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Chapter 3

Yeast as a Biocatalyst in Microbial Fuel Cell

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Additional information is available at the end of the chapter

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Abstract

Microbial fuel cells (MFCs) are fascinating bioelectrochemical devices that use the catalytic activity of living microorganisms to draw electric energy from organic matter present naturally in the environment or in the waste. Yeasts are eukaryotic microorganisms, classified as members of the fungus kingdom. Several yeast strains have been studied as biocatalysts in MFC with or without external mediator such as *Saccharomyces cerevisiae, Candida melibiosica, Hansenula anomala, Hansenula polymorpha, Arxula adeninvorans and Kluyveromyces marxianus*. In this chapter, we will focus on the use of yeast as a biocatalyst in the anode of microbial fuel cells (MFCs). How different yeast strains transfer electrons to the anode of the microbial fuel cells, advantages and challenges of the use of yeasts in MFCs, how to improve the performance and sustainability of the yeast-based MFCs through the modification of the anode electrode surface, and the application of the yeast-based MFCs in continuous wastewater treatment were discussed.

Keywords: yeast, microbial fuel cell, biocatalyst, electron transfer, mediator

1. Introduction

Microbial production of energy and/or chemicals from renewable carbohydrate feedstocks, and other organic-based wastes such as wastewater, is an attractive alternative to the current common fossil fuels. Microbial fuel cells (MFCs) are among the fast-growing microbial electrochemical systems (MESs) that offer a promising way for simultaneous wastewater treatment and electricity production [1–3]. Although MFCs showed promising features such as simultaneous wastewater treatment and electricity generation, low sludge production, wide range of substrates and operating at room temperature, the low power output and high cost especially that of the Pt cathode are the main challenges facing their commercialization [4–6].

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In MFCs, the exo-electrogenic microorganisms act as biocatalysts in anaerobic oxidation of the organic materials that exist in different wastes, liberating electrons that can be collected by a conductive electrode, i.e., anode, generating an external power-producing circuit, and protons transferred through an electrolyte to a cathode surface. At the cathode, electrons react with protons and oxygen producing water [7–9]. The exo-electrogenic microorganisms that can be used in MFCs can be a prokaryote or eukaryote. Although prokaryotic microorganisms showed promising results in the MFCs and a lot of research has been carried out using them due to their ease in the electron transfer mechanism, yeast, as a eukaryote, attracted researchers' attention and was extensively studied as a biocatalyst in MFCs [4–6].

2. Microbial fuel cells: structure, components and mechanism

Microbial electrochemical systems (MESs) are innovative technology, recently implemented for numerous applications [10–15] such as (i) the simultaneous wastewater treatment and electricity production by MFCs, (ii) bio-hydrogen and/or other chemical production by microbial electrolysis cells (MECs), (iii) water desalination by microbial dialysis cells (MDCs) and (iv) electricity production in sediments or plant MFCs.

In case of MFCs, microorganisms oxidize organic matter, producing electrons that travel through a series of respiratory enzymes in the cell and make energy for the cell in the form of ATP. The electrons are then released to a terminal electron acceptor (TEA) that becomes reduced. Many TEAs such as oxygen, nitrate, sulfate and others readily diffuse into the cell where they accept electrons forming products that can diffuse out of the cell. However, it is now known that some microorganisms can transfer electrons exogenously (i.e., outside the cell) to a TEA such as metal oxides like iron oxide. This is the case of bacteria called exo-electrogens, which can be used to produce electricity in MFC [16].

Figure 1 shows a schematic diagram of an air-cathode MFC that consists of anode and cathode electrodes separated by a separator (if needed). The anode compartment composed of anode and carbon source (organic materials), with or without exogenous mediator. At the cathode, an electron acceptor (O_2 from air) reacts with protons that pass from the anode to the cathode through the electrolyte, and the electrons produce water.

2.1. Anode material

Anode material is considered as an important parameter that affects the performance of MFCs. The anode of the MFCs should have high electrical, mechanical and chemical stability, be biocompatible and have high surface area [20]. Carbon materials (conventional and nonconventional) are the best materials that are applied as anode in the MFCs showing high power output. The conventional carbon materials such as carbon paper, carbon cloth, carbon brush and carbon felt, and the nonconventional ones such as carbon nanotubes (CNTs), carbon nanofibers and graphene have been extensively applied in MFCs. Little work have been carried out using noncarbonaceous materials such as stainless steel, gold and titanium [17–19], which showed a lower performance compared to that obtained in case of using carbon.

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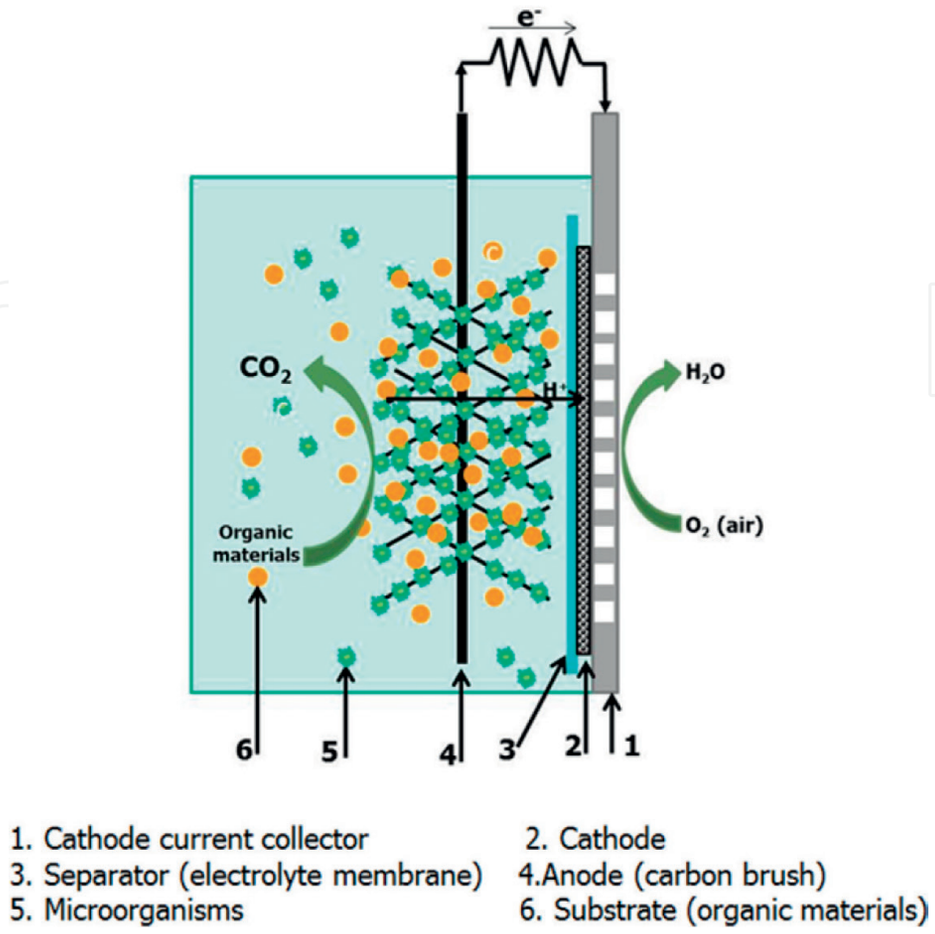


Figure 1. A schematic diagram showing the main components of an air-cathode MFC.

2.2. Cathode material

Cathode material has a significant impact on the overall cell voltage and it should have a high redox potential. Carbon materials such as carbon paper and carbon cloth modified with high active catalyst such as Pt catalyst are among the most common cathodes of the MFCs [20]. Although modifying the carbon cloth and/or carbon paper with Pt significantly decreased the oxygen reduction activation energy and increased the reaction rate, the high cost and scarcity of the Pt are the main challenges facing the application of such cathode. Recently, a wide range of non–Pt-based catalysts were investigated as cathodes in MFCs and showed promising results that gave them a potential to replace Pt catalyst in the near future such as carbon nitrogen alloys and metal carbides [18, 20–29].

2.3. Separator

As anode is working under anaerobic conditions, while cathode is working under aerobic conditions, the addition of separator with high ionic conductivity and low permeability could improve the MFC performance [30]. A large number of separators have been extensively studied in MFCs such as anion and cation exchange membranes, salt bridge, glass fibers, microfiltration membrane, porous fabrics, and coarse-pore filters [31–37]. It is worth mentioning that some MFCs showed better performance even without using the separator [3].

2.4. Microbes and electron transfer in microbial fuel cells

Microorganisms are generally divided into two main categories, prokaryotes and eukaryotes. Prokaryotes are simpler (no distinct nucleus) and smaller in size (around 1 μ in diameter) compared to eukaryotes that have larger size (5–10 μ or more) and are complex (possessing a distinct nucleus and subcellular organelles such as plastids and mitochondria) [4, 6]. All microorganisms that are capable of exo-cellular electron transfer (exo-electrogens) can be effectively used in MFCs without adding soluble exogenous mediators [4, 22, 30–38].

The possible electron transfer mechanisms in MFCs are shown in **Figure 2** and can be summarized in the following:

1. Direct electron transfer (DET) whether by direct cell attachment or through nanowires (pili)

   DET requires a direct contact between the anode surface and the outer membrane of the microorganism. Pili are nanowires that are formed out to connect the microorganism's membrane to the anode surface. The merits of the pili formation that multiple layers biofilm microorganisms can participate in the electron transfer while bulk ones do not participate in the electron transfer [4, 39–43].

2. Indirect electron transfer through external or internal mediators

   In this type, a redox active material (mediator) is responsible for the electron transfer between the microorganism and the anode surface. This redox can either be exerted naturally by the

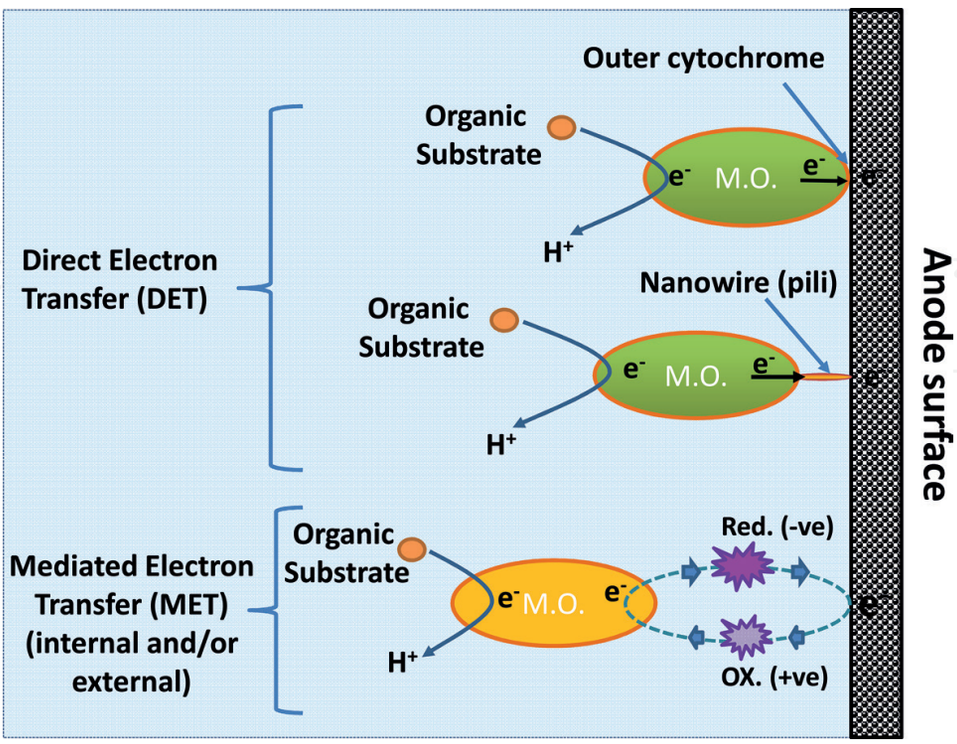


Figure 2. Schematic diagram showing different electron transfer mechanisms in MFCs.

microorganisms (internal) or can be added from outside (external). These mediators whether internal or external will be responsible for the electron transfer from the bulk microorganisms to the anode surface. The electron transfer in the mediated electron transfer is higher than that in the DET [4, 44–51].

Internal mediators have several advantages over the external ones such as they are cheap as they are exerted by the microorganism and have no toxic effect on the microorganism. **Figure 3** shows a schematic diagram of the disadvantages of external mediators and some types of the internal and external mediators.

Several external mediators have been investigated in MFCs such as methylene blue (MB), methyl red, methanyl yellow, methyl orange, bromocresol purple, bromocresol green (BcG), romothymol blue, bromophenol blue, Congo red, cresol red, eriochrome black T, murexide, neutral red (NR), yeast extract, etc.

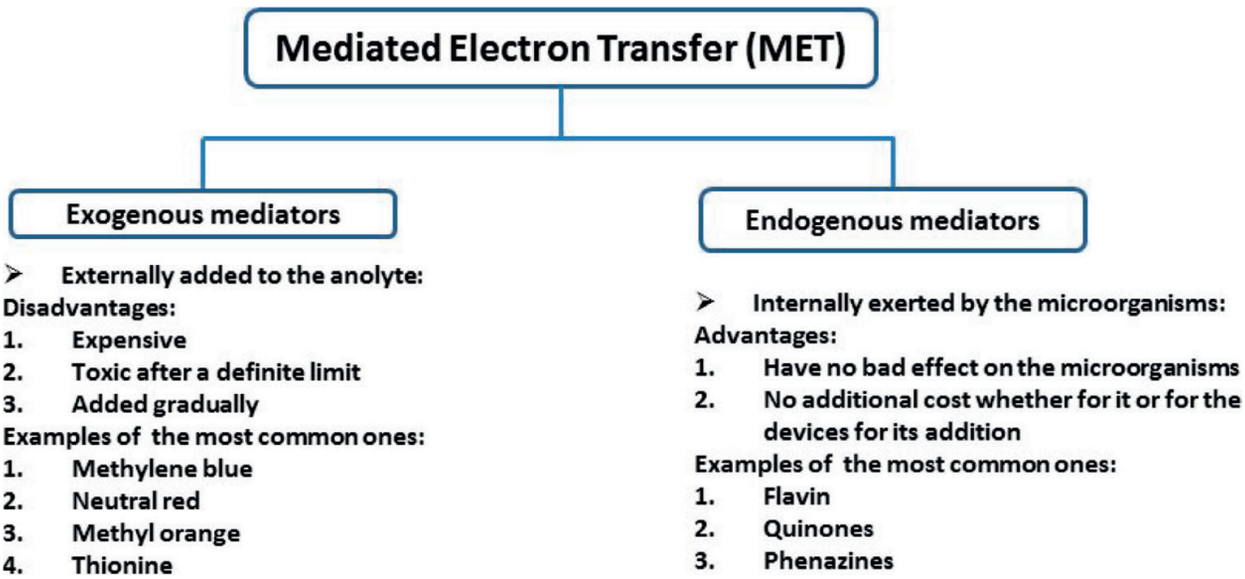


Figure 3. External and internal mediators in MFCs.

3. Yeast as a biocatalyst in MFCs

Yeast is a eukaryote with cell compartmentalization and has more complicated architecture compared to prokaryotes. Yeast is considered as an ideal biocatalyst for microbial fuel cell applications as most strains are nonpathogens, can metabolize wide range of substrates, are robust, and are easily handled. The bio-catalytic activity of the yeast would be related to the existence of different natural electron shuttles, mediators, such as azurin, ferredoxin and cytochromes, which could be used by redox enzymes for electron transfer from the yeast cells to the anode surface. This is in addition to the high extent of proteins in the yeast cell membrane, which is an important characteristic of electroactive species [4, 6]. Yeast cells also have a thick (100–200 nm) cell wall constructed of polysaccharides and proteins [43, 52]. Yeast cytochromes are located in the mitochondria, and transmembrane proteins (tPMETs) are

located in the cell membrane, which are enclosed by the cell wall. Hence, to obtain an electrochemical response from the yeast cells, it has been assumed that a mediator must traverse the cell wall and interact with the membrane and/or internal redox sites such as NAD+/NADH [41, 42], or that the response originates from the soluble electroactive species exported from the cell [4, 45].

The electron transfer during the metabolism of the organic materials in the yeast cell is shown in **Figure 4**. Electrons liberate during the oxidation of the substrate into pyruvate in the glycolysis process, which takes place in the cytosol of the cell. These electrons received by the NAD+ forming NADH, which is recycled through its oxidation by the liberation of the electrons to the anode surface whether directly through the tPMETs or through the mediator to form NAD+ again — cycle of NADH to NAD^+. In mitochondria, oxidation of pyruvate into organic acids is associated with the liberation of the electrons that are received by the NAD+ forming NADH, which in turn are oxidized by releasing electrons to the mediator to form the NAD+ again. The reduced form of the mediator lost electrons to the anode surface to complete the cycle [38, 46].

Several yeast strains have been studied as biocatalysts in MFC with or without external mediator such as *Saccharomyces cerevisiae* (*S. cerevisiae*) [41–52], *Candida melibiosica* 2491 (*C. melibiosica*) [53–56], *Hansenula anomala* (*H. anomala*) [40], *Hansenula polymorpha* (*Hansenula polymorpha*) [57], *Arxula adeninivorans* (*A. adeninivorans*) [58] and *Kluyveromyces marxianus* (*K. marxianus*) [59].

3.1. *S. cerevisiae*

Baker's yeast (*S. cerevisiae*) is a single cell-based organism used in bread-making and beer production industry. *S. cerevisiae* is a simple eukaryotic cell, which serves as a model system

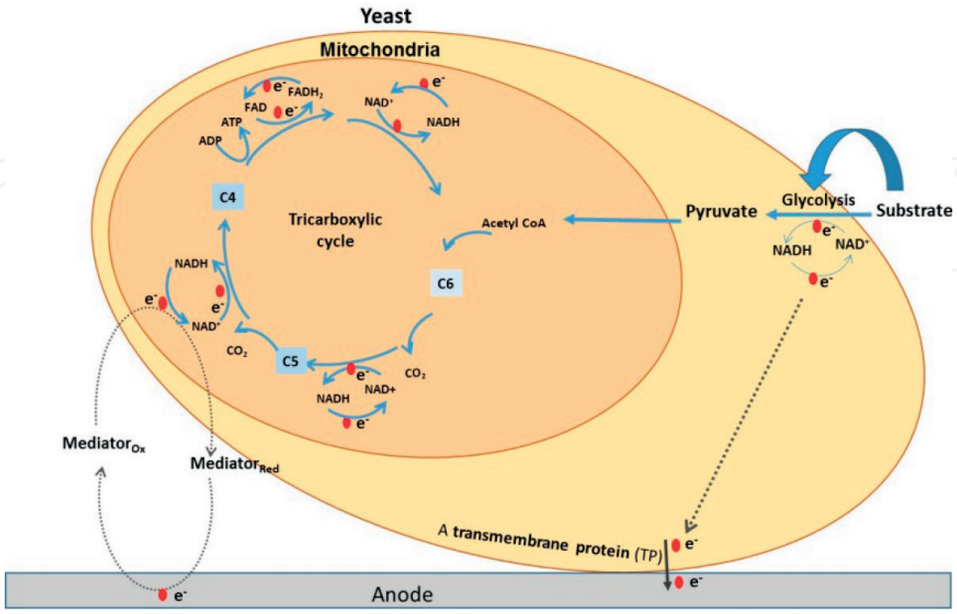


Figure 4. Schematic diagram shows the possible electrons' origin and transfer of yeast cells to MFC.

[59, 60] for many eukaryotes, including human cells, for the study of fundamental cellular processes such as the cell cycle, DNA replication, recombination, cell division and metabolism [60, 61]. *S. cerevisiae* is considered to be a good biocatalyst in MFC due to its broad substrate spectrum, easy and fast mass cultivation, nonpathogenic, cheap and can be maintained for a long time in the dried state [9, 60–62]. Due to these features, *S. cerevisiae* was recently used in a large-scale MFC [63].

3.1.1. Mediator-less MFC

Mediator-less MFCs are those that operate without the addition of any external mediator. Sayed et al. [6] studied the mechanism by which *S. cerevisiae* transfers the electrons to the anode surface whether through the solution species or through the surface-confined species in a mediator-less MFC. *S. cerevisiae* was cultivated outside the MFC and then applied in an air-cathode mediator-less MFC using glucose as a substrate. Carbon paper was used as an anode and carbon paper with Pt/C as a cathode. When the MFC was operated with the yeast cells, the anode potential decreased from 0.4 to 0.1 V (vs. NHE pH 7) during 45 h. At the same time, the open circuit voltage (OCV) increased from 0.25 to 0.65 V. A maximum power output above 3 mW/m^2 was attained during the linear sweep voltammetry (LSV). At the end of the MFC operation, when the anolyte was replaced with a fresh one without yeast cells, i.e., just glucose, into the anode chamber, the cell attained the same maximum cell voltage within 1 h of cell operation. The same maximum power generation during the LSV was also attained. On the other hand, when another MFC using a fresh anode was operated with the filtered anolyte solution, i.e., no yeast cells, neither cell voltage nor anode potential changed, **Figures 5** and **6**. The ex-situ cyclic voltammetry of the filtered anolyte at the end of the experiments showed no redox peaks; i.e., no mediator existed in the anolyte. These measurements showed that the electron transfer was done through the surface-confined species, and there was no role of the solution species in it.

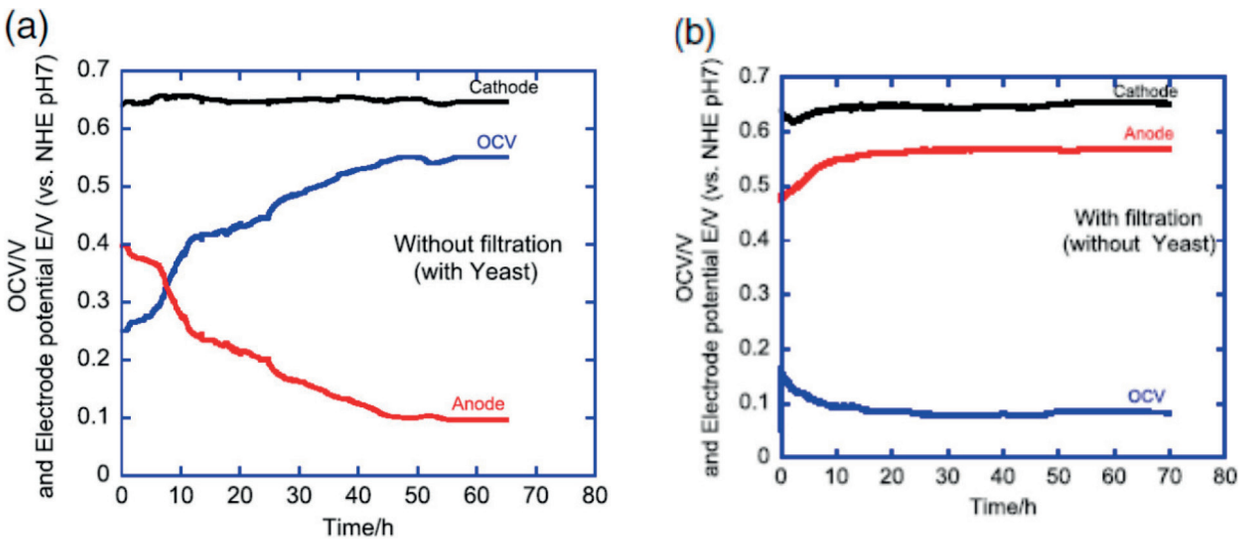


Figure 5. The OCV and the electrode potentials vs. time of the MFC using carbon paper (CP) as the anode material. (a) Without filtration and (b) with filtration [6].

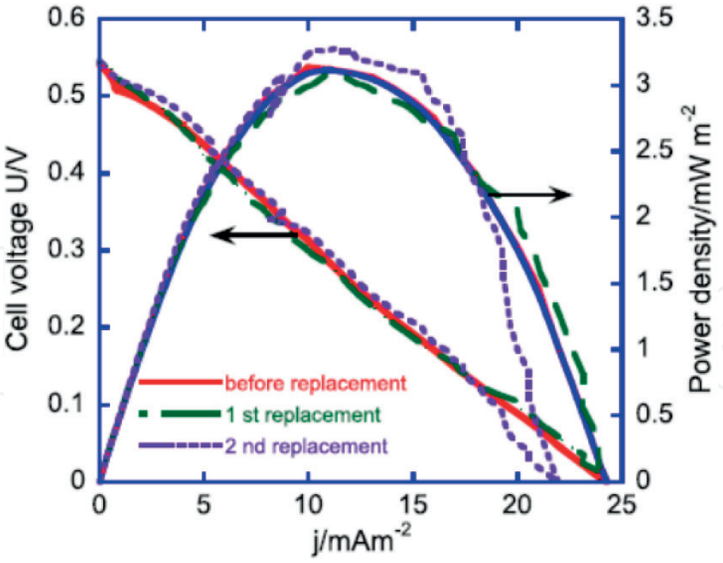


Figure 6. The i-V and i-p curves measured before and after the replacements of the anolyte solution [6].

The same conclusions for the direct electron transfer and no role of the mediator in the electron transfer of the *S. cerevisiae* were confirmed by Rawson et al. [41] who studied the direct electron transfer from the *S. cerevisiae* cells attached to the anode surface. The authors modified the anode surface with a mediator, osmium bipyridine complex, layer that hindered the mediator from penetrating the cell wall and reacting with the internal redox species. Results showed that the electron transfered from the yeast cells to the electrode surface through the yeast cell wall and no involvement of the endogenous mediator in this electron transfer.

In another study, the performance of air-cathode MFC using *S. cerevisiae* as an anodic biocatalyst under different redox conditions and organic loading was investigated [38]. The MFC was operated with synthetic wastewater at organic loading rate (OLR) of 0.91 kg COD/m^3-day and the performance of yeast-based MFC along with wastewater treatment was investigated at different feeding pH of 5.0, 6.0 and 7.0. Using cyclic voltammetry, which is an effective tool to identify the electron transfer mechanism in MFCs [64], the MFC performance was dependent on the OLR and the pH. Cyclic voltammetry confirmed the existence of the NADH/NAD+ and FADH/FAD+.

Although *S. cerevisiae* could be effectively used as a biocatalyst in mediator-less MFC, the power output was limited by the low electron transfer rate from the microorganism to the anode surface. The performance of the *S. cerevisiae*-based MFC could be improved by enhancing the rate of electron from the yeast cell to the anode surface by one or more of the following techniques:

i. Anode modification [42].

ii. Immobilization of the yeast cells on carbon nanotube [43].

iii. Yeast surface display of dehydrogenases [52].

iv. Addition of exogenous mediators [44–51].

3.1.1.1. Enhancement of electron transfer in a mediator-less MFC

The electrical conductivity of the anode plays an important role in the performance of the MFCs. The effect of the modification of carbon paper with thin layer of different transition metals, i.e., cobalt and gold, on the performance of air-cathode MFCs using *S. cerevisiae* as a biocatalyst was investigated [42]. Sputtering technique was used for preparing different thin layers of Co and Au with thicknesses of 5 and 30 nm on the surface of carbon electrodes. The 5-nm layer showed no significant effect on the cell performance, and this was related to the rare existence of the metals detected by the energy dispersive x-ray (EDX) measurements. On the other hand, 30 nm of Co significantly improved the performance where the power output increased from 12.8 to 20.2 mW/m^2 while the steady current discharge at 0.2 V increased from 8 to 27 mA/m^2, **Figure 7**. On the other hand, 30 nm of Au-modified electrode showed a negative effect on the cell performance. The positive effect of the Co on the performance was related to the enhancement of the electron transfer by the Co and the stimulation of the yeast growth on the modified electrode surface as confirmed by the SEM images. While Au suppressed the growth of the yeast cells as proved from the SEM images due to its poisoning effect, decreasing the performance (**Table 1**) [42].

The electron transfer of *S. cerevisiae* based MFC was enhanced by immobilizing *S. cerevisiae* on carbon nanotube (yeast/CNT) to be used as a catalyst in a membrane-less MFC [43]. The effect of the entrapping polymer (EP) and cross-linker (glutaraldehyde, GA) addition on the performance and stability of the MFC using *laccase* as cathodic catalyst was investigated. GA was selected as cross-liner due to its ability to promote cross-linking between yeast cells and poly(ethylenimine) (PEI), which used as the entrapping polymer due to its positive charge property. Bare CNT showed only C═C (sp^2) bonds indicating that CNT had not any functional group. In case of the immobilized yeast cells, C─N (C═N) bond peak appeared indicating that yeast cell and CNT were properly bonded. The immobilization of the yeast enhanced the power by 150% where it increased from 138 to 344 mW/m^2.

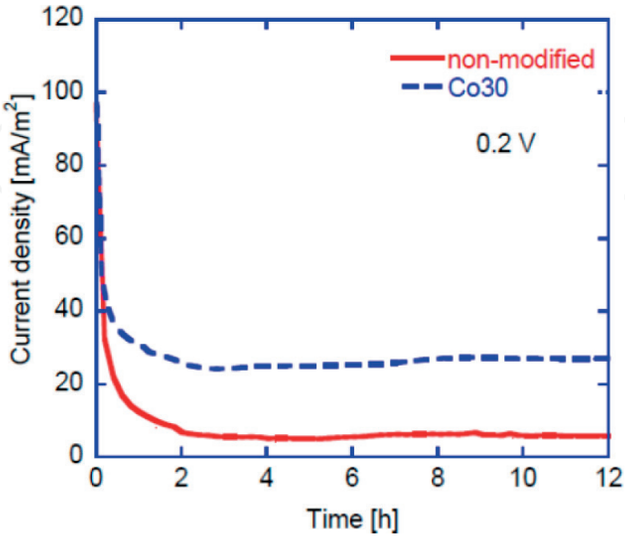


Figure 7. The i-t measurements at 0.2 V for a mediator-less yeast-based MFC using nonmodified (NME) carbon paper and Co, 30 nm, modified one [42].

Ref.	Max. power		Anode chamber (WV)	Separator	Cathode		Anode material	Carbon source	MFC type
	mW/m^2	mW/m^3			Electron acceptor	Electrode			
6	3.	17	84 mL (70 mL WV)	NRE 212	O_2 (air)	Pt/C over carbon paper	Carbon paper	Glucose	Air cathode
42	12.9 20.2 2		(70 mL WV)	Nafion 117	O_2 (air)	Pt/C over carbon paper	Carbon paper Co sputtered carbon paper Au-sputtered carbon paper	Glucose	Air cathode
38	25.51		350 mL (320 mL WV)	Nafion 117	O_2 (air)	Graphite plate	Graphite plate	Synthetic wastewater	Air cathode
52	2.7 2.8 33		8–10 mL (5 mL WV)	Nafion 117	O_2 (air)	A graphite plate	A graphite plate/ MWCNT	Lactose D-glucose lactose	Dual chamber
46		40	500 mL	Nafion 117	Potassium ferricyanide	Reticulated Vitreous carbon	Reticulated Vitreous carbon	Glucose	Dual chamber
47	28		850 mL (760 mL WV)	Nafion 117	-	Graphite plates	Graphite plates	Glucose	Dual chamber

Table 1. Summary of the studies done on the mediator-less *S. cerevisiae* yeast-based MFC.

The performance of *S. cerevisiae* based MFC was improved by displaying dehydrogenases, cellobiose dehydrogenase from *Corynascus thermophilus* (CtCDH) on the surface of *S. cerevisiae* using the yeast surface display system [52]. The surface displayed dehydrogenases were used in mediator-less two compartments MFCs. The MFCs were operated using unmodified *S. cerevisiae*, CtCDH-displaying *S. cerevisiae* and glucose oxidase (GOx) was used for comparison. Graphite plates modified with multi-walled carbon nanotubes (MWCNT) were used as electrodes in the anode and cathode compartments that were separated by Nafion 117. A maximum power output of CtCDH-displaying *S. cerevisiae* MFC was 33 mW/m^2 which was around 12 times higher than those obtained in case of GOx, and unmodified *S. cerevisiae*, 2.8 and, 2.7 mW/m^2, respectively.

3.1.2. Mediated yeast-based MFC

Several studies have been carried out to enhance the electron transfer through the addition of an external mediator. A candidate external mediator must satisfy several requirements such as being electrochemically active, fast release of electrons on the electrode surface, biocompatible to the microorganisms, soluble and chemically stable in the anolyte media, easily penetrate the cell membrane, and has a prober redox potential that is sufficiently positive to provide fast electron transfer from microorganisms to the anode while not too strong to avoid a big loss of potential [2, 14, 16]. Different mediators such as MB, NR, thionine, yeast extract, and others enhanced the electron transfer in *S. cerevisiae* yeast-based MFCs, and their power output are shown in **Table 2**.

Using copper electrodes and a sulfonated polyether ether ketone (SPEEK) as proton exchange membrane, Permana et al. [48] studied the performance of dual chamber *S. cerevisiae* yeast-based MFCs with and without MB using glucose as substrate. The MFC operated with MB showed higher cell voltage, higher power and energy outputs, and slightly lower glucose consumption without affecting the bioethanol production compared to the mediator-less MFC. Using rotating disc electrodes (RDEs), Ganguli and Dunn [45] were able to simultaneously determine the catalytic current under quiescent conditions along with the reduced mediator concentration that not adsorbed by the yeast. Based on the results from the anode kinetics study, a yeast powered microbial fuel cell successfully produced power density of ~1500 mW/m^2 once the reduced mediator concentration stabilized.

The effect of the anode modification on the performance of the mediated *S. cerevisiae* yeast-based MFC that used glucose as a substrate and MB as a mediator was investigated [50]. The anode carbon paper was sputtered with a thin layer of 30 nm of Co (Co30) or Au (Au30). The modification of the anode significantly improved the performance from 80 to 148 mW/m^2 and 120 mW/m^2 in case of Co30 and Au30, respectively, as shown in **Figure 8**. Although the cell resistance in case of Au is lower than that in Co, the performance of the latter was better and this was related to the poisons effect of the Au on the growth of the yeast cell on the anode surface; therefore, only the yeast in the anolyte took part in the performance, while in case of the Co, the yeast cells in the anolyte and those formed as biofilm on the anode surface took part in the electron transfer. The cell resistance decreased from 25 μΩ cm^2 in the case of nonmodified (NME) anode to 4 and 3 μΩ cm^2 in case of Co30 and Au30, respectively. The better performance

Ref.	Max. power		Mediator	Anode chamber (WV)	Separator	Cathode		Anode material	Carbon source	MFC type
	mW/m^2	mW/m^3				Electron acceptor	Electrode			
59	22	850×10^3	2-hydroxy-1,4-naphthoquinone	WV, 7.5 cm^3	Gore-Tex, 30 μm	$K_3[Fe(CN)_6]$	Carbon rods	Carbon rods and carbon fiber bundles	Glucose	Dual-chamber
50	80 148 120		MB	(70 mL WV)	Nafion 117	O_2 (air)	Pt/C over carbon paper	Carbon paper Co-sputtered carbon paper Au-sputtered carbon paper	Glucose	Air cathode
45	150		MB	10 mL	Nafion	Potassium ferricyanide	Carbon felt	Carbon felt	Glucose	Dual chamber
46		146.71 ± 7.7	MB	500 mL	Nafion 117	Potassium ferricyanide	Reticulated vitreous carbon	Reticulated vitreous carbon	Glucose	Dual chamber
52	39 31 32 22 14		MB (0.1 M)	25 mL		O_2 (air)	Pt/C over carbon cloth	Graphite plate	D-xylose D-glucose L-arabinose D-cellobiose D-galactose	Air cathode
44	400 80 500		MB NR MB &NR	32 mL	Nafion 115		Reticulated vitreous carbon	Reticulated vitreous carbon	Dextrose	Dual chamber

Ref.	Max. power		Mediator	Anode chamber (WV)	Separator	Cathode		Anode material	Carbon source	MFC type
	mW/m^2	mW/m^3				Electron acceptor	Electrode			
45	1500		MB	10 mL	Nafion	Potassium ferricyanide	Carbon felt,	Carbon felt	Glucose	Dual chamber MFC
46		145	MB	500 mL	Nafion 117	Potassium ferricyanide	Reticulated vitreous carbon,	Reticulated vitreous carbon	Glucose	Dual chamber MFC
47				850 mL (760 mL WV)	Nafion 117	-	Graphite plates	Graphite plates		Dual chamber
51	36 70	36	YE	70 mL WV	Nafion 117		Pt/C over carbon paper	Carbon paper Au-plated carbon paper	Glucose	Air cathode

Table 2. Summary of the studies done on the mediated *S. cerevisiae* yeast-based MFC.

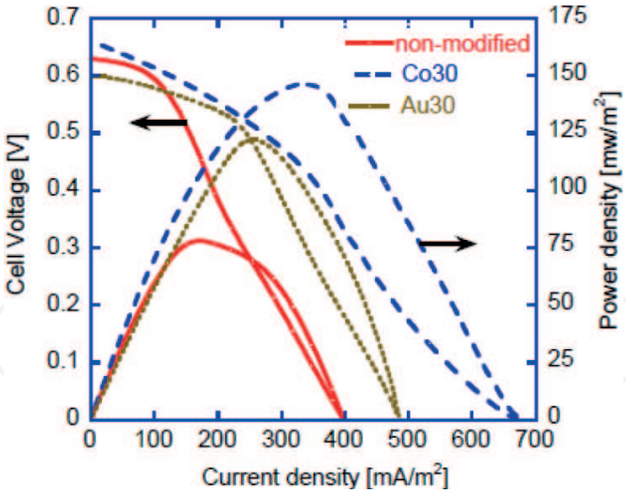


Figure 8. The i-V and i-p curves of the yeast-based MFC with 0.1 mM MB using nonmodified carbon paper, and Co30 and Au30 as anodes [50].

in both cases was related to the metal-modified surface that significantly enhanced the electron transfer via the exogenous mediator. It was also considered that the highly conductive surface of the Co or Au on the anode surface increased the efficiency of the electron transfer by contacting a part of the mediator with an electric charge on the anode.

MB was also used in air-cathode MFC that used modified *S. cerevisiae* using yeast surface display system [52]. Pyranose dehydrogenase from *Agaricus meleagri*s (AmPDH) was displayed on the surface of *S. cerevisiae*. The MFCs were operated using unmodified *S. cerevisiae* or AmPDH-displaying *S. cerevisiae* with various fuels, D-xylose, D-glucose, L-arabinose, D-cellobiose and D-galactose using 0.1 mM MB. AmPDH displaying *S. cerevisiae* generated high power outputs using the different substrates, 3.1, 3.9, 3.2, 2.2, and 1.4 μW/cm^2 in case of using D-glucose, D-xylose, L-arabinose, D-cellobiose and D-galactose, respectively, compared with a maximum power output of 0.8 μW/cm^2 in case of the unmodified *S. cerevisiae* using D-xylose as a fuel [52].

Compared to MB, NR showed promising results in a two-compartment *S. cerevisiae* yeast-based MFC for degradation of whey. With a fixed concentration of the two mediators of 100 μmol/l, the maximum power and current densities increased from 1.43 μW and 11.5 μA to 50 μW and 470 μA in case of the NR compared to 11.3 μW and 120 μA in case of MB. These results showed that NR served as a suitable mediator and enhanced the electrical energy by 5 folds compared to that of MB [49]. When NR (0.5 mM) was added to the MB (0.5 mM), the *S. cerevisiae* yeast-based MFC showed a maximum power output of 500 mW/m^2 compared to 400 mW/m^2 in case of 1 mM of MB [44]. This increase in the performance was related to the role of the MB in the enhancement of the anaerobic respiration, while NR involved with fermentation only. This study [44] showed that the addition of the MB was effective than NR, which is in contradiction to that reported by Najafpour et al. [49]. This might be related to the difference in the operation conditions, and/or any other reasons that is not clear for the authors right now.

Thionine is another mediator that worked effectively in *S. cerevisiae* yeast-based MFC [47]. Thionine addition significantly increased the performance from 3 to 28 mW/m^2. An optimum concentration of thionine was 500 mM, giving a maximum voltage of 420 mV and a maximum current of 700 mA/m^2. Cyclic voltammetry measurements showed a redox peak of −0.1 V vs. Ag/AgCl.

Yeast extract, which is one of the main components of the biological cultivating media, was effectively used as a mediator in *S. cerevisiae* yeast-based MFC [51]. Using two different anodes, plain carbon paper and gold-plated carbon paper, the current density increased from 94 and to 190 and 300 mA/m^2, respectively, by yeast extract addition as shown in **Figures 9a** and **10a**. While the power density increased from 12.9 and 2 to 32.6 and 70 mW/m^2 with the yeast extract addition for the plain and the gold-plated electrodes, respectively, as shown in **Figures 9b** and **10b**. The role of the yeast extract as an electron transfer mediator was confirmed using the gold-plated carbon paper where no cells were detected on its surface (as confirmed from the scanning electron microscopic [SEM] images); therefore, the role of the surface-confined species in the cell performance was denied.

3.2. *C. melibiosica*

C. melibiosica is a yeast strain that possess high phytase activity, which existed in plant wastes. This yeast strain was used in numerous studies as a biocatalyst in MFC with and without mediator as can be seen in **Table 3**. The catalytic activity of *C. melibiosica* was studied in a dual chamber MFC with and without the addition of MB using different carbon sources, i.e., fructose, glucose and sucrose [53]. Results showed that *C. melibiosica* could be used as a biocatalyst in a mediator-less MFC giving a maximum power output of 60 mW/m^3 in case of fructose. This power increased three times either by the addition of yeast extract and peptone or by the MB addition [53].

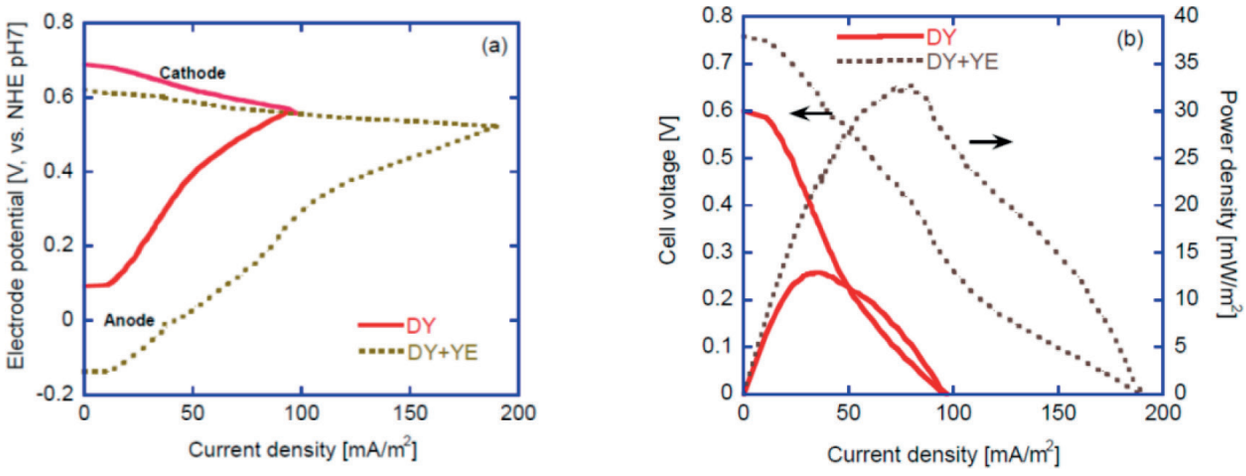


Figure 9. The effect of the yeast extract (YE) addition to *S. cerevisiae* (DY)-based MFC using nonmodified carbon paper as anode on (a) the electrode potentials, and (b) the current-voltage and current-power curves [51].

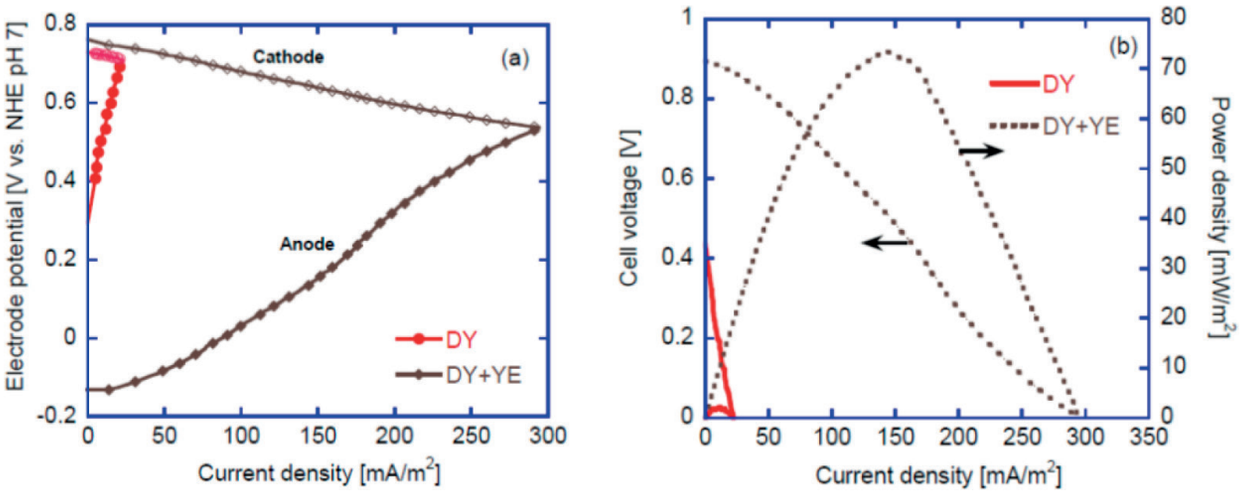


Figure 10. The effect of the yeast extract (YE) addition on *S. cerevisiae* (DY)-based MFC in case of using gold-sputtered carbon paper as anode on (a) the electrode potentials, and (b) the current-voltage and current-power curves [51].

The effect of the mediator type, i.e., bromocresol green (BcG), bromocresol purple, romothymol blue, bromophenol blue, Congo red, cresol red, eosin, eriochrome black T, methyl red, methanyl yellow, MB, methyl orange, murexide and NR on the performance of *C. melibiosica*-based MFC was investigated [54]. Results showed that among the investigated mediators, MB, methyl orange, methyl red and NR increased the performance compared to the mediatorless MFC. MB showed the best among all of them where the performance increased from 20 to 640 mW/m^2 with MB concentration of 0.8 mM. This was related to its ability not only to increase the electron transfer rate but also forcing the living cells to switch on various catabolic pathways and divert electrons from different energetic levels, thus increasing the energy production. This had been confirmed by measuring the ethanol production. where the MFC that operated using MO and MR produced trace amounts of ethanol, while in case of MB, ethanol was not detected. These indicated that the aerobic respiration processes were predominant in these cases. On the other hand, ethanol was produced in large quantities when NR and BcG were used, demonstrating that these mediators stopped the respiratory processes and displaced them with alcoholic fermentation.

The performance of *C. melibiosica*-based MFC was investigated using modified and nonmodified (NME) carbon felt [55]. The carbon felt was modified by Ni using two different techniques, i.e., galvanostatic pulse (GME) and potentiostatic pulse (PME). Carbon felt was used as the cathode, Nafion 117 as the separator, fructose, yeast extract and peptone (YP_{fru}) as the anolyte, and potassium ferricyanide as the catholyte. The power output of the MFC significantly increased using the modified electrodes where it increased from 36 mW/m^2 in case of the NME to 390 and 720 mW/m^2 in case of PME and GME, respectively. These values were even higher than that obtained in case of using the NME with addition of the external mediator, MB. The authors related the improvement in the cell performance to the existence of Ni ions, which acted as an electron acceptor and/or due to adaptive mechanism which enhanced electron transfer through the yeast membrane. In another study, the authors prepared carbon felt modified with NiFe and NiFeP using the same preparation method [56]. They found

Ref.	Max. power		Electron transfer mechanism		Anode chamber (WV)	Separator	Cathode		Anode material	Carbon source	MFC type
	mW/m^2	mW/m^3	Mediator less	Mediator			Electron acceptor	Electrode			
53		60	Mediator less		100 mL	Salt bridge	Potassium ferricyanide	Graphite rods,	Graphite rods	Fructose, YP_{fru} Fructose	Dual chamber
		180	Mediator less								
		185		MB							
54	640			MB	13 mL	Nafion 117	Potassium ferricyanide	Carbon felt	Carbon felt	YP_{fru}	Two chamber
55	36		Mediator less		13 mL	Nafion 117	Potassium ferricyanide	Carbon felt	Carbon felt NME	Fructose	Dual chamber
	720								Ni-nanomodified carbon felts galvanostatic pulse deposition (GME)		
56	390		Mediator less		13 mL	Nafion 117	Potassium ferricyanide	Carbon felt	Ni-nanomodified carbon felts potentiostatic pulse technique (PME)	Fructose	Dual chamber
	83								NiFe(g.)		
	93								NiFe(p.)		
	155								NiFeP(g.)		

Table 3. Summary of the studies done on the *C. melibiosica* yeast-based MFC.

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that among the different tested electrodes, NiFeP-modified electrodes showed the best performance of 260 ± 8 and 155 ± 6 mW/m^2 prepared potentiostatically and galvanostatically, respectively. The authors related the improvement in the performance to same reasons that were described above [55].

3.3. Other yeast strains

3.3.1. H. anomala

The catalytic activity of *H. anomala* in a mediator-less MFC using glucose as the substrate was investigated [40]. The *H. anomala* cells were immobilized on the surface of the anode by physical adsorption and covalent linkage. The results showed that *H. anomala* could transfer the electrons through the redox proteins, i.e., *ferricyanide reductase* and *lactate dehydrogenase* exist in their outer membrane. Moreover, the MFC was operated using different anodes, i.e., graphite, graphite felt and polyaniline–Pt-composite-coated graphite. A maximum power output of 2.34, 2.9 and 0.69 W/m^3 was obtained in case of graphite felt, graphite modified with PANI and Pt, and graphite, respectively. The high performance was related to the high surface of the graphite felt and the presence of the catalytic active Pt in case of the graphite modified with PANI and Pt, respectively.

3.3.2. H. polymorpha

The electron transfer pathways between the cytosolic redox enzymes of *H. polymorpha*, overexpressing flavocytochrome b2 (FC b2), and the electrode surface was studied [57]. Both wild and genetic *H. polymorpha* yeast cells were entrapped in osmium-complex-modified redox polymers (OsRP), which are essential for the electron transfer communication, on the surface of graphite electrodes. With the addition of L-lactate, current generation was noticeable when genetic modified one was used and it was in direct contact with the redox polymer, i.e., OsRP. The results suggested that the overexpression of FC b2 and the related amplification of the FC b2/ L-lactate reaction cycle were essential to provide enough charge to the electron-exchange network in order to facilitate sufficient electrochemical coupling between the cells, via the redox polymer, and the electrode. Also they suggested that the intimate contact between the cell walls and the redox polymer is a prerequisite for electrically wiring the cytosolic FC b2/ L-lactate redox activity.

3.3.3. A. adeninvorans

The biocatalytic activity of the nonconventional yeast *A. adeninvorans* in a mediator-less dual chamber MFC was investigated [58]. Results showed that *A. adeninvorans* was effectively used as a biocatalyst ion in the MFC, generating a power of more than 0.025 W/m^2. The electron transfer was confirmed to be through the secretion of an endogenous mediator in the solution. This was confirmed using cyclic voltammetry of the supernatant from the *A. adeninvorans*. An irreversible oxidation peak at +0.45 V appeared. An *A. adeninvorans* yeast-based MFC showed a better performance than that obtained in case of *S. cerevisiae* yeast-based MFC, and this was related to the exertion of endogenous mediator in case of *A. adeninvorans*.

3.3.4. K. marxianus

Kaneshiro et al. [59] have investigated the catalytic activity of six different yeast strains in a dual chamber MFC with glucose as the substrate including *K. marxianus*, *S. cerevisiae*, *Pichia pastoris*, *H. polymorpha*, *Kluyveromyces lactis*, *Schizosaccharomyces pombe*, *Candida glabrata* and yeast strains isolated from soil [59]. Among the different tested yeast strains, *K. marxianus* showed the highest cell performance followed by *S. cerevisiae* and *P. pastoris*. Although *K. marxianus* showed the lowest glucose consumption, it showed the lowest ethanol production indicating highest efficiency. Furthermore, *K. marxianus* showed catalytic activity for the metabolism of fructose and xylose; therefore, the authors suggested that *K. marxianus* could be effectively used for of woody biomass. *K. marxianus* is one of the robust yeast strains that could be used at high temperature; therefore, the authors investigated its catalytic activity under different temperatures, 37, 45 and 50°C. The results showed that *K. marxianus* had the highest activity at 45°C. This could be used for the treatment of high-temperature effluents that are produced in some industries.

4. Large-scale yeast-based MFC

A novel yeast-based MFC stack that composed of 4 units of total capacity of 1840 mL was designed and operated using glucose as the carbon source, graphite plates as the electrodes and Nafion 117 as the separator [63]. The stack was operated under continuous mode with a hydraulic retention time of 6.7 h. Single cell and cells connected in parallel and/or series connections were investigated to achieve the best operating conditions. A maximum current of 6447 mA/m^2 and maximum power of 2003 mW/m^2 were obtained. A Columbic efficiency of 22% was obtained in the parallel connection. **Figure 11** showed that the stack could be operated for more than 3 days with stable voltage and power output. The results obtained in this study proved the potential of yeast for scaling up. **Table 4** showed summary of the materials and operating conditions used in the stack.

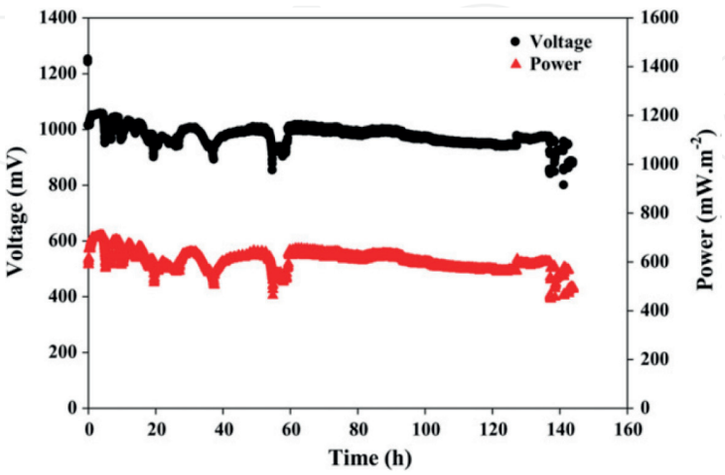


Figure 11. Close circuit voltage and produced power from staked MFC at parallel mode with 1 KΩ resistances in external circuit for 148 h [63].

MFC material	Plexiglas
MFC type	MFCs stack composed of 4 anodes and 3 cathodes compartments
Anode	Graphite plates, size of 40 × 60 × 1.2 mm
Cathode	Graphite plates, size of 40 × 60 × 1.2 mm
Membrane	Nafion 117.32 cm^2
Catholyte	Potassium permanganate (400 μmol/L)
Anode media	Yeast (*S. cerevisiae* PTCC 5269). NR (200 μmol/L)
Fuel	Glucose, 30 g/L
Anode chamber (volume)	460 mL
Working volume	350 mL
Current collector	Copper wire
Mode	Continuous up flow mode
HRT	6.7 h

Table 4. A summary of the stack materials and operating conditions.

5. Conclusions and recommendations

Yeast is successfully used as a biocatalyst in MFC, which exhibits different electron transfer mechanisms according to its strains. In *S. cerevisiae* and *H. anomala*, the electron transfer takes place through the surface-confined species; in *C. melibiosica, H. polymorpha and A. adeninivorans*, the transfer of electrons from yeast cells to the anode is both by the secretion of redox molecules and by the direct electron transfer. The modification of the anode and the addition of external mediator significantly enhanced the cell performance. *K. marxianus* is one of the most promising yeast strains as it could effectively metabolize the complex organic materials with high power output even under high operating temperature conditions; therefore, it could be a best choice for wastes with fluctuated temperature. Further studies on this type and other types are required. Moreover, the surface modification of the carbon material with graphene could improve the performance.

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