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Role of Electrochemically Active Bacteria in the Treatment of Piggery and Poultry Wastewaters from Umuagwo in Ohaji Egbema Local Government Area of Imo State, Nigeria

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ABSTRACT: In this study, piggery and poultry wastewaters were used as agro-based industrial wastewaters to evaluate the role of electrochemically active bacteria in an anodic chamber of microbial fuel cell (MFC) with 0.1M potassium permanganate cathodic cell using salt bridge preparation. The BOD_5, COD, TDS, TSS, nitrogen, phosphates, pH and conductivity served as indicative parameters for determining the wastewater treatment efficiencies (WWTE) of the MFCs. Results obtained from the WWTE reveal that the MFCs were able to reduce the piggery wastewater parameters, BOD, COD, TDS, TSS, nitrogen, phosphate, pH, conductivity by 89.66, 69.57, 52.20, 69.04, 70.27, 59.57, - 4.41 and 0.99 %, respectively while the same parameters for the poultry wastewater were reduced by 82.61, 78.59, 58.03, 67.13, 70.49, 64.52, 2.70 and 28.04 %, respectively. Statistically, there were significant differences before and after treatments and between wastewater samples revealing that that the effect of treatment before and after on physicochemical parameters were different for piggery wastewater than they were for poultry wastewater. Microbes in the biofilms on the electrodes (potential exoelectrogens) include *Staphylococcus aureus, Bacillus cereus, Micrococcus luteus, Corynebacterium* sp., *Enterococcus* sp., *Pseudomonas aeruginosa, Escherichia coli, Klebsiella* sp., *Salmonella* sp. and *Shigella* sp. while the fungal isolates include *Mucor* sp., *Saccharomyces* sp. and *Aspergillus* sp. in both piggery and poultry wastewaters. Thus, microbial fuel cell bacteria oxidize the organic matter leading to decontamination of the wastewater – a significant approach in addressing the public health threats of these wastes in our country.

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Methods of wastewater treatment were first developed in response to the adverse conditions caused by the discharge of wastewater to the environment and the concern for public health. Further, as cities became larger, limited land was available for wastewater treatment and disposal, principally by irrigation and intermittent filtration. Also, as populations grew, the quantity of wastewater generated rose rapidly and the deteriorating quality of this huge amount of wastewater exceeded the self-purification capacity of the streams and river bodies (Rajasulochana and Preethy, 2016). Conventional methods for removing metals are either becoming inadequate to meet current stringent regulatory effluent limits or are increasing in cost. As a result, alternative, cost effective technologies are in high demand (Rajasulochana and Preethy, 2016). Microbial Fuel Cell (MFC) technology allows electricity generation while simultaneously treating wastewater. Microbial fuel cells use electrochemically active bacteria to oxidize substrates and separate protons from electrons. The separated electrons travel through the anode and external circuit

to generate a current. The released protons simultaneously travel through a Proton Exchange Membrane (PEM) into the cathode chamber where they combine with the electrons from the completed circuit to form water. When used for wastewater treatment, an effluent stream with a lower organic loading is discharged from the anode, which can be discharged to a municipal sewer or, if required, treated further. MFC technology can be applied as a renewable energy source with applications in power generation, wastewater treatment and water quality monitoring (Dannys *et al*., 2016). Microorganisms act as biocatalysts in MFCs. Therefore, understanding their behavior is critical to improving MFC performance. Microbiological studies have attempted to explain why microbes can transfer electrons from/to an electrode and how electrons are transferred. Three mechanisms are proposed based on pure culture studies such as: direct electron transfer through membrane-binding proteins mediated electron transfer with the aid of soluble electron shuttles; and electron transfer via bacterial nanowires (Katz et al., 2003; Gorby et al., 2006). Electrochemically active organisms such as *Geobacter* spp. and *Shewanella* spp. are widely used as model organisms to study electron transfer (Lovley, 2006; Bretschger et al., 2006). Microbiological studies of mixed culture are used to map microbial community on the electrode, identify the dominant species, and isolate new strains that are electrochemically active (Logan, 2009). This study was carried out so as to proffer solutions to the teeming environmental problems caused by waste water discharge to the environment. The proffered solution is cheaper, easy to manage and have the capacity to produce energy simultaneously as it treats waste water. This technology can substitute the aeration chamber in the waste water treatment plant. The effluent from this technology is also safe for discharge to the environment. Therefore, the objective of this study was to investigate the role of electrochemically active bacteria in the treatment of piggery and poultry wastewaters from Umuagwo in Ohaji Egbema Local Government Area of Imo State, Nigeria.

MATERIALS AND METHODS

Sample Collection and Preparation: Wastewater sample was collected from a poultry farm in the early morning hours during the routine clean-up of the farm. The sample was adequately labelled. Another well labelled sample was obtained from a piggery farm at the same hour which the poultry effluent was collected. Both sampling sites were located at Bvisoug Limited (farm division) Umuagwo in Ohaji Egbema Local Government Area of Imo State. Each sample was collected in sterile a 10 L gallon, with the sample occupying 9/10 of its volume so as to allow for agitation and homogenization of samples. The sample was agitated vigorously in order to homogenize it before transporting it to the laboratory for analyses. The organic content is the target substrate for the microbial oxidation which will produce the electrons desired in MFC set up.

Physicochemical Characteristics of Wastewater Prior to Treatment: The physicochemical analyses were done according to standard procedures. The following physicochemical analyses were carried out in the Anthony van Leeuwenhoek research laboratory; biological oxygen demand (BOD), chemical oxygen demand (COD), pH, phosphate, conductivity, total dissolved solid (TDS), total nitrogen and total suspended solid (TSS) according to American Public Health Association manual (1998) and AOAC (2010).

Microbiological Analysis of Wastewater: Prior to use in MFC, the wastewater was serially diluted ten-fold and plated out on Nutrient agar, Eosine methylene blue agar, MacConkey agar, Cetrimide agar and Mannitol salt agar in duplicates using the spread plate technique. The plates were incubated at 37 °C for 24 - 48 hr. The viable plate count was determined as Colony Forming Unit (CFU) per ml and colonial characteristics recorded. Colonies found on the plates were subcultured on fresh sterile nutrient agar plates at 37 ^{o}C for 24 hr (Uba *et al*., 2020).

Components and Construction of the Microbial Fuel Cell: Microbial fuel cells were constructed in line with the H-type design adopted by Adeleye and Okorondu (2015). Each microbial fuel cell was done in triplicates and the mean of the three values was reported for waste water treatment capacity, respectively. The microbial fuel cell design consisted a cathode, an anode, interconnected by a proton exchange membrane (salt bridge).

Preparation of the salt bridge: Analyses were done according to Adeleye and Okorondu (2015). The salt bridge was prepared using 2 % sterile agar-agar and 1 M NaCl. A 12 cm salt bridge was made using a PVC 1-inch diameter pipe. One end of a PVC tube was sealed with aluminum foil in an easily detachable way and held standing vertically using a soft support. The sterile cooled mixture was emptied into the PVC tube held in the soft support and allow to solidify thereby forming the salt bridge which was used for the MFC set up.

Preparing the chamber: The chambers were prepared according to the method illustrated by Adeleye and Okorondu (2015). An equal volume plastic container each with a 1-litre volume, served as cathode and

anode chamber. A hole, equal in diameter to the 1-inch Adopter was made 6 cm from the base of the 1-liter tank base. The 1-inch adopter served as a point of attachment for the salt bridge which was posed to interconnect the two chambers. After the hole had been made, the 1-inch adopter was glued using Abro sealant. Next, a hole each was drilled into the lid of both chambers to allow the passage of wire. The set-up was allowed to dry and solidify.

Electrode preparation: According to methods illustrated by Logan *et al*. (2006) and Adeleye and Okorondu (2015), equal-diameter, equal-length Graphite rods were purchased and the height of the rod was adjusted to a length of 15.7 cm; the diameter of the rod was also 1.8 cm. The total surface area of 95.01 cm^2 and the surface area density of 95.01 cm^2/L were obtained, respectively. These electrodes were prepared and points for wire attachments were formed to aid a tight connection and conductivity.

Preparing the catholyte: Chemicals were standardized according to methods illustrated by AOAC (2010). $KMnO_4$ was used as preferred catholyte. It has a relative molecular mass of 158.05 $gmol^{-1}$. The catholyte was standardized using chemical methods so as to obtain a concentration of 0.1 Mol. With the molar mass and the target concentration known, the mass of $KMnO_4$ measured becomes 15.8 g.

Coupling the microbial fuel cell: The salt bridge was made a day before the set-up was coupled. The sample was collected the same day the microbial fuel cell was to be coupled. The set-up was coupled by joining the two chambers using the salt bridge with the aid of the adopter inch and Abro sealant.

Thereafter, 900 mL waste water was placed in the anode as the anolyte, and the catholyte which was also 900 mL of 0.1 M potassium permanganate was introduced into the cathode. A multimeter was connected to the cathode and the anode with the aid of the low resistance copper wire before they were inserted into the chambers as shown in Figure 1 below. The triplicate set ups were left for 18 days at room temperature (Adeleye and Okorondu, 2015).

Physicochemical and Microbial Analyses of Wastewater after Treatment: After treatment, the physicochemical parameters and microbiological characteristics of the waste water were also assessed. The physicochemical and microbial analyses were done according to standard procedures of APHA (1998) and AOAC (2010) as previously described above.

Fig 1: A simplified microbial fuel cell

Characterization and Identification of Bacterial Isolates from Biofilm on the Cathode and Anode of the Microbial Fuel Cell: Sample collection and Isolation of microorganisms from MFCS: By adopting the method of Uba *et al*. (2020), the MFC was decoupled and a well-labelled sterile swab was used to scrape the electrodes in order to collect the microbial community in the biofilms after 18 days. This was used to prepare a stock solution by dissolving scrappings collected into 10 mL of distilled water, and a tenfold serial dilution was done up to 10^{-7}. Under aseptic conditions, aliquot volumes (0.1 mL) of 10^{-7} dilution were transferred and inoculated into freshly prepared and surface dried Nutrient agar and MacConkey agar while 0.1 mL of 10^{-4} dilution was used for the Mannitol salt, Cetrimide and the Eosin methylene blue agars inoculations. The inoculated plates were incubated at a temperature of 37 °C for 24 hr for the aerobic culture while for the anaerobic culture, incubation was done in an anaerobic jar at room temperature for 5 days using nutrient agar.

Characterization and identification of microbial isolates: Microorganisms isolated from the samples were characterized based on the colonial, morphological, microscopic and biochemical characteristics of the pure cultures (Cheesbrough, 2006). The identities of the isolates were cross matched with features obtained in Standard Microbiological Manuals (Buchannan and Gibbon, 1974).

Waste water treatment ability: The waste water treatment capacity of the microbial fuel cell was assessed and measured as wastewater treatment efficiency in line with previous work published by Akaluka *et al*. (2015). The BOD, COD, TDS, TSS, nitrogen, phosphate, pH, and conductivity were used as determinant parameters and was monitored at 2 days interval over 18 days. Therefore, the ability of the microbial fuel cell to treat waste water was examined.

These was obtained in efficiencies (percentages) and calculated as:

$$Efficiency = \frac{PV_i - PV_f}{PV_i} \times 100 \ldots 1$$

Where PV_i = initial parameter value and PV_f = final parameter value

Aliquot of the wastewater undergoing treatment was collected and key wastewater parameters were examined so as to assess the efficiency of the MFC.

Statistical Analysis: The data obtained in this study were expressed as mean ± S.D. with experiments being conducted in triplicate. The statistical significance was determined by analysis of variance (ANOVA) to determine if the physicochemical parameters of waste water before and after treatment data obtained significantly varied ($P < 0.05$) from one another. Regression and coefficient of determination were also conducted (using SPSS) to compare the effect of substrate type on the Waste water Treatment Capacity (WWTC) and output of voltage produced by the microbial fuel cell. Where necessary, the t-statistics were conducted to test whether the physicochemical parameters of piggery wastewater do significantly differ from that of the poultry wastewater using SPSS version 20.

RESULTS AND DISCUSSION

Poultry and piggery farms and slaughter houses have originally been a source of nuisance from the time past. The results indicated that the physicochemical parameters assessed before and after treatment for the poultry waste water were BOD_5 4.6 and 0.8 (mg/mL), COD 2896 and 620 (mg/mL), TDS 3,426 and 1438 (mg/mL), TSS 870 and 286 (mg/mL), nitrogen 122 and 36 (mg/mL), phosphates 124 and 44 (mg/mL), pH was 7.4 and 7.2 and conductivity was 2946 and 2120 (mV/mL) respectively. The results for the piggery waste water were BOD_5 5.8 and 0.6 (mg/mL), COD 4,600 and 1400 (mg/mL), TDS 3,946 and 1886 (mg/mL), TSS 1,150 and 356 (mg/mL), nitrogen 296 and 88 (mg/mL), phosphates 188 and 76 (mg/mL), pH was 6.8 and 7.1 and conductivity was 3026 and 2996 (mV/mL) (Table 1). The ANOVA result in Table 2 showed significant differences in the treatment (before and after) for all the physicochemical parameters except pH. The pH of this study was near neutral. 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. The abundance and activity of microbial community are controlled by pH (Elakkiya and Matheswaran, 2013). Also, all the physicochemical parameters showed significant differences between the wastewaters. The physicochemical parameters in piggery wastewater are significantly greater than the ones in the poultry wastewater. There were significant interactions/effects between the wastewaters and treatments (before and after). This implied that the effect of treatment (before and after) on physicochemical parameters were different for piggery wastewater than they were for poultry wastewater. This study reported high waste water parameters which is in line with previous investigations. Poultry waste water consists of various constituents the form of particulates, organics, and nutrients. This wastewater is the cumulative wastewater generated from uncollected blood, feathers, eviscerations, and cleaning of the live haul area at a slaughter plant (Kiepper *et al*., 2008). Screens are the most popular form of preliminary physical treatment process used in on-site poultry wastewater treatment systems to remove poultry processing wastewater constituents (Kiepper, 2003). Wastes from piggery farms are difficult to manage compared with the volume of waste produced in each day (Noophan, 2009; Department of Livestock, 2007). BOD_5 and COD of waste water are reported to be between 1500 – 3000 mg/L and 4000 - 7000 mg/L, respectively. Piggery farm waste water has also been reported to have high Nitrate-nitrogen and Ammonious-Nitrogen as well as a high phosphorus content (Deng *et al*., 2006).

The result of the physicochemical variation of piggery wastewater undergoing treatment in the microbial fuel cell over 18 days using the BOD, COD, TDS, and TSS as key waste water parameters is shown in Figure 1. Result obtained from the waste water treatment efficiency revealed that the microbial fuel cell was able to reduce the piggery waste water parameters BOD, COD, TDS, TSS by 89.66, 69.57, 52.20 and 69.04 %, respectively (Figure 1). The parameters showed a trending decrease in the values of the parameters monitored. The coefficient of determination was found to be 0.989 (98.9 %) for BOD, 0.968 (96.8 %) for COD, 0.685 (68.5 %) for TDS, and 0.937 (93.7 %) for TSS. This means that 98.9 %, 96.8 %, 68.5 %, and 93.7 % of the relationships between time and the observed values of BOD, COD, TDS, and TSS generated, respectively could be mathematically determinable, leaving the rest 1.1 %,3.2 %, 31.5 %, and 6.3 %, respectively to errors. In other words, the mathematical model concerned has been found to be very good. All the physicochemical parameters of piggery wastewater undergoing treatment in the microbial fuel cell over 18 days showed negative relationship with time. This implied that the more the piggery wastewater received treatment by the day, the physicochemical parameters (BOD, COD, TDS, and

TSS) decrease. All the physicochemical parameters showed significant relationships with time. This showed that the linear relationship between physicochemical parameters and time is indeed very highly acceptable.

Table 1: Physicochemical parameters of waste water before and after treatment

	Piggery waste water			Poultry waste water		
Parameter	initial	final	WWTE	Initial	Final	WWTE
BOD_5	5.8^a	0.6^a	89.65517	4.6^b	0.8^b	82.6087
COD	4600^a	1400^a	69.56522	2896^a	620^a	78.59116
TDS	3946^a	1886^a	52.20476	3426^a	1438^a	58.02685
TSS	1150^a	356^a	69.04348	870^a	286^a	67.12644
Nitrogen	296^a	88^a	70.27027	122^b	36^b	70.4918
phosphate	188^a	76^a	59.57447	124^b	44^b	64.51613
pH	6.8^a	7.1^a	-4.41176	7.4^b	7.2^a	2.702703
Conductivity	3026^a	2996^a	0.991408	2946^a	2120^a	28.03802

Key: The averages followed by the same letter do not differ statistically between themselves; WWTE = Wastewater treatment efficiencies

Table 2: ANOVA of physicochemical parameters of waste water before and after treatment

Parameter	Treatment (Before and After)		Wastewater (Poultry and Piggery)		Interaction effect (Treatment versus Wastewater)	
	F-calculated	F-tabulated	F-calculated	F-tabulated	F-calculate d	F-tabulated
BOD	6075.00 **	11.2586	75.00 **	11.2586	147.00**	11.2586
COD	2248.99**	11.2586	462.77**	11.2586	64.03 **	11.2586
TDS	125920.11 **	11.2586	7206.68 **	11.2586	40.2126 **	11.2586
TSS	14241.63 **	11.2586	918.75 **	11.2586	330.75 **	11.2586
Nitrogen	7203.00 **	11.2586	4256.33 **	11.2586	1240.33 **	11.2586
phosphates	3072.00**	11.2586	768.00**	11.2586	85.33**	11.2586
pH	0.7500^{ns}	11.2586	36.75 **	11.2586	18.75 **	11.2586
conductivity	5495.52**	11.2586	6854.52 **	11.2586	4752.12**	11.2586

*Key: ** Significant at a level of 1 % of probability ($p < 0.01$); ^{ns}Non-significant ($p > = 0.05$)*

The result of the physicochemical variation of poultry wastewater undergoing treatment in the microbial fuel cell over 18 days using the BOD, COD, TDS, and TSS as key waste water parameters is shown in Figure 2. The same parameters for the poultry waste water were reduced by 82.61, 78.59, 58.03 and 67.13 %, respectively also demonstrating a trending decrease in the values of the parameters monitored. The coefficient of determination was found to be 0.925 (92.5 %) for BOD, 0.994 (99.4 %) for COD, 0.622 (62.2 %) for TDS, and 0.571 (57.1 %) for TSS. This means 92.5 %, 99.4 %, 62.2 %, and 57.1 % of the relationships between time and the observed values of BOD, COD, TDS, and TSS generated, respectively could be mathematically determinable, leaving the rest 7.5 %, 0.6 %, 37.8 %, and 42.9 % respectively to errors. In other words, the mathematical model concerned has been found to be very good. All the physicochemical parameters of poultry wastewater undergoing treatment in the microbial fuel cell over 18 days showed negative relationship with time. This implied that the more the poultry wastewater receives treatment by the day the physicochemical parameters (BOD, COD, TDS, and TSS) decrease. All the physicochemical parameters showed significant relationships with time. This showed that the linear relationship between physicochemical parameters and time is indeed very highly acceptable. Statistical analysis using T-test revealed that all physicochemical parameters except BOD in the two wastewater samples had their levels of probability to be less than 0.05. This indicated that the COD, TDS and TSS of the wastewaters statistically differ significantly except BOD. This implied that the COD, TDS and TSS of piggery wastewater is significantly greater than that of the poultry wastewater as can be deduced from their averages. From the data obtained, the microbial fuel cell was more efficient in treating the piggery waste water with respect to parameters such as BOD and TSS while all other parameters were removed better in the poultry waste water at the end of the 18 days of experimentation. Akaluka *et al.* (2015) obtained a chemical oxygen demand (COD) decrease of 84.03 % and 57.9% decrease in BOD_5 of abattoir waste water. Ghangrekar and Shinde (2007) reported a COD removal efficiency of 88 % between 16 - 35 days in their study. Elakkiya and Matheswaran (2013) in their research reported a 91 % removal of COD using a dairy wastewater in a dual chamber MFC. Liu *et al.* (2004) reported a COD removal efficiency of 80 % for domestic wastewater. Thus, the COD result of this study was within the range of the previous studies by other workers or researchers. The reduction in the BOD and COD in the MFCs were as a result of the dissolved oxygen consumed by the indigenous microorganisms therefore utilizing the organics and producing more biomass (Akaluka *et al.*, 2015).

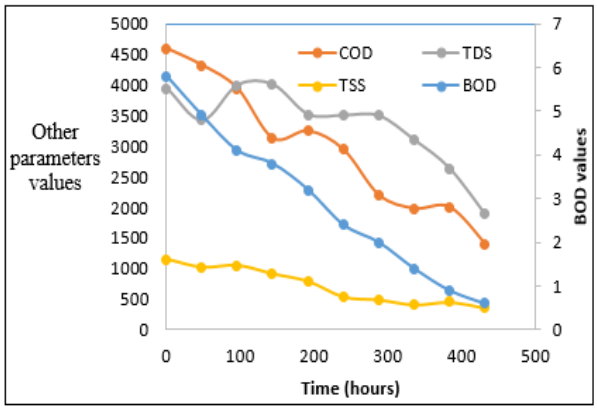

Fig 1: Physicochemical variation of poultry wastewater undergoing treatment in the Microbial fuel cell over 18 days

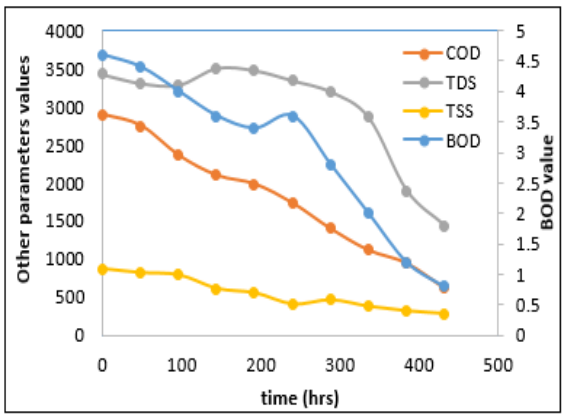

Fig 2: Physicochemical variation of poultry wastewater undergoing treatment in the Microbial fuel cell over 18 days

The microbial community of the pretreated waste water consisted of bacteria which include *Staphylococcus aureus, Bacillus cereus, Micrococcus luteus, Corynebacterium* sp., *Enterococcus* sp., *Pseudomonas aeruginosa, Escherichia coli, Klebsiella* sp., *Salmonella* sp. and *Shigella* sp. while the fungal isolates include *Mucor* sp., *Saccharomyces* sp. and *Aspergillus* sp. in both piggery and poultry waste water (Table 3).

The treated waste water samples from piggery and poultry recorded presence of *Staphylococcus aureus, Bacillus cereus, Micrococcus luteus, Escherichia coli, Enterococcus* sp, *Salmonella* sp. and *Shigella* sp. (Table 4). The fungal isolates include *Saccharomyces* sp., *Aspergillus* sp. in the poultry waste water while the piggery waste water recorded *Mucor* sp., *Saccharomyces* sp., *Aspergillus* sp., and *Penicillium* sp. (Table 5). The biofilm on the surface of the electrodes was also assessed. Results revealed that the anodes of the microbial fuel cell in the poultry waste water consisted of *Staphylococcus aureus, Bacillus cereus, Micrococcus luteus, Escherichia coli, Enterococcus* sp., *Salmonella* sp., *Shigella* sp., *Saccharomyces* sp., and *Aspergillus* sp. On the other hand, the piggery waste water electrodes had *Staphylococcus aureus, Bacillus cereus, Micrococcus luteus, Escherichia coli, Enterococcus* sp., *Salmonella* sp., *Shigella* sp., *Saccharomyces* sp., and *Aspergillus* sp. (Table 3).

Table 3: Microbial community in the microbial fuel cell before and after treatment

Pre treated waste water		Treated waste water		Electrodes	
Poultry	Piggery	Poultry	Piggery	Poultry	Piggery
Staphylococcus aureus, Bacillus cereus, Micrococcus luteus, Corynebacterium sp. , *Enterococcus* sp., *Pseudomonas aeruginosa, Escherichia coli, Klebsiella* sp., *Salmonella sp, Shigella* sp. *Mucor* sp., *Saccharomyces* sp. *Aspergillus* sp.	*Staphylococcus aureus, Bacillus cereus, Micrococcus luteus, Corynebacterium* sp. , *Enterococcus* sp. *Escherichia coli, Klebsiella* sp., *Salmonella* sp., *Shigella* sp., *Mucor* sp. *Saccharomyces* sp *Aspergillus* sp.	*Staphylococcus aureus, Bacillus cereus, Micrococcus luteus, Escherichia coli, Enterococcus* sp. *Pseudomonas aeruginosa.* *Salmonella* sp. *Shigella* sp., *Saccharomyces* sp. *Aspergillus* sp.	*Staphylococcus aureus, Bacillus cereus, Micrococcus luteus, Escherichia coli Enterococcus* sp. *Pseudomonas aeruginosa,* *Salmonella* sp. *Shigella* sp., *Mucor* sp., *Saccharomyces* sp. *Aspergillus* sp. *Penicillium* sp.	*Staphylococcus aureus, Bacillus cereus, Micrococcus luteus, Escherichia coli Enterococcus* sp. *Salmonella* sp., *Shigella* sp. *Saccharomyces* *Aspergillus* sp.	*Staphylococcus aureus, Bacillus cereus, Micrococcus luteus, Escherichia coli, Enterococcus* sp., *Salmonella* sp., *Shigella* sp. sp.,*Saccharomyces* sp. *Aspergillus* sp.

The microbial populations on the biofilms are the potential electricigens that are capable of treating waste water according to Adeleye and Okorondu (2015). Previous studies revealed that *Escherichia coli* (Chin-Tsan *et al*., 2010), *Bacillus* spp. (Adeleye and Okorondu, 2015) and *Saccharomyces cerevisiae* (Logan *et al*., 2006) have been identified with microbial fuel cells. Organisms associated with microbial fuel cells are usually associated with biofilm formation. They may or may not require a mediator (Logan *et al.*, 2005). Akaluka *et al*. (2015) also revealed presence of microbes similar to those reported in this research.

Table 4: Colonial, microscopic and biochemical characteristics of bacterial isolates

Bacterial isolates	Colonial morphology	G	Mot	Spo	Cat	Oxi	Coag	In	MR	VP	Cit	Ure	NO_3	H_3S	S	L	M	Mn	G
Staphylococcus aureus	Smooth golden yellow colonies	+S	-	-	+	-	+	-	-	+	-	+	+	-	+	+	+	+	+
Micrococcus luteus	Small smooth moist and shiny yellow colonies	+S	-	-	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-
Corynebacterium sp.	Circular dull and dry umbonate cream colonies	-R	-	-	+	-	-	-	-	+	+	-	+	-	-	-	+	-	+
Pseudomonas sp.	Bluish-green moist and shiny colonies	-R	+	-	+	-	-	-	+	-	+	+	+	-	-	-	-	+	+
Bacillus cereus	Dull and dry flat serrated cream colonies	-R	+	+	+	-	-	-	-	+	+	-	+	-	-	-	-	-	+
Enterococcus fecalis	Smooth shiny low convex cream colonies	+S	-	-	+	-	-	-	+	-	+	-	+	-	+	+	+	+	+
Bacillus subtilis	Rough slimy cream colonies	-R	+	+	+	-	-	-	-	+	+	-	+	-	-	-	-	+	+
Salmonella sp.	Black central colonies	-R	+	-	+	-	-	-	+	-	+	-	+	+	-	-	+	+	
Shigella sp.	Moist mucoid and shiny light pink colonies	-R	-	-	+	-	-	-	+	-	-	+	-	-	-	-	+	-	-
Escherichia coli	Purple metallic sheen	-R	+	-	+	-	-	+	+	-	-	+	+	-	+	+	+	+	+
Klebsiella sp.	Mucoid shiny pink colonies	-R	-	-	+	-	-	-	-	+	+	+	+	-	+	+	+	+	+

G = Gram reaction, + = Positive, - = negative; S = Spherical, R = Rod, Mot = Motility, Spo = Spore, Cap = Capsule, Cat = Catalase, Oxi = Oxidase, Coag = Coagulase, In = Indole, Vp = Voges Proskaeur, Cit = Citrate, Ure = Urease, No_3 = Nitrate reduction, G = Glucose, S = Sucrose, L= Lactose, M = Mannitol, H_2S = Hydrogen sulphide reduction

Table 5: Heterotrophic counts and characteristics of fungal isolates

Sample code	Total count	Colony code	Colonial characteristics	Microscopic characteristics	Identity of isolates
A	$1.9x10^6$	AK	Circular umbonate cream colonies	Large gram positive spherical budding cells	*Saccharomyces* sp.
		AL	Rough slimy revised cream colonies	Gram positive oval budding cell	*Saccharomyces* sp.
		AO	Black spores attached on short white hyphae	Hyphae septate condia globosed	*Aspergillus* sp.
B	$2.8x10^6$	BK	Circular umbonate cream colonies	Large gram positive spherical budding cells	*Saccharomyces* sp.
		BL	Black spores attached on short white hyphae	Hyphae septate condia globosed	*Aspergillus* sp.
		BO	Rough slimy cream colonies	Large gram positive spherical budding cells	*Saccharomyces* sp.
		BT	Short white mycellium	Non-septate hyphae	*Mucor* sp.
		BQ	Green spore enclosed in white mycelium	Septate hyphae conidia mopshaped	*Penicillium* sp.
X	$1.2x10^6$	XK	Circular umbonate cream colonies	Large gram positive spherical budding cells	*Saccharomyces* sp.
		XL	Short white mycellium	Non-septate hyphae	*Mucor* sp.
		XO	Black spores attached on short white hyphae	Hyphae septate condia globosed	*Aspergillus* sp.
Y	$1.9x10^6$	YK	Circular umbonate cream colonies	Large gram positive spherical budding cells	*Saccharomyces* sp.
		YL	Black spores attached on short white hyphae	Hyphae septate condia globosed	*Aspergillus* sp.
		YO	Green spore enclosed in white mycelium	Septate hyphae conidia mopshaped	*Penicillium* sp.
		YT	Rough slimy cream colonies	Large gram positive spherical budding cells	*Saccharomyces* sp.

Conclusion: The whole study has revealed that physicochemical parameters in piggery wastewater are significantly greater than the ones in the poultry wastewater. There were significant interactions/effects between the wastewaters and treatments (before and after). All the physicochemical parameters of piggery

and poultry wastewaters undergoing treatment over 18 days showed negative relationship with time. Also, the piggery and poultry wastewaters from Umuagwo in Ohaji Egbema LGA of Imo State, Nigeria are excellent reservoirs of exoelectrogens.

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