

Alternative Energy by Bioelectrogenesis from the Bacteria *Pseudomonas Aeruginosa* and *Aeromonas Hydrophila*

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Bioelectrogenesis allows the transformation of chemical energy into electrical energy by means of microbial fuel cells. The research aimed to determine the amount of energy generated by bioelectrogenesis using *Pseudomonas aeruginosa* and *Aeromonas hydrophila* bacteria. Four double chamber H-type glass microbial fuel cells with a capacity of 500 mL and 2 control cells were constructed, using graphite or aluminium rod electrodes at the anode and graphite rod at the cathode for all the cells. 325 mL of anaerobic sludge and 75 mL of wastewater from a wastewater treatment plant were inoculated as substrate for the bacteria in the anode, where 50 mL of the aforementioned bacteria strain were inoculated, respectively. The experimental part was carried out in 20 days; the conditions of the cells were evaluated in terms of temperature and pH, characteristics of the bacteria and the behaviour of the voltage generated. It was established that the bacteria that generated the highest voltage was *Pseudomonas aeruginosa* with 0.8960V, using an aluminum electrode in the anode chamber. The results indicate that bioelectrogenesis using bacteria in anaerobic sludge and wastewater is a promising technology for obtaining clean and low-cost energy.

1. Introduction

Fossil energy sources in the world are becoming increasingly scarce, so there is a need to replace them with sustainable and renewable energy, one of the alternatives is bioenergy, which according to data only represents 10% of primary energy worldwide (IEA, 2022). An emerging biotechnology application is the generation of energy using microbial fuel cells where microorganisms are used to convert the chemical energy in a substrate into electrical energy through metabolic activity by transferring electrons to an anode electrode which, when placed in circuit with a cathode chamber separated by a proton membrane, produces electrical energy (Revelo et al., 2013).

Research has been carried out on the use of bioelectrogenesis, it is indicated that the substrate has a relevant role in the anodic behavior of the microbiological fuel cell, it has been tested with acetate as a substrate in the cells, giving a significant generation of electricity; In the same way, the characterization of the microorganisms in suspension and the biofilm by means of the Illumina technique, showed that *Desulfuromonas*, *Solitalea*, *Acholeplasma*, *Desulfobacula* and *Sphaerochaeta* are the main responsible for the generation of energy (Mateo S., 2018).

Electrical energy has been obtained from wastewater from the Rio Seco industrial park, with the syntrophic association of the microalgae *Chlorella vulgaris* and *Scenedesmus obliquus* and a consortium of anaerobic bacteria native to the wastewater, this bioelectrochemical system generated an average voltage of 66.50 ± 1.70 mV, an average current density of 0.02 ± 0.00 mA/mm² and an average power density of 2.43 ± 0.33 mW/mm², up to 14 days of evaluation (Terán, 2017).

The design of a microbial fuel cell with electrodes such as copper and zinc, allowed the generation of bioelectricity whose average circuit voltage was 0.507 V, current of 476.7 μ A and maximum power density of 0.24 mW/cm²; Also, the conductivity in the anode chamber was around 14.5 to 7.75 μ S/cm with an average pH of 8.0. (Rojas, S. et al., 2019).

The selection of materials, measurements and components of a fuel cell have a positive impact on energy generation; in a microbial fuel cell process with temperatures ranging from 45 °C to 28 °C for 30 hours of evaluation, the optimum temperature was 38.31 °C using *Pseudomonas aeruginosa* bacteria and 37.9 °C for *Escherichia coli*; In addition, pH values of 6.3 and 6.5 with *Escherichia coli* bacteria generate higher energy, while using *Pseudomonas aeruginosa* the values should be close to 7. The average voltage generated by *Pseudomonas aeruginosa* was 294.17 mV, while for *Escherichia coli* produced an average voltage of 186.44 mV (Bermúdez and Bernal, 2018).

2. Methodology

The research was experimental in nature and was carried out according to the following steps:

2.1 Obtaining the bacteria and adaptation.

Bacterial strains of *Pseudomonas aeruginosa* and *Aeromonas hydrophila* species were seeded to verify their adaptability and reproduction using Mc Hillton and Mac Conkey agar culture media, respectively.

2.2 Obtaining and characterization of the substrate

The substrate was obtained consisting of anaerobic sludge with water from the CITRAR-UNI domestic wastewater treatment plant. These sludge and water were characterized for their physicochemical properties at the César Vallejo University Laboratory before and after being used as substrate in the microbial fuel cell process, see Table 1.

Table 1: Characteristics of the sludge, initial and in the treatment cells

Parameter	Initial	Cell 1: <i>Aeromonas hydrophila</i> bacteria	Cell 2: <i>Pseudomonas aeruginosa</i> bacteria	Cell 3: <i>Aeromonas hydrophila</i> bacteria	Cell 4: <i>Pseudomonas aeruginosa</i> bacteria	Standard deviation
pH	8.0	8.05	7.90	8.04	8.15	0.10
Temperature (°C)	22.3	22.4	22.5	22.7	22.7	0.15
Conductivity (µS/cm)	3.10	13.80	8.40	7.8	3.70	4.14
Turbidity (NTU)	77.0	50.0	61.0	33.6	35.1	13.02
Dissolved oxygen (ppm)	5.19	0.60	8.34	5.59	5.56	3.22

2.3 Microbial Cell Design and Construction

Six 500-mL double-chamber H-type glass microbial cells joined by a 10-cm glass bridge were designed and constructed, as shown in Figure 1. A graphite electrode was used as cathode, a graphite electrode or aluminum as anode (depending on the assay) and proton exchange membrane bridge with agar-agar solution (a 100 mL syringe was used). The experimental design and the description of the cells for the investigation is presented in Table 2.

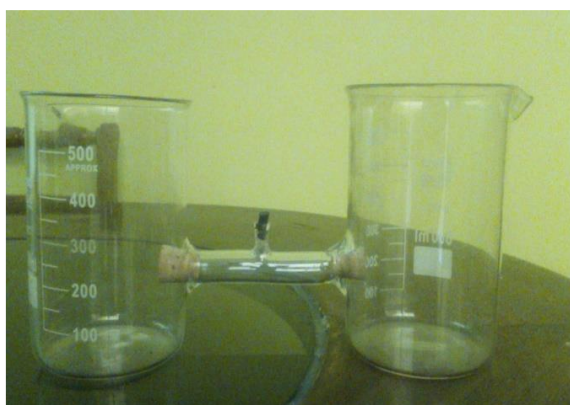


Figura 1: Microbial cell

Table 2: Description of the anodic and cathodic cells for each design

	Cell 1 design	Cell 2 design	Cell 3 design	Cell 4 design	Cell 5 Design (Control)	Cell 6 Design (Control)	
Anodic Cell	Bacteria:	50 mL of inoculum of <i>Aeromonas hydrophila</i>	50 mL of inoculum of <i>Aeromonas hydrophila</i>	50 mL of inoculum of <i>Pseudomonas aeruginosa</i>	50 mL of inoculum of <i>Pseudomonas aeruginosa</i>	-	
	Electrode type:	Graphite (44.37 cm ²)	Aluminum (36 cm ²)	Graphite (44.37 cm ²)	Aluminum (36 cm ²)	Graphite (44.37 cm ²)	Aluminum (36 cm ²)
	Sustrato:	Residual water: 75 mL	Residual water: 75 mL	Residual water: 75 mL	Residual water: 75 mL	Residual water: 75 mL	Residual water: 75 mL
Cathodic Cell	Electrode type:	Graphite (22 cm ²)	Graphite (22 cm ²)	Graphite (22 cm ²)	Graphite (22 cm ²)	Graphite (22 cm ²)	
	Medio:	Copper sulfate solution (1 M): 400 mL	Copper sulfate solution (1 M): 400 mL	Copper sulfate solution (1 M): 400 mL	Copper sulfate solution (1 M): 400 mL	400 mL of distilled water	400 mL of distilled water

2.4 Power generation process

In the process of energy generation with the cells with the different designs, it was monitored for 20 days, 64 measurements of the voltage generated were made; in the first 8 days it was measured twice a day every 12 hours and in the following days, 4 times a day every 6 hours. The pH, temperature and bacterial population of the anodes of the experimental cells were also recorded.

3. Results and discussion

3.1 Cell temperature during the process

The cells maintained a temperature between 20.8 °C and 21.8 °C, those in the anode were inoculated with *Aeromonas hydrophila* and *Pseudomonas aeruginosa* bacteria, as well as in the control cells. (Figure 2); these temperatures were relatively low and could affect the optimal generation of energy because scientific literature indicates that taking into account that the presence of substrates and their concentration requires more time to obtain a constant voltage of electric current in a microbial cell, the appropriate temperature for the generation of electric energy and removal of organic matter in the wastewater is 25°C reaching high coulombic efficiencies between 28.4% and 70.69%, for chemical demands of 1980 mg/L and 3200 mg/L; therefore, the oxidation process in the anode substrate, is directly related to the temperature, in the generation of electric power (Valencia, 2018).

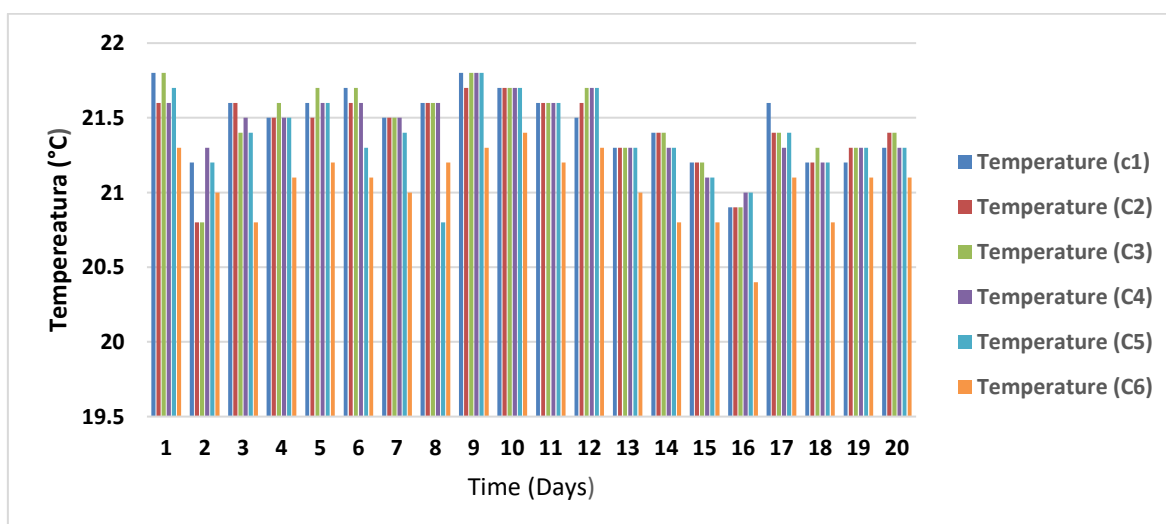


Figure 2: Cell temperatures

3.2 Energy generated in Microbial Cell 1 (C1)

This cell consisted of an anode cell where *Aeromonas hydrophila* bacteria were inoculated in a sludge medium with wastewater and a graphite electrode was placed, according to the Cell 1 design mentioned in Table 2. As shown in Figure 3, in this cell the energy generation was increasing with a logarithmic trend as time progressed, possibly due to the growth of the bacterial population and was higher than the energy generated in the control cell where no bacteria were inoculated (Cell 5), by 6.31 % on day 20.

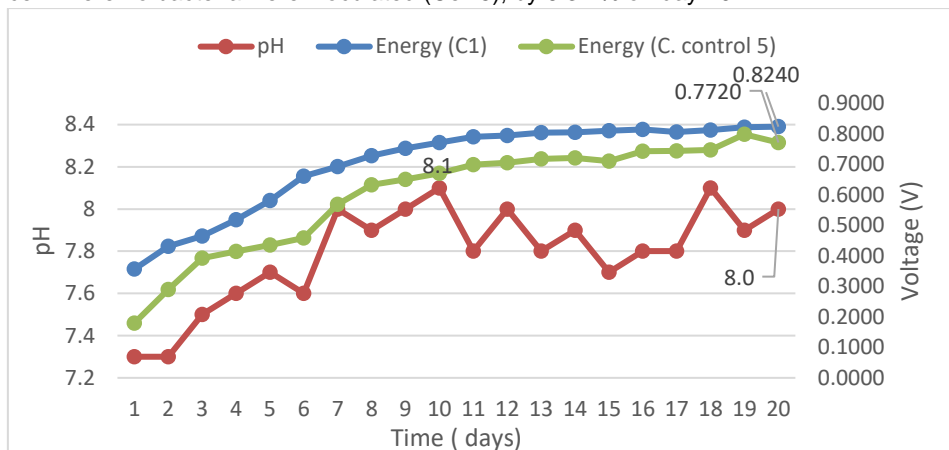


Figure 3: Energy generated in microbial cell 1

3.3 Energy generated in Microbial Cell 2 (C2)

This cell corresponded to cell design 2 (see Table 2), it consisted of an anodic cell where *Pseudomonas aeruginosa* bacteria were inoculated in a sludge medium with wastewater and an aluminum electrode was placed.

The energy generated in this cell on day 5 was 0.4484 V, higher than the energy generated by the control cell 5 (control), from day 6 the energy generated had a very slow growth so that the energy generated in the control cell (without bacteria) was higher (Figure 4). So, in this cell the inoculated bacteria did not improve the generation of energy in the way that, if it happened with the other microbial cells, it is very likely that this result was influenced by the temperature that in some cases inhibits the generation of energy (Valencia, 2018).

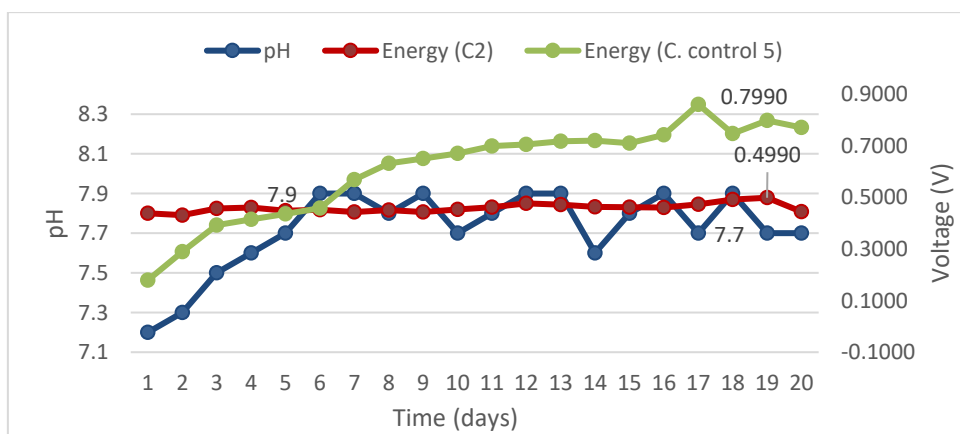


Figure 4: Energy generated in Microbial Cell 2

Comparing Cell 1 with *Aeromonas hydrophila* bacteria and Cell 2 with *Pseudomonas aeruginosa* bacteria, where in both anode chambers graphite electrode was used, it was in cell 2 that the energy generated was lower. In cell 2 the pH was in the range of 7.2 to 7.9 and presented optimal conditions for population growth of the bacteria that was higher than cell 1 and the others; it is important to keep in mind this parameter because it can affect the solubilization of organic compounds (such as phosphates) for the performance of microorganisms (Acosta-Suárez et al., 2019).

3.4 Energy generated in Microbial Cell 3 (C3)

Cell 3 with an anode chamber where *Aeromonas hydrophila* bacteria were inoculated in a sludge medium with wastewater and an aluminum electrode was placed. The energy generated from day 7 when it reached 0.8366 V was almost similar until day 19 when it was 0.8410 V, see Figure 5. The energy generated by this cell, in the same time interval, was higher than that generated in the control cell by approximately 10 %.

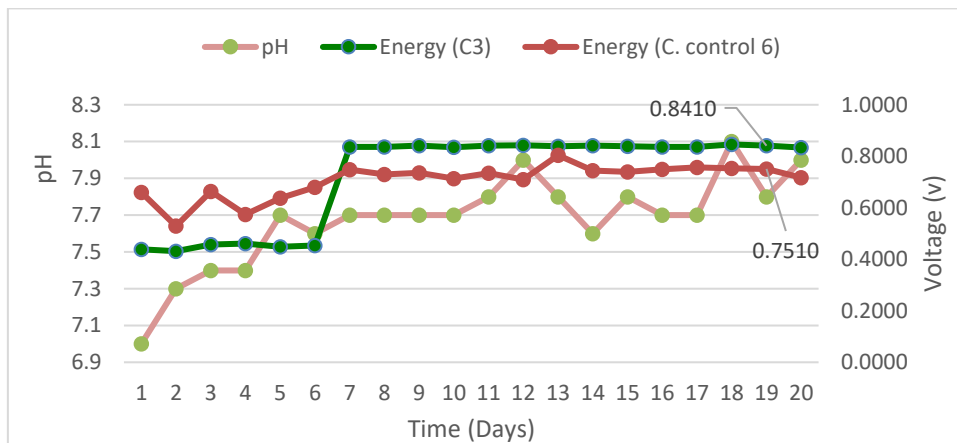


Figure 5: Energy generated in Microbial Cell 3

3.5 Energy generated in Microbial Cell 4 (C4)

In Cell 4 with an anode cell inoculated with *Pseudomonas aeruginosa* bacteria in a sludge medium with wastewater and an aluminum electrode was placed. The energy obtained in cell 4 from day 2 was higher than the energy generated in the control cell until day 20 of monitoring, reaching the highest level on day 18 with the value of 0.8960 V while in the control cell it was 0.7530 V, i.e., with a margin of 15.95 % higher, see Figure 6. The pH was progressively increasing in the interval from 7.3 to 8.44.

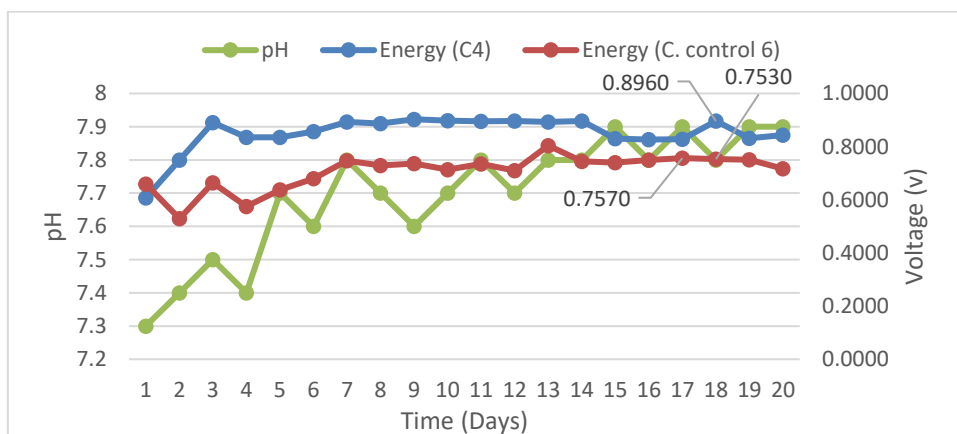


Figure 6: Energy generated in Microbial Cell 4

It was determined that using the same aluminum electrode in the anodic cell and the cathodic cell with graphite electrode, when *Pseudomonas aeruginosa* was inoculated, the microbial cell produced more energy than when *Aeromonas hydrophila* bacteria were inoculated.

Of the 4 microbial cells, it resulted that cell 4 generated more energy with 0.8960 V, then cell 3 with 0.8410 V, cell C1 with 0.8240 V and finally cell C2 with 0.4920 V; that is when using in the anionic cell *Pseudomonas aeruginosa* bacteria in sludge substrate with wastewater, with aluminum electrode and in the cathodic cell a graphene electrode in copper sulphate solution, proved to be the most optimal in obtaining energy; therefore, microbial fuel cells allows obtaining bioelectricity while in this process decreases organic pollutants with the presence of carbon and nitrogen (Sawasdee V. and Pisutpaisal N., 2018) that in high levels are found in sewage

sludge or wastewater treatment plants and require convenient handling such as also use of fixed bed gasifiers to produce hydrogen (Zaccariello and Mastellone, 2020).

3.6 Bacterial growth in anionic cells

The bacteria inoculated in the anionic chambers of the microbial cells had the population growth shown in Table 3, where it was found that bacteria cell C2 reached day 20 with a higher population than the other cells. This bacterium, due to its great capacity for adaptability and metabolizing various types of substrates, allows its growth, as graphite does not interfere with the transfer of protons through the selective membrane, favouring microbial activity.

Table 3: Bacterial growth in the cells

Time (Days)	Cell 1:	Cell 2:	Cell 3:	Cell 4:
	Number of	Number of	Number of	Number of
	<i>Aeromonas hydrophila</i> bacteria	<i>Pseudomonas aeruginosa</i> bacteria	<i>Aeromonas hydrophila</i> bacteria	<i>Pseudomonas aeruginosa</i> bacteria
0	0.019	0.019	0.019	0.019
4	1.44E+10	3.42E+10	1.52E+10	8.55E+09
8	8.59E+10	1.216E+11	4.8336E+11	1.52E+11
20	5.244E+11	6.3232E+11	3.61E+09	9.12E+10

4. Conclusion

The generation of energy in microbial fuel cells is a feasible alternative to be used and perfected to generate sustainable renewable energy, so it requires further research into the conditions conducive to making this method of bioelectrogenesis efficient; this research verifies this possibility, finding that *Pseudomonas aeruginosa* bacteria with sludge support with wastewater and aluminum electrode in the anode chamber generated 0.8960 V, higher than in cells where *Aeromonas hydrophila* and graphite electrodes were used.

Acknowledgments

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