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Sustainable Use of the Fungus *Aspergillus* sp. to Simultaneously Generate Electricity and Reduce Plastic through Microbial Fuel Cells

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Abstract: The improper disposal of plastic waste has become a significant problem, with only a small amount recycled and the rest ending up in landfills or being burned, leading to environmental pollution. In addition, the cost of electric energy has risen by over 100% in the last 20 years, making it unaffordable for remote areas to access this service due to high installation costs, leaving people living far from major cities without electricity. This study proposes an innovative solution to these issues using microbial fuel cell (MFC) technology to simultaneously reduce plastic waste and generate electric energy by utilizing the fungus *Aspergillus* sp. As a substrate for 45 days. The MFCs reached maximum values of 0.572 ± 0.024 V and 3.608 ± 0.249 mA of voltage and electric current on the thirty-first day, with the substrate operating at a pH of 6.57 ± 0.27 and an electrical conductivity of 257.12 ± 20.9 mS/cm. Furthermore, it was possible to reduce the chemical oxygen demand by 73.77% over the 45 days of MFC operation, while the recorded internal resistance was 27.417 ± 9.810 Ω, indicating a power density of 0.124 ± 0.006 mW/cm^2. The initial and final transmittance spectra, obtained using FTIR (Fourier Transform Infrared), showed the characteristic peaks of polyethylene (plastic), with a noticeable reduction in the final spectrum, particularly in the vibration of the C-H compound. After 45 days of fungus operation, the plastic surface used as a sample exhibited perforations and cracks, resulting in a thickness reduction of 313.56 µm. This research represents an initial step in using fungi for plastic reduction and electric energy generation in an alternative and sustainable manner.

Keywords: plastic reduction; bioenergy; fungus; microbial fuel cells; *Aspergillus*

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1. Introduction

In recent decades, the use of plastic has increased significantly and has become a fundamental part of various human activities. From 1950 to 2015, production increased from approximately 2 million metric tons to 320 million, reaching 8.3 billion metric tons in 2017 [1–3]. As a result, environmental sustainability has become a danger due to the large amount of plastic waste, which must be collected or reused correctly to avoid severe pollution caused by burning waste or dumping it in landfills or rivers [4]. Only a tiny amount of plastic is recycled globally sustainably, while most of it pollutes the environment, leading to health and environmental problems such as pollution of agricultural soils and oceans [5]. In addition, increased plastic use has been observed due to COVID-19 restrictions [6].

On the other hand, a significant problem that threatens the sustainability of the environment and the human population is the sources of conventional fuels, such as fossil fuels, used for producing electric power [7,8]. It has been reported that if the consumption of fossil fuels continues at the same rate as today or increases, they will be depleted in the next 40 years [9]. In addition, it is estimated that the consumption of this type of source releases 21 million tons of CO_2 (carbon dioxide) annually, gradually increasing the greenhouse effect and significantly damaging the environment [10,11]. Although many countries have increased electric power (sustainable) supply to homes, many families still need this service. For example, in South American countries such as Ecuador, Bolivia, Brazil, Argentina, and Peru, the percentages of households with access to electricity for cooking are 94.70%, 88.30%, 96.50%, 99.90%, and 85.50%, respectively [12,13]. In this challenging scenario, renewable sources have emerged as an innovative, sustainable solution to meet energy needs, offering a reassuring path forward. In 2020, 1179 GW (gigawatts) of renewable energy were accumulated using sustainable methods, representing an essential and reassuring source of usable electrical energy for society [14].

In the search for new ways to generate electrical energy sustainably, since 1960, microbial fuel cell (MFC) technologies have been evolving to offer society a new alternative source of sustainable energy [15,16]. MFCs are characterized by the electrochemical phenomena within the anodic and cathodic chambers, which convert chemical energy into electrical energy by releasing electrons in the oxidation and reduction process, using different types of organic waste as fuel or substrate [17,18]. In a study by Rincón et al. (2022), banana waste was investigated as a fuel in MFCs, where the results showed peaks of power density of 41.3 mW/m^2 and with 580.99 Ω of internal resistance [19]. Similarly, Agudelo et al. (2022) utilized coffee wastewater as a substrate, leading to peaks of 400 mV and a 70% reduction in the concentration of organic matter present in the substrate [20]. Fungi have also been explored as substrates in MFCs to assess their potential. For instance, Abdallah et al. (2019) utilized the fungus *Aspergillus sydowii* and banana waste as substrates, resulting in the generation of 0.76 V and a power density of 160 mW/m^2 [21].

For the improvement of MFCs, different types of microorganisms have been used as biocatalysts for various applications, such as reducing toxic heavy metals and eliminating toxic dyes in wastewater, while generating electrical energy [22]. The literature mentions that in the last ten years, fungi without mediators have begun to be used, observing the tremendous electrogenic potential of these microorganisms [23]. These fungi produce short-lived electroactive compounds that act as mediators, helping to generate energy. In addition, by involving redox enzymes in the fungal cell walls, electrons can be transmitted directly [24]. One of the fungi that are characterized by biodegrading plastic is the fungus *Aspergillus* sp. Because the cutinases they produce as subclasses of esterase enzymes can hydrolyze polyesters with high molar mass, breaking the chains of carboxylic esters [25,26]. There are reports that the fungus *Aspergillus* sp. Has managed to penetrate polymeric structures through its hyphae and degrade PET (polyethylene terephthalate) foams [27].

This research observed the potential of the fungus *Aspergillus* to impressively and simultaneously reduce a plastic sample and obtain electrical energy through microbial fuel cells at a laboratory scale over 45 days. The values of electrical conductivity, electric current, pH, voltage, chemical oxygen demand, current density, power density, and internal resistance were monitored during the operation period of the MFCs. The plastic sample in its initial and final state was measured through FTIR (Fourier Transform Infrared) and SEM (Scanning Electron Microscope) to observe the transmittance spectrum and micrographs of the samples. The research presents a sustainable, innovative, and highly environmentally friendly way of using microbial fuel cells and fungi, as they can generate electrical energy and reduce plastic waste efficiently and economically.

2. Materials and Methods

a. Design and assembly of MFCs

Single-chamber MFCs were obtained from Xin Tester (Shanghai, China, volume 100 mL). The electrode materials used were activated carbon (AC) and zinc (Zn) as anode and cathode, whose areas were 26.5 and 22 cm^2, respectively. An external resistor of 100 Ω was used for the external circuit, which was connected to a 0.5 mm thick copper wire and with Nafion as separated between the chambers (see Figure 1).

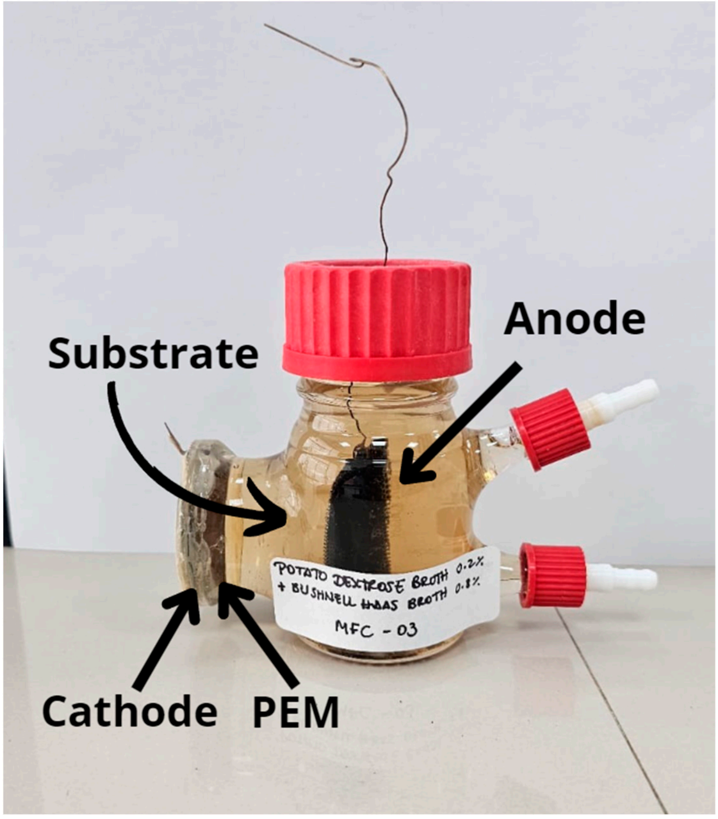

Figure 1. Schematic of the MFC with the substrate of potato broth and *Aspergillus* sp.

b. Electrochemical and morphological tests.

A Truper multimeter (MUT-830 Digital Multimeter) was used to monitor the current (I) and voltage (V) values of the MFCs. The chemical oxygen demand (COD) values were determined using the closed reflux colorimetric method (NTP standard 360.502:2016) [28]. The internal resistance of the MFC was calculated using Ohm's law and an energy sensor (Vernier- ±30 V and ±1000 mA). Segundo et al.'s method determined the values of power density (PD) and current density (CD) (2024) using external resistors of 0.2 (±0.05), 5 (±0.50), 20 (±2.4), 50 (±6.52), 120 (±10.55), 240 (±15.62), 480 (±20.64), 520 (±30.88), 780 (±50.75), and 1000 (±60.55) Ω [28]. Micrographs of the plastics were taken using SEM (TESCAN USA, Tallahassee, FL, USA). Fourier measured transmittance spectra of the plastic films with transform infrared spectrometer (FTIR, Thermo Scientific IS50, Austin, TX, USA).

c. Collection of samples, isolation, and selection of *Aspergillus* sp.

The plastic remains used as samples were obtained from the municipal landfill of the El Milagro population center, Trujillo, Peru; they were placed in sterile containers and labeled for transport to the Cesar Vallejo University laboratory. Small fragments of the plastic sample were placed in tubes with 10 mL of Sterile Physiological Saline Solution (SSFS), from which 100 µL of the suspension was extracted and surface-sown on plates with potato dextrose agar (PDA) supplemented with Chloramphenicol. These plates were incubated at 28 °C for five days [29]. Then, the fungi were replicated in PDA for identification by comparing their morphology and fungal structure with the taxonomic keys of Barnett S. A.'s guide (1972) [30], as shown in Figure 2.

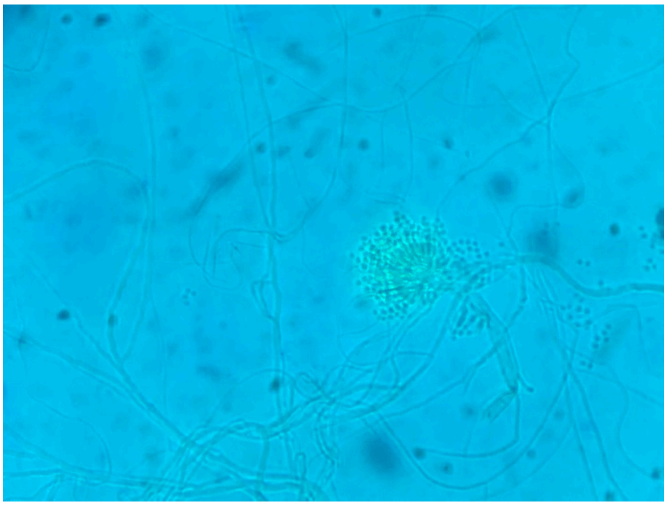

Figure 2. Microscopic image of the fungus *Aspergillus* sp.

d. Microbial fuel cell operation

The experiment was performed in a single-chamber Microbial Combustion Cell (MFC), in which the sterile substrate and the *Aspergillus* sp. Fungus were placed. The substrate was sterilized in an autoclave before being used and consisted of 80% of minimal salt medium and 20% of potato broth; the inoculum was composed of 4 fractions of the *Aspergillus* sp. Culture (5 mm in diameter) [31]. With the components ready for use and in sterile conditions, the sterile substrate, the microbial inoculum, and a 1.5 × 1.5 cm sheet of low-density polyethylene were placed inside the MFC.

3. Results and Analysis

The data presented in Figure 3a show the voltage data over time. Voltage started at 0.039 ± 0.001 V on day 4 and peaked at 0.572 ± 0.024 V on day 31 before gradually decreasing to 0.431 ± 0.032 V by day 45. The initial voltage increase is mainly attributed to oxidation reactions in the anodic chamber and reduction in the cathodic chamber. As Zhao et al. (2019) explain, these reactions create a potential difference that peaks as the chemical compounds are used up, resulting in a decrease in voltage. In similar systems, peak voltages of 0.38 V can be achieved, with the redox potential attributed to the crucial activity of fungal redox enzymes, such as laccase (enzymes), which oxidize aromatic phenolic compounds and amines, and play a significant role in the process, impressively contributing to the generation of peak voltages [32]. Other study also revealed that the fungus *Irpex lacteus* can reach peaks of 0.35 V, highlighting the superior redox potential of fungal laccase compared to bacterial laccase. Furthermore, Aspergillus, a filamentous fungus, shows significant biotechnological potential for the industrial production of chemical products [33]. Monitoring of the electric current showed that the values increased from day 4 (0.105 ± 0.084 mA) progressively until day 31 with a maximum value of 3.608 ± 0.249 mA, and then decreased in the current values until day 45 (2.562 ± 0.291 mA). The electric current values are caused by the release of electrons by the fungus *Aspergillus* sp. These electrons are captured by the anodic electrode and transported to the cathode. The delay in the increase in current values for four days is due to the acclimatization time of the fungus and the formation of the mediating biofilm. The fungus *Psathyrella candolleana* is used as a substrate in the MFC, and the generated electrons come from the organic content of the native microflora. This microflora is responsible for transferring the electrons to the surface of the electrode, leading to an increase in current values in the initial days. At the same time, the COD is reduced due to the activity of the microflora. Thulasinathan et al. (2024) used the fungus *T. harzianum* and a biodegradable crystal violet, mentioning that the ability to biodegrade some substances depends on each fungus and its compatibility [34]. It is crucial to note that electron production and energy generation are not always directly proportional. They depend on the resistance of the MFCs, a complex system that plays a significant role in their operation [35].

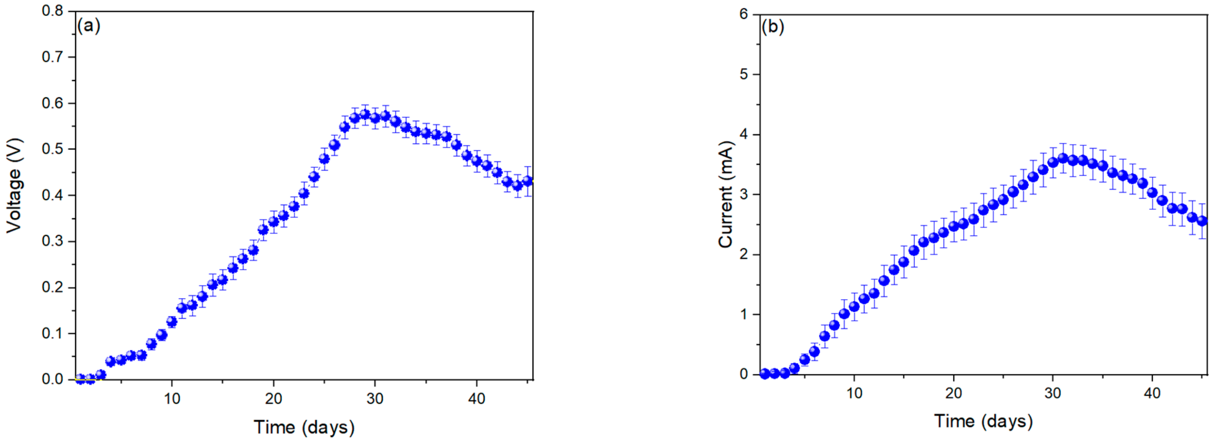

Figure 3. Report on monitoring the values of (**a**) voltage and (**b**) electric current.

The pH values increased progressively from day 1 from a slightly acidic region to the last day to a slightly alkaline region, with the optimum operating pH value being 6.57 ± 0.27 on day 31 (Figure 4a). The performance of microbial fuel cells (MFCs) is greatly influenced by the proliferation of microorganisms at standard pH levels. Tiwari et al. (2021) highlighted in their research that pH variations can affect proton transmission to the cathode, leading to a decrease in oxygen reactions and subsequently reducing the electrical output of the MFC [36]. Certain bacteria, such as *Pseudomonas aeruginosa*, Acinetobacter Schindler, and *Pseudomonas*, have been utilized as biocatalysts in MFCs, generating peak I and V of 0.110 mA and 110 mV, respectively, when operating at a pH of 7 [37]. These findings represent a novel approach in the field of microbiology and biochemistry. Additionally, *Lysinibacillus xylanolytic* has been used as a substrate in H-type MFCs, resulting in a peak voltage of 1127 mV using aluminum and graphite electrodes [38]. The electrical conductivity values (Figure 4b) showed a similar behavior to that reported in Figure 3a,b. On the first day, the values increased from 50.60 ± 1.03 mS/cm until day 31 with a value of 257.12 ± 20.9 mS/cm and then decreased until day 45 with 207.40 ± 4.16 mS/cm. The literature indicates that the initial increase in electrical conductivity values is attributed to the significant release of ions resulting from numerous redox reactions. Over time, this trend gradually decreases until the final day [39,40]. The high electrical conductivity explains the high current and voltage values produced in Figure 2 because the anodic electrode had greater freedom to move and capture the electrons. Figure 4c shows the values of the chemical oxygen demand (COD) recorded in the MFCs, where a decrease of 73.77% is observed concerning the values recorded on the first day (756.86 mg/L) and the last day (198.54 ± 38.95 mg/L); the most significant reduction of the COD is observed in the first 30 days (59.16%). The initial thirty days saw the most significant decrease in organic matter, indicating the high metabolic activity of the fungus *Aspergillus* sp. This early activity in the MFCs resulted in the highest electric current values; similar behavior was observed with the fungus *Aspergillus niger* in MFCs, which managed to reduce COD values by 77.9% and generate 0.814 V [41]. Research by Kongthale et al. (2023) showed that yeast strains used in winery wastewater produced maximum voltages of 219.33 ± 6.43 mV and simultaneously reduced COD values by 79.14 ± 0.92% [42]. These findings have important implications for the practical application of MFCs in areas such as bioenergy and environmental science.

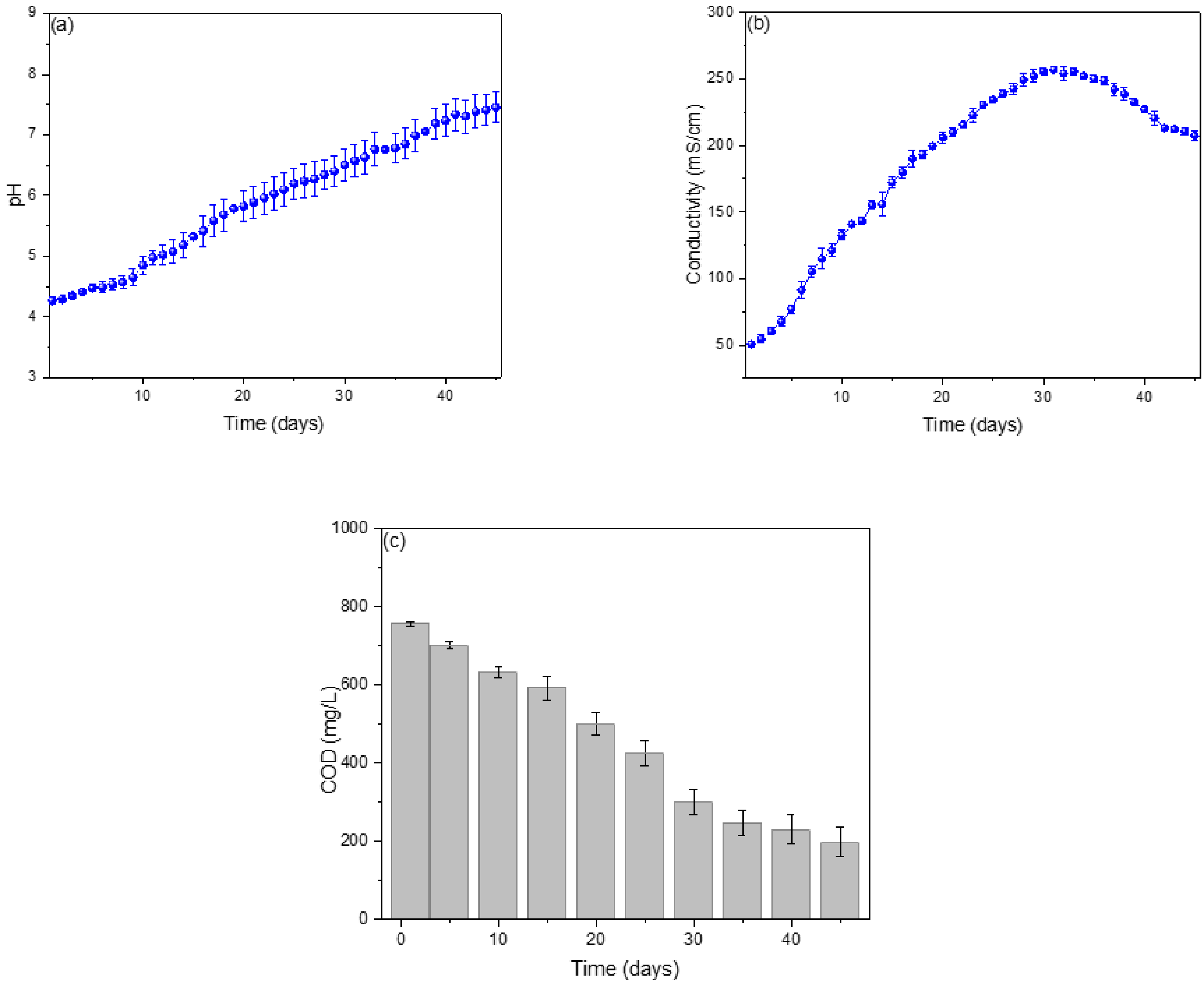

Figure 4. Parameters shown for (**a**) pH, (**b**) conductivity, and (**c**) COD.

On day 31, the maximum power density values observed were 0.124 ± 0.006 mW/cm^2 at a current density of 5.201 mA/cm^2 with a voltage of 530.619 ± 25.155 mV (refer to Figure 5a). According to Andirukonis et al. (2021), one crucial factor for achieving good power density results is the adhesion of microorganisms on the surface of the anodic electrode, as it plays a vital role in electron transfer [43]. Also, the literature has reported that the designs used in MFCs are influential in the power density efficiency and that marine sludge has excellent potential as a biocatalyst [44]. *Rhizobium anhuiense* bacteria has been used as a substrate in MFC, achieving power density peaks of 4.93 mW/cm^2, where the authors mention that redox mediators in the proton exchange membrane influence the power density results [45]. Žalnėravičius et al. (2022) also used *Rhizobium anhuiense* bacteria as a substrate in MFCs, generating a power density of 4.93 mW/cm^2 when operating at pH 7, and observed that by introducing glucose, the power density values improved significantly [46]. Figure 5b shows the internal resistance calculated using Ohm's Law, achieving an internal resistance of 27.417 ± 9.810 Ω on day 31. In their research, Islam et al. (2020) emphasized the collaborative nature of understanding the internal resistance of MFCs, where the resistance is influenced by the biofilm formed and has adhered to the anodic electrode and the synergism between the microorganisms in the substrate [47]. Considering various factors such as the MFCs' geometry, the electrodes' structure, the substrate used, and the diffusion rate of protons and electrons is crucial for this collaborative effort [48]. Bhatt et al. (2024) contributed to this collective knowledge by using the fungus *Aspergillus niger* in their research. They demonstrated how variations in resistance values can impact power density. Additionally, they noted that high resistance values can impede electron flow, thereby reducing the electric current of the MFCs [49].

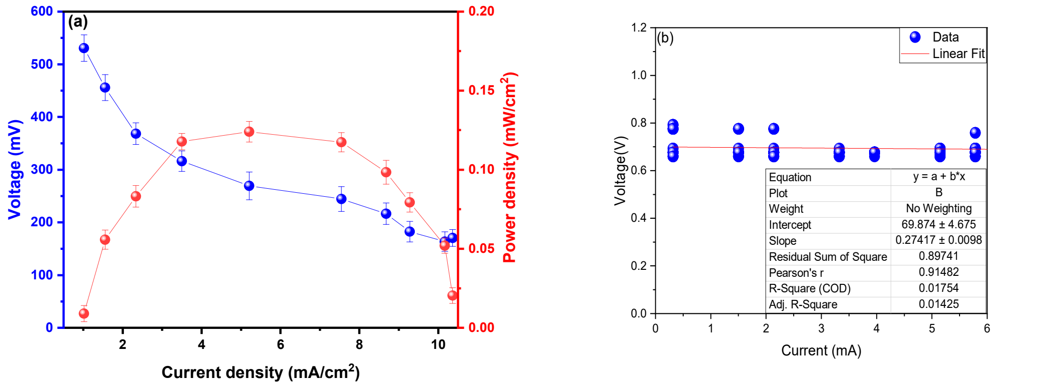

Figure 5. Values of (**a**) power density as a function of current density and (**b**) internal resistance.

The initial and final FTIR transmittance spectra are shown in Figure 6, where characteristic plastic peaks are observed. The peak at 3405 cm^{-1} shows the stretching of the OH group, the vibration bands between 2844 and 2919 cm^{-1} belong to the C-H compounds of the aliphatic group, while the peak at 1646 cm^{-1} is characteristic of the stretching of the C=O group, and the bands of the peak at 1477 cm^{-1} show the existence of the COO- groups and the peak at the wavelength 723 cm^{-1} belongs to the stretching of the C=C group [50,51]. It has been observed that the initial peaks of the spectra decreased in intensity. Previous studies have shown that the fungus *Aspergillus* can reduce plastic waste by covering the plastic with degradative enzymes and using polyethylene for growth. This demonstrates the fungus's ability to break down plastic [52]. The fungus *Aspergillus* is capable of producing hydrolytic and oxidative enzymes and adheres well to plastic substrates [53]. The initial and final micrographs are shown in Figure 7, where a smooth surface without imperfections is shown with an initial thickness of the sample of 751.46 µm. The latest micrograph reveals a surface with irregular porous structures, indicating the strong activity of the fungus *Aspergillus*. The final thickness is only 437.90 µm, which shows a significant reduction of 313.56 µm. This is a groundbreaking discovery, as it suggests that these irregular structures are formed directly as a result of the activity of the fungal hyphae. These hyphae create a biofilm on the plastic surface, causing streaks and penetrations in the plastic waste. This discovery brings to light the potential damage caused by the fungus Aspergillus and its role in degrading plastic waste [26,54,55].

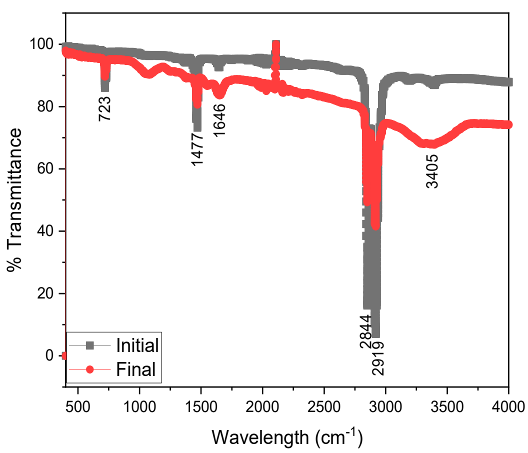

Figure 6. FTIR spectrum of the plastic samples in initial and final states.

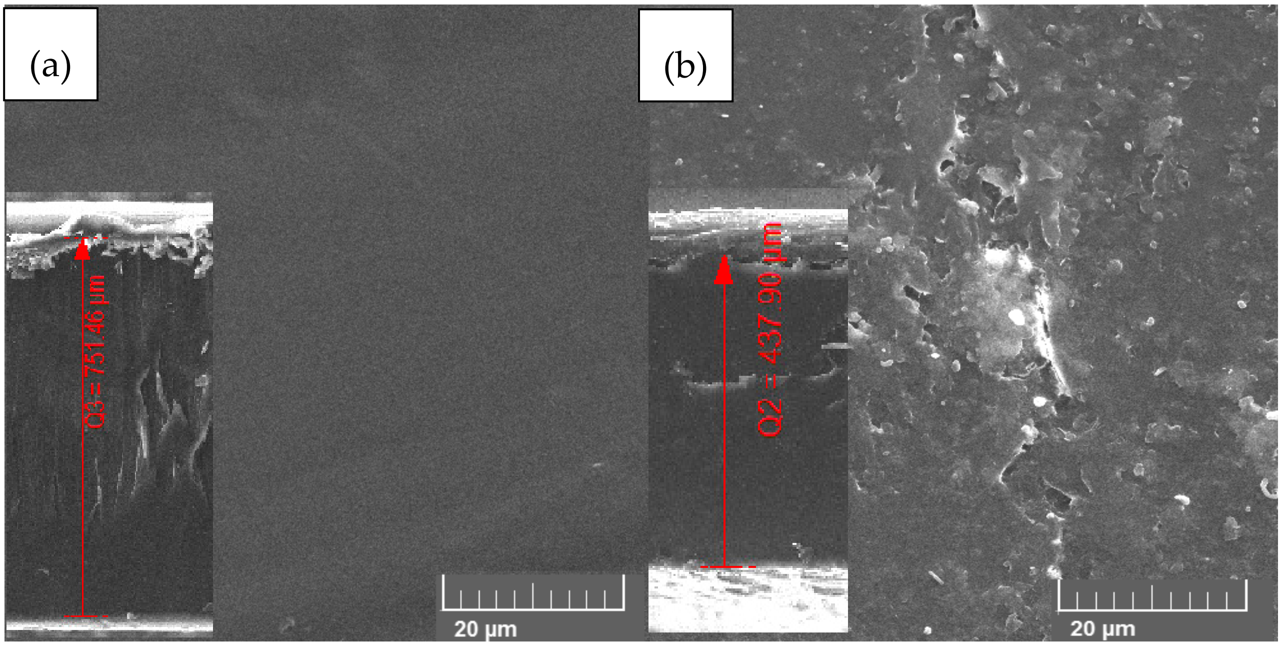

Figure 7. Micrographs of the plastic samples in their (**a**) initial and (**b**) final state after 45 days.

4. Conclusions

The research results were successful, indicating that the fungus *Aspergillus* sp. effectively generates electrical energy and reduces plastic waste simultaneously through laboratory-scale microbial fuel cells for 45 days of operation. Maximum values of electric current and voltage were generated on day 31, with values of 3.608 ± 0.249 mA and 0.572 ± 0.024 V. On this day, the substrate showed a pH of 6.57 ± 0.27 and an electrical conductivity of 257.12 ± 20.9 mS/cm. The chemical oxygen demand (BOD) was also reduced by 73.77% from 756.85 mg/L to 198.54 ± 38.95 mg/L. The maximum power density shown on day 31 was 0.124 ± 0.006 mW/cm^2 with an internal resistance of the MFC of 27.417 ± 9.810 Ω. In addition, the initial and final FTIR spectra showed the characteristic peaks of the plastic (polyethylene), observing a significant reduction in the final peaks. The peaks that suffered the most significant reduction were 2844 and 2919 cm^{-1} of the C-H compound, indicating a substantial degradation of the plastic. Finally, the final micrographs showed deformations and perforations on the surface of the plastic used as a substrate, reducing the plastic's thickness by 313.56 µm.

This research, the first of its kind, holds the potential to revolutionize the fields of microbiology, environmental science, and renewable energy. The successful use of *Aspergillus* sp. in generating electrical energy and reducing plastic waste simultaneously in MFCs is a significant step forward. The need for standardization of parameters is a clear call to action, emphasizing the importance of ongoing research and development in these areas.

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