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The Dynamics of Electrochemicals and Microbial Populations during Anaerobic Treatment of Human Urine in Soil Microbial Fuel Cells

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Authors' contributions

This work was carried out in collaboration between both authors. Author HOS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors HOS and CJO managed the analyses of the study. Author CJU managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The dynamics of electrochemicals and microbial populations during anaerobic treatment of human urine in soil microbial fuel cells (MFCs) were investigated. The experimental MFC was supplemented with daily urine input while the control MFC was without urine. During the treatment process, electrochemical and microbiological parameters in effluent of the urine-supplemented MFC were monitored using standard methods. The pH of the urine increased from 5.70 to 7.16 after 15 days of treatment in the urine supplemented MFC. The concentration of phosphorus, potassium, sodium, calcium, magnesium, total nitrogen and total organic carbon of the urine reduced from 0.76 g/l to 0.07 g/l, 1.91 g/l to 0.17 g/l, 2.24 g/l to 0.09 g/l, 0.14 g/l to 0.003 g/l, 0.08 g/l to 0.00 g/l, 8.25 g/l to 0.74 g/l and 7.10 g/l to 0.53 g/l respectively after 15 days of treatment. Furthermore, Open voltage of the urine supplemented MFC ranged from 5.63 V to 10.34 V while Open voltage of the control ranged from 1.84 V to 5.02 V after 15 days of operation. The

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population of facultative bacteria (FAB) and strict anaerobic bacteria (SAB) ranged from 64.2 x 10^4 CFU to 36.2 x 10^4 CFU and 21.2 x10^4 CFU to 61.3 x10^4 CFU respectively with time. The urine supplemented MFC performed significantly ($p < 0.05$) better than the control with respect to voltage output while significantly reduced concentrations of organic carbon, nitrogen and metallic (salt) species were found. Therefore, the soil MFC may be applied as a waste management option to treat human urine while generating electricity before disposal.

Keywords: Human urine; anaerobic treatment; electrochemicals; microbial fuel cell.

1. INTRODUCTION

Microbial fuel cell (MFC) is a technology that directly converts the chemical energy stored in organic matter to electricity [1,2]. Driven by the increasing concern over the energy–climate crisis and environment pollution, MFC technology has been developed rapidly in the past decade. This is because MFC is not only an alternative approach for electricity generation; but can also be used to treat wastewater [1-5]. It is quite evident that most of the studies of MFCs are performed for the electricity generation and it is the prime application of the technology [6]. In the anode chamber of the MFC, the microorganisms oxidize the substrate into protons and electrons that are passed through a proton exchange membrane (PEM) and electrical connection, respectively to the cathode [7-9]. The two chambers of the MFC can be electrically connected to a multimeter and with an external resistor box, to measure the voltage, and subsequently the power can be calculated using Ohm's law [10,11].

The electrical output of the MFC depends on many factors including the design of MFC, electrode materials, microbial inoculum (pure or mixed culture), proton exchange membrane (if applicable) and other operational conditions [12]. Many approaches are already employed to increase the electrical output in the MFCs. The amendments in MFCs are basically focused on new MFC designs to reduce the internal resistance of the system, cost-effective electrode materials with high surface area, cheaper cation exchange membranes, modifications of the electrode material with nanomaterials (e.g. gold nanoparticles, nickel nanoparticles) and other physical (e.g. heat treatment of stainless steel electrode) or chemical (nitrogen-doped electrodes) treatment methods [13-15].

Microbial fuel cells (MFCs) have shown the potential to treat different industrial, urban or domestic wastewaters [13,16,17]. Though, highly toxic wastewaters cannot be completely treated in MFCs, however MFCs are able to reduce the Chemical Oxygen Demand (COD) of wastewaters much enough to meet discharge regulations before it is released into the environment. MFCs have proved up to 98% COD removal from wastewater [16]. Usually, wastewaters rich in organic materials (such as carbohydrates, proteins, lipids, minerals, fatty acids, etc.) provide the substrate for microbial metabolism to produce electrons and protons. Moreover, wastewaters are also the source of microbial inoculum. Basic wastewater treatment assays (COD, Biochemical Oxygen Demand, total solids, nitrogen removal) could be employed to monitor the treatment efficiency of MFCs before and after operation [13]. MFC studies operated for wastewater treatment are usually coupled with power generation; however, the columbic efficiency obtained in such cases is still relatively low [13]. The aim of this study was to investigate the dynamics of electrochemicals and microbial populations during anaerobic treatment of human urine in soil microbial fuel cells.

2. MATERIALS AND METHODS

2.1 Design and Set-Up of the Soil Microbial Fuel Cells (Mfcs)

The MFCs consisted of a rectangular plexiglass chamber with a length of 40 cm, width of 30 cm and a depth of 20 cm. The rectangular chamber was further divided into 24 single-chambered air-cathode cells of equal dimensions, consisting of an anode and a cathode placed on opposite ends. The anodes and cathodes were made of carbon cloth and graphite sheets respectively. Both electrodes had the same dimension of 12 cm x 3 cm x 0.01 cm in length, width and thickness respectively. In a cell, the anode was placed at the bottom while the cathode was placed at the top where it had access to air (oxygen). The space between the anode and cathode was filled with urine contaminated clay-sand mix. The dimension of each of the 24 cells was 8 cm (length) x 4 cm (width) x 15 cm

(depth). The remaining 5 cm depth of the MFC was filled with small gravels to allow for easy exit of the treated urine out of the 40 cm x 30 cm x20 cm rectangular MFC chamber. Furthermore, the zone (containing the clay-sand mix) was separated from the lower zone (containing the small gravels) by a sieve cloth with pores of 0.10mm in diameter. The MFCs were designed so that there was continuous inflow and outflow of the urine through the clay-sand mix which separated the anode from the cathode. To increase both voltage and current, the 24 cells inside the 40 cm x 30 cm x 20 cm rectangular MFC chamber were connected to each other in series-parallel fashion.

2.2 Preparation of the Clay-Sand Mix

Top and sub soil samples of loam which were heavily contaminated with urine over long period were sourced and collected at different locations around Wellspring University Benin City, Nigeria. This was done because we thought that the soil may contain potential electrogenic bacterial populations which may have adapted to high concentrations of urine and have possessed the ability to degrade the urine over time. After collection, the soil samples were taken to the laboratory in plastic bags. In the lab, the soil samples were thoroughly mixed to obtain a composite sample. The composite soil was mixed with water in a 60 cm x 40 cm x 30 cm plexiglass chamber in order to suspend clay particles contained in the loam soil samples. Afterwards, the clay-water mix was filtered through a carbon cloth filter into another chamber to separate the clay from the sand. The diameter of the filter pores was wide enough to allow some sand particles pass to through it so as to form the clay-sand mix. The clay-water mix was allowed to stand for 24 h in order to settle clay and remaining sand particles at the bottom of the chamber. Afterwards, the excess water was decanted and the wet clay-sand mix was placed in a carbon cloth with much smaller diameter which allowed only water to leave the mix. After 72 h, when the water had been removed, the wet solid clay-sand mix was ready for use. The reason for allowing some sand particles in the clay-sand mix was to slightly increase its porosity and drainage capacity enough to allow for a slow movement of the urine from the point of entry to the exit of the MFC chamber. This would also allow enough contact time between the microbes and their substrate (urine). Samples of the clay-sand mix were collected to determine its electrochemical and microbiological properties using standard methods.

2.3 Collection of Urine Samples and Operation of the MFCs

A total of 15L of fresh composite urine were taken from 150 healthy volunteers using sterile 150 ml containers as described by Simeon et al. [18]. After collection, urine samples were taken to the lab and stored in urine bank used for feeding the MFCs on a daily basis. Before subjecting the urine to anaerobic treatment in the MFCs, electrochemical and microbiological properties of the composite urine were estimated using standard methods. The MFCs were operated in open-circuit mode as described by Yan et al. [19]. A total of 720 ml of urine was fed into the experimental MFC on a daily basis. Each of the 24 smaller MFCs which made up the experimental MFC was fed with 30 ml of urine daily. In the control, sterile de-ionized water was used instead of urine. The control and experimental set-ups were operated at room (laboratory) temperature (28.3°C) for a period of 15 days. During this period, electrochemical and microbiological properties of the effluent coming out of the Experimental MFC (with urine input) were monitored on a daily basis using standard methods.

2.4 Determination of Electrochemical Parameters

The pH of the clay-sand mix and effluent from the urine supplemented MFC was estimated using a digital hand-held pH meter (SCT-lilliput, Scichem Tech) as described in Standard Methods [20]. A total organic carbon (TOC) combustion analyser (TEKMAR DOHRMANN APOLLO 9000 model) was used to analyse TOC in the clay-sand mix and effluent samples of the urine supplemented MFC as described by Mahlangu et al. [21]. Calcium (Ca) was determined using the ethylene-diamine-tetra-acetic acid (EDTA) titrimetric protocol described in Standard methods [20]. Potassium (K) and sodium (Na) were determined using the flame emission photometric protocol described in Standard Methods [20]. Total nitrogen (N) and phosphorus (P) were estimated using the spectrophotometric protocols described in Standard Methods [20]. The voltages (V) in open circuit mode of both MFCs were measured using a digital multi-meter (RadioShack) as described by Yan et al. [19].

2.5 Determination of Microbiological Parameters

The culture media used to enumerate and isolate facultative anaerobic bacteria (FAB) and strict anaerobic bacteria (SAB) in the urine-contaminated clay-sand mix and effluent samples from the urine supplemented MFC were the agar media as described by Ogbonna et al. [22] and Ogbonna et al. [23] respectively. Facultative anaerobic bacteria (FAB) were enumerated and isolated using the Agar Crack Technique and Spread Plate Technique described by Abdulkadir and Waliyu [24] and Wolf [25] respectively. Strict anaerobic bacteria (SAB) were enumerated and isolated using the Agar Roll-tube Technique described by Holdman and Moore [26] and Wolf [25] respectively. Bacterial isolates were identified according to Bergey's Manual of Determinative Bacteriology [27], using morphological and metabolic/biochemical tests [28,29].

2.6 Statistical Analysis

Within the Microsoft Excel (Version 2016) environment, temporal relationship between the bacterial populations and open-circuit voltage generation in the urine supplemented MFC was determined using correlation analysis. The Student's t-test was used to determine if there was a significant difference in open-circuit voltage generation between the Control MFC (with de-ionized water input) and the Experimental MFC (with urine input).

3. RESULTS AND DISCUSSION

3.1 Dynamics of Electrochemical Parameters

Average pH of the composite urine sample before anaerobic treatment in the MFC was 5.70. During the treatment process, average pH of the effluent from the urine supplemented MFC increased slightly with time to 7.16 after 15 days (Fig. 1). The concentration of phosphorus, potassium, sodium, calcium and magnesium in the composite urine sample before treatment was 0.76 g/l, 1.91 g/l, 2.24 g/l, 0.14 g/l and 0.08 g/l respectively. After 15 days of anaerobic treatment in the urine supplemented MFC, phosphorus, potassium, sodium, calcium and magnesium reduced to 0.07 g/l, 0.17 g/l, 0.09 g/l, 0.003 g/l and 0.00 g/l respectively (Fig. 2). Total nitrogen and total organic carbon reduced from 8.25 g/l and 7.10 g/l to 0.74 g/l and 0.53 g/l respectively after 15 days of anaerobic treatment of the composite urine sample inside the urine supplemented MFC (Fig. 3). Open voltage of the Experimental MFC (with urine input) ranged from 5.63 V to 10.34 V after 15 days of operation (Fig. 4). Open voltage of the control MFC (without urine input) ranged from 1.84 V to 5.02 V after 15 days of operation (Fig. 4). There was a significant difference between open voltage output of the MFC with and without urine input with respect to time.

The result in Fig. 1 suggests that the pH of effluent samples of the urine supplemented MFC increased with time of operation. This result agrees with the findings of Yan et al. [19] who also reported an increase in the pH of effluents from MFCs treating urine. The reason for the increase in pH of the effluent from the urine supplemented may be associated with the fact that the some of the bacteria species may be using the urea/uric acid present in the urine as a source of carbon/nitrogen thereby removing them from the urine MFC system with time [14,30,31]. Of course urea hydrolysis under anaerobic condition leads to the production of CO_2 and NH_3 [19]. Ammonia (NH_3) is slightly basic in nature and may have also contributed to the increase in pH of the effluent of the urine supplemented MFC with time [19].

The concentration of phosphorus, potassium, sodium, calcium, magnesium, total nitrogen and total organic carbon reduced with time of anaerobic treatment of the composite urine sample as shown in Fig. 2 and Fig. 3 respectively. This result is in line with the findings of Yan et al. [19] who also reported reductions in the concentrations of these parameters in effluents of MFCs operated under various conditions. Phosphorus, potassium, sodium, calcium and magnesium are usually present in urine in the form of salt which may help the urine to be more electrically conductive than pure water [18]. Therefore, their removal/disappearance in the effluent of the urine MFC suggests that the MFC may be employed to desalinate urine (or salt and waste waters in general) before disposal in to the environment [19].

In addition, these elements may have been used for growth or bio-accumulated by the bacteria present in the urine supplemented MFC which can cause their concentrations to decrease in the effluent of the urine MFC with time [32]. The decrease in total nitrogen and total organic

carbon in the urine MFC may also be attributed to the activities of the bacteria present in the urine MFC [19,32]. This is because electroactive/non-electroactive bacteria may consume the organic matter (urea/uric acid) present in the urine for their growth activities which may then lead to the removal of both carbon and nitrogen in the form of ammonia, nitrate, carbon dioxide, methane and organic acids from the system [5,33,34].

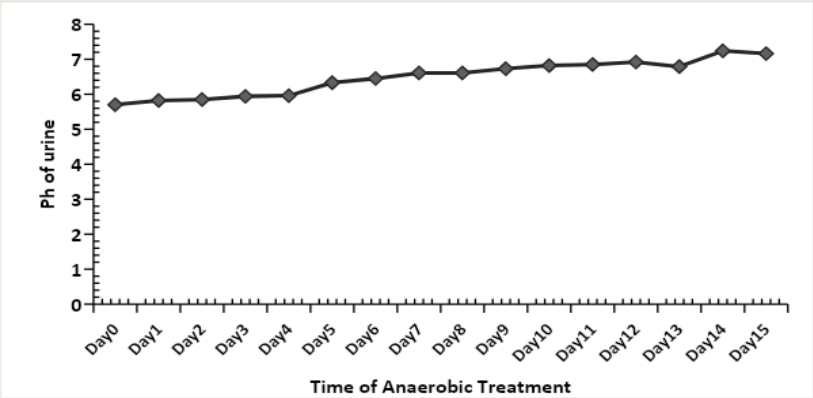

Fig. 1. pH of effluent from the urine MFC

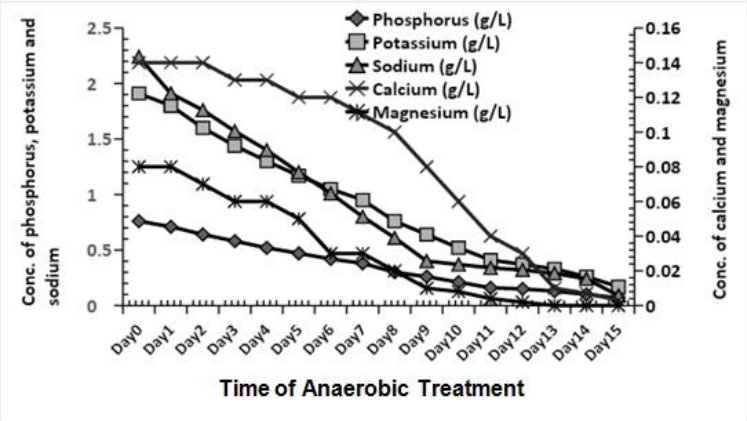

Fig. 2. Concentration of metallic species in effluent of the urine MFC

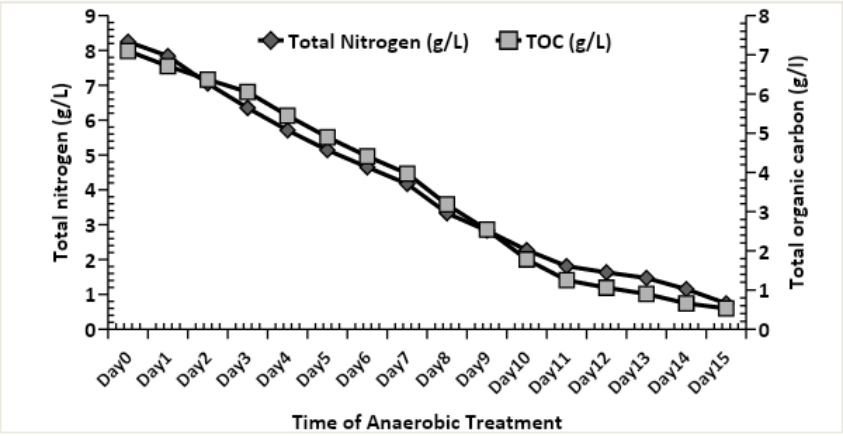

Fig. 3. Nitrogen and Organic carbon in effluent of the urine MFC

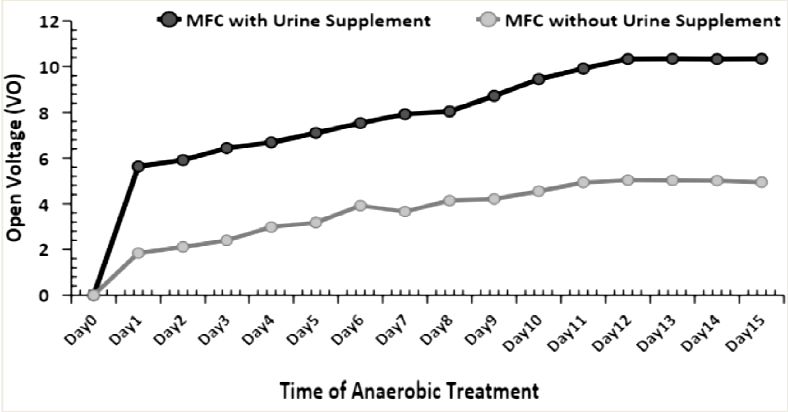

Fig. 4. Open voltage generated by the MFCs

Open voltage of the urine supplemented MFC was shown to be significantly higher than the open voltage of the control MFC which was without urine input with time (Fig. 4). This result is in line with the findings of Simeon et al. [18] who also reported higher voltage generation in MFCs with urine input than without urine input. This may be because of the fact that addition of urine served as a source of carbon and nitrogen to both electroactive and non-electroactive bacteria respectively. A major part of the organic carbon present in the urine may have been converted to electricity by electroactive bacteria in the urine supplemented MFC in addition to that initially present in the clay-sand mix. Furthermore, urine is more electrically conductive than deionized water and clay-sand mix used in this study due to the salts that are usually dissolved in it [18]. This allows it to further lower the internal resistance of the urine supplemented MFC compared to the control MFC which was without urine input.

3.2 Dynamics of Microbial Populations

The average population of facultative anaerobic bacteria (FAB) and strict anaerobic bacteria (SAB) in the urine-contaminated clay-sand mix used to construct the MFCs was 64.2 x 10^4 CFU/g and 36.3 x 10^4 CFU/g respectively (Fig. 5). No bacterial species was detected in the urine sample before it was fed in to the urine supplemented MFC. At the initial stage of the anaerobic treatment process, the population of FAB in effluent samples of the urine supplemented MFC decreased from 52.6 x 10^4 CFU/ml at around day one (1) to 50.2 x 10^4 CFU/ml at around day four (4). From day five (5), the population of FAB increased from 54.8 x 10^4 CFU/ml to peak at 62.5 x 10^4 CFU/ml at around day ten (10) and then decreased progressively with time to 36.2 x10^4 CFU/ml around day 15 of the treatment process (Fig. 5). Furthermore, the population of SAB in the urine supplemented MFC initially decreased slightly from 32.4 x10^4 CFU/ml on day one (1) to 21.2 x10^4 CFU/ml at around day three (3). However, from day four (4), the population of SAB in the effluent samples of the urine supplemented MFC increased progressively with time to 61.3 x 10^4 CFU/ml at around day 15 (Fig. 5).

There was a positively moderate correlation (r = 0.633) between the population of FAB and open voltage in the urine supplemented MFC with time (Fig. 6). However, the correlation (r = 0.934) between open voltage and the population of SAB was positively very strong with time (Fig. 7). A total of fourteen (14) bacterial species were isolated from the urine-contaminated clay-sand mix used in constructing both MFCs (Fig. 8) as shown in Table 1. The bacteria species identified were probably belonging to genera such as *Bacillus sp., Clostridium sp., Lactobacillus sp., Pseudomonas sp., Flavobacterium sp., Micrococcus sp., Enterobacter sp.* and *Staphylococcus* sp. (Fig. 8). A total of nine bacteria probably belonging to species such as *Bacillus sp., Clostridium sp., Lactobacillus sp., Pseudomonas sp., Micrococcus sp., Enterobacter sp.* and *Staphylococcus sp.* were isolated from effluent samples of the urine-supplemented MFC (Fig. 8).

The result in Fig. 5 shows that the population of strict anaerobic bacteria (SAB) increased with time while the population of facultative anaerobic bacteria increased initially but later decreased progressively with time in effluent samples of the urine supplemented MFC. The population of SAB

may have increased due to the presence of organic carbon (such as urea/uric acid) and the anaerobic nature of the urine MFC [32]. Initially, pockets of oxygen which may have been present in the urine MFC may have favoured the population of FAB but as they consumed the oxygen, they would have improved the environment for the population of SAB to proliferate. When the oxygen reduced beyond their carrying capacity, the population of FAB would have been affected negatively thereby resulting to the progressive fall in their population with time of operation.

Bacteria probably identified as *Bacillus sp., Clostridium sp., Lactobacillus sp., Pseudomonas sp., Flavobacterium sp., Micrococcus sp., Enterobacter sp.* and *Staphylococcus* sp. were isolated from the urine-contaminated clay-sand mix used in building the MFCs. Bacteria species classified under these genera have been isolated from urine-contaminated soils in various locations within Nigeria as reported by several authors [35]. Their presence in the soil suggests that they may have adapted well enough to the high concentration of urine (in the soil) which has been reported to be toxic to many bacteria species due to its high saline content [35]. Bacteria species which was found to be associated with the effluent of the urine supplemented MFC included *Bacillus sp., Clostridium sp., Lactobacillus sp., Pseudomonas sp., Micrococcus sp., Enterobacter sp.* and *Staphylococcus* sp. (Fig. 8). These bacteria species appear to have been washed out of the clay-sand mix inside the urine supplemented MFC because the untreated composite urine samples fed into the urine MFC lacked any bacteria at the time of sampling. These bacteria species appear to be the same species that were initially isolated from the urine-contaminated clay-sand mix used as the matrix in the MFCs. Bacteria species belonging to these genera have been isolated from MFCs in other studies [32]. Their presence in the effluent of the urine supplemented MFC suggests that they may have been involved in the generation of electricity inside the MFCs in some capacity [32].

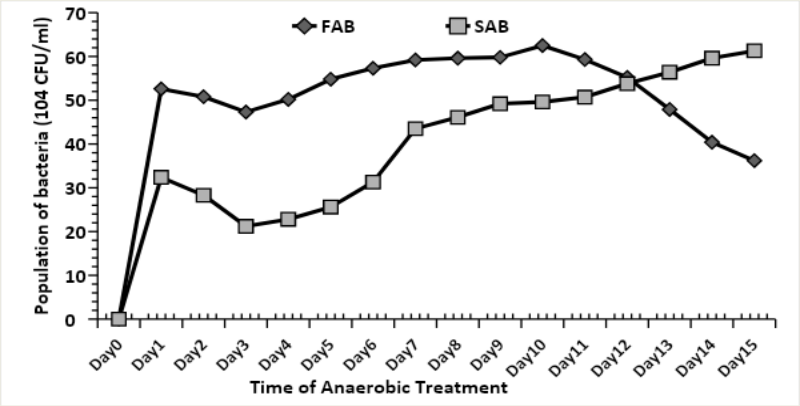

Fig. 5. Population of FAB and SAB in effluent of the urine MFC

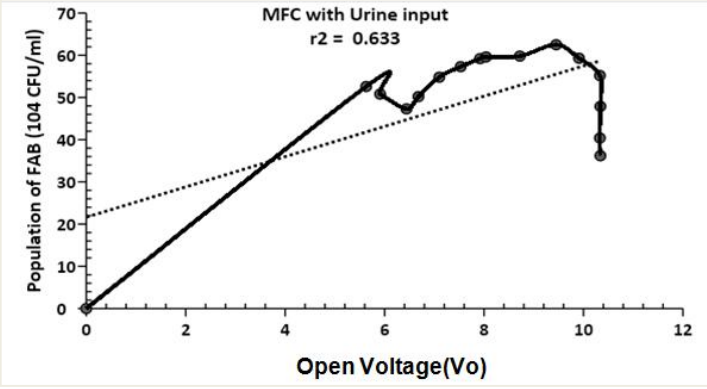

Fig. 6. Temporal relationship between FAB and open voltage

Table 1. Biochemical characteristics of bacteria isolated from the clay-sand mix and effluent of the urine supplemented MFC

Biochemical Tests	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14
Gram stain	+	+	+	+	-	+	+	+	+	+	+	+	+	-
Shape	Rod	Rod	Rod	Cocci	Rod	Cocci	Cocci	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Arrangement	Single	Single	Single	Cluster	Single	Cluster	Cluster	Chain	Chain	Chain	Chain	Single	Single	Paired
Spore	+	+	+	-	-	-	-	+	+	+	-	-	-	-
Acid fast	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Motility	+	+	-	+	+	-	-	+	+	+	+	+	+	+
O_2 requirement	SA	SA	SA	FA	FA	OA	OA	FA	FA	FA	OAN	FA	OA	FA
Oxidase	-	-	-	+	-	+	+	-	+	+	-	+	+	+
Coagulase	-	-	-	+	-	-	-	-	+	-	-	+	-	-
Citrate	-	-	-	-	+	-	+	+	-	+	+	+	+	+
Catalase	+	-	-	+	+	+	+	+	+	+	-	+	+	+
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urease	-	-	-	+	-	-	+	+	+	-	+	+	+	-
H_2S Production	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitrate red.	-	+	-	-	+	+	-	+	+	+	-	+	-	+
Methyl red	+	+	+	-	-	+	-	-	-	-	-	-	-	-
Voges Proskauer	-	-	-	-	+	+	+	+	+	+	-	-	-	-
Ornithinedecarboxylase	-	-	-	-	+	-	-	-	-	-	-	-	-	-
D-glucose	+/+	+/+	+/+	+/+	+/+	+/-	+/-	+/+	+/+	+/+	+/+	-	+/-	+/+
D-mannitol	-	-	+/+	+/-	+/+	+/-	-	-	+/+	-	+/+	+/-	+/+	+/+
D-sucrose	+/+	-	+/+	+/+	+/+	+/-	-	+/+	+/+	+/+	+/+	-	-	+/-
Lactose	+/+	-	+/+	+/+	+/+	-	-	-	-	-	+/+	-	+/+	+/-
D-maltose	+/+	+/+	+/+	+/+	+/+	+/-	-	+/+	+/+	+/+	+/+	-	+/+	+/-
Glycerol	-	-	+/+	-	-	-	-	-	+/+	-	-	+/+	-	+/-
Cellulose	+/+	-	+/+	-	-	-	-	+/-	+/-	+/-	-	-	-	-
Starch	+/-	-	+/-	-	-	-	-	+/-	+/-	+/-	-	-	-	-
Gelatin	-	+/-	+/-	+/-	-	+/-	+/-	+/-	+/-	+/-	-	+/-	+/-	+/-
Esculin	+/-	-	-	+/-	-	-	-	+/-	+/-	+/-	-	+/-	-	-
Lipid	-	+/+	+/-	-	-	-	-	-	+/+	+/+	-	+/+	-	+
Probably identify	*Clostridium sp*	*Clostridium sp*	*Clostridium sp*	*Staphylococcus sp*	*Enterobacter sp*	*Micrococcus sp*	*Micrococcus sp*	*Bacillus sp*	*Bacillus sp*	*Bacillus sp*	*Lactobacillus sp*	*Pseudomonas sp*	*Pseudomonas sp*	*Flavobacterium sp*

OA = Obligate aerobe, SA = Strict anaerobe, FA = Facultative anaerobe, +/+ = Acid and gas production; +/- = Acid production without gas production, - = No fermentation

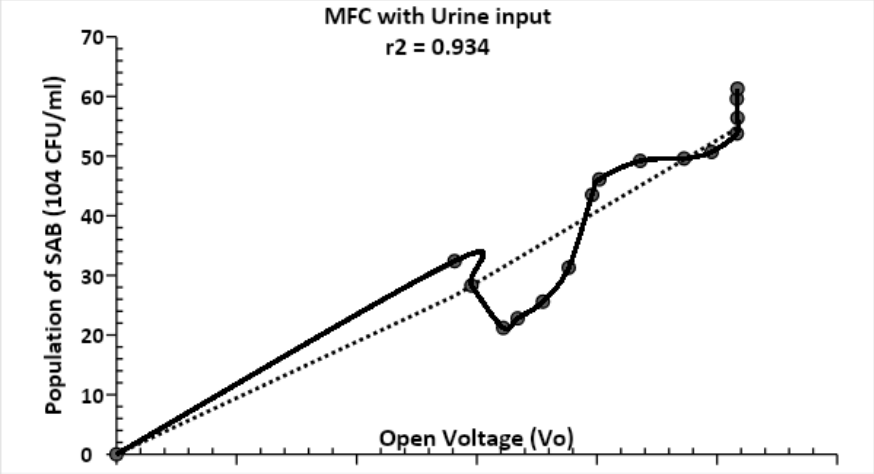

Fig. 7. Temporal relationship between SAB and open voltage

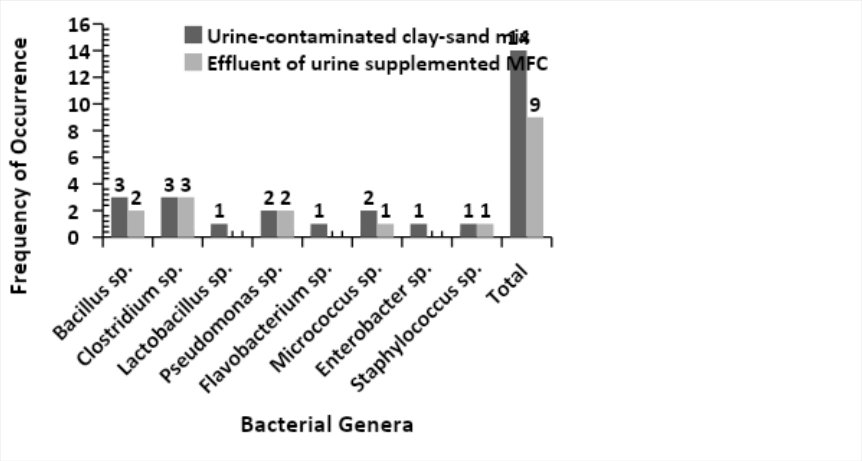

Fig. 8. Distribution of bacteria isolates

4. CONCLUSION

This study evaluated the dynamics of electrochemicals and microbial populations during anaerobic treatment of human urine in soil microbial fuel cells. Result from the study showed that the MFC with urine input performed significantly ($p < 0.05$) better than the MFC without urine input with respect to voltage generation while significantly reduced concentrations of total organic carbon, total nitrogen and metallic (salt) species found in the urine samples with time. Therefore, the MFC used in this study may be applied as a waste management option especially in a developing country like Nigeria where waste disposal has posed serious challenge to successive government. Furthermore, the outcome of this study may serve as an alternative source of power in developing economies like ours where there is erratic power supply and over dependence on fossil fuels.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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