Journal of Power Sources 396 (2018) 1–11

Contents lists available at ScienceDirect

Journal of Power Sources

journal homepage: www.elsevier.com/locate/jpowsour

Effects of methylene blue and methyl red mediators on performance of yeast based microbial fuel cells adopting polyethylenimine coated carbon felt as anode

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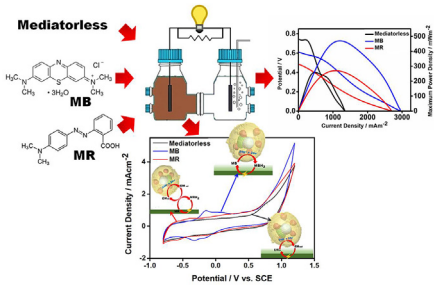
HIGHLIGHTS

- Methylene blue and methyl red as mediators in yeast-MFC are investigated.
- Electron transfer by methylene blue is mostly promoted.
- MPD of yeast-MFC using methylene blue is 430 mW m^2.
- Absorption of methylene blue inside yeast is optically inspected.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Microbial fuel cell
Methylene blue
Methyl red
Mediator
Polyethylenimine
Yeast

ABSTRACT

The electron transfer mechanisms of yeast *Saccharomyces cerevisiae* employing two different mediators, methylene blue (MB) and methyl red (MR), are suggested. The effects of the mediators on Microbial fuel cells (MFCs) performances are investigated when yeast and glucose are the biocatalyst and the substrate, respectively. Yeast tends to stand as floating cell rather than attached to supporting electrode. Therefore, to combine direct and mediated electron transfer mechanisms of yeast, two mediators and carbon felt modified with polyethyleneimine (PEI) (CF-PEI) are adopted and their roles are evaluated. As a result, CF-PEI surface is functionalized with amino groups that can attract and entrap more yeast cells. The cyclic voltammetry (CV) curves representing the mechanisms demonstrate that electron transfer rate constant of MB (0.44 s^{-1}) is higher than MR (0.37 s^{-1}). In addition, the performances of the yeast-MFC adopting MB (429.29 ± 42.75 mW m^{-2} at ~1200 mA m^{-2}) are better than those of the yeast-MFC adopting MR and the yeast-MFC without mediator. The reason is that MB is effectively adsorbed by yeast and collects more electrons than MR. These benefits of MB are reflected in a more efficient electron transfer chain and minimize the side reactions deactivating the catalyst.

1. Introduction

Bio-electrochemical systems (BESs) have inspired new biotechnologies that can generate energy from biomass [1]. The BESs can be fed by pure organic compounds or waste biomass, for example glucose [2], food waste [3] and many industrial wastewater [4], to

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https://doi.org/10.1016/j.jpowsour.2018.06.005
Received 17 February 2018; Received in revised form 12 May 2018; Accepted 1 June 2018

directly produce electricity or value-added molecules [5].

Microbial fuel cells (MFCs) are the most renowned BESs. These devices, compared to other electrochemical devices, can theoretically achieve more efficient energy extraction and conversion at low temperature (~20 °C), low substrate concentration and without side-heat production [6]. Physical, chemical, biocompatibility and electrochemical properties of involved components are important for the precise operation of MFCs, while the design and external conditions have a significant influence on the performance of MFCs [7,8]. The biological environment inside the MFCs and the external load or the temperature can change the colonization of electrode surface, the rate of electron production and the growth of the biofilm.

At the current stage of development, the profitability and convenience of MFCs should be improved by finding more cost-effective materials [9], and optimizing the time-to-stable-power [10,11] because the acclimation and start-up of MFCs can range from 5-6 days to 5–6 weeks, depending on several conditions [12,13]. Similarly, a proper management strategy of MFCs leads to higher electricity production [14].

One of the possible solutions to alleviate these issues is to use yeast as biocatalyst. Many species of yeast are already well-known, largely available at commercial level, and used in several biotechnology processes for energy applications [15–17]. Particularly, *Saccharomyces cerevisiae* is fast-growing, facultative anaerobic and a temperature resistant yeast that is easy to manage. Unfortunately, it is difficult to grow it in form of biofilm and entrapment or immobilization by anchorages is needed to promote exo-electrogenesis. Additionally, open circuit potential (OCP) is usually lower than that from other biocatalysts that are usually employed in MFCs because the electron transfer rate is low [18,19].

Therefore, it is important to analyze the interaction between biocatalyst and conductive electrode [20], because direct adsorption of mediator can be considered as one of the parameters that determine the electron transfer rate. Particularly, the biocompatibility and reversibility of mediator play a key role in shuttling the electrons from donor microorganisms to acceptor electrode in order to overhaul electrons transfer [1]. For MFCs application, some research groups reported that *S. cerevisiae* could be used for both direct extracellular electron transfer mediated by endogenous flavins [21,22] and indirect electron transfer by artificial mediators [23,24]. This is possible because the formation of a biofilm-like layer of yeast was facilitated by the surface modification of electrode with amine-based compounds and may induce physical entrapment, weak electrostatic interactions, and chemical bonding with yeast. As a result, the modified structure provides to yeast cells an artificial anchorage to the carbon surface [25–28]. Hence, by combining these two strategies, i.e. entrapment and mediator, the yeast can be better exploited as biocatalyst.

In a yeast-based MFC, the biocatalyst can be found as floating biomass and/or deposited on the carbon felt anode. If a specific entrapment or immobilization is not used, the direct electron transfer of yeast is limited. Thus, it is better to use an artificial mediator to boost electron transfer via the indirect mechanism. However, the complete entrapment of yeast for exclusive direct electron transfer is difficult. Therefore, the combination of the two strategies, i.e. the attachment of yeast cells through amine or hydrogen bonds to improve the electron transfer directly to the anode and the use of a dissolved mediator to harvest electrons from floating cells, can be attempted to maximize a synergistic effect [29].

Recently, several mediators and amine-based compounds were considered as eligible candidates: methylene blue (MB), methyl red (MR) and methyl orange (MO) for the role of mediator and polydopamine (PD), polyethyleneimine (PEI) [30–32] or complex amine-terminated compounds for the role of artificial anchorage in yeast-based MFCs. For instance, Rossi et al. [33] obtained an OCP of 0.4 V and a maximum current of 500 μA using a graphite anode with yeast immobilized on a cellulose acetate membrane and MB as mediator; Wei et al. [25] functionalized carbon nanotubes with a ionic liquid containing primary amine groups (-NH_2) on carbon cloth to increase adhesion and interfacial direct electron transfer between microorganism and anode in a MFC; Jiang et al. [27] used polydopamine with carbon foam as anode for a MFC. There were various merits in these works with mediators and amine-based modifications for MFCs with non-yeast biocatalysts, but there are still not enough studies focused to develop MFCs using yeast cultivated from *S. cerevisiae*.

In this work, we investigate and propose a mechanism to elucidate the interaction among yeast, two mediators, i.e. MB and MR, and the carbon felt (CF) modified with PEI as source of amine groups for adhesion (CF-PEI). The convenience of yeast, mediators and modified surface are exploited together to enhance the yeast-based MFC performance. These interactions are electrochemically evaluated using cyclic voltammetry (CV) measurements, while chemical and optical investigations are done by Scanning Electron Microscopy (SEM) and X-ray Photoelectron Spectroscopy (XPS). For full cell operation, the polarization curves of H-type MFCs are used to determine performance. The structure of anode electrode after test was inspected by Digital Optical Microscopy (DOM).

2. Experimental

2.1. Preparation of yeast medium and modified anode

Commercial yeast cultivated from *Saccharomyces cerevisiae* was purchased from Sigma Aldrich (St. Louis, USA), and it was used in semi-aerobic batch reactors with a modified yeast extract-peptone-D-glucose (YPD) medium that consists of 5 mg mL^{-1} of yeast extract, 2.5 mg mL^{-1} of peptone, and 5 mg mL^{-1} of D-glucose, according to [32]. All nutrients were prepared in 0.1 M PBS (pH 7.4) with an initial yeast concentration of 0.7 mg mL^{-1}. The appropriate volume of yeast/YPD medium was then used to conduct of half-cell or full cell experiments.

Carbon felt (XF30A–3.5T), purchased from Toyobo Co. (Osaka, Japan), was treated with 5 mg mL^{-1} of 50% w/v PEI (Sigma Aldrich) for 3 h at room temperature. Afterward, the carbon felt was rinsed with de-ionized (DI) water until a neutral pH was detected to prevent other bonding or polymerization in advance and then it was vacuum-dried in oven at 80 °C for 12 h before use. SEM (InspectF, FEI Co.) was used to investigate morphology of untreated and PEI-treated carbon felts while XPS analysis (K-alpha+, Thermoscientific Co.) was conducted to evaluate chemical bonding and surface functionalization.

2.2. MFC configuration and half-cell test voltammetry setup

For the MFCs configuration, Schott's glass H-type bottles (Adams and Chittenden, USA) were used as the MFC reactor, while the electrodes had 7 cm^2 of geometric area. A PEI-treated carbon felt and an untreated carbon felt were placed in anode and cathode side, respectively. Nafion 117 membrane (treated with 3% w/w H_2O_2, 0.5 M H_2SO_4, and DI water) was used to split anode and cathode chambers. A volume of 150 mL of yeast/YPD medium with 0.1 mM MB or MR mediator (Sigma Aldrich, St. Louis, USA) and same volume of 0.1 M PBS (pH 7.4) were fed respectively in anode and cathode chambers. A magnetic stirrer was then placed in anode chamber to keep solution in continuous and homogenous agitation, while an orifice valve in the cap of anode chamber was used to evacuate eventual gases. On the other side, air was fed to the cathode chamber to provide oxygen as terminal acceptor and induce the oxygen reduction reaction (ORR). The complete experimental setup is included in the supplementary section (Fig. S1).

The yeast-MFCs were operated in triplicate for 120 h until stable OCP was reached. A WonaTech Zive SP-2 potentiostat (Seoul, Korea) was used for electrochemical measurements. The potentiostat was also connected to a Frequency Response Analyzer (FRA). By coupling the