



# Effect of Salt Enrichment on Electricity Generation and Waste Water Treatment in a Microbial Fuel Using Oxygen as Electron Acceptors

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**Abstract:** One way to reduce treatment cost while producing useful products from wastewater is the microbial fuel cell (MFC) technology. It provides a means of treating wastewater with simultaneous production of energy. In the present study, the H-type MFC was used to study the effect of salt enrichment on electricity generation using carbon and copper electrodes with oxygen as electron acceptor. Wastewater from septic tank was used as substrate, one set of which was enriched with 1M NaCl. The voltage and current generated were monitored using a multimeter which was connected to the anode and cathode by a copper wire. Oxygen was used as electron acceptor at the cathode chamber. Voltage and current readings were taken per hour per day for 15 days. Wastewater treatability was determined by comparing the biological oxygen demand (BOD), dissolved oxygen (DO), chemical oxygen demand (COD) and total dissolved solid (TDS) of the wastewaters before and after treatment. The results show that the fuel cells generated voltages and currents that varied according to the electrode used. Salt enrichment enhanced the efficiency of both current and voltage generation in the MFCs. Carbon electrode MFC performed better than copper electrode MFC. Both the enriched and the un-enriched MFCs had very high percentage removal of BOD, DO, COD and TDS in both carbon and copper electrode MFCs. DO and BOD percentage removals were at least 60% in all the MFCs while the CODs removals were at least 50% in all the treatments. The least percentage wastewater parameter removal was observed in TDS of salt-enriched copper electrode MFC. The study proved that wastewater can be conveniently treated using MFC. The best option would be to use salt-enrichment in a carbon-electrode microbial fuel cell.

**Keywords:** MFC, Waste Water Treatment, Electricity Generation, Salt Enrichment

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## 1. Introduction

Production of electrical energy using microorganisms through microbial fuel cells (MFC) is one such renewable and sustainable technology that is considered to be one of the most efficient [1, 2] and carbon neutral energy sources [3]. MFCs are fuel cells that are capable of converting chemical energy available in organic substrates into electrical energy using bacteria as a biocatalyst to oxidize the biodegradable substrates. The fact that bacteria can oxidize the substrates to produce electricity makes MFCs an ideal solution for wastewater treatment and domestic energy production. Logan

[4] reported that MFCs can generate power densities as much as  $1\text{kW/m}^3$  of reactor volume. MFC as a source of bioenergy production has accelerated the research worldwide and the technical aspects of MFCs have been reviewed extensively [5].

MFCs are a promising technology for organic waste treatment and sustainable bioelectricity production [6, 3, 7]. Functionally, MFCs are bio-electrochemical reactors where microbes convert the chemical energy contained in organic substrates into electric energy by transferring electrons from the substrate directly or indirectly to the anode in the cell [7, 8, 9]. Such fuel cells can be operated in batch-mode or in continuous mode. Diverse MFCs designs and set-ups have

been described with different anode and cathode arrangements and they can be fed with varying substrates, such as carbohydrate, acetate and other volatile fatty acids (VFAs), H<sub>2</sub> or more complex and mixed feedstock, for example wastewaters, or even more recalcitrant biomaterials such as lignocellulose and chitin [10, 11, 12]. They can be inoculated with a monoculture, or with a natural inoculum containing an unknown mix of species [9, 6, 13]. Different feedstock and operating conditions will typically lead to the development of different microbial consortia [14, 15], and even with identical starting and operating conditions consortia may diverge towards different phylogenetic compositions [16, 17, 18].

It is well known that various species of bacteria called exoelectrogens can transfer electrons of substrates to anode [19, 5]. Previous studies showed that *E. coli* [20], *Shewanella putrefaciens* [21], *Shewanella oneidensis* [22], *Geobacteraceae sulferreducens* [23], *Rhodospirillum rubrum* [24] could be used for electricity generation. Several studies showed that organic compounds such as glucose [24], acetate or butyrate [25], domestic wastewater [26, 27], swine wastewater [28], beer brewery wastewater [29], chocolate industry wastewater [30] were used as substrate in MFC. In 2004 the research studies showed that there is a directly relationship between electricity production from MFC and wastewater treatment [19]. Ahn and Logan [26] in a study conducted on domestic wastewater treatment with MFC found out that Power density and COD removal were 422 mW/m<sup>2</sup> and 25.8% respectively. According to Junqiu Jiang, MFC can generate electricity from sewage sludge and Total chemical oxygen demand (TCOD) of the sludge was reduced to 46.4% [27]. Rismani-Yazdi *et al* [31] reported that in a bioconversion of cellulose into electrical energy in microbial fuel cells, maximum power density reached 55 mW/m<sup>2</sup> (1.5 mA, 313mV). In a study conducted on the capability of converting glucose to electricity at high rate, it was found out that power density was 3.6 W/m<sup>2</sup> and electron recovery was up to 89% [32].

The present study was conducted to verify the effect of salt enrichment on microbial fuel cell using oxygen as electron acceptor in an H-type cell whose electrodes were made of either copper or carbon rod.

## 2. Materials and Method

### 2.1. Sample Collection

The wastewater was collected from the hostel area of Federal University of Technology, Owerri, Nigeria. It was from the hostel septic tank that was evacuated for disposal. The wastewater sample was collected using sterile container and transported to laboratory on ice. Analysis of the sample was done within 1hr of collection.

### 2.2. Microbial Fuel Cell Preparation

The salt bridge was prepared using a mixture of molten 2% Agar-agar and 1M NaCl in a 12cm length by 1-inch diameter

PVC pipe. This was used as proton exchange membrane (PEM). The electrodes were made up of copper and graphite rods of 15.5cm length and 1.4cm diameter. Both the anode and cathode chambers were made with 1 litre containers and connected to each other with the salt bridge.

The sample was collected the same day the set-up was coupled. The set-up was coupled by joining the two chambers using the salt bridge with the aid of the adopter using topgit gum. The wastewater was placed into the anode as the anolyte while water was placed in the cathode. The anode chambers of one set of the MFC were enriched with 1M NaCl while the other set was left un-enriched. The lids of the cathode chambers were used to passively aerate the cathode using oxygen as the terminal electron acceptor. The multimeter was connected to the cathode and the anode with the aid of the low resistance copper wire before they were inserted into the chambers. The initial voltage and current readings were taken at time 00 and allowed to acclimatize for 30minutes to 1 hour before subsequent readings were taken. Readings were taken on hourly basis for 15 days and the average was taken as the daily readings.

### 2.3. Microbiological Analysis

The setup was allowed to run for 15days, after which the MFC was decoupled and a sterile swab was used to scrape the electrodes in order to collect the microbial community in the biofilms. This was used to prepare a stock solution. A tenfold serial dilution was done and aliquot volumes (0.1ml) of the dilution was inoculated into freshly prepared nutrient agar, mackonkey agar, manittol salt agar, and the Eiosin methylene blue agar. The inoculated plates were incubated at a temperature of 37°C for 24 hours for the aerobic culture while for the anaerobic culture, incubation was done in an anaerobic jar at room temperature for 5-7days using nutrient agar.

Microorganisms isolated from the samples were characterized based on the colonial, morphological and biochemical characteristics of the pure cultures.

### 2.4. Waste Water Treatment Ability

The ability of the microbial fuel cell to treat waste water was examined by determining the BOD, DO, COD, and TDS of the waste water before and after 15 days of treatment using the HANA multiparameter analyzer. The efficiencies (percentages) of the microbial fuel cell to remove any of the parameters were calculated using the following expression:

$$efficiency = \frac{initial\ parameter\ value - final\ parameter\ value}{initial\ parameter\ value} \times 100 \quad (1)$$

## 3. Result

Voltage and current of the microbial fuel cell (MFC) were measured at hourly intervals and the average of the readings was taken on daily basis. The result of the copper electrode MFC shows that the un-enriched MFC had higher voltage on the first day which reduced sharply with increase in the number of days and came to a halt on the sixth day of the

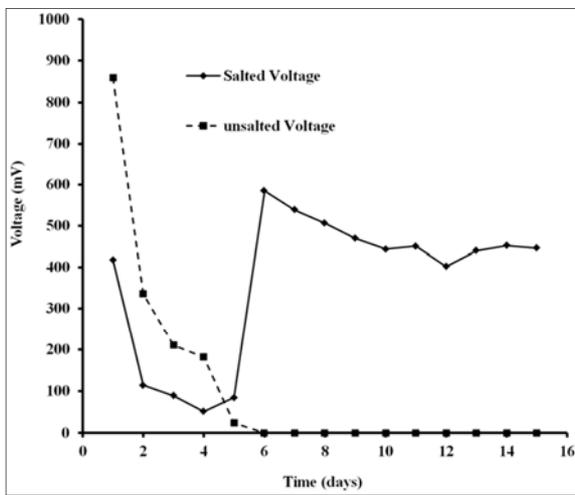
study (Figure 1). The curve fitted into a sigmoid 5 parametric model with an R<sup>2</sup> value of 0.74696. The equation of the curve is stated below:

$$y = y_0 + \frac{a}{1 + e^{-(x-x_0/b)}} \quad (2)$$

The parametric values are as follows:

**Table 1.** Parametric values of the sigmoid 5 parametric model for the unenriched NFC.

Parameter	Value
A	3.22E+02
B	1.40E-02
C	1.31E+00
x0	5.40E+00
y0	1.51E+02



**Figure 1.** Effect of salt enrichment on the voltage of an MFC using copper electrode.

For the salt-enriched voltage, the voltage was equally high on the first day of the study but lower than the voltage of the un-enriched MFC. The voltage also reduced sharply to the lowest value on the 4<sup>th</sup> day as depicted in figure 1. After the fourth day, the voltage increased exponentially to reach the peak value of 584.57 mV on the sixth day, after which, the voltage reduced gradually to 445.92 mV at the end of the study. The curve also fitted into a sigmoid 5 parametric model with an R<sup>2</sup> value of 0.9814 and the equation of the curve is as stated in equation 1.0 above. The parametric constants are as follows:

**Table 2.** Parametric values of the sigmoid 5 parametric model for the salt-enriched NFC

Parameter	Value
A	87770
B	-0.4954
C	0.3679
x0	-5.262
y0	0.4982

The current generated in both the salt-enriched and the un-enriched MFC also followed the same pattern as the voltage

as presented in figure 2. Both cells had high current values at the beginning of the study which reduced sharply from the second day of study. While the un-enriched MFC generated no more current after the fifth day of study, the current value in the salt-enriched MFC increased exponentially from the fifth day to a peak current value of 0.517 mA on the sixth day of study. The value started reducing gradually from the seventh day to the thirteenth day, after which, it increased slightly on the fourteenth day and reduced to the value of 0.263 mA on the final day of study. Both the salt-enriched and the un-enriched curves followed a Rational 8 parametric model with the equation of the curve as shown below:

$$y = \frac{a+bx+cx^2+dx^3}{1+ex+fx^2+gx^3+hx^4} \quad (3)$$

The R<sup>2</sup> value for the salt-enriched MFC is 0.912693 while the parametric constants are as follows:

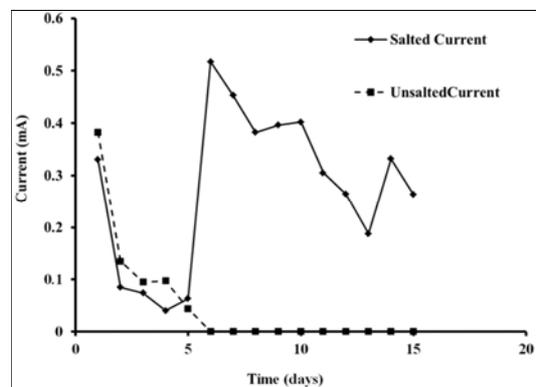
**Table 3.** Parametric values of the rational 8 parametric model for the salt-enriched MFC.

Parameter	Value
A	3.11E-01
B	-1.68E-01
C	2.75E-02
D	-1.25E-03
E	-3.55E-01
F	2.42E-02
G	2.75E-03
H	-2.24E-04

For the un-enriched MFC, the R<sup>2</sup> value is 0.999571 while the parametric constants are as follows:

**Table 4.** Parametric values of the rational 8 parametric model for the unenriched MFC.

Parameter	Value
a	-2.190e-1
b	9.161e-2
c	-1.256e-2
d	5.640e-4
e	-2.196e+0
f	9.881e-1
g	-1.670e-1
h	9.685e-3



**Figure 2.** Effect of salt enrichment on the current of an MFC using copper electrode.

Figure 3 shows the voltage generated by both salt-enriched and un-enriched MFCs using carbon electrodes. The voltage of the salt-enriched cell decreased slightly from the first day till the fifth day and increased exponentially till the tenth day. It reduced progressively till the end of the study. The un-enriched cell voltage dropped sharply from the first day to the second day, and remained relatively steady till the fourth day. It dropped sharply to a halt on the 6<sup>th</sup> day of study. The curve generated by the salt-enriched carbon electrode cell followed a rational 5 parametric model which was as stated below:

$$y = \frac{a+bx+cx^2}{1+dx+ex^2} \tag{4}$$

The R<sup>2</sup> value of the curve is given as 0.9885 while the parametric values are as follows:

**Table 5.** Parametric values of the rational 5 parametric model for the salt-enriched MFC.

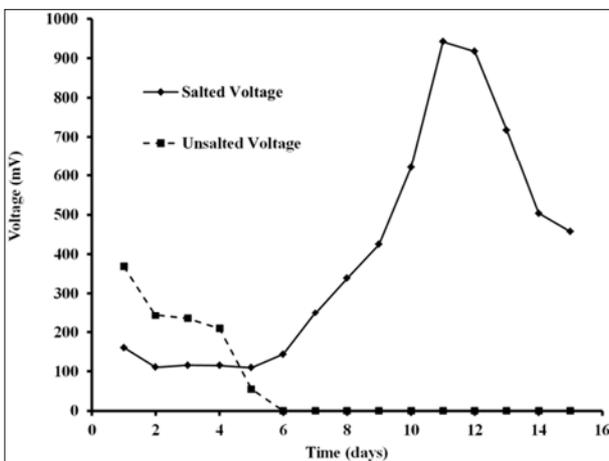
Parameter	Value
a	1.161e+2
b	-1.801e+1
c	9.548e-1
d	-1.718e-1
e	7.652e-3

The curve generated by the un-enriched carbon electrode cell followed a sigmoid 3 parametric model with an R<sup>2</sup> value of 0.956596 and the equation of the curve and the parametric values are as follows:

$$y = \frac{a}{1+e^{-\left(\frac{x-x_0}{b}\right)}} \tag{5}$$

**Table 6.** Parametric values of the sigmoid 3 parametric model for the un-enriched MFC.

Parameter	Value
a	3.246e+2
b	-8.068e-1
x <sub>0</sub>	4.019e+0



**Figure 3.** Effect of salt enrichment on the voltage of an MFC using carbon electrode.

The current generated by both salt-enriched and un-enriched cells using carbon electrodes are presented in figure

4. The salt-enriched cell current was relatively steady from the first day till the 4<sup>th</sup> day. On the 5<sup>th</sup> day the current reduced slightly and increased abruptly till the 8<sup>th</sup> day before it reduced slightly on the 9<sup>th</sup> day. It increased exponentially after the 9<sup>th</sup> day and reached its peak on the 11<sup>th</sup> day before reducing sharply on the 12<sup>th</sup> day. It increased again slightly on the 13<sup>th</sup> day and reduced progressively till the end of the study. The curve followed a rational 8 parametric model as presented in equation (2.0) with an R<sup>2</sup> value of 0.964333 and the parametric values were as follows:

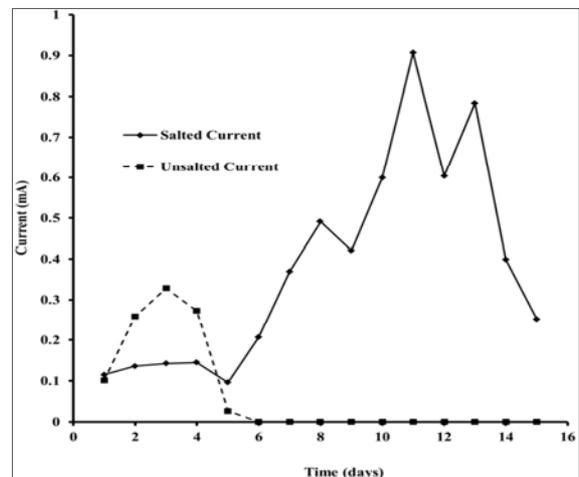
**Table 7.** Parametric values of the rational 8 parametric model for the salt-enriched MFC.

Parameter	Value
a	1.078e-1
b	-3.660e-2
c	3.549e-3
d	-1.037e-4
e	-4.326e-1
f	6.538e-2
g	-4.224e-3
h	1.001e-4

The un-enriched carbon electrode cell current increased exponentially until it reached its peak on the 4<sup>th</sup> day, it then reduced progressively till the 6<sup>th</sup> day when it came to a halt. The curve also followed a rational 8 parametric model (equation 2.0) with an R<sup>2</sup> value of 1 and the parametric values were as follows:

**Table 8.** Parametric values of the rational 8 parametric model for the un-enriched MFC.

Parameter	Value
a	4.297e-2
b	-1.640e-2
c	2.013e-3
d	-7.888e-5
e	-1.123e+0
f	4.962e-1
g	-9.861e-2
h	7.343e-3



**Figure 4.** Effect of salt enrichment on the current of an MFC using carbon electrode.

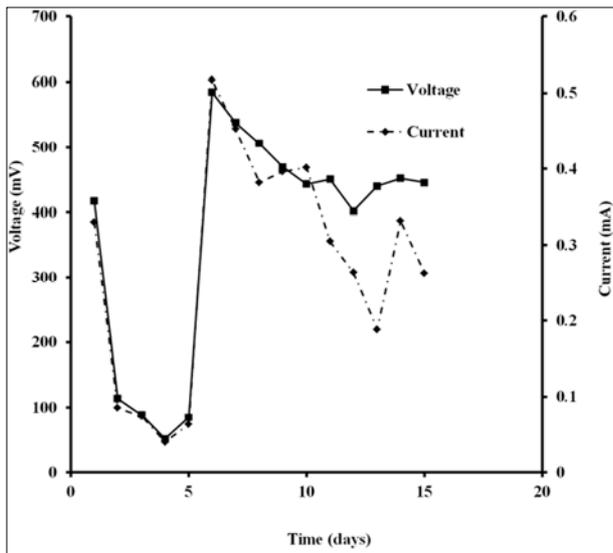


Figure 5. Changes in current and voltage with time in a salt-enriched copper electrode MFC.

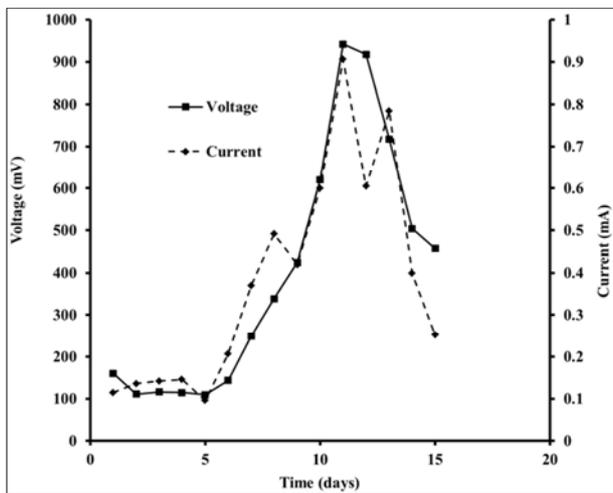


Figure 6. Changes in current and voltage with time in a salt-enriched carbon electrode MFC.

The changes in current and voltage with time in both salt-enriched copper and carbon electrodes of the microbial fuel cells are presented in figure 5 and 6, respectively. In the copper electrode cell, the voltages and currents curves followed the same pattern. There were high voltages and currents on the first day of the study which both progressively reduced to their lowest values on the 4<sup>th</sup> day. There were slight increases in the values on the 5<sup>th</sup> day, after which, there were exponential increase in both values till they reach their peaks on the 6<sup>th</sup> day. Both values decreased irregularly afterwards till the end of the study. In the carbon electrode cell, the curves also followed almost the same pattern. The values were relatively steady till the 5<sup>th</sup> day, the values then increased exponentially till both of them reached their peaks on the 10<sup>th</sup> day. After the 10<sup>th</sup> day, their values decreased irregularly till the end of the study. In the un-enriched copper cell, the voltage and current values followed the same pattern as well. Both values started at very high

values on the first day and decreased exponentially till the 6<sup>th</sup> day when both values came to zero, (figure 7).

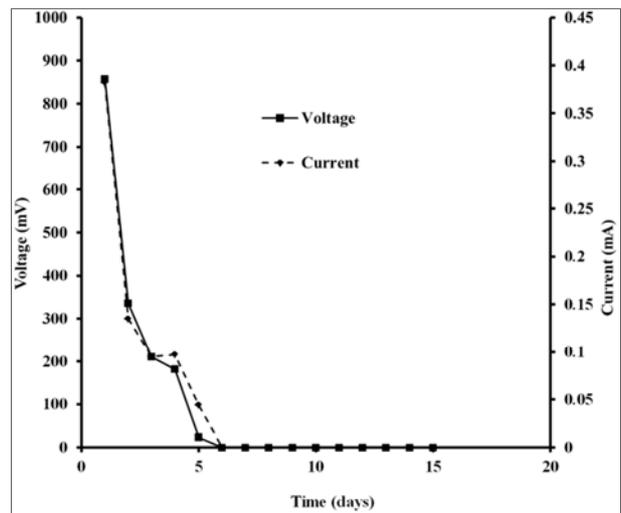


Figure 7. Changes in current and voltage with time in an unenriched copper electrode MFC.

In the un-enriched carbon cell, the current generated was low on the first day of the study and it increased exponentially till it reached its peak on the 3<sup>rd</sup> day. After the 3<sup>rd</sup> day, the current value decreased progressively till the 6<sup>th</sup> day when the value came to zero. The voltage had the highest value on the first day and progressively decreased till the 6<sup>th</sup> day when the value also came to zero as depicted in figure 8.

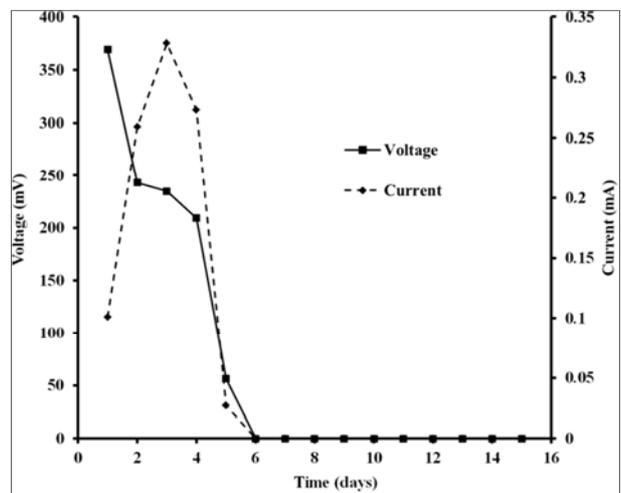


Figure 8. Changes in current and voltage with time in an unenriched carbon electrode MFC.

The percentage waste water parameters removal in the salt-enriched microbial fuel cell is presented in figure 9. The parameters under consideration are Total Dissolved Solid (TDS), Dissolved Oxygen (DO), Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD). There were very high percentage removal of DO, COD and BOD in the carbon electrode while there were very high percentage removals of DO and BOD in the copper electrode. There were low percentage removal of TDS in both cells but carbon

electrode had higher percentage removal of TDS than copper electrode cell. In the un-enriched microbial fuel cell, the highest percentage removal was observed in DO of the carbon electrode. The percentage removal of TDS and COD were higher in the copper electrode cell than the carbon electrode cell. The carbon electrode cell had slightly higher percentage BOD removal than the copper electrode cell, (figure 10).

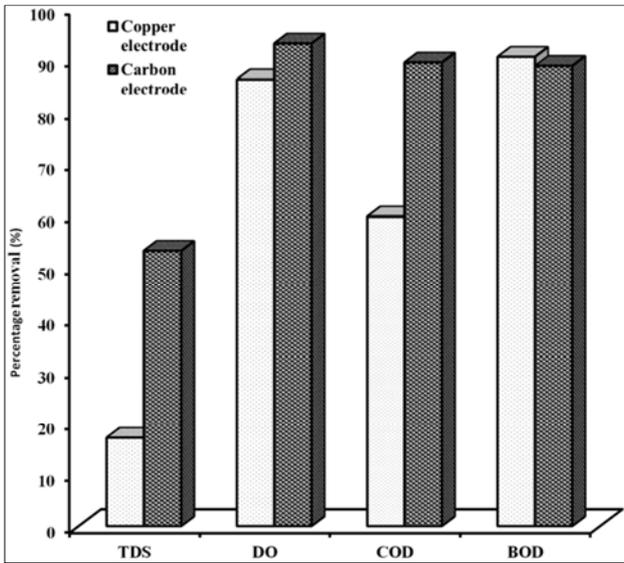


Figure 9. Percentage wastewater parameters removal in salt-enriched MFC.

Key:  
 TDS = Total Dissolved Solid  
 DO = Dissolved Oxygen  
 COD = Chemical Oxygen Demand  
 BOD = Biological Oxygen Demand

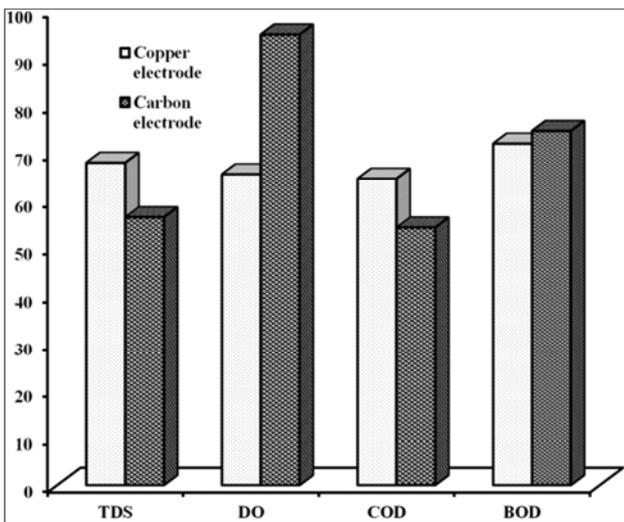


Figure 10. Percentage wastewater parameters removal in the unenriched MFC.

Key:  
 TDS = Total Dissolved Solid  
 DO = Dissolved Oxygen  
 COD = Chemical Oxygen Demand  
 BOD = Biological Oxygen Demand

The comparative percentage wastewater parameter removal in the salt-enriched and un-enriched copper electrode MFC is presented in figure 11.

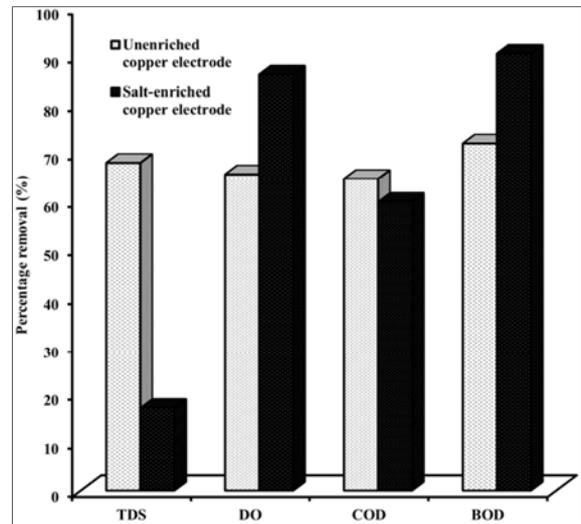


Figure 11. Comparative percentage wastewater parameters removal in the enriched and unenriched copper electrode MFC.

Key:  
 TDS = Total Dissolved Solid  
 DO = Dissolved Oxygen  
 COD = Chemical Oxygen Demand  
 BOD = Biological Oxygen Demand

Highest percentage removals were observed in DO and BOD of the salt-enriched copper electrode cell while the least percentage removal was observed in TDS of the same cell. All the parameters had appreciable percentage removal in the un-enriched copper electrode cell. In the carbon electrode cell presented in figure 12, the percentage removal of DO, COD and BOD were very high in the salt-enriched electrode. However, the highest percentage removal was observed in DO of the un-enriched electrode cell. Other parameters had fairly high percentage removal in the un-enriched carbon electrode cell.

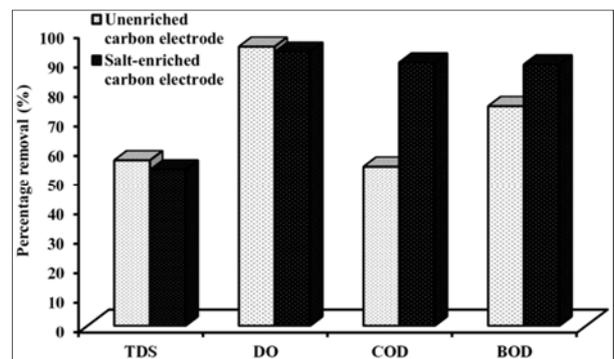


Figure 12. Comparative percentage wastewater parameters removal in the enriched and unenriched carbon electrode MFC.

Key:  
 TDS = Total Dissolved Solid  
 DO = Dissolved Oxygen  
 COD = Chemical Oxygen Demand  
 BOD = Biological Oxygen Demand

The organisms isolated from the anode electrode were *Bacillus* species, *Streptococcus* species, *Escherichia coli*, and *Staphylococcus aureus*.

## 4. Discussion

The H-shaped MFC is a widely used two-chamber MFC design consisting usually of two bottles or cylindrical chambers connected by a membrane, usually cation exchange membrane (CEM) such as Nafion [33]. In the present study, a proton exchange membrane made of agar agar was used. This is due to the high cost of Nafion CEM.

In MFCs, substrate is regarded as one of the most important biological factors affecting electricity generation [34]. A great variety of substrates can be used for electricity production ranging from pure compounds to complex mixture of organic matter present in wastewater. So far the only objective of various treatment processes is to remove pollutants from waste streams before their safe discharge to the environment. In the last century, activated sludge process (ASP) has been the mainstay of wastewater treatment. However, it is a very energy intensive process and according to an estimate, the amount of electricity needed to provide oxygen in ASP in USA is equivalent to almost 2% of the total US electricity consumption [35]. The emphasis of today's waste management is on reuse and recovery of energy, which has led to new views on how these streams can be dealt with. In the present study, wastewater from septic tank was used as substrate for the recovery of energy and waste treatment. The result can be extrapolated for any wastewater.

The production of current in an MFC is directly linked to the ability of the bacteria to oxidize a substrate and transfer electrons resulting from this oxidation to the anode electrode. The current, power density and pollutant removal efficiencies differ between the various studies according to the experimental conditions. This is in accordance with the result of the present study. Different experimental conditions were made such as the use of different electrodes and salt enrichment, and these conditions affected the currents and voltages generated in the MFCs together with pollutant removal in the wastewater. Salt-enrichment resulted in enhanced and sustained current and voltage generation in both copper and carbon electrode MFCs. The percentage pollutant removal (as measured by TDS, DO, COD and BOD) also varied according to the experimental setup. In the salt-enriched MFC, there were higher percentage reductions of TDS, DO and COD in carbon electrode cell than copper electrode cell. However, although BOD reductions were high in both electrode cells, copper electrode cell had higher percentage reduction than carbon electrode MFC. In the un-enriched MFC, both carbon and copper electrode MFC had high percentage reduction of the wastewater parameters but the highest reduction was observed in DO of the carbon electrode cell. Comparatively, in the copper electrode MFC, salt-enriched cell performed better in DO and BOD removal while un-enriched cell performed better in TDS and COD removal. But in carbon electrode cell, the salt-enriched cell

had higher percentage reduction of COD and BOD while the un-enriched cell had higher percentage reduction of TDS and DO. Copper being a heavy metal, it may have dissolved in the MFC anode chamber thereby inhibiting the growth and performance of the microbial biofilm, which eventually affected the overall performance of the MFC.

An important issue when the anode and cathode are connected is the conductivity of the electrolyte [36]. Waste streams used as substrates have typically low conductivity of 1-2mS/cm and can be improved to achieve high current densities by addition of salts [37]. Salt enrichment in the present study helped to enhance the overall performance of the MFC in both the copper and carbon electrode cells.

It has been known for almost one hundred years that bacteria could generate electricity [38]. But only in the past few years has this capability become more than a laboratory novelty [39]. Microorganisms have the ability to produce electrochemically active substances that may be either the metabolic intermediaries, or the final products of anaerobic respiration. For the purpose of energy generation, these fuel substances can be produced in one place and transported to a microbial fuel cell to be used as fuel [39]. Bacteria are so far known to transfer electrons to a surface but most of the organisms studied were *Geobacter* and *Shewanella* species [40] although *E. coli* has been used as exogenous mediator in MFC [41]. In the present study, bacterial species were isolated from the anode electrode and one of the organisms isolated was *E. coli*. *E. coli* may have acted as an exogenous mediator for the transfer of electrons by the other bacterial species isolated in the study.

## 5. Conclusion

In conclusion, it is worthy of note that wastewater treatment based on MFC offers a great opportunity to develop the technology, as the fuel for electricity production is free 'wastewater' which must be treated. The present study has shown that wastewater could be treated to appreciable and satisfactory level using the microbial fuel cell while at the same time generating electricity. More research into this area of study is hereby solicited mainly in developing countries like Nigeria where wastewater treatment is much of a burden to companies and environmental protection agencies.

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