

Abattoir wastewater treatment and energy recovery using a Ferricyanide-catholyte Microbial Fuel Cell.

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Abstract. The capacity of Microbial fuel cells (MFCs) to produce voltage and concurrently treat abattoir waste water was investigated in MFCs that used 0.1M potassium ferricyanide ($K_3[Fe(CN)_6]$) as catholytes. Physicochemical, electrochemical and Microbiological properties of the MFCs were monitored. The open circuit voltage (OCV) readings were taken at 3 hours interval and maximum OCV of 965mV was recorded. Also, The physicochemical characteristics of the MFCs revealed that the pH decreased by 0.2 after treatment; Chemical Oxygen demand, biochemical oxygen demand, total suspended solids, ammonia, and total nitrogen reduced by 88.4%, 65.56%, 43.88%, 60% and 60% respectively. However, Phosphate increased by 54%. The bacterial isolates from the raw abattoir wastewater were *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Enterobacter aerogenes*, *Escherichia coli* and *Micrococcus luteus* while *Enterococcus faecalis*, *Bacillus cereus* and *Escherichia coli* were isolated from the biofilms on the anode. Microbial fuel cells therefore have capacities for simultaneous waste water treatment and electricity generation.

Introduction

Microbial fuel cells are unique devices that produce energy as well as treat wastes [1, 2, 3]. Electricity can be generated in microbial fuel cells using mixed cultures enriched from several natural sources [3, 4]. MFCs utilize a wide range of substrates. Several configurations and outputs has also been reviewed [5].

In a microbial fuel cell, bacteria that degrade (oxidize) organic matter, are kept physically separated from the electron acceptor by a proton exchange membrane (PEM). The PEM allows only protons to pass from the anode to the cathode but it keeps the electrons from passing through. The bacteria produces electrons and pass them to the anode in the MFC chamber and then via a circuit to the cathode where they combine with protons and an electron acceptor (becomes reduced) [6]. One of the key factors to producing better outputs from MFCs is the strength and type of Catholyte [7].

The use of chemical oxidizers as catholytes such as potassium ferricyanide, potassium permanganate and Manganese (IV) as other electron acceptor in a MFCs has been adopted by many [8]. However, Potassium ferricyanide ($K_3[Fe(CN)_6]$) is the most common electron acceptor in experimental microbial fuel cell due to its high performance.

Abattoir waste water contains large loads of organic matter coming from the cow blood, rumen contents and wash water. Proper treatment of animal waste and resource recycling to reduce its environmental impact are currently important issues for the livestock industry [9]. The process of power generation and simultaneous treatment of abattoir wastewater in large scale systems can become practicable with proper design and reactor configuration [10]. In this research, the potential of MFCs to generate electricity and treat abattoir waste water was investigated by using Potassium ferricyanide ($K_3[Fe(CN)_6]$) as catholyte.

Materials and Method

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 bijoux 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 [11]. The abattoir wastewater collected aseptically in sterile bijoux 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, Eosine methylene 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.

Characterisation of bacterial isolates

Distinct colonies from the plates were purified by subculturing 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 [12]. The identities of the isolates were determined using standard manuals [13].

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 [14]. 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 millilitres 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 $20 \pm 1^\circ\text{C}$ 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 [14].

Chemical Oxygen Demand

The Chemical Oxygen Demand (COD) was determined using the closed reflux colorimetric method [14]. The Tubetests heater was calibrated and the control was set to 150°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 [14].

pH

The pH meter was calibrated with a pH 7 buffer solution. The electrodes were rinsed with deionized water, dampened lightly and inserted into the abattoir wastewater in a clean beaker. The pH reading was taken and recorded [14].

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 [14].

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/cuvette and used to zero the photometer. Then the sample readings were taken with the photometer [14].

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 ± 0.1°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 [14].

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:

$$100(A-B)/ \text{sample volume}$$

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 [16]. Two H shaped (dual chamber) microbial fuel cells (DCMFC) were constructed with four transparent polyacrylic containers (1litre volume each). The containers were perforated and attached with an inner adapter using epoxy glue. Two agar 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 [15]. 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 [7]. 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 $30 \pm 3^\circ\text{C}$. Nine hundred millilitres 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 millilitres of 0.1M potassium ferricyanide ($\text{K}_3[\text{Fe}(\text{CN})_6]$) solution were added to the cathode chambers 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.

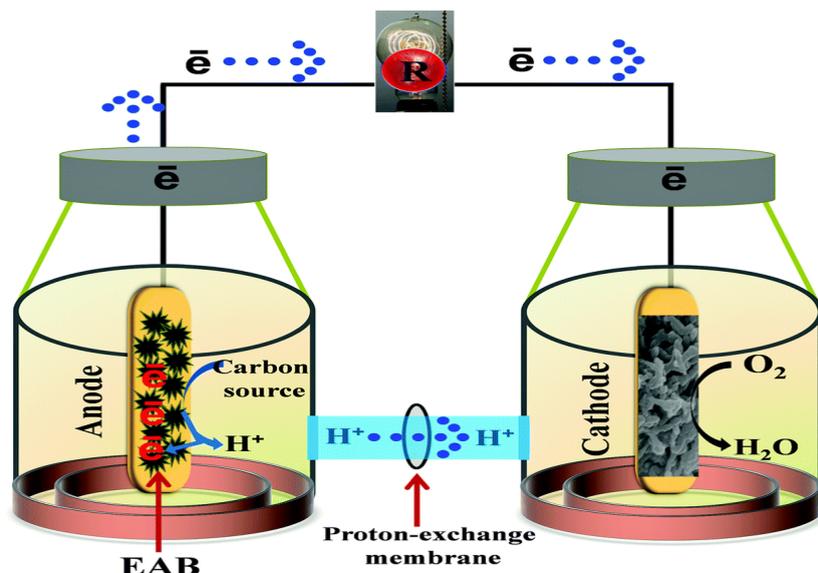


Figure 1: Schematic diagram of a H-shaped (dual Chamber) Microbial Fuel Cell set up. [17]
Sourced from Sajid et al., 2016

RESULTS

Table 4.8 shows the percentage changes in physicochemical properties of abattoir wastewater after MFC treatment with potassium ferricyanide catholyte. Chemical Oxygen demand, biochemical oxygen demand, total suspended solids, ammonia, and total nitrogen reduced by 88.4%, 65.56%, 43.88%, 60% and 60%. The pH of the abattoir wastewater remained constant.

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

Parameters of physicochemical analysis	Before MFC treatment (mg/ml)	After MFC treatment (0.1M Potassium ferricyanide) mg/ml
COD	9350	1079
BOD	2600	895
TSS	3250	1824
pH	7.2	7.2
Phosphate	480	739
Conductivity	5.5 μ s	12.4 μ s
Ammonia	1.2	0.48
TDS	2.8ppm	6.25ppm
Total Nitrogen	1.05	0.42

Keys:

COD= Chemical oxygen demand

BOD= Biochemical oxygen demand

TSS= Total suspended solids

TDS= Total dissolved solids

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) generated are shown in Figure 2. The maximum OCV reading for potassium ferricyanide catholyte MFC is 965 mV.

The OCV of the MFC rose from 347 mV to a maximum peak of 965mV in the 93rd hour. After the maximum peak of 965mV was attained, the voltage fluctuated before stabilising and on the 20th day, it ends with an OCV value of 781mV.

At the end of the 20 days, the potassium ferricyanide solution (which was orange-red) accepted electrons that came from the anode chamber and the solution turned dark green.

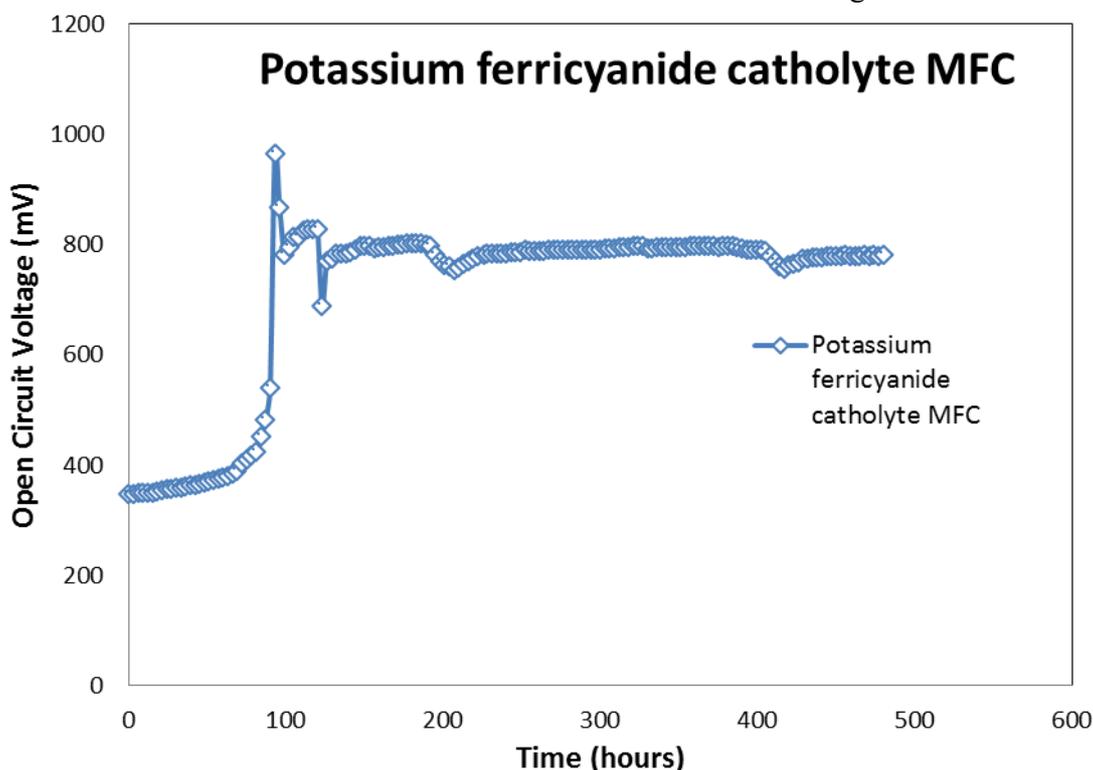


Figure 2: OCV in Millivolts Generated from Abattoir Wastewater Using MFC and ferricyanide catholyte

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 also shows all the bacteria isolated from the abattoir wastewater before and the after use in the MFC.

Table 2: Comparison of the microbiological properties of the raw abattoir wastewater and the used abattoir wastewater

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

DISCUSSIONS

The MFC was operated at room temperature of $30 \pm 3^\circ\text{C}$ 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 recorded was 965 mV. This could be as the result of the difference in potential between the anode (where the indigenous exoelectrogenic organisms donated electrons to the graphite) and the cathode (where potassium ferricyanide acted as electron acceptor). The OCV gradually increased from 347 mV and peaked in the 93rd hour with the value 965mV, then the voltage dropped and fluctuated. Later on, the MFC maintained fairly stable reading towards the end of the experiment (19th and 20th day). The fluctuations are caused by the different potentials produced by mixed cultures while utilizing the substrate until a stable community is attained [16, 7].

The maximum OCV generated in this study was low when compared to the report of Momoh and Naeyor [18] but higher than that of Adeleye and Okorundu [16]. This low OCV will translate to low power density [7]. 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 [18]. Also, H shape or dual chamber MFC has high internal resistance [7] which may have contributed to the low OCV. More work is needed to combat the MFC internal resistance and produce better electrodes and devices that will trap the electricity for upscaling purposes [5].

From the physicochemical results, the pH was initially at 7.2(near neutral) and remained constant. 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 [19]. The abundance and activity of microbial community are controlled by pH.

The chemical oxygen demand was 88.46% for the used abattoir wastewater in potassium. This parameter can be compared with the report by Ghangrekar and Shinde [20] which gave a COD removal efficiency of 88% within 16-35 days. Up to 80% of the COD has been removed in some other cases [6, 11]. Elakkiya and Matheswaran [19] reported a COD removal of 91% using a dairy wastewater in a dual chamber MFC. Thus, the COD result of this study was within the range of the previous studies by other workers or researchers.

The totals suspended solids, biochemical oxygen demand, ammonia and total nitrogen of the used wastewater decreased by 43.88%, 65%, 60% and 60% respectively. The 65.56% decrease in BOD is as a result of the dissolved oxygen consumed by the indigenous microorganisms. Total nitrogen and ammonia decreased by 60%. This large decrease in values may have been as a result of denitrification and ammonia oxidation activities that took place in the anode chamber under reduced oxygen conditions [11].

Contrary to nitrogen, phosphate increased by 54% for ferricyanide. An explanation for this may be that the low redox potential in the anode cells lead to a release of inorganic phosphate from organic matter [11]. 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. Libin et al. [21] suggested that the anodic microbes were influenced significantly by TDS of the electrolytes. Notably, according to their observations, anodic microbes of MFCs operated at TDS 30 g/L were sharply different from others. They suggest that the high TDS condition was not suitable for the stable operation of the MFCs.

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. [22] in which *Bacillus spp* and *Micrococcus luteus* were both isolated from the abattoir wastewater. *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 [23], thus this organism does not thrive under anaerobic conditions.

CONCLUSION

Microbial fuel cells are promising technologies for the simultaneous waste water treatment and energy recovery. This paper demonstrated that using a ferricyanide-catholyte-MFC, power could be obtained and also waste water can be treated simultaneously. Microbes usually termed exoelectrogens can power the process by converting the substrates present in the waste water into electricity. By doing this, the substrate is used up and therefore treatment of the waste occurs

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