Environmental Biosensor Potential of Microbial Fuel Cells for Nitrate Reduction

Alex Guambo, Silvia Paña, Cristina Calderón, Magdy Echeverría and Celso Recalde

Centro de Investigación de Energías Alternativas y Ambiente, Escuela Superior Politécnica de Chimborazo Riobamba (ESPOCH), Panamericana Sur Km 1 ½, Chimborazo EC060155, Ecuador
Tel.: + 593 992861566, fax: (03) 2317-001
E-mail: alexfernag@gmail.com

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Abstract: The present study focuses on the production of bioelectricity through the use of microbial fuel cells (SMFC) using a pure culture of Pseudomonas aeruginosa extracted from Andean soils at 3850 m above sea level. This biosensor has been developed from cell systems of bioelectrochemical microbial fuels that are used for the control of toxic compounds such as nitrate. In the study two treatments were applied each with different concentration of NO3 - which were examined individually by microbial fuel cells in batches built from a single chamber partially exposed to air, at the end of the complete investigation of the analyzes presented the nitrate in each of the cells.

According to the results obtained from bioelectricity. The bacterial culture of Pseudomonas spp. generates a considerable production of bioelectricity, a concentration of 30 mg/L in the microbial fuel cell 1 (SMFC1) produced 0.083 mV, and at 60 mg/L in the microbial fuel cell 2 (SMFC2) produced 0.109 mV, on average per day. This production increases proportionally if nitrate is added to the cells. With these findings, this system is proposed as a potential biosensor.

Keywords: SMFC, Inorganic Compounds, Nitrates, Biosensor, Pseudomonas spp.

1. Introduction

In recent years the new technology and its development allows to exercise a control of the environmental quality, the need to have systems of detection, analysis and rapid controls that allow to reach high levels of specificity and sensitivity with the purpose of detecting the presence of contaminants with different physical and chemical characteristics present in increasingly lower concentrations [1] in different sectors, make it an environmental challenge that uses the evolution of electronics, telecommunications and the possibility of miniaturization of technologies make possible the development and implementation of New detection systems such as environmental biosensors as an analytical system linking a sensitive biological element with a transducer to obtain a fast, accurate and sensitive detection of individual or combined substances.

The biosensors have been implemented in environmental safety systems in two ways [2]. The first as a monitoring method that predicts a possible biological effect, such as toxicity, and the second a screening method that serves as an alert for the presence of a pollutant compound by measuring them in a short time [2].
The biosensors that use complete cells are an important option, due to the non-use of enzymes, allow the detection of large number of chemical compounds within temperature and pH intervals greater for their versatility can compromise the selectivity of the compound to detect, Which is solved by the use of molecular techniques to make detection specific [3].

Microbial Fuel Cells (MFCs) are devices that can use bacterial metabolism to produce an electrical current from a wide range of organic substrates. Many groups of bacteria have been experimented in MFC and they were found to be electrochemically active in the anode region. Biosensors have been developed based on bioelectrochemical systems MFCs are used for the monitoring of toxic compounds, bacteria show a low metabolic activity when they are inhibited by toxic compounds, which causes a low transfer of electrons towards the electrode, this response being the Base to construct a biosensor immobilizing a bacterium in the electrode of a MFCs and protecting it behind a membrane.

Today, there is a growing global energy expenditure due to accelerated population growth. In addition to a large exploitation of fossil resources and environmental damage that causes its use.

For this reason there are several studies based on the topic of energy production, using microbial fuel cells, or MFCs.

These cells have several applications, the sustainable production of electrical energy from biodegradable compounds, the industrial biosynthesis of compounds interest at low cost, and their main application as biosensor.

Biosensors using microbial systems arise from the combination of a microorganism in intimate contact with a suitable physical transducer, which generates a measurable signal [4].

Studies on this biotechnology indicate that the power density generated by an MFC could not be equal to or better than that of a chemical fuel cell. However, the possibility of decontaminating bodies of water or degrading waste compounds at low cost with the possibility of producing electrical energy in the process without the generation of new pollutants and its use as a biosensor represents a cost-benefit of great importance [5].

Thus, if a toxic compound diffuses through the membrane, it can be measured by the change in the sensor potential, which may be useful as indicators of toxic substances in rivers or at the entrance of water treatment plants [6].

Several bacterial strains have been tested for the production of energy, for example the bacterial genera Geobacteraceae spp. as dominant microorganisms with a high electrogenic activity, have also been studied in Rhodobacter ferrireducens, Aeromonas hydrophila, Clostridium butyricum and Enterococcus gallinarum, in this article we are based on the study of a microbial strain of Andean highlands in the Chimborazo-Ecuador province.

The Andean highlands, contains a significant amount of microorganisms and remains a favorable candidate to provide bacterial strains with possible biotechnological interest. The current study focuses on as environmental biosensor by MFC developed with pure culture of Pseudomonas aeruginosa in the anodic chamber. This will help us in determining the electrochemical activity of the bacterium when it is cultured as pure. There are very few reports in this area as biosensor.

Pseudomonas spp., bacteria gram negative bacilli, motile, and due to the polar flagella that possess are aerobic bacteria strict, do not form spores and morphologically are similar to Enterobacteria, which is a producer of phenazines as Redox mediators giving the capacity to produce bioelectricity [7].

The gender used in this study that belongs to the Pseudomonas can develop and grow in liquid media, observing the formation of a biofilm, which reflects the preference of this microorganism for aerobic conditions. It can degrade glucose oxidatively and convert nitrogen to nitrite or nitrogen gas. The species that belong to the gender Pseudomonas, in general, present fast growth and a great ability to metabolize a wide variety of substrates. In the gender of Pseudomonas you can find some species such as; P. fluorescens, P. putida, P. syringae and P. alcaligenes. P. aeruginosa which are widely distributed in nature [8].

According to certain studies it has been observed that these bacteria produce 310 mV and 20 mV as pure culture, and 450 mV and 40 mA as a crop together with Bacillus tequilensis [9].

2.1. Methodology

The bacteria extracted at Andean in the recharge zone of Lake Mapahuíña (9742946 N, 747817 E) in the Sangay National Park- Ecuador, which belongs to the micro basin of the Zula River, having an oval-oblong morphometry of 281,542 ha of mainly sandy loam soil. The average daily temperature is between 6-12 °C, with a daytime maximum of 15 °C and a night-time minimum of 3 °C recorded. The annual rainfall of the zone is between 700-1000 mm [10]. The life zones in its paramo grassland ecosystem include lower montane dry and wet forests which contribute to its vast ecological diversity [11-12].

Bacteria were isolated from the soil sample, the sampling included taking a portion of soil (200 g, depth: ~20 cm) using a plot (1 × 1 m) from the study area, which had an average altitude of 4130 meters above sea level. Sample integrity was maintained with the use of resalable plastic bags and cold storage during transportation to the laboratory for analysis.

They were isolated by serial dilution method and colonies were taken from dilution 10^6 to 10^-10 based on the colony morphology. The bacteria were then sub cultured many times to obtain pure culture which was maintained in nutrient agar slants for further use.
Four isolates with their respective replica which were taken for the study in Microbial fuel cell was tested in various biochemical tests sequencing was done to identify the genus and species of the bacteria using API 20E test system, and then tested using four culture media: Sim, Simmons Citrato, Kliger and Urea [13]. The bacterial morphology was studied with the help of microscope.

Five cubic microbial fuel cells made of acrylic with a volume of 125 mL, were constructed and open circuit voltage were recorded at the time interval of 1 min for 15 days with a data acquisition system (DAQ NI 6009), at room temperature. The MFCs were inoculated with 2 mL of Pseudomonas aeruginosa culture grown with Nutritive Agar, it is observed in Fig. 1.

Fig. 1. Image of the fabricated SMFC and NI 6009 DAQ processor.

Two concentrations of NO₃⁻ (30 mg L⁻¹, and 60 mg L⁻¹) were examined individually in batch operation mode SCMFCs constructed of single chamber partially air exposed, for duplicated and one control, thereafter, the synthetic waste water made from potassium nitrate was analyzed using a photometer HACH DR 2800™.

The anodes and cathodes were separated with cellophane paper [14]. With internal contact surface area of the electrodes of 5 cm × 5 cm, both electrodes (carbon fiber) were pre-treated prior to usage in the SMFCs in order to improve the biofilm formation and remove impurities.

2.2. Electrochemical Characteristics in SMFCs

Analysis inside the SMFC:

Reagent used for the production of synthetic nitrate waters:

\[ \text{KNO}_3 \rightarrow K^+ + NO_3^- \]

Response:

Anode:

\[ \text{NO}_3^- + 2H^+ \leftrightarrow NO_2 + H_2O \]

\[ E^- = 0.78 \text{V} \]

Catode:

\[ K^+ + 1e \rightarrow K^0 \]

\[ E^- = 2.92 \text{V} \]

\[ E_{SMFC} = E_{CATODE} - E_{ANODE} \]

\[ E_{SMFC} = 2.92 - 0.78 \]

\[ E_{SMFC} = 2.14 \text{V} \]

3. Figures and Tables

In Table 1, all the average values are shown for 15 days per minute that were obtained with the DAQ NI 6009 device, and these were used for the analysis and final representation.

According to the monitored data of each minute, it shows a relation and incidence of nitrate concentrations in each cell, as seen in Fig. 2, the CCM 2 generated the highest voltage production with a total average value of 0.1055.

Table 1. Voltages generated in the SMFC average per day.

<table>
<thead>
<tr>
<th>Days</th>
<th>SMFC1 (V) 30 ppm</th>
<th>SMFC2 (V) 60 ppm</th>
<th>SMFC3 (V) Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>****</td>
<td>****</td>
<td>****</td>
</tr>
<tr>
<td>5</td>
<td>0.083943434</td>
<td>0.109870222</td>
<td>0.06134446</td>
</tr>
<tr>
<td>6</td>
<td>0.057038656</td>
<td>0.098874673</td>
<td>0.017525387</td>
</tr>
<tr>
<td>7</td>
<td>0.021077097</td>
<td>0.108348751</td>
<td>0.021601513</td>
</tr>
<tr>
<td>8</td>
<td>0.049541685</td>
<td>0.115261055</td>
<td>0.010331115</td>
</tr>
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<td>0.051622134</td>
<td>0.127245255</td>
<td>0.008823311</td>
</tr>
<tr>
<td>10</td>
<td>0.058086988</td>
<td>0.146089256</td>
<td>0.018268492</td>
</tr>
<tr>
<td>11</td>
<td>0.063563488</td>
<td>0.161457082</td>
<td>0.009034066</td>
</tr>
</tbody>
</table>

Fig. 2. Voltage production as a function of time by the Pseudomonas aeruginosa bacterium.

Efficiency in the Elimination of Nitrates:

The content was measured at the beginning and at the end of the experiment with a nitrate selective electrode HACH DR 2800™ for MFC: the nitrate content decreased from an initial value of 30 mg/L.
to 20.7 mg·L$^{-1}$, giving a 31% nitrate removal efficiency. For the case of the CCM2 the initial concentration of 60 mg/L was reduced to 40.6 mg·L$^{-1}$, giving a 32.3% nitrate removal efficiency. This indicates that the microbial culture of Pseudomonas aeruginosa contains denitrification, combining the oxidation of organic matter in the reduction of nitrates [15].

From the total of data shows the production from day 4 to day 11 as the period in which the production of voltage has a relation significantly proportional to the concentration supplied, this stage is considered as stage of monitoring of information of process of use of inorganic compounds in the production of bioelectricity being considered as an environmental biosensor.

The SMFCs were monitored for 15 days, saving the voltage data generated by the cells in a processor through the LABVIEW system (Fig. 1). Before to final assembly, assay tests were performed on two CCMs providing each with a different substrate, CCM1 possessed simply Pseudomonas Aeruginosa bacteria in liquid H2O (MB), CCM2 possessed glucose (MQ); (See Fig. 3) to understand the need to provide a stimulus of bacterial growth in an inorganic medium in order to produce bioelectricity for longer periods of time.

The first three days the cells tend to undergo a change in voltage generation which is presumed to be the time of adaptation of the bacteria to a new medium and the cells react according to the concentration of nitrates from day four, so it is observed in Fig. 4.

The glucose was injected as an incitement for the bacteria in a quantity of 5 mL at the beginning of the experiment, no more glucose was applied during the monitoring [16].

The 30 ppm SMFC1 produced 0.083 mV, the 60 ppm SMFC2 produced 0.109 mV, and the SMFC3 which is control produced 0.061 mV. In the course of the days it can be observed that the cells have a detection time of nitrates of about eight days then they tend to decay and interact different.

This may be due to the fact that the Pseudomonas aeruginosa species meets all its growth stages, considering that there was no substrate renewal during the experiment, so that from day 12 the relationship changes and the voltage generation behavior is indistinct to the concentration of nitrates (view Fig. 4 and Table 2).

The relationship between the concentration supplied and the amount of output voltage undergoes a deterioration in its last stage, it is assumed that the

<table>
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<tr>
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<th>SMFC2 (V) 60 ppm</th>
<th>SMFC3 (V) Control</th>
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<tr>
<td>1</td>
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<tr>
<td>15</td>
<td>0.107191508</td>
<td>0.033670948</td>
<td>0.073837544</td>
</tr>
</tbody>
</table>

The relationship between the concentration supplied and the amount of output voltage undergoes a deterioration in its last stage, it is assumed that the
nutrients of the inorganic compound were depleted, in addition the resistance that presents the SMFC could be another influencing factor, besides this type of devices Could reach the stable stage in a prolonged time and achieve a longer durability of monitoring without nutrient recharge even though a lower voltage output performance was achieved. [17].

4. Conclusions

Our research demonstrated the production of electrical energy through the biodegradation of nitrates present as synthetic wastewater, using a microbial fuel cell (MFC) inoculated with Pseudomonas Aeruginosa grown naturally, collected from the Laguna de Mapahuía located in the interiors of Sangay National Park - Province of Chimborazo - Ecuador. Using a single chamber SMFC, we managed to generate maximum voltages of up to 619 mV and to reduce the concentration of nitrates with an efficiency of 32.3 % in the best of cases.

This experiment emphasizes that the bacterial culture of Pseudomonas spp., Has a considerable production of bioelectricity, which increases gradually if a contaminant (nitrate) is added to the cells, so this system could monitor the nitrates in the wastewater.

Our projections will focus on understanding the scalability problems of MFCs to function as mobile devices in the monitoring of water quality parameters that involve the delay when analyzing in a laboratory, through the understanding that could be found between the relationships of the production of bioelectricity with various potentially contaminating compounds in wastewater.

Acknowledgements

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