Urea oxidation in a paper-based microfluidic fuel cell using *Escherichia coli* anode electrode

L C Castillo-Martínez¹, D M Amaya-Cruz+², J Gachuz², D Ortega-Díaz³, J M Olivares-Ramírez², D Dector⁴, A. Duarte-Moller⁴, A L Villa⁵, A Dector*⁶

¹Universidad Tecnológica de San Juan del Río, San Juan del Río, Querétaro, México
²Universidad Autónoma de Querétaro, Querétaro, Mexico
³Centro de Investigación y Desarrollo Tecnológico en Electroquímica, Pedro Escobedo, Mexico
⁴Centro de Investigación en Materiales Avanzados, Chihuahua, Mexico
⁵Universidad de Antioquia UdeA, Medellín, Colombia
⁶Conacyt-Universidad Tecnológica de San Juan del Río, San Juan del Río, Querétaro, Mexico

+dmamayacruz@hotmail.com and *andres.dector@live.com

Abstract. This paper presents the use of *Escherichia coli* as an anode in a paper-based microfluidic urea fuel cell (P-based μUFC). For the construction of this P-based μUFC, a saline solution with *E. coli* bacteria was deposited on carbon cloth and used as an anode for urea oxidation, furthermore, a piece of Toray® carbon paper was impregnated with Pt/C and used as an cathode to reduce the oxygen present in the air. The P-based μUFC proved with a urea solution of 0.33 M, showed an open-circuit voltage of 0.83 V, a maximum current density of 3.253 mA cm⁻² and a maximum power density of 0.608 mW cm⁻², these values were similar to report when human urine was used as fuel. The microfluidic fuel cell developed was evaluated for 20 consecutive days at room temperature in order to observe the lifetime of the bacteria with respect to the power generated. This work represents an advance in the possible use of physiological fluids such as urine to generate electrical energy in non-implantable medical devices.

1. Introduction

An increasing demand for portable medical devices has originated a search for energy sources because the principal problem is that a lithium-ion battery limits their functionality. A solution towards power sources for portable medical devices has been the use of micro fuel cells [1-3]. In this sense, paper-based microfluidic fuel cells (P-based μFC) have been proposed as a power supply for non-implantable medical devices [4-6]. The P-based μFC have presented some advantages as a very low-cost and disposable; the independence of pumps and a membrane between the anode and cathode is not necessary.

On the other hand, the use of urine for its urea content as fuel has shown the greatest potential for electricity generation [7, 8]. Different materials such as nickel [9, 10] and bacteria have been proposed to show great potential in the oxidation of urea for energy production. In this sense, one interest
publication shows that urea can be used as fuel in micro fuel cells that used *Escherichia coli* as an anode.

Mardanpour and Yaghmaei [11] reported a microfluidic microbial fuel cell using nickel as electrode and *Escherichia coli* as biocatalyst. The authors employed urine as fuel recollecting 14 W cm\(^{-3}\). This work established the perspective of using the microbial fuel cell for implantable medical devices. However, this report has the disadvantage of using external pumps for the flow of urea, which turn out to be very expensive in addition to the complex operating conditions, for that reason in this work is proposed a P-based µFC that use *Escherichia coli* catalysts and urea as fuel, as a possible backup power supply. Although the intention is not to replace or compete with a lithium-ion battery, it arises as another option of emergency power that can serve as a power source for a non-implantable medical device while a sample of urine is used as fuel.

2. Methodology

2.1. Electrode fabrication

*Escherichia coli* ATCC25922 was cultured in lactose broth (3 g L\(^{-1}\) beef extract, 5 g L\(^{-1}\) peptone, and 5 g L\(^{-1}\) casein) at 35 °C for 24 hours. Then, sowing was done on Mac Conkey agar (Britania) and later a reseed in liquid medium (3 g L\(^{-1}\) peptone, 3 g L\(^{-1}\) peptone gelatin, 5 g L\(^{-1}\) NaCl and 5 g L\(^{-1}\) urea), which had the function to adapt the bacteria to the components of the medium, to 35 °C for 24 hours. Subsequently, the *E. coli* bacterium was reseeded in the medium containing urea on BAB agar (BRITANIA) at 35 °C for 24 hours. Finally, a suspension based on McFarland turbidity standard 2 (6.0x10\(^{8}\) CFU) was prepared using a 0.9% saline solution as a diluent. A square of 1 x 1 cm of carbon cloth placed in a vial with *E. coli* suspension was subjected to lyophilization in a lyophilizer (Edwards mod. Modulyo).

2.2. Physicochemical characterization of *Escherichia coli*

Physicochemical characterization of *Escherichia coli* consisted of obtaining the scanning electron microscope (SEM) images with a coupled EDS instrument (Hitachi SU3500 SEM).

2.3. Device fabrication

![Figure 1. Schematic of the paper-based microfluidic urea fuel cell.](image)

The construction of the device is similar to a typical lateral flow test. It consisted of a 5 mm wide paper (Fusion 1 Whatman) in which the anode and cathode electrodes have been placed at the bottom and top of the paper strip in sandwich mode both with dimensions of 1 x 1 cm. The anode was placed under the paper strip, while the cathode was attached on top to facilitate oxygen access from the atmosphere, so the contact area was of 0.5 x 0.5 cm. This paper strip was placed between a
poly(methyl methacrylate) (PMMA) shell fabricated using a CNC micro milling system and homogeneously tightened. Gold-pins were used to improve the electrical conductivity. Finally, a window in the PMMA tap (0.5 cm x 0.5 cm) was opened as an air intake for the air-breathing cathode. Figure 1 shows a schematic of the P-based µUFC.

2.4. Performance of the paper-based microfluidic urea fuel cell

The electrochemical evaluation of the developed P-based µUFC allowed the validation of the proposed fuel cell approach and was used to perform the characterization under physiological near operating conditions. In fact, this configuration would be easy to use in a real application environment in which a lateral flow test could be wetted with a single fluid (generally the fluid under analysis).

In this study, the paper-based microfluidic urea fuel cell was evaluated with a solution containing 0.3M of KOH and 0.33M urea to simulate the fluid under analysis. The fuel cell tests were performed using a micropipette with 25 µL for the urea solution. The voltage and current were measured using a Zahner Zenium potentiostat/galvanostat. The reported current and power densities were calculated according to the planar geometric area of the electrodes exposed to the microchannel (0.25 cm²). In order to observe the lifetime of the bacteria with respect to the power generated the P-based µUFC was evaluated for 20 consecutive days at room temperature. In all the cases, a set of polarisation and power density curves were created, and the one that best described the performance was shown for each test.

3. Results and discussions

3.1. Physicochemical characterization of Escherichia coli

SEM micrograph of Escherichia coli on Toray® carbon paper electrode is shown in Figure 2; where is observed that a cluster of bacteria is present over the surface of the electrode, which indicates that, through lyophilisation, E. Coli bacteria is deposited on the carbon-based surface. The Escherichia coli bacteria were observed as straight bacillus with a size of 0.5 to 2 µm. In addition, according to Fig. 2b, saline solution used in the bacteria adaptation process is also deposited on the structure of Toray® carbon paper electrode.

This suggests that the lyophilisation as drying method could create electrodes that on its surface do not contain only bacteria, however, this salt could be convenient as a means of adaptation when the bacteria hydrate again.

![Figure 2. SEM images of Escherichia coli bacteria lyophilized on Toray® carbon paper electrode.](image)

3.2. Performance of the paper-based microfluidic urea fuel cell

When the urea solution was collocated on the paper strip immediately was absorbed by capillary action and make contact with electrodes creating the polarization curve shown in Figure 3. The open circuit voltage (OCV) of the fuel cell was 0.83 V. The maximum current density and power density that is delivered by the P-based µUFC were 3.253 mA cm⁻² and 0.608 mW cm⁻², respectively.
Figure 3. Polarization and power density curves for the paper-based microfluidic urea fuel cell with E. coli anode and Pt/C cathode in 0.33 M urea at 25 °C.

These values are higher than reported by others paper-based microbial fuel cells [11, 12]. Nevertheless, to observe the lifetime of the bacteria with respect to the power generated, the P-based μUFC was evaluated for 20 consecutive days at room temperature.

Figure 4 shows the evolution of the P-based μUFC output power density at the prolonged use of 20 days. The curve shows that in the start process, the power density of 0.60 mW cm⁻² that was increment with respect to time, resulting in maximum power density after 8 days of operation of 1.58 mW cm⁻². Two days later (in the 10-day) is observed a large decrease in the power density response. A prolonged use of 20 days causes a reduction in the power density of 66% respect to initial value. Is interesting to observe that power density vs days curve has the same tendency as a growth curve of bacteria: adaptation, exponential, stationary and cell death phases are presented.

Figure 4. Paper-based microfluidic urea fuel cell power density stability after 20 days

The same behavior described in Figure 4 can also observed with the voltage respect to the days of measurements for the P-based μUFC in Figure 5. All the stages mentioned above are observed. The
initial OCV in the day 1 was 0.83 V; after 0.9 V, the OCV increased in the range of 1.06 V in the day 6 to day 16, finally rebounded to its initial value (0.84 V) over 19 day.

![Paper-based microfluidic urea fuel cell voltage stability after 20 days.](image)

Figure 5. Paper-based microfluidic urea fuel cell voltage stability after 20 days.

4. Conclusions

The lyophilization of bacteria resulted as an option for their deposition in Toray paper electrode and its subsequent use as an electrode in the oxidation of urea. The E. coli bacteria represents an organic catalyst option, showing excellent results in voltage, current density, and power density. In addition, the bacterium showed an excellent behavior when it was evaluated as an anode in a paper-based fuel cell for 20 consecutive days in the oxidation of urea. Finally, this work represents an advance in the possible use of physiological fluids such as urine to generate electrical energy in non-implantable medical devices.

Acknowledgments

Authors want to thank to CONACYT and CONCYTEQ through projects: Cátedra CONACyT project 513 and Nuevos Talentes 2018 CONCYTEQ, respectively.

References

[1] Torres N, Santander J, Esquivel J P, Sabaté N, Figueras E, Ivanov P, Fonseca L, Gràcia I and Cané C 2008 Sens actuators B 132 540-544
[2] Verjulio R W, Santander J, Sabaté N, Esquivel J P, Torres-Herrero N, Habrioux A and Alonso-Vante N 2014 Int J Hydrogen Energy 39 5406-5413
[3] Morales-Acosta D, Rodríguez GH, Godínez L A and Arriaga L G 2010 J Power Sources 195 1862-1865
[4] Esquivel J P, Del Campo F J, Gómez de la Fuente J L, Rojas S and Sabaté N 2014 Energy Environ Sci 7 1744-1749
[5] Dector A, Galindo-de-la-Rosa J, Amaya-Cruz D M, Ortiz-Verdín A, Guerra-Balcázar M, Olivares-Ramírez J M, Arriaga L G and Ledesma-Garcia J 2017 Int J Hydrogen Energy 42 27979-27986
[6] González-Guerrero M J, Javier del Campo F, Esquivel J P, Leech D and Sabaté N 2017 Biosens Bioelectron 90 475-480
[7] Lan R, Tao S and Irvine J T S 2010 A direct urea fuel cell - power from fertiliser and waste Energy Environment Science 3 438–441
[8] Rollinson A N, Jones J, Dupont V and Twigg M V 2011 Energy Environment Science 4 1216–1224
[9] Yan W, Wang D and Botte G G 2012 Applied Catalys B: Environmental 127 221–226
[10] Wang D, Yan W, Vijapur S H and Botte G G 2012 J Power Sources 217 498–502
[11] Mohammad M M and Soheila Y 2016 Biosens Bioelectron 79 327–333
[12] Fraiwan A, Mukherjee S, Sundermier S, Lee H S and Choi S 2013 Biosens Bioelectron 49 410–414