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Potential use of pepper waste and microalgae *Spirulina* sp. for bioelectricity generation

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Abstract

The research aimed to generate bioelectricity using pepper waste and the microalgae *Spirulina* sp by a double-chamber microbial fuel cell (dcMFC). A dcMFC was constructed with Cu and Zn electrodes, where organic waste and microalgae were placed in the anodic and cathodic chambers, respectively. Also, electrochemical parameters were measured for 35 days. Finally, possible electrogenic microorganisms were isolated and identified. It was possible to generate maximum values of current (6.04414 \pm 0.2145 mA) and voltage (0.77328 \pm 0.213 V). The maximum conductivity value was 134.1636 \pm 7.121 mS/cm, while the internal resistance value was 83.784 \pm 7.147 Ω . The values of power and current density reached were 584.45 \pm 19.14 mW/cm² and 5.983 A/cm², respectively. The optimal operating pH was 4.59 \pm 0.14. From the microbial growth on the anode, the yeast *Yarrowia phangngaensis* (1) and *Pseudomonas stutzeri* (2) were identified, which may be involved in the transfer of electrons to the electrode. In conclusion, it was possible to generate clean energy in a laboratory-scale dcMFC when pepper waste and *Spirulina* sp. were used. These results are promising because organic waste can generate sustainable and environmentally friendly energy.

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Keywords: Pepper waste; Spirulina sp; Bioelectricity; Microbial fuel cells; Microalgae; Yeast

1. Introduction

In recent years, economic growth, urbanization, and increased energy demand for multiple development activities have generated a negative impact on the environment [1]. The use of fossil fuels to generate energy contributes to

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global warming by releasing toxic gases (mainly carbon dioxide) into the atmosphere [2,3]. On the other hand, 13×10^9 tons of organic waste are generated per year, which is not properly managed, becoming a problem of environmental importance [4–7]. These wastes represent potential sources of energy, only the forest and agricultural residues generated in Europe contain 4.5×10^{12} MJ per year [8,9]. This has led to the emergence of new technologies to take advantage of this waste and make them environmentally friendly, such as microbial fuel cells (MFCs). These are bioelectrochemical devices where microorganisms are responsible for breaking down organic matter and generating electrons to generate bioelectricity [10–12].

MFCs are primarily categorized according to their configuration into single-chamber MFCs, double-chamber MFCs, and stacked MFCs, as well as other more complex configurations. Double-chamber MFCs stand out due to their high-power output compared to other designs. This type of MFC consists of two chambers (anodic and cathodic) with their respective electrodes and a proton exchange bridge (PEB) [13–15]. The efficiency of MFCs depends on several factors, such as their configuration, the substrate, and the type of electrogenic microorganisms. These microorganisms can produce electrons and protons from the metabolic breakdown of the substrate [16]. In this sense, the microorganisms can be used in MFCs for electricity generation, for example, some species of bacteria are mentioned such as *Geobacter sulphurreducens, Shewanella putrefaciens, Escherichia coli, Proteus vulgaris, Enterobacter* sp., and the yeast *Saccharomyces cerevisiae*, among others [17]. These microorganisms have three mechanisms for extracellularly transferring electrons: (i) short-range electron transfer through a redox-active protein, such as cytochrome type C, which is associated with the outer surface or extracellular matrix, (ii) electron transfer through oxidized shuttles, which are released by microbial cells, and (iii) long-range electron transport through conductive biofilms [18].

The substrate is another factor affecting MFC performance, as they require long-term stability and serve as a carbon and energy source for microorganisms. Diverse substrates can be used within MFC as fuels, for example, wastewater, inorganic substrates, lignocellulosic biomass, etc. [19]. Biodegradable compounds from organic waste may represent a promising energy source in the future [20]. Research has shown that various organic wastes can be used in MFCs and that various chemical components such as flavonoids and carotenoids can be involved in the generation of bioelectricity [21]. Vegetables such as pepper (*Capsicum annuun*) are economically important crops worldwide, with some species found in Peru [22]. The pepper is processed in agro-industrial companies, generating waste such as husk, seeds, and leaves [23]. There is evidence that red pepper powder has been used to generate bioenergy in MFCs [24].

Advances have been made in the study of the use of microalgae in MFCs both as substrates in the anodic and cathodic chamber [25]. The photosynthetic capacity of microalgae offers advantages due to they can take CO₂ and generate oxygen. This sustainable oxygen production in the cathodic chamber of MFCs is useful as an electron acceptor and can reduce aeration costs [25–28]. Some research has shown that when microalgae are used in MFCs where the voltage can be improved [29,30]. In Huarachi-Olivera et al. (2018), through the use of *Chlorella vulgaris* and a microbial community, it was possible to increase the bioelectrogenic activity and the potential [29]. In another study, organic residues of mango were used in the anode and the microalgae *Spirulina* sp. at the cathode, obtaining voltages of 0.84546 ± 0.314 V [30].

Pepper is one of the main agro-export products that contribute to the growth of the agricultural sector in Peru. This vegetable is planted, harvested, and processed in different agro-industrial companies in the La Libertad region in order to be exported. However, those batches that do not meet the specifications may end up as waste, these are collected by the company CUC SAC. This company is specialized in the integral management of organic and inorganic, hazardous, and non-hazardous solid waste. Based on the aforementioned background, the objective of generating bioelectricity through a double-chamber MFC with pepper waste as a substrate of the anode and the microalgae *Spirulina* sp. in the cathodic chamber was proposed. In this way, it seeks to take advantage of this waste as a sustainable energy source and in an environmentally friendly way.

2. Method and materials

2.1. Sample of pepper waste

The sample consists in preserve pepper (See Fig. 1) present in the CUC SAC company (Trujillo, Peru). Approximately, 5 kg was collected. In the laboratory, the waste extract (500 mL) was obtained using an extractor (Maqorito- 400 rpm).



Fig. 1. Pepper waste.

2.2. Preparation of microalgae Spirulina sp. cultures for the cathodic chamber

The microalgae *Spirulina* sp. was maintained in an Erlenmeyer flask (1000 mL) with a medium based on salts with an uncontrolled temperature [30]. An air pump (160 H/L) was used to homogeneous the culture. The illumination of culture was made with a 6-watt LED focus. Finally, the microalgae biomass concentration in the cultivations was monitored daily by optical density at a wavelength of 600 nm using a digital spectrophotometer (Jenway, UK). The volume of microalgae in the cathodic chamber was 500 mL

2.3. Design of double-chamber microbial fuel cells (dcMFC)

The dcMFC design (see Fig. 2) consisted of two chambers (anodic and cathodic), electrodes (anode and cathode), a saline fluid (such as a proton exchange membrane (PEM)), and an external resistor. For the construction of the dcMFC, two polyvinyl chloride bottles of 1000 mL capacity were used. The two chambers were joined by the salt bridge, which consists of agarose (35 g/L) and KCl (15 g/L). This saline medium was placed inside a container made from the body of a syringe (6.8 cm \times 2.1 cm). The electrodes were made from Copper and Zinc (10 cm \times 5 cm). These were arranged at the anode and cathode respectively and were joined by a copper wire to external resistance. Finally, an air pump (160 H/L) injected oxygen into the cathode. Three double-chamber dcMFCs were manufactured.



Fig. 2. Double-chamber MFC design.

2.4. Characterization and operation of the dcMFC

The electrical parameters (voltage and current) were measured with a multimeter (Prasek Premium PR-85) and an external resistance of 100 Ω , during 35 days of monitoring. Likewise, the pH and electrical conductivity values were measured using a pH meter (Series 110 Oakton, USA) and a conductivity meter (CD-4301, USA), for 35 days.

While the power density (DP) and current (CD) values were measured using the formula mentioned in Rojas-Flores et al. (2021) [31], with an area (of $100 \pm 4.94 \text{ cm}^2$). While the values of the internal resistance of the MFCs an energy sensor (Vernier- \pm 30 V & \pm 1000 mA) was used.

2.5. Isolation and molecular identification of microorganisms

After the measurements were completed, the electrode (anode) was removed from the anode chamber. To isolate possible electrogenic microorganisms, a sample was taken at the anode using the swab technique. For the isolation of bacteria, MacConckey agar and Nutrient agar were used, while for the isolation of fungi, Sabouraud agar with 4% glucose plus antibiotic was used. The incubation time for the bacterial cultures was 24 h at 35 °C, while for the fungi it was 24 h at 30 °C. After the incubation time, the characteristics of the culture were observed, while Gram and methylene blue staining was used to observe its microscopic characteristics. Finally, axenic (or pure) cultures were performed.

For molecular identification, axenic cultures were sent to the Ecobiotechnology Laboratory SAC (Perú). The CTAB method was used for genomic DNA extraction, while For the amplification of the rRNA 16S of the genomic DNA, the universal primers 1492R/27F and ITS (Internal Transcribed Spacer) regions were used for bacteria and fungi, respectively. The amplified fragments were sequenced by the Sanger method. The MEGA X program (https://www.megasoftware.net/) and Blast Software (https://blast.ncbi.nlm.nih.gov/Blast.cgi) were necessary to analyze the sequences. Finally, the identification of the microorganisms was possible by the percentage of identification in Blast.

3. Results and analysis

Voltage values increased from the first operating day $(0.17124 \pm 0.001 \text{ V})$ until day 21 when it showed a maximum value of $0.77328 \pm 0.213 \text{ V}$, which declined until the last day to $0.32635 \pm 0.3014 \text{ V}$ (see Fig. 3(a)). These values show an increase of approximately 45% compared to the initial and maximum values. This is due to the increased hydrolysis and conversion rate of nutrients for the metabolism of microorganisms [32]. On the other hand, in the last few days, discoloration was observed in the cathode chamber of the algae, which would be due to the death of microorganisms for the presence of the zinc electrode, which is a toxic material for them [33].

Fig. 3(b) shows the values of electric current generated during monitoring, where the maximum value of 6.04414 ± 0.2145 mA was observed on day 22, which gradually declined until the last day (1.53367 \pm 0.2791 mA). The results obtained are greater than those shown in previous studies, in which a small air pump was used in the cathode chamber to enhance the electron reaction. This may occur because the inflow of O_2 helps reduce reactions in the chamber [34]. The increase in the initial current values is due to the good adhesion of electron-generating microorganisms in the process of their metabolism to the anode electrode [35,36]. Fig. 3(c) shows the pH means values of the dcMFCs monitored during the 35 operating days, observing that, although the values increased from 3.22 to 5.292 ± 0.134 on the first and last day, respectively, they always remained in the acidic range. These values are some of the most important parameters for the correct operation of the cells because they help the acclimatization of electron-generating microorganisms, although the pH shown was acidic and previous studies show that the pH of these ranges is not favorable for bacteria to breathe and grow easily. This research shows high voltage and current values; being its optimum operating pH of approximately 4.59 ± 0.14 [37–39]. Similarly, Fig. 3 (d) shows the values of electrical conductivity of the substrate, showing an increase of 134.1636 ± 7.121 mS/cm until day 21 and, then decreasing slowly until the last day (91.3105 \pm 8.155 mS/cm). The values of electrical conductivity are strongly linked to the ionic strength, and the variation of this parameter influences the transport of ions generated from one chamber to another and the power of the system [40,41]. Fermentation and sedimentation of the substrate in the last days of monitoring influenced the decrease in electrical conductivity [42].

Fig. 4(a) shows the voltage vs resistance graph which is based on Ohm's Law, V = IR, where V, is the voltage, I, is the electric current, and R is the resistance. As V is on the Y-axis and I is on the X-axis, the slope of the graph represents the value of internal resistance of the microbial fuel cell, in this case, 83.784 \pm 7.147 Ω . The low resistance value is one of the parameters influencing the high values of electric current because it presents low opposition to the passage of electrons from the anode chamber to the cathode chamber [43]. The low value found is due to the metal electrodes used, which, by their characteristics, have a low resistance to the passage of electric current [44].



Fig. 3. Values of (a) voltage, (b) electric current, (c) pH, and (d) electrical conductivity of the microbial fuel cells during monitoring.



Fig. 4. Values of (a) internal resistance and (b) power density and current density of microbial fuel cells.

Fig. 4(b) shows the values of power density (PD) and voltage (V) according to current density (CD), with a $PD_{MAX.}$ of 584.45 ± 19.14 mW/cm² at a CD of 5.983 A/cm² and a maximum voltage of 727.39 ± 19.34 V. The values obtained in this research are higher in comparison with previous works, for example, Priya and Setty (2019) used apple waste in their double-chamber microbial fuel cells with carbon electrodes, managing to generate a $PD_{MAX.}$ of 31.58 mW/cm² at a CD of 350 mA/cm² [45]. Similarly, Asefi et al. (2019) used food waste as substrate in their double-chamber MFC with carbon felt electrodes, managing to generate approximately 775 ± 21 mV and 422 mW/cm² voltage and PD, respectively [34].



Fig. 5. Microorganisms isolated from the surface of the anode with pepper waste. (a) copper anode. (b) *Yarrowia phangngaensis* colony in Sabouraud Agar medium with 4% glucose, and (c) *Pseudomona stutzeri* colony on nutritive agar. (d) Microscopic (1000x) observation of *P. stutzeri* stained with Gram stain, and (e) vegetative cells of *Y. phangngaensis*. colored with lactophenol blue.

In Fig. 5, microbial growth on the copper anode of the anodic chamber is shown (Fig. 5(a)), which is an important step for the transfer of electrons and therefore for the generation of electric current. [46–48]. On the other hand, the cultures of the isolates grew the nutrient and Sabouraud media (see Fig. 5 (b and c)), while the microscopic characteristic can be observed such as Gram-negative bacteria (see Fig. 5(d)) and yeasts that behave as Gram-positive (see Fig. 5(e)).

lable	1.	Species	identified	in	the	anode	(electrode) in	contact	with	pepper	waste.	

Identified species	Phylum	% Identity	Access number
Yarrowia phangngaensis	Ascomycota	100.00	MH793861.1
Pseudomonas stutzeri	Proteobacteria	100.00	MT027239.1
Pseudomonas stutzeri	Proteobacteria	99.86	MT027239.1

Regarding molecular identification, Table 1 shows that for the molecular identification of bacteria, the 16S rRNA gene was used, whose sequence is highly conserved and specific for this group [49]. The regions sequenced and analyzed in the BLAST bioinformatics software obtained an identity percentage of 100.00% and 99.86% for the two isolates belonging to the species of *Pseudomonas stutzeri*. In the identification of yeasts, ITS sequences were used, which is the marker par excellence of fungi [50]. Likewise, BLAST analysis of the fungal sequence yielded a percentage of identity of 100% for the species *Y. phangngaensis*.

As for the transfer of electrons, several methods vary according to the electrogenic species involved [51,52]. *Y. phangngaensis* could participate in electron transfer through direct contact with the anode since no external mediators were used. Therefore, transmembrane proteins and cytochromes C can facilitate the transfer of electrons in this yeast [53]. However, a recent study indicates that the yeast species, *Zygosaccharomyces bailii*, produces electroactive metabolites that are related to flavins and that they may play an important role in mediated electron transfer [40]. Regarding *Pseudomonas* species, they are proteobacteria that easily form biofilms and can be electrogenic such as reported in another proteobacterium [54]. Furthermore, the transfer of electrons to the anode may be due to the production of mediators such as pyocyanin in *Pseudomonas*. On the other hand, a study showed that *P. aeruginosa* produces a type IV conductive pili (PaT4P), which functions as a conductive nanomaterial [51].

Finally, the results obtained from organic waste used as substrates in an MFC contribute to achieving the seventh objective of sustainable development, affordable and non-polluting energy, as mentioned in an article by [55], where it refers that MFCs and the science of microbiology contribute to achieving this objective due to the potential of

bioelectrochemical systems and the metabolic capabilities of microorganisms, however, more studies are required to improve bioelectricity production.

As a limitation of the research, it is possible that other species of non-cultivable microorganisms could contribute to the transfer of electrons. Therefore, it suggests for future research a metagenomic analysis to identify the microbial communities developed on the electrode (anode).

4. Conclusion

This research successfully generated bioelectricity through laboratory-scale microbial fuel cells, using copper and zinc electrodes, pepper waste (anode chamber), and microalgae *Spirulina* sp. (cathode chamber) as substrates. A maximum voltage and current value of 0.77328 ± 0.213 V and 6.04414 ± 0.2145 mA was observed on days 21 and 22, respectively; with an optimal operating pH value of 4.59 ± 0.14 and a maximum substrate electrical conductivity of 134.1636 ± 7.121 mS/cm on day 21. The high current and voltage values are mainly due to the low resistance of $83,784 \pm 7,147 \Omega$ displayed by microbial fuel cells. A maximum power density of 584.45 ± 19.14 mW/cm²at a current density of 5.983 A/cm² was also shown. The microorganisms *Y. phangngaensis* and *P. stutzeri* were molecularly identified. In summary, this research shows the capacity of pepper residues and the use of the microalgae *Spirulina* sp. with great potential to be used as a fuel on a large scale and with the capacity to compete with other alternative energies.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Segundo Jonathan Rojas Flores reports financial support, administrative support, article publishing charges, statistical analysis, travel, and writing assistance were provided by Autonomous University of Peru. Segundo Jonathan Rojas Flores reports a relationship with Autonomous University of Peru that includes: employment. Segundo Jonathan Rojas Flores has patent pending to Segundo Rojas Flores.

Data availability

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

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