Thionine Increases Electricity Generation from Microbial Fuel Cell Using Saccharomyces cerevisiae and Exoelectrogenic Mixed Culture

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Microbial fuel cells (MFCs) have been shown to be capable of clean energy production through the oxidation of biodegradable organic waste using various bacterial species as biocatalysts. In this study we found Saccharomyces cerevisiae, previously known electrochemically inactive or less active species, can be acclimated with an electron mediator thionine for electrogenic biofilm formation in MFC, and electricity production is improved with facilitation of electron transfer. Power generation of MFC was also significantly increased by thionine with both aerated and non-aerated cathode. With electrochemically active biofilm enriched with swine wastewater, MFC power increased more significantly by addition of thionine. The optimum mediator concentration was 500 mM of thionine with cyanin from Pseudomonas aeruginosa in MFC with the maximum voltage and current generation in the microbial fuel cell were 420 mV and 700 mA/m², respectively. Cyclic volatametry shows that thionine improves oxidizing and reducing capability in both pure culture and acclimated biofilm as compared to non-medicated cell. The results obtained indicated that thionine has great potential to enhance power generation from unmediated yeast or electrochemically active biofilm in MFC.

**Keywords**: microbial fuel cell, mediator, electron shuttle, electricity generation, Saccharomyces cerevisiae

Introduction

Production of energy from renewable resources is now a widely accepted and utilized sustainable concept, which reduces global carbon dioxide emissions and thus decrease the environmental burden derived from fossil fuel. The microbial fuel cell (MFC) is a novel technology that can recover bioenergy in the form of hydrogen and/or electricity directly from organic matter, while simultaneously treating biodegradable contaminants in wastewaters (Oh and Logan, 2005; Oh et al., 2010). It has been extensively investigated and shown that the MFC performance mainly depend on operational and design factors, such as system architecture, electrode material and surface area, catalytic bacterial species on the electrode, types of substrate, and operating conditions (pH, conductivity and flow rate) in the anode chamber, as well as cathode catalyst and electrolyte (Oh et al., 2004, 2009, 2010; Kim et al., 2005, 2007a; Liu et al., 2005; Oh and Logan, 2006).

Bacteria have been presented as key catalysts in MFCs and therefore the improvement of biocatalyst on the electrode has been widely investigated from this perspective in relation to other features; increase electrode surface area by brush carbon electrode (Logan et al., 2007), activation of electrode surface by chemical treatment (e.g. ammonia (Kim et al., 2007b); active selection of electrogenic species (Kim et al., 2005). The combined electron transport mechanisms between bacterial cell membrane and electrode surfaces is believed to be a rate limiting factor which determines the whole MFC system performance. The bacterial electron transfer mechanisms reported so far are; direct electron transfer from outer cell membrane to electrode; electrically conductive nanowire (Beveridge, 2004; Reugera et al., 2005); electron shuttles using externally added or self produced chemicals (e.g. procyanin from Pseudomonas aeruginosa) (Rabaey et al., 2004)

A significant improvement in cell current has been observed with the addition of electrochemical mediators that facilitate the electron transfer between bacteria and electrode (Allen and Bennetto, 1993; Park and Zeikus, 2000; Rabaey et al., 2005). Typically, MFCs performances have been known to be enhanced by the addition of electron shuttles with e.g. Shewanella, Pseudomonas, and Escherichia coli; particularly also in Gram-positive bacteria, Bacillus, which were otherwise inefficient to transfer electrons from their internal electron transport chain to outer electron acceptor. Electron mediator which has a redox potential close to that of NADH/NAD⁺ can facilitate electron shuttling between the reaction center inside of the cell and terminal electron acceptor (anode electrode). Several exogeneous electron mediators such as methyl viologen, methylene blue, neutral red, and thionine have been used in MFCs (Table 1). Choi et al. (2003) suggested that the desirable characteristics of mediators are facilitation of reversible electron transfer and minimal mediator accumulation inside of the
cell membrane. They reported that the thionine can easily penetrate through the phosphatidylcholine (PC) layer in the cell membrane during the process, compared to other mediators, namely HQN, phenothiazine, quinone, and azo family mediators. It is also reported that thionine is not involved in any assimilatory biochemical reaction thus it cannot be accumulated in the cell membrane, and can therefore facilitate reversible electron transfer between cell and terminal electron acceptor. The increased electron transfer rate with thionine should result in higher current generation and therefore could be used in MFCs for sustaining improved performance.

Park and Zeikus (2000) have also shown the interactions between bacterial cultures and electron mediators. Incorporation of mediators onto the graphite electrode increased power output 10-100 fold because of the facilitated interaction of these mediators with NAD+ (Park and Zeikus, 2003; Ringeisen et al., 2008; Najafpour et al., 2007). The soluble redox mediators used in MFCs for the improvement of electron transfer have been summarized in Table 1.

In this paper, we demonstrate the yeast, *Saccharomyces cerevisiae*, which has previously been known to be exoelectrogenically inactive, can produce electricity in an MFC and power can be increased by using thionine as mediator. *S. cerevisiae* has been used as biocatalyst for biofuel cells with using methylene blue as mediator in the previous studies (Gunawardena et al., 2008; Najafpour et al., 2010b). However, its metabolic and genetic information has been widely investigated, which would be helpful in studying its performance in MFCs. The main objectives of the study were to determine an optimum concentration of thionine as electron mediator in the anode chamber and its electrochemical characteristics, determined by adding electron shuttle and considering sensitivity to operational parameter such as aeration in the cathode chamber.

### Materials and Methods

#### Microorganism and cultivation

*Saccharomyces cerevisiae* PTCC 5269 supplied by the Iranian Research Organization for Science and Technology (Tehran, Iran) was used as active biocatalysts in this study. The microorganism was grown anaerobically in a jar vessel. The medium for the pure culture consisted of glucose, yeast extract, NH₄Cl, NaH₂PO₄, MgSO₄, and MnSO₄; 10, 3, 0.2, 0.6, 0.2, and 0.05 g/L, respectively. The medium was autoclaved at 121°C and 15 psi for 20 min. The pH was initially adjusted to 6.5. The *S. cerevisiae* were inoculated into sterilized media at ambient temperature and incubated at 30°C. The pure culture was fully grown in a 100 ml flask without any agitation for the experiment duration of 24 h. In a MFC operation 1 g/L of glucose was used in an anode chamber. For a mixed culture inoculum, swine wastewater (0.3 ml) in Chuncheon city, Korea was used. After that MFCs were inoculated with anode medium of a working MFC initially inoculated with swine wastewater. Glucose (20 mM) was used as an energy source in a nutrient solution (pH=7.0) containing (per L of deionized water): NaHCO₃ (3.13 g/L), NH₄Cl (0.31 g/L), MgSO₄ (0.2 g/L), KCl (0.13 g/L), NaH₂PO₄ (4.22 g/L), Na₂HPO₄ (2.75 g/L), and trace metal (12.5 ml) and vitamin (12.5 ml) solutions. (Kim et al., 2005)

#### Chemicals and analyses

All chemicals and reagents used for the experiments were analytically grades and supplied by Merck (Darmstadt, Germany). The pH meter (Model HANA 211, Romania) was employed to measure pH values of the aqueous phase. The initial pH of the working solution was adjusted by addition of diluted deionized water: NaHCO₃ (3.13 g/L), NH₄Cl (0.31 g/L), KCl (0.13 g/L), NaH₂PO₄ (4.22 g/L), Na₂HPO₄ (2.75 g/L), and trace metal (12.5 ml) and vitamin (12.5 ml) solutions. (Kim et al., 2005)

#### MFC construction and operation

The fabricated cells made of glass (Pyrex) material at laboratory scale were used for the MFC. The volume of each chamber (anode and cathode) was 850 ml with a working volume of 760 ml. A sampling port, wire point inputs and inlet port were provided for the anode chamber. The se-