Fungi-Based Microbial Fuel Cells

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Abstract: Fungi are among the microorganisms able to generate electricity as a result of their metabolic processes. Throughout the last several years, a large number of papers on various microorganisms for current production in microbial fuel cells (MFCs) have been published; however, fungi still lack sufficient evaluation in this regard. In this review, we focus on fungi, paying special attention to their potential applicability to MFCs. Fungi used as anodic or cathodic catalysts, in different reactor configurations, with or without the addition of an exogenous mediator, are described. Contrary to bacteria, in which the mechanism of electron transfer is pretty well known, the mechanism of electron transfer in fungi-based MFCs has not been studied intensively. Thus, here we describe the main findings, which can be used as the starting point for future investigations. We show that fungi have the potential to act as electrogens or cathode catalysts, but MFCs based on bacteria–fungus interactions are especially interesting. The review presents the current state-of-the-art in the field of MFC systems exploiting fungi.

Keywords: fungi; microbial fuel cell; biocatalyst

1. Introduction

The technology known as microbial fuel cells (MFCs) has been intensively developed over the last two decades, due to its great potential for clean energy production in the form of electric current [1,2]. MFCs owe their popularity to various microorganisms: Bacteria, fungi, or algae whose catalytic activity allows for current generation from a wide range of substrates, from simple sugars to toxic, highly polluted wastewaters [3]. Usually, microorganisms are used in MFCs in the anode compartment, where they transfer electrons released from the microbial oxidation of an organic substrate. The protons formed during oxidation pass through a proton-selective membrane to the cathode, where they combine with oxygen to form water. The spontaneous movement of electrons from anode to cathode results in the production of electric current in the system. Nowadays, the most popular MFCs are those exploiting bacteria, which were initiated after the first electrogenic strains, such as Shewanella sp. or Geobacter sp., were discovered in the late 1990s [4,5]. Electrogens were found to transfer electrons directly to the anode through outer membrane transporting proteins, like cytochrome c, or through membrane appendages called nanowires [6]. Other electrogenic strains, like Pseudomonas aeruginosa, have been proved to self-produce endogenous mediators, such as pyocyanin, that can shuttle electrons to the anode [7]. Electrogenic bacteria are exploited in MFC systems as single strains or in bacterial consortia, which are especially favorable when complex substrates are used for current production [8].

Although the breakthrough discovery of the electrical effects accompanying organic matter decomposition was made in 1911 on yeast and bacteria simultaneously, fungi-based MFCs were less investigated than bacterial ones [9]. This lower interest in fungal MFCs was connected with the lack of proved fungal electrogens and the lower power production of MFCs working on single fungi
strains in comparison to bacteria. However, over the 10 last years, investigations especially devoted to mediatorless fungi-based MFCs have appeared, and their results have revealed the electrogenic potential of fungi [10]. Recent findings suggest that the mechanism of electron transfer realized by fungi may be direct, similar to bacteria, when the redox enzymes present in the fungal cell membrane, e.g., ferricyanide reductase or lactate dehydrogenase, are involved [11]. It has been also supposed that some short-lived electroactive compounds behaving like mediators may also be included in the process [12].

The most intensively studied systems among fungal MFCs are yeast-based ones, where direct electron transfer was proved via cytochrome c [13]. However, recent findings indicate that other fungi also have redox-active enzymes that can assure electrogenic activity in MFC systems. Fungi belonging to the group of white-rot—well-known wood degraders—were found to have an extracellular oxidative ligninolytic enzymatic system that enables them to degrade lignin [14,15]. Enzymes of this system also allow for the degradation of various xenobiotic compounds and dyes [16]. The major extracellular enzymes are oxido-reductases, i.e., laccases (EC 1.10.3.2), manganese peroxidases (MnP, EC 1.11.1.13), and lignin peroxidases (LiP, EC 1.11.1.14). Additionally, some auxiliary enzymes, such as cellulose dehydrogenase (CDH, EC 1.1.99.18) and glucose oxidase, have been also isolated from white-rot fungi. Laccases are a group of N-glycosylated blue oxidases that contain four copper atoms localized in distinct binding sites within the active site. These enzymes are able to catalyze the oxidation of phenolic compounds and aromatic amines by utilizing atmospheric oxygen as the electron acceptor [17]. Laccase has been frequently applied as a cathodic biocatalyst in enzymatic fuel cells, due to its high oxidation/reduction potential [18–21]. Moreover, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) is known as an effective mediator to transfer electrons from the electrode to laccase [22,23].

Next, MnPs are glycosylated glycoproteins with a heme prosthetic group, which preferentially oxidizes Mn$^{2+}$ into Mn$^{3+}$. Mn$^{3+}$ ions are stabilized by chelating agents, such as oxalic acid. These chelators can be introduced as exogenous chemical reagents or can be secreted by fungi. Mn$^{3+}$ stabilized with a chelator demonstrates higher reactivity and can also act as an additional redox mediator [24–29]. LiPs are also N-glycosylated proteins containing heme in the active site and have a mechanism of action typical for peroxidases. CDH is also a N-glycosylated peptide and consists of two domains. It is worth noting that the CDH structure is recognized as being the only known extracellular flavocytochrome [30–32]. The first domain is a small cytochrome domain (CYT), which is located at the N-terminus and includes a heme b redox cofactor. The second is a C-terminal flavodehydrogenase domain (DH), which uses flavin adenine dinucleotide (FAD) as a redox cofactor [33,34]. CDH is involved in the reaction of carbohydrate oxidation—mainly of cellulose and lactose, followed by maltose and glucose [35,36]. During the oxidation of carbohydrates, two electrons are obtained in the DH domain. Further, the obtained electrons can be moved to a two-electron acceptor by the DH domain. Alternatively, electrons can be transferred to a one-electron acceptor by either the DH or the CYT domain. Transport of electrons by the CYT domain requires prior internal electron transfer (IET) from the DH domain to the CYT domain [37]. Additionally, it was found that CDH uses the CYT domain for direct electron transfer from substrate to anode. In this regard, the CYT domain plays the role of a mediator and thus eliminates the need for adding an exogenous mediator during the oxidation processes [38,39].

Fungi have been used in the MFC systems in two main modes (Figure 1): In the anode (electron transfer is realized directly, through redox-active fungal proteins or through chemical mediators facilitating the electron transport) or in the cathode (fungi are the source of enzymes catalyzing the reduction of a terminal electron acceptor, mainly oxygen). This article reviews the current state-of-the-art in the field of fungi-based MFCs, presenting the use of fungi as anode and cathode catalysts or as a supporting factor in bacterial MFCs.
2. Fungi as Biocatalysts in the Anode of MFCs

The anode is a crucial element of each MFC owing to its direct contact with the electron-producing microorganisms. The anode not only ensures electron conductivity, but also strongly influences the adhesion of microorganisms, which determines the biofilm formation needed for ensuring the electron transfer from the microorganism to the electrode. Various fungi species have been used as anode microorganisms, producing electrons from different substrates and transferring them to the anode directly via redox-active enzymes or due to chemical mediators. The power production obtained from fungi-based MFCs is from several mWm\(^{-2}\) to as high as several Wm\(^{-2}\), depending on the anode material and construction, the fungi type, and the presence of a mediator.

2.1. Saccharomyces cerevisiae

Yeasts are among the best-studied eukaryotic cells, as they are easy to grow and susceptible to biological and genetic modifications. Most strains are not pathogenic and can metabolize a wide range of substrates. For these reasons, yeast has also been most widely considered as a biocatalyst in MFCs. During the last two decades, several papers reporting the utilization of Saccharomyces cerevisiae as an anode biocatalyst in yeast-based MFCs, with or without an external mediator, have been published. A variety of exogenous mediators, e.g., methylene blue (MB), neutral red (NR), or thionine, were applied in order to enhance electron transfer between the microorganism and the anode (Table 1).
Bennetto et al. reported that Baker’s yeast could be utilized as a biocatalyst in the anode of an MFC where two mediators—thionine and resorufin—were used [40]. The results showed that an MFC employing \( \text{S. cerevisiae} \) immobilized on the anode with resorufin as a mediator obtained a maximum power density of 155 mWm\(^{-2}\). Yeast-based MFCs with the addition of MB as a mediator were also studied by Walker et al. [41] and Permana et al. [42]. They used \( \text{S. cerevisiae} \) to determine optimal conditions for electricity generation with respect to initial fungi concentration, temperature, substrate, and oxygen concentration in double-chamber yeast-based MFCs. However, power production lower than that for thionine and resorufin was obtained [40]. The effect of MB on the performance of a \( \text{S. cerevisiae} \)-based two-compartment glucose-fed MFC was examined elsewhere [43]. A maximum power density of \( 146 \pm 7.7 \text{ mWm}^{-3} \) was achieved at a 1000 \( \Omega \) resistance. The low yield of the yeast-based fuel cell in this experiment was assigned to the \( \text{O}_2 \) reduction overpotential and inefficient electron transfer between the mediator and cell walls of \( \text{S. cerevisiae} \). The authors reported cytotoxicity of the mediator as a factor limiting the power output.

As the construction of the anode significantly affects the performance of an MFC, modification of its surface was proposed to improve \( \text{S. cerevisiae} \)-based MFC performance. The goal was to enhance microorganism adhesion and electron transfer from the biocatalyst to the anode surface. For these investigations, carbon paper electrodes with a thin (5 nm and 30 nm) layer of cobalt or gold were used in \( \text{S. cerevisiae} \)-based MFCs [44,45]. It was observed that application of a Co layer significantly increased the adhesion of \( \text{S. cerevisiae} \) cells to the anode surface and enhanced the performance of the MFC. Another modification of the anode surface was the immobilization of yeast cells on carbon nanotubes (CNTs). Christwardana and Kwon studied the performance of mediatorless MFCs, employing \( \text{S. cerevisiae} \) cells immobilized on CNTs as the anodic catalyst (yeast/CNT) and laccase as the biocatalyst in the cathode [46]. Yeast cells were bonded to CNTs covalently (C–N), and electron transfer was enhanced by the hydrophobic interaction between carbon nanotubes and yeast. Additionally, the effect of a yeast-based catalyst structure containing poly(ethyleneimine) (PEI) and glutaraldehyde (GA) on MFC performance was investigated. PEI was used as an entrapping polymer to support the adhesion of yeast cells to CNTs (positive charge on PEI and negative charge of yeast), while GA acted as a cross-linker between \( \text{S. cerevisiae} \) cells and PEI. The use of both investigated yeast-based anodic catalysts significantly improved the stability and performance of the MFCs. The maximum power density increased from 138 mWm\(^{-2}\) for the MFC employing bare CNTs to 344 mWm\(^{-2}\) with yeast-based anode catalysts. Enhanced power generation in MFCs using a yeast/CNT catalyst was explained by facilitated electron transport via cytochrome c and cytochrome a3 when this catalyst was applied. The obtained power production here was significantly higher than for other \( \text{S. cerevisiae} \)-based MFCs. In other studies, the application of \( \text{S. cerevisiae} \) immobilized on reticulated vitreous carbon (RVC) with neutral red (NR) as a mediator achieved a much lower power density, i.e., ca. 80 mWm\(^{-2}\) [13], and the maximum power density in the MFC with \( \text{S. cerevisiae} \) immobilized on a graphite electrode with thionine as a mediator peaked at 60 mWm\(^{-2}\) [47]. However, the value of production for the yeast/CNT catalyst-based MFC was lower than that of an MFC employing \( \text{S. cerevisiae} \) immobilized on carbon felt with an MB mediator, where the maximum power density was 1500 mWm\(^{-2}\) [48].
| Fungus                  | MFC Type                  | Electron Acceptor | Cathode Material | Anode Material | Separator | Substrate | Electron Transfer Mechanism | Max. Power Density |
|------------------------|---------------------------|-------------------|------------------|----------------|----------|-----------|----------------------------|------------------|
| **Saccharomyces cerevisiae** | Double chamber            | Potassium ferricyanide | Platinum mesh    | Platinum mesh  | Nafion   | glucose   | Mediatortless               | 65               |
| **S. cerevisiae**       | Dual chamber              | Potassium permanganate | Copper electrode | Copper electrode | SPEEK   | glucose   | Exogenous Mediator          | 4.48             |
| **S. cerevisiae**       | Double chamber            | Potassium ferricyanide | RVC              | RVC            | Nafion   | Glucose   |                           | 40.00 ± 7.7      |
| **S. cerevisiae**       | Single chamber            | O<sub>2</sub> (air) | Pt/C over carbon paper | Carbon paper   | Nafion   | glucose   | +                          | 12.9             |
| **S. cerevisiae**       | Single chamber            | O<sub>2</sub> (air) | Pt/C over carbon paper | Carbon paper   | Nafion   | glucose   | N/A                        | 20.2             |
| **S. cerevisiae**       | Single chamber            | O<sub>2</sub> (air) | CNT              | Bare CNT/GA/PEI/CNT | Membrane less | glucose   | +                          | 138              |
| **S. cerevisiae**       | Double chamber            | Potassium ferricyanide | RVC              | RVC            | Nafion   | dextrose  | N/A                        | 40.00             |
| **S. cerevisiae**       | Double chamber            | O<sub>2</sub> (air) | Graphite plate   | Graphite plate | Nafion   | glucose   | Thionine                   | 3                 |
| **S. cerevisiae**       | Double chamber            | Potassium ferricyanide | Carbon felt      | Carbon felt    | Nafion   | glucose   | 1500                       | N/A               |
| **S. cerevisiae**       | Double chamber            | O<sub>2</sub> (air) | Graphite plate   | Graphite plate | Nafion   | glucose   | 7                          | N/A               |
| **S. cerevisiae**       | Double chamber            | O<sub>2</sub> (air) | Graphite rods    | Graphite rods/MWCNT | Nafion   | lactose   | 2.7                        | N/A               |
| **S. cerevisiae**       | Double chamber            | O<sub>2</sub> (air) | Graphite rods    | Graphite rods/MWCNT | Nafion   | lactose   | 33                         | N/A               |
| **S. cerevisiae**       | Single chamber air cathode | O<sub>2</sub> (air) | Pt/C over carbon cloth | Graphite plate | Teflon   | d-glucose | 8                          | N/A               |
| **S. cerevisiae**       | Single chamber air cathode | O<sub>2</sub> (air) | Pt/C over carbon cloth | Graphite plate | Teflon   | d-glucose | 31                         | N/A               |
| **S. cerevisiae**       | Single chamber air cathode | O<sub>2</sub> (air) | Graphite plate   | Graphite plate | Nafion   | Synthetic wastewater | +                | 25.51            |
| **S. cerevisiae**       | Single chamber air cathode | O<sub>2</sub> (air) | Graphite plate   | Graphite plate | Nafion   | glucose   | +                          | 3                |
| Fungus                  | MFC Type            | Cathode Material  | Anode Material  | Separator | Substrate         | Electron Transfer Mechanism | Max. Power Density |
|------------------------|---------------------|-------------------|-----------------|-----------|-------------------|-----------------------------|-------------------|
| **Table 1. Cont.**     |                     |                   |                 |           |                   |                             |                   |
| **Electron Acceptor**  | **Cathode Material**| **Anode Material**| **Separator**   | **Substrate** | **Max. Power Density** |
| *S. cerevisiae*        | Double chamber      | $O_2$ (air)       | Graphite plates | Nafion    | glucose            | N/A                         | 60 mW/m²          |
|                        |                     | Potassium permanganate | Graphite plates | Nafion    |                    | NR                          | 133 mW/m²         |
|                        |                     |                    |                 |           |                   |                             | N/A               |
| *S. cerevisiae*        | Double chamber      | $O_2$ (air)       | Graphite plates | Nafion    | glucose            | N/A                         | 0.414 mW/m²       |
|                        |                     | Potassium ferricyanide | Graphite plates | Nafion    |                    | NR                          | 2 mW/m²           |
|                        |                     |                    |                 |           |                   |                             | N/A               |
| *S. cerevisiae*        | Double chamber      | Potassium ferricyanide | Graphite plates | Nafion    | glucose            | N/A                         | 0.33 mW/m²        |
|                        |                     | Carbon cloth       | Carbon cloth    | Nafion    |                    | NR                          | 123.4 mW/m²       |
|                        |                     |                    |                 |           |                   |                             | N/A               |
| Candida melibiosica    | Double chamber      | Potassium ferricyanide | Plane graphite rods | Salt bridge | Fructose            | N/A                         | 60 mW/m²          |
|                        |                     |                    |                 |           |                   | NR                          | 180 mW/m²         |
|                        |                     |                    |                 |           |                   |                             | 185 mW/m²         |
|                        |                     |                    |                 |           |                   |                             | 185 mW/m²         |
|                        |                     |                    |                 |           |                   |                             | 185 mW/m²         |
| Candida melibiosica    | Double chamber      | Potassium ferricyanide | Carbon felt | Carbon felt | Nafion              | YP$_{fru}$ + | 20 mW/m²          |
|                        |                     |                    |                 |           |                   | N/A                         | 46 mW/m²          |
|                        |                     |                    |                 |           |                   |                             | 89 mW/m²          |
|                        |                     |                    |                 |           |                   |                             | 113 mW/m²         |
|                        |                     |                    |                 |           |                   |                             | 137 mW/m²         |
|                        |                     |                    |                 |           |                   |                             | 140 mW/m²         |
| Candida melibiosica    | Double chamber      | Potassium ferricyanide | Carbon felt | Carbon felt | Nafion              | YP$_{fru}$ + | 36 mW/m²          |
|                        |                     |                    |                 |           |                   | N/A                         | 720 mW/m²         |
|                        |                     |                    |                 |           |                   |                             | 390 mW/m²         |
| Azotobacter vinelandii | Continuous flow, dual chamber | Potassium permanganate | Carbon fibre cloth | Carbon fibre cloth | Nafion | YP$_{fru}$ + | N/A | N/A | 28 mW/m²          |
|                        |                     |                    |                 |           |                   |                             | 100 mW/m²         |
| Candida sp. IR11       | Single chamber      | $O_2$ (air)       | ADE 75          | Carbon felt | N/A | glucose rejected wastewater | + | N/A | 20.6 ± 1.52 mW/m² |
|                        |                     |                    |                 |           |                   |                             | N/A               |
| *C. melibiosica*       | Double chamber      | Potassium ferricyanide | Carbon felt | Carbon felt | Nafion | YP$_{fru}$ + | N/A | N/A | 52 ± 9 mW/m²     |
|                        |                     |                    |                 |           |                   |                             | 83 ± 8 mW/m²       |
|                        |                     |                    |                 |           |                   |                             | 89 ± 15 mW/m²      |
|                        |                     |                    |                 |           |                   |                             | 135 ± 6 mW/m²      |
|                        |                     |                    |                 |           |                   |                             | 260 ± 8 mW/m²      |
| Hansenula anomala      | Double chamber      | Potassium ferricyanide | Plane graphite | Plane graphite/polyaniline-Pt | N/A | Glucose, dextrose, malt extract | + | N/A | 45.6 × 9 mW/m² |
|                        |                     |                    |                 |           |                   |                             | 89 × 4 mW/m²       |
|                        |                     |                    |                 |           |                   |                             | 135 × 6 mW/m²      |
|                        |                     |                    |                 |           |                   |                             | 260 × 8 mW/m²      |
| Kluyveromyces marxianus | Dual chamber        | Potassium ferricyanide | Carbon rods | Carbon fiber bundles | Cation-specific membrane | glucose | YP$_{fru}$ + | N/A | N/A | 690–2900 mW/m² |
|                        |                     |                    |                 |           |                   |                             | 850,000 mW/m²      |
| Lipomyces starkeyi     | Dual chamber        | Potassium permanganate | Stainless steel | Stainless steel | Nafion | Palm oil mill effluent | + | N/A | 12,870 mW/m² |

RVC—reticulated vitreous carbon is abbreviated, CNT—carbon nanotubes MWCNT—multi-walled carbon nanotube, SPEEEK—sulphonatedpoliether ether ketone, ADE 75—air diffusion electrode, MB—methylene blue, NR—neutral red, BcG—bromocresol green, MR—methyl red, MO—methyl orange, GA—glutaraldehyde, PEI—poly(ethylenimine), GOx—glucose oxidase, CDH—cellobiose dehydrogenase, PDH—pyranose dehydrogenase, YP$_{fru}$—medium containing yeast extract, peptone and fructose.
In another study, Fishilevich et al. investigated the performance of a dual-chamber glucose-fed MFC employing *S. cerevisiae* with glucose oxidase (GOx) from *Aspergillus niger* [49]. The applied enzyme is a highly efficient redox enzyme, which converts β-D-glucose to glucono-δ-lactone and hydrogen peroxide. The anode compartment comprised GOx surface-displaying yeast cells, MB as a redox mediator, and glucose as a substrate. The cathode compartment contained a laccase from *Trametes versicolor* as a catalyst and ABTS as a redox mediator. However, the obtained maximum power density obtained in this MFC was relatively low: ca. 13.6 mWm\(^{-2}\). Gal et al. studied the performance of a *S. cerevisiae*-based MFC using two different surface-displayed dehydrogenases [50]. They were cellobiose dehydrogenase from *Corynascus thermophilus* and pyranose dehydrogenase (PDH, EC 1.1.99.29) from *Agaricus meleagris* and were displayed on the *S. cerevisiae* surface using a yeast display system. *S. cerevisiae* cells with PDH were used as an anodic biocatalyst in a two-compartment mediatorless MFC using lactose as a substrate. The MFC employing PDH-displaying *S. cerevisiae* produced a maximal power output of 33 mWm\(^{-2}\), which was ca. 12 times higher than that obtained in the MFC using unmodified *S. cerevisiae* (2.7 mWm\(^{-2}\)). In another study, *S. cerevisiae* was used as an anodic biocatalyst in an air-cathode MFC using synthetic wastewater as a substrate and graphite as electrodes [51]. It was shown that the yeast-based MFC power production was associated with the substrate concentration and the pH. The highest current density was observed at pH 6.0–160.36 and 282.83 mA/m\(^2\) at an organic loading rate of 0.91 and 1.43 kg COD/m\(^3\), respectively. The investigations of the electron transfer mechanism revealed the presence of redox mediators, such as NADH/NAD\(^+\) and FADH/FAD\(^+\), and suggested that the higher rate of electron transfer at pH 6.0 was connected with enhanced proton shuffling by different redox mediators.

The utilization of complex substrates in MFCs requires the application of microbial consortia wherein various species are able to accomplish diverse processes. It is commonly known that the fermentative microorganisms of the consortium can transform large organic molecules into smaller fermentation products that can be used by electrogenic species. Lin et al. designed an MFC that employed a microbial consortium, including the electrogen *Shewanella oneidensis* MR-1 and genetically engineered *S. cerevisiae* as a fermenter, with glucose as a carbon source [56]. The ethanol pathway was knocked out in *S. cerevisiae* cells, while the lactic acid pathway was programmed in the cells. As a result, *S. cerevisiae* metabolized glucose to lactic acid, which was further used by *S. oneidensis* for power production. Additionally, the efficiency of such a fermenter–exoelectrogen consortium was increased by achieving an optimal relation between the carbon metabolism of *S. cerevisiae* and the extracellular electron transfer of *S. oneidensis*. Moreover, the use of *S. cerevisiae*, which did not form a biofilm on the anode, allowed the anode surface to be entirely occupied by the exoelectrogen, which also enhanced MFC performance. The maximum power density obtained from that glucose-fed MFC was 123.4 mWm\(^{-2}\).

2.2. *Candida* sp.

2.2.1. *Candida melibiosica*

*Candida melibiosica* 2491 is a yeast strain with high phytase activity that digests the hardly biodegradable phosphorous compounds of plant tissues. Hubenova and Mitov found the oxidation–reduction potential for *C. melibiosica* without the chemical addition of artificial mediators, which indicates its ability to transfer electrons in MFCs [57]. To examine the possibility of using *C. melibiosica* as an electron shuttle, the performance of an MFC employing this yeast strain was investigated. Results of experiments with different carbohydrates, i.e., glucose, fructose, and sucrose, demonstrated that the *C. melibiosica*-based MFC could produce bioelectricity even in the absence of an exogenous mediator, with a maximum power output of 60 mWm\(^{-3}\) when fructose was used as a substrate. It has been observed that there is a correlation between the produced current, yeast cell growth phases, and the rate of the substrate assimilation, which was demonstrated by the rate of in vivo produced electrons. Additionally, the influence of other exogenous mediators with various formal
potentials (bromocresol green (BcG), bromocresol purple, bromothymol blue, bromophenol blue, Congo red, cresol red, eosin, Eriochrome Black T, methyl red (MR), methylan yellow, methyl orange (MO), murexide, neutral red (NR), and tropaeolin) on the performance of an C. melibiosica-based MFC was investigated [57,58]. It was demonstrated that MB, MO, MR, and NR increased the performance of the MFC in comparison to a mediatorless system. The highest achieved enhancement in power density was from 20 to 640 mWm$^{-2}$ with MB at a concentration of 0.8 mM. The authors related the improvement in the MFC performance to the ability of the mediator to increase electron transfer kinetics.

C. melibiosica-based MFC performance was also studied with the use of modified carbon felt as the anode material [59]. The carbon felt anode was modified by the electrodeposition of nickel using agalvanostatic (Ni$_{(g)}$—galvanostatically modified Ni–carbon felt) or a potentiostatic (Ni$_{(p)}$—potentiostatically modified Ni–carbon felt) pulse plating technique. The use of Ni-modified carbon felt in a dual-chamber mediatorless C. melibiosica-based MFC allowed for the increase in power output from 36 mWm$^{-2}$ for the nonmodified electrode (NME) to 390 and 720 mWm for Ni$_{(p)}$ and Ni$_{(g)}$, respectively. Interestingly, these values were even higher than that achieved in an MFC using nonmodified carbon felt as the anode and MB as the electron shuttle. The improvement of C. melibiosica-based MFC performance using these modified anodes was related to Ni, which served as an electron acceptor or initiated an adaptive mechanism with an increased electron transfer rate through the yeast cell membrane. In another report, the authors used nanomodified NiFe and NiFeP–carbon felt materials as the anode in a C. melibiosica-based MFC. Anodes were modified by the pulse electrodeposition technique, as mentioned above. The highest power production was achieved for the NiFeP-modified electrode and peaked at 260 ± 8 and 155 ± 6 mW in the case of the potentiostatic and galvanostatic carbon felt modification, respectively [62].

2.2.2. Candida sp. IR11

Lee et al. isolated Candida sp. IR11 from a biofilm formed on the anode of a single-chamber glucose-fed MFC inoculated with anaerobic sludge [61]. Since the ability to reduce ferric iron by this new yeast strain has been demonstrated, it was supposed that Candida sp. IR11 has electrogenic potential. Candida sp. IR11 was inoculated into a single-chamber MFC where the substrate was wastewater from upflow anaerobic sludge, and a maximum power density of 20.6 ± 1.52 mWm$^{-2}$ was observed, which was accompanied by COD removal at a level of 91.3 ± 5.29%.

2.3. Arxula adeninivorans

Arxula adeninivorans is a yeast strain that can grow at high temperatures (up to 48 °C) with a wide pH and high salinity tolerance. The bioelectrochemical activity of Arxula adeninivorans was investigated using a mediatorless double-chamber MFC operated in continuous mode [60]. The maximum power density in the MFC employing Arxula adeninivorans was ca. 28 mWm$^{-2}$, and it was observed that A. adeninivorans secretes a soluble electroactive molecule, which is believed to provide current generation in MFCs. Further, the use of 2,3,5,6-tetramethyl-1,4-phenylenediamine (TMPD) as a redox mediator was examined. Application of TMPD as an anodic mediator and KMnO$_4$ as the cathodic reducing agent in an A. adeninivorans-based MFC led to a significant increase in maximum power density, i.e., 1.03 ± 0.06 Wm$^{-2}$. The obtained values were close to the highest power output reported by Ganguli and Dunn for their yeast-based MFC [49].

2.4. Hansenula anomala

Prasad et al. investigated a mediatorless MFC with H. anomala as a biocatalyst and glucose as a substrate [11]. The H. anomala cells were immobilized on a plain graphite anode by physical adsorption and covalent bonds. It was found that H. anomala used enzymes present in their outer membrane, i.e., lactate dehydrogenase and ferricyanide reductase, in the electron transfer process. Additional experiments with different types of anodes, i.e., plain graphite, graphite felt, and polyaniline (PANI)-coated graphite modified with Pt catalyst, revealed a maximum power output of 2.9 Wm$^{-3}$. 
obtained for the anode modified with PANI and Pt. However, the use of the graphite felt anode, which had a surface area bigger than that of plain graphite, resulted in a maximum power output similar to the level of 2.3 Wm$^{-3}$, which was apparently higher than that for the plain graphite electrodes (0.69 Wm$^{-3}$).

2.5. Other Species

The catalytic activity of seven different yeast strains—S. cerevisiae, Kluyveromyces marxianus, Pichia pastoris, Hansenula polymorpha, Kluyveromyces lactis, Schizosaccharomyces pombe, and Candida glabrata—in a mediatorless double-chamber MFC was investigated by Kaneshiro et al. [63]. The highest power density was obtained when carbon fiber was used as an anode with glucose as a substrate. The MFC employing K. marxianus rendered the highest power output of 850 Wm$^{-3}$. Moreover, it was found that K. marxianus can utilize fructose and xylose as a carbon source in addition to glucose. The three highest power levels were obtained for glucose, fructose, and xylose, respectively. Since glucose and xylose are produced from lignocellulosic biomass and the degradation of both is required for complete degradation of the biomass, the authors proposed that K. marxianus could be useful for the development of MFCs using waste products from the wood industry and forestry.

Enhanced current generation using yeast–bacterial coculture was observed by Islam et al. [64]. The power production in MFCs, which inoculated the yeast Lipomyces starkeyi with Klebsiella pneumoniae was 3–6 times higher (12.87 Wm$^{-3}$) in comparison to MFCs using bacteria or yeast separately. It was observed that the yeast utilized electron shuttles produced by the bacteria, which is encouraging for future investigations on the mutualistic interactions of fungi and bacteria for increasing the power production of MFCs.

3. Fungi Used as a Cathode Catalyst

Cathode construction and the type of the final electron acceptor used in the cathodic compartment have an essential influence on an MFC’s electrical output. Oxygen is a common electron acceptor, due to its easy accessibility, high redox potential, and the absence of waste or toxic chemical end products. However, the oxygen reduction process at the cathode limits the performance of the MFCs, because of high overpotential and low reaction kinetics. The application of a suitable catalyst can improve the efficiency of oxygen reduction by lowering the activation energy and enhancing the reaction rate. The most common cathodes used for MFCs are Pt-coated carbon electrodes that use dissolved oxygen as the electron acceptor. Carbon electrodes modified with Pt exhibited significantly decreased oxygen reduction activation energy and increased the reaction rate. However, the use of a platinum catalyst has serious drawbacks, such as high costs and toxicity. Enzymes have considerable advantages over chemical catalysts, such as biocompatibility and higher specific selectivity, transformation efficiency, and activity under mild conditions. Although enzymes have better electrochemical catalytic performances in comparison to microbes, the use of enzymatic fuel cells is limited, due to the high costs of enzyme production and purification, as well as the short-term enzyme activity in relation to its inactivation. A microbial community placed in the cathode chamber or inoculated directly on the cathode produces enzymes that effectively catalyze the reduction of oxygen, which results in significantly improved cathode performance [65,66]. A great advantage of using the whole microbial cells for catalyzing the oxygen reduction reaction is an eliminated need for enzyme isolation and purification. Such an approach also allows the enzymatic cycle to occur in its natural environment, i.e., within a living organism.

3.1. Trametes versicolor (Coriolus versicolor)

Trametes versicolor is a filamentous fungus known for its ability to produce oxidative enzymes. These enzymes enable the exchange of electrons between the electron donor and the acceptor. Wu et al. [67] investigated the performance of an MFC employing the laccase-secreting white-rot fungus T. versicolor, where this white-rot fungus was able to continuously secrete laccase, which could
help to avoid the cost-consuming laccase isolation and purification (Table 2). Moreover, production of laccase enabled the elimination of disruptions of the system by the irreversible inactivation of the enzyme. *C. versicolor* was inoculated in a cathode chamber of an MFC filled with a medium containing glucose as a carbon source. ABTS was used as a redox mediator, since it had been previously used as an effective mediator, which allowed for transferring electrons between the electrode and laccase [22,23]. Two additional MFCs—one with a catholyte enriched with commercially available laccase and one with a carbon fiber cathode—were operated as the controls. The MFC inoculated with the white-rot fungus obtained higher values of maximum power density ($320 \pm 30 \text{ mWm}^{-3}$) in comparison to the control MFCs with conventional abiotic cathodes (40–50 mWm$^{-3}$). However, the MFC employing the laccase-based cathode demonstrated a maximum power density of $480 \pm 30 \text{ mWm}^{-3}$. In order to maintain a high voltage level, a dose of ABTS was reapplied. However, the maximum power density of the MFC inoculated with *C. versicolor* was 2/3 of that for the laccase-based MFC, which the authors attributed to the limited electron transfer, due to adhesion of the white-rot fungus to the carbon fiber. Increasing the pH in the cathode chamber and reducing it in the anode chamber led to a decrease in MFC performance and stability [68,69]. In the MFC inoculated with the white-rot fungus, pH variation of the catholyte was observed, which was connected with the metabolism of the fungus. Experimental results showed that the white-rot fungus *T. versicolor* inoculated in the cathode chamber converted glucose to acetate and thus attenuated the deactivation of laccase.
| Microorganism          | MFC Type                      | Electron Acceptor | Cathode Material | Anode Material          | Separator                  | Anolyte                          | Max. Power Density mW/m² | Ref.  |
|------------------------|-------------------------------|-------------------|------------------|-------------------------|---------------------------|----------------------------------|--------------------------|-------|
| *Trametes versicolor*  | Double chamber/H-type         | O₂ (air)          | Activated carbon fiber | Activated carbon fiber | Proton exchange membrane  | Potassium ferricyanide          | N/A 320 ± 30             | [67] |
| *T. versicolor-S. oneidensis* | Double chamber/H-type | O₂ (air)          | Graphite rods     | Graphite rods            | Sterion cation exchange membrane | Acetate                        | N/A 1200                | [70] |
| *Ganoderma lucidum*    | Single chamber                | O₂ (air)          | N/A               | N/A                      | PVA gel                   | Acid orange 7                    | 13.38 N/A               | [71] |
| *G. lucidum*           | Double chamber                | O₂ (air)          | CPC rings         | CPC rings                | SAP-containing PVA gel    | Sludge from a wastewater treatment plant from the dying industry | 207.74 N/A              | [72] |
| *Galactomyces reessii* | Double chamber                | O₂ (air)          | Vulcan-carbon cloth coated with Pt | Plain carbon cloth with coconut coir | Nafion                      | Rubber wastewater sludge         | 59 1163                  | [73] |
| *Rhizopus sp.*         | Double chamber                | O₂ (air)          | Pt-black carbon PTFE carbon felt | Pt-free-Pt carbon PTFE carbon felt | Graphite plates            | Potassium ferricyanide          | N/A 317.3 197.8          | [74] |
| *Aspergillus sp.*      | Double chamber                | O₂ (air)          | Pt-black carbon PTFE carbon felt | Pt-free-Pt carbon PTFE carbon felt | Graphite plates            | Potassium ferricyanide          | N/A 438.16 328.73        | [74] |
| *Penicillium sp.*      | Double chamber                | O₂ (air)          | Pt-black carbon PTFE carbon felt | Pt-free-Pt carbon PTFE carbon felt | Graphite plates            | Potassium ferricyanide          | N/A 344.1 288.9          | [74] |

PVA—polyvinyl alcohol, SAP—superabsorbent polymer, CPC—carbide porous ceramic, PTFE—polytetrafluoroethylene.
Interesting investigations were conducted by Fernandez de Dios et al., who developed a fungus–bacterium-based MFC for energy production from wastewaters. Additionally, the authors applied the electro-Fenton process in the cathode chamber to increase the degradation efficiency of the organic compounds present in the wastewater [70]. During the Fenton process, hydroxide radicals are produced to oxidize organic pollutants, either completely into carbon dioxide, water, and inorganic salts, or incompletely into less hazardous intermediates [75]. The traditional Fenton process is widely used as a suitable treatment method for highly concentrated wastewaters and sludge. Although the reaction components Fe$^{2+}$ and H$_2$O$_2$ are comparatively affordable, the high consumption and chemical instability of H$_2$O$_2$ significantly reduce the efficiency of this process. Moreover, removal of iron after the treatment is required. Recently, it was reported that H$_2$O$_2$ can be synthesized from acetate or wastewaters at the cathode of an MFC [76,77]. It was observed that fungal hyphae provided a type of scaffolding through which bacteria could effectively move and spread over a larger surface area. Thus, they could overcome difficulties related to movement in the surrounding environment [78].

Moreover, there is a catabolic cooperation between these microorganisms when they coexist in one medium. These interactions between bacteria and fungi may indicate that the physical interaction between them can play an important role in organic substrate degradation and electron transport during MFC operation. Fernandez de Dios et al. have conducted research in which the fungus 

T. versicolor

was grown in the presence of 

S. oneidensis

in order to allow the bacterium taking advantage of the fungus network to transport the electrons to the anode. It was shown that after 1 month of MFC operation, a homogenous biofilm of the bacterium and fungus developed over the electrode. The bacteria were anchored on the surface of the fungal hyphae, which allowed for transport of electrons. Examination of the biofilm structure by SEM analysis indicated that the combination of the fungus and bacterium is a suitable system that permits a high bacterium concentration in the electrode chamber. The ability to produce power in a fungus–bacteria-based MFC was demonstrated using an H-type reactor in a minimal medium with acetate as the carbon substrate. A maximum volumetric power density of 1.2 Wm$^{-3}$ per anode liquid volume was obtained, which was higher than that achieved with the S. oneidensis MR1 H-type reactor (0.24 mWm$^{-3}$). The enhanced electricity generation of the MFC was related to Fenton’s reactions, which promoted electron consumption. Additionally, it was shown that during this MFC operation, approximately 94% of Lissamine Green B and 83% of Crystal Violet were removed. Although the decolorization grade was on a similar level to the present and previous studies, utilization of in situ electro-Fenton reactions in the MFC greatly reduced the operation cost by eliminating the external energy supply requirement.

### 3.2. *Ganoderma lucidum*

Lai et al. investigated a single-chamber MFC with the laccase-secreting white-rot fungus 

Ganoderma lucidum

BCRC 36123 inoculated on the cathode surface to improve power production and degrade the azo dye acid orange 7 (AO7) [71]. Azo dyes are synthetic pigments that abundantly appear in wastewaters produced by the textile industry and paper manufacturing. These chemical compounds are poorly biodegradable and can be completely degraded only by white-rot fungi. The ability of white-rot fungi to break down azo dyes is attributed to laccase activity. Laccase was presumed to simultaneously act as a cathode catalyst in the MFC and, in conjunction with an anaerobic microbial community in the anode chamber, collectively degrade azo dye pollutants. The availability of AO7 to the fungal mycelium on the cathode was assured by substitution of the proton exchange membrane for polyvinyl alcohol hydrogel (PVA-H) film, which allowed AO7 diffusion from the anode chamber to the cathode. Application of fungal mycelium to the cathode provided a constant delivery of fresh laccase, and additional microorganisms enabled the degradation of the azo dyes. The decolorization of the azo dye AO7 coupled with the generation of electricity was investigated in a double-chamber MFC with a fungal biocathode [72]. The laccase-producing G. lucidum was cultivated under solid-state fermentation on wood chips. Utilization of this solid substrate supported fungal growth and stimulated the steady production of laccase. Wood chips inoculated with G. lucidum were placed around the MFC.
cathode and the performance of three types of MFCs—blank (potato dextrose broth (PDB) and fungus, no wood chips), substrate only (wood chips and PDB, no fungus), and both substrate and fungus (wood chips, PBD, and fungus)—were studied. MFC employing \textit{G. lucidum} inoculated on wood chips exhibited a power density of 207.74 mWm\(^{-2}\), and the efficiency of AO7 decolorization was 96.7\%. It was found that the laccase secreted by the white-rot fungus diffused into the anode chamber, where, together with the microbial community, it enhanced the dye removal from wastewaters. In order to examine the influence of AO7 concentration on AO7 removal efficiency and power generation, the performance of a \textit{G. lucidum}-based MFC was investigated using various AO7 concentrations in the anolyte, from 30 to 1000 mgL\(^{-1}\). The results showed that the power density increased with AO7 concentration up to 500 mgL\(^{-1}\). Further increases in AO7 concentration (up to 1000 mg/L) caused a reduction in power density. The phenomenon was assigned to the inhibitory effect of the azo dye acid orange 7 on bacterial growth. The maximum power density and maximum current density at an AO7 concentration of 500 mgL\(^{-1}\) were 207.74 mWm\(^{-2}\) and 585.18 mAm\(^{-2}\), respectively. The obtained values were significantly higher than those reported in previous studies [71,79,80].

3.3. \textit{Galactomyces reessii}

\textit{Galactomyces reessii} is a yeast strain which is able to degrade a wood matrix by secreting laccase. Chaijak et al. [73] investigated the performance of a two-chamber MFC employing the fungus \textit{G. reessii} as a biocatalyst on the cathode. The fungus cultured on coconut coir was placed in the cathode chamber so that it came into physical contact with the cathode. Plain carbon cloth was used both in the anode and cathode. Additionally, two other types of cathodes were used: Vulcan carbon cloth coated with Pt and plain carbon cloth with coconut coir as a positive and negative control, respectively. A mixture of sludge from the rubber industry and synthetic wastewater containing sulfate and ethyl acetate was used as a substrate. It was found that coconut coir supported the growth of \textit{G. reessii} and the production of laccase without the use of additional chemicals or culture media. The maximum current densities and power densities produced by the MFC using \textit{G. reessii} were 59 mWm\(^{-2}\) and 253 mAm\(^{-2}\), respectively. It is worth noting that the MFC employing Pt as a cathodic catalyst and the MFC using a biocathode with \textit{G. reessii} generated similar values of maximum voltage, current, and power. Therefore, the authors suggested that \textit{G. reessii} could be utilized as a biocatalyst, instead of Pt.

3.4. Other Species

Three strains of filamentous fungi—\textit{Rhizopus} sp., \textit{Aspergillus} sp., and \textit{Penicillium} sp.—isolated from Caatinga’s soil were involved as biocatalysts in the cathode compartment of a double-chamber MFC [74]. The power outputs for the examined fungi were obtained using two different cathode materials, i.e., (1) carbon felt electrodes coated with carbon Black Vulcan\textsuperscript{®} and (2) Pt in polytetrafluoroethylene (PTFE) and Pt-free Black Vulcan\textsuperscript{®}-coated carbon felts. A plate of graphite immersed in potassium ferricyanide was used as the anode. The maximum power outputs, both in the case of the platinum-free carbon cathode and in the case of the Pt-coated cathode in the MFC employing \textit{Aspergillus}, were 328.73 and 438.16 mWm\(^{-3}\), respectively.

4. Summary

Among all the investigated fungi strains, \textit{S. cerevisiae} has been the most popular. The highest power densities obtained for \textit{S. cerevisiae}-based MFCs reached 1.5 Wm\(^{-2}\) when MB was used as a mediator in a dual-chamber reactor. In a mediatorless system, the maximum power density for a \textit{S. cerevisiae}-based single-chamber MFC was 334 mWm\(^{-2}\) when a carbon nanotube-based electrode modified with PEI was used. However, the highest power production for fungi-based MFCs was 720 mWm\(^{-2}\) in a mediatorless system when \textit{C. melibiosica} was used as an anode biocatalyst, with the use of Ni-modified electrodes. Though it is hard to compare the results of investigations where different reactor and electrode arrangements were applied, we can conclude today that the power production in MFCs using a single strain of fungi seems to be comparable to power production in
MFCs using a single bacteria strain. For example, the maximum power production in a dual-chamber system was on the level of 41 mWm$^{-2}$ where the single strain *S. oneidensis* MR-1 was used [81], or 4–45 mWm$^{-2}$ for *Geobacter* strains in early two-chamber reactors [82]. These values are close to the power densities obtained for most mediatorless, fungi-based dual-chamber MFCs, collected in Table 1. The power production in electrogenic bacteria-based MFCs was remarkably increased to ca. 860 and 461 mWm$^{-2}$ for *S. oneidensis* and *G. sulfurreducens*, respectively, when a mixed-culture or single-chamber reactor was applied [81,82]. A similar effect was observed for fungi-based MFCs where the application of bacteria–fungi consortia allowed for an increase in power production to 12.87 Wm$^{-3}$ or even 850 Wm$^{-2}$ for milliliter-scale reactors (Tables 1 and 2).

The years of investigation into MFC technology has allowed for the development of commercially available bioreactors able to produce power of up to 300 kW [83]. Until now, most investigations on MFCs have been conducted with the use of various bacteria strains and consortia. However, the present review indicates that fungi can be considered very promising catalytic microorganisms for MFC technology. The highest power density obtained for fungi-based MFCs was 1.5 Wm$^{-2}$, which provides researchers with a foundation for future investigations, especially with the application of bacteria–fungi mixed consortia in MFCs. The recent findings showed that the application of such a consortium (*S. oneidensis*–*S. cerevisiae*, *L. starkeyi*–*K. pneumoniae*, *S. oneidensis*–*T. versicolor*) enhanced the power generation in the system and allowed for power production from complex substrates. The application of fungi in MFC systems can be especially valuable when complex, difficult substrates need to be used in the MFC. The high capacity of fungi for the biodegradation of difficult, toxic substances (e.g., biodegradation of azo dyes at the level of 97%) can be used for current production with the simultaneous treatment of difficult waste.

5. Challenges and Perspectives for Fungi-Based MFCs

Similar to bacterial MFCs, the main obstacle that needs to be overcome in fungi-based MFCs is a low power production that is insufficient to assure the energetic self-sufficiency of such systems. A serious drawback of MFCs using fungi is the use, in most studies, of dual-chamber reactors, which are known for higher internal resistance in comparison to single-chamber ones. To date, in fungi-based MFCs, ferricyanide has been used as the main electron acceptor, which is not feasible to apply at a larger scale. Also, in many studies, mediators are still used, which excludes such an arrangement for commercial purposes. Future studies with fungi-based MFCs should be focused on enhancing the power production in such systems. This can be realized through investigations using single-chamber reactors with various electrode designs and materials. The practical application of fungi-based MFCs demands the elimination of chemical catholytes, such as ferricyanide, and mediators. Thus, searching for new strains of fungi that can produce power with a high efficiency is crucial. A very promising direction for investigations seems to be MFCs based on bacteria–fungi consortia, whose diverse properties can lead to the multiple-times enhancement of power production in the system, especially when complex substrates are used.

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