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Bioelectricity generation through Microbial Fuel Cells using *Serratia fonticola* bacteria and *Rhodotorula glutinis* yeast

F. Silva-Palacios[a], A. Salvador-Salinas[a], M.A. Quezada-Alvarez[b], M. Rodriguez-Yupanqui[a], Rojas-Flores Segundo[c,*], Nazario-Naveda Renny[c], Luis Cabanillas-Chirinos[d]

[a] *Escuela de Ingeniería Ambiental, Facultad de Ingeniería, Universidad Cesar Vallejo, Trujillo, 13007, Peru*
[b] *Departamento de Ingeniería Ambiental. Universidad Nacional de Trujillo, Trujillo, 13011, Peru*
[c] *Vicerrectorado de Investigación, Universidad Autónoma del Perú, Lima 15842, Peru*
[d] *Instituto de Investigación en Ciencias y Tecnologia, Universidad Cesar Vallejo, Trujillo 13001, Peru*

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Abstract

Nowadays, there is great interest in microbial fuel cells because of the different substrates that can be used in them for electric energy generation. In order to find an alternative and contribute with eco-friendly technologies, this research used the *Serratia fonticola* bacteria and *Rhotula glutinis* yeast as a fuel source through laboratory scale microbial fuel cells. A single chamber microbial fuel cell with air cathode was fabricated with a copper foil and a graphite plate as anode and cathode electrode respectively. For the characterization of the cells, physicochemical parameters such as voltage, electric current, pH and electrical conductivity were measured for 30 days and at room temperature (18 ± 2.2 °C). It was possible to generate peak voltage and current values of 0.53 ± 0.01 V and 0.55 ± 0.02 V and current values of 1.76 ± 0.16 mA and 1.52 ± 0.02 mA, for MFCs with bacteria and yeast respectively. In addition, acidic operating pH was observed, and its conductivity peak values around 242 mS/cm. Finally, this work demonstrates the great potential that microorganisms have for the generation of electric current, giving a new and promising way to generate electricity

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Keywords: Bioelectricity; Electric current; Bacteria; Yeast; Microbial fuel cells

1. Introduction

The undeniable growth of the need for energy production and its negative impact on the environment due to the use of fossil fuels has led in recent decades to a growing interest in the use of alternative sources, such as microbial fuel cells (MFCs). The function of this technology consists in the conversion of chemical energy into

* Corresponding author.
E-mail address: segundo.rojas.89@gmail.com (R.-F. Segundo).

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electricity through redox reactions catalyzed by microorganisms [1]. So, the operation of the MFCs is affected by the materials used for the construction of the system, structure, microorganisms present, substrate and environmental conditions [2]. In obtaining energy, the electrodes drive the development of the potential difference that acts as a net propelling force in the transfer of electrons from the anode to the cathode and in such a way cause reducing equivalents such as electrons in the form of redox carriers [3].

Electroactive microorganisms possess the ability to donate (electrogenic) or accept (electrotrophic) electrons from a substrate. Electrogenic microorganisms release electrons on the anode surface, being represented and quantifiable as a positive electric current, however, electrotrophic microorganisms are in charge of recovering these electrons from the cathode surface [4]. Extracellular electron transfer occurs through two mechanisms [5]. The first is direct electron transfer, where the electrogene accepts an electron donor for oxidative metabolism [4]. During this process, the microorganisms carry out the transfer of electrons through the cell membrane that they possess; that assists the cytoplasm to carry out the transfer of electrons to the outer membrane of the cell and then to form biofilms on the appliance [4]. These mechanisms will vary in each species of microorganism, due to the development and characteristics of ease of adaptation to the environment in which they are found. Among them are *Geobacter sulfurreducens*, *Aeromonas hydrophilia*, *Clostridium sp. Arcobacter sp. Rhosofoferax sp., Orchrobactrum sp. and Shewanella putrefaciens* [6]. And the second mechanism is the indirect or mediated transfer of electrons, where the microorganism receives the electron from inside the cell membrane, reducing and transferring the electrons to the anode [3]. This mechanism has two categories, which are mediators by redox shuttles and mediators by extracellular enzymes secreted by other microorganisms that generate diffusible chemical substances [7].

The transfer with mediators by redox shuttles proceeds as electron carriers, including flavins, phenazines and soluble menaquinone, linking the reactions inside and outside the cell; however, they are more effective depending on the distance between electrodes and the richness of organic matter in the substrate [8]. As an example of this, there are the gram-positive bacteria *Listeria monocytogenes* and the gram-negative bacteria such as Pseudomonas, *Shewanella* and *Serratia sp.* which have peritrichous flagella that through their movement fulfill the function of transporting electrons [6]. The *Bacillus sp*. *Chlorobium limicola, Rhodobacter sphaeroides, Rhodopseudomonas palustris, Chlamydomonas reinhardtii* and *Escherichia coli* are also exampling of this [4]. In addition, the species *Enterobacter cloacae*, *Geothrix fermentans, Micrococcus luteus, Proteus vulgaris, Shewanella japonica, Shewanella loihica and Shewanella oneidensi* have both electron transfer mechanisms [9]. On the other hand, yeasts are particular and special due to their rapid unicellular growth and at the same time their adaptation to growing in liquid media [10], such as *Rhodotorula mucilaginosa, Rhodotorula slooffiae, Rhodotorula glutinis, Rhodosporium babievae*, among others that according to studies affirm that the wall cells of these are capable of binding significant amounts of Calcium (Ca^{2+}) in addition to a wide range of heavy metal ions that are accumulated in their system; likewise, yeast biomass is capable of absorbing considerable amounts of cations and other chemical elements in the growth medium [11].

Based on the above, this work evaluates the use of the *Serratia fonticola* bacteria and *Rhodotorula glutinis* yeast for the generation of electrical energy through single-chamber microbial fuel cells, to which the values of conductivity, pH, voltage and current were monitored for 30 days. This research will give potential use of bacteria and yeast for future applications as aggregates in MFCs to increase their electrical values in an environmentally friendly way.

2. Materials and methods

2.1. Microbial fuel cells fabrication

For this investigation, the MFCs were designed with a single air cathode chamber and electrodes (anode and cathode). For the construction of the MFCs, a prototype of transparent borosilicate glass of 100 ml capacity was used. The anode consisted of a 3 cm copper plate. wide and 5.50 cm long and the cathode of a graphite plate of 1 cm radius *x* 2 cm length. Each electrode was connected to a copper wire 0.5 mm thick at the ends, previously sanded to remove the plastic protection. 8 single-chamber MFCs with air cathode were fabricated, which were subsequently sterilized under a UV light lamp in a biosafety cabinet (JSR Biological Safety Level Class II Type A2) for a period of 20 min [12].

2.2. Isolation and identification of microorganisms

Microorganisms were isolated by the swab method [13] from a sample of mine tailings obtained from Santa Catalina tailings dam in the town of Shorey, Quiruvilca District, Santiago de Chuco Province, in the La Libertad Region, at an altitude of 3,818 meters above sea level, on the slopes of the eastern flank of the Western Andes mountain range, the source area of the River Moche. Cultures were carried out in BHI broth and minimal medium salt broth (MMS), which contained 6 g/L of $Na_2(HPO_4)$, 3 g/L. KH_2PO_4, 1 gr/L NH_4Cl, 0.5 gr/L NaCl, 0.246 gr/L $MgSO_4.7H_2O$, 0.01 gr/L $CaCl_2$ and 0.5% glucose [14] and they were incubated at 33 °C for 24 h. Subsequently, the sowing was carried out by means of the method of striation in Petri dishes on a plate with MMS Agar for the isolation of bacteria and 2% modified Sabouraud Agar, which contained in its composition 10 gr/L of peptone, 20 gr/L of glucose and 20 gr/L of agar and the modification lay in the addition of 4% MMS [15] for the isolation of fungi and yeasts. The incubation period was 24 h at 33 °C.

Once the microorganisms were isolated, Gram staining and methylene blue were performed for identification through microscopic morphological characteristics. Consequently, axenic or pure cultures were identified by means of the VITEK 2 compact 15 automated system. It was possible to identified species: *Serratia fonticola* bacteria and *Rhodotorula glutinis* yeast. The microorganisms isolated are shown in Fig. 1, it can be evidenced that (A) is the *Serratia fonticola* colony on 2% modified Sabouraud agar, (B) is the *Rhodotorula glutinis* colony on 2% modified Sabouraud agar, (C) the microscopic observation of *Serratia fonticola* colony and (D) Microscopic observation of *Rhodotorula glutinis* colony. As Koch [9] points out, species from different phylogenetic groups that include bacteria, archaea, yeasts and algae may be present within the MFCs.

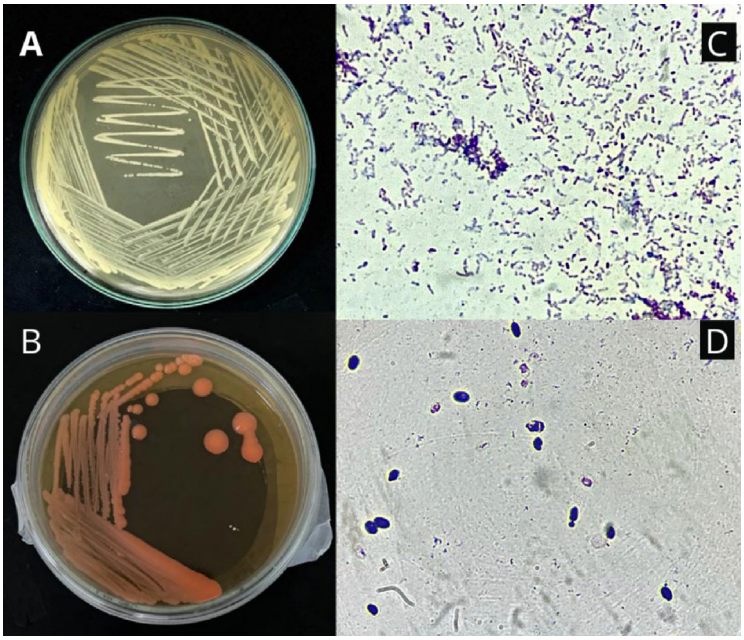

Fig. 1. Microorganisms isolated from mine tailings substrate.

2.3. Inoculation of strain in MFCs for their electrogenic evaluation

The identified strain was inoculated in a CCM containing a minimum salt medium, according to the following composition: 6 gr/L of $Na_2(HPO_4)$, 3 gr/L. KH_2PO_4, 1 gr/L NH_4Cl, 0.5 gr/L NaCl, 0.246 gr/L $MgSO_4.7H_2O$, 0.01 gr/L $CaCl_2$ and 0.5% glucose [14].

2.4. Characterization of microbial fuel cells

Daily monitoring of voltage and electric current was carried out by means of a Prasek Premium PR-85 brand multimeter; pH (pH-meter 110 Series Oakton), electrical conductivity (conductivity meter CD-4301), and resistance using an energy sensor (Vernier ± 30 V & ± 1000 mA) [16].

3. Results and analysis

Fig. 2(a) shows the average values of the generated voltages of MFCs during a period of 30 days. The graph show that the maximum voltage generated by the *Serratia fonticola* bacteria is 0.53 ± 0.01 V from day 4 to 8 and the minimum voltage values recorded were 0.37 ± 0.01 V on days 29 and 30. In the other hand, with the *Rhodotorula glutinis* yeast, values of 0.55 ± 0.02 V were generated on day 4 and minimum voltage values of 0.31 ± 0.04 V were generated on day 30. The voltage losses observed are related to the metabolism of the microorganisms, this decrease is inevitable because the obtaining of energy is given by the oxidation of the substrates [17]. In addition, the *Serratia fonticola* bacteria and the *Rhodotorula glutinis* yeast generated similar values, since they presented the same operating conditions, in addition these are a species of electron donors that are oxidized at the anode, this transfer of electrons generated to the cathode and migration from them to the anode produces a voltage difference and therefore is responsible for the generation of electrical energy [18]. According to the potential of the anode, being the final receptor of electrons, it establishes the energy gain for the bacteria. The greater the difference between the redox potential of the substrate and the anode, the greater the metabolic energy obtained by the bacteria, but the lower it is, the greater the voltage obtained in the MFCs; therefore, the anode potential must be kept lowest as long as possible. However, if the anode potential becomes too low, electron transport will be inhibited and substrate fermentation can provide higher energy for microorganisms [19].

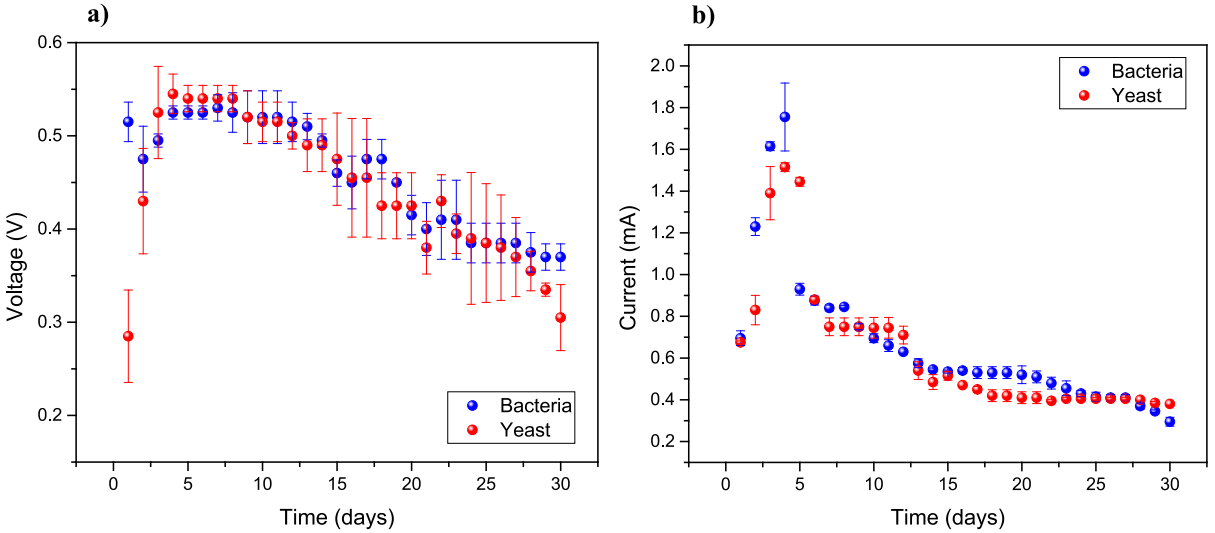

Fig. 2. Values of (a) voltage and (b) current of MFCs.

In the recording of the monitoring of the electric current observed in Fig. 2 (b), the maximum values produced by the *Serratia fonticola* bacteria was 1.76 ± 0.16 mA on the fourth day and with the *Rhodotorula glutinis* yeast, 1.52 ± 0.02 mA generated on the fourth day. Regarding the minimum values reached, a current of 0.30 ± 0.02 mA was achieved on day 30 by the *Serratia fonticola* bacteria, and 0.38 ± 0.00 mA by *Rhodotorula glutinis* yeast, generated on same day. The values obtained in the MFCs with bacteria and yeast generated similar values, since the substrates in both cases was MMS. As Hernandez mentioned, [20] about 95% of electrogenic microorganisms produce pyocyanin, a redox metabolite. This simple or complex intermediate molecule that is formed during various metabolic processes that occur in microbial organisms, is involved in essential metabolic functions. which is directly involved in the development, microbial growth and in the generation of electrical current in microbial fuel cells to configure bioelectrochemical systems.

Another important parameter is the pH, where the values obtained in the monitoring of the MFCs are found in Fig. 3(a). MFCs with *Serratia fonticola* bacteria show a maximum pH of 6.24 ± 0.01 on 3 days consecutively (26, 27 and 28) and the minimum value was 2.98 ± 0.00 on first and second day; while, with *Rhodotorula glutinis* yeast a maximum value of 6.17 ± 0.01 and a minimum of 2.97 ± 0.00 on the first day of data collection were obtained. As observed pH values tend to rise for both samples; microorganisms have an influence on the increase of pH values due to the generation of electrons in oxidation of the substrate [17]. Because, these microorganisms, as a last resort, feed on the dead biomass at the end of the substrate nutrients; as dead microorganisms can accumulate on the anode during operation over time and this resulting dead microbial layer can also provide resistance, limiting power generation [21]. Likewise, Li et al. states that these dead microorganisms generate a layer at the bottom of the biofilm, which promotes the feeding and proliferation of live bacteria in the upper layer, improving the performance of the MFCs and raising the pH value [22].

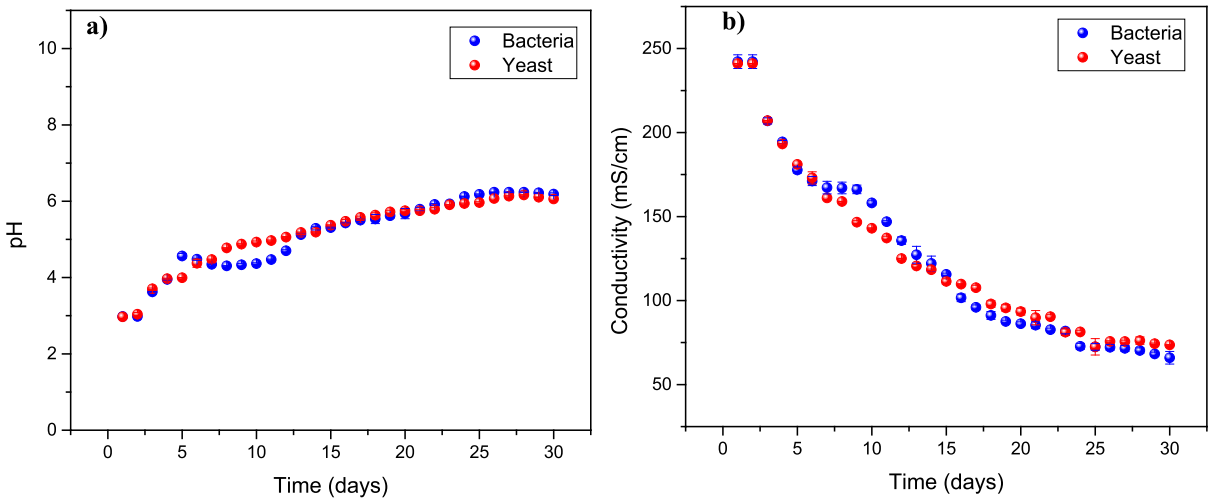

Fig. 3. Monitoring of the values of (a) pH and (b) conductivity of MFCs.

Fig. 3(b) shows the electrical conductivity values of the substrate, achieving the maximum electrical conductivity in the first 2 days of monitoring, 242.20 ± 4.10 mS/cm in the MFCs inoculated with the *Serratia fonticola* bacteria, followed by the *Rhodotorula glutinis* yeast, with values of 241.05 ± 0.07 mS/cm in the first 2 days. On the other hand, the minimum values of, on the last day, 65.95 ± 3.75 mS/cm were recorded in the *Serratia fonticola* bacteria, followed by the *Rhodotorula glutinis* yeast having values of 73.60 ± 0.28 mS/cm on day 30. In MFCs with these microorganisms, the conductivity decreases, which could be due to the reduction of salt ions present in the medium, agreeing with [23], since the most effective substrates for increasing conductivity are glucose, acetate, and wastewater, as possible fuels in the microbial combustion chamber. On the other hand, there is not much information on inorganic substrates, but the authors prefer to use substrates of reduced sulfur and iron thiosulfate ions, where stabilization was achieved using iron and thiosulfate ions to improve conductivity; however, organic substrates are more effective than inorganic ones [24]. Therefore, direct transmission occurs due to the presence of bioelectrically active species of microorganisms in the culture medium; For example, outer membrane cytochromes, droplet conductance, and extracellular secretions play a role in the transfer of electrons between microorganisms and the anolyte electrodes.

Fig. 4 shows the graph that is adjusted by Ohm's law., which can be expressed by the formula $V = RI$, where V is the potential difference or voltage (V), R is the resistance (Ω), and I is the current (mA) [25]. Referring to this formula, the voltage is directly proportional to the current, so a linear function ($y = mx + b$) can be established [26], and from the slope (m) the resistance (Ω) of the MFC can be calculated [27]. This methodology uses polarization curves (graph of electric current (I) against voltage (V)) and is the most widely accepted to analyze the electrical efficiency of MFC [28].

In the MFCs with *Serratia fonticola* bacteria, the resistance is 215.2151 ±11.251 Ω (Fig. 4(a)). While the resistance in the MFCs with *Rhodotorula glutinis* yeast is 198.5621 ±16.325 Ω (Fig. 4(b)). The internal resistance

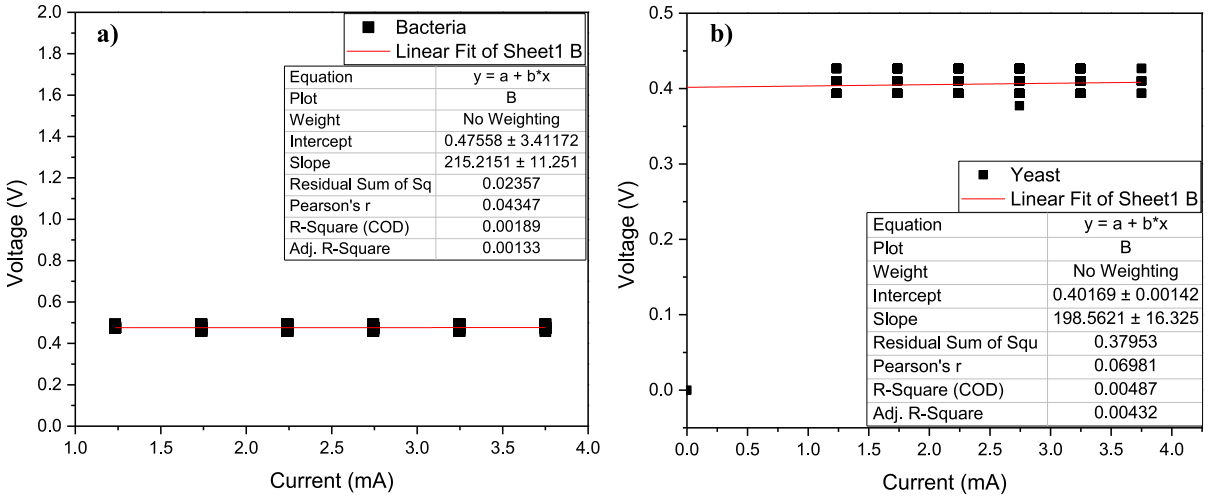

Fig. 4. Internal resistance of (a) pristine and (b) sterile mine tailings substrate MFCs.

of MFCs depends on the decomposition of the substrates used for power generation because the electrons that are released in the oxidation process in the anode chamber flow freely throughout the system when the internal resistance is low [29]. So, the low internal resistance exposed can be affected by the adhesion of the microorganisms present in the substrate with the anode electrode and confirms the high current values shown by the cells [30,31]. In some studies, copper anodes were used, which is a metallic material that has low resistance and good conductivity, allowing the flow of electrons through it [29]; however, despite show good results in the generation of bioelectricity, its main limitation is its corrosion [32].

4. Conclusions

Bioelectricity was successfully generated using *Serratia fonticola* bacteria and *Rhodotorula glutinis* yeast as fuel in laboratory-scale microbial fuel cells, which was operated and monitored for 30 days at room temperature. A maximum voltage values of 0.53 ± 0.01 V and 0.55 ± 0.02 V and current values of 1.76 ± 0.16 mA and 1.52 ± 0.02 mA, were observed for bacteria and yeast respectively. While MFCs operated at an acidic pH, and its conductivity values decreased from about 242 mS/cm. These results confirm the excellent electrical properties of these microorganisms as fuel for electricity generation. The use of proton exchange membranes is recommended for further work to improve the electrochemical properties of the substrates.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Rojas Flores Segundo Jonathan reports financial support was provided by Universidad Autonoma del Peru. Rojas Flores Segundo Jonathan reports a relationship with Autonomous University of Peru that includes: employment. Rojas Flores Segundo Jonathan has patent pending to Licensee. This manuscript has no conflicts of interest.

Data availability

No data was used for the research described in the article.

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