# Application of urine as fuel in a soil-based membrane-less single chamber microbial fuel cell

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Abstract: Microbial fuel cell (MFC) technology is a promising bio-technology that utilizes the microorganism in organic wastes to generate electricity. Although human urine has been identified as a suitable substrate in MFCs, its possible utilization in a soil-based membrane-less single chamber microbial fuel cell (MSCMFC) for constant power generation has, hitherto, not been reported. In this study, a MFC was set up with mud as inoculums in a plastic cylindrical vessel using carbon felt electrodes. It was operated for 19 days (456 hours) without extra substrate. Then, the MFC was treated with human urine (as substrate) four times (Days 19, 24, 32 and 36) each time the MFC output stabilized across external loads. A control MFC (MFC<sub>control</sub>) was made the same way and operated under the same conditions, but without addition of urine. Both MFCs were operated for 40 days. The initial open circuit voltage (OCV) of the MFC treated with urine (MFC<sub>urine</sub>) was 227 mV and that of MFC<sub>control</sub> was 219 mV. Both MFCs produced overlapping OCVs to the point of adding urine. The maximum OCVs of MFC<sub>control</sub> and MFC<sub>urine</sub> prior to treatment were 729 mV and 740 mV respectively. The OCV of MFC<sub>urine</sub> increased to a maximum value of 755 mV, four days after the initial treatment (day 23). At the final stage (Day 40), OCV of MFCurine was 474.64 mV; whereas the corresponding value for MFC<sub>control</sub> was 7.31 mV. A micro chip was used to amplify the output of the MFCs to power a light emitting diode. In addition, MFCurine was used to power a digital clock/thermometer. This study showed that human urine can be successfully utilized as fuel in a soil-based MSCMFC for the production of electrical energy which can be boosted to power low energy utility devices in farms or homes. Keywords: soil, urine, microorganism, power, fuel cell

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# 1 Introduction

Renewable energy technologies are generally, clean sources of energy that have a much lower environmental impact than conventional energy technologies. Apart from the common renewable energy sources, there has been a recent wide research interest in developing microbial fuel cell (MFC) technology. The reasons for this recent interest in using bacteria to generate electricity are a combination of the growing needs for new sources of energy (Logan and Regan, 2006) and concerns about environmental pollution associated with the fossil fuel based methods of electricity generation.

Apart from being environmentally friendly, MFC technology allows direct conversion of substrate energy to electricity, and thus ensures wastes to energy conversion. In addition, microbes are found virtually in all soils, sediments, and streams on the planet (Simeon et al., 2016a). This makes soil MFCs very attractive for applications that only require low power but where replacing batteries may be time consuming and expensive. MFCs can possibly be used to power sensors particularly in the river and deep water environments where it is difficult to replace batteries. Powered by MFCs, the sensors can be left alone in remote areas for many years

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without maintenance (Li, 2013). As long as conditions remain favourable for current production by the anode-associated microbes, an MFC has the potential to produce electricity indefinitely (Franks and Nevin, 2010).

Substrates, in the form of organic materials which the microbes are able to degrade, must be sufficiently available in an MFC for efficient production of electricity. A spectrum of substrates suitable in MFCs ranges from simple to complex mixture of organic matter present in wastewater. Even though substrates rich in complex organic content may assist the growth of diverse active microbes, simple substrates are generally considered most suitable for immediate electricity generation. The most commonly used substrates which have been reported as successfully utilized for basic MFC operations and electricity generation are acetate and glucose (Das and Mangwani, 2010). Brewery wastewater has been successfully used as substrate as it is supplemented with growth promoting organic matter and devoid of inhibitory substances (Feng et al., 2010). Lignocellulosic biomass from agriculture residues as hydrolysis product (monosaccharide) are good substrates for electricity production in MFCs (Catal et al., 2008). Starch processing water is also a suitable source of substrates for microbes in MFC (Kim et al., 2004). High level of removal efficiency, with supplementary benefit of generating electricity, has been achieved with MFCs connected in series to treat leachate (Gálvez et al., 2009). One such global and abundant waste product is human or animal urine, which has already been demonstrated to be an efficient fuel for direct electricity production via single MFCs with efficiency greater than 50% (Ieropoulos et al., 2012).

Urine is a complex fluid that contains various amounts of electrolytes, urea, and other metabolic products (Chambers and Kunin, 1985). Basically, human urine consists of 95 % of water with organic compounds which include urea, uric acid, creatinine, carbohydrates, hormones, fatty acids, pigments and enzymes. The electrolytes contain cations  $K^+$ , Na<sup>+</sup>, Ca<sup>2+</sup>, NH4<sup>+</sup>, and anions, Cl<sup>-</sup>, HCO<sub>3</sub><sup>-</sup>, PO4<sup>3-</sup>, SO4<sup>2-</sup> (Kirchmann and Pettersson, 1995, Pedro, 2012). The high concentration of sodium chloride and urea (rich in nitrogen and phosphorus) in human urine, including its organic contents, enhance its suitability as substrate in MFCs. According to Ieropoulos et al. (2012), the compositions of the normal human urine include urea (6-18 g day<sup>-1</sup>), uric acid (1.8 g day<sup>-1</sup>), creatinine (0.5-0.8 g day<sup>-1</sup>), amino acids (0.12 g day-1) and peptides (0.5 g day<sup>-1</sup>). Variable amounts of lactic acid, citric acid, bilirubin and porphyrins, ketone bodies (aceto-acetic acid:  $\beta$ -hydroxybutyrate; acetone), and small amounts of hexose (glucose) and pentose (arabinose) sugars may also be present in normal urine. Compounds such as urea and uric acid cannot be utilized as carbon-energy (C/E) sources by the microbial community inside an MFC. Ieropoulos et al. (2012) pointed out that these are not included when considering the bio-available organic content of excreted urine. In total, the dry weight content of metabolisable organic substrates, within excreted urine, has been estimated to be 0.78 g/human/day (White et al., 1968). Lipid is also not included owing to its insignificant amount in urine. Therefore, the mean calorific value of 1 g of carbohydrates, peptides, proteins or amino-acids (as metabolizable substrates within urine) has been estimated to be 2.08 kcal (Rodriguez et al., 2005, Ieropoulos et al., 2012). Despite the organic content in urine that microbes can metabolize, only a tacit reference has hitherto been made to its direct utilization in MFCs to enhance electricity generation.

Ieropoulos et al. (2012) reported, for the first time, the direct utilization of neat (unprocessed) urine in MFCs for the production of electricity. Similarly, Santoro et al. (2013) investigated a treatment process for human urine in membrane-less single-chamber MFC. The results of both experiments clearly demonstrated that human urine can be effectively degraded in single chamber microbial fuel cells (SCMFCs) with stable current generation, and increased conductivity of the solution.

Although human urine has been identified as a suitable substrate for recharging soil MFCs (Simeon and Raji, 2016), its direct utilization in a soil-based membrane-less single chamber microbial fuel cell (MSCMFC) for practical implementation has, hitherto, not been reported. In view of this, the present study is basically designed to explore the possibility of its adoption as a suitable substrate to improve the performance of soil MFCs for real practical applications.

#### 2 Methodology

## 2.1 Soil sampling and MFC set up

Soil was sampled from the University of Ibadan where crops have been previously cultivated and was prepared into mud by sieving to remove pebbles and by adding water. The Mudwatt Microbial fuel cell kit (Keego Technologies LLC, Stanford, USA) was used in this study. Soil was patted down in MFC vessel up to 1 cm to make a smooth surface and anode was placed on the top of the soil, finally soil sample was added up to 4 cm line as shown in Figure 1. The cathode was placed on the top of the soil and the vessel was covered with the electrodes passed through the appropriate holes on the lid as described by Simeon et al. (2016a). The hacker board was inserted in the indentation provided on the lid for easy insertion of other components. The MFC was set in a mode to power a light emitting diode (LED) using a voltage amplifier micro-chip and a 10 µF/50volts capacitor provided with the hacker board.

#### 2.2 Urine collection, preparation and utilization

According to the method described by Ieropoulos et al. (2012), neat (unprocessed) urine samples were taken from a single healthy volunteer (height 1.75 m, and average weight for a young adult) on a normal diet with no prior history of urinary tract or renal disease.

After 19 days (456 hours) of continuous operation of the cell, when a fairly stable voltage output had been obtained for three consecutive days, the anode and the cathode wires were unplugged from the hacker board. With the nitrile gloves put on, the cathode was gently lifted with proper precaution so as not to get any mud on top of the cathode. A clean 3 mL medicine dropper was used to suck up fresh urine from the container. The urine was added in drops to the top of the mud, spread out evenly across the mud's surface. The cell was kept undisturbed for 5 minutes to allow the urine soak into the mud. Then, the fuel cell was re-assembled and left for 24 hours, after which the voltage drops across seven resistors were measured and the power output computed. Addition of the same volume of urine was repeated on days 24, 32 and 36 (after 576, 768 and 864 hours of operation respectively); each time a stable power output was obtained for at least two days. This was done in order to

investigate the response of the power output to the same substrate feeding at different time (Simeon et al., 2016b).

## 2.3 MFC operation

Initially the MFC was operated for 19 days (456 hours), when the power output reached a stable maximum, without adding any substrate to the soil. After the voltage measurement of day 19, 3 mL of freshly collected human urine was fed into the cell at four duration times (Days 19, 24, 32 and 36). This volume of urine, which was just sufficient to saturate the soil with water, was added each time, a decline in voltage was observed after a fairly stable voltage output had been obtained for at least 2 days. The MFC was operated for 40 days (960 hours), for enough duration for experimentation and data acquisition.

For comparison, a control MFC (MFC<sub>control</sub>) was made and operated for 40 days also, under the same conditions as the first one; but without the addition of urine. Both MFCs were operated at ambient temperature range of  $27^{\circ}C\pm 3^{\circ}C$ 

# 2.4 Data acquisition and calculations

Crocodile clips were used to hold the multi-meter probes and the resistor's lead to the cell anode and cathode for voltage measurement (Plate 1). Voltage drops of the MFC across seven external loads (4670, 2190, 1000, 470, 220, 100 and 47  $\Omega$ ), starting from the highest to the lowest, were noted after stabilisation (5 to 10 minutes intervals). This measurement was repeated every 24 hours, for the whole duration of the experiment. With the measured values of voltage, the current and power were determined from Equation (1) and (2) respectively, according to Ohm's law.

$$I = \frac{V}{R} \tag{1}$$

$$P = IV \tag{2}$$

where, I = current, A; P = Power W; V = voltage across each resistor, V; R = resistance of each resistor,  $\Omega$ .



Plate 1 Voltage measurement from the MFC



Figure 1 Schematic diagram of the MFC set-up (Source: Simeon et al., 2016a)

#### **3** Results and discussion

#### 3.1 MFC performance across external loads

The results of the daily voltage measured across seven external loads for 19 days prior to urine addition to the MFC are presented in Figures 2-6; while Figure 7 presents the voltage measured across the external loads 24 hours before and after the addition of urine. Figure 8 presents the percentage change in voltage across the external loads 24 hours and 48 hours after urine addition to the MSCMFC.

The first set of voltages measured across the external loads 4670, 2190, 1000, 470, 220, 100 and 47  $\Omega$  were 162, 105, 64, 25, 12, 5 and 2 mV, respectively. The daily peak voltage increased from 162 mV on day 1 (Figure 2) to a maximum of 686 mV on day 18 (Figure 6).

The voltage drops across the external loads were fairly stable for three consecutive days for all the resistors (as shown in Figure 6). A slight drop below the stable values was observed on day 19 indicating a drop in substrate concentration of the soil. Hence, urine was fed into the MFC on day 19 after the day's measurement. The steady increase in voltages across the external loads, from day 1 up to day 18, as presented in Figures (2-6) before the addition of urine is an indication of the growth of the soil indigenous microorganisms. These results suggested the establishment of a microbial community conducive to extracellular electron transfer.

There was a sharp increase in voltage measured across the 470  $\Omega$ , 24 hours after the first treatment with urine; whereas there was only a slight change in the voltages measured across the other external loads (Figure 8). The voltage measured across the 470  $\Omega$  load indicated

an overshoot of 21.5% 24 hours after urine was added (Figure 8. Higher voltages were measured across the 1000, 470, 220, 100 and 47  $\Omega$  loads respectively, compared to the values recorded on day 19, just before the addition of urine. On the other hand, there was a drop in voltage across the 4670  $\Omega$  and 2190  $\Omega$  external loads, compared to the values recorded on day 19. As shown in Figure 8, this initial drop in voltage was, however, accompanied by increase in voltage across the two loads respectively, after 48 hours of substrate feeding. There was an increase in the voltages measured across all the external loads, (except 470  $\Omega$  and 220  $\Omega$ ) after 48 hours of adding urine compared to the values obtained on day 19 (Figure 7).



Figure 2 Voltage measured across external loads for the first four





Figure 3 Measured voltages across external loads prior to urine addition (Days 5-8)



addition (Days 9-12)



Figure 5 Voltage drop across external loads prior to urine addition (Days 13-16)



Figure 6 Voltage drops across the External Loads prior to substrate feeding (Days 16-19)



Figure 7 Voltage measured across external loads before (days 18 and 19) and after (days 20 and 21) urine addition



Figure 8 Percentage change in voltage across the external loads 24 hours and 48hours after urine addition to the MSCMFC

#### 3.2 Daily open circuit voltages of the MSCMFC

A comparison of the daily open circuit voltages (OCVs) of  $MFC_{urine}$  and  $MFC_{control}$  measured throughout the period of experiment is presented in Figure 8.

The OCVs obtained from both MFCs, in this study

(Figure 9), had overlapping values prior to addition of urine. This clearly demonstrated that the same species of microorganisms were present in the MFCs and the conditions in the two cells were similar. The higher values obtained from MFC<sub>urine</sub> is attributed to enhancement of the soil conductivity due to increased ionic strength and sustained metabolism of the microbes owing to availability of substrate.



Figure 9 Daily OCV of urine treated MFC and the control MFC

The maximum OCVs of 729 mV and 755 mV (for MFC<sub>control</sub> and MFC<sub>urine</sub> respectively) achieved from the MFCs in this study are comparable to the values reported by Samuel et al. (2013); although the life-span of the MSCMFC reported was shorter compared to this present study. This discrepancy in the length of operating time may be attributed to the different sources of soil samples, different operating conditions or difference in the species of active microbial community in the soil samples used. In a similar study, Jenna (2010) reported a peak voltage of 635 mV from an air-cathode SCMFC treating leachate from a land-fill. Conversely, using a double chamber MFC with a proton exchange membrane (PEM), Barua and Deka (2010) reported a maximum voltage of 197 µV from a combination of two or more bio-wastes (slurry, cow dung, drain water, rice washing water, and vermin-compost). This value is far below the minimum voltage obtained from this study without adding any substrate. This difference may be attributed to the different substrates utilised in the different studies and the conditions under which they were operated. It is however worthy of note that this discrepancy points to the efficiency of the soil-based MFCs and the advantages of MSCMFCs over the double chamber MFCs as has been pointed out by many researchers (Liu et al., 2005; Jenna 2010; Samuel et al., 2013).

# **3.3** Practical implementation of the MSCMFC: led and electronic clock/thermometer

To show the feasibility of the soil-based MFCs for real applications, the MFCurine was connected to a DC-DC voltage booster (Mudwatt's hacker board) to constantly charge a 10  $\mu$ F/50 V electrolytic capacitor which was used to operate a red LED. The LED started blinking after 30 hours of the set up at an OCV of 343 mV. It started initially with one blink every 8 seconds and the frequency increased up to 6 blinks in a second before the addition of urine. The blinking frequency increased above 6 sec<sup>-1</sup> after the first addition of urine for at least three days and then gradually decreased until the blinking rate was 3 sec<sup>-1</sup> on day 30 of the set up. It remained constant at this frequency up to day 40. Similarly, the MFC<sub>control</sub> was used to power the LED but not beyond day 24. Apart from the LED, the MFC<sub>urine</sub> was used to power an electronic clock/digital thermometer. This was achieved by connecting two capacitors (10  $\mu$ F/50 V and 47  $\mu$ F/ 16 V) in parallel to the booster and then the clock/ thermometer was connected to the terminals of the capacitors. These practical implementations are depicted in Plates 2 and 3, respectively.



Plate 2 MSCMFC (a) before powering a LED (b) powering the LED



Plate 3 MFC<sub>urine</sub> powering (a) electronic clock (b) digital thermometer

## 4 Conclusions

Fresh human urine has been successfully utilised to enhance electricity generation in soil MFC. The better performance of the urine treated MFC over the 40-day operational period compared to the control MFC showed that unprocessed human urine from healthy individuals is a well-matched substrate for use in soil-based MFCs. In this study, urine did not only improve the MFC electricity generation but also produced fairly constant power output and enhanced the longevity of the MFC. The better performance of the urine treated MFC is attributed to the high amount of organics, conductivity, and buffering capacity of urine. Although, initial addition of urine reduced the power output of the cell across some external loads, due to decay resulting from urine hydrolysis, this was only transient as it was overcome by proper aeration of the cathode. These results ported a robust potential for the development of MFCs for power generation from agricultural soil and urine. In addition, the application of a micro-chip to power a LED and electronic clock/thermometer in this study is a clear indication that the outputs of soil MFCs can be amplified for practical applications.

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