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Extracellular electron transfer in yeast-based biofuel cells: A review

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ABSTRACT

This paper reviews the state-of-the art of the yeast-based biofuel cell research and development. The established extracellular electron transfer (EET) mechanisms in the presence and absence of exogenous mediators are summarized and discussed. The approaches applied for improvement of mediator-less yeast-based biofuel cells performance are also presented. The overview of the literature shows that biofuel cells utilizing yeasts as biocatalysts generate power density in the range of 20 to 2440 mW/m^2, which values are comparable with the power achieved when bacteria are used instead. The electrons' origin and the contribution of the glycolysis, fermentation, aerobic respiration, and phosphorylation to the EET are commented. The reported enhanced current generation in aerobic conditions presumes reconsideration of some basic MFC principles. The challenges towards the practical application of the yeast-based biofuel cells are outlined.

1. Introduction

The biofuel cells, exploiting whole microorganisms as biocatalysts, attract an increasing research interest during the last decade as a perspective technology for simultaneous electricity generation and wastewater purification. As far as most of the investigations in the field are performed by utilization of bacteria (prokaryotes) [1–5], these bioelectrochemical systems (BES) are more popular as microbial fuel cells (MFCs). The MFCs are electrochemical devices, by which the chemical energy obtained in the intracellular biochemical metabolic pathways is converted into electrical one. For each microorganism applied as a biocatalyst, the clarification of the mechanisms, by which the intracellular electrons, derived from the oxidation of the electron donors (substrates), are transported to the MFC-anode, is of significant importance. The extracellular electron transfer (EET) mechanisms are well established for bacterial species belonging to the *Geobacter* and *Shewanella* genera [6]. The natural habitats of these bacteria are soils and aquatic sediments, where in the absence of oxygen they couple their metabolism with a reduction of available external electron acceptors such as Fe(III) or Mn(IV) oxides. Inoculated in MFC, the exoelectrogenic bacteria use in a similar manner the anode as an extracellular terminal electron acceptor for optimization of their energy gain that is why they are often referred to as anode respiring bacteria [7–9]. The mechanisms of EET, established for bacteria, are classified as two types: direct EET — with the participation of outer membrane bounded cytochromes and/or electroconductive nanowires (pili), and indirect EET — accomplished by an endogenous or exogenous mediator of electron transport (electron shuttles). It is recognized that *Shewanella putrefaciens* [10], *Geobacteraceae sulfurreducens* [11], *Geobacter metallireducens* [12,13], and *Rhodoferax ferrireducens* [14] are capable of forming electrochemically active biofilms on the anode surface, which facilitate the direct EET. Despite the indisputable advantages, intensive laboratory investigations and realized pilot projects, the MFCs have not been widely applied in the practice mainly because of the lower electrical outputs in comparison with other power sources [1]. Until now the MFCs are reported to produce power density in the range of 20 to 6860 mW/m^2 [1–5,15–17] (see also Supplementary data, Table S1). The value of 6.86 W/m^2, achieved with an anode/cathode area ratio of 1:14 [16], reveals the potentials for further improvement of the biofuel cell performance. The technological challenges are connected with the optimization of MFC-components and design, as well as the transition of the devices from laboratory to industrial scale.

During the last few years, the utilization of yeast species such as *Saccharomyces cerevisiae* [18–24], *Hansenula anomala* [25], *Hansenula polymorpha* [26], *Arxula adeninivorans* [27,28] and *Candida melibiosica* [29–36] as biocatalysts in fuel cells has also been reported. According to the taxonomy of the living organisms, yeasts belong to the eukaryotes and they are classified in the kingdom Fungi. For this reason, although these bioelectrochemical systems belong to the class of the microbial fuel cells, for avoiding the semantic and taxonomic incompatible term "yeast microbial fuel cell" (often jargonized in lab) they are referred to as yeast-based biofuel cells.

The yeasts are utilized for centuries in numerous biotechnological processes of the food industry, which generate huge amounts of wastewaters rich of organic matter. Biofuel cells are considered as an

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alternative for purification of such wastewaters. From this point of view, the yeasts, used in the respective production, could be used as biocatalysts, instead of additional inoculation of the wastewater with other microbial species. The easy cultivation, broad substrate spectrum, fast growth and tolerance of the yeasts to a wide range of environmental conditions are advantageous for the development of yeast-based biofuel cells. The more sophisticated organization, richer genome and compartmentalization of the eukaryotic cells, however, complicate the analyses and the mechanisms of EET performed by yeasts still remain unclear.

This review summarizes for the first time the state-of-the art of the yeast-based biofuel cell research and development, emphasizing on the elucidation of EET mechanism. The necessity for future detailed investigations is also discussed.

2. Investigations with the use of exogenous mediators of electron transfer

Early examples of yeast-based biofuel cells used a variety of mediators as a means to overcome slow kinetics of electron transport across the semi-permeable cell membrane. These mediators are mainly organic dyes, possessing chemically stable and reversibly oxidized and reduced forms, easily penetrating the cell being biocompatible.

Seventy years after the pioneer's work of Potter [37], Benetto et al. [38] reported the utilization of Backer's yeast as biocatalysts in biofuel cells, in which the electron transfer from the yeast cells to the anode was mediated by resorufin (Table 1). It has been proved that the electrons reaching the anode originate from the intracellular oxidation of the glucose. Experiments with ^{14}C-labelled glucose demonstrated that the current generation was related to the glucose degradation to $^{14}CO_2$.

Several papers, reporting results achieved with *Saccharomyces cerevisiae* and methylene blue (MB) as an exogenous electron shuttle, were published in the last decade [20,22,24,29,31,38–41].

Gunawardena et al. [20] investigated the effect of MB on the electrical outputs (open circuit voltage, current and power under different loads), the internal resistance and the efficiency of *S. cerevisiae* yeast-based biofuel cell using reticulated vitreous carbon (RVC) electrodes and potassium ferricyanide as a final electron acceptor in the cathode compartment. Under anaerobic conditions, the maximum power of 146.7 ± 7.7 mW/m^3 (with respect to the electrode volume) was recorded at 1000 Ω load. The low performance of the explored biofuel cell has been attributed to the high overpotential for the reduction of O_2 at the RVC cathode, and the electron transfer inefficiencies between the mediator and the microorganism's cell walls. Probably, a cytotoxicity of the mediator at the applied high concentration (50 mM MB) is another reason for the low outputs.

Optimizing the conditions for electricity generation in a double-chamber yeast-based biofuel cell, Walker and Walker [22] have established that higher current outputs are produced when a moderate amount of oxygen is available in the anolyte in comparison with the use of deoxygenated or oxygen-saturated solutions. The observed enhanced current production has been associated, on the one hand, with more available net electrons, generated via the energetically favourable aerobic respiration, and on the other hand, with a minimum competition for the produced electrons between the concentration-limited oxygen and the reduced form of the exogenous mediator (methylene blue).

Performing chronoamperometric studies with rotating disc electrodes, Ganguli and Dunn [24] established that both the mediator convection and adsorption affect the kinetics of the anodic reaction in yeast-based biofuel cell. Increasing the MB concentrations and controlling the mediator adsorption, a power density of ca. 1500 mW/m^2 was achieved, which represented a significant increase from the prior reports on yeast-catalyzed fuel cells. High power densities were also obtained by immobilizing baker's yeast in electrically conductive alginate–carbon composite anodes and addition of 1 mM MB as an electron mediator [42]. A power density of ca. 700 mW/m^2 was recorded at the optimal for the yeast development pH. Increasing the pH of the anolyte to 10 has resulted in a considerable increase of the power density to 2440 mW/m^2, which is the highest reported for yeast-catalyzed biofuel cells until now. The observed performance has been linked to the enhanced proton transport out of the biofilm at the higher pH [42]. However, no information for the repeatability and durability of the reported data has been provided.

Exploring *C. melibiosica* 2491 yeast strain as a potential biocatalyst [29], it has been established that the biofuel cell could generate electricity even in the absence of an artificial mediator. It has been found that the produced current correlates with the yeast development status and the rate of the substrate assimilation, demonstrating its connection with the in vivo produced electrons. A significant increase of the current and power outputs was achieved by the addition of MB up to concentrations of 0.9 mM. Further increase of the MB concentration resulted in a decrease of the power due to the cytotoxic effect of the mediator.

In addition to the improvement of the electron transfer kinetics (resp. electrical outputs), the utilization of exogenous mediators could be also a useful approach to modulate the cell catabolism and examine the EET mechanism in the biofuel cells. Investigating the influence of exogenous mediators with different formal potentials on the performance of *C. melibiosica* yeast-based biofuel cell [31], it has been established that

Table 1
Summary of the reported yeast-based biofuel cells with the use of artificial electron transfer mediators.

Biocatalyst	Carbohydrate source	Conditions	Anodes used	Exogenous mediator	Maximum power density	Ref.
S. cerevisiae	Glucose	Anaerobic (N_2)	Glassy carbon	Resorufin	155 mW/m^2	[38]
S. cerevisiae	Dextrose	Anaerobic (N_2)	RVC	MB	400 mW/m^2	[39]
				NR	100 mW/m^2	
				MB + NR	500 mW/m^2	
S. cerevisiae	Glucose	Anaerobic (CO_2)	RVC	MB + $K_3[Fe(CN)_6]$	147 mW/m^3	[20]
S. cerevisiae	Dextrose	Anaerobic (N_2)	Carbon felt	MB	300 mW/m^2	[24]
S. cerevisiae	Glucose	Anaerobic	Graphite plate	NR	30 mW/m^2	[40]
S. cerevisiae	Glucose	Anaerobic	Graphite	Thionine	60 mW/m^2	[41]
S. cerevisiae	Glucose	Semi-aerobic	Graphite	Riboflavin	33 mW/m^2	[44]
S. cerevisiae	Glucose	Semi-aerobic	Pt-mesh	MB	65 mW/m^2	[22]
C. melibiosica	Fructose	Semi-aerobic	Graphite rods	MB	185 mW/m^3	[29]
C. melibiosica	Fructose	Semi-aerobic	Carbon felt	BcG	46 mW/m^2	[31]
				NR	89 mW/m^2	
				MR	113 mW/m^2	
				MO	137 mW/m^2	
				MB	640 mW/m^2	
A. adeninivorans	Dextrose + glucose	Aerobic	Carbon fibre cloth	TMPD	1 W/m^2	[27]

Methylene blue is abbreviated as MB, methyl orange — as MO, methyl red — as MR, bromocresol green — as BcG, neutral red — as NR, tetramethyl-phenylenediamine — as TMPD, and reticulated vitreous carbon — as RVC. The term "aerobic conditions" means aeration of the suspension by shaking, semi-aerobic — presence of oxygen from the air without shaking, anaerobic — purging the anodic compartment was with nitrogen gas or carbon dioxide.