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Self-Sustaining Bioelectrochemical Cell from Fungal Degradation of Lignin-Rich Agrowaste

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Abstract: The present work describes a self-sustaining bioelectrochemical system that adopts simple cell configurations and operates in uncontrolled ambient surroundings. The microbial fuel cell (MFC) was comprised of white-rot fungus of *Phanaerochaete chrysosporium* fed with oil palm empty fruit bunch (EFB) as the substrate. This fungal strain degrades lignin by producing ligninolytic enzymes such as laccase, which demonstrates a specific affinity for oxygen as its electron acceptor. By simply pairing zinc and the air electrode in a membraneless, single-chamber, 250-mL enclosure, electricity could be harvested. The microbial zinc/air cell is capable of sustaining a 1 mA discharge current continuously for 44 days (i.e., discharge capacity of 1056 mAh). The role of the metabolic activities of *P. chrysosporium* on EFB towards the MFC's performance is supported by linear sweep voltammetry measurement and scanning electron microscopy observations. The ability of the MFC to sustain its discharge for a prolonged duration despite the fungal microbes not being attached to the air electrode is attributed to the formation of a network of filamentous hyphae under the submerged culture. Further, gradual lignin decomposition by fungal inocula ensures a continuous supply of laccase enzyme and radical oxidants to the MFC. These factors promote a self-sustaining MFC devoid of any control features.

Keywords: microbial zinc/air cell; *Phanaerochaete chrysosporium*; white-rot fungus; membraneless MFC; lignin-rich agrowaste

1. Introduction

Biofuel cells (BFC) are popularly dubbed nature's solution in the quest for sustainable energy substitutes for fossil fuels. They represent a clean, renewable energy source which produces benign by-products as it produces energy. BFCs are bioelectrochemical devices that generate emf and subsequently electricity from oxidoreductase enzyme activities on the organic substrate. The oxidoreductase enzymes that catalyse the redox reactions are either supplied in pure isolated forms or released in situ by cultured microbes.

BFC inherently possess low energy gain yields (i.e., low output/high cost) since the biocatalysed redox reactions are complex and occur under controlled surroundings. Typically, the bioelectrochemical system comprises a dual chamber with a semi-permeable polymer such as cellulose, which separates the anodic and cathodic compartments. In the anodic compartment, microbes are cultured as a biofilm on the catalysed anode [1]. If a pure enzyme is employed, it is "wired" onto the current collector employing immobilisation procedures [2]. The anolyte is buffered to a specific pH depending on the active nature of microbes or enzymes. The anolyte is also incorporated with several other constituents, such as the electron transfer mediators [3], nutrients for microbe growth [4], carbon nanotubes (CNT) [5], and co-enzymes/co-factors (in the case of the enzymatic system) [6]. In a simple setup, the cathodic compartment comprises a catalysed air electrode in an oxygen-saturated electrolyte, added with an electron transfer mediator such as phenazines, phenothiazines, phenoxazines, and quinones [7]. Otherwise, similar requirements are applied to the catholyte.

Recent developments in BFC technology have pushed the maximum power density in the watt range [8,9], though the technology's technical and economic viability remains far from the sustainability criteria [10]. Accordingly, this work proposes a hybrid microbial electrochemical system that simplifies the complexity of the bioelectrochemical system design and consequently its cost, while markedly enhancing the energy output, paving the way towards high energy gain yields. In this study, the bioelectrochemical system employs fungal microbes that are not immobilised but, rather, suspended freely in an unbuffered electrolyte, enclosed in a membraneless, single-chamber cell, and it operates in a passive mode (uncontrolled ambient surroundings) for a prolonged duration, despite being a microbial system.

Regardless of the bioelectrochemical system's components and design, the contemporary interest is to utilise it to convert waste (i.e., complex organic substrate) into non-polluting, benign by-products when producing energy. Extensive efforts have been directed towards wastewater treatment in particular [10,11]. The hybrid system studied in this work is developed with the same interest, in which the system employs the naturally occurring white-rot fungi (i.e., *Phanaerochaete chrysosporium* fungus (Basidiomycete)). This fungal strain degrades lignin and other biopolymers by producing ligninolytic enzymes such as laccase [12,13]. Laccase is an oxidoreductase that belongs to the copper-containing enzyme family and demonstrates a specific affinity for oxygen as its electron acceptor. The fungal microbes are cultured in a carbohydrate-based electrolyte and fed with oil palm empty fruit bunch (EFB) as the organic substrate. As the fungal microbes degrade the lignin-rich waste (EFB), the laccase concomitantly catalyses the reduction of molecular oxygen. Therefore, coupling an electropositive metal anode such as zinc and an air electrode in an enclosure filled with fungal microbes fed with lignin-rich agrowaste constitutes a microbial zinc/air cell that degrades waste as it generates electricity. This paper describes the hybrid system's design, components, performance advantages, and promising prospects as a waste-decomposing cell.

2. Materials and Methods

2.1. Cell Components and Design

The cell simply comprised two electrodes (zinc anode and air cathode) inserted into a membraneless, single-compartment, 500-mL polystyrene jar, which was filled with 250 mL of electrolyte, 5 g of the dried specimen of *P. chrysosporium*, and 2 g of EFB as the organic substrate (see Figure 1). The electrolyte comprised 24 g/L potato dextrose broth (PDB). The PDB electrolyte containing the dried fungus specimen and EFB was first incubated for four days prior to being employed in the microbial fuel cell (MFC). The zinc anode (99% purity) was a 250-μm-thick foil cut into a 3 cm × 3 cm size. The air cathode used was a commercial E4/E4A EFL air electrode strip cut into the same size of 3 cm × 3 cm.
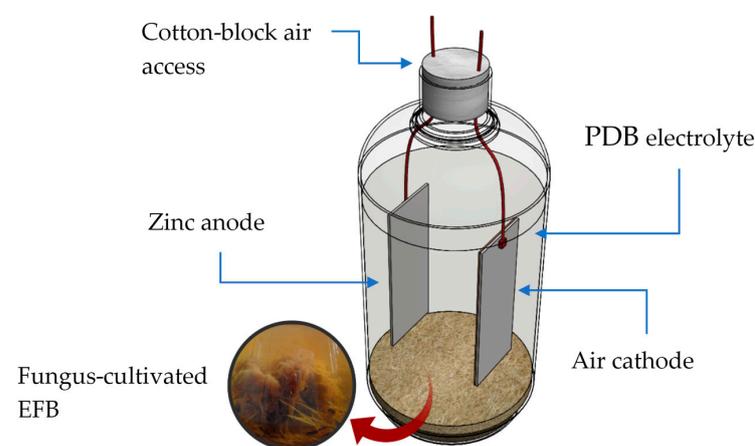

Figure 1. Schematic illustration of the fungal biofuel cell. Note that the air cathode is totally submerged in the potato dextrose broth (PDB) electrolyte.

In the MFC, the fungal microbes were left to be freely suspended in the electrolyte. They eventually grew and attached to the EFB at the bottom of the cell enclosure (refer to Figure 1). The electrolyte was not buffered, and no additives, such as electron transfer mediator, microbe's growth enhancer, or special nutrients, were added into the electrolyte. The MFC was left to operate in the uncontrolled ambient surroundings.

2.2. Microbes and Organic Substrates

A white-rot fungus strain, *Phanerochaete chrysosporium*, was used as the organic waste-degrading microbe in the microbial fuel cell. The fungal strain was initially grown by solid culture onto potato dextrose agar (PDA) for 6 days before it was recultivated by submerged culture in potato dextrose broth (PDB) at 37 °C for 14 days. Under submerged culture in PDB, the fungus spores underwent mycelial growth, forming spherical white lumps. The specimens were then filtered out from the PDB and heat-dried until the moisture content was less than 15%. The quantity of fungal microbes employed in the MFC was determined from these dried specimens.

Oil palm empty fruit bunch was obtained in its raw form from the plantation, manually shredded, and sieved into small-sized pieces. It was then soaked overnight, rinsed in distilled water, and dried under the sunlight. EFB is a lignocellulosic material which consists of cellulose, hemicellulose, and lignin [14].

2.3. MFC and Specimen Characterisations

The MFC was characterised according to its power density profile and discharge at a constant current using a Neware Battery Tester (BTS 4000, NEWARE, Shenzhen, China). Linear sweep voltammetry (LSV) was performed to correlate the *P. chrysosporium* metabolism activities with the oxygen reduction reaction (ORR) on the air cathode. LSV measurement was performed using the Eco Chemie Autolab (PGSTAT302N, Metrohm, Utrecht, The Netherlands) against a stainless-steel counter electrode (vs. Ag/AgCl) at a scan rate of 0.05 V/s. All measurements were conducted at an ambient temperature of 28 °C.

The morphology of the *P. chrysosporium* fungal colony under submerged culture (PDB) and solid-state culture (PDA) was observed under an optical microscope (Carl Zeiss GmbH). Fungal metabolic activities on EFB were examined using scanning electron microscopy (SEM) (JSM-6700F, JEOL Ltd., Tokyo, Japan). The lignin content of EFB was determined from the thermogravimetric analysis (TGA4000, Perkin Elmer, Inc., Waltham, MA, USA).

3. Results and Discussion

White-rot fungi such as *Phanerochaete chrysosporium* decomposes lignocellulosic biomass. *P. chrysosporium* has been widely researched, given its ability to produce a more complete lignin-degrading enzyme complex than most other strains [15]. As such, the fungus can decompose a broad range of organic substrates, even in aquatic environments [16]. Biodegradation of lignocellulosic fibers proceeds first with the degradation of lignin. Lignin constitutes the three-dimensional structural matrix of the plant cell wall and forms the natural protection of hemicellulose and cellulose within the cell wall. Lignin-degrading enzyme complexes (ligninolytic enzymes) comprise mainly lignin peroxidases (LiP), manganese peroxidases (MP), versatile peroxidases (VA), and laccases [17]. Among these, laccase is often singled out to be paired with an oxidative enzyme (such as pyranose oxidase) in enzymatic fuel cells [6,18]. This is because laccase only requires molecular oxygen as its co-substrate for catalysis. The most common type of lignin degradation catalysed by laccase described to date is the one-electron oxidation of hydrogen-donating phenolic substrates, forming an oxygen-centred free radical, and concomitantly the four-electron reduction of molecular oxygen to water [19].

Most biofuel cells are dual chambers separated by a polymeric, semi-permeable membrane. The oxidation of organic substrate typically occurs in the anodic chamber and the cathodic chamber is filled with a reducing agent such as ferric cyanides or supplied with an air electrode to complete the redox couple. Some researchers have coined the

term "air cathode MFC" to refer to cases where the air cathode is paired against a bio-anode (i.e., electrochemically active bacteria colony grown on the catalytic anode) [20,21]. In an enzymatic fuel cell employing laccase and bilirubin oxidase, zinc is occasionally paired as an anode against the enzyme-immobilised cathode; such a pairing is referred to as semi-EFC [22,23]. In both embodiments, the bioelectrochemical systems are not self-sustainable despite the low current output. The anodic biofilm of the air cathode MFC needs to be replenished for fed-batch mode, while, for a continuous loading operation, the MFC must be equipped with alternate pumping and retention mechanisms. As for the semi-EFC, the immobilised or wired enzyme on the air cathode needs to be replenished as the protein enzymes degrade over time. Therefore, self-sustainability and inherent cost and system complexity issues remain formidable challenges for the practical implementation of bioelectrochemical systems. The present work demonstrates a low-cost, stand-alone, and self-sustaining MFC that generates electricity out of agrowaste at the constant current output without external energy input.

The as-prepared PDB electrolyte was slightly acidic (around pH 6.5) and was not buffered. As such, in the absence of fungi microbes and EFB substrate, the control cell is essentially an acidic zinc/air cell in a weak electrolyte. The MFC registered a stable open-circuit voltage of around 1.2 V. The polarisation profile of the cell depicts the typical ohmic loss region and mass transfer loss region [24] (see Figure 2). The activation loss region is not obviously seen due to the small voltage drop as the current was withdrawn. From the ohmic region, the internal resistance of the cell was estimated to be 331 Ω. The contribution of lignin-degrading *P. chrysosporium* is evident from the subsequent power density profile. The MFC power output peaked at a current density of 3.1 A/m^2 (1.9 W/m^2) and tripled the control cell (0.6 W/m^2). MFCs have been commonly characterised under a constant load discharge, typically 1000 Ω [4,8,21,25,26]. As such, the current output is around 0.6 mA and deteriorates as the cell voltage decreases, often rapidly. A galvanostatic discharge test would be a better measure to assess the cell's capacity to generate electricity. It underlines the bioelectrochemical system's ability to sustain the charge transfer rate between electroactive species and the conducting electrode and substantiates the stability of the charge transfer mechanisms, as, otherwise, the discharge will cease.
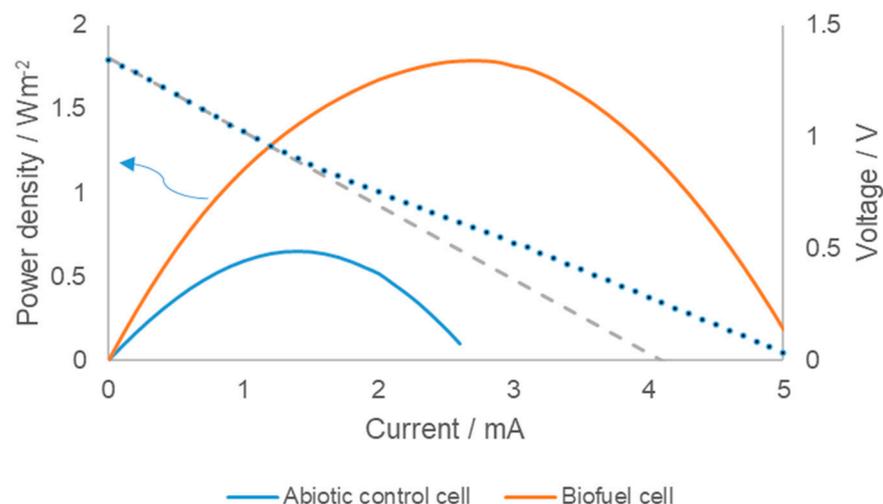

Figure 2. Polarisation profile and the subsequent power density profile of the biofuel cell. The abiotic control cell comprising a zinc anode and air cathode in a PDB electrolyte, which was slightly acidic (pH 6.5).

The microbial zinc/air cell was discharged at a constant current of 1 mA and left to operate in the uncontrolled ambient surroundings. The discharge profile resembled the metal–air characteristic profile—a flat plateau and an abrupt voltage drop at the end of discharge. The flat plateau suggests stable oxygen reduction reaction catalysis from the

fungal microbes' metabolic activities. The discharge lasted for 44 days, with an average operating voltage of 0.67 V (see Figure 3). Thus, on average, the MFC could sustain a continuous output of 0.67 mW, and the resulting discharge capacity of 1056 mAh is the highest recorded by a biocatalysed, single electrochemical cell, according to our literature survey. Notably, the control cell could not sustain the discharge current, although its polarisation profile projected a peak output at 1.4 mA. Researchers commonly use the polarisation profile as one of the performance criteria of an electrochemical cell. The measurement essentially records instantaneous power output and is best for rapid comparative purposes. However, it is not an accurate indicator to substantiate the ability of the system to sustain the desired energy output.
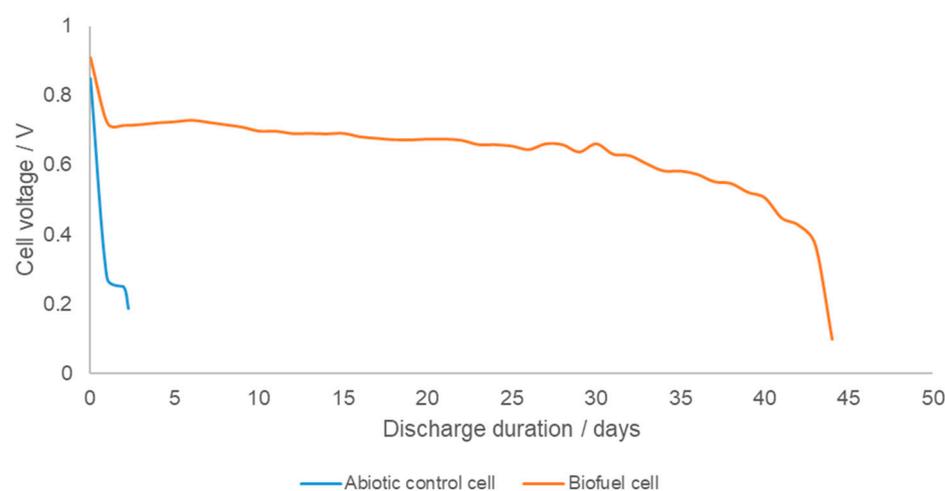

Figure 3. Discharge capacity profile of the biofuel cell rated at 1 mA.

Recently, Liu and coworkers [8] reported an MFC with a peak power output in the watt range, i.e., 3.26 Wm^{-2}, estimated from the polarisation profile; this could be the highest value reported to date. The MFC was a 200-mL dual chamber cell. The anode comprised tungsten carbide nanoparticles pasted onto carbon cloth and was inoculated with a mixture of bacteria-enriched effluent and anaerobic sludge. Sodium acetate served as the fuel in the anolyte, while potassium ferric cyanide served as the reducing agent in the catholyte. The MFC operated in batch mode, in which the system had to be replenished approximately every 6 days. In view of waste degradation, the system demonstrated excellent performance, i.e., high coulombic efficiency (83.2%) and chemical oxygen demand (COD) removal rate (95.5%). However, its discharge capacity was far lower than the value obtained in the present work. The MFC was discharged under a constant load of 1000 Ω, registered a working voltage of 0.6 V at the beginning of discharge, and ceased to discharge at around 0.5 V. Thus, during the intermittent discharge of 6 days, the average output was approximately 1.51 Wm^{-2} and the resulting average discharge capacity was only 79.2 mAh.

Nevertheless, it must also be pointed out that this work utilised a submerged air electrode in a non-air circulated cell type. MFCs that employ an air electrode either adopt an open-air air electrode (i.e., one side is exposed to air) or the cell is air/oxygen-circulated [27–29]. However, the microbial zinc/air cell studied operates solely on dissolved oxygen in the electrolyte. This was intended to substantiate the role of lignin-degrading enzymes excreted by fungal *P. chrysosporium* in catalysing the reduction of dissolved oxygen on the air electrode. Atmospheric air has approximately 20.9% oxygen by volume (209 mL/Litre), but only 35% of this proportion dissolves in water (i.e., 7–8 mL/Litre at STP) [30]. In such an oxygen-scarce environment, high catalytic activities are required to sustain the ORR. In addition, only Pt-based abiotic catalysts are durable in acidic surroundings [31]. The ability of the MFC to generate and sustain electricity with such limitations only substantiates the biocatalytic ORR on the air electrode. Linear sweep voltammetry measurement was also performed on the air

electrode in the electrolyte incubated with *P. chrysosporium* fed with EFB. The results further support the above conjecture (refer to Figure 4). In the presence of the fungal inocula, the submerged air electrode showed a higher current response over the applied potential range, which suggests increased ORR activity.
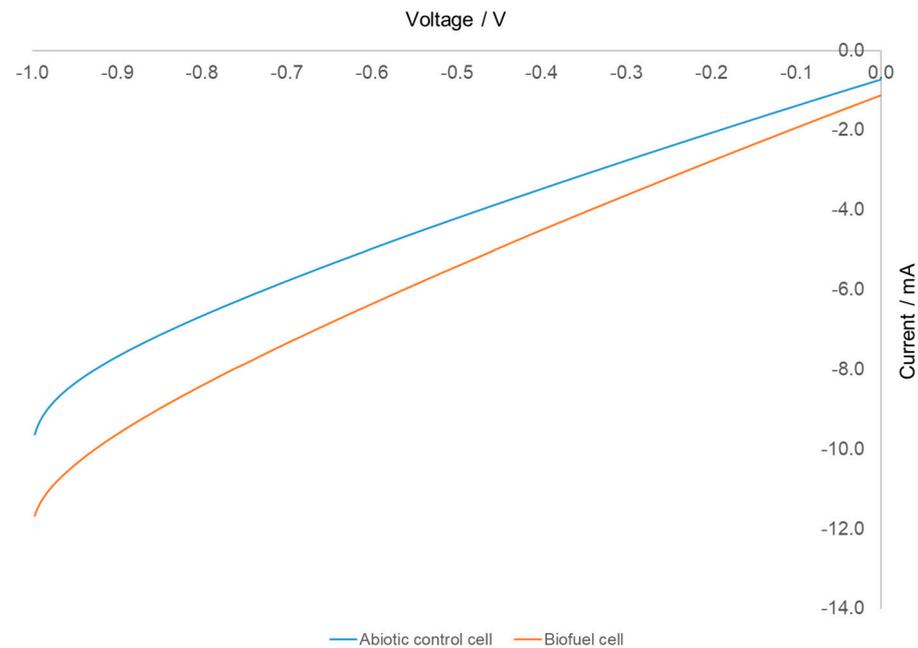

Figure 4. Linear sweep voltammetry (LSV) of the air cathode in the presence and absence of *P. crysosporium* inocula in PDB electrolyte.

This work adopted a simple cell design to establish the viability of a Zn–*P. chrysosporium* biofuel cell as a low-cost and sustainable bioenergy technology. Its performance could be further enhanced through the following approaches, at the expense of a more complex cell design and, of course, the cost. First, the use of an open-air air electrode would provide a higher concentration oxygen supply as compared to dissolved oxygen in the electrolyte. This could increase the energy output by approximately 14% [32]. Second, the separation of the anolyte and catholyte using an ionic exchange membrane could prevent current leakage. The current leakage in the absence of a membrane separator has been estimated at around 10% [33]. Finally, an air cathode biofuel cell is cathode-limiting. Enhancing the air cathode surface area would definitely increase cell performance. However, both the membrane separator and air cathode are expensive components in a biofuel cell. Both components account for at least 70% of the overall system cost [11]. Thus, the increment in cost would far exceed the energy gain, making it not viable for capacity enhancement.

The biotransformation of lignin is complex and requires the synergistic action of several enzymes. *P. chrysosporium* produces two classes of extracellular oxidative enzymes during lignin degradation (i.e., peroxidases and laccases) [34]. This process occurs simultaneously and is interrelated with cellulose depolymerisation. Laccases are considered the most important component of the ligninolytic enzyme complex [17,35] as they catalyse the oxidation of a wide range of aromatic compounds and use molecular oxygen as an electron acceptor. In the presence of a redox mediator such as syringaldehyde or acetosyringone, laccase is further capable of oxidising non-phenolic compounds [35]. Therefore, laccase may act on another compound to produce a stable radical that serves as its mediator. On the other hand, peroxidases are H_2O_2-requiring enzymes [36]. Unlike laccases, which use molecular oxygen that is readily accessible, hydrogen peroxide needs to be generated in the reaction pathways. In *P. chrysosporium*, glyoxal oxidase provides H_2O_2 for the peroxidase [35]. Peroxidases also produce secondary metabolites that serve as a reaction enhancer or mediator. Lignin peroxidases (LiP), for example, produce veratryl alcohol (VA), which is

a high-redox-potential substrate that functions as a redox mediator to oxidise lignin [37]. Often, the reaction mechanisms are focussed solely on the laccase catalytic reduction of molecular oxygen. Figure 5 below illustrates several probable redox reactions that may occur on the air cathode since it is an electron donor site. The presence of these oxidants in the vicinity of the EFB is thought to render the bioelectrochemical system self-sustainable for an extended duration.
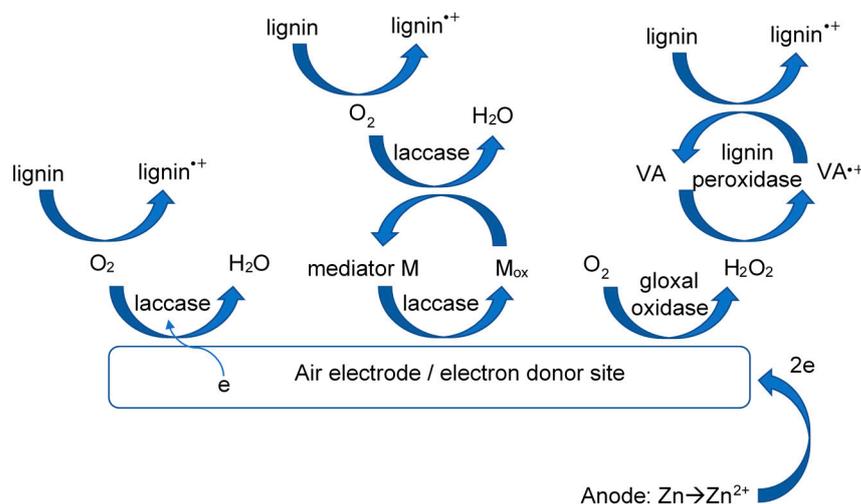

Figure 5. The illustration indicates the probable interactions between the air electrode as a charge donor site and several established reactions [34,35] involving extracellular enzymes during lignin oxidation.

The next issue that needs to be addressed is that, unlike other MFCs, the fungal microbes in the present work were not cultured on the air cathode or current collector. Therefore, the likelihood of the stated reactions may seem to be low or difficult to sustain. However, the discharge capacity of the fungal biofuel cell obtained proved otherwise. Aside from the oxidant-rich surroundings induced by the lignin degradation, what made the fungal biofuel cell sustainable was the morphology of the fungal inocula. Under a submerged culture, *P. chrysosporium* inocula developed into a mycelial colony. A further, close-up view of the mycelial colony revealed a submicrometer-sized (~0.2 μm), filamentous network of hyphae that branched out from the mycelial cells (see Figure 6a,b). As such, the whole cell electrolyte, particularly in the vicinity of the air cathode, was most likely entangled with a network of long filamentous hyphae, promoting the aforementioned redox reaction pathways. When cultured onto potato dextrose agar (PDA), *P. chrysosporium* propagates as white sporangium colonies (see Figure 6c), which were of completely different morphology to the mycelial colonies.

Fungal degradation of the EFB substrate was examined under SEM. Observations were made before and after exposure to *P. chrysosporium* under submerged culture for a period of 10 days. The SEM images are shown in Figure 7. Following 10 days, the rugged lignin external surface had been smoothed and thinned, resulting from the metabolic activities of the fungal microbes. Shallower silica body craters can be observed in Figure 7b. On day 20, the outer protective lignin layer started to collapse, as shown in Figure 7c. Finally, after one month, the innermost lignin structure was exposed, revealing the fibre's unique structural core matrix (see Figure 7d). The lignin composition in EFB was approximately 42%, as estimated from the thermogravimetric analysis (results not shown). The recalcitrant nature of lignin is evident from the SEM images. It took around one month for the fungal microbes to penetrate deeply into the lignin structure's inner core. From the viewpoint of the MFC's performance, the gradual degradation of lignin is favourable. Indeed, this ensures that the electrochemical system has a continuous supply of biocatalysts and radical oxidants, provided that the discharge current does not exceed the limiting charge transfer rate of the redox reactions involved. For the MFC studied in this work, the discharge data

suggest that 1 mA is a well-balanced charge transfer rate. Attempts at a higher discharge rate only lasted several days.
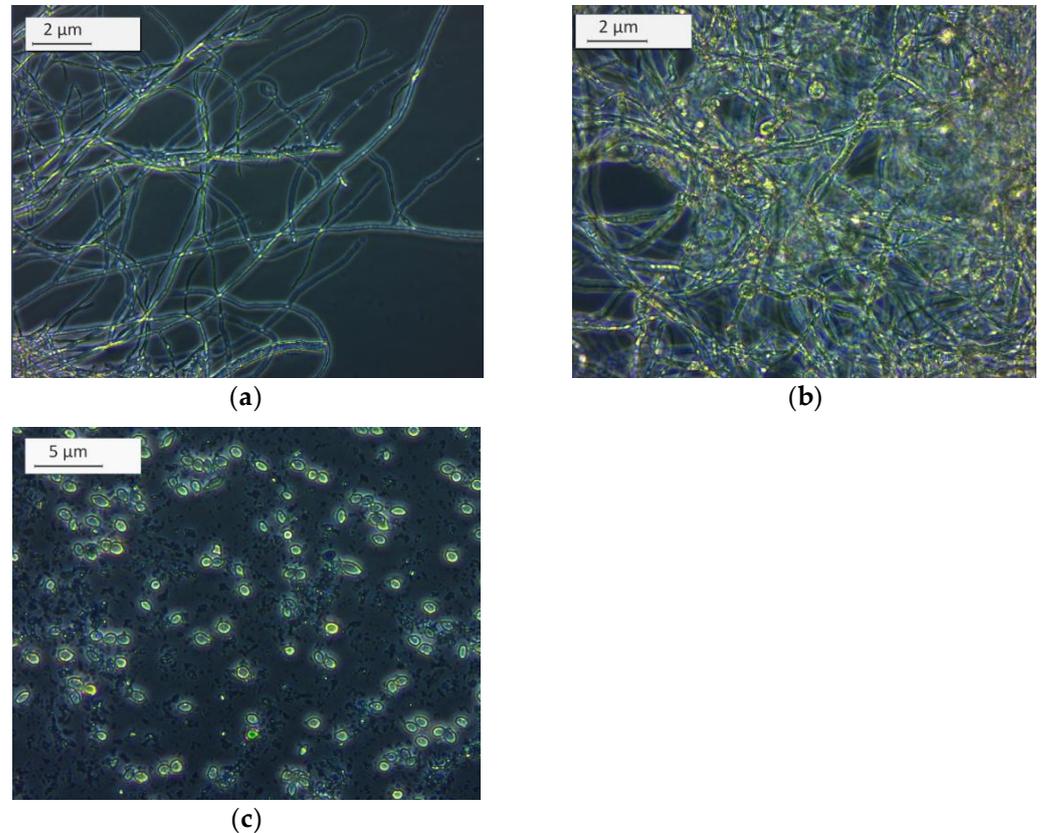

Figure 6. Optical microscopy images (100×) of *P. chrysosporium* filamentous hyphae network formed under submerged culture (**a**,**b**), as compared to the sporangium cells' morphology when grown onto potato dextrose agar (PDA) (**c**).

Bacterial MFCs have been investigated more than fungal MFCs. Among fungal MFCs, yeasts have been the most widely employed microbes since they are easy to grow and susceptible to biological and genetic manipulation [38,39]. There have been at least two reports on the use of white-rot fungus as the source for the cathodic catalyst. Wu and coworkers [29] paired a hexacyanoferrate anolyte and catholyte inoculated with a white-rot strain of *Coriolus versicolor* in a 100-mL H-shaped MFC. Both anolyte and catholyte were buffered and incorporated with activated carbon fibres. The catholyte was continuously stirred and air-saturated. Upon activation by an ABTS chemical mediator (2,2′-Azi-no-bis(3-ethylbenzthiazoline-6-sulfonic acid)), the MFC could only generate a discharge current of around 180 µA, fading within 24 h, and needed to be dosed repeatedly with ABTS to induce the electricity. Separately, Lai and coworkers [40] studied the synergistic two-stage degradation of azo dye acid orange 7 (AO7) in a single-chamber MFC. However, the single-chamber cell needed to be physically separated by a 10-cm-thick polyvinyl alcohol hydrogel since the anodic bacteria colony degrades AO7 under anaerobic surroundings (stage 1), while the white-rot fungus of *Ganoderma lucidum* requires oxygen for the second stage of degradation. The fungus was first cultured on PDA, overlaid onto a carbon cloth air electrode, and incubated for 14 days for further growth. Once assembled as a complete MFC, the setup required a further 20 days' stabilisation, though the current output from the system was less than 150 µA. In both works, the system designs were complicated, not self-sufficient, and could only generate current in the µA range.
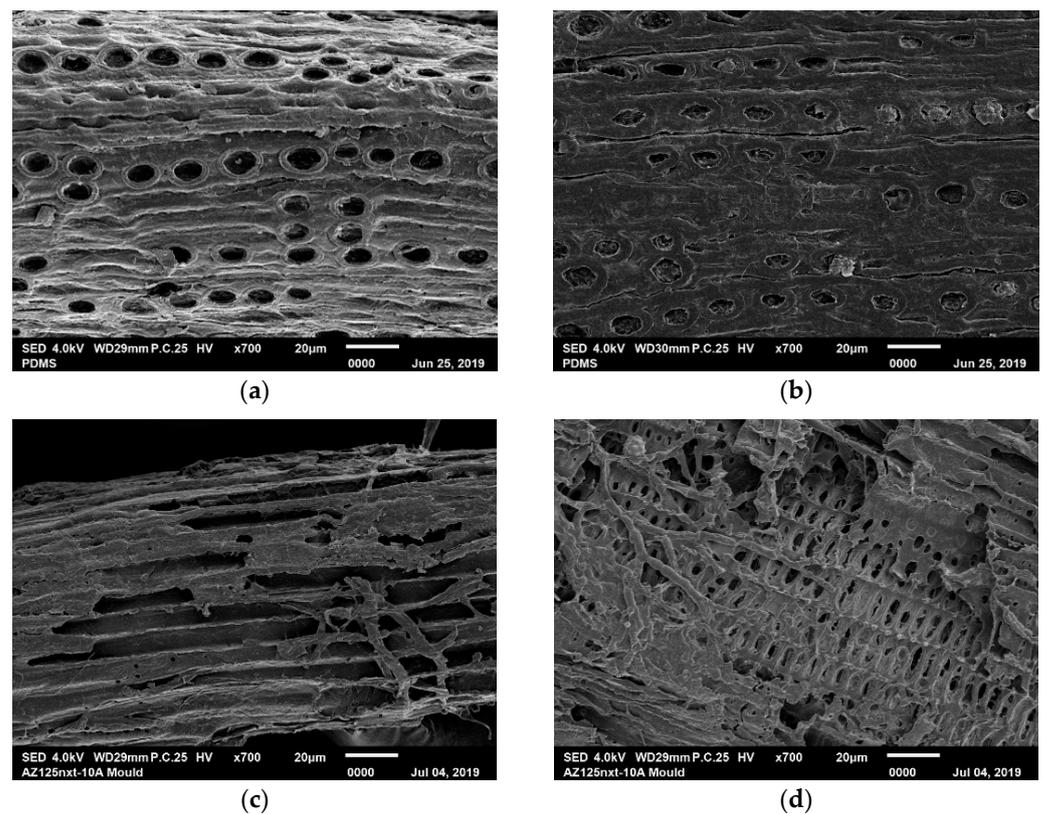
Figure 7. SEM images showing degradation stages of EFB lignin under continuous exposure to *P. chrysosporium* in PDB electrolyte: (**a**) day 0; (**b**) day 10; (**c**) day 20; (**d**) day 30.

A self-sustaining MFC is a combination of robust microbes, a suitable substrate, and optimal system design. White-rot fungi are robust, ubiquitous microorganisms, having remarkably high adaptive behaviour and tolerance to changing environments. As such, they have been extensively studied for bioremediation [41]. Providing *P. chrysosporium* with lignin-rich EFB yields versatile a machinery of enzymes, laccase, and peroxidase, which complement each other in ligninolysis [42]. Consequently, the MFC is not only supplied with oxidoreductase but is enriched with oxidants that could serve as electron shuttle mediators. Further, the hyphae network of *P. chrysosporium* developed under a submerged culture promotes a simple MFC design. The integration of these factors produces a self-sustaining MFC devoid of any control features and capable of sustaining a constant discharge current over a prolonged period.

4. Conclusions

In this paper, a simple MFC design configuration was introduced by pairing a zinc anode with the biocatalytic activities of white-rot *P. chrysosporium* fungi fed with agrowaste (EFB) substrate. The cell was a single-chamber, membraneless enclosure left to operate in uncontrolled ambient surroundings. However, as demonstrated in this paper, the MFC was capable of sustaining a 1-mA discharge current for 44 days continuously, with an average operating voltage of 0.67 V (1056 mhA capacity). Unlike most MFCs, the fungal microbes were not cultured on the current collector but left to be freely suspended in the unbuffered electrolyte. *P. chrysosporium*'s long filamentous hyphae network is thought to contribute significantly to sustaining stable charge transfer processes between the air electrode and fungal microbes. Moreover, the gradual fungal degradation of EFB ensures that the MFC is not only supplied with biocatalysts but is enriched with oxidants that serve as charge transfer mediators. In essence, these results could possibly pave the way towards a high-yield biofuel cell for practical implementation in bioenergy harvesting in the near future.

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