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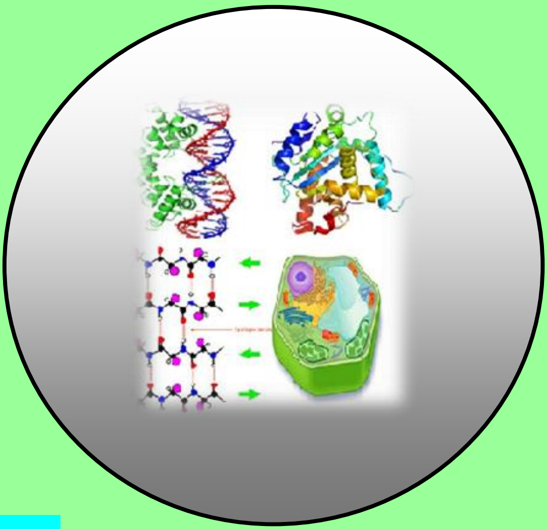

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E.O. Egbadon
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jbiolchemres@gmail.com

RESEARCH PAPER

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Egbadon, E.O., Nweke, C.O., Akujobi, C.O., Braide, W., Akaluka, C.K. and Adeleye, S.A.
Department of Microbiology, Federal University of Technology Owerri, Imo state, Nigeria

ABSTRACT

Dual-chamber Microbial Fuel Cells (MFCs) were constructed using non-reactive polyacrylic containers of 1100ml with a working volume of 1000ml. 1000ml of the abattoir wastewater was fed into the anode chamber while equal volume 100mM Potassium Ferricyanide solution was fed into the cathode chamber. An Agar-salt Bridge (2% Agar and 1% NaCl) with dimension 10cm×3cm (length and radius) served as Proton Exchange Membrane. Rod-shaped carbon electrodes of length and diameter 12 cm × 1.2 cm were used. The Open circuit voltage, current, power density and physicochemical parameters were monitored. An initial Open circuit voltage of 459 mV, Current of 0.22 mA, and Power density of 22.10mW/m^2 were recorded, which increased to give maximum Open Circuit Voltages of 736 mV, Current of 0.46mA, and Power density of 66.43mW/m^2. The results also shows a 56.09%, 92.31%, 56.27%, 89.92%, 73.29% and 75.46% decrease for Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Organic Carbon, Total Soluble solids (TSS), Total Dissolved Solids (TDS), Nitrate, and Nitrate-Nitrogen respectively, while a -3.58%, -3.51%, -4.21%, -228.76%, -226.07% and -226.16% increase was observed for Phosphates, Phosphorus, Orthophosphates, Ammonia, Ammonium-Nitrogen and Ammonium respectively. The bacterial isolates identified were Bacillus species, Streptococcus species, Escherichia coli and Staphylococcus aureus.
Keywords: Abattoir wastewater treatment, Bioelectricity, Microbial Fuel Cell

INTRODUCTION

Recently, much research is focused on alternative and renewable energy sources to replace our current reliance on fossil fuels. It is evident that mankind is increasingly dependent on energy with the advancement of science and technology (Mathuriya and Sharma, 2009) and also increase in economic growth and social development. Energy needs in the world continue to increase, driving demand at an unsustainable pace. There are several alternatives to oil that are also carbon-based such as methane hydrates and the conversion of coal into methane gas and the use of readily available oil reservoirs. Available alternatives are accompanied with an increase in the emission of carbon (iv) oxide which affects the ozone layer and an impending global energy crisis, thus, there is need for an alternative and renewable energy sources.

On the other hand, waste generation is a continuous aspect of life (Adeleye and Okorondu, 2015) and large volumes of high-strength wastewaters are produced from domestic and agricultural processes (Min *et al.,* 2005). It is necessary to treat wastewater prior to being released into the environment to avoid ground water pollution and other problems that may have negative implications on the environment. Bitton (2005) reiterates the objectives of waste water treatment. Anaerobic treatment technologies provide potential for reducing treatment costs, but these technologies are generally only suitable for high-strength wastewaters streams typically produced by industries. In addition, conventional anaerobic treatment produces high level of methane-gas which when released into the atmosphere contributes to global warming. One method has been advocated to reduce wastewater treatment cost, finding ways to produce useful products from wastewater is the Microbial Fuel Cell (MFC) technology (Liu *et al.,* 2004), which provides a means of treating wastewater with a simultaneous production of energy.

In a MFC, bacteria present in the wastewater are used as catalyst to oxidize organic and inorganic matter present in the wastewater to generate current (Logan *et al.,* 2006). Electrons produced by the bacteria from these substrates are transferred to the anode (negative terminal) and flow to the cathode (positive terminal) linked by a conductive material containing a resistor, or operated under a load (i.e, producing electricity that runs a device). By convention, positive current flows from the positive to the negative terminal, a direction opposite to that of electron flow. The device must be capable of having the substrate oxidized at the anode replenished, either continuously or intermittently; otherwise, the system is considered to be a biobattery (Logan *et al.,* 2006). Electrons can be transferred to the anode by electrons mediators or shuttles by direct membrane associated electron transfer or by so-called nanowires produced by the bacteria or perhaps by other as yet undiscovered means (Rabaey *et al.,* 2005). In most MFCs the electrons that reach the cathode combine with protons that diffuse from the anode through a separator and oxygen provided from air; the resulting product is water (Bond *et al.,* 2002).

Microbially catalyzed electron liberation at the anode and subsequent electron consumption at the cathode, when both processes are sustainable, are the defining characteristics of an MFC (Logan *et al.,* 2006).MFC have been constructed using mixed cultures as well as pure cultures. However, MFCs operated using mixed cultures have given substantially greater power densities than those with pure cultures (Rabaey*et al.,* 2005).MFCs are being constructed using a variety of materials, and in an ever increasing diversity of configurations. These systems are operated under a range of conditions that include differences in temperature, pH, electron acceptor, electrode surface areas, reactor size, and operation time (Logan *et al.,* 2006) as this conditions have proven to have a great effect on the power density as well as the wastewater cleanup capacity of a MFC.

The aim of this research study is to generate electricity from abattoir wastewater and simultaneously treat the wastewater using the Microbial Fuel Cell technology.

MATERIAL AND METHODS

Collection of Swine and Abattoir Wastewater Sample

The abattoir wastewater samples were collected from a cow slaughter house at relief market, Egbu road, Owerri, Imo state, Nigeria. The samples were collected according to the methods described by (Lokhande *et al.,* 2011). The wastewater samples were collected in a four (4) litre container that has been washed with alcohol, thoroughly washed with water, again rinsed with the swine and abattoir wastewater sample to be collected and then filled up with the wastewater samples. The sample containers were then covered. Wastewater samples were carefully taken to the microbiology laboratory for isolation and identification using selective media.

Identification of Potential Isolates

Isolates obtained from selective media are then identified on the basis of staining procedures and biochemical tests as prescribed by Bergey' Manual of Systematic Bacteriology 10^{th} Edition.

Physicochemical analysis

The physicochemical analyses were carried out using HANA instruments (USA). Parameters monitored include; pH, Temperature, Conductivity (µS/cm), Chemical Oxygen Demand (COD) (mg/l), Biochemical Oxygen Demand (BOD) (mg/l), Organic Carbon (%), Total Soluble Solids (mg/l), Total Dissolved Solids (mg/l), Nitrate (mg/l), Nitrate-Nitrogen (mg/l), Phosphate (PO_4^{3-}) (mg/l), Phosphorus (P) (mg/l), Phosphate (P_2O_5) (mg/l), Ammonia (mg/l), Ammonia-Nitrogen (mg/l) and Ammonium (mg/l).

Construction of Microbial Fuel Cell

The traditional Dual-chamber MFC was constructed using non-reactive polyacrylic containers of 1100ml with a working volume of 1000ml. One plastic container was used as the anode chamber and the other plastic container used as the cathode chamber. 1000ml of the wastewater was fed into the anode chamber while the catholyte (100mM Potassium Ferricyanide solution) was fed into the cathode chamber. The Agar-salt Bridge (Proton exchange membrane) was used as the connector between the anode and cathode chamber and was prepared according to methods described in Min *et al.,* (2005). The Agar-salt Bridge (Proton exchange membrane) was prepared using 2% Agar and 1% NaCl. The length and diameter of the agar-salt bridge 10cm×3cm respectively. The electrodes used were rod-shaped carbon electrodes of length and diameter 12 cm × 1.2 cm respectively. The electrodes were placed in the anode and cathode chamber and connected to a copper wire (Logan, 2005), then sealed and made air-tight. A schematic drawing of the set-up is shown in Fig 1.

Operation Condition of MFC

The Abattoir wastewaters was used as the substrate as well as the source of the inoculum such that the bacteria present will utilize the nutrient present and attach to the electrodes to produce electricity. No other form of nutrients was added except the nutrients present in the wastewater and the pH was not adjusted.

The rod-shaped carbon electrodes were sanded lightly to facilitate bacterial attachment to the surface of the electrodes (Logan. 2007). The ambient temperature varied between 27^{o}C to 32^{o}C throughout the period of the experiments. The setup were allowed to stand for 21 days and open circuit voltage and current measured at intervals of 3 hours each day using a digital multimeter (Model: DT830).

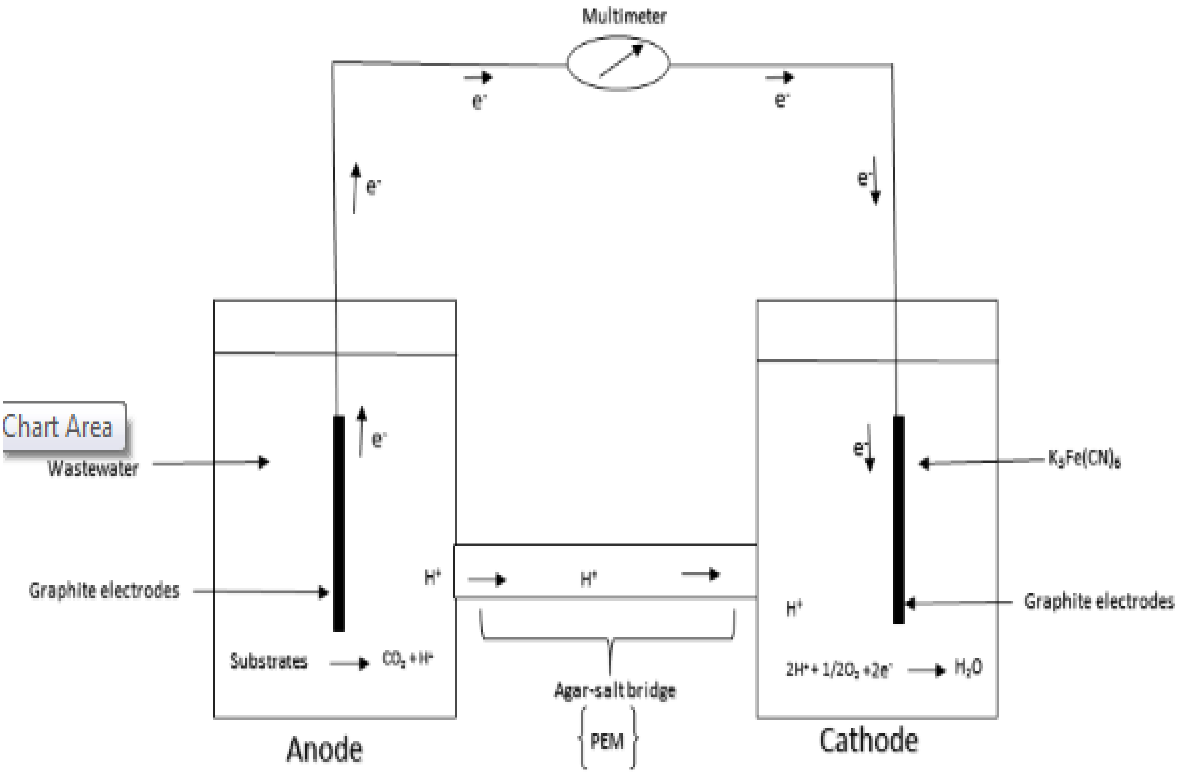

Figure 1. Schematic drawing of the Dual-chamber MFC used.

Determination of Percentage (%) change of Physicochemical Parameters

The percentage change of physicochemical parameters before and after operation of the MFC was determined using the formula below:

$$Percentage\ (\%)\ Change = \frac{initial - final}{initial} \times 100$$

The percentage change in physicochemical parameter gives an indication of increase or decrease of the physicochemical measured in percentage usually termed Waste water treatment efficiency (WWTE) after 21 days (504 hours) of operation of MFC.

Preparation of Catholyte

The catholyte used was Potassium Ferricyanide at a concentration of 100mM (Park and Zeikus, 2002). It was prepared by dissolving 32.92 grams of the salt in 1000 ml of distilled water.

Determination of Power Density

The power density (P) obtained from this experiment normalized by the projected surface area of the graphite rod anode (m^2) was determined using the formular; (Rabaey *et al*., 2005; Momoh and Nyeayor. 2010).

$$Power\ density = \frac{Current\ (mA)\ \times Volts\ (V)}{\text{Surface area of projected anode } (\text{m}^2)}$$

Where P is expressed in mW/m^2.

RESULT AND DISCUSSIONS

Microbiological Analysis

The isolates identified were *Bacillus* species*, Streptococcus* species*, Escherichia coli* and *Staphylococcus aureus.* However, there was a decrease in the number of bacterial population after treatment with MFC.

Physicochemical Analysis of Abattoir Wastewater before and After Treatment

The results of changes in physicochemical characteristics are shown in Table 1. The Chemical Oxygen Demand (COD) decreased from 8200mg/l to 3600mg/l to give a percentage decrease of 56.09%. A higher decrease was recorded for Biochemical Oxygen Demand (BOD) after it decreased from 1300mg/l to 100mg/l giving a 92.31%. Organic carbon concentration in abattoir wastewater also gave a 56.27% decrease after the concentration reduced to 0.621% from 1.42%. Nitrate and Nitrate-Nitrogen present in the wastewater gave a percentage decrease of 73.29% and 75.46% after showing decrease from 1917mg/l to 512mg/l and 432mg/l to 106mg/l respectively. After treatment, some parameters however increased Such as Phosphates, Phosphorus, Orthophosphates, Ammonia, Ammonia-Nitrogen and Ammonium which increased from 699mg/l to 724mg/l, 228mg/l to 236mg/l, 522mg/l to 544mg/l, 142mg/l to 464mg/l, 117mg/l to 381.5mg/l and 151mg/l to 492.5mg/l respectively. The results shows a 56.09%, 92.31%, 56.27%, 89.92%, 73.29% and 75.46% decrease for Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Organic Carbon, Total Soluble solids (TSS), Total Dissolved Solids (TDS), Nitrate, and Nitrate-Nitrogen respectively, while also showing a -3.58%, -3.51%, -4.21%, -228.76%, -226.07% and -226.16% increase for Phosphate, Phosphorus, Orthophosphates, Ammonia, Ammonium-Nitrogen and Ammonium respectively.

Open Circuit Voltage Generated During MFC Operation of Abattoir Wastewater.

The results for the Open Circuit Voltage (OCV) generated during the 21 day operation of MFC for treatment of Abattoir wastewater is shown in Figure 2. The Open Circuit Voltage (OCV) was recorded at an interval of 3 hours per day and the maximum voltage generated was 758 mV (0.758 V). The initial voltage recorded on the first three hours after pitching was 459 mV which slowly increased within 156 hours to peak at 758 mV 156. There was however a drop in voltage to 426 mV after 300 hours further decreasing to 297 mV after about 420 hours, then to 150 mV after 504 hours. There was a steady increase in the voltage within the first few days. This steady increase also continued after the voltage peaked but followed by a sharp drop in voltage from 644 mV to 436 mV between 294 hours and 300 hours.

Table 1. Physicochemical characteristic of Abattoir wastewater.

Physicochemical Parameter	Before Treatment	After Treatment	Percentage (%) Change
pH	7.6	7.9	
Conductivity (µS/cm)	1777	11550	
Chemical Oxygen Demand (COD) (mg/l)	8200	3600	56.09
Biochemical Oxygen Demand (BOD) (mg/l)	1300	100	92.31
Organic Carbon (%)	1.42	0.621	56.27
Total Soluble Solids (mg/l)	9298	937	89.92
Total Dissolved Solids (mg/l)	1155.05	7507.5	
Nitrate (mg/l)	1917	512	73.29
Nitrate-Nitrogen (mg/l)	432	106	75.46
Phosphate (PO_4^{3-}) (mg/l)	699	724	-3.58
Phosphorus (P) (mg/l)	228	236	-3.51
Phosphate (P_2O_5) (mg/l)	522	544	-4.22
Ammonia (mg/l)	142	464	-226.76
Ammonia-Nitrogen (mg/l)	117	381.5	-226.07
Ammonium (mg/l)	151	492.5	-226.16

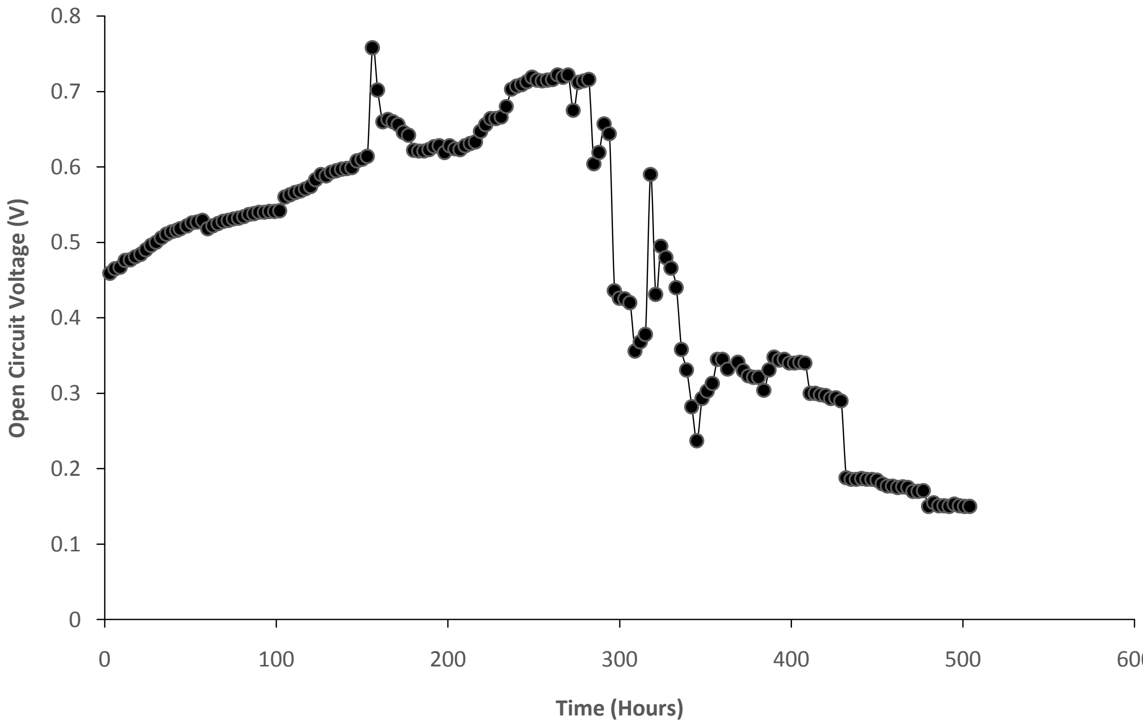

Figure 2. Open circuit voltage (OCV) generated per Time during treatment of Abattoir wastewater.

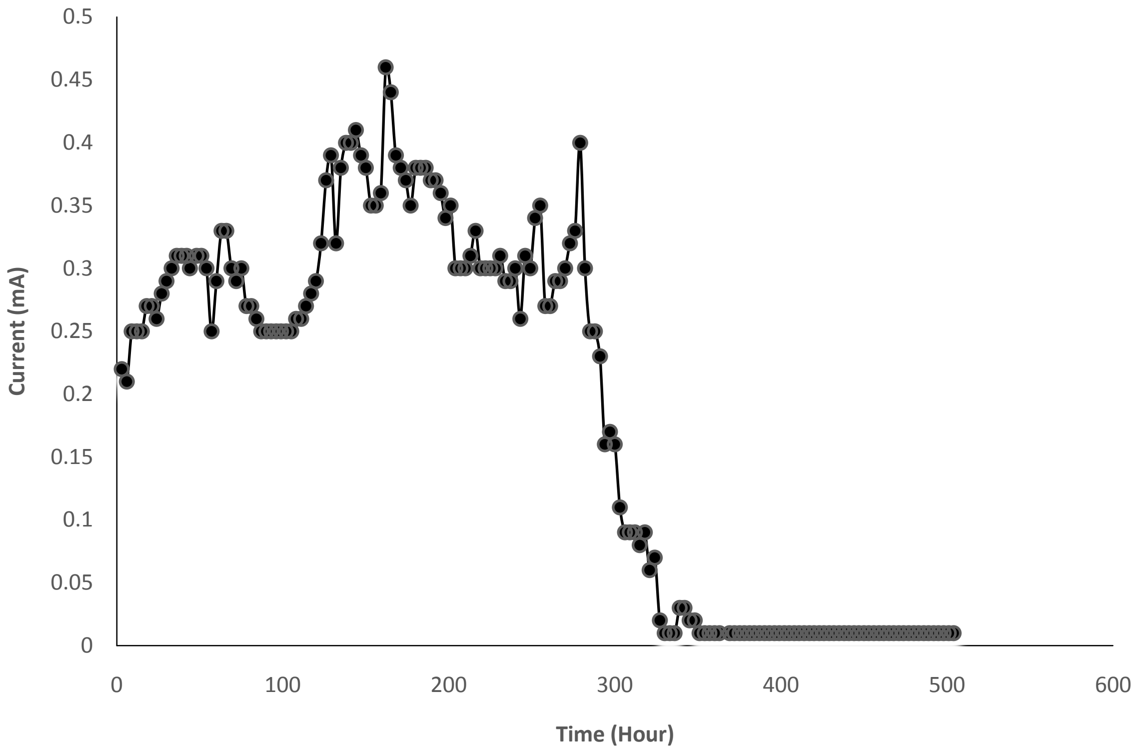

Figure 3. Current generated per time obtained during treatment of Abattoir wastewater.

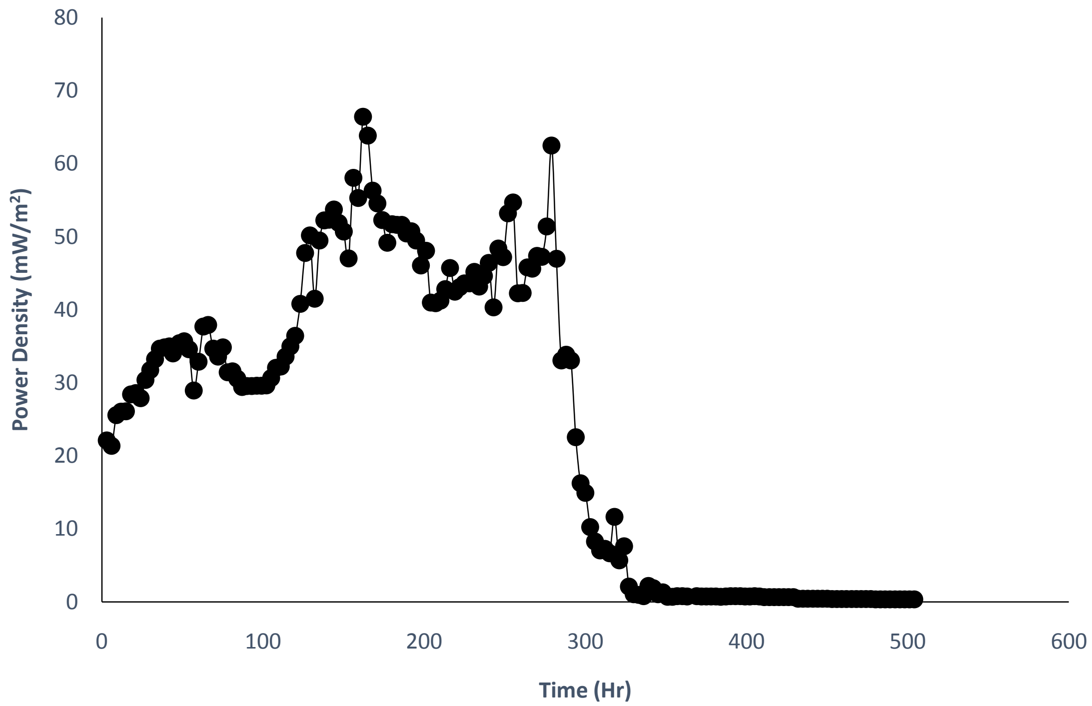

Figure 4. Power Density generated per Time during Treatment of Abattoir wastewater.

Current generated during MFC Operation of Abattoir Wastewater

The results for the Current generated per Time (Hours) during operation of MFC for the treatment of Abattoir wastewater is shown in Figure 3.The Maximum Current in mA (milli Amperes) generated was 0.46 mA. Abattoir wastewater recorded 0.22mA after pitching on the first day. The current increased to 0.46mA after 162 hours. The current however fluctuated over time then decreasing to 0.01mA after 330 hours. It remained constant at 0.01mA for the remaining 72 hours of operation of the MFC.

Power Density Results during MFC Operation of Abattoir Wastewater

Power density results recorded during operation of Abattoir wastewater is shown in Fig 4. Power density in (mW/m^2) recorded on the first day was $22.1mW/m^2$. It however increased over time to $37.91mW/m^2$ after 66 hours then decreasing gradually to $29.43mW/m^2$ after 87 hours. The power density then peaked to a maximum value of $66.83mW/m^2$ and then fluctuated over time to reach a high value of $62.49mW/m^2$. The value dropped to less than 1.0 mW/m^2 after 348 hours of operation.

DISCUSSION

An important aim in this study was to identify potential bioelectricity generating bacteria from both abattoir wastewaters. The potential isolates from abattoir wastewater from this study were *Bacillus* species, *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus faecalis*. These isolates were identified on the basis of biochemical characteristics and other staining techniques in accordance with Bergey's manual of systematic bacteriology. Potential bioelectricity generating isolates used in previous studies includes *Clostridium species* (Mathuriya and Sharma, 2009; Finch *et al.,* 2011), *Geobacter sulfureducens* (Benneto, 1990), *Shewanella japonica* (Biffinger *et al.,* 2011), *Shewanella putrefaciens* (Kim *et al.,* 2002), *Klebsiella* specie (Xia *et al.,* 2010), *Corynebacterium* species (Liu *et al.,* 2010), *Enterobacter cloacae* (Samrot *et al.,* 2010), *Lactococcus lactis* (Freguia *et al.,* 2009) and *Bacillus megaterium* (Borah *et al.,* 2013). The bioelectrogens isolated from these experiments have also been reported as possible electrogenic bacteria that can drive the generation of electricity using the technology of MFC as reported in studies carried out by (Liu *et al.,* 2010). The total bacteria population decreased after the 21 day period of operation of the dual-chamber MFC. This decrease in bacteria population could however have resulted from decrease in organic substrate present as organic carbon, organic forms of nitrogen and phosphorus.

In this study, the addition of Abattoir wastewater to their respective chambers gave an initial Open circuit voltage of 459 mV, Current of 0.22 mA, and Power density of $22.10mW/m^2$ respectively, which increased and peaked within 5-7 days of operation to give maximum Open Circuit Voltages of 736 mV, Current of 0.46mA, and Power density of $66.43mW/m^2$ respectively. This initial increase in voltages, currents and power densities might have resulted from both biological and chemical factors, based on the differences of the potential between the two chambers (Min *et al.,* 2005). The voltage and currents were seen to decrease with time. This decrease in OCV can be attributed to the rate of utilization of the organic substrate in the medium (wastewater) (Patil *et al.,* 2013). Also, reduction of Ferricyanide from Fe $(CN)_6^{3-}$ to $Fe(CN)_6^{4-}$ (Logan, 2006) can also be responsible for the decrease in Open Circuit Voltage, current and power density.

The results from this study conducted using a dual-chamber MFC demonstrated that bioelectricity can be generated from abattoir and swine wastewaters. This is in agreement with previous studies carried out by Min *et al.,* (2005) and Momoh and Neayor, (2010) who conducted similar studies using Swine wastewater and Abattoir wastewater respectively. The results from this study revealed a maximum Open Circuit Voltage (OCV) of 736 mV, a Current of 0.46mA, and Power density of 66.43mW/m^2 for Abattoir wastewater respectively.

The Open Circuit Voltage (OCV) value of 736mV generated during treatment of Abattoir wastewater in this study is lower compared to Voltages generated in the study carried out by Momoh and Neayor, (2010) using Abattoir wastewater recording a maximum voltage of 1560mV. The increase in Open Circuit Voltage could have resulted from Calcium Hypochlorite (Bleaching powder) which was the source of catholyte used in the study. The low Open Circuit Voltage can however be compared to a similar Open Circuit Voltage of 710mV generated in a study carried out by Guerrero-Rangel *et al.,* (2010) using the same source of catholyte used in this study ($K_3[Fe(CN)_6]$).

The low open circuit voltage recorded in this study could have resulted from a difference in the Proton exchange membrane used in various studies. Agar-salt bridge has a high internal resistance (Logan, 2006) and could be responsible for the low open circuit voltage and current. The reactor design could also have acted as a factor in the maximum open circuit voltage generated. Min *et al.,* (2005) using a single chamber MFC reported a maximum power density of 261 mW/m^2 which is quite higher compared to 88.46mW/m^2 generated in this present study. The difference in power density could have resulted from the use of different design and configuration. Momoh and Neayor, (2010), reported a maximum open circuit voltage of 1560 mV, 1400 mV and 2890 mV for the single dual-chambered, and the double dual-chambered in parallel and series respectively.

Furthermore, this study demonstrates the ability of a Microbial Fuel Cell to remove some nutrients from the wastewaters. A COD and BOD removal efficiency of 56.09% and 92.31%was recorded respectively for Abattoir wastewater. This can be correlated to 86 % COD removal efficiency reported in a study by Min *et al.,* (2005). In this study, Ammonia, Ammonia-Nitrogen, Ammonium, Nitrate, Nitrate-Nitrogen, Phosphorus, Orthophosphates and Phosphates were also examined to determine the effect of MFC on these parameters present in the wastewater. The Nitrate, Nitrate-Nitrogen parameters were seen to decrease by 73.29% and 75.46% for Abattoir wastewater. Decrease in Nitrate and Nitrate-Nitrogen level observed in Abattoir wastewater may be due to the process of denitrification (Clauwert *et al.,* 2008). From the results of the physicochemical parameters, Phosphates, Phosphorus and Orthophosphates value increased by -3.58%, -3.51% and -4.22% respectively in Abattoir wastewater. The increase in Phosphates, Phosphorus and Orthophosphates could have been a result of stored phosphates in the bacteria (Liu *et al.,* 2002), or the conversion of organic phosphorus in the wastewaters to different forms of phosphates (Min *et al.,* 2005).

Ammonia, Ammonia-Nitrogen and Ammonium rates increased by -226.76%, -226.07% and -226.16% in abattoir wastewater. The increase observed in abattoir wastewater could have resulted from the process of ammonification when organic form of Nitrogen present in the wastewater is converted to ammonium and ammonia (Britton, 2005).

It should be emphasized that this high rate of ammonification could have resulted from the high protein content of abattoir wastewater and high waste material generated from the slaughter house which includes material such as offal, hides, blood, garbage from stomach and intestine, and sanitary septage (Ping and Gauri, 2011).

CONCLUSION

The results from this study demonstrates that the dual-chamber MFC was able to treat abattoir and swine wastewaters demonstrating a COD and BOD removal efficiency of 56.09% and 92.31%, for the abattoir wastewater.

The result from this study also underscores the role bacteria species such as *Bacillus* specie, *Escherichia coli, Staphylococcus aureus, Streptococcus* specie and *Corynebacterium* specie play roles in the generation of electricity from wastewater.

Electricity was generated using the wastewater as substrates with the dual-chamber MFC producing a maximum open circuit voltage of 758 mV, current of 0.46mA and power density of 66.43mW/m^2 for abattoir wastewaters. The electricity generated was not constant throughout the 21 day period of operation. Reduction of the catholyte which contributed to the low open circuit voltage and current, the system design also acted as a factor in voltage production. Thus, it is recommended that further studies be carried out using laboratory scale reactors to treat wastewater and generate electricity from the wastewater.

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Corresponding author: Mr. Egbadon, E.O., Department of Microbiology, Federal University of Technology Owerri, Imo state, Nigeria

Email: emmanuelegbadon@gmail.com / adeleyesamuela@gmail.com

(+2347038088249); (+2347031297068)