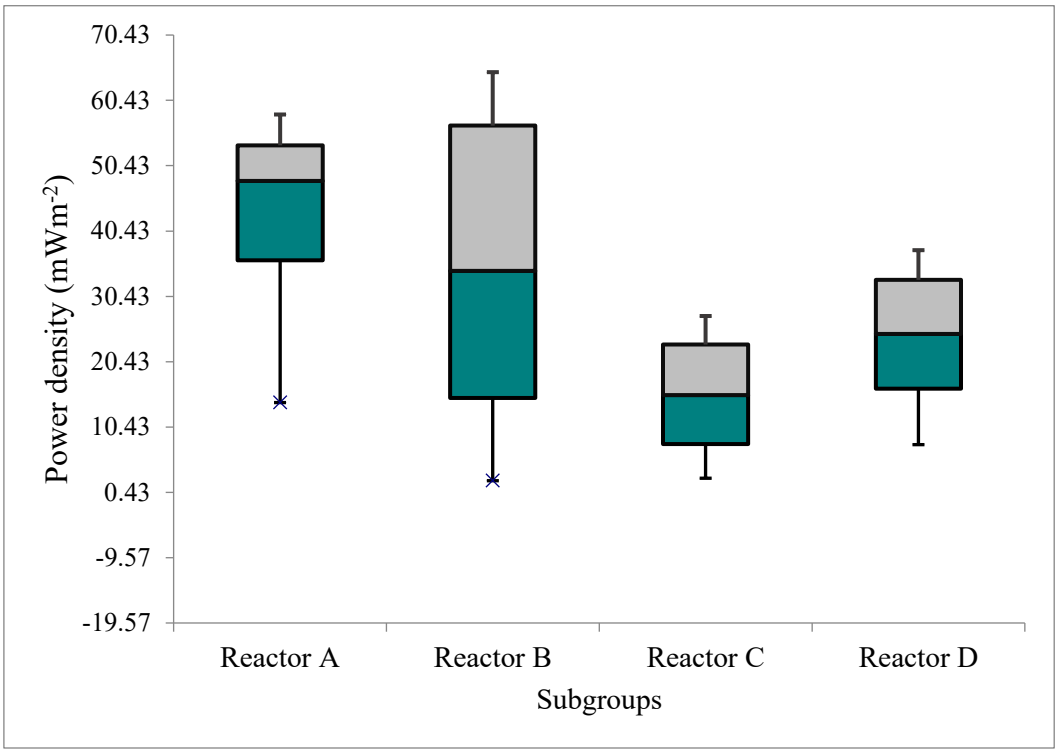


Fig. 9: Boxplot of the Reactor A-D dataset for power density with outliers in A and B

3.2.3 Volumetric power

Analysis of power per volume shows the normality of the data and Reactor B with an outlier (Fig. 10). Standard Deviation, Variance, Skewness, and Kurtosis (0.086; 0.007; -0.222; and -1.155). The p-value is greater than 0.05, and the calculated value of F= 2.550 is less than a critical value of 2.798 (Table 9). Null hypotheses were accepted in this case at a 5 percent level of significance. Therefore, there was no significant difference in power output per volume between the MFCs, and it was concluded with 95 percent confidence; thus, their performance is relatively the same. This makes it unnecessary to conduct a post hoc test.

Table 9: One factorial analysis of variance table

Source of Variation	SS	Df	MS	F	P-Value	F critical
Between Groups	0.052	3	0.017	2.550	0.067	2.798
Within Groups	0.329	48	0.007			
Total	0.381	51				

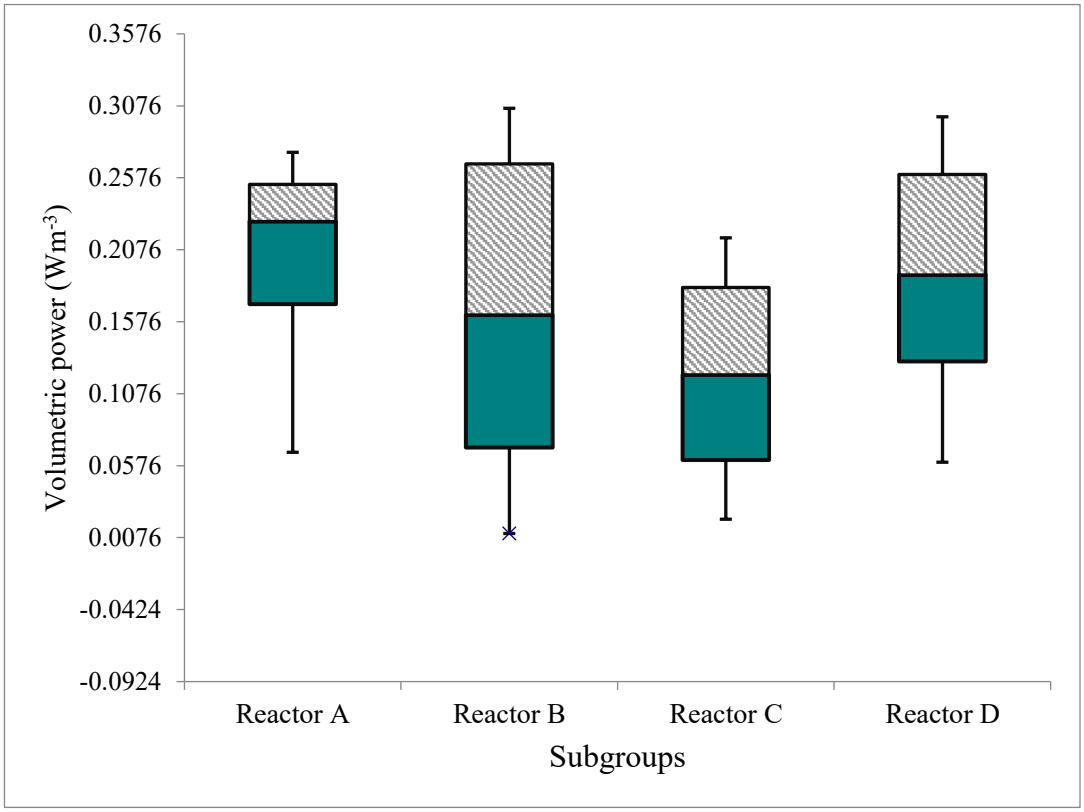


Fig. 10: Box plot for power per volume showing an outlier in reactor B

3.3 Treatment Efficiency

For first stage, the initial COD was 1008 mgL^{-1}, which is still within the range of 340-1550 mgL^{-1} from abattoir wastewater (Metropolis et al., 2010). The percentage of organic matter removal from wastewater in this study was calculated with equation 7. The influent COD was analyzed at the beginning and end of the cycle time. Removal rates were 23%, 27%, 15%, and 32% in A, B, C, and D, respectively. Reactor C exhibits the slowest organic removal due to its lower volume compared to A and B, or its lower electrode surface area compared to B and D, which may have impacted the treatment efficiency of the MFC. For the second stage, the highest percentage removal is 25.14% (Fig. 11).

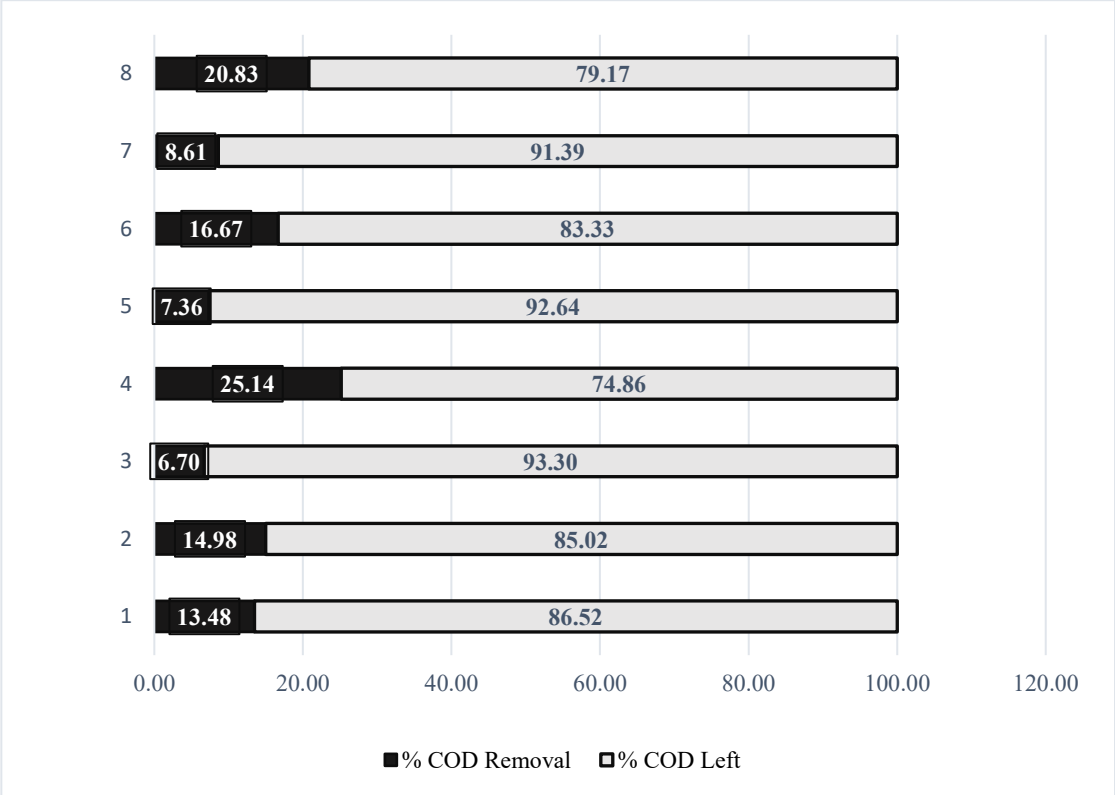


Fig. 11: COD Removal rate

3.4 Coulombic Efficiency

Equations 5 and 6 were used to calculate Coulombic Efficiency (CE) for complex substrates, such as wastewater. The current against time curves generated in each of the AWMFCs were integrated to obtain 130.25, 186.90, 63.23, and 111.28, respectively, for A, B, C, and D.
The overall performance, based on CE, of Reactor A (31%) and B (36%) is similar, despite the different general electrode surface areas. Likewise, Reactors C and D produced efficiencies of 59% and 56%, respectively. The higher CE achieved in reactor B, out of the 250 mL, signifies the effect of increased surface area on the power output of the membrane-less Microbial Fuel Cell. This phenomenon was also observed in Reactors A and B, where the anaerobic B produced a higher CE than its counterpart; however, this was not the case in the 150 mL Reactors.

3.5 Experimental Design and Optimization

Design of Experiment (DoE) was carried out in eight (8) reactors using mean voltage as the response. The optimal condition for voltage production was determined by using the QI Macros and Minitab DoE application (Table 10). The model summary and results of the voltage generation based on the Full Factorial Design are presented in Tables 11 and 12. It includes the actual experimental voltage, the predicted voltage, and their residuals. The linear regression models that best described the process are as follows:

$$Voltage = 65.184 + 0.807\,U + 8.054\,V - 2.755\,W - 4.834\,UV + 1.594\,UW - 1.15\,1VW \quad (8)$$

Table 10: The factorial design with voltage as a response

Standard Order	Run Order	Projected Surface Area	Volume	Category	Mean Voltage
2	1	1	-1	-1	62.93
3	2	-1	1	-1	82.91
1	3	-1	-1	-1	54.54
4	4	1	1	-1	71.38
7	5	-1	1	1	71.62
6	6	1	-1	1	62.61
8	7	1	1	1	67.05
5	8	-1	-1	1	48.44

Where U is the Projected Surface Area, V is the Volume, and W is the Category, these three are the main effects, while the interactive effects include UV, UW, and VW. The positive T-Value for the overall model terms of PSA (5.49), Volume (54.75), PSA and Category (10.83) showed favorable effects on voltage generation. In contrast, the negative T-values for the model Categories (-18.7), PSA and Volume (-32.86), and Volume and Category (-7.82) revealed unfavorable effects on Voltage.

According to the model summary table, at an alpha value of 0.05, the interactive effect between Projected Surface Area and Category ($p = 0.059$) is not statistically significant. However, Volume and Category had a p-value of 0.081, which is significant, and thus they were removed from the model. Equation 8 is now reduced to equation 9:

$$Voltage = 65.18 + 0.81\,U + 8.05\,V - 2.75\,W - 4.83\,UV \quad (9)$$

3.6 Optimization of Voltage Generation

The experimental value was 82.91 mV, while the optimum conditions, as determined by the Projected Surface Area of the electrode, Volume of reactors, and Category (+1, +1, and -1),

remained at 81.62 mV. The close values confirm the model developed for voltage generation in MFCs. A Pareto chart was used to test the standardized effects. At the same time, the primary and interactive factors were displayed (Fig. 12, Fig. 13, and Fig. 14). Significant effects were found in PSA and Volume (p = 0.019), Volume (p = 0.012), and Category (p = 0.024), all at a 95% confidence level.

Table 11: Model summary table

Term	Effect	Coeff	T-Value	P-Value
Constant		65.184	443.1	0.001
Projected Surface Area	1.614	0.807	5.49	0.115
Volume	16.107	8.054	54.75	0.012
Category	-5.509	-2.755	-18.73	0.034
Projected Surface Area*Volume	-9.668	-4.834	-32.86	0.019
Projected Surface Area*Category	3.187	1.594	10.83	0.059
Volume*Category	-2.302	-1.151	-7.82	0.081

Table 12: Factorial experimental design results

Actual Voltage	Predicted Voltage	Residuals
62.929	62.781	0.147
82.912	82.764	0.147
54.540	54.687	-0.147
71.376	71.524	-0.147
71.619	71.766	-0.147
62.614	62.761	-0.147
67.046	66.899	0.147
48.440	48.293	0.147

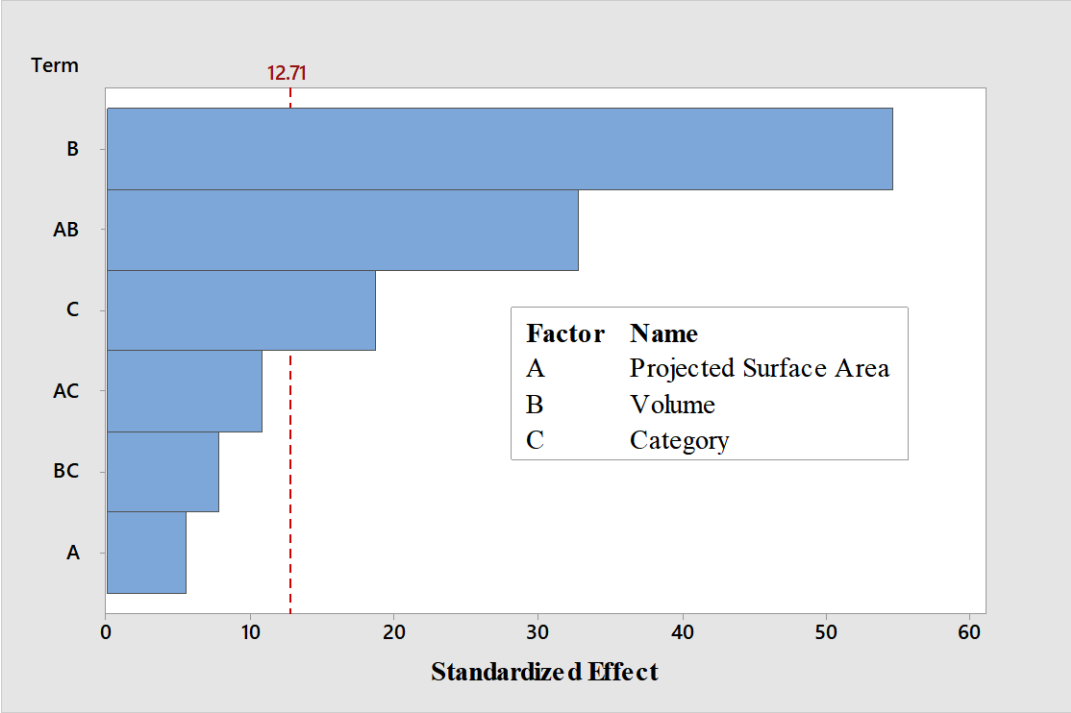


Fig. 12: Pareto chart of the standardized effects (Response is Voltage, α=0.05)

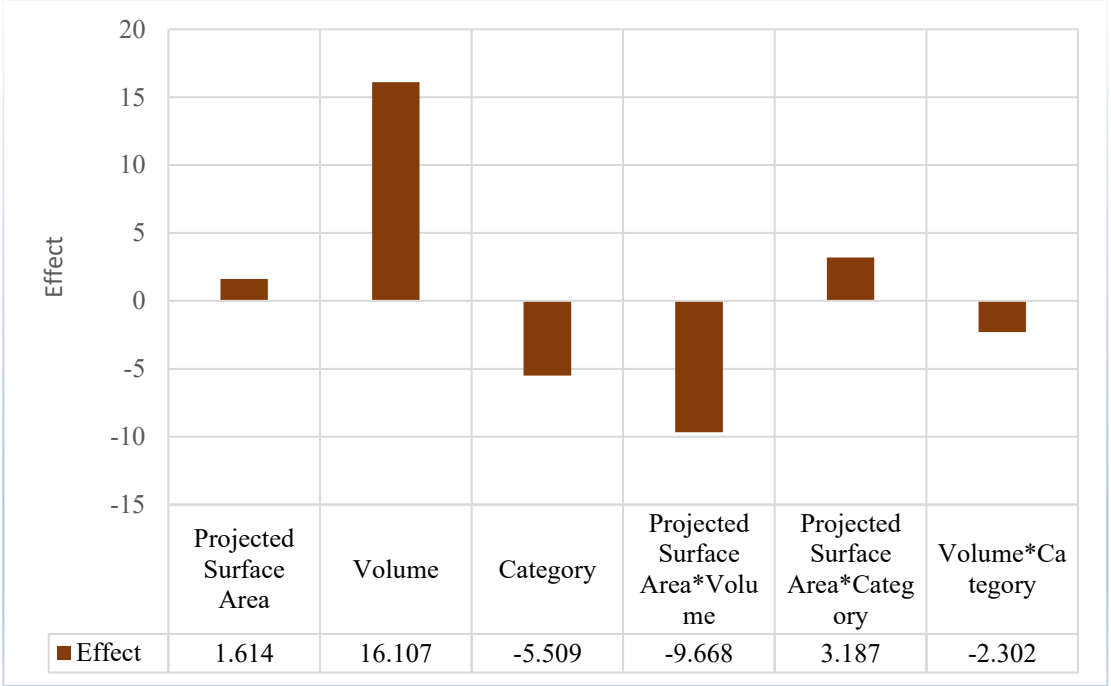


Fig. 13: Effect of the primary and interactive factors

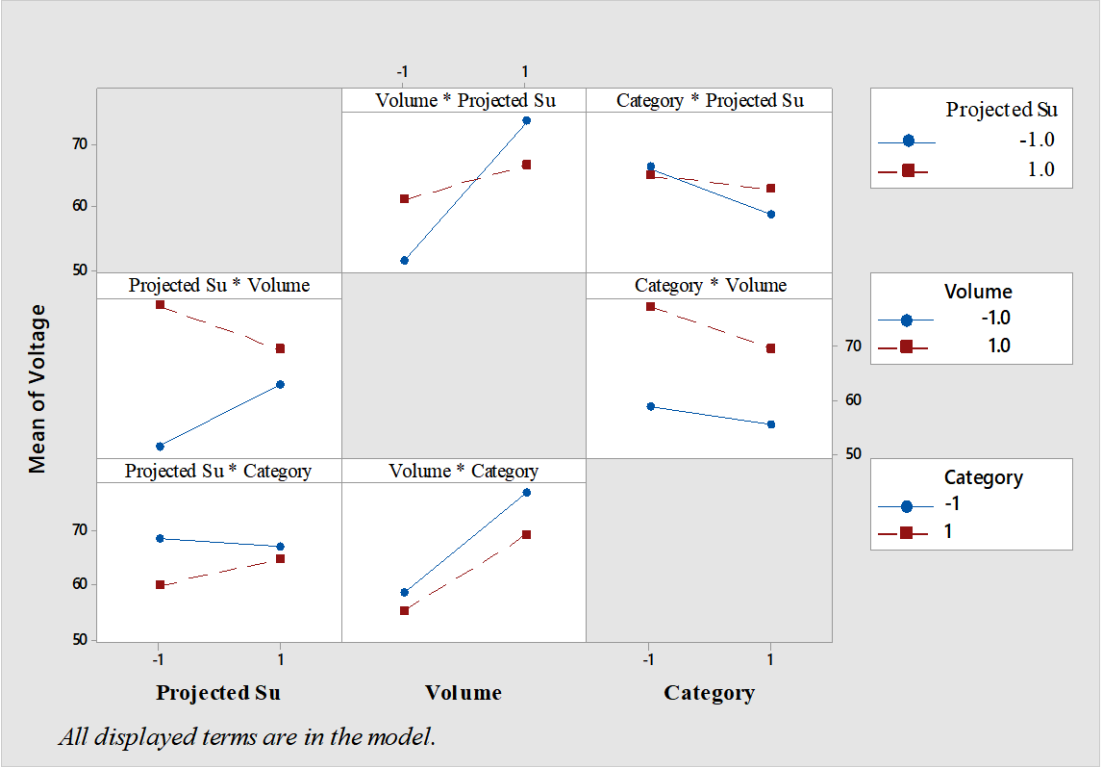


Fig. 14: Interactive plots

4.0 Conclusion

The feasibility of treating and recovering energy from abattoir wastewater was evaluated using mediator-free single-chamber membrane-less AWMFCs of varying volumes and electrode surface areas. The maximum power generation was 64.75 mWm^{-2}, while a 32% COD removal was the highest achieved. The reactors reduced the organic matter of the wastewater and simultaneously generated bioenergy. Power and organic content were inversely proportional until certain stages, after which this assertion was no longer held.

Also, a predictive model for the reactors' performance was developed. Therefore, MFC technology can be designed and optimized to achieve increased power output. It has prospects to enhance energy access and become part of the Wastewater Treatment Plant (WWTP).

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