Abattoir Wastewater Treatment and Energy Recovery using a Biocathode Microbial Fuel Cell

By

Akaluka, C. K., Braide, W., Orji, J. C., Adeleye, S.A, Egbadon, E. O. and Okey Mbata, C.C.

ISSN 2319-3077 Online/Electronic ISSN 0970-4973 Print

Journal Impact Factor: 4.275 Global Impact factor of Journal: 0.876 Scientific Journals Impact Factor: 3.285 InfoBase Impact Factor: 2.93 Index Copernicus International Value IC Value of Journal 6.01 Poland, Europe

J. Biol. Chem. Research Volume 32 (2) 2015 Pages No. 1036-1049

Journal of Biological and Chemical Research

An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in various International and National Scientific Databases

Published by Society for Advancement of Sciences®

 J. Biol. Chem. Research. Vol. 32, No. 2: 1036-1049, 2015

 (An International Peer Reviewed / Refereed Journal of Life

 Sciences and Chemistry)

 Ms 32/2/138/2015

 All rights reserved

 ISSN 0970-4973 (Print)

 ISSN 2319-3077 (Online/Electronic)

 Akaluka, C.K. , Ada

 http:// www.sasjour



Received: 23/10/2015 Revised: 17/11/2015

RESEARCH PAPER Accepted: 21/11/2015

Abattoir Wastewater Treatment and Energy Recovery using a Biocathode Microbial Fuel Cell

Akaluka, C. K., Braide, W., Orji, J. C., Adeleye, S.A, Egbadon, E. O. and

*Okey Mbata, C.C.

Department of Microbiology, Federal University of Technology, P.M.B 1526, Owerri, Imo State, Nigeria.

*Department of Biochemistry, Federal University of Technology, P.M.B 1526, Owerri, Imo State, Nigeria.

The capacity of Microbial fuel cells to produce voltage and concurrently treat abattoir waste water was investigated. Two H-shaped (dual chamber) microbial fuel cells (DCMFC) were constructed with four transparent polyacrylic containers (1litre volume each), Two agar salt bridges were prepared in 10cm (length) by 3cm (diameter) polyvinylchloride (PVC) pipes each containing 2% molten agar and 1M sodium chloride. Graphite rod shaped electrodes for anode and cathode with dimension 12cm length by 1.2cm diameter where used. The waste water served as anolyte while the catholyte was made up of 1% glucose thereby modifying the cathode to become a biocathode. The open circuit voltage (OCV) and current readings were taken at 3 hours interval and maximum OCV of 557mV was recorded. Also, The physicochemical and microbial characteristics of the MFCs revealed that the pH decreased by 0.2 after treatment; COD, BOD, Ammonia, TSS, and Total Nitrogen decreased by 84.03%, 57.9%, 99.9%, 12.6% and 99.9% respectively. However, Phosphate increased by 16.88%. The bacterial isolates from the raw abattoir wastewater were Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Enterococcus faecalis, Enterobacteraerogenes, Escherichia coli and Micrococcus luteus while Enterococcus faecalis and Staphylococcus aureus were isolated from the biocathode.

Keywords: Biocathode, Microbial Fuel Cells, Physicochemical, Anolyte and Catholyte.

INTRODUCTION

More than 1.6 billion people live without access to electricity and an eco friendly environment globally. Experts estimate that 60-70% of the Nigerian population lack access

to clean environment and electricity (CREDC, 2007). Although oil, natural gas and coal are the resources for energy production today, these cannot sustain the teaming population in the future and also are not environmentally friendly as they contribute to air pollution (Logan, 2008). Currently, the solution to the looming energy crisis is to develop alternative sources of energy that is renewable and sustainable. On the other hand, waste water treatment is another global issue that is eating up the finances of industries and Nations (Ghanapriya et al., 2012). Existing treatment cost is estimated to be very expensive that some developing countries discharge the waste water untreated to the environment. Microbial fuel cells are unique in that they can produce energy as well as treat wastes (Rahimnejad et al., 2012; Logan et al., 2007; Liu et al., 2005). Electricity can be generated in microbial fuel cells using mixed cultures enriched from domestic waste water (Liu et al., 2005), animal waste (Min and Logan, 2004) and anaerobic sewage sludge (Liu et al., 2005). It has been demonstrated that a wide range of substrates (cellulose, sugars and acetone) can be used to capture electricity from organic matter using bacteria. Several configurations and outputs has also been reviewed (Logan et al., 2015). The flexibility of microorganisms to use a range of fuels especially wastes makes microbial fuel cell an ideal technology for renewable bioelectricity generation from biomass (Logan, 2009). Although the power densities currently generated from MFCs are too low to be considered as a viable alternative energy source (Franket al., 2010). Much research efforts have been directed in this field in recent years (Liu et al., 2012; Wang and Ren, 2013; Rahimnejad et al., 2012). Abattoir waste water contains large loads of organic matter coming from the cow blood, rumen contents and wash water. The use of abattoir wastewater as a substrate for MFC that has a potential for degrading has been investigated (Momoh and Neayor, 2010a; Ghanapriya et al., 2012). Proper treatment of animal waste and resource recycling to reduce its environmental impact are currently important issues for the livestock industry (Ghanapriya et al., 2012). The process of power generation and simultaneous treatment of abattoir wastewater in large scale systems can become practicable with proper design and reactor configuration (Momoh and Naeyor, 2010a). In this research, the potential of MFCs to generate electricity and treat abattoir waste water was investigated by enriching the catholyte with analytical glucose so as to encourage growth of microorganisms thereby converting it to a biocathode.

MATERIAL AND METHODS

Collection of Abattoir Wastewater

Wastewater was collected from an abattoir at Relief market, Egbu road, Owerri, Imo state, Nigeria. They were collected aseptically into sterile bijou bottles for microbiological analysis and clean plastic containers for physicochemical analysis. The wastewater served as inoculum and organic substrate sources for the Microbial Fuel Cell without any modification such as adjustment of pH or addition of nutrients (Min *et al.*, 2005a). The abattoir wastewater collected aseptically in sterile bijou bottles were transported to the microbiology laboratory immediately for analysis.

Microbiological analysis of raw Abattoir Wastewater

Prior to use in MFC, the abattoir wastewater was serially diluted ten-fold and plated out on Nutrient agar, EosinMethylene Blue agar, MacConkey agar and Mannitol Salt agar in duplicates using the spread plate technique. The plates were incubated at 37°C for 24-48 hours.

J. Biol. Chem. Research

Vol. 32 (2): 1036-1049 (2015)

Characterization of bacterial isolates

Distinct colonies from the plates were purified by sub culturing on Nutrient agar at 37°C for 24 hours. The isolates were then identified through microscopy (gram stain reaction, motility, sporulation and capsules were determined) and the following biochemical tests; catalase, oxidase, coagulase, methyl red, indole, Vogues Proskauer, citrate and carbohydrate fermentation tests; glucose, fructose, maltose, lactose and sucrose (Cheesbrough, 2000). The identities of the isolates were determined using standard manuals (Buchanan and Gibbon, 1984).

Physicochemical analysis

The following physicochemical analysis were carried out in a chemistry laboratory; conductivity, pH, total dissolved solid (TDS), chemical oxygen demand (COD), biological oxygen demand (BOD), phosphate, ammonia, total nitrogen and total suspended solid (TSS) according to American Public Health Association manual (1998). The physicochemical parameters were determined before and after the MFC experiment.

Estimation of biochemical oxygen demand

The respirometer was calibrated according to manufacturer's instruction. The abattoir wastewater was diluted with distilled water at a ratio of 1:10. One hundred milliliters of the diluted abattoir wastewater was transferred into the respirometer with the magnetic stirrer in place. The alkaline bung was filled with potassium hydroxide and the BOD sensor head was placed to cover the respirometer container. The BOD sensor was set and the initial dissolved oxygen (DO) value taken. Another respirometer containing 100ml of distilled water was set up which served as control. The respirometers (sample and control) were incubated at $20 \pm 1^{\circ}$ C in an incubator that had a stirring device. The time for incubation was taken. Readings of the BOD sensor was taken after 5 days. Difference in the dissolved oxygen (DO) between the final reading and initial reading was corrected for the 1:10 ratio dilution and recorded as BOD (APHA, 1998).

Chemical Oxygen Demand

The Chemical Oxygen Demand (COD) was determined using the closed reflux colorimetric method (APHA, 1998). The Tubetests heater was calibrated and the control was set to150°C and the safety shield was put in position. Two milliliter of the abattoir wastewater was transferred into the COD Tubetests tube using a clean standard laboratory pipette. Each Tubetests tube contained standard sulphuric acid, potassium dichromate and silver sulphate catalyst as prepared by the manufacturer. The caps of the COD Tubetests tubes were replaced tightly and each tube was gently inverted to mix the contents until all the precipitate was suspended. The Tubetest was labelled properly and placed in the Tubetests heater at 150°C with the safety screen in position.

Reagents blank was prepared by adding 2ml of deionized water into the Tubetests tube and placing it in the heater at the same time and temperature as the sample. The tubes were digested for two hours at 150°C and then the heater turned off. The tubes were removed and allowed to cool to room temperature. The reagent blank was used to zero the photometer at 490nm wavelength before the sample tubes were read and recorded (APHA, 1998).

pН

The pH meter was calibrated with a pH 7 buffer solution. The electrodes were rinsed with deionized water, damped lightly and inserted into the abattoir wastewater in a clean beaker. The pH reading was taken and recorded (APHA, 1998).

Phosphate

The abattoir wastewater was diluted with deionized water at a ratio of 1:20. Using a clean pipette, 5ml of the diluted wastewater was transferred into Tubetest cuvette and one Wagtech phosphate test tablet added into the tube and crushed with an applicator. Each phosphate test tablet contains the reagents; ammonia molybdate, ammonium metavanadate, hydrochloric acid, activated carbon and phenolphthalein indicator. After the test tablet had dissolved in the wastewater, it was allowed to stand for 15 minutes for the reaction to take place.

A blank was prepared along with the sample which contained the crushed test tablet in distilled water, allowed to stand for 15 minutes. The reagent blank was inserted in the photometer set at 490nm with phosphate test selected on the machine. It was zeroed with the reagent blank and reading of the sample tube was taken at the same wavelength (APHA, 1998).

Ammonia

The abattoir wastewater was diluted in distilled water at a ratio of 1:5. Using a clean pipette, 5ml of the diluted waste water was transferred into a test tube. 0.6ml of the NH4 reagent 1 (containing sodium chloride) was added to the sample. Then cap measurement of NH4 reagent 2 (containing a chlorinating agent) was added and the mixture was shaken vigorously and allowed for 5 minutes. Then 4 drops of NH4 reagent 3 (containing thymol) was added, mixed and allowed to stand for 5 minutes.

Reagent blank was prepared including all the reagents mentioned above while the sample was replaced with distilled water. Then it was poured into a cell/curvette and used to zero the photometer. Then the sample readings were taken with the photometer (APHA, 1998).

Conductivity

The conductivity meter was calibrated using potassium chloride of 0.01M (745.6mg of anhydrous KCL was dissolved in 200ml distilled water and diluted to 1000ml in a volumetric flask). The conductivity cell was rinsed with 0.01M KCL solution three times and in the fourth time, the temperature was adjusted to $25.0 \pm 0.1^{\circ}$ C. The probe in the standard KCl solution was adjusted to read 1412µmho/cm.

The probe was then inserted in a beaker containing the sample (wastewater), the temperature was adjusted to 25°C and the sample conductivity was taken (APHA, 1998).

Total Dissolved Solids (TDS)

The total dissolved solid was derived from half of the value of the conductivity measurement of the abattoir wastewater.

Total Nitrogen

Abattoir wastewater was dispensed into the alkaline persulfate digestion reagent pyrex tube at a volume ratio of 2 to 1. It was capped tightly and mixed properly then digested on a heater at 100-110°C for 1 hour. When the digestion cycle was complete, the alkaline persulfate pyrex tube was removed from the heater and allowed to cool. After cooling, the total nitrogen was determined using a colorimeter at 540nm.

Total Suspended Solids (TSS)

A glass-fiber filter disk of a filtration apparatus was washed and vacuumed three times with 20 ml portions of reagent grade water. The filter was then inserted on an aluminum weighing dish and weighed. The filter apparatus was assembled and wet with the reagent-grade water. The abattoir wastewater was stirred to homogeneity and 10ml of the wastewater was pipetted onto the glass-fiber filter. The filter was then washed with successive 10-ml volume of reagent-grade water allowing complete drainage between washings and it was continuously suctioned for 3 minutes after filtration was complete. The filter was removed and weighed in the aluminum weighing dish. The total suspended solid (mg/ml) was determined as follows:

100(A-B)/ sample volume

Where A= weight of filter + dried residue in mg and

B= weight of filter in mg

Construction of H-shaped Microbial Fuel Cell

The microbial fuel cell was constructed according to methods described by Adeleye and Okorondu, (2015). It consists of an anode, a cathode and sometimes a membrane or separator between the electrodes (Rahimnejah *et al.*, 2012). The anode chambers containing abattoir wastewater and the cathode chambers of the MFCs containing glucose water. In an MFC, the bacteria that oxidise a substrate are kept physically separated from the electron acceptor by a proton exchange membrane (Liu *et al.*, 2004). The Proton Exchange membrane that separated the bacteria from oxygen, allows charge transfer between the electrodes (Logan, 2008). Organic matter (carbon source) is oxidized at the anode by microorganisms, called exoelectrogens, which transfer electrons to the electrodes (Werner *et al.*, 2013). Through anaerobic metabolism of organic substances, by microorganisms electrons and protons are liberated (Banik *et al.*, 2012).

Two H-shaped (dual chamber) microbial fuel cells (DCMFC) were constructed with eight transparent polyacrylic containers (1litre volume each). The containers were perforated and attached with an inner adapter using epoxy glue. Twoagar salt bridges were prepared in 10cm (length) by 3cm (diameter) polyvinylchloride (PVC) pipes each. The agar salt bridges contained 2% molten agar and 1M sodium chloride (Min *et al.*, 2005b). Two containers were linked with an agar salt bridge interconnection. One chamber served as the anode and the other linked chamber served as the cathode.

Graphite electrodes for anode and cathode with dimension 12cm by 1.2cm where used. The electrodes were sanded lightly to increase the surface area for bacteria growth and attachment (Logan, 2008). Holes were bored in the lid of the anode and cathode chambers to allow the passage of coated copper wires which were connected to the stainless steel wires wound around the graphite electrodes. A second hole was bored on the lid of the cathode chambers to contain glucose solution; this was done to allow for aerobic respiration.

Determination of Open Circuit Voltage (OCV) and current generated in the MFC using different catholytes at room temperature

A total of two MFCs were set up at room temperature $30\pm 3^{\circ}$ C. Nine hundred milliliters of abattoir wastewater were added into each anode chambers of the MFC set up (A and B) as shown in figure 1. Then Nine hundred milliliters of 1% glucose solution were added to the cathode chambers of the MFCs (A and B) as shown in figure 1.

Graphite electrodes were submerged in the anolytes and catholytes up till 10.5cm height and were connected with copper wires passing through the MFC lids. The anode chambers were covered tightly to enable an aseptic anaerobic microbiological condition while the cathode chambers for glucose were loosely tightened to enable aerobic respiration.

The copper wires connecting the electrodes from the MFC were connected to the probes of a multimeter and open circuit voltage (OCV) and current readings were taken at 3 hours interval for 20 days. A digital air thermometer was kept in the room housing the MFC setups, the air temperatures were monitored and recorded every 3 hours for 20 days.



Figure 1. H-shaped (dual Chamber) Microbial Fuel Cell set up.

RESULTS

The physicochemical parameters obtained from the experiment was used to calculate waste water treatment efficiency (WWTE) using the relation

$$WWTE = \frac{initial - final}{initial} \times 100$$

In this research, the chemical oxygen demand, biochemical oxygen demand, total suspended solids, ammonia, and total nitrogen reduced by 84.03%, 57.9%, 12.62%, 99.99% and 97.14% respectively. The pH of the abattoir wastewater decreased by 0.2.

Table 1. Initial and final physicochemical parameters of the abattoir waste water.

Parameters of physicochemical analysis	Before MFC treatment	After MFC treatment (glucose catholyte)
COD (mg/ml)	9350	1493
BOD (mg/ml)	2600	1095
TSS (mg/ml)	3250	2840
рН	7.2	7.0
Phosphate (mg/ml)	480	561
Conductivity (µs)	5.5	8.0 μs
Ammonia (mg/ml)	1.2	<0.01
TDS (ppm)	2.8	3.98
Total Nitrogen (mg/ml)	1.05	0.03

J. Biol. Chem. Research

Vol. 32 (2): 1036-1049 (2015)

Determination of Open Circuit Voltage (OCV) and Current Generated in the MFC Using Different Catholytes at Room Temperature

The results of the estimation of the Open circuit voltage (OCV) using glucose catholyteis shown in Figure 2. The maximum open circuit voltage obtained was 557 mV. The OCV was 256 mV on the first day and rose gradually to give a maximum value of 557 mV on the 234th hour (\approx 10th day). After the 234th hour, the voltage dropped gradually to 433 mV on the 20th day.Using glucose as a catholyte, a biofilm was formed on the cathode which fed on the glucose solution in the cathode chamber. The biofilm accepted electrons from the anode chamber through the copper wires in the MFC. The glucose solution became cloudy at the end of the 20 days.



Figure 2. OCV in Millivolts Generated from Abattoir Wastewater Using MFC and Glucose Catholytes.

Comparison of the Microbiological Properties of the Abattoir Wastewater before and After Use in the MFC

The comparison of the microbiological properties of the raw abattoir wastewater and the used abattoir wastewater (after use on the H shape MFC for 20 days) is shown in Table 2. A large reduction in the population of microorganisms in the abattoir wastewater was observed in this study. Table 2 shows all the bacteria isolated from the abattoir wastewater before and the after use in the MFC. The bacteria isolated from the biofilms on the anode and biocathode of the MFC were alsoreported. The biochemical tests used to identify specific isolates in this study is presented in Tables 3, 4 and 5.

Bacterial Isolates	Bacterial isolates	Bacterial isolates on	Bacterial Isolates			
before MFC	after MFC	Anode (Exoelectrogenic	on Cathode			
treatment	treatment	bacteria)	(Electron acceptor)			
Staphylococcus aureus	Staphylococcus	Enterococcus	Enterococcus			
	aureus	faecalis	faecalis			
Bacillus cereus	Bacillus cereus	Bacillus cereus	Staphylococcus			
			aureus			
Bacillus subtilis	Bacillus subtilis	Escherichia coli				
Micrococcus luteus	Enterococcus					
	faecalis					
Enterococcus	Escherichia coli					
faecalis						
Enterobacter	Enterobacter					
aerogenes	aerogenes aerogenes					
Escherichia coli	Klebsiella					
	pneumoniae					

Table 2. Comparison of the microbiological properties of the raw abattoir wastewater andthe used abattoir wastewater.

DISCUSSIONS

The bacterial isolates from the raw abattoir wastewater were *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Enterobacter aerogenes*, *Escherichia coli* and *Micrococcus luteus*. The bacterial isolates from this study can be compared with some of the isolates from the abattoir wastewater research work done by Adesemoye *et al.*, (2006) in which *Bacillus* spp and *Micrococcus luteus* were both isolated from the abattoir wastewater. In this study, *Escherichia coli* was isolated and this organism serves as an indicator of faecal contamination of abattoir wastewater and yet was not eliminated by the process. *Micrococcus luteus* which was previously isolated from the raw wastewater did not emerge after MFC treatment for 20 days. This may be because of the effect of reduced oxygen treatment for 20 days on the aerobic bacteria. *M. luteus* is an obligate aerobe (Woodward and Kell, 1991), thus this organism does not strive under anaerobic conditions.

The results of the physicochemical parameters represented in Table 1 presents the treatment capacity of the MFCs using glucose ascatholytes. The pH was initially at 7.2(near neutral) but decreased by 0.2 after treatment. The pH has been shown to be vital for the performance of an MFC. MFC performance peaks at pH 7 which is due to the microbial requirement for adaptation at that pH (Elakkiya and Matheswaran, 2013). The abundance and activity of microbial community are controlled by pH.

The chemical oxygen demand was9350 mg/mlbut finally became 1493mg/ml therefore obtaining about 84.03% treatment efficiency by the MFC. This parameter can be compared with the report by Ghangrekar and Shinde (2007) which gave a COD removal efficiency of 88% within 16-35 days. Elakkiya and Matheswaran (2013) reported a COD removal of 91% using a dairy wastewater in a dual chamber MFC. Liu *et al.*, 2004 reported a COD removal efficiency of 80% for domestic wastewater.

TSC		TCC		TEC					THBC			Grou
MSA	MCA	MCA	EMA	EMA	NAW	NAW	NAW	NAM	NAW	Code		p Colo
W1	W2	W1	W2	W1	5	14	13	12	1			Ŋ
Golden yellow low convex	Pink mucoid	Pink moist and shiny	Pink with purple dot	Metallic sheen	Creamy tiny circular	Yellow moist and shiny	Creamy and Low convex	Creamy and flat	Golden yellow moist and shiny	Morphology		Colonial
S+	₽	-R	'n	₽	S+	S+	₽	₽₽	C+	Gram	charac	Micros
'	+	+	+	+		'	+	+		Mot	teristi	copic
	•	•		•		1	+	+	I	Spore	S	
'	1	'	'	1	'	'	'	•		Cap		
+	+6	9 +	+ ⁶	+G	+		+	+	+	G	Fer	ណ្ឌ
+	+G	+6	+G	+ ₆	+		+	+	+		men	boh
+	+G	+ ₆	+ ⁶	+G	+	1		•	+	\leq	tatio	drat
+	÷6	+ ₆	+6	+ ₆	+		ť	•	+	-	-	æ
	+	+	+	+	•	1	+	•		×		
+	+	+	+	+	'	+	+	+	+	ഹ	Tests	Bioch
			•	1						Oxi		emical
+	1		'	1	'	'		1	+	Coa		
1	1	+		+				1		Ind		
'	1	+	'	+	'	'	'	•		MR		
+	+		+	1	+	+	+	+	+	VP		
'	+		+	1	+	+	+	+		C i		
S. aureus	E. aerogenes	E. coli	E. aerogenes	E. coli	E. faecalis	M. luteus	B. subtilis	B. cereus	S. aureus		Isolates	Identity of

Table 3. Characteristics of Bacterial Isolates from Abattoir Wastewater Prior to its Use inMFC (Before Electricity Generation).

Keys: THBC = Total heterotrophic bacteria count, TEC = Total enterobacteriaceae count, TCA= Total coliform count, TSC= Total *Staphylococcus aureus* count, Gram= Gram staining reaction, Mot= Motility, Spore= Spore formation, Cap= Capsule, G- Glucose, F=Fructose, M=Maltose, L= Lactose, X=Xylose, Ca= Catalase, Coa=Coagulase, Oxi= Oxidase, Ind=Indole, MR=Methyl Red, VP=Vogues Proskauer, Cit=Citrate Utilisation, +^G = positive plus gas production, +S = positive and spherical, +R= positive and rod-shaped.

TSC			TCC		TEC				THBC		Group
MSAWA1	MCAWA3	MCAWA2	MCAWA1	EMAWA2	EMAWA1	NAWA4	NAWA3	NAWA2	NAWA1	Code	Colony
Golden yellow low convex	Creamy raised convex	Pink moist and shiny	Pink mucoid	Pink with purple dot	Metallic sheen	Creamy tiny circular	Creamy and Low convex	Creamy and flat	Golden yellow moist and shiny	Morphology	Colonial
S+	÷₽	-R	ӡ	Ъ	ӡ	S+	ħ	÷	S +	Gram	Micro
•	•	+	+	+	+		+	+		Mot)scopic cteristi
•	•	'	1	I	•	1	+	+	1	Spore	S
	+	'	1		•	'		•		Cap	
+	-te	+6	+ ₆	+6	+ ₆	+	+	+	+	G	ଳ ଜ୍ମ
+	-6 -6	+G	+ ₆	+6	+ ₆	+	+	+	+		men.
+	9 +	<mark>6</mark> +	<mark>6</mark>	+6	+ ₆	+	ı	•	+	\leq	/drat tatic
+	9 ⁺	+ ⁶	+ ₆	9 +	+ ₆	+	÷.		+	-	3 6
	+	+	+	+	+		+	•	•	\times	
+	+	+	+	+	+	'	+	+	+	ြ	Bioche Tests
	•		1		•			•		X.	emical
+			1	'	1		'	'	+	Coa	
•		+	•	•	+	I	•	•	1	Ind	
'	· ·	+	1		+	+		1		MR	
+	+	•	+	+			+	+	+	<₽	
1	+		+	+	•	+	+	+	1	<u>C</u> t	
S. aureus	K. pneumoniae	E. coli	E. gerogenes	E. aerogenes	E. coli	E. faecalis	B. subtilis	B. cereus	S. aureus	Isolates	Identity of

Table 4. Characteristics of Bacterial Isolates from Abattoir Wastewater after Use in the MFC.

TSC	Cathode THBC	TCC	TEC		Anode THBC		Group
7.0 x 10 ⁵	9.8 x 10 ⁶	6.5 x 10 ⁵	3.5 x 10 ⁵		8.2 x 10 ⁵	Count(cfu/ml)	Total
MSA/CA1	NA/CA1	MCA/AN1	EMB/AN1	NA/AN2	NA/AN1	Code	Colony
Golden yellow low convex	Creamy Low convex	Pink moist and shiny	Metallic sheen	Creamy dull and dry	Creamy Low convex	Morphology	Colonial
S+	St	-₽	∽	ħ	S+	Gram	Micro chara
		+	+	+	'	Mot	scopic cterist
				+		Spore	3
•	•	•	•	•		Cap	
+	+	<mark>6</mark> +	<mark>6</mark> +	+	+	G	Fer Gar
+	+	<mark>6</mark> +	<mark>6</mark> +	+	+		bohy
+	+	+6	÷		+	\leq	l drat
+	+	-te	÷	•	+	-	3 0
'		+	+	•	•	×	
+	ı	+	+	+		G	Bioc
'	1					Oxi	hemic
+	ı		•	•	•	Coa	al Test
		+	+	1		Ind	S.
	+	+	+		+	MR	
+				+		≤P	
'	+	•	•	+	+	Cit	
S. aureus	E. faecalis	E. coli	E. coli	B. cereus	E. faecalis		Identity of Isolates

Table 5. Characteristics of Bacterial Isolates from Anode and Glucose Biocathode MFC.

The totals suspended solids, biochemical oxygen demand, ammonia and total nitrogen of the used wastewater also decreased. The 57.9% decrease in BOD MFCs were as a result of the dissolved oxygen consumed by the indigenous microorganisms. Total nitrogen and ammonia for decreased by 99.9% respectively. This large decrease in values may have been as a result of dentrification and ammonia oxidation activities that took place in the anode chamber under reduced oxygen conditions (Min *et al.*, 2005a).

Contrary to nitrogen, phosphate increased by 16.88%. An explanation for this may be that the low redox potential in the anode cells leads to a release of inorganic phosphate from organic matter (Min et al., 2005a). Conductivity and total dissolved solids (TDS) increased in the abattoir wastewater which may be as a result of mineralization that took place during the running of the MFC. The MFC was operated at room temperature of $30 \pm 3^{\circ}$ C for 20 days in a batch mode. The abattoir wastewater was not buffered and no external microorganism was introduced into the anode. The indigenous exoelectrogenic organisms in the abattoir wastewater broke down the organic matter in the abattoir wastewater and thereby generated electricity. The open circuit voltage (OCV) and current readings were taken at 3 hours interval. The maximum OCV readings were 557mV. The maximum OCV for glucose biocathode (557 mV) was as a result of the difference between the potential at the anode (where exoelectrogenic microorganisms donated electrons to the graphite) and the cathode (where microorganisms that formed biofilm on the graphite accepted electrons while feeding on glucose) (Logan, 2008). The OCV started at 256 mV and gradually climbed until the 234th hour when the maximum was 557 mV. Followed by a constant dropping towards the end of the MFC experiment. This also shows that the glucose may have been depleted and organisms in the biocathode needed more nutrients to function effectively as electron acceptors.

The maximum OCV generated in this study (Figure 2) was low when compared to the report of Momoh and Naeyor (2010b) and Adeleye and Okorondu, (2015). This low OCV will translate to low power density (Logan, 2008). The possible cause of the low OCV and current readings observed in this study may be as a result of the high strength abattoir wastewater which has an COD of 9350 mg/l used in the set-up and poor utilization of the graphite anodes (Momoh and Naeyor, 2010b). Also, H shape or dual chamber MFC has high internal resistance (Logan, 2008) which may have contributed to the low OCV. The electricity supply in the H shape microbial fuel cell was erratic. Therefore, research is needed to combat the internal resistance and produce better electrodes and devices that will trap the electricity for upscaling purposes (Logan *et al.*, 2015).

ACKNOWLEDGEMENTS

Special thanks goes to the Federal university of Technology Owerri for her different means of assistance; human, financial and academic. More so, my supervisors, Prof (Mrs) J.C. Orji and Dr. Wesley Braide for their tireless contribution in achieving this work....Akaluka Cynthia Kelechi(Corresponding author).

REFERENCES

Adeleye S.A. and Okorondu S. I. 2015. Bioelectricity from students' hostel waste water using microbial fuel cell. Int. J. Biol. Chem. Sci. 9 2 : 1038-1049.

- Adesemoye, O. A., Opere, B. O. and Makinde, S. C. O. 2006 . Microbial Content of Abattoir Wastewater and its Contaminated Soil in Lagos. African Journal of Biotechnology, 5 10: 1963-1968.
- American Public Health Association APHA, 1998. Standard Methods for the Examination of Water and Wastewater 20th ed. American Public Health Association, Washington DC, USA. pp 4-95- 5-30.
- Banik, A., Jana, N. K., Maiti, B. R. and Ghosh, T. K. 2012. Development of Microbial Fuel Cells and Electrode Designs with Waste Water Anaerobes. Greener Journal of Biological Sciences, 2 2:13-19.
- Bruce E. Logan, Maxwell J. Wallack, Kyoung-Yeol Kim, Weihua He, Yujie Feng, and Pascal E. Saikaly 2015 . Assessment of Microbial Fuel Cell Configurations and Power Densities.Environ. Sci. Technol. Lett., 2, 206–214.
- Buchanan, R. E. and Gibbon, N. E. 1984. Bergey's Manual of Determinative Bacteriology. 8th ed. . The Williams and Wilkin's Co. Baltimore. pp 1246-1249.
- Cheesbrough, M. 2000. District Laboratory Practise in Tropical Countries Part 2. Cambridge University Press, Cambridge, United Kingdom. pp 38- 219.
- CREDC Community Research and Development Centre. 2007. Promoting Renewable Energy and Energy Efficiency in Nigeria. A Report of a One-day Conference Held at the University of Calabar Hotel and Conference Centre on 21st of November, 2007. pp 1-50.
- Elakkiya, E. and Matheswaran, M. 2013. Comparison of Anodic Metabolisms in Bioelectricity Production during Treatment of Dairy Wastewater in Microbial Fuel Cell. Bioresource Technology, 136:407–412.
- Frank, A. E., Malvankar, N. and Nevin, K. P. 2010. Bacterial Biofilm: the Powerhouse of a Microbial Fuel Cell. Biofuels, 14:589-604.
- Ghanapriya, K., Rana, S. and Kalaichalvan, P. T. 2012 . Electricity Generation from Slaughterhouse Wastewater Using Microbial Fuel Cell Technology. Advanced Biotechnology, 11 9 :20-23.
- Ghangrekar, M. M. and Shinde, V. B. 2007. Performance of membrane-Less Microbial Fuel Cell Treating Wastewater and Effect of Electrode Distance and Area on Electricity Production. Bioresource Technology, 98 15: 2879–2885.
- Liu, H., Grot, S. and Logan, B. E. 2005. Electrochemically Assisted Microbial Production of Hydrogen from Acetate. Environmental Science Technology, 39 11 :4317-4320.
- Liu, L., Tsyganova, O., Lee, D. J., Su, A. and Change, J. S. 2012 . Anodic Biofilm in Singlechamber Microbial Fuel Cells Cultivated under Different temperatures. International Journal of Hydrogen Energy, 37: 15792-15800.
- Logan, B. E. 2008. Microbial Fuel Cells. 1st edition, John Wiley and Sons. Hoboken, New Jersey. pp 1-120.
- Logan, B. E. 2009 . Exoelectrogenic Bacteria That Power Microbial Fuel Cells. Nature Reviews of Microbiology, 7:375-381.
- Logan, B. E., Cheng, S., Watson, V. and Estadt, G. 2007. Graphite Fiber Brush Anodes for Increased Power Production in Air-Cathode Microbial Fuel Cells. Environmental Science Technology, 41:3341-3346.

- 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 and Technology, 38 21: 5809-5814.
- Min, B., Cheng, S. and Logan, B. E. 2005b. Electricity Generation Using Membrane and Salt Bridge Microbial Fuel Cells. Water Research, 39:1675-1686.
- Min, B., Kim, J. R., Oh, S., Regan, J. M. and Logan, B. E. 2005 . Electricity Generation from Swine Wastewater Using Microbial Fuel Cells. Water Research, 39:4961–4968.
- Momoh, O. L. Y. and Naeyor, B. 2010a. A Novel Electron Acceptor for Microbial Fuel Cells: Nature of Circuit Connection in Internal Resistance. Journal of Biochemistry and Technology, 2 4 :216-220.
- Momoh, O. L. Y. and Naeyor, B. 2010b. Generation of Electricity from Abattoir Wastewater with the Aid of a Relatively Cheap Source of Catholyte. Journal of Applied Science and Environmental Management, 14 2 :21-27.
- Rahimnejad, M., Ghoreyshi, A. A., Najafpour, G. D., Younesi, H. and Shakari, M. 2012 . A Novel Microbial Fuel Cell Stack for Continuous Production of Clean Energy. International Journal of Hydrogen Energy, 37:5992-6000.
- Wang, H. and Ren, Z. J. 2013 . A Comprehensive Review of Microbial Electrochemical Systems as a Platform Technology. Biotechnological Advances, 31:1796-1807.
- Werner, C. M., Logan, B. E., Saikaly, P. E. and Amy, G. L. 2013 . Wastewater Treatment, Energy Recovery and Desalination Using a Forward Osmosis Membrane in an Air-Cathode Microbial Osmotic Fuel Cell. Journal of Membrane Science. 42 8:116-122.
- Woodward, A. M. and Kell, D. B. 1991. On the Relationship Between the Nonlinear Dielectric Properties and Respiratory Activity of the Obligately Aerobic Bacterium *Micrococcus luteus* Journal of Electro analytical Chemistry and Interfacial Electrochemistry. 321 3: 423-439.

Corresponding author: Adeleye, S.A., Department of Microbiology, Federal University of Technology, P.M.B 1526, Owerri, Imo State, Nigeria.

Email: cynthia.akaluka@futo.edu.ng adeleyesamuela@gmail.com