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Optimization of glucose concentration and glucose/yeast ratio in yeast microbial fuel cell using response surface methodology approach

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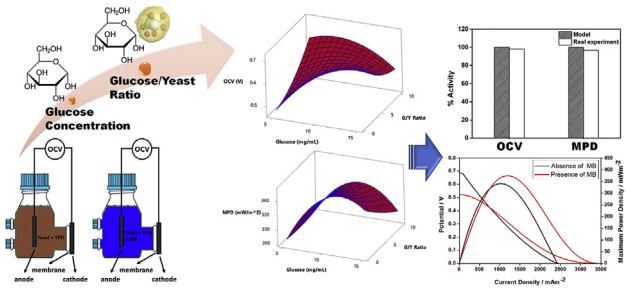
HIGHLIGHTS

- Single chamber yeast microbial fuel cell with a novel anode is optimized.
- Response Surface Methodology was used to tune 2 operational parameters.
- Dataset of experimental response with or without methylene blue was created.
- The shape of response surface shows that a non-trivial optimum point exists.
- The optimal point was experimentally checked and error was only 3–5%.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work the influence of two practical parameters, i.e. glucose concentration and glucose/yeast ratio, on performance of yeast-based microbial fuel cells (yeast-MFC) is investigated. The novel carbon felt pretreated with polyethylenimine is adopted as anode in open-air single chamber yeast-MFCs. The combination of the two parameters is optimized using response surface methodology with statistical approach. The optional presence of methylene blue as mediator is also included for comparison. Experimental dataset is initially built as reference and 4 mathematical equations are derived to predict the response regarding open circuit voltage (OCV) and maximum power density (MPD). By varying glucose concentration and glucose/yeast ratio, computed response surfaces show different responses are obtained and an optimum point exists within the range investigated. Finally, the optimized combinations for yeast-MFCs with/without mediator are predicted and response is verified in real experiment. The model tends to slightly overestimate the response, but accuracy is within confident range for both OCV and MPD. In fact, MPD obtained for the optimized yeast-MFC without mediator is 340.9 mW m^{-2}, 3.2% lower than model, while it is 374.4 mW m^{-2}, 5% lower than model, for the case including mediator. The discrepancy of OCV prediction is below 3%, making the approach reliable.

1. Introduction

Microbial fuel cell (MFC) is a bio-device of actual interest in many application fields like energy recovery, biosynthesis of secondary nutrients, wastewater treatment, residual biomass valorization, medicine and environmental sensing [1]. They are essentially based on the

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capability of some microorganisms to oxidize organic compounds at the anode and to extract protons and electrons from the substrate at a relatively high rate [2,3], depending on several environmental conditions and on the interactions between living cells and electrode surface [4–8]. The most important concept of MFCs is to find out the best electron transfer pathway from microorganism to anode. The electron transfer can occur as the direct transfer by the immediate delivery of electrons from cellular membrane to anode, or as the mediated transfer by the intermediate exogenous redox species that can shuttle electrons from cellular membrane, via anodic medium, to anode [9,10]. Therefore, the discovery of a highly biocompatible anode environment and a suitable 3D electrode material for enhancing the colonization of anode by the microorganisms is of primary interest [11].

Particularly, many efforts have been made for developing suitable and cheap 3D electrode materials based on carbon felt (CF) [12–14], graphite [15], graphitic carbon [7,16,17], nanostructured carbon [18–20]. Among these materials, CF is very attractive because it possesses low density ($\sim 0.14\ g\ cm^{-3}$), high porosity (84–95%), high surface area ($0.09–0.6\ m^2\ g^{-1}$), flexible pore size (from 100 nm to 50 μm) accessible to microbes, substrates and anolyte [12] and the cost is lower than carbon nanotubes of graphene (i.e. 70 $\$\cdot kg^{-1}$ vs 100–1000 $\$\cdot kg^{-1}$) and more effective if considering the actual performance of MFCs [21]. This material has a porous structure, from macro to nano level, that can be further decorated with precious/non-precious metal particles or modified with different amino-terminated molecules to increase adhesion and biocompatibility [8,22–24], thus becoming the cheapest, most effective and commercially reliable anode material for MFCs.

Hidalgo et al. [13] proposed a layer-by-layer procedure to depose polyaniline on CF and tripled the power density with respect to the pristine CF. Zhu et al. [25] obtained similar increase by treating CF with nitric acid and ethylenediamine, while Han et al. [26] applied CFs treated with HCl in a photoelectrocatalytic microbial fuel cell and measured improved current generation and tolerance to low pH. Zou et al. [27] functionalized CF with nanoporous Mo_2C particles to introduce a robust interface between carbon fibers and endogenous flavins of microorganisms to promote the direct transfer of electrons.

In this regard, the development of performing anode materials for MFCs is an important issue [28,29]. However, the development is a general topic that is not related to the specific optimization of anodic environment for a precise microorganism. Some relevant environmental parameters to consider in anode side, when operating a MFC with a specific inoculum, are temperature, pH, concentration of the substrate [6,30,31], presence and concentration of inhibiting species or not-degradable compounds [32], ratio between initial biomass and the useful substrate.

The effects of these parameters can determine the biological activity of microorganism and the type of current extraction [33]. Recently suggested numerical and statistical models have considered the biological, chemical and operational factors [34]. Also, the factors are coupled with the geometry and architecture of MFC reactors [35], and the response and performance of MFCs can be tuned and regulated.

As an efficient and practical approach to determine the effects of these parameters, the response surface methodology (RSM) can be proposed. The use of RSM was already applied in some biotechnology processes [36–39] and even in MFCs, experimental modeling and design using the RSM has been used for maximizing power output [40]. In spite of that, more studies are still needed to consolidate this method, to enhance the understanding of empirical phenomena involved in MFCs and to predict and manipulate the performance of MFCs [3,41–43]. These kinds of study can contribute to increase the market share of this technology and to realize scaled-up systems [44].

The novelty of this work is the focus on yeast-MFCs [45,46], with *Saccharomyces Cerevisiae* as biocatalyst and glucose as model substrate, and the follow-up of two previous works in which firstly a CF pretreated with polyethylenimine (CF-PEI) demonstrated higher capacity to attach yeast cells [47] and secondly the electron transfer mechanisms between CF-PEI, methylene blue (MB) and yeast cells was studied [48]. The combination of yeast-MFC, CF-PEI and RSM is studied for the first time. The influence of two major parameters on the performance response of a single chamber open-air yeast-MFC is experimentally determined and then modeled according to RSM to optimize the parameters and getting the highest possible performance within a suitable range for real conditions. More than two parameters could be studied in RSM but, to have a complete and comprehensive visual representation of the response surfaces, two is the optimal number of tunable parameters to study the response of each output. Glucose concentration and glucose to yeast ratio (G/Y) are considered as the tunable parameters, and open circuit voltage (OCV) and maximum power density (MPD) are the responses modeled for the yeast-MFC. Other parameters as pH and temperature can be considered as tunable parameters, but in real situations they act more like external factors rather than real manipulable parameters. The optional adoption of MB as redox mediator is also included to demonstrate the feasibility and reliability of the RSM approach.

2. Materials and methods

2.1. MFC configuration and measurements

For the MFC configuration, Schott's glass half H-type bottles (Adams and Chittenden, USA) were used as MFC reactor, while the CF electrodes have 7 cm^2 of geometric area. A PEI treated CF acted as anodic electrode while untreated CF acted as cathodic electrode.

Carbon felt (XF30A–3.5T) was purchased from Toyobo Co. (Osaka, Japan) and treated using $5\ mg\ mL^{-1}$ of 50% w/v polyethylenimine (PEI) solution (Sigma Aldrich, St. Louis, USA) for 3 h at room temperature, then washed with de-ionized (DI) water until a neutral pH was detected in the water to prevent other bonding or polymerization. Finally, the modified CF was vacuum dried in oven at 80 °C for 12 h before being used for single cell tests.

Nafion 117 membranes were treated with 3% w/w H_2O_2, 0.5 M H_2SO_4, and DI water. It was used as the separator and placed in between anode chamber and open-air cathode.

Commercial yeast from *Saccharomyces cerevisiae* (Sigma Aldrich, St. Louis, USA) was used as biocatalyst for bioelectricity generation. The yeast was prepared in the batch anodic chamber using modified yeast extract-peptone-D-glucose (YPD) medium [16,47] that consists of $5\ mg\ mL^{-1}$ of yeast extract, $2.5\ mg\ mL^{-1}$ of peptone, and various concentration of D-glucose and yeast amount, following the design of experiments and the imposed glucose/yeast ratio, i.e. mg of glucose/mg of yeast. All nutrients were prepared in 0.1 M PBS (pH 7.4) and 140 mL of yeast in YPD medium was fed to the anode chamber, while Methylene Blue (MB) was optionally fed into the anode solution with the final concentration of 0.1 mM. A magnetic stirrer was then placed in anode chamber to keep anolyte in homogenous state and a magnetic hot plate was used to keep temperature in range between 25 and 27 °C, while an orifice valve in the cap of anode chamber was used to evacuate eventual gases. On the other side, environmental air was used as oxidizer to the open-air cathode and provides oxygen as terminal acceptor. The complete experimental setup is shown in Fig. 1.

The yeast-MFC was operated for 72 h until stable OCV was reached, while 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 frequency response analyzer with the potentiostat, the power output was analyzed as a product of the current and potential. Polarization curves were measured with a scan rate of $10\ mV\ s^{-1}$ from the OCV until the voltage reached 0 V.

2.2. Design of experiments

The optimization study was conducted to verify the influence of two major parameters, i.e. glucose concentration and glucose/yeast ratio G/