

# Bioelectricity Production from Abattoir Wastewater Using Microbial Fuel Cells Connected in Series

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Abstract— In a bid to further study the possibility of producing bioelectricity from microbial fuel cell (MFC), this study aims at increasing the production of bioelectricity in abattoir wastewater. The wastewater from abattoir was used to construct three different microbial fuel cells and they were connected in series. The voltage of electricity produced was recorded over the period of three days. The physiochemical property of the wastewater was determined. Also the microorganisms present in the wastewater was quantified using pour plate method and the bacteria was identified. The MFC connected in series gave a high voltage, 1.98V. The wastewater had a pH of 6.08 and temperature of 31.4°C. The result of the physiochemical properties of the wastewater which included; electrical conductivity, total dissolved solids, dissolved oxygen and BOD are 15,150  $\mu/cm^3$ , 10,230, 8.20 mg/L, 6.00 mg/L respectively. The abattoir wastewater has a high average aerobic count of  $2.56 \times 10^{23}$  cfu/ml and average anaerobic count of 1.35×10<sup>23</sup> cfu/ml. Isolated bacterial species includes; Actinobacillus, Aeromonas, Bacillus, Citrobacter, Clostridium, Enterobacter, Enterococcus, Escherichia coli. Proteus, Klebsiella. Micrococcus. Neisseria, Psuedomonas aeruginosa, Samonella, Serrentia, Shigella, Staphylococcus and Streptococcus. This study recommends newer means of harvesting electrons from the microbes.

**Keywords**— Abattoir: Wastewater: Bioelectricity: Microbial Fuel Cell: Series: Microorganisms

# I. INTRODUCTION

Bioelectricity is electric potentials and currents produced by or occurring within living organisms. Bioelectric potentials are generated by a variety of biological processes and generally range in strength from one to a few hundred millivolts.

#### 1.0 Aim of the study

The aim of the study is to study bioelectricity production from abattoir wastewater using microbial fuel cells connected in series.

# 1.1 Significance of the study

This study will provide a template for generating higher amount of clean and cheap electricity from the activities of microorganisms in bio wastes. This will in turn lead to increase in the study of electricity to the power grid of the nation.

#### II. LITERATURE REVIEW

# 2.1 Bioelectricity

Bioelectricity was first discovered in 1789 by an Italian physicist Luigi Galvani from the use of electricity as a signal

between the nerves and muscles in a frog leg. He touched an exposed sciatic nerve with a charged metal scalpel and observed the dead frog's leg flex as if it were alive. Galvani believed that the muscular contractions were due to electrical energy emanating from the animal. However, Allesandro Volta was convinced that the electricity in Galvani's experiments originated from the presence of the dissimilar metals (Enderle, 2004; Grimnes and Martinsen, 2008).

# 2.1.1 Abattoir Wastewater

Abattoir wastewater is gotten from the abattoir site where farm animals, goat, cows, sheep, rams, are slaughtered. Abattoir wastewater consist of several pollutants which includes animal blood, faeces, urine and fat. Organic matter present in abattoir wastewater is usually very high (Bustillo-Lecompte and Mehrvar, 2015)

#### 2.1.2 Microorganisms involved

Volumes of wastewater termed sewage are generated from many sources. This sewage represents thousands of tons of organic matter. A wastewater treatment plant is a microbiological zoo that houses bacteria, protozoa, metazoan and other micro life. The microorganisms do the actual breakdown and removal of nutrients and organic material in the wastewater. Many microorganisms possess the ability to transfer electrons derived from the metabolism of organic matters to the anode. Marine sediment, soil, wastewater and fresh water are all rich sources for these microorganisms. Almost all microorganisms have the ability to transfer electrons derived from the metabolism of organic matters which includes soil, wastewater, fresh water sediment, cow dung and activated sludge to the anode (Oh and Logan, 2005). Microbes transfer electrons to the electrode through an electron transport system that either consists of a series of components in the bacterial extracellular matrix or together with electron shuttles dissolved in the bulk solution. Examples of these microbes are listed in Table 2.1 (Pant et al., 2010, Choi et al., 2007, Ringeisen et al., 2006; Shin et al., 2006). 2.1.3 Substrates for bioelectricity

Substrate is an important factor in any biological process. It serves as carbon and energy sources. The ability of microorganisms to oxidize substrates and transfer electrons results in the production of current (Rozendal *et al.*, 2007). Substrate influences both the integral composition of the bacterial community in the anode biofilm, and also the production of bioelectricity. The categories of substrates that can be used for the generation of bioelectricity may include non-fermentable substrates which may include acetate,

butyrate, a fermentable substrate; glucose, xylose, sucrose and complex substrates; domestic wastewater (Sun *et al.*, 2008).

TABLE 2.1: Microbes and substrate they synthesiz	ze
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Microbes	Substrate
Actinobacillus succinogenes	Glucose
Aeromonas hydrophila	Acetate
Alcaligenes feacalis	Glucose
Clostridium beijerinckii	Starch, glucose, lactate, molasses
Clostridium butyricum	Starch, glucose, lactate, molasses
Desulfovibrio desulfuricans	Sucrose
Enterococcus faecium	Glucose
Enterococcus gallinarum	Starch
Erwinia dissolven	Glucose
Escherichia coli	Glucose
Geobacter metallireducens	Acetate
Geobacter sulfurreducens	Acetate
Gluconobacter oxydans	Glucose
Klebsiella pneumonia	Glucose
Lactobacillus plantarum	Glucose
Micrococcus luteus	Glucose
Proteus mirabilis	Glucose
Proteus mirabilis	Glucose
Proteus vulgaris	Glucose, maltose, galactose
Pseudomonas aeruginosa	Starch, glucose
Rhodoferax ferrireducens	Glucose, xylose, sucrose, altose
Saccharomyces cerevisiae	Lactose, glucose
Shewanella oneidensis	Lactate
Shewanella putrefaciens	Lactate
Streptococcus lactis	Glucose

Complex substrates include domestic wastewater, food process wastewaters, paper recycled wastewater (Sun *et al.*, 2008 and Sun *et al.*, 2009). Examples of substrates that are used in MFCs include the following;

# 2.1.3.1 Glucose

Glucose is the most commonly used substrate in MFCs. According to Zhou *et al.*, (2011), MFCs with glucose exhibit higher power output than anaerobic sludge, lower energy conversion efficiency than acetate (Rabaey, 2003) and lower columbic efficiency.

# 2.1.3.2 Acetate

Acetate is the end product of several metabolic pathways for higher order carbon sources. Bond *et al.* (2002) claimed that acetate could be a carbon source to induce electro active bacteria. This made acetate to be widely used extensively in the study of MFCs.

# 2.1.3.3 Starch

Starch is a polymer of carbohydrate which consists of a large number of glucose. This glucose is joined together by glycosidic bonds. Many bacteria are capable of decomposing starch through the production of the enzyme amylase which is able to break the bond. Pant *et al.*, 2010, Choi *et al.*, 2007, Ringeisen *et al.*, 2006; Shin *et al.*, 2006

# 2.1.4 Microbial metabolism

All living cells depend on biochemical reactions to maintain homeostasis. All the biochemical reactions in an organism are collectively referred to as metabolism. This is of two basic types which include catabolism which involves the breakdown of molecules and anabolism which includes the building up of new molecules. Bacterial metabolism focuses on the chemical diversity of substrate oxidation and dissimilation reactions (reactions by which substrate molecules are broken down), which normally function in bacteria to generate energy. Sewage contains nutrients of every type; phosphorus, nitrogen, sodium, potassium, iron, calcium and compounds such as fats, sugars and proteins. Microorganisms use these substances as a "food" source for energy, for the synthesis of cell components and to maintain life processes. In microbial fuel cells, microbes convert organic matter to electricity through anaerobic metabolism through fermentation. The products of fermentation do not react readily with electrodes. Effective anaerobic oxidation of complex assemblages of organic matter requires the fermentation products from the metabolism of substances as sugars, amino acids and related compounds. Anaerobic microbes oxidize fermentation products and organic compounds to carbon dioxide.

# 2.1.5 Pathway involved

Many microorganisms possess the ability to transfer the electrons derived from the metabolism of organic matters to the anode. Marine sediment, soil, wastewater, fresh water sediment cow dung and activated sludge are all rich sources for these microorganisms (Zhang et al., 2006). The bacterial cell is a highly specialized energy transformer. Chemical energy generated by substrate oxidation is conserved by formation of high-energy compounds such as adenosine diphosphate (ADP) and adenosine triphosphate (ATP) or compounds containing the thioester bond. The oxygen requirement of bacteria reflects the mechanism used by those particular bacteria to satisfy their energy needs. Obligate anaerobes do not carry out oxidative phosphorylation. Furthermore, they are killed by oxygen, they lack enzymes such as catalase; which breaks down hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) to water and oxygen, peroxidase; by which 1NADH +  $H_2O_2$  are converted to 2NAD and  $O_2$  and superoxide dismutase; by which superoxide,  $O_2$ , is converted to  $H_2O_2$ (Boundless, 2015). These enzymes detoxify peroxide and oxygen free radicals produced during metabolism in the presence of oxygen. Anaerobic respiration includes glycolysis and fermentation. During the latter stages of this process NADH, generated during glycolysis, is converted back to NAD by losing a hydrogen. The hydrogen is added to pyruvate and, depending on the bacterial species, a variety of metabolic end-products are produced (Boundless, 2015). According to Gorby et al., (2009) and Lovely, (2006) Geobacter and Shewanella species accounts for the majority of the microbial population that have been utilized in MFC technology. Photosynthetic bacteria can also be used effectively in MFC for electric power generation (Rosenbaum et al., 2007). Cyanobacterial strains of Anabaena and Nostoc also have been used as biocatalysts in MFCs. Pseudomonas aeruginosa strains have also been used along with manipulation of NAD co-factor thereby increasing the metabolic rate and potential of the bacteria toward enhanced biofuel production (Zhao et al., 2004).

# 2.1.6.1 Mediated electron transfer (MET)

Electrons are transferred to an electrode through the use of mediators (Davis and Higson, 2007). Mediators provide a platform for the microorganisms to generate electrochemically



active reduced products. It relies on the redox cycling of mediators between the microbes and the electrode. These mediators can either be naturally present compounds such as humic acids and sulfur species or compounds produced by the microorganisms themselves (Stams *et al.*, 2006; Rabaey et al., 2005). When mediator is in an oxidized state, they are reduced in the cytoplasm or periplasm by absorbing electrons released by enzyme catalyzed organic carbon oxidation inside the bacteria. The reduced mediators migrate to an anode and release the electrons to the anode to become oxidized again (Schroder, 2007). Usually neutral red, thionine, methylene blue, anthraquinone-2, 6-disulfonate, phenazines and iron chelates are added to the reactor as redox mediators (Du *et al.*, 2007).

#### 2.1.6.2 Direct electron transfer (DET)

This mechanism depends on the direct physical contact between the microbe and the electrode. Some microbes possess the ability to transport electrons from the inside of the cell to the external through the cell membrane or special conductive pili rather than mediators. They use a series of membrane redox proteins, such as ctype cytochromes and heme proteins that transport the electrons from the inside of the cell to the electrode (Lovely, 2006) and others utilize electrically conductive pili on the surface of their cells to transfer electrons (Du *et al.*, 2007; Gorby et al., 2006).

# 2.2 Fuel cell

Fuel cell is an electrochemical cell that converts the chemical potential energy from a fuel into electricity through an electrochemical reaction of hydrogen fuel with oxygen or another oxidizing agent. The chemical potential energy can be energy stored in molecular bonds. Fuel cells can produce electricity continuously for as long as fuel and oxygen are supplied. The first fuel cells were invented in 1838. The first commercial use of fuel cells was in NASA space programs to generate power for satellites and space capsules. They are also used to power fuel cell vehicles, including forklifts, automobiles, buses, boats, motocycles and submarines.

# 2.2.1 Mechanism

All fuel cells work in the same manner. Fuel cells consist of four basic elements; an anode, the electrolyte, the cathode and the catalyst.

# 2.2.2 Anode

The anode is the negative post of the fuel cell. It conducts the electrons that are released from the hydrogen molecules so that they can be used in an external circuit. Oxidation reaction occurs in the anode side.  $2H_2 \longrightarrow 4H^++4e^-$ 

# 2.2.3 Cathode

The cathode is the positive post of the fuel cell that has channels etched into it that distribute the oxygen to the surface of the catalyst. It also conducts the electron back from the external circuit to the catalyst where they can recombine with the hydrogen ions and oxygen from water. Reduction reaction occurs in the cathode.

 $O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$ 2.2.3 Electrolyte The electrolyte is the proton exchange membrane. It conducts only positive charged ions. It is designed so ions can pass through it but the electrons cannot.

# 2.2.4 Catalyst

The catalyst is a special material that facilitates the reaction of oxygen and hydrogen. A catalyst oxidizes the fuel at the anode, usually hydrogen. This turns the fuel into a positively charged ion and a negatively charged electron. The electrons released travel through a wire creating the electric current. The ions travel though the electrolyte to the cathode. Once they reach the cathode, the ions are reunited with the electrons and the two react with a third chemical usually oxygen to create water or carbon dioxide. The net reaction is:

 $2H_2O$ 

 $2H_2 + O_2 \longrightarrow$ 

# 2.3 Microbial fuel cell

A microbial fuel cell (MFC) is a device that converts chemical energy released as a result of oxidation of complex organic carbon sources which are utilized as substrates by microorganisms to produce electrical energy thereby proving to be an efficient means of sustainable energy production. The electrons that are released due to the microbial metabolism in MFC are captured continuously to maintain a constant power density. The microbial fuel cell (MFC) has gained much attention because of its ability to generate power from organic or inorganic compounds via microorganisms. The electrons released due to the microbial metabolism are captured to maintain a constant power density, without an effective carbon emission to the ecosystem. The various parameters involved in MFC technology toward power generation include maximum power density, columbic efficiencies and sometimes chemical oxygen demand removal rate which evaluate the effectiveness of the device. Application of microbes toward bioremediation at the same time resulting in generation of electricity makes MFC technology a highly advantageous proposition which can be applied in various sectors of industrial, municipal and agricultural waste management. Although the efficiency of MFCs in power generation initially was low, recent modifications in the design, components and working have enhanced the power output to a significant level thereby enabling application of MFCs in various fields including wastewater treatment, biosensors and bioremediation (Tharali et al., 2016). Microbial fuel cell is made up of two compartments, anode and cathode, separated with proton/cation exchange membrane. Microorganisms oxidize the substrate and produce electrons and protons in the anode chamber of MFC. Electrons collected on the anode are transported to cathode by external circuit and protons are transferred through the membrane internally. The bacteria thrive on the surface of the anode and convert the substrate present in the wastewater which may include glucose, acetate, starch, maltase and lactase into carbondioxide, protons and electrons.

 $C_{12}H_{22}O_{11} + 13H_2O \longrightarrow 12CO_2 + 48H^+ + 48e^-$ Under aerobic conditions, bacteria use oxygen or nitrate as a final electron acceptor to produce water but in the anode of the MFC, oxygen is absent and bacteria transfer electrons from their natural electron acceptor to an insoluble acceptor,



such as the MFC anode. Due to the ability of bacteria to transfer electrons to an insoluble electron acceptor, MFC can be used to collect the electrons originating from the microbial metabolism. This can be transferred through membraneassociated components. The electrons then flow through an electrical circuit with a load or a resistor to the cathode. The potential difference (Volt) between the anode and the cathode, together with the flow of electrons (Ampere) results in the generation of electrical power (Watt). The protons flow through the proton or cation exchange membrane (selective permeable membrane) to the cathode (Oh and Logan, 2004). Gil et al., (2017), reported that some factors can affect the performance of MFCs which includes the rates of substrate oxidation, electron transfer to the electrode by the microbes, the resistance of the circuit, proton transport to the cathode through the membrane, oxygen supply and reduction in the cathode.

#### 2.3.1 Mechanism of microbial fuel cell

Du et al., (2007), reported that the electrode reaction is the breakdown of the biodegradable substrate to carbondioxide and water along with production of electricity using acetate as a substrate. During microbial catabolism, microorganisms convert chemical energy present in carbohydrates including sugars and alcohol to electrical energy. Fermentation is a wellknown mechanism for anaerobic metabolism of organic matter and many microbial fuel-cell studies till now relied solely on fermentative microorganisms (Shukla et al., 2004). The basic MFC design according to Logan, (2004), consists of an anode, cathode, proton exchange membrane (PEM) and an electrical circuit. In an MFC, bacteria present in the anode compartment uses organic substrates in form of glucose, acetate, starch, maltase and lactase as fuels to produce electrons and protons through their metabolism (Rabaey and Verstraete, 2005). The anode compartment is maintained under anaerobic conditions as oxygen inhibits electricity generation whereas the cathode is exposed to oxygen (Logan, 2004; Rahimnejah, 2009). These electrons are accepted by nicotinamide adenine dinucleotide (NADH) in the electron transport chain and subsequently transferred to terminal electron acceptors such as nitrate, sulphate and oxygen and then reaches the outer membrane proteins (Logan and Regan, 2006; Salgado, 2009). These electrons are being transferred by the bacteria to the anode from where the electrons reach the cathode through the external circuit, thus producing electric current, which is measured by a voltmeter or ammeter connected to the device (Salgado, 2009). The protons generated are diffused through the PEM to the cathode and subsequently combine with the electrons and oxygen to form water.

# 2.3.2 Two chamber MFC

This type of microbial fuel cell is the most widely used. It is built in a classic H-shaped and it consists of two chambers connected with a proton pump as shown in Figure 2.1. Here one chamber serves as the anode and the other serves as the cathode. The two chambers are connected to an external circuit. This type of MFC is used basically for research. The reactor was utilized to study the effect of different membranes on internal resistance (Kim *et al.*, 2007).

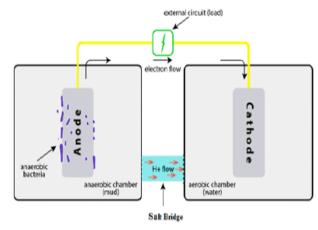


Figure 2.1: Two Chamber MFC with salt bridge Source: Parkash A. (2015)

The general power output is generally low due to their complex design, high internal resistance and electrode based losses (Du *et al.*, 2007; Logan and Regan, 2006; Nwogu, 2007).

#### Mechanism

In order to reduce the internal resistance, this MFC uses larger membrane area and a shorter electrode distance which reduces internal resistance substantially and increases power generation. The anodic chamber is fed with wastewater or other organic and the cathodic chamber contains dry air (Min and Logan, 2004). *Advantages* 

Internal resistance is reduced in two chamber MFC.

2.4.3 Application of microbial fuel cell

#### 2.4.3.1 Electricity generator

Due to the ability of MFCs to convert chemical energy stored in the chemical bonds of organic compounds into electricity, MFC possess much higher efficiency (Du *et al.*, 2007). However, the power produced by MFCs is still too low to be useful in most applications but to ensure enough power was supplied, energy produced by the MFC was stored in a capacitor and used in short bursts when needed (Shantaram *et al.*, 2005) and Tender *et al.*, 2008).

#### 2.4.3.2 Waste treatment

The most successful and widely used biological technology for wastewater treatment is the activated sludge process. MFCs are able to harvest energy from organic matters and treat wastewaters at the same time. The amount of power generated by MFCs in the wastewater treatment process reduces the power input needed in the aerobic treatment stage (Du et al., 2007). The ability of MFCs to degrade organic matters result in the production of less solids to be disposed and organic compounds are broken down to carbon dioxide and water (Holzman, 2005). MFC can be used to treat a wide range of organic wastewaters if proper electricity-producing bacteria are enriched in the anode. The first demonstration of MFCs using domestic wastewater as the substrate was reported by Liu et al. (2004), they used single-chamber MFCs, which did not need any oxygen aeration into the cathode chamber, and the COD removing rate of domestic wastewater



was up to 80%. Then Rabaey et al., (2004), demonstrated up to 96% of the organic matter in wastewater was converted to electricity by a single-chamber MFC, demonstrated soluble COD removal efficiency was over 90% in an up-flow MFC using artificial wastewater as substrate.

#### 2.4.3.3 Bioremediation

#### 2.4.3.4 Biosensors for pollution analysis

Kim *et al.*, (2007), found that there was a proportional correlation between the Columbic yield of MFCs and the strength of the wastewater. Therefore, MFCs are a good approach to examine biological oxygen demand (BOD) in wastewater. Moon *et al.*, (2004), made some efforts to optimize the response time and sensitivity of MFCs used as continuous BOD sensor.

#### 2.4.3.6 Sediment MFCs

More research on microbial fuel cell has shown that electricity can be harvested from organic matter in aquatic sediments (Liu *et al.*, 2005). The purpose of the sediment MFC design is to power devices placed on seafloor or under water environment where it is expensive and technically difficult to exchange traditional batteries routinely (Kim *et al.*, 2003). The idea is to place the anode into the anaerobic sediment and place the cathode into the overlying water containing dissolved oxygen. As the exoelectrogenic bacteria are rich in the sediments, the sediment MFCs are ready to produce electricity.

# 2.5 Problems and challenges of microbial fuel cell

#### 2.5.1 Low power

The major challenge of MFC is the production of low voltage which can only be used in limited applications. The current generated by Saldago, (2009) and Kim, (2007) was very low and could power only small devices.

# 2.5.2 Production of electricity in large scale

According to Schwartz, (2007), implementing MFC on a large scale is difficult while maintaining low costs and minimizing hazards while maximizing power generation. The performance is affected by factors such as pH, temperature, substrate, and microbial activity, resistance of circuit and electrode materials.

# 2.5.3 Microbe/electron interaction

Current production by bacteria in MFC is a complex process. It requires more insight into the process of electron transfer (Franks and Nevin, 2010). Biofouling of cathode affects MFC performance, also electrode properties affects microorganism wiring and MFC performance (Cheng *et al.*, 2008). Development of higher catalytic materials with superior performance is important to avoid biofouling or corrosion of electrodes (Huang *et al.*, 2011).

# III. MATERIALS AND METHODS

# 3.1.0 MATERIALS

#### 3.1.1 Collection Area

Samples were collected at early hours of the day from Lafia Modern Abattoir, Shingeh, Lafia, Nasarawa State. *3.1.2 Samples Collection* 

Three thousand milliliters of wastewater samples and sediments were collected from the abattoir using a shovel into the bowl in the morning to avoid influence of anthropogenic activities around the sample.

3.1.3 Processing of Wastewaters

The collected samples contained large amount of wastes from slaughtered farm animals which includes cows, sheep, goats, rams and chicken. These wastes include; feacal wastes, blood and normal flora from the skin of the animals, channeled into the abattoir dumping site where they are used as manure where the sample was collected.

3.1.4 Construction of Microbial Fuel Cell

MFC was constructed as described by Cheng *et al.*, (2006). MFC assembly consists of two containers connected with the external circuit and proton pump.

Apparatus used includes the following;

#### 3.1.4.1 Plastic containers

One thousand milliliters plastic containers were used for each microbial fuel cell. One of the containers served as the anode jar which contains the wastewater which the second container served as the cathode jar which contains distilled water.

# 3.1.4.2 Copper wire

Copper wires with the diameter 1.40mm were used at both the anode and the cathode. The copper wires used were measured using micrometer screw jack with minimum measurement of 0.1mm.

#### 3.1.4.3 Proton pump

The proton pumps used are molded hollow plastic tube filled with wick soaked in salt water solution for 2 hours.

# 3.1.4.4 Digital multimeter

Digital multimeter is a test tool used to measure two or more electrical values, principally voltage (volts), current (amps) and resistance (ohms). It is a standard diagnostic tool for technicians in the electrical/electronic industries. The digital multimeter model ALDA DT-830D was used. The digital multimeter is made up of four components; display; where measurement readout is viewed, buttons, for selecting various functions, dial or rotatory switch for selecting primary measurement values which includes volts, amps and ohms and input jacks where test leads are inserted. The meter was turned on, and the probes were inserted into the correct connections. The switch was set to the volts. The range was optimized for the best reading. Readings were recorded.

#### 3.1.5 MFC set up:

Step 1: Two plastic containers are needed for the setup of each pair. One of the containers is constructed as anaerobic jar with tightly sealed cover and serves as the anode which is the negative terminal, while the other container is constructed as an aerobic jar with air supply to the cathode.

Step 2: The proton exchange bridge is constructed by soaking cotton wick in salt water. The soaked wick is placed into the plastic pipe. This serves as a passage for the movement of ions from the anode to the cathode.

Step 3: The proton exchange bridge in connected to the plastic containers by boring hole by the side of the container and fixing the proton exchange bridge tightly into the bored hole. The proton exchange bridge is tightly fixed to hold it in place and to prevent leaking.



Step 4: Copper wires are coiled up inside each container with a point at the top for external connection.

Step 5: The two containers were connected with an external circuit which is made up of copper wires connected to the point at the top of each container and connected to a digital multimeter.

Step 6: The sludge containing the microorganisms and glucose is added to the anaerobic chamber and the cover is sealed. While regular water is added to the aerobic chamber. Conductivity is increased by adding a pinch of salt.

Step 7: This was replicated into three MFCs and was connected in series, that is, the cathode of MFC1 was connected to the anode of MFC2 and the cathode of MFC2 was connected to the anode of MFC3 as shown in Figure 3.1. The digital ammeter was connected to both anodes of MFC1 and the cathode of MFC3.

Step 8: Voltage current is measured every two hours and results obtained were recorded.



Figure 3.1: MFCs connected in series

#### 3.5.1 Measurement of electricity

The bioelectricity produced is determined by measuring the electricity produced per day and using the digital multimeter and the voltage is measured in millivolts.

#### IV. RESULT

#### 4.1 Physiochemical properties of the wastewater

As shown in Table 4.1, electrical conductivity, total dissolved solids, dissolved oxygen, biochemical oxygen demand and chlorine were very high in the abattoir wastewater.

Table 4.1: Physicochemical properties of the wastewater				
Parameters	Abattoir			
Electrical conductivity (μ/cm <sup>3</sup> )	15,550			
Total dissolved solids (TDS)	10,230			
Dissolved oxygen (DO) (mg/L) 10 <sup>th</sup> dilution	8.20			
Biochemical oxygen demand (BOD) (mg/L)	6.00			
Chlorine (mg/L) 5 <sup>th</sup> dilution	50.00			
Ph	6.08			
Temperature (°C)	31.4			

# 4.2 Electricity generated when three MFCs were connected in series

Three MFCs containing wastewater from abattoir wastewater to its ability to produce high amount of electricity, were connected in series. The electricity generated is listed in Figure 4.1. There was an exponential increase in the amount of electricity produced.

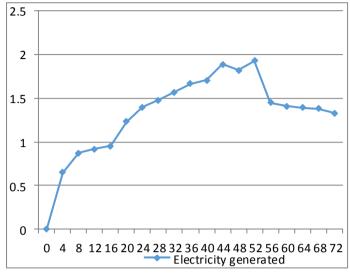


Figure 4.1: Electricity generated when connected in series

#### 4.3 Mean electricity generated

The mean electricity generated was calculated by dividing the total electricity produced by each wastewater by the total hours it was left to stand. The mean electricity as shown in Table 4.2, shows a steady increase in electricity produced.

Table 4.2: Mean electricity generated			
Hour	Abattoir (volts)		
0-12	0.813±0.0130		
13 – 24	1.183±0.0133		
25 - 36	1.57±0.0333		
37 - 48	$1.806 \pm 0.0667$		
49 - 60	1.935±0.0341		
61 – 72	$1.610 \pm 0.0423$		

4.4 Number of colonies in each wastewater

Sequel to growth on nutrient agar for day 1 and colonies were counted. Table 4.3 shows the number of growth on the agar. Day 1 Aerobic plate had the highest number of colonies.

Ta	Table 4.3: Number of colonies in each wastewater					
Wastewater	Colony Forming Units					
-	Da	Day 1		Day 2		
-	Aerobic	Anaerobic	Aerobic	Anaerobic		
	(cfu/ml)	(cfu/ml)	(cfu/ml)	(cfu/ml)		
Abattoir	$2.84 \times 10^{23}$	$1.02 \times 10^{23}$	$2.27 \times 10^{23}$	$1.68 \times 10^{23}$		

#### 4.5 Biochemical properties of isolated microorganisms

Samples of the wastewater were allowed to grow on nutrient agar and morphological characterization was taken, and colonies isolated were subjected to various biochemical tests which include their Gram's reaction, catalase, oxidase, citrate, coagulase, indole and hemolysis tests. Table 4.4 below shows the reactions of the isolated microbes to the different biochemical tests.



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Suspected Organisms	Gram's stain	Aerobic/Anaerobic	Catalase	Oxidase	Citrate	Coagulase	Indole	Hemolysis
Actinobacillus sp.	-ve rods	A/AN	+ve	+ve	Weak	-ve	+ve	Gamma
Aeromonas sp.	-ve rods	A/AN	+ve	+ve	+ve	-ve	+ve	Beta
Bacillus sp.	+ve rods	A/AN	+ve	+ve	+ve	-ve	-ve	Gamma
Chromobacterium sp.	-ve rods	AN	+ve	+ve	-ve	-ve	-ve	Gamma
Citrobacter sp.	-ve rods	A/AN	+ve	-ve	+ve	-ve	-ve	Alpha
Clostridium sp.	+ve rods	AN	-ve	-ve	+ve	-ve	-ve	Gamma
Enterobacter sp.	-ve rods	A/AN	+ve	-ve	+ve	-ve	-ve	Gamma
Enterococcus sp.	+ve cocci	A/AN	-ve	-ve	-ve	-ve	-ve	Alpha
Escherichia coli	-ve rods	A/AN	+ve	-ve	-ve	-ve	+ve	Beta
Klebsiella sp.	-ve rods	A/AN	+ve	+ve	+ve	-ve	-ve	Gamma
Micrococcus sp.	+ve cocci	А	+ve	+ve	-ve	-ve	-ve	Beta
Neissera sp.	-ve diplococci	А	+ve	+ve	+ve	-ve	-ve	Gamma
Proteus mirabilis	-ve rods	A/AN	+ve	-ve	+ve	-ve	-ve	Beta
Pseudomonas aeruginosa	-ve rods	А	+ve	+ve	+ve	-ve	-ve	Gamma
Samonella sp.	-ve rods	A/AN	+ve	-ve	-ve	-ve	-ve	Gamma
Serrentia sp.	-ve rods	A/AN	+ve	-ve	+ve	-ve	-ve	Beta
Shewanella sp.	-ve rods	AN	+ve	+ve	-ve	-ve	-ve	Gamma
Shigella sp.	-ve rods	A/AN	+ve	-ve	-ve	-ve	-ve	Beta
Staphyococcus sp.	+ve cocci	A/AN	+ve	-ve	+ve	+ve	-ve	Beta
Streptococcus sp.	+ve cocci in chain	A/AN	- ve	-ve	+ ve	-ve	-ve	Alpha

Table 4.4: Biochemical properties of isolated organisms

#### V. CONCLUSION AND RECOMMENDATIONS

#### 5.1 Discussion

This study assessed the ability of microorganisms to act as catalyst in the production of electricity from different wastewaters. The main goal of the research work is to check the ability of microorganisms to utilize wastewater as a substrate to produce current in an MFC connected in series. The MFC produced current which proved Nimje *et al.* (2011) which proved the ability of MFC with high microbial content to produce high electric voltage.

This present study showed the presence of high amount of coliform in the wastewater sample. It is proposed that this is responsible for the difference in the amount of electricity generated. Bacteria like Escherichia coli and Staphylococcus aureus and other bacterial species Actinobacillus, Aeromonas, Chromabacterium, Citrobacter, Bacillus, Clostridium, Enterobacter, Enterococcus. Klebsiella, Micrococcus, Neissera, Proteus, Pseudomonas, Samonella, Serrentia, Shewanella, Shigella and Streptococcus isolated from the wastewater were in agreement with the work done by Javalkar and Alam (2013). Similar results of microbes used for microbial fuel cells have been reported by Sengodon and Hays (2012).

The detection of faecal coliform such as Escherichia coli, Salmonella and Shigella indicates the faecal pollution in the wastewater and hence the presence of organic matter that encouraged the increased bacterial growth recorded in the study. It is implied that high amount of organic matter in the wastewater aided high amount of bacteria in the wastewater as observed by Nimje *et al.* (2011).

Some of these bacteria like Shewanella sp. are proven examples of bacteria with ability to produce current (Briffinger *et al.*, 2008; Nimje *et al.*, 2011). Most of these bacteria can produce electric current during their metabolic activities.

The abattoir wastewater also has a very high value of electrical conductivity of 15,500  $\mu/cm^3$ . According to Allison

*et al.* (1938), electrical conductivity depends on the concentration and mobility of charged particles of varying sizes and the substances in the solution were chemically disintegrated and synthesized. Electrical conductivity is also dependent on the metabolic processes of living organisms in a solution. The presence of high organic matter supports the metabolic processes of the living organisms in a solution. The presence of high organic matter supports the metabolic processes of the living organisms and the abattoir wastewater had a lot of organic matter which supports the metabolic processes of living organisms. As a result of this, abattoir wastewater had high amount of electrical conductivity. This study agreed with the work done by Malvankar *et al.* (2012) which proved that high electrical conductivity is found in mixed-species found in wastewater sludge.

The pH values obtained in this study showed that the water sample was slightly acidic due to organic contamination. Udom *et al.* (2002) attributed the low pH to abundance of organic matter in the wastewaters and decontamination of these organic matter results in the continual decrease of pH. Authors like Gaboriaund *et al.* (2006), emphasized that microbes can be involved in electrical activity and generate currents at neutral pH. This agrees with the work of Biffinger *et al.* (2008) which showed that microbes such as Shewanella have the ability to produce current at slightly acidic pH. According to Gaboriaud *et al.* (2006) and Dague *et al.* (2006), a continual decrement leads to decreased voltage production by the microbes because the outer membrane of microbes swells and softens which affects the electron transfer rate through the membrane.

Biological oxygen demand, chlorine, total dissolved solids, electrical conductivity and dissolved oxygen in the wastewater evaluated showed an interesting result with high parameters. The biological oxygen demand generated from the wastewater signifies high level oxygen for use by the bacteria present in the wastewater. The presence of high organic waste in the wastewater led to an increase in the amount of decomposing bacteria and this reduced the BOD level. The high BOD



values are indicatives of the presence of high organic pollutants. When there is a high amount of BOD levels, there is a low level of dissolved oxygen in the water. This allows the growth of microorganisms that are more tolerant of lower dissolved oxygen and discourages the growth of macro organisms. The high Total Dissolved Solids in the abattoir wastewater are indicatives of materials carried in solids (Oladiji et al., 2004).

The abattoir wastewater had very high amount of TDS. Low chlorine content in the wastewater recorded in the study aided the ability of microorganisms to thrive well in the wastewaters. Increase in amount of chlorine in the wastewaters slows down oxygen consumption to degrade the organic matter present in the effluent. Low amount of chlorine increases the amount of microbes. This work agreed with the experiment done on wastewater by Collivignarelli et al. (2017) where high chlorine content in the wastewater.

#### 5.2 Conclusion

Voltage is produced in microbial fuel cell through the action of bacteria and is passed through electrons to an anode through a proton pump. Abattoir wastewater had a lot of endogenous microbes which enabled it to generate a high amount of electricity when connected in series.

#### 5.3 Recommendation

- ✓ Electricity generated from the wastewater is low compared to the amount generated by non-renewable sources hence newer means of harvesting electrons from the microbes is encouraged.
- ✓ Further research is needed to discover how electric voltage can be increased in the production in microbial fuel cell.

#### REFERENCES

- Allison, J. B., Anderson, J. A. and Cole, W. H. (1938). The method of electrical conductivity studies on Bacterial metabolism. ResearchGate. 571-585.
- [2] Bond D. R., Tender L. M., Reimers C. E., Stecher H. A., and Holmes D. E. (2002). Harnessing microbially generated power on the seafloor. *Natural Biotechnology*, 20: 821-825.
- [3] Boundless (2015). "Special Culture Techniques". Boundless Microbiology, 21 July 2015.
- [4] Brigginger, J. C., Pietrona, J., Bretschger, O., Nadeaue, L. J., Johson, G. R., Williams, O., Nealson, K. H., Ringeisen, B. R. (2008). The influence of acidity on microbial fuel cell containing Shewanella oneidensis. *Biosensor Bioelectron*, 24: 906 911.
- [5] Bustillo-Lecompte C. F. and Mehrvar M. (2015). Slaugterhouse wastewater characteristics, treatment and management in the meat processing industry: a review on trends and advances. *Journal of Environmental Managemet*, 161:287 – 302.
- [6] Chen G. W., Choi S. J., Lee T. H., Lee G. Y., Cha J. H., and Kim C. W. (2008). Application of biocathode in microbial fuel cells: cell performance and microbial community. *Applied Microbiology and Biotechnology*, 79: 379-388.
- [7] Cheng S., Liu H. and Logan B. E. (2006). Power densities using different cathode catalysts (Pt and CoTMPP) and polymer binders (Nafion and PTFE) in single chamber microbial fuel cells. *Environmental Science & Technology*, 40: 364-369.
- [8] Choi Y., Jung E., Park H., Jung S. and Kim S. (2007). Effect of initial carbon sources on the performance of a microbial fuel cell containing environmental microorganism *Micrococcus luteus*. *Notes*, 28: 1591.

- [9] Dague, E., Gaboriaud, F., Duval, J., Jorand, F. and Thomas, F. (2006). Probing surface structure of Shewanella sp. by Microelectrophoresis. Biophysics Journal, 90: 2620 – 2621.
- [10] Davis F. and Higson S. P. (2007). Biofuel cells recent advances and applications. *Biosensor and Bioelectronics*, 22: 1224-1235.
- [11] Du Z., Li H., and Gu T. (2007). A state of the art review on microbial fuel cells: A promising technology for wastewater treatment and bioenergy. *Biotechnology Advances* 25, 464-482.
- [12] Enderle J. (2004). Bioelectric phenomenon: Introduction to biomedical engineering. *Elsevier*, 627–692.
- [13] Franks A. E., and Nevin K. P. (2010). Microbial Fuel Cells. A Current Review. Energies, 3, 899-919.
- [14] Gaboriaud, F., Dague, E., Bailet, S., Jorand, F., Duval, J. and Thomas F. (2006). Multiscale dynamics of the cell envelope of Shewanella putrefaciens as a response to pH change. *Colloids Surgace B*; *Biointerfaces*, 52:108 – 116.
- [15] Gil A., Siegel D., Bonsing-Vedelaar S., Permentier H., Reijngoud D. J., Dekker F. and Bischoff R. (2017). The degradation of nucleotide triphosphates extracted under boiling ethanol conditions is prevented by the yeast cellular matrix. *Metabolomics*, 13(1):1.
- [16] Gorby A. Y., Yanina S., McLean J. S., Rosso K. M., Dianne M., Alice D., Beveridge J., Chang I. S., Kim H. B., Culley D. E., Reed S. B., Romine M. F., Saffarini D. A., Hill A. E., Shi, L., Elias D. A., Shi L., Elias D., Kennedy D. W., Pinchuk G., Watanave K., Ishii S., Logan B., Nealson K. H. and Fredrickson J. K. (2006). Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. *Proceedings of Natural Academy Science* USA, 106(23): 9535.
- [17] Gorby Y. A., Yanina S., McLean J. S., Rosso K. M., Moyles D., Dohnalkova A., Beveridge T. J., Chang I. S., Kim B. H., Kim K. S., Culley D. E., Reed S. B., Romine M. F., Saffarini D. A., Hill E. A., Shi L., Elias D. A., Kennedy D. W., Pinchuk G., Watanabe K., Ishii S., Logan B., Nealson K. H. and Fredrickson J. K. (2006). Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. *Proceedings of the National Academy of Sciences*, 103, 11358-11363.
- [18] Grimnes S. and Martinsen O. G. (2008). Bioimpedance and bioelectricity basics. Academic Press, 2: 99.
- [19] Holzman D. C. (2005). Microbe power. Environmental Health Perspectives, 113(11), 754-757.
- [20] Huang L., Regan J. M., and Quan X. (2011). Electron transfer mechanisms, new applications, and performance of biocathode microbial fuel cells. *Bioresource Technology*, 102, 316-323.
- [21] Javalkar, P. D. and Alam, J. (2013). Generation from Microbial Fuel Cell using Bio-waste as Fuel. *International Journal of Scientific and Research Publications*. 3(8).
- [22] Kim B., Chang I., and Gadd G. (2007). Challenges in microbial fuel cell development and operation. *Applied Microbiology and Biotechnology* 76, 485-494.
- [23] Kim B. H., Chang I. S., Gil G.C., Park H. S. and Kim H. J. (2003). Novel BOD (biological oxygen demand) sensor using mediator-less microbial fuel cell. *Biotechnology Letter* 25: 541-545.
- [24] Kim J. R., Cheng S. and Logan B. E. (2007). Power generation using different cation, anion and ultrafiltration membranes in microbial fuel cells. *Environment Science Technology* 4: 1004-1009.
- [25] Liu H., Cheng S. and Logan B. E. (2004). Power generation in fed-batch microbial fuel cells as a function of ionic strength, temperature and reactor configuration. *Environment Science Technology* 39: 5488-5493.
- [26] Liu H., Grot S. and Logan B. E. (2005). Electrochemically assisted microbial production of hydrogen from acetate. *Environmental Science Technology* 39: 4317–4320.
- [27] Logan B. E. (2004) Extracting hydrogen and electricity from renewable resources. *Environment Science Technology* 38: 160A-167A.
- [28] Logan B. E., and Regan J. M. (2006). Microbial Fuel: Cells— Challenges and Applications. *Environmental Science Technology* 40: 5172-5180.
- [29] Lovley D. R. (2006). Bug juice: harvesting electricity with microorganisms. *Nature Reviews Microbiology* 4: 497–508.
- [30] Malvankar, N. S., Lau, J., Nevin, K. P., Franks, A. E., Tuominen, M. T. and Lovley, D. R. (2012). Electrical conductivity in a mixed-species biofilm. *Applied and Environmental Microbiology*, 84(16).



- [31] Min B. and Logan B. E. (2004). Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell. *Environmental Science Technology* 38(21): 5809-5814.
- [32] Moon B. and Logan B.E. (2004). Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell. *Environmental Science Technology*, 38(21): 5809-5814.
- [33] Nimje, V. R., Chen C., Chen, H., Chen, C., Huang, Y. M., Tseng, M., Cheng, K. and Chang, Y. (2011). Comparative Bioelectricity production from various wastewaters in Microbial fuel cells using mixed cultures and a pure strain of *Shewanella oneidensis*. *Bioresource Technology*, 23:12.
- [34] Nwogu N. G. (2007). Microbial fuel cells and parameters affecting performance when generating electricity. *Basic Biotechnology*, 73-79.
- [35] Oh S. E. and Logan B. E. (2005). Hydrogen and electricity production from a food processing wastewater using fermentation and microbial fuel cell technologies. *Water Resources* 2005; 39:4673–82.
- [36] Oladiji, T., Adeyemi, O. and Abiola, O. O. (2005). Toxicological evaluation of the surface water of Amilegbe River using Rats. *ResearchGate*, 15.
- [37] Pant P., Deepak C., Van B., Gilbert T., Diels O., Ludo L., Vanbroekhoven J. and Karolien P. (2010). "A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production ", *Bioresource Technology* 101:1533-1543.
- [38] Rabaey K., Boon N., Höfte M. and Verstraete W. (2004). Microbial phenazine production enhances electron transfer in biofuel cells. *Environmental Science & Technology* 39: 3401-3408.
- [39] Rabey K and Verstraete W. (2005). Microbial fuel cells: novel biotechnology for energy generation. *Trends in Biotechnology* 23:291-298.
- [40] Rahimnejah M., Najafpour M., Daud R. and Ghoreysh W. (2009). Low voltage power generation in a biofuel cell using anaerobic culture. *World Applied Sciences* 6(11): 1585-1588.
- [41] Ringeisen B. R., Henderson E., Wu P. K., Pietron J., Ray R., Little B., Biffinger J. C., JonesMeehan J. M. (2006). High power density from a miniature microbial fuel cell using Shewanella oneidensis DSP10. *Environmental Science and Technology* 40: 2629-2634.
- [42] Rosenbaum P., Paneth N., Leviton A., Goldstein M., Bax M., Damiano D., Dan B. and Jacobsson B. (2007). A report: the definition and classification of cerebral palsy Development. *Medical Child Neurology Supplement* Feburary; 109:8-14.
- [43] Rozendal R., Hamelers H. V. M., Molenkamp R. J. and Buisman C. J. N. (2007). Performance of single chamber biocatalyzed electrolysis with different types of ion exchange membranes. *Water Resource* 11: 1984-1994.
- [44] Salgado C. A. (2009). Microbial fuel cells powered by *Geobacter* sulfurreducens. Basic Biotechology. Volume 5:1.
- [45] Schroder U. (2007). Anodic electron transfers mechanisms in microbial fuel cells and their energy efficiency. *Physical Chemistry Chemical Physics* 9, 2619–2629.
- [46] Schwartz K. (2007). Microbial fuel cells: Design elements and application of a novel renewable energy sources. *Basic biotechology Cells: Enzyme and Microbial Technology* 47, 179-188.
- [47] Segodon, P. and Hay, D. B. (2012). Microbial Fuel Cells. Future Fuel Technologies, National Petroleum Council (NPC) Study, 13.
- [48] Shantaram A., Beyenal H., Veluchamy R. R. A. and Lewandowski Z. (2005). Wireless sensors powered by microbial fuel cells. *Environmental Science & Technology* 39, 5037-5042.
- [49] Shin S., Choi, Y., Na, S., Jung, S. and Kim, S. (2006). Development of bipolar plate stack type microbial fuel cells. *Bulletin-Korean Chemical Society* 27, 281.
- [50] Shukla A. C., Shukla A., Mishra R. C. and Dikshit A. (2004). Broad spectrum herbal fumigant formulation for the management of stored grain pests. *Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products* 56(56).
- [51] Stams A. J., De Bok F. A., Plugge C. M., van Eekert M. H., Dolfing, J. and Schraa, G. (2006). Exocellular electron transfer in anaerobic microbial communities. *Environmental Microbiology* 8, 371-382.
- [52] Sun M., Sheng G. P., Zhang L., Xia C. R. and Mu Z. X. (2008). An MEC-MFCcoupled system for biohydrogen production from acetate. *Environment Science Technology* 42: 8095-8100.
- [53] Sun M., Sheng G., Mu Z., Liu X. and Chen Y. (2009). Manipulating the hydrogen production from acetate in a microbial electrolysis cellmicrobial fuel cell-coupled system. *Power Sources* 19: 338-343.

- [54] Tharali A. D., Sain N. and Osborne W. J. (2016). Microbial fuel cells in bioelectricity production. *Journal of Frontier in Life Science*. Volume 9, 2016 Issue 4.
- [55] Udom, G. J., Ushie, F. A. and Esu, E. O. (2002). A geochemical survey of groundwater in Khana and Gokana local government oarea of Rivers State, Nigeria. *Journal Applied Science Environment Mangement*, 6:53– 59.
- [56] Zhang E., Xu W., Diao G. and Shuang, C. (2006). Electricity generation from acetate and glucose by sedimentary bacterium attached to electrode in microbial-anode fuel cells. *Journal of Power Sources*; 161:820–5.
- [57] Zhao S., Zhoa X., Zou H., Fu J., Du G., Zhou J. and Chen J. (2014). Comparative proteomic analysis of Saccharomyces cerevisae under different nitrogen sources. *Journal Proteomics* 101:102-12.
- [58] Zhou M., Chi M., Luo J., He H. and Jin T. (2011). An overview of electrode materials in microbial fuel cells. *Journal Power Sources* 196: 4427-4435.