Generation of Voltage by means of Microbial Fuel Cells using the bacteriae *Actinomycetes, Bacillus sp* and *Escherichia coli.*

Rojas Flores, Segundo
Grupo de Investigación en Ciencias Aplicadas y Nuevas Tecnologías
Universidad Privada del Norte
Trujillo 13007, Perú
segundo.rojas.89@gmail.com

Rodríguez Yupanqui- Magda, De La Cruz Noriega- Magaly, Agüero Quiñones-Rickelmi, Enríquez León-Martin, Contreras Cáceda-Cynthia
Department of Environmental Engineering
Universidad Privada César Vallejo
Víctor Larco Herrera, Trujillo, Perú
maguii623@gmail.com, rickelmi2001@gmail.com, martin1258_@hotmail.com, criscaconca09@gmail.com, mrodriguez@ucvedu.pe

Abstract

The energy problems of countries and global warming is one of the most necessary difficulties to be solved by society. In this research, microbial fuel cells (MFCs), a new way of generating voltage using as fuel the bacteria *Actinomycetes, Bacillus sp* and *Escherichia coli.* The bacterium *Bacillus sp* showed higher voltage generation with a peak of 0.65 volts, during the five days of data collection; the pH and conductivity parameters of this bacterium were 7 and 24 mS/cm respectively. Due to the voltage generated from this bacterium the pH was adjusted to 4, 7 and 10 to observe the influence that this parameter has on the voltage produced during twelve days. The pH 7 was the one that showed the highest voltage during the whole sampling. The pH of these cells remained constant as well as their conductivity which was 41.7, 24 and 52.6 mS / cm for the cell of pH 4, 7 and 10 respectively; the data were taken at room temperature (~21°C).

Keywords

Bacteria, bioelectricity, microbial fuel cell, *Actinomycetes, Bacillus sp* and *Escherichia coli.*

1. Introduction

Energy demand worldwide is increasing, and even more so in rural areas. Therefore, in order to meet these needs, the use of both non-renewable and renewable energy sources, which are environmentally friendly, has become a necessity. On the other hand, the problem of climate change is being driven by the release of greenhouse gases such as CO_2, in the use of some types of energy. Due to this problem, it is not possible to continue generating this type of electricity in this way, that is where the environmental challenge facing humanity comes in. For this, there are renewable energies (wind, solar, biomass), all of them can meet our future energy needs, but are not used in our country. While a microbial fuel cell (MFC) is a bioelectrochemical system that generates electrical energy from the interaction of microorganisms in different aqueous media dissolved by oxidation of organic matter (biomass). An MFC comprises a cathode, an anode and almost always a cationic or protonic exchange membrane accompanied by an electrical circuit; the fuels can be glucose, acetate, lactose among other components that can be used, as long as they have a biodegradability factor that can be used by different microorganisms. This type of technology is currently used in energy production and wastewater treatment.

Ibáñez (2010) in his research "Wastewater treatment and simultaneous generation of energy electrical using microbial fuel cells", could confirm that in the manure of some animals such as pigs, chickens and cows, as well as in river muds there are microorganisms that, when degraded in organic matter present in the wastewater, produce small electrical currents. In the first phases of the research they obtained currents around the microamperes, the prototypes presented high values of internal resistances of the order of 76Ω and 76Ω, which meant losses of voltage in the cell; with the prototype of the phase 3 the internal resistance was reduced to 10kΩ, this reduced the losses and increased the power in the cell; with the prototype of the phase 4 the best electrical results were obtained, the internal resistance was reduced to 111,76Ω and the maximum current delivered by the cell was of 1.8 mA. Buitrón and Pérez (2011) conducted a study entitled "Electricity Production in Microbial Fuel Cells Using Waste Water: Effect of Distance Between Electrodes," which evaluated the influence of electrode separation on electricity production and organic matter disposal in microbial fuel cells using wastewater. For this purpose, three cells of similar geometry, but with different volumes were built. On average, an organic matter removal efficiency of 71% was achieved. The increase in the distance between the electrodes (4, 8 and 12 cm) did not cause any negative effect on the generation of electricity, since in the largest separation (120 mL cell) a maximum voltage of 660 mV was obtained, while for the 40 and 80 mL cells it was 540 mV and 532 mV respectively. The maximum power density was present in the cell with a separation of 12 cm (408 mW/m^2).

The purpose of this research is to demonstrate the performance of bacteria inside microbial fuel cells to obtain voltage, to show that bacteria are the best alternatives to produce energy in a profitable, ecological and economic way for the society, and to contribute to the care for the environment.

2. Materials and Methods

2.1. Construction of the microbial fuel cell: The MFCs were manufactured with tubes (PVC) of 5 and 10 cm in diameter and length respectively; they were coated by an acrylic box of 25 cm2 area and 10 cm long. Three holes were made, two of which (at the ends) served to get copper wires out of the MFC to the outside, on one side, the wires were attached inside the cell to zinc and copper electrodes (anode and cathode) 5 cm in diameter and 2 mm thick. In the cells covers, 4 inlet and outlet holes were made for the entry of screws of 4 and 110 mm in diameter and length respectively, to allow a better adjustment of the cell, as shown in Figure 1.

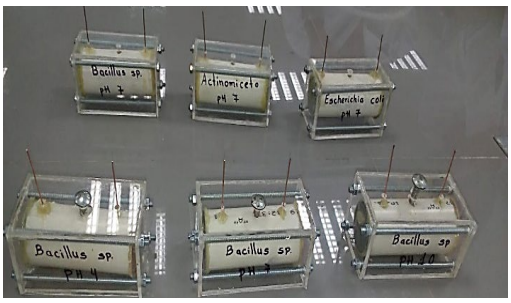

Figure 1. Prototypes of the six Microbial Fuel Cells.

2.2. Isolation of bacteria

2.2.1 Isolation and identification of *Bacillus sp*

2.2.2.1 solation of *Bacillus sp*: A suspension of the culture soil was prepared in sterile physiological saline solution s.p.s.s (1g / 10 ml) was subjected to a heat treatment at 80° C for 10 minutes. Subsequently, 0.1 ml of the last 10-5 dilution in Petri dishes containing Nutritive Agar was surface plated. The plates were incubated at 37°C for 24 h. After the incubation time, the colonies, which according to their macroscopic morphology corresponded to the genus Bacillus, were isolated in Nutritive Agar (Vos et al 2011).

2.2.2.2 Identification of Bacillus sp: The identification of the genus Bacillus sp. was carried out from a 24-hour incubation culture through the Gram staining technique, to verify the

bacillary form with characteristic spore and the positive catalase test, were classified as belonging to the genus *Bacillus sp*.

2.2.2 Isolation and identification of Actynomycetes

2.2.2.1 Isolation of Actinomycetes: A suspension of culture soil sample was prepared in s.p.s.s. 1g /10 ml in a test tube. Dilutions up to 10-6 were made in a battery of tubes with 9ml of s.p.s.s. Of the last three dilutions 100 uL were taken with a micropipette and poured into plates with soy tripticasa agar (STA). From there, it was spread in 3 plates with a Drigalsky aza. It was incubated at 30°C for 24 to 72 hours until the appearance of colonies.

2.2.2.2 Identification of Actynomycetes: It was carried out from a culture of 24 hours of incubation through the Gram staining technique, with the result being long gram-positive Bacilli in the form of hyphae.

2.2.3 Isolation and identification of *Escherichia coli*

2.2.3.1 Isolation of *Escherichia coli*: Sowing was carried out by exhaustion of stria of a sample of residual water, in a selective MacConkey Agar medium, it was incubated at 35 ° for 24 hours; after the time lapsed, positive red colonies were observed. The colonies, which according to their macroscopic morphology corresponded to the Escherichia genus, were isolated in Nutritive Agar.

2.2.3.2 Identification of *Escherichia coli*: A 24-hour incubation culture was performed using the Gram staining technique to determine the form of gram-negative bacilli. From these colonies a presumptive biochemistry was performed in TSI and LIA media, incubated at 35° for 24 hours, after the time lapsed, the reading was made: TSI (A/A) LIA gas (K/K).

2.2.4 Preparation of the inoculum of *Bacillus sp* in Brain Heart Infusion broth (BHI)

The culture of Bacillus sp was reactivated in test tubes containing broth (BHI), incubated at 37°C during 24 hours, pH 7. From the pure culture of *Bacillus sp,* suspensions were prepared in sterile distilled water (SDW) at a concentration of 108 cfu/ml. A BHI broth was also prepared at pH 10, which was adjusted with NaOH to 2%, and at Ph 4 37% HCl was added. They were incubated at 37° for 24 hours.

2.2.5 Incorporation of the cultures *Bacillus sp, Escherichia coli*, and *Actinomiceto* in broth BHI to the Microbial Cells:

We incorporated 100 ml of broth BHI that contained each strain respectively, and we used 3 sterile syringes of 10 ml, for each bacterium (*Bacillus sp, Escherichia coli* and *Actinomiceto*) and other 3 syringes of 10 ml, which served for our bacterium *Bacillius* with pH 4.7 y 10.

2.3 Characterization

The voltage of the microbial fuel cells was measured by a multimeter (Prasek Premium PR-85) daily for a period of 5 days, for 30 minutes daily, then proceeded to measure the MFC of the bacterium *Bacillus sp* varying its pH due to the higher voltage it showed in the first days. Changes in conductivity (conductivity meter CD-4301) and pH (pH-meter 110Series Oakton) were also monitored. The data were taken over a 12-day period.

3. Results and Discussion

Figure 2 (a) shows colonies 2mm in diameter, irregular edges of creamy consistency, and in the microphotography (figure 2 (b)) positive Gram cocci with formation of endospores by means of Gram Staining, characteristics of the genus *Bacillus sp* (Mandic-Mulec et al 2016). Figure 3 (a) shows small, soft, white colonies, irregular edges, in 4 or 5 days and in the microphotography gram-positive filamentous bacteria are observed by means of Gram Staining, characteristics of the genus *Actinomyceto* (Part, 2012). For the *Escherichia coli* bacterium in figure 3

(a) the growth of red lactose fermenting colonies in Mac Conkey Agar is observed after 24 hours at 37° C and from those colonies a biochemistry was performed where *Escherichia coli* in TSI presents a yellow beak and yellow base (A/A), producing gas in the test tube and LIA when the tube presents a lilac beak and background (K/K) without production of hydrogen sulfide. (Khan et al ,2011).

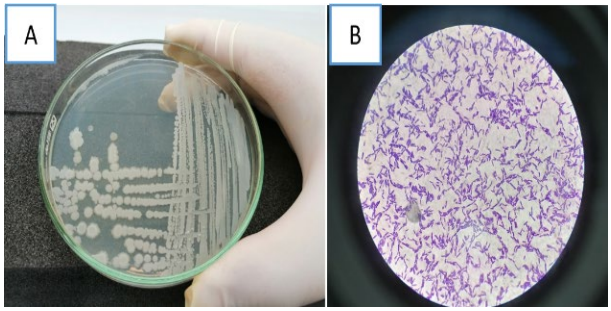

Figure 2. (a)Growth of *Bacillus sp* in Nutritional Agar at 37°C for 24 hours and (b) microscopic growth of *Bacillus sp* by Gram Staining.

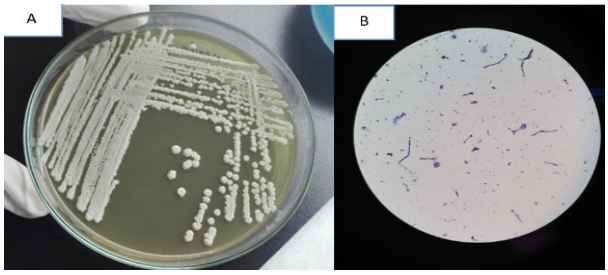

Figure 3. (a) Growth of *Actinomyceto* in Agar Soy Tripticasa at 30°C for 72 hours and (b) microscopic of *Actinomyceto* by Gram Staining.

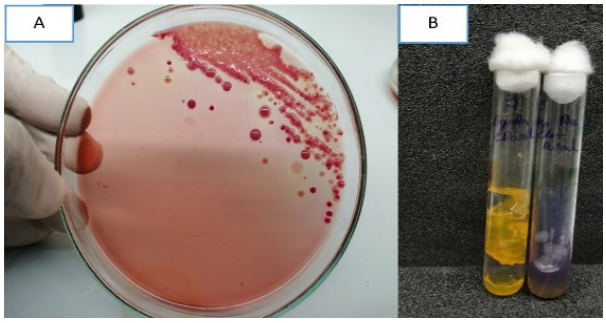

Figure 4. (a) Growth of *Escherichia coli* in Mac Conkey Agar at 35°C for 24 hours and (b) growth of *Escherichia coli* in TSI and LIA Agar.

The yields of the MFCs with the three bacteria were measured during five days (figure 5 (a)), in which it can be observed that the *Escherichia coli* bacteria begins to decrease its voltage compared to the other two, from 0.64 to 0.29 volts. The bacteria *Actinomiceto* decreases slightly between 0.51 to 0.46 volts. This may be because bacterial cells can easily detach from the outer layer of the biofilm created at the liquid-phase anode of the substrate. During this event, the device loses bacterial cells as a biocatalyst at the anode, decreasing the voltage. (Mohammadifar, M., & Choi, S.; 2019), or that some organic substrate compounds are used for bacterial growth and not for electricity generation (Sharma and Li, 2010). While the *Bacillus sp* the voltage increases through time, being its maximum peak in the fifth approximately 0.73 volts; this would be due to the fact that this genus are electrotrophs that can accept electrons from the surface of the cathode in a direct or mediated way (Rittmann, 2006), reason why gram-negative and gram-positive bacteria such as *Micrococcus luteus, Bacillus subtilis and Staphylococcus carnosus* stand out (Huang et al., 2011). In figure 5 (b) the initial pH values were adjusted to 7 for the three bacteria, in the five days small variations between 6.5 and 7.5 were observed. The conductivity of the bacteria

Actinomycetes, Bacillus sp and *Escherichia coli* can be observed in figure 5 (c) which were 41.7, 24 and 52.6 mS/cm respectively, remaining constant during all days.

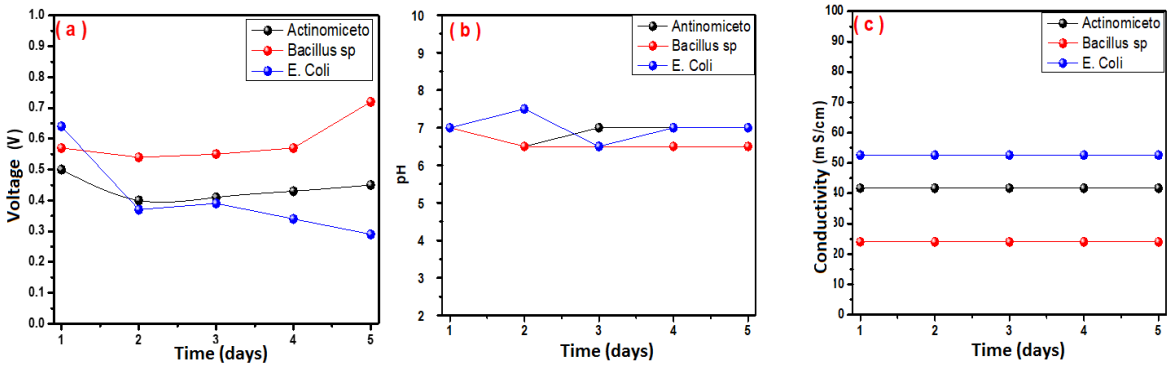

Figure 5. Obtaining a) Voltage, b) conductivity and c) pH in 5 days of the MFC with three different bacteria (*Actinomycetes, Bacillus sp* and *Escherichia coli*).

The initial voltage generation for *Baccillus sp* bacteria with different pHs (4, 7 and 10) is shown in figure 06, in which it is observed that *Bacillus sp* with pH 7 shows an initial voltage of 0.65; this being the highest generation in comparison with the other two that were 0.52 and 0.06 volts for pH 4 and 10 respectively. Although after 12 days the voltage decreased of the MFC with pH 7 always showed greater voltage generation (figure 7 (a)) in comparison with the other two. The decrease in voltage over the days may be due to the decrease in nutrients. (Barua, E et al. 2018). Figure 7 (b) shows the monitoring of pH values during all 12 days, in which small fluctuations can be observed, but stabilizing again in the last three days of data collection. While the conductivity values of the MFCs (figure 7 (c)) remain constant during the 12 days at 23.8, 24.01 and 24.8 mS/cm for pH 4, 7 and 10 respectively.

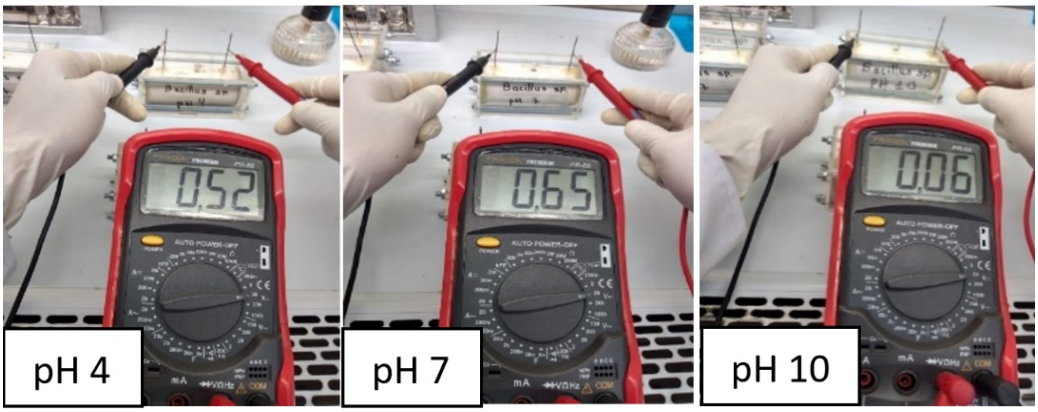

Figure 6. Initial voltage values of MFCs with different pH of bacterium *Bacillus sp*.

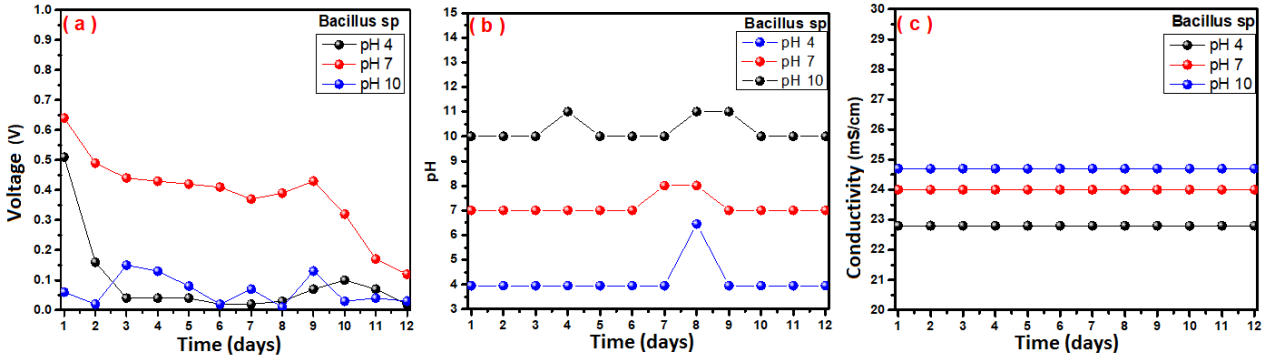

Figure 7. Obtaining a) Voltage, b) conductivity and c) pH for 12 days of the MFC of bacterium *Bacillus sp* with different pH (4, 7 and 10).

4. Conclusions

In this research, low-cost, agar-based microbial fuel cell type biobattery prototypes were created. The bacteria used as substrates (fuel) were the *Actinomicetros, Bacillus sp* and *Escherichia coli*. The Bacillus sp was the one that showed greater voltage in 5 days with a maximum peak of 0.73 volts. The pH and conductivity values for this bacterium were 6.5 and 24 mS/cm respectively. In the constructed MFCs the pH of the bacterium *Bacillus sp* was varied because this was the one that showed the highest voltage in the first 5 days. The MFC with pH 7 showed the highest voltage generation in 12 days, which reached a peak of 0.65 volts on the first day. pH and conductivity (24.01 mS/cm) remained constant throughout this period.

5. References

Agurto, Tomás. 2009.Microbiología: Bioquímica Bacteriana. Lima: Ed Imprenta Unión.

Barua, E., Hossain, M. S., Shaha, M., Islam, E., Zohora, F. T., Protity, A. T., ... & Hashem, A. (2018). Generation of Electricity Using Microbial Fuel Cell (MFC) from Sludge. Bangladesh Journal of Microbiology, 35(1), 23-26.

C. Harwood. 1989."Biotechnology Handbooks 2: Bacillus. Eds Plinum Press, New York

Huang, L., Regan, J. M., & Quan, X. (2011). Electron transfer mechanisms, new applications, and performance of biocathode microbial fuel cells. *Bioresource Technology*, *102*(1), 316-323.

Khan, F., Rizvi, M., Shukla, I., & Malik, A. (2011). A novel approach for identification of members of Enterobacteriaceae isolated from clinical samples. *Biol Med*, *3*(2), 313-319.

Mandic-Mulec, I., Stefanic, P., & van Elsas, J. D. (2016). Ecology of bacillaceae. In The Bacterial Spore: from Molecules to Systems (pp. 59-85). American Society of Microbiology.

Mandic-Mulec, I., Stefanic, P., & van Elsas, J. D. (2016). Ecology of bacillaceae. In *The Bacterial Spore: from Molecules to Systems* (pp. 59-85). American Society of Microbiology.

Mohammadifar, M., & Choi, S. (2019). A solid phase bacteria-powered biobattery for low-power, low-cost, internet of Disposable Things. Journal of Power Sources, 429, 105-110.

Parte, A. (2012). *Bergey's manual of systematic bacteriology: Volume 5: The actinobacteria*. Springer Science & Business Media

. Doi, M. Mc Gloughlin, M .1999 "Biology of Baci li: Aplications to Industry". Eds. Roy H. Doi Hir eman, USA.

Rittmann, B. E. (2006). Microbial ecology to manage processes in environmental biotechnology. *TRENDS in Biotechnology*, *24*(6), 261-266.

Sharma, V., & Kundu, P. P. (2010). Biocatalysts in microbial fuel cells. *Enzyme and Microbial Technology*, *47*(5), 179-188.

Vos P, Garrity G, Jones D, Krieg N, Ludwig W, Rainey F et al. 2009 . Bergey's Manual of Systematic Bacteriology. Vol. 3. 2 a ed. Atenas: Springer;

Vos, P., Garrity, G., Jones, D., Krieg, N. R., Ludwig, W., Rainey, F. A., ... & Whitman, W. B. (Eds.). (2011). *Bergey's manual of systematic bacteriology: Volume 3: The Firmicutes* (Vol. 3). Springer Science & Business Media.