Bioelectricity generation from Abattoir wastewater by *Enterobacter agglomerans*

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Abstract

This study investigated generation of bioelectricity from Abattoir wastewater (AWW) in microbial fuel cell (MFC) by *Enterobacter agglomerans*. Potassium permanganate, sodium chloride and oxygen as catholytes were used to improve the efficiency of the Microbial fuel cell. Furthermore, addition of sodium acetate (booster) in the anode chamber also enhanced electric current generation. The highest voltage readings of 1163.4 mV was from *E agglomerans* MFC incorporating potassium permanganate, followed by sodium chloride (538.6 mV) and lastly distilled water (325.9 mV). This study showed that *Enterobacter agglomerans* is an electrochemically active organism that has the potential to generate electricity from Abattoir wastewater.

Keywords: Diffuse radiation, Clearness Index, Global Solar Radiation, Sunshine Hour.

1. Introduction

The world's limited supply of fossil fuels and the impact of it on climate change require us to develop alternative energy sources. Among the next generation energy sources, microbial fuel cell (MFC) is attracting wide attention due to its intended use to recover energy in the form of electricity. MFCs are fuel cells that convert chemical or solar energy to electrical energy using microorganisms as the catalysts (Allen and Bennetto 1993)

Most electricity generating bacteria belong to the phylum Proteobacteria with a few among firmicuites (Cao et al., 2009). Notable among which include *Enterobacter* sp , which is a facultative anaerobic, Gram positive rod (Olga et al 2016).

Bacteria gain energy by the transferring electrons from an electron donor (glucose or acetate) to an electron acceptor (oxygen). The larger the difference in potential between donor and acceptor the bigger growth of the organism which can have proportionally effect on the columbic efficiency and on the electricity generation. Hence microbial fuel cells make use of potential microbial energy to generate electricity (Olga et al., 2016**)**.

Although, this technology seems promising, microbial fuel cells are not deficient of their own challenges. Power output from microbial fuel cells has been affected by high internal resistance inherent in these systems. However, in a bid to improve power density in MFC's, the use of electron acceptors (Catholytes) apart from oxygen has been extensively explored (Rabaey et al., 2004; Rabaey et al., 2003)

A range of organic substrates can be used by the microbes in bioelectricity production. Notable among these substrates are: domestic wastewater (Choi & Ahn, 2013), swine wastewater (Min et al., 2005),Oil wastewater (Jiang *et al*. 2013; Choi & Liu 2014), Waste sludge (Ge *et al*., 2013; Choi & Ahn, 2014), Fruit and vegetable wastes (Logroño *et al*., 2015), food waste leachate (Choi and Ahn, 2015) sediments from marine and lake, brewery wastewater (Logan 2005; Rabaey et al., 2005; Feng et al., (2008) and abattoir wastewater (Momoh and Neayor 2010).

Abattoir wastewater has been identified as one of complex wastewater generated in most under develop, developing and developed countries. Abattoir wastewater is known to contain: carbohydrate, lipid, organic acid, protein, nitrogen, cellulose, phosphorus, high BOD and COD which is consistent for a product with high organic matter (Jeffrey et al., 2009).

Abattoir water has been used in MFC (Momoh and Neayor et al., 2010) with catholytes (Akaluka et al., (2016). Though *Enterobacter* has been used in MFC but not with these sets of catholyte. In this study, the use of Potassium permanganate, Sodium chloride and oxygen as electron acceptor (catholyte) in MFC with *Enterobacter* sp as electron generator was investigated.

2.0 Materials and Methods

2.1 Wastewater Collection

Abattoir wastewater was collected from Odo eran, abbatoir at Lafenwa, Abeokuta, Nigeria. Collection of sample was done using clean and sterile 10L plastic containers

2.2 Sterilization of wastewater

Filtration of the wastewater samples was carried out with the use of filter paper (Whatman No 1). Solid particles in the fluid were removed. All the filtration processes were carried out in 2L measuring cylinder.. A portion of abattoir wastewater was sterilized with autoclave so as to kill all the microorganism present at, 121 ºC for 15 minutes. It was allowed to cool down to room temperature (Nasirahmadi *et al.*, 2010) and exactly 1.2 L was introduced into the anode chamber.

2.3 Physicochemical properties of abattoir wastewater

The physiochemical properties of the Abattoir wastewater determined were: pH, Total solid, Total Dissolved Solids, Total Suspended Solids, Determination of Ammonia, Total Organic Carbon, Biological Oxygen Demand (BOD) determination, Chemical Oxygen Demand (COD). Determination and Elemental Analysis of Mg, Fe, P, Na, K, Ca. COD and BOD removal was calculated according to Abhilasha *et al.*, 2009 as:

$$E_{COD} = \frac{CODin - CODout}{CODin} x100\%$$

Where:COD_{in} = is the influent COD and

COD_{out} = is the effluent COD.

2.4 Aerobic Bacterial Count of Abbatoir waste water

Standard techniques were carried out by using the method of Sudarshan, (2000). One milliliter from the wastewater was serially diluted in ten-fold with sterile distilled water and an aliquot of appropriate diluents of 1ml was aseptically dispensed into petri-dishes. Pour plate method was employed using Plate Count Agar (Lab M, UK) and was aseptically poured on the plates. All plates were inoculated in replicates and incubated at 30 ºC for 24 hrs, after which colony count was determined. Colonies were sub-cultured for pure culture.

2.5 Construction and Operation of Microbial Fuel Cell

Microbial fuel Cells (MFCs) were constructed from plastic material of 1500 ml capacity and working volume of 1200 ml. The anode and cathode were separated by a plastic of (3_X10 cm) which house proton exchange membrane (salt-bridge). Two graphite electrode of surface area of 17.6 cm^2 were used in the anode and cathode chambers. The electrodes were attached to copper wires which was glued to the lid of each chamber by a nonconductive epoxy. The anode chambers were filled with 1200 ml sterilized AWW abattoir wastewater for the separate experiment. The anode chamber was completely sealed with epoxy to maintain anaerobic condition. The cathode chambers were filled differently with sodium chloride, potassium permanganate ($KMnO_4$) (catholytes) and distilled water respectively. The pH was adjusted to 7 using 0.5 N NaOH (Aishwarya *et al.*, 2011). Small holes were drilled on the lid of the Cathode chamber to allow for exchange of air (aerobic condition). The external circuits were completed by connecting a resistor (1 kΩ) between the two heads of the graphite electrodes. The salt bridge which forms a bridge between cathodic and anodic chamber facilitates the transfer of ions (protons). Graphite electrodes were used for anode and cathode while the salt bridge consist of 1 M concentrations of NaCl and 10% Agar (Mali *et al.*, 2012). Voltage and current reading of MFC was taken using voltmeter and ammeter. Sodium acetate ($C_2H_3NaO_2$) of 500 mM was introduced into all the MFCs. Readings were taken at 4 hrs interval for a period of 10 days. The mean current and voltage reading for each day was determined. Colony forming unit (CFU/mL) for each day was monitored as well as the effect of pH variation on the current and voltage generated

2.6 Screening for electrogenic microorganisms from Wastewater

Screening test for electrogenic microorganisms was done by culturing an aliquot portion of AWW from MFC. Organisms isolated repeatedly from the MFC at the beginning and end of the experiment were selected to be electrogenic (Zhang et al., 2009). Abbatoir wastewater was introduced into the anode chamber of the MFC which contained a graphite electrode . The MFC was allowed to run for 5 days. On the fifth day, an aliquot portion of wastewater from the anode chamber of the MFC was cult.ured. A comparison of microorganisms present before and after running the MFC was made..

2.7 Characterisation and Identification of Electrogenic microorganisms

Then the selected organism *Enterobacter agglomerans* was biochemically characterized using API 20E Kit. Furthermore it was quantify using spectrophotometer.

Statistical Analysis

The data obtained from voltage and current hourly reading which constituted daily readings were subjected to statistical analysis using statistical package for social sciences (SPSS) version 16.0. Comparison of mean was done using analysis of variance (ANOVA) while post-hoc test was conducted using Duncan Multiple Range test (DMRT). P-value was set at 0.05.

3.0 Results and Discussion

3.1 Results

Physiochemical properties of Abattoir wastewater (AWW) were: pH, 6.9; calcium content, 26 mg/L; Magnesium ion, 24 mg/L; Iron 14.50 mg/L; Potassium ion, 92.50 mg/L; Sodium ion 85.30 mg/L; phosphorus, 15.67 mg/L; Ammonia, 768 mg/L; TDS, 57 mg/L; TSS, 615 mg/L;, TOC, 70 mg/L; conductivity, 837.80 S/m, Dissolved Oxygen 1.8 mg/L, Biochemical Oxygen Demand, 4123.4 mg/L and Chemical Oxygen Demand, 7346.5 mg/L was displayed in Table 1.

Biochemical characteristics of the screened microorganisms was presented in Table 2. The results showed one gram positive and two gram negative organisms from abbatoir waste water. The selected organism *Enterobacter agglomerans,* is a gram negative, motile, non-spore former, catalase positive, coagulase positive, indole negative, oxidase negative, citrate negative, urease negative, no hydrogen sulphide production, methyl red positive and Vogues-Proskauer negative. Positive results for glucose, lactose, mannitol utilization while negative for sucrose utilisation. Furthermore, *Enterobacter agglomerans* was further identified using API 20E kit (Table 3).

Table 1: Physicochemical properties of Abbatoir wastewaters

Test	Abattoir wastewater (AWW)
pH	6.9
Calcium (mg/L)	26.10
Magnesium (mg/L)	24.00
Iron (mg/L)	14.50
Potassium (mg/L)	92.50
Sodium (mg/L)	85.30
Phosphorus (mg/L)	15.67
Ammonia (mg/L)	768
Total Dissolved Solid (mg/L)	57
Total Suspended Solid (mg/L)	615
Total Organic Carbon (mg/L)	70
Conductivity (S/m)	837.80
Dissolved Oxygen (mg/L)	1.8
Biological Oxygen Demand (mg/L)	4123.4
Chemical Oxygen Demand (mg/L)	7346.5

Table 2: Biochemical characterization of isolates from Abbatoir wastewater

SERIAL NO	SOURCES	GRAM RXN	SHAPE	SPORE	CAPSULE STAIN	CATALASE	COAGULASE	MOTILITY	INDOLE	OXIDASE	CITRATE	UREASE	H_2S
1	AWW	+	Rod	+	+	+	-	+	-	+	-	-	-
2	AWW	-	Rod	-	-	+	-	+	-	-	-	-	-
3	AWW	-	Rod	-	-	+	-	+	-	-	-	-	-
4	AWW	+	Rod	+	+	+	-	+	-	+	-	-	-
5	AWW	-	Rod	+	+	+	-	+	+	-	-	-	-
6	AWW	-	Rod	-	-	+	-	+	-	-	-	-	-

Contd.

SERIAL NO	SOURCES	METHYL RED	VP	GLUCOSE	LACTOSE	SACROSE	MANNITOL	PROBABLE ORGANISM
1	AWW	+	+	A	A	A	A	*Bacillus subtilis*
2	AWW	+	-	A	A	-	A	*Enterobacter agglomeran*
3	AWW	+	-	A	A	-	A	*Enterobacter agglomeran*
4	AWW	+	-	A	-	-	A	*Bacillus subtilis*
5	AWW	+	-	A	-	-	A	*Escherichia coli*
6	AWW	+	-	A	A	-	A	*Enterobacter agglomeran*

Keys:

\+ Positive; - Negative; AWW – Abbatoir waste water

Table 3: Further biochemical tests for *Enterobacter agglomerans* using API 20E Kit

Tests	*Enterobacter agglomerans*
β-galactosidase	+
Arginine Dihydrolase	-
Lysine Decarboxylase	-
Ornithine Decarboxylase	-
Citrate Utilization	+
Hydrogen sulphide production	-
Urease	-
Tryptophane Deaminase	-
Indole production	-
Voges–Proskauer	+
Gelatine test	-
Glucose oxidation	+
Mannitol oxidation	+
Inositol oxidation	+
Sorbitol oxidation	+
Rhamnose oxidation	+
Saccharose oxidation	-
Melibiose oxidation	-
Amygdalin oxidation	+
Arabinose oxidation	+

Key: - Negative; + Positive

The comparison of the different catholyte used in the Figure 1 and 2 shows that $KMnO_4$ had the highest voltage and current generating capability value of 1163.4 mV, 23.6 µA. Followed by NaCl, 538.6 mV, 8.4 µA and the lowest is 325.9 mV, 6.4 µA for H_2O . The addition of sodium acetate on day 6 increased voltage readings. Also, the current and pH relationship over time was presented in Fig 3 and 4 with the use of NaCl and $KMnO_4$. The peak current of 8.4 µA on day 2 corresponding to pH 7 and lowest current of 1 µA was on day 9 at pH of 7.8 in NaCl. The current peak of 23.6 µA was on day 1 at pH 6.8 (Figure 4). The highest colony forming unit of 21.7 CFU/ mL was on day 10 (Figure 5). The electrolytic relationship between the voltage and current generated had the peak of 1163.4mV, 23.6 µA respectively (Figure 6).

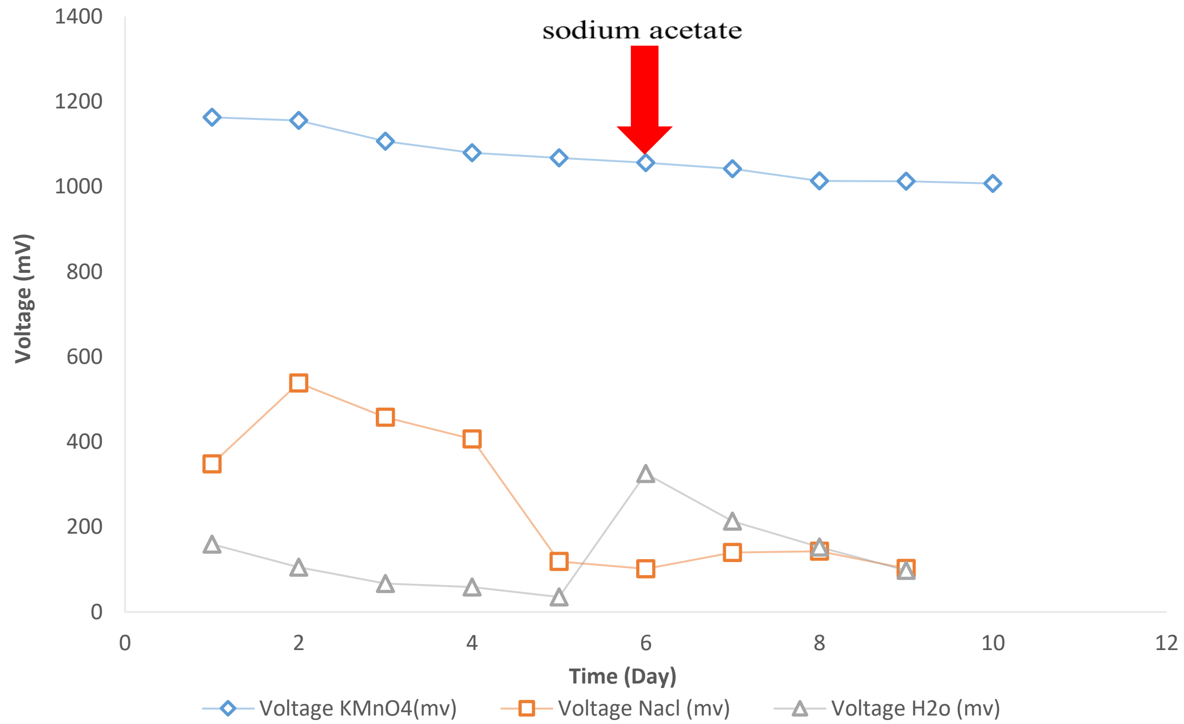

Figure 1: Voltage generated using $KMnO_4$, NaCl and H_2O as catholyte in the MFC of Abattoir Wastewater

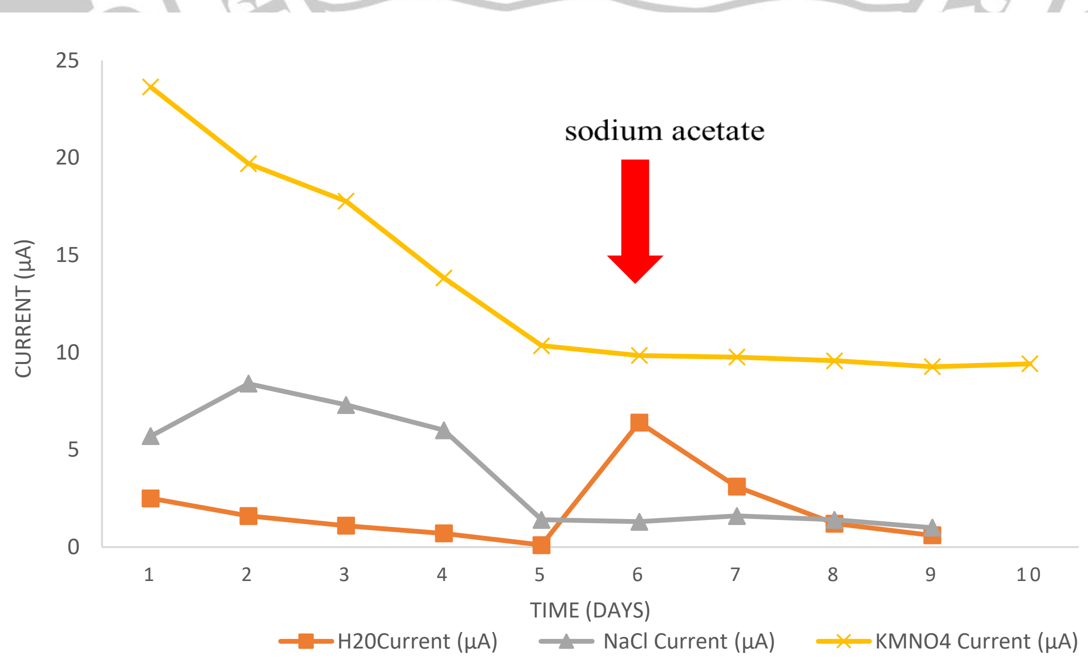

Figure 2: Current generated using $KMnO_4$, NaCl and H_2O as catholyte in the MFC of Abattoir Wastewater.

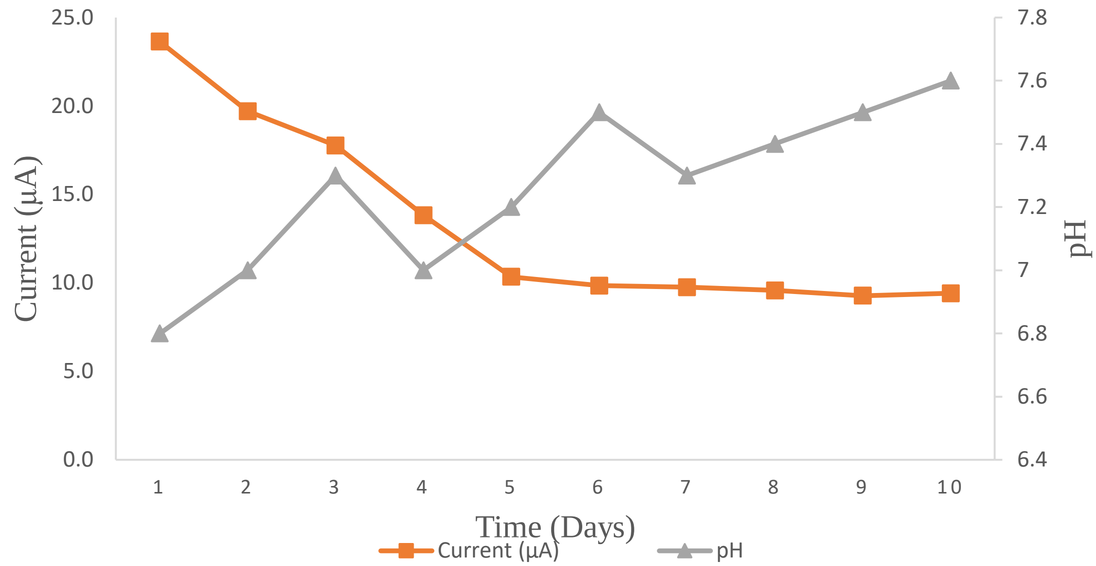

Figure 3: The effect of pH on current generated with time by *Enterobacter agglomerans* from Abattoir Wastewater with $KMnO_4$

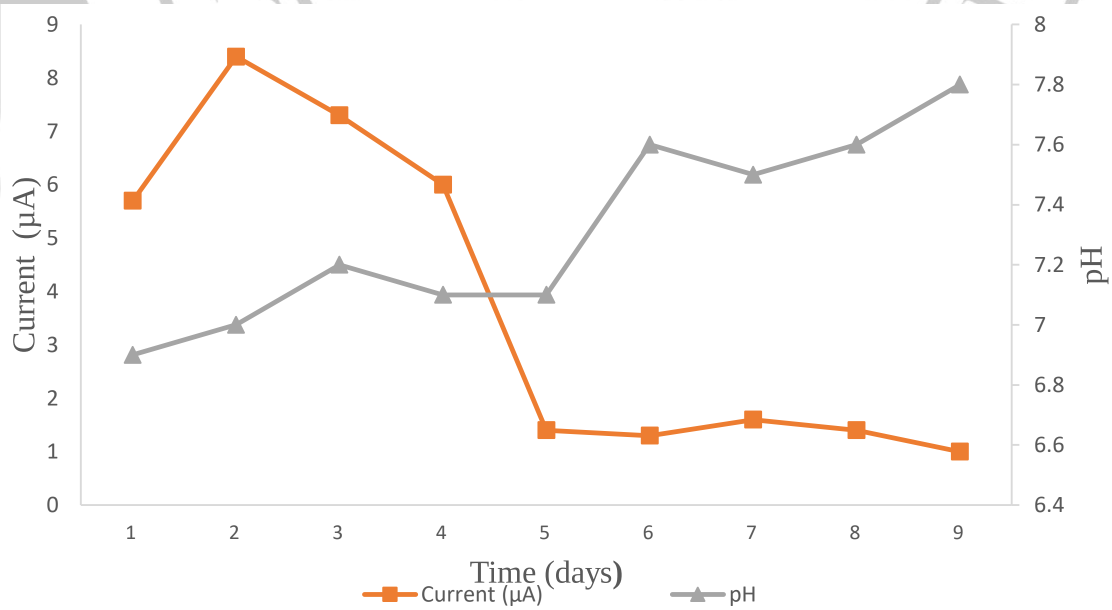

Figure 4: Effect of pH on the current generated with time by *Enterobacter agglomerans in* Abattoir wastewater with NaCl as catholyte.

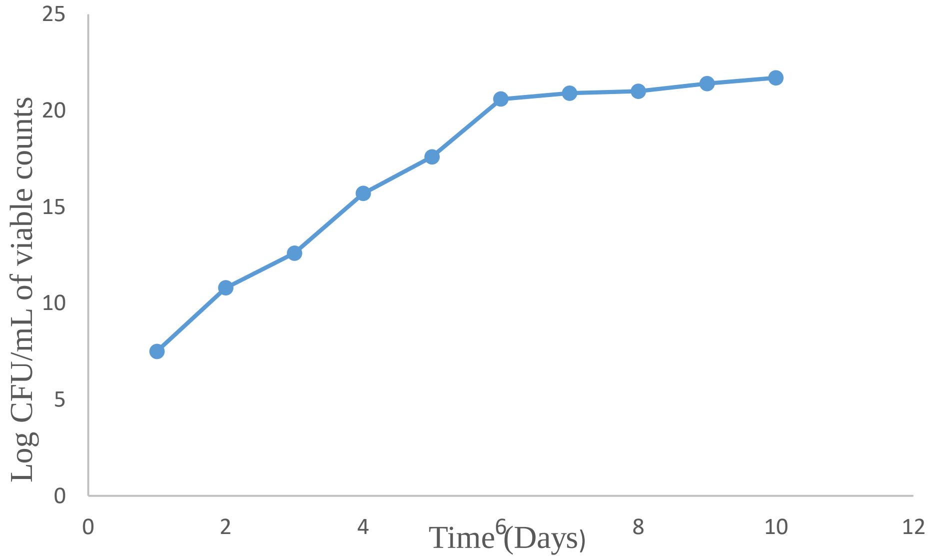

Figure 5: The growth curve of *Enterobacter agglomerans* in Abattoir Wastewater.

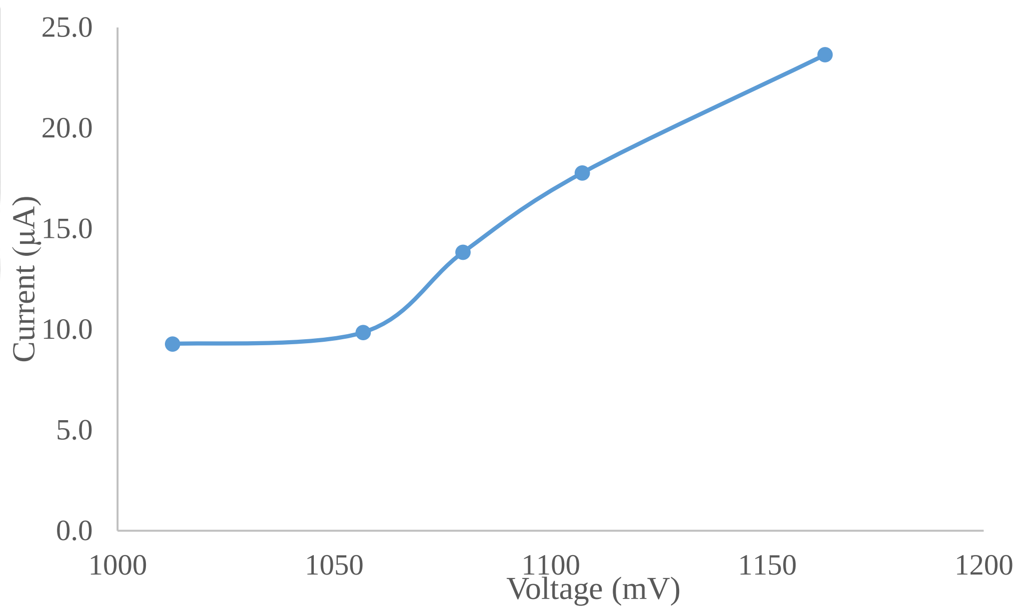

Figure 6: Current and voltage relationship generated by *Enterobacter agglomerans* from Abattoir wastewater with NaCl as catholyte.

3.2 Discussion

Physiochemical characteristic of Abattoir wastewater showed that it is a complex form of wastewater with the presence of phosphorus, calcium, potassium, iron, ammonia, magnesium and high value of BOD and COD.

Microbial consortia analysis of the microbial fuel cell has indicated a range of organisms used in microbial fuel cell with representatives from the divisions: *Enterobacteriaceae* (Angenent *et al.*, 2004; Chen *et al.*, 2008). Although, *Enterobacter agglomerans* has not been used in any MFC experiment, their ability to persist during the screening test showed that they are capable of generating bioelectricity.

According to Carmen *et al.* (2011), oscillation in the voltage and current generated can be linked to external perturbations such as oxygen diffusion from water surface and temperature instabilities among other parameters. Furthermore, MFC voltages decreases rapidly in this research because of continue nutrient depletion of the Abattoir wastewater by *Enterobacter agglomerans*. This is similar to the work done by Saravanan *et al.* (2010) who worked on dairy wastewater and the result obtained showed a progressive decrease in current and voltage generation with time.

The geometric growth curve of the microbes in this experiment showed that the as microorganism' population increases there was a simultaneous decrease in the voltage and current generated from each MFC. This contradict the work of Chin-Tsan *et al.* (2010) who claim that the highest power generation of microorganisms was at the stationary phase of the microbial growth curve.

The catholytes used in this study showed that it can enhance MFC operation . The comparative study of the three catholyte showed that potassium permanganate proved to be the best catholyte for MFC voltage and current generation followed by sodium chloride and lastly oxygen

Furthermore, voltage and current production was boosted in all the catholyte with the introduction of sodium acetate. This indicates that sodium acetate can be used to boost MFC performance.

High voltage and current readings can be related to the high BOD and COD of the abattoir wastewater (Momoh *et al.*, 2010). High COD removal values were recorded in all the wastewater samples. This agreed with Venkata *et al.* (2008); Zhang *et al.* (2009); Mathuriya and Sharma, (2010); Elakkiya *et al.*, (2013). This is a good reason why MFC system should be incorporated into the wastewater treatment plant. This will enhance simultaneous electricity generation while treating abbatoir wastewater.

4.0 Conclusion

The result showed that $KMnO_4$ and NaCl as catholyte can increase the bioelectricity generation from Abattoir wastewater by *E. agglomerans*.

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