



Fungal-mediated electrochemical system: Prospects, applications and challenges

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ABSTRACT

Microbial fuel cells (MFCs) that generate bioelectricity from biodegradable waste have received considerable attention from biologists. Fungi play a significant role as both anodic and cathodic catalysts in MFCs. *Saccharomyces cerevisiae* is a fungus with an ability to transfer electrons through mediators such as methylene blue (MB), neutral red (NR) or even without a mediator. This unique role of fungal cells in exocellular electron transfer (EET) and their interactions with electrodes hold a lot of promise in areas such as wastewater treatment where yeast cell-based MFCs can be used. The present article highlights the physico-chemical factors affecting the performance of fungal-mediated MFCs in terms of power output and degradation of organic pollutants, along with the challenges associated with fungal MFCs. In addition, to this comparative assessment of fungal-mediated bio-electrochemical systems, their development, possible applications and potential challenges are also discussed.

1. Introduction

The overwhelming demand for energy generated by the exponential increase in global population and rapid industrial growth has led to an excessive consumption of fossil fuels that has drastically depleted these resources. Generating energy from organic material/biomass is a sustainable alternative approach to address this crisis. The traditional methods of bioenergy production have certain limitations such as the need for large spaces, high capital investment and complexities associated with the production process. Several studies have reported the potential of microorganisms to reduce the cost of the bioconversion process and help to generate bioelectricity from biodegradable waste (Ban et al., 2001; Narita et al., 2006). Research work on bioenergy generation and sustainability has, thus, increased throughout the world to address alternative, non-chemical approaches for power generation. Biotic means of bioenergy production usually exploits different types of microbial species like algae and bacteria. However, very little

information is available on the use of fungal-mediated electrochemical system for energy generation. since certain fungi have only been reported for their potential to use their novel cell factories for energy generation. Considering their rapid growth and metabolism, their ability to degrade and convert waste materials for the production of bioenergy within a short period, and the presence of complex enzymatic systems in them, the prospect of using fungi in bioremediation is quite promising. For instance, microbial electrochemical technology (MET) using fungi to generate energy using wastewater as a substrate. This has a great deal of potential in future for the development of alternative renewable energy sources like utilization of oleaginous microorganisms for biodiesel production and product formulation.

Biodiesel is a renewable source of energy that is of particular interest to researchers in the field of biofuel production. Biofuel can be obtained from various plant biomass, such as cereals, sugar crops and edible oil seeds, etc. Various categories of fungi have recently been reported for the production of biofuels from such biomass. Fungal strains such as

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lignolytic and hydrolytic are commonly being utilized for the production of bioethanol. Certain types of basidiomycetes fungi have also been reported for their potential to produce extracellular enzymes that degrade the lignocellulosic materials. The use of oleaginous microorganisms like fungi has many advantages such as reduced land requirement, short cultivation period and their production of high lipid / fatty acids like plant or vegetable oils (Beopoulos and Nicaud, 2012; Ratledge et al., 2004). Fungal cells can be used for environmental management and bioelectricity generation since they produce certain enzymes such as peroxidases (lignin peroxidase and manganese-dependent peroxidase) that degrade hemicellulose, lignin and polyaromatic phenols (Hofrichter, 2002). In recent years, yeast and filamentous fungi have been identified as oleaginous microbes of significant importance. Oleaginous fungal species within *Zygomycetes* are an excellent source of oleic and palmitic acids that may be used for biodiesel production. Furthermore, anaerobic fungi have an arsenal of extracellular multi-enzyme complexes that aid in the digestion of various biomasses for biogas production. Recent studies have highlighted the efficacy of fungal-based MFCs in biodiesel production. These potential strains can transfer electrons via cytochrome C. The *Candida* sp., *Saccharomyces cerevisiae*, *Colletotrichum* sp., *Alternaria* sp., *Penicillium* sp., *Rhizopus* sp. and *Aspergillus* sp. which have been identified for their prominent role in MFC for electricity generation (Belniak and Maminska, 2018). Fungi become oleaginous when various organic substrates like glucose and sucrose are added to their growth medium. Each oleaginous microorganism has specific abilities to utilize organic substrates and enhancing the lipid yield by maximizing the bioconversion of organic substrates. It has been observed that, in a microbial consortium, a less productive strain always tends to follow a more productive strain during the co-metabolism, resulting in the production of a higher total biomass than those of single cultures. To extract lipids, the entire fungal biomass may be utilized which has an added advantage. This microbial lipid can be directly converted to fatty acid methyl esters (FAME) using low-cost methods as mentioned in Bautista et al. (2012). Catalysts, on the other hand, are required in large-scale biodiesel production facilities in order to accelerate the process.

The lower-chain carbon compounds produced during the enzymatic treatment to wastewater can be utilized as substrate for microbial oxidation on MES. Biofuel production involves the production of bio-hydrogen, biodiesel and bioethanol. The *zygomycetes* fungi biomass, such as *Mortierella isabellina* and *Cunninghamella echinulate* have been reported to contain 60–70% lipid content and 40–57% of dry cell weight respectively (Fakas et al., 2009). Certain fungal genera, such as *Aspergillus* and *Mucor* have recently been recognised for their ability to store oils in their cells under specific conditions, with a maximum oil content of up to 80% (Dhanasekaran et al., 2017). Fungal strains with high lipid content are usually preferred, for their greater efficiency in the production of biofuels and the metabolism of triacylglycerides (TAG). Kurosawa et al. (2013) isolated a few oleaginous microorganisms that have the ability to metabolize xylose and thereby assist in lipid production from lignocellulosic hydrolysates.

Yeasts are eukaryotic fungi that have a wide range of economic and environmental application. For example, *Candida melibiosica*, *Blastobotrys adenivorans*, *Kluyveromyces marxianus*, *Pichia polymorpha*, *P. anomala* and *Saccharomyces cerevisiae* have been examined as biocatalysts in MFCs with or without an external mediator. Yeast, as a eukaryote, has gained the significant interest of researchers mainly due to its ease of use in MFCs. *Kluyveromyces marxianus*, one of the most promising yeast strains, produces high power output under relatively high temperature conditions when grown in natural organic substrates. The fungal biocatalysts used in energy generation primarily increase the rate of electron transmission due to increased fungal hyphae networking and thereby produce stable electricity which may contribute to external electrochemical operations. Due to this unique property, yeast and other fungi have been preferred over prokaryotes like bacterial cells for electricity generation and wastewater treatment (Sayed and

Abdelkareem, 2018). Fungal cells are not only useful in the production of bioelectricity and wastewater treatment processes, but in quality biofuel production as well. Fungal species such as *Rhodospiridium toruloides*, *Yarrowia lipolytica*, *Cryptococcus* sp., *Aspergillus* sp., *Penicillium* sp., and *Trichoderma reesei* play an important role in biofuel production. Biodiesel production using microbial lipids is popularly known as single cell oils (SCOs), that, has attracted the immense attention of researchers. Huang et al. (2009) reported microbial oil production from sulphuric acid-treated rice straw hydrolysate (SARSH) by cultivation of *Trichosporon fermentans*. Few studies have recorded the potential of white-rot fungus and soft rot fungus in the degradation of lignocellulosic materials (Anderson and Akin, 2008; Sun and Cheng, 2002). There are reports on the existence of endophytic microorganisms in some oleaginous plants (including their seeds) that have a capacity for microbial lipid production (Peng and Chen, 2007; Dey et al., 2018). *Gliocladium roseum*, an endophytic fungus, is known for its ability to commercially produce biodiesel (mycodiesel) from a variety of substances. Because of its low carbon content, biodiesel produced by yeast or other fungi is more environment friendly and of high value. Recently, metabolic engineering has strived to increase the synthesis of lipids in fungal cells. In *S. cerevisiae* strain YPH499, glyceraldehyde-3-phosphate pathway is modified by over-expressing genes namely - *glycerol kinase*, *diacylglycerol acyltransferase* and *phospholipid diacylglycerol acyltransferase*. The alteration of genetic makeup resulted in the accumulation of large number of TAGs in that particular strain (Yu et al., 2013). Besides, metabolic engineering, global transcription machinery engineering (GTME), enzyme engineering and metagenomics may be exploited together to manipulate cells in order to improve the target strain and its performance in energy generation.

2. Fungi and fungi-mediated fuel cells

Microbial fuel cells (MFCs) are typically operated in a closed-system mode with the anodic compartment maintained in an anaerobic environment (Slate et al., 2019). Exploiting the potential of MFCs is a non-chemical, sustainable and promising alternative technology that uses living micro-organisms to convert the organic substrates to generate electricity. In addition, MFC exhibits certain other advantages such as low sludge generation, use of a wide range of substrates, low power consumption and optimum temperature performance (Sayed et al., 2012; Schaetzle et al., 2008). Wastewater treatment, degradation of organic compounds and generation of bioenergy in the MFC technology seem to be promising strategies for the future. The recent increase in global warming as well as in the price of fossil fuels and the diminishing trends of the available sources have attracted the attention of scientists towards alternative renewable energy sources like microbial cell factories including fungi. Apart from wastewater treatment and power generation, MFCs have a wide range of application in the production of bio-hydrogens and other useful chemicals as well as in water desalination processes. MFC works on the principle of an oxidation–reduction (redox) reaction through a series of electrochemical and microbial pathways. The production of electrons and protons in an anodic chamber through the oxidation of the substrate by microbes and reduction of oxygen as terminal electron acceptor occurs in the cathode chamber. The performance of the MFC depends on various factors like the configuration of the MFC, the choice of substrate, the types of biocatalyst, anodic material, environmental conditions and electrocatalyst at the cathode (Fig. 1).

2.1. Components of MFCs

Microorganisms and electrodes are the functional units of MFCs that play a significant role in microbial-mediated electrochemical system. A typical MFC consists of two chambers separated by a proton exchange membrane (PEM) that acts as a membrane separator. This membrane separator divides the MFC into two distinct anodic and cathodic

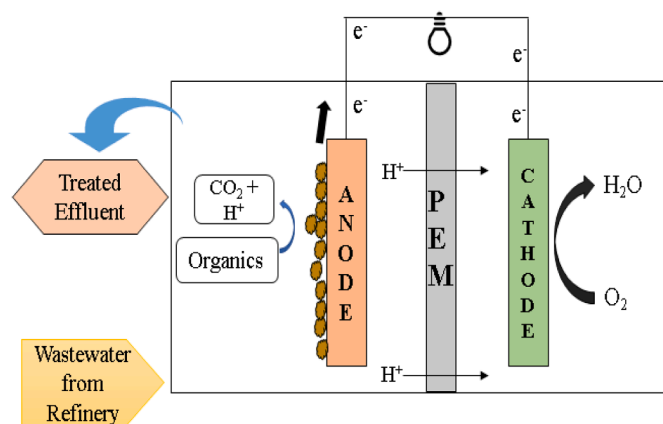


Fig. 1. Microbial fuel cells (MFCs).

sections. Typically, an exoelectrogen is a microorganism that possesses the ability to transfer electrons extracellularly. Exoelectrogens act as biocatalysts that oxidize organic material resulting in the delivery of electrons which are transferred from anode to cathode, generating electricity, while protons are transferred to the cathode via PEM. Subsequently, the combination of proton and electron produces a water molecule at the cathode to complete the bioelectrochemical reaction.

2.1.1. Anodic aspects

In the anodic compartment, microorganisms are known to transfer the electrons to an anode, which are then liberated through the oxidation of organic waste, generating energy. Biodegradable waste materials such as sewage wastewater and brewery wastewater, which are naturally rich in organic substances like glucose, sucrose, acetate, lignocellulose, biomass materials etc., can be used in MFC for generation of bioelectricity.

Modifications in the anodic surface increases the performance of MFCs as the anodic electrode serves as one of the driving features affecting power generation in MFCs. Thus, the alteration of anodic material seems to be a suitable strategy to increase performance. The anode of the MFC should have properties that result in a high electrical and chemical stability, biocompatibility and high surface area (Watanabe, 2008). Carbon paper, carbon cloth, carbon felt, graphene and carbon nanotubes are recently being exploited for their potential to modify the anode surface. Carbon-based materials are non-corrosive and cost-effective due to which they are widely being used in the modification of electrodes. Richter et al. (2008) indicated the use of stainless-steel, gold and titanium as other materials to improve the surface characteristics of anodic material and providing a suitable platform to microbes for biofilm formation on anodic surfaces.

Microorganisms that transfer electrons extracellularly are normally used as biocatalysts in MFCs. The electron transfer (ET) mechanism in MFCs is of two types: direct and indirect. Direct ET, again, comprises two types of transfer (through outer cytochrome and through nanowire) while indirect ET is a mediated electron transfer, that is, the transfer of electrons through mediators. Microbial consortia demonstrate a better electron transfer mechanism as compared to single microorganisms. *Geothrix* sp., and *Shewanella* sp., can generate electricity through direct ET if used as biocatalysts in MFCs. Additional microbial species such as *Pseudomonas* sp., *E. coli*, *Gamma proteobacteria* are being introduced in MFCs to improve the energy efficacy of power generating microbial species.

A wide range of membrane separators including the PEM, anion and cation exchange membrane, salt bridge, glass fiber, microfiltration membrane are recently being used in MFCs. According to Kim et al. (2008) the exploited PEM must have features like high conductivity and low permeability for optimum MFC performance towards energy generation.

2.1.2. Cathodic aspects

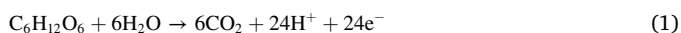
Cathode compartment involves oxygen reduction reaction (ORR), which is one of the key interactions in biological respiration and energy conversion systems such as MFCs (Jadhav et al., 2014). Electrons received by the cathode compartment through the external circuit and protons transported via PEM plays an essential role in a reduction reaction. The reduction reaction involves the reaction between protons and electrons and results in the formation of water molecules (H_2O). The cathode has a vital influence, affecting the total cell voltage output and should have high redox potential. To increase the reduction rate, cathode modifications with carbon materials such as carbon paper and carbon cloth modified with an active catalyst such as platinum (Pt) has been suggested (Watanabe, 2008). However, the use of Pt as active catalyst is not a cost-effective approach. Biocathode intervention is an alternative non-chemical option used in MFCs due to its low cost, stability and sustainability. Microorganisms embedded in an anaerobic anodic chamber as well as in an oxygenated cathodic chamber constitute a mutual configuration of a dual compartment-based yeast fuel cell. According to Cetinkaya et al. (2016) and Zou et al. (2010) the displacement of charge between the chambers is essential as well since it should be linked through an ion i.e. proton or cation transfer through the membrane.

The biocatalyst used in a yeast-based MFC accumulated into the carbon felt anode acts as floating biomass (Christwardana et al., 2018). The use of yeast-based MFCs has certain advantages since yeasts (i) have to degrade extremely complicated substrates such as starch as well as cellulose-based substrates into simple organic molecules (Mao and Verwoerd, 2013; Schaeztle et al., 2008); (ii) can thrive inside the anaerobic environment (Mao and Verwoerd, 2013). Other benefits in using yeast-based MFCs include their easy and simple production procedure, broader substrate consumption, rapid development and sensitivity of the yeast strains etc. In addition to yeast cells, other fungal stains may also be exploited as potent biocatalysts for both electrochemical systems and wastewater treatment approaches. However, only a few studies on the use of fungal strains in energy generation has still been conducted over the last few decades. According to Sayed and Abdelkareem (2018), scientists are interested in working with the fungi-based MFCs (mediator less) and based on the published results it may be more useful to exploit fungi as an exoelectrode to generate electricity. In the presence of redox enzymes such as ferricyanide reductase or lactate dehydrogenase (Prasad et al., 2007), fungi exhibit similar electron transfer mechanism (ETM) that occurs in bacteria. The extracellular oxidative ligninolytic enzyme system allows fungal cells to degrade xenobiotic compounds and dyes (Wesenberg et al., 2003). Fungi produce extracellular oxidoreductase enzymes i.e., laccases, manganese peroxide and lignin peroxide. Laccase can oxidize the phenolic mixtures and aromatic amines by using atmospheric oxygen as a terminal electron acceptor (Leonowicz et al., 2001). Recently, fungi have been found to play a dual role in MFCs (Shabani, 2021). In the anode, they facilitate electron transfer through their respiratory proteins or chemical mediators and in the cathode, they facilitate the reduction of terminal electron acceptors, mainly oxygen. Recent studies have shown that direct electron transfer is carried out by cytochrome C (Wilkinson et al., 2006).

Fungi-based MFCs (yeast-based fuel cell) have, thus, gained significant attention from global scientists and are being widely used in wastewater treatment systems. Yeasts, such as *Saccharomyces cerevisiae*, are considered model organisms to be used as biocatalysts in MFCs. *S. cerevisiae* is easy to culture and can grow in anaerobic environments. Room temperatures of around 30 ± 1 °C are found to be suitable for its growth. Similar findings were reported by Gunawardena et al. (2008a) and Schaeztle et al. (2008) who observed that pure cultures of yeast cells are more effective in wastewater treatment (Hubernova and Mitov, 2015). Pure cultures of yeast cells are potent biocatalysts for MFCs due to their non-pathogenic nature, high growth rate and easy to culture conditions. The yeast cell wall is naturally very thick and the external part of the cell membrane is separated from the cell wall. As a result,

direct contact between the cell membrane and the electrode is quite difficult to achieve. Yeast has a trans-plasma membrane electron transfer system (tPMET), commonly known as plasma membrane oxidoreductase (PMOR). The plasma membrane (PM) NADH-oxidoreductase (PMOR) system is present throughout the membrane and is involved in the transport of electrons by means of molecules such as NADH and NADPH to an external electron acceptor or anode. Glucose oxidation is observed as the main source of energy production for the yeast cell factories. According to Gunawardena et al. (2008b) certain reactions are involved in the anodic and cathodic compartments of the yeast-based fuel cell as mentioned below.

In anodic chamber



In cathodic chamber



In MFCs, different yeast species may be used. Fungi have been used in MFCs from two major aspects namely, the anode (e^- transport performed precisely, using redox-active fungal protein, while synthetic mediators for transfer of electrons) and the cathode (fungus is an origin for an enzyme that catalyze decreases in the final e^- receiver, especially O_2).

2.2. Electron transfer by fungi in MFCs

The involvement of mediator-less fungal MFCs and their analyses with electrogenic efficiency of the fungi have been examined (Sayed and Abdelkareem, 2018) and studies on fungal-mediated electron transport have received a boost from reports on the presence of redox protein such as ferricyanide reductase or lactic acid dehydrogenase in fungi. Based on the findings of Prasad et al. (2007), it has been observed that fungi are more active than bacteria in MFCs. The direct transfer of electrons via cytochrome-c has been demonstrated in widely studied models of fungi-based MFC (Wilkinson et al., 2006).

Yeast-based fuel cells perform an active role in electron transfer during the synthesis of ATP, along with the reduction of NAD^+ into NADH (Fig. 2).

Production of energy requires extra cellular electron transport movement to be directed towards the electrode. Such interactions proceed through a continuous transfer from the membrane surrounded by cytochromes, exogenous redox mediators. This is because exoelectrogens are inadequate to provide transmission of electrons without redox mediators (Patil et al., 2012). Exoelectrogens are currently being investigated for their use in the development of MFCs, which have the potential to convert diverse organic substances such as activated sludge from waste water treatment into ethanol, hydrogen gas and electricity.

The white rot fungus, *Sporotrichum pruniosum* is known for its

efficiency in degrading chemical substances, organic biomass and pollutants using an extracellular enzyme (Bugg et al., 2011; Martinez et al., 2005). The basic extracellular enzymes commonly reported in *S. pruniosum* are oxidoreductases, which include laccases (LC), manganese peroxidases (MnPs) and lignin peroxidases (LiPs). In contrast, other auxiliary enzymes, such as cellobioses dehydrogenase (CDH) and glucose oxidase have also been screened from white-rot fungus. The wide range of wood-degrading fungi produces cellobiose dehydrogenase (CDH), an N-glycosylated peptide. It oxidizes soluble cellodextrins, mannodextrins and lactose to lactones via a ping-pong mechanism involving a wide range of electron acceptors such as quinones, phenoxyl radicals, Fe^{3+} , Cu^{2+} and triiodide ion. Initially, a short cytochrome domain i.e., CYT domain situated near N-terminus carries cofactor heme-b redox. Ludwig et al. (2010) reported the alternative role of C-terminus flavoprotein dehydrogenase (FDH), which utilizes flavin adenine dinucleotide (FAD) as a cofactor of redox reaction (Ludwig et al., 2010). CDH is primarily concerned with the degradation kinetics of carbohydrates in cellulose molecules as well as disaccharides including lactose, glucose and maltose (Tasca et al., 2010).

2.3. Possible methods of electron transfer in MFCs

The cytochromes and transmembrane proteins (tPMETs) of yeast are found within its mitochondria and cell membrane. A mediator is usually required to pass electrons through the cell wall or membrane as well as redox locations such as $NAD^+/NADH$ in the cell. The bio-catalytic behavior of yeast cells is, thus, dependent on the occurrence of various distinct natural electron shuttles or mediators. For instance, azurin, cytochromes and ferredoxin could be used via redox enzyme to increase electrical current from the cell to the anode surface. Raghavulu et al. (2011) and Gunawardena et al. (2008) demonstrated that the electron transport system (ETS) of yeast cells was defined during the degradation of organic compounds. The glycolytic pathway is a series of enzymatic reactions that convert glucose (glycolysis) to pyruvate, thereby generating the energy sources like adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NADH). In the pathway, one glucose compound is split into two pyruvate compounds which are then converted into two acetaldehyde compounds by pyruvate decarboxylase. A NADH-dependent enzyme that converts acetaldehyde to alcohol is alcohol dehydrogenase. The electrons released by this method are taken up by NAD^+ which is then recovered by generating electrons on the electrode surface via NADH oxidation, either to form NAD^+ again via tPMETs or internal/external mediators. However, the use of exogenous mediators raises operating costs and has the potential to pollute the environment. For these purposes, recent research has been focused on the use and development of bioelectrochemical systems using MFCs without artificial mediators.

3. Different fungal strains used in MFCs

Certain yeast strains like *Saccharomyces cerevisiae* (Gunawardena et al., 2008), *Candida melibiosica* (Babanova et al., 2011; Hubenova and Mitov, 2015), *Pichia anomala* (Prasad et al., 2007), *P. polymorpha* [referred to as *Hansenula polymorpha* in the original paper] (Shkil et al., 2011) and *Blastobotrys adenivorans* (Haslett et al., 2011) are being identified as potential catalysts in MFCs. The classification of certain fungal strains based on electron transfer mechanisms in MFC (a) mediator-driven MFC and (b) mediator-less MFC has been depicted in Table 1.

3.1. *Saccharomyces cerevisiae* [Meyen ex E.C. Hansen]

Saccharomyces cerevisiae is a yeast being used widely in modern biological tools and techniques due to its unique physiology and important roles in bakeries, food processing industries, beer and wine making activities. Yeast, also serves as a good biocatalyst in MFCs

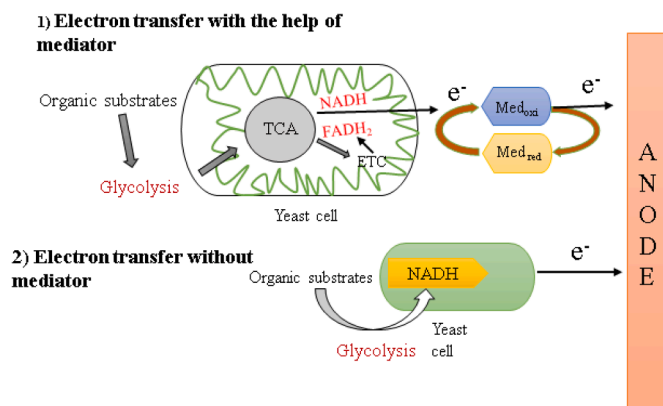


Fig. 2. Electron transfer in *Saccharomyces cerevisiae* based MFC.

Table 1

Classification of certain fungal strains based on electron transfer mechanism in MFC (a) mediator-driven MFC and (b) mediator-less MFC (Sekrecka-Belniak and Toczyłowska-Maminska 2018).

Fungi	Electron acceptor	Mediator-driven MFC	Mediator-less MFC
<i>Saccharomyces cerevisiae</i>	Potassium ferricyanide	Methylene blue, neutral red, thionine, riboflavin	N/A
<i>Candida melibiosica</i> 2491	Potassium ferricyanide	Methylene blue, bromocresol green, neutral red, methyl red, methyl orange	YES
<i>Blastobotrys adenivorans</i>	Potassium permanganate	2,3,5,6-tetramethyl-1,4-phenylenediamine	N/A
<i>Candida</i> sp. IR11	O ₂ (air)	N/A	YES
<i>Pichia anomala</i>	Potassium ferricyanide	N/A	YES
<i>Kluyveromyces marxianus</i>	Potassium ferricyanide	2-hydroxy-1,4-naphthoquinone	N/A

because of its potential to utilize a wide range of substrates, its conduciveness to easy cultivation and microbial mass production methodologies, zero pathogenicity to non-target organisms including human beings, low cost, rapid multiplication and ability to survive and remain active even in a dried environment for a longer period (He et al., 2017). Christwardana et al. (2018) indicated the significance of yeast cells in MFCs due to its unique features and sustainability. *S. cerevisiae* has been extensively studied and characterized as a biocatalyst in biological fuel cells (Ganguli and Dunn, 2009; Gunawardana et al., 2008; Raghavulu et al., 2011). Exogenous mediators like methylene blue (MB) and neutral red (NR) are used to increase the transport of electrons between the anodes and microbes. The application of yeast cell surface-displayed dehydrogenase includes cellobiose dehydrogenase (CDH) and pyranose dehydrogenase (PDH) (Gal et al., 2016a). Both CDH and PDH based biocatalysts were useful in the anodic compartment of MFCs.

4. MFCs without mediators

In a mediator-less MFC, *S. cerevisiae* transports electrons to the anodic surface either via the surface-confined species or through the solution species (Sayed et al., 2012a). Without any mediators, the ability of the anode is observed to reduce from 0.4 to 0.1 V within 45 hrs. However, the open-circuit voltage (V_{OC}) improves from 0.25 to 0.65 V at the same period. On the other hand, in linear sweep voltammetry (LSV), the methodology which normally operates and measures electricity in a working electrode, a peak power production happens above 3 mW/m². In this case, the power output could be restricted by slowing down the transport rate of electrons from the microorganism to the surface of the anode (Christwardana and Kwon, 2017). Examples of mediator-less MFC are shown in Table 1.

4.1. The effect of electrode modification on the performance of yeast-based MFCs

MFCs are a novel technology that has the potential to purify various types of wastewater while converting their chemical energy to electrical energy via active biocatalysts. Electrode materials are vital in influencing the performance and efficacy of MFCs. Various electrode materials, including graphite, carbon cloth, carbon paper (CP) and carbon nanotube platinum (CNT/Pt)-coated CP significantly affect the performance of a dual-chambered MFC.

For example, the performance of yeast-based MFCs was investigated by coating carbon paper with a thin layer of cobalt (Co) or gold (Au). The modification was carried out using a sputtering technique to deposit a thin layer of Co or Au with a thickness of 5 nm or 30 nm. The electrode performance was measured in terms of the electrode half cell potential

and fuel cell power output activities. The Co modification significantly enhanced the fuel cell's performance, whereas the Au modification decreased it. The scanning electron microscopy (SEM) analysis revealed that the metals had an effect on the adhesion density of yeast cells to the electrode surface. Kasem et al. (2013) observed that the electron transfer occurred via surface confined species at the mediator less anode. The effect of anode modification on the performance of *S. cerevisiae* based MFC has been investigated using glucose as a substrate and MB as mediators (Kasem et al., 2013). Carbon anode fibers were prepared from a thin 30 nm layer of Co30 and of Au30 coated material respectively. When the maximum power density was calculated by multiplying the current density with the corresponding operating cell voltage, it was observed that while the power density was 20.2 mW/m² for Co30, it was less for Au30 at 12.9 mW/m². For the unmodified anode this reduced more drastically to less than 2 mW/m² for Au30. It has been noticed that *S. cerevisiae* cell immobilization on carbon nanotubes (yeast/CNT), used as a biocatalyst in membrane-less MFC, subsequently, improved electron transfer in *S. cerevisiae*-based MFCs (Christwardana and Kwon, 2017).

5. Use of mediators in yeast based MFCs

The effect of mediators that influence the efficiency of MFCs, using yeast and glucose as biocatalyst and substrate respectively, is being analysed. Yeast is a free-floating cell that does not get attached to a supporting electrode. Thus, various mediators are required to combine the direct and mediated electron transfer mechanisms of yeast. Electron transport in MFCs using *S. cerevisiae* can be improved using mediators like methylene blue (MB), neutral red (NR), thionine and yeast extract that eventually result in increased power generation (Permana et al., 2015). NR reported positive results with *S. cerevisiae* in a dual-chamber system. Thionine increased the efficiency in *S. cerevisiae* MFC from 3 to 28 mW/m² (M. Rahimnejad et al., 2012). The maximum thionine concentration used was 500 mM that resulted in a power output of 420 mV and a peak thionine voltage of 700 mA/m² (ET Sayed et al., 2015). ET Sayed et al. (2015) mentioned the unique significance of *S. cerevisiae* that was being used as an anodic biocatalyst in air-cathode MFCs with sewage water as a substrate and graphite as an electrode.

6. Different yeast strains used in MFCs

S. cerevisiae, *C. melibiosica*, *Pichia anomala*, *P. polymorpha* and *Blastobotrys adenivorans* are some prominent yeast strains that are being utilized as biocatalysts in anode chambers (Table 2), as mentioned previously in Section 3.

6.1. *Candida melibiosica* [H.R. Buckley & Uden]

The strain *Candida melibiosica* 2491 was picked from certain examined microbes because it had potential in phytase action (Hubenova and Mitov, 2010), which is the reason for the strain's continued experimental use as an active catalyst. *Candida melibiosica* 2491 is a yeast strain that has been deployed in a number of biological fuel cells as a catalyst either in the presence or the absence of a mediator. Catalytic activity of *C. melibiosica* was observed in the dual-chamber MFC using various carbon sources, namely fructose, glucose and starch with or without the addition of MB (Hubenova and Mitov, 2010). *C. melibiosica* can generate bioelectricity even in the absence of an extracellular mediator when used as a fructose substrate, with a peak current of 60 mWm³. This establishes a link between bioelectricity generation, yeast growth factor and substrate availability. The effect of various mediators such as bromothymol blue (BTB), bromophenol blue (BPB), cresol red (CR), bromocresol green (BcG), bromocresol purple eosin, methyl yellow, MB, methyl orange, eriochrome black T, methyl red, murexide and NR on *C. melibiosica* performance in MFCs has also been extensively studied to improve the efficiency of MFCs. The maximum power density improvement with MB at 0.8 mM concentration was observed from 20 to

Table 2
MFCs with potent fungal strains as the anode catalyst

Types of MFC used	Strains of yeast used	e- Receiver	Anode components	Cathode components	Substrate used	Extracellular mediator	Peak electricity frequency (mW/m ²)	References
Dual compartment	<i>S. cerevisiae</i>	Potassium ferricyanide	Platinum mesh	Platinum mesh	Glucose	Methylene blue	65	(Walker and Walker, 2006)
Single compartment	<i>S. cerevisiae</i>	O ₂	Carbon paper	Pt/C wrapped carbon cloth	Glucose	Methylene blue	80	(Kasem et al., 2013)
Dual compartment	<i>S. cerevisiae</i>	Potassium ferricyanide	RVC	RVC	Dextrose	Methylene blue and neutral red	500	(Wilkinson et al., 2006)
Dual compartment	<i>S. cerevisiae</i> with GOx	O ₂	Graphite	Graphite	Glucose	Methylene blue	13.6	(Fishilevich et al., 2009)
Dual compartment	<i>S. cerevisiae</i> with CDH	O ₂	Graphite	Graphite rod CNT/ MWCNT	Lactose	N/A	33	(Gal et al., 2016a)
Single compartment air-cathode	<i>S. cerevisiae</i> with CDH	O ₂	Pt/C upon Carbon fabric	Graphite plate	D-Glucose, L-Arabinose, D-Galactose	Methylene blue	31, 32, 14	(Gal et al., 2016a)
Dual compartment	<i>C. melibiosica</i>	Potassium ferricyanide	Carbon paper	Carbon paper	Y _P _{fru}	Neutral red, Methylene red, Methylene blue	89, 113, 640	(Babanova et al., 2011)
Continue discharge double compartment	<i>Blastobotrys adeninivorans</i>	Potassium permanganate	Carbon fiber cloth	Carbon fiber cloth	Dextrose and glucose	N/A and TMPD	281,030	(Haslett et al., 2011)
Single compartment	<i>Candida</i> sp. IR11	O ₂	ADE 75	Carbon felt	Glucose denied wastewater	N/A	20.6	(Lee et al., 2015)
Dual compartment	<i>P. anomala</i>	Potassium ferricyanide	Plain graphite	Plain graphite	glucose	2-Hydroxy-1,4 Naphthoquinone	N/A	(Kaneshiro et al., 2014)

640 mWm². According to Babanova et al. (2011) exogenous mediators, in addition to their active role in improving the kinetics of electron transfer (electrical deliverables), might also help to reduce cell catabolism (Babanova et al., 2011).

6.2. *Candida* species IR11

The ability of *Candida* sp. IR11 to pass electrons and generate electrical energy has been addressed in a pure culture MFC. Lee et al. (2015) isolated *Candida* sp. IR11 from a biofilm consisting of a single-chambered glucose fed with an MFC and further inoculated in an anaerobic digester. Although, the ability of this yeast strain is to reduce the ferric iron concentration, it was believed to retain its electrogenic ability. It was experimented by placing *Candida* sp. IR11 in a separate compartment of MFC, with the substrate as anaerobic crossflow effluent sludge water. During the investigation, a peak power intensity up to 20.6 ± 1.52 mWm⁻² was measured followed by COD elimination (91.3 ± 5.29%).

6.3. *Pichia* spp.

Pichia anomala is a fungi under Saccharomycetaceae, known for its abilities in electricity production. Prasad et al. (2007) studied an MFC without a mediator using *P. anomala* as a biological catalyst together with glucose as a precursor molecule. The *P. anomala* cells were typically incapacitated on the anode surface through the adsorption process by physical means and covalent connection. *P. anomala* was identified to have an enzyme available throughout the bacterial extracellular membrane, such as ferricyanide reductase and lactate dehydrogenase in the process of transfer of electrons. In comparison, the MFC was controlled utilizing abundant anodes like graphite, polyaniline-Pt co-mixture wrapped with graphite and graphite felt.

Due to its distinct features, *P. polymorpha* (in addition to *P. anomala*) is often utilized as a good source of power generation. It is a thermo-tolerant fungus, able to survive at temperatures ranging from 30 to 50°C. *P. polymorpha* can be embedded over the graphite electrodes to work in MFCs.

6.4. *Blastobotrys adeninivorans* [(Middelhoven, Hoogk. Niet & Kreger-van Rij) Kurtzman & Robnett (2007)]

Blastobotrys adeninivorans is a dimorphic yeast that can grow with a limited pH and high alkaline sensitivity under extreme temperatures even up to 48°C. The biocatalytic action of non-traditional yeast, *B. adeninivorans* in the dual-chamber MFC without a mediator was examined (Haslett et al., 2011). In the MFC utilizing *B. adeninivorans*, the peak energy density was recorded as approximately 28 mWm⁻². The electron transfer was achieved by an endogenous mediator which was naturally produced in the solution. Similarly, 2,3,5,6-tetramethyl-1,4 phenylenediamine (TMPd) was used as a redox mediator in MFCs. Implementation using TMPd as an anode mediator or KMnO₄ as the cathode reducing reagent in an *B. adeninivorans* mediated MFC resulted in a significant improvement in peak energy voltage of up to 1.03 ± 0.06 Vm⁻². This was probably due to the *B. adeninivorans* employing the cyclic voltammetry of a supernatant. An unavoidable oxidation level arose to near ±0.45 V. The MFC centered on *B. adeninivorans* revealed better results than the MFC based on *S. cerevisiae*. In a mediator-less dual compartment of MFC, the catalysed action of different fungal species like *S. cerevisiae*, *P. polymorpha*, *K. marxianus*, *P. pastoris*, *Kluyveromyces lactis*, *C. glabrata* as well as *Schizosaccharomyces pombe* were examined by Kaneshiro et al. (2014). *Kluyveromyces marxianus* is an aerobic yeast capable of respiro-fermentative metabolism, which involves the simultaneous generation of energy from levulose (fructose) and xylose as the sole carbon source in MFC, which could be beneficial for wood waste management (Fig. 3).

7. Fungi as a catalyst in cathodes

In MFCs, the configuration of the cathode side, as well as the electron receiver at the terminal utilized in the cathode chamber have a significant impact on electric production. Due to the easy access to oxygen, lack of harmful substances and its own higher catalytic prospects, oxygen is acknowledged to be the primary electron acceptor. In fungi based MFCs, different fungal species are being utilized as cathodic catalysts. White rot fungi (*Trametes versicolor* and *Ganoderma lucidum*) are certain prominent fungal species used in MFCs and show a better electricity output. Platinum-coated carbon electrodes that use dispersed oxygen as an electron receiver appear to be the most common cathode for MFCs.

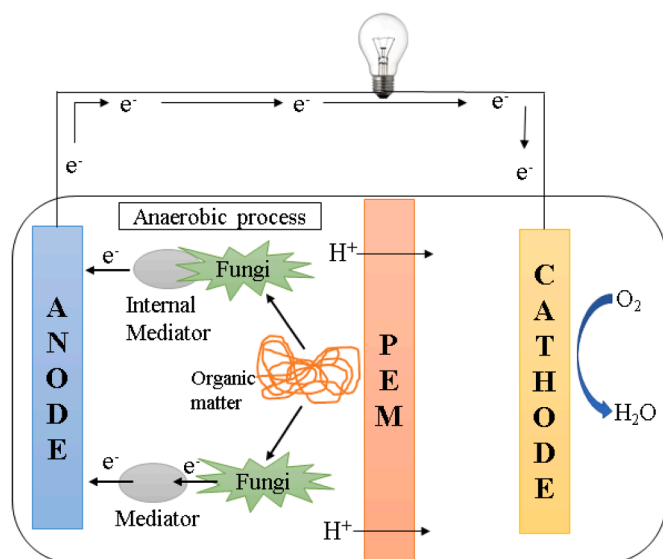


Fig. 3. Diagrammatic representation of fungi used as anode catalyst in MFC.

The rate of reaction is enhanced while the stimulation of oxygen reduction is reduced due to Pt altered with carbon electrolytes. According to Carbajosa et al. (2010), the improvement of cathode efficiency is dependent on a bacterial population located within the cathode compartment that produces enzymes that essentially catalyze redox reactions.

7.1. *Trametes versicolor* [(L.) Lloyd]

Trametes versicolor is recognized for its efficient production of laccase and ligninolytic enzymes. The efficiency of an MFC by using white-rot fungus, *T. versicolor* has been reported by Wu et al., 2012. In the cathode compartment of the two MFCs, *T. versicolor* was used with glucose as the sole source of carbon for production of bioenergy. In order to make a controlled experiment, one of the two MFCs was filled with laccase as an enhanced catholyte and the other with a cathode of carbon fiber. The MFC with the abiotic carbon cathode showed a current of 40–50 mWm^{-3} . In contrast, the MFC inserted with the white-rot yeast generated an extremely high yield of energy of up to 320 (± 30) mWm^{-3} . The highest conversion efficiency [480 (± 30) mWm^{-3}] was after using the laccase catholyte in the MFC. For the MFC based on carbon fiber, the average energy output introduced with *C. versicolor* was 2/3. The minimal transport of electrons was due to the limited conductivity of white-rot yeast to the carbon fiber. The strength and capabilities of MFCs are directly related to parameters like alkalinity, pH at the cathode compartment (Oh and Logan, 2006; Watanabe, 2008). Fernandez de Dios et al. (2013) investigated the role of fungi and bacteria in MFCs for energy generation via water and sewage wastewater treatment. The biodegradation process is accelerated by the introduction of efficient microbial species which contribute to the remediation of harmful toxicants and to the production of energy. Potent microbial fuel cell associations can influence and improve electron transfer as well as biological substrate deterioration. For example, in the presence of *Shewanella oneidensis*, the fungus *T. versicolor* develops hyphae networks that allows for the efficient transfer of electrons into the anode. It takes nearly 30 days of MFC activity for the formation of homogeneous biofilms of the microorganisms as well as fungi on the electrode. MFCs fortified with fungi thus appear to be more useful.

7.2. *Ganoderma lucidum* [(W. Curt.: Fr.) P. Karsten] (Lingzhi or Reishi)

Ganoderma lucidum is an ornamental fungus belonging to the Ganodermataceae family. It is a widespread white rot fungus that degrades a

wide variety of hardwoods. Lai et al. (2017b) investigated an MFC with the inoculation of laccase producing white-rot fungi, *Ganoderma lucidum* BCRC 36123, over the surface of the cathode to increase electricity generation by degrading an azo dye acid orange 7 (AO7), also known as 2-naphthol orange, and Orange II. Likewise, the fungus, *Phanerochaete chrysosporium* (white-rot), degrades the azo dyes via laccase action. Laccase was assumed to function as a catholyte in the MFC, and degraded azo dyes (Lai et al., 2017). The anode compartment was allowed to disperse dyes (AO7) to the cathode compartment. The replacement of PEM to PVA-Hs [Poly(vinyl alcohol) hydrogels] improved fungus accessibility of AO dyes through mycelium at the cathode. The experimental results indicate that the fungus-based biocathode outperforms the conventional abiotic cathode by approximately seven orders of magnitude (Wu et al. 2012, Lai et al., 2017a).

7.3. *Galactomyces reessii* [(Van der Walt) Redhead & Malloch]

Galactomyces reessii is a fungal species with the ability to degrade wood by laccase production (Chaijak et al. 2018a). Chaijak et al. (2018b) investigated the efficiency of dual compartment MFCs with *G. reessii* and confirmed its importance as a fungus-based biocathode. Two different types of cathode materials, namely, Pt-coated vulcan carbon fabric and coconut coated with pure carbon fiber, were used during the experimentation. Laccase production was observed in both cases. It was observed that the use of coconut fiber stimulated *G. reessii* development. Using *G. reessii*, optimum power generation efficiency of up to 59 mWm^{-2} and voltage intensities of 253 mAm^{-2} were achieved in the MFC. It was observed that using *G. reessii* as a biocathode material produced significantly higher peak strength, intensity and energy values than using Pt coated cathode in the MFC.

7.5. Other yeast species involved in cathodes in MFCs

Morant et al. (2014) investigated certain fungal species like *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp., which they isolated from soil samples and used in the cathode chamber of dual compartment MFCs, as cathodic biomaterial. Cathodic components such as (1) Pt with polytetrafluoroethylene (PTFE), (2) Pt- without black vulcan wrapped with fiber of carbon components, and (3) carbon fiber wrapped with black vulcan electrolyte were utilized for analysing energy outcomes using various fungal systems.

In the MFC, Pt covered with cathode recorded 438.16 mWm^{-3} power generation while Pt free cathodic carbon had 328.73 mWm^{-3} . However, the peak in energy output levels were obtained after utilizing the fungus, *Aspergillus* sp. Table 3 indicates different yeast species that have promising roles in MFCs. Laccase is a biocatalyst that can move electrons from the cathode electrode to the bacterial fuel cell layer. As laccase is a copper-containing oxidoreductase enzyme, most observations suggest that it plays an important role since it has the ability to catalyze oxidation reactions and thereby increase their chances for a smooth participation in biological degradation machineries. A 'multicopper oxide-reductase' enzyme is known to secrete laccase. Shleev et al. (2005) reported that laccase accepts electrons from molecular oxygen, assisting in the catabolization of organic compounds. White-rot fungus makes and metabolizes laccase to restore nutrients through organic components to the soils via lignin degeneration in the ecosystem. Fungi laccases are composed of four copper molecules, which have a significantly higher capacity for the redox reactions of organic as well as aromatic compounds using enzyme-mediated system. It may be mentioned that in MFCs, for the generation of power, *in situ* elimination of laccase by white-rot fungi may be considered as a suitable and cost-effective strategy for sustainability development. The viability of using white-rot *in situ* fungi for the secretion of laccase was tested to increase the efficiency of the MFC. When laccase generates, lignin is degraded. Here, rubber wastewater sludge was used in the anode as a substrate with laccase placed in the cathodic compartment under optimal

Table 3
MFC with fungi as the cathode catalyst.

Type of MFC used	Yeast used	Cathode components	Electron acceptor	Anode components	Anolyte used	Peak energy density in mW/m ³	Reference
Dual-Compartment/ H-Type	<i>Trametes versicolor</i>	Activated carbon fiber	O ₂	Activated carbon fiber	Potassium ferricyanide	320±30	Wu et al., 2012
Dual-compartment	<i>Ganoderma lucidum</i>	CPC ring	O ₂	CPC ring	Effluent from wastewater treatment plant	N/A	Lai et al., 2017a
Dual-compartment	<i>Galactomyces reessii</i>	Vulcan-carbon cloth wrapped with Pt	O ₂	Plain carbon cloth	Rubber wastewater effluent	1162	Chaijak et al., 2018b
Dual compartment	<i>Rhizopus</i> sp.	Pt-black carbon PTFE carbon felt and Pt-free-Pt carbon PTFE carbon felt	O ₂	Graphite plate	Potassium ferricyanide	313.3 and 197.8	Morant et al., 2014
Dual compartment	<i>Aspergillus</i> sp.	Pt-black carbon PTFE carbon felt and Pt-free-Pt carbon PTFE carbon felt	O ₂	Graphite plate	Potassium ferricyanide	438.16 and 328.73	Morant et al., 2014
Dual compartment	<i>Penicillium</i> sp.	Pt-black carbon PTFE carbon felt and Pt-free-Pt carbon PTFE carbon felt	O ₂	Graphite plate	Potassium ferricyanide	344.1 and 288.9	Morant et al., 2014

environmental conditions. The results, thereby, suggest the potential of microbial enzyme systems in electricity generation. Laccase derived from fungal strains like *G. lucidum* strain BCRC 36123, *T. versicolor* as well as *Pleurotus ostreatus*, has shown significant achievement in energy production through the incapitate fungal enzymes upon a layer of the cathode (Lai et al., 2017b; Mani et al., 2017; Wu et al., 2012). Laccase returns nutrients to the soil through the degradation of lignin from plants. MFCs along with laccase as a biocathode are predicted to minimize the MFC manufacturing cost significantly. Laccase, with a dual-chamber MFC, generates a large amount of power as compared to the single-chamber MFC. In one study, it produced a maximum open-circuit voltage of 250 mV, a power density of 59 mW/m², and also increased the half-cell potential up to 70%. Similarly, ABTS 2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) is recorded as an effective mediator to transfer the electrons from electrode to laccase (Wu et al., 2012). In a dual-chamber MFC, an airtight anodic chamber filled with 100 mM hexacyanoferrate at pH 6.5 and a cathodic chamber filled with laccase secreting white-rot fungus with sufficient growth medium is required for the optimum growth of the fungal cells. White rot fungus starts oxidizing ABTS as measured using spectrophotometer at 420 nm. The results indicated that the maximum voltage and maximum power density increased up to 180 mV and 320 mW/m³ respectively during this process. The voltage of the system was initially low in the absence of ABTS; however, it increased after the addition of certain amounts of ABTS (Fig. 4). It can, hence, be concluded that ABTS assists in improving the performance of fungus-based MFCs.

The concept of 'biocathode' has been developed by the use of certain biological components in the cathode (Fig. 5).

8. Factors affecting the performance of fungi based MFCs

In MFC technology using fungi as a biocatalyst, the chemical energy present in the substrate gets converted into electrical energy. Fungal cells at the anodic side oxidize the substrates, producing electrons and protons. The electrons are absorbed by the electrode and protons flow through the PEM towards the cathodic side and finally H₂O molecule formation takes place. The performance of MFCs is affected by certain physical and biological factors that contribute to their functioning (Bhagchandani et al., 2020). Physical factors include reactor configuration, electrode material and separator while substrate type and concentration, choice of inoculum, external resistance and so on, fall under potent biological factors influencing the working conditions of MFCs.

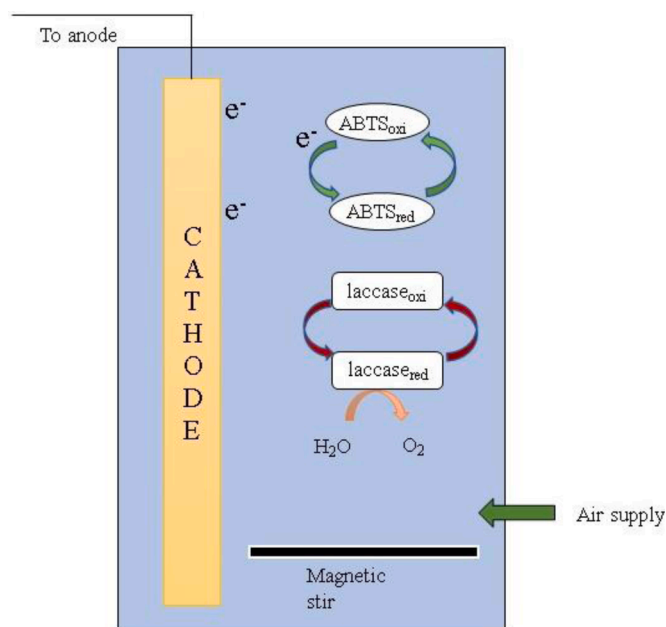


Fig. 4. Activity of the laccase -ABTS in the cathodic chamber that transfers electrons from the electrode to the laccase.

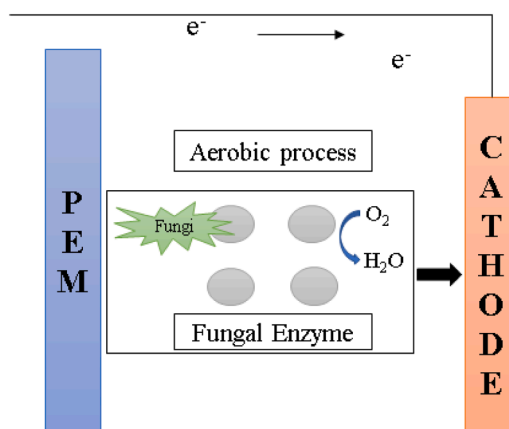


Fig. 5. Fungi as cathode catalyst (Biocathode).

8.1. Anode

For optimum MFC operation, a suitable anodic material with properties like high conductivity, resistance to corrosion, biocompatibility, ability to withstand different chemical conditions and pH is necessary. In addition, the material in use must possess high mechanical strength and enough surface area for possible cell attachment. The most widely used anodic material involves the carbonaceous materials that are of low cost, easily available and possess an anti-corrosive property, parameters that are considered essential to improve the performance of MFCs. Besides carbonaceous materials, Cheng and Logan (2007) mentioned the use of certain other types such as graphite fiber brush, carbon cloth, graphite foil, carbon nanotube, graphene doped with nickel and printed porous carbon as a 3-D anode for their use in MFCs. It has been reported that by using a phosphate buffer there is an increase in solution conductivity and ammonia gas treatment of a carbon cloth anode substantially increased the surface charge of the electrode (0.38 to 3.99mWm⁻²) and thereby enhanced MFC performance. The combined effects of phosphate buffer and ammonia-treated electrode estimated more power generation (power increased from 1640mWm⁻² to 1970mWm⁻²) of up to 48% as compared to earlier experiments that used the air-cathode MFC (Guo et al., 2015). It has also been reported that carbon cloth anode with pre-treatment of ammonia gas increased the charge of the electrode and the performance of the MFC. Cheng and Logan (2007) stated that the treatment is useful to increase the performance of MFCs to up to 20% as compared to the untreated electrode. Pham et al. (2009) recorded the use of 3D electrodes as another alternative to improve the attachment of electroactive microbes to enhance power generation. In another electrochemical study, by using acetaminophen (APAP) as substrate, four different strains of *Scenedosporium dehoogii* on the carbon paste electrode were slightly modified by cellulose fibers and Nafion 117© (that acted as proton exchange membrane). The optimal resistance attained was around 3000 Ohm at the maximum power density. After 40 hrs, the power density was increased to 50mWm⁻² and the biodegradation of APAP was successfully observed. Fungal cells require mediators for electron transport. The use of mediators also influences the performance of the MFC. de Oliveira (2019) observed that the addition of the electron mediators improves the current and voltage that substantially plays a vital role in cell to electrode transfer. It is stated that the use of different exogenous compounds with different toxicity stages impact directly on energy generation (Adebule et al., 2018).

8.2. Cathode

Cathode plays an essential role in power generation. In the cathode chamber, the final electron acceptor (EA) is reduced via electrons generated in the anode while protons flow from the anode through PEM (Jadhav et al., 2020). Several cathodic aspects influence the performance of the materials. Carbonaceous choices of cathode catalyst, cathode surface material, cathode operating conditions such as pH are crucial factors affecting the cathode performance. Altering the design configuration is sometimes used to improve the surface area of the cathode. Modification of the cathode with a highly active catalyst such as platinum is used to improve the performance that also increases the reaction rate. The cathodic environment includes the catholyte pH, concentration of EAs, buffering capacity of catholyte and temperature etc., which are responsible for performance variations. It has been noticed that dissolved oxygen concentration increases with an increase in power density in the dual-chamber MFC. Liu and Logan (2004) stated that at the temperature range of 20 to 32 °C, the increased power output was calculated to be 9%. The study conducted by Wu (2012) suggested that when the MFC was injected with the white-rot fungus as cathode catalyst, the maximum voltage and power density obtained was 180 ± 20 mV and 320 ± 30 mW/m³ respectively. In comparison, with carbon fiber (that acts as control) it was 40 ± 10 mV and 50 ± 10 mW/m³

respectively. The growth of the fungal cells is directly proportional to the performance of the MFC. The composition of the growth medium thus plays a significant role in the power output and overall performance of MFCs.

9. Membrane separators in MFCs

The membrane separator or PEM serves as a salt bridge between the two-compartments of an MFC. The PEM holds the capacity to transfer the protons generated at the anode compartment to the cathode compartment and thereby inhibits oxygen diffusion into the anode chamber and prevents short circuit via insulation of the electrode in the MFC (Leong et al., 2013). PEMs used in MFCs should have a high capacity for cation transfer. Cation exchange membrane, anion exchange membrane, bipolar membrane, ultrafiltration membrane, micro-filtration membrane, etc., serve as diverse membrane separators used as PEMs (Kim et al., 2007). Nafion is the most common and widely used PEM due to its highly selective permeability of protons and high electrical conductivity. As biofouling reduces the activity of a membrane fouling, therefore, it is required to develop novel PEMs with efficient capabilities. However, one of the disadvantages in using PEMs in MFCs is the slow transfer of protons from the anodic to the cathodic chamber that undergoes the pH alteration. This problem eventually interferes with the system stability and adversely affects the power output, i.e., it increases the resistance of the cell and overall cost of the MFC. Development of active PEMs to improve charge transfer in fungal based fuel cells is essential for improving the capabilities of biological-based alternative renewable energy sources for future exploitation and research.

10. Choice of substrate

The choice of substrate is one of the vital parameters for determining the performance of MFCs in electricity generation. Power production and energy build-up in an MFC depends on the abilities of the fungal cell to oxidize diverse substrates of varying natures and concentrations. A higher concentration of the substrate increases the rate of electron transfer, resulting in a high power output. Various simple as well as complex organic compounds released from diverse environmental sources like municipal, domestic, dairy, slaughter, refinery, rice mill wastewater can be used as substrates in fungal fuel cells. It has been observed that simpler forms of substrates are easier to degrade than large and complex substrates. Additionally, simpler substrates produce more power output as compared to complex substrates. Growing fungal cells efficiently use a variety of foods, nutrients and specific nutrient ingredients, such as a high proportion of carbohydrate, nitrogen, cornmeal, herbaceous and wood stems, seeds, leaves and so on, which are typically found in high concentrations in wastewater bodies. In addition, the amount of electricity produced and stored by each substrate is directly influenced by MFC configuration as well as other factors such as pH, temperature, salinity or salt stress and so on.

11. Fungal metabolism

The metabolic pathway of the microorganism is considered as a pivotal parameter that determines the cell potential. In MFCs, the performance of a system is influenced by the growth and metabolism of the microbes that are being used. Fungi are eukaryotic and have a complex cellular organization. There are two pathways involved in electron transfer in fungi. Oxidation of the substrate e.g., glucose oxidation results in the production of two NADH molecules, per glucose molecule (glycolysis). While on the other hand, mediators such as MB interact with a component of ETC that keeps the ETC functioning and generates electrons from the TCA. These two metabolic pathways seemed to be essential in providing electrons simultaneously for the removal of waste from the substrates. However, mutation in these pathways disturbs the

system and lowers the power output. Reactor configuration also seems to influence the performance of biological fuel cells. There are basically two construction designs—single and dual-chambers—to evaluate the performance of MFCs *in vitro*. In addition, sufficient space within the chamber, airtight compartments, and inlet and outlets for various purposes are certain prerequisites to arrange the electrodes and the PEM in the system.

12. Environmental factors

Temperature, pH, ionic strength, salinity etc., are believed to be key factors affecting the growth and metabolism of the microorganisms in MFC. Fungal cells normally grow in low pH (pH 5–7). Even a slight increase in the pH of an MFC can affect fungal cell growth. Ionic strength is also another parameter influencing the performance of MFCs. Increased ionic conductivity can reduce the internal resistance and lead to power generation. Ionic conductivity is directly proportional to the power output, i.e., increase in the ionic conductivity increases the power output of the system. Additionally, temperature plays a critical role in electricity generation. The temperature of the system facilitates the metabolism of the cells and their enzymatic reactions. Optimum temperature is always favoured to maintain the system as even a slight increase or decrease in temperature leads to the denaturation and inactivation of the cell components. The organic load in the substrate is essential since it eventually affects the substrate conversion in the anodic compartment of the MFC. Another parameter that critically affects the performance of the MFC in the generation of electricity is external resistance.

13. Application of yeast cells in wastewater treatment

Yeast cells that are commonly being used in MFCs consist mainly of two type: fermented and oxidized (Rozone, 2021). Fermented yeast uses six-carbon sugar to ferment into alcohol and CO₂ that are of prime use in most food and beverage industries. Yeast possesses the ability to metabolize most of the organic and inorganic compounds as well as hazardous materials. Yeast cells normally grow and proliferate in acidic environments, at optimum temperatures of 25–30°C. However, yeast exhibits a strong metabolic efficiency and also possesses the ability to adapt in diverse environmental conditions such as high acid conditions and temperatures, osmotic pressure resistance due to the presence of certain enzymes in their cells. Exploitation of yeast in wastewater remediation dates back to 1970, when Yoshizawa developed a yeast for wastewater treatment. As yeast cells are known to produce diverse lipids, glycolipids and enzymes of significance they are found to be suitable for the treatment of wastewater since it usually contains high concentrations of organic matter, heavy metals, ion wastewater and domestic sewage. Chigusa et al. (1996) observed that a high concentration of organic matter is more quickly degraded after the use of yeast consortium. He reported this after applying mixtures of nine effective yeast strains for the treatment of soybean oil wastewater the effects of which remained for an entire year. The substrate of oily wastewater with high COD and BOD when treated with yeast can reduce the oil content from 10,000 mg/L to 100 mg/L. Similar findings were made by Zheng et al. (2001) who reported the efficiency of yeast cells in removing high concentration oil to up to 98% in wastewater. Han et al. (2005) demonstrated the ability of yeast cells to remove dyes from wastewater. Yeast cells also work more efficiently in the treatment of heavy metal contaminated ion wastewater. Fungi have been reported to act as biocatalysts in the anode chamber, allowing wastewater to be used as a substrate. Biodegradable organic wastewater can be used as anolyte feed. Because of the presence of a diverse range of enzymes (both exocellular and endocellular), this fungus-based MFC can be used for any type of waste biodegradation activity, as opposed to fermentation. The treatment of wastewater using fungal degraders is possible with the consumption of substrate. It has recently been reported that fungi in

combination with algae is effective in MFCs to produce electricity by using molasses as a substrate. Microporous tubes embedded in activated bleaching earth acted as an ion exchange medium during the experiment to transport protons from the yeast to algae, resulting electricity production. A particular strain, *Galactomyces reessii*, generated electricity at a rate of 59.0 mW/m² on substrates such as rubber industry sludge and synthetic wastewater. As anolytes, ferricyanide potassium, acetate and acid orange were used. The process made use of oxygen as an electron acceptor. In the experiment, a double chambered H-type MFC was used with a salt bridge and a nafion membrane as separators. The anode was made of graphite plates and activated carbon fiber (Wu et al., 2012; Morant et al., 2014, Belniak and Maminska, 2018; Chaijak et al., 2018b). Unique yeast species with their ability to transfer electrons extracellularly can help to improve fuel cell-based technologies. They can also be used to purify wastewater as a biocatalyst. The exploitation of natural and genetically modified yeast species of improved enzyme activities may be useful to degrade toxic substances along with their capacity for electricity generation. For instance, *C. melibiosica* cell is functional for electricity generation as well as phytate remediation because of its high phytase activity (Hubenova et al., 2014a), which opens up novel avenues for utilizing non-chemical, eco-friendly and more sustainable approaches for the purification of phosphate polluted wastewater areas (Hubenova et al., 2014) as well as other emerging contaminants and xenobiotics.

14. Challenges and prospects

Analogous to bacterial MFCs, fungal based MFCs use fungi as a biocatalyst. Yeast has been successfully used as a biocatalyst in MFCs in both chambers, i.e., anodic and cathodic chambers. Although different microbial strains are known for their abilities to transfer electrons, till date, very little information is available on the potential of yeast-based MFCs for power generation.

Kluyveromyces marxianus is a promising fungus known for its abilities in wastewater treatment. It has been contended that the addition of mediators eventually improves the performance of MFCs. However, the greatest challenge in the use of MFC-mediated fungi is that enough power generation is still not possible through fungal MFCs. In commercial electricity generation processes using fungi-based MFCs, electron acceptors such as ferricyanide cannot be used in such a way as to identify the invention or the use of novel electron acceptors. Further, extensive research is required to explore novel and efficient fungal strains that may be utilized in biological fuel cells to produce high-efficiency power and fuel generation by utilizing wastewater as well as other organic and inorganic complexes for the benefit of mankind. Electrode modification is required to improve the performance of the fungal based MFCs. Microbial based biofuels now-a-days seem to be viable alternatives to petroleum-based fuels. Reducing the cost of biofuel production will help in the production of as many co-products as possible. Diverse enzymatic reactions and the generation of fungal enzymes required for bioconversion are primarily identified by advanced molecular biology and biotechnological tools for system expansion and ethanol production. Future efforts to improve fungal-based biofuel production on a larger scale for maximum productivity and quality product recovery should focus on the use of optimal genetic and metabolic alteration of strains, protein engineering methods, and so on. Further research on heterotrophic oleaginous fungus species and their fatty acid profiles, such as triacylglycerols and sterol biosynthesis characteristics, is required to gain a better understanding of their functions and regulations for future waste to energy applications. The use of whole fungal cells as biocatalysts for the production of biofuel still remains a major challenge in MFCs due to their considerably slower response rate. This will have to be enhanced through extensive and novel works in the area mentioned to enable a significant increase in the quantity and maintenance of the system. As global demands energy have risen rapidly it has led to a rapid depletion of traditional energy sources.

Furthermore, global warming together with the dangerous effects of conventional energy sources on the climate as well as the increase in the prices of fossil fuels necessarily compels us to explore the potential of fungi in generating biofuels as an economically feasible, eco-friendly and sustainable solution. Although German researchers began producing ethanol from plant materials in 1898 and continued in the United States during World War I, research into the combined ability of various fungi and bacteria to degrade cellulose and other plant polymers has increased in the twenty-first century. Recent advances in biofuel research have focused on the ability of certain microbial species to biosynthesize and store large amounts of fatty acids in their biomass, which could be used to replace conventional oil in biodiesel production (Xiong et al., 2008). Analysis for the fatty acid profile of the microbial lipids is essential in using microbial species as feedstock for biodiesel production. Transition of plant biomass production using microbial communities offers a considerable commercial solution for biofuel production. Recent technologies like protein engineering, genetic and metabolic modifications of microbial strains can be of immense importance in the development and design of fungal-based biofuels (FBB) in future. Efforts to improve the advancement of biofuels have prompted researchers to allow them to produce extra ethanol from unique nutritional resources to identify genomes in micro fungi that enhance their resistance to ethanol.

15. Conclusion

Fungi based MFCs are a recent advancement in wastewater treatment technology. Electrodes and microorganisms are the key players in MFCs, whose improvement and modification is directly related to the increase of the output of the system. The present review provides evidence that fungi can be explored and measured as capable microorganisms for biological fuel cells. Some yeast species can efficiently digest complex organic substances with excellent power output even at high working temperatures making them better alternatives for waste conversion. Furthermore, adding graphene to the surface of the carbon material can increase efficacy and may be commercialized in the near future because of its carbon neutral characteristics. The use of potent fungal strains as anodes and cathodes (biocathodes) is also summarized in this review. Electron transfer system in the fungal cell and various types of fungi with their electron transport mechanisms is also highlighted for the benefit of stakeholders. *S. cerevisiae*, with modifications in the electrode and optimization of the environmental condition have shown promising results for MFCs. Various experiments have also been summarized to prove the efficiency of fungi in the degradation of organic matter in wastewater and the simultaneous generation of bioelectricity. However, to improve the activity of fungi based MFCs, extensive research is required to design the particular alterations in electrode material and to find and improve the electron transfer mechanism of different fungal strains that are of use in energy generation. This review traces the chronological development of microbial fuel cell technology with fungi as catalysts and the different operational factors in optimizing this technology to enhance overall power production. The review also provides measures to overcome the limitations associated with this technology by developing fungal mediated MFCs including various potent strains and utilization of diverse nanomaterials for enhanced electricity generation. Nevertheless, the estimated cost of fungal mediated MFCs and cell life cycles need to be addressed more precisely in comparison to conventional methods in order to obtain more valuable information on the efficiency of this technology.

Declaration of Competing Interest

The authors declare no conflict of interest.

Author Contributions

Prajakta Pawar, Mayur Thakare: Collection of data from published papers, review and assistance in the creation of Figures and the draft manuscript; **Ram Prasad:** Concept, advice on scientific research and proofreading; **Hemen Sarma, P. N. Bhattacharyya, Dipak A. Jadhav, Abhilasha Singh Mathuriya, Soumya Pandit:** The design concept, the content, original draft, as well as the visualization have been prepared and the manuscript revised.

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