Biosensors and Bioelectronics 56 (2014) 19–25

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Enhancement of bioelectricity generation by cofactor manipulation in microbial fuel cell

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ARTICLE INFO

Article history:
Received 11 September 2013
Received in revised form
25 November 2013
Accepted 9 December 2013
Available online 2 January 2014

Keywords:
Microbial fuel cell
Pseudomonas aeruginosa
NAD synthetase
NADH/NAD$^+$
Metabolic engineering

ABSTRACT

Microbial fuel cells (MFCs) are promising for harnessing bioenergy from various organic wastes. However, low electricity power output (EPT) is one of the major bottlenecks in the practical application of MFCs. In this study, EPT improvement by cofactor manipulation was explored in the *Pseudomonas aeruginosa*-inoculated MFCs. By overexpression of *nadE* (NAD synthetase gene), the availability of the intracellular cofactor pool (NAD(H/$^+$)) significantly increased, and delivered approximately three times higher power output than the original strain (increased from 10.86 μW/cm^2 to 40.13 μW/cm^2). The *nadE* overexpression strain showed about a onefold decrease in charge transfer resistance and higher electrochemical activity than the original strain, which should underlie the power output improvement. Furthermore, cyclic voltammetry, HPLC, and LC–MS analysis showed that the concentration of the electron shuttle (pyocyanin) increased approximately 1.5 fold upon *nadE* overexpression, which was responsible for the enhanced electrochemical activity. Thus, the results substantiated that the manipulation of intracellular cofactor could be an efficient approach to improve the EPT of MFCs, and implied metabolic engineering is of great potential for EPT improvement.

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1. Introduction

Microbial fuel cells (MFCs), which are devices that convert chemical energy into electricity by catalyzing the oxidation of various carbon sources in biomass or even organic waste in wastewater by a series of electrochemically active bacteria (EAB), have outstanding prospects for generating new bioenergy (Aelterman et al., 2008; Angenent et al., 2004; Logan, 2009; Lovley, 2008). With the worsening global warming and energy crisis triggered by fossil fuels, MFCs have attracted increasing attention in recent years because of electric power or biohydrogen generation without net carbon dioxide emissions into the ecosystem (Gil et al., 2003; Moon et al., 2006). MFCs are also promising for generating clean energy and treating organic wastewater pollutants simultaneously (Chang et al., 2005; Oh and Logan, 2005). However, low electricity power output (EPT) is one of the major bottlenecks in the practical application of MFCs (Yong et al., 2012).

Many researchers have attempted to increase the EPT and scale up of the bioelectricity generation by optimizing the electrode material (Lai et al., 2011; Logan et al., 2007; Schröder et al., 2003), operation parameters and components of MFCs (Du et al., 2007; Hou et al., 2011). Another logical method for increasing bioenergy production is the improvement of bacteria to enhance bioelectricity generation (Alfonta, 2010), in which bacteria are genetically modified to increase the amount of releasable electrons (Yong et al., 2012) and improve the efficiency of transferring released electrons to the anodes (Lai et al., 2011; Shkil et al., 2011; Yu et al., 2011; Zhang et al., 2006).

EAB generate electrons by metabolizing organic substrates and transferring them via extracellular transport to an electrode surface in MFCs, which have a function in bacterial metabolism and internal reactions (Franks and Nevin, 2010). It is reported that a transposition mutant of *Shewanella oneidensis* MR-1 deficient in the biosynthesis of cell surface polysaccharides showed an increased ability to adhere to a graphite anode and to generate 50% more current in an MFC than the wild strain, which suggested

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http://dx.doi.org/10.1016/j.bios.2013.12.058

that cell surface engineering is a possible scheme to improve bacterial current generation in MFCs (Kouzuma et al., 2010). However, not all of the electrochemically active bacteria (EAB) can synthesis capsular polysaccharides. Cofactors play important roles in most biosynthetic reactions, catabolic reactions and act as redox carriers in transfer of energy for the cell (Wang et al., 2013b). Nicotinamide adenine dinucleotide (NAD^+) and its reduced form NADH are essential cofactors and electron carriers that are primarily involved with cellular metabolic reactions and energy production (Förster et al., 2003). Particularly, NAD(H/$^+$), which represent the intracellular redox state of cells, has central functions in extracellular electron transfer and metabolic pathways (Berrios-Rivera et al., 2002a, 2002b; Girbal et al., 1995; Nielsen, 2003; Vemuri et al., 2007). One strategy to change NAD(H/$^+$) is to alter the ratio of NADH to NAD^+. Yong et al. (2012) reported that disruption of the lactate biosynthesis pathway in *Escherichia coli* increases the NADH/NAD^+ ratio in anaerobic cultures, thus generating higher current output. Nevertheless, amplification, addition, or deletion of a particular genes in target metabolic pathways, which have the potential to considerably improve process productivity for some target products such as organic acids, amino acids, biofuels, and pharmaceuticals (Ji et al., 2013), do not always result in the desired phenotype (Jang et al., 2012). In the meanwhile, overexpression, deletion, or introduction of heterologous genes in target metabolic pathways always contributed to the NAD(H/$^+$) regeneration, which altered the intracellular ration of NADH/NAD^+ and changed the metabolite distribution (Ji et al., 2013; Liang et al., 2012). However, these methods could not increase the intracellular pool of NAD(H/$^+$). To date, limited studies have been made on changing the NAD(H/$^+$) concentration by directly increasing the NAD(H/$^+$) pool (Balzer et al., 2013; Gao et al., 2012; Ji et al., 2013; Wang et al., 2013b).

Genetic engineering is a feasible approach to construct efficient bioelectrocatalysis and rewire gene regulatory circuits for high-performance MFCs (Alfonta, 2010). Overexpression of the *pncB* gene (encoding Nicotinic acidphosphoribosyl transferase, NAPRTase), which is involved in the NAD salvage pathway, increased the total NAD levels (Berrios-Rivera et al., 2002a, 2002b). Meanwhile, NAD synthetase, encoded by the *nadE* gene, catalyzes the final step in de novo synthesis and a salvage pathway for NAD biosynthesis (Lai et al., 2011; Ozment et al., 1999). The NAD^+ level increases with *nadE* overexpression, thereby up-regulating genes whose products catalyze NADH synthesis (as the carrier of electrons). One hypothesis is that *nadE* overexpression can increase the NAD(H/$^+$) pool at the source and cause disturbances in the metabolic network, thus changing the EPT in EAB.

In this study, *nadE* was overexpressed manuplated in *Pseudomonas aeruginosa* to examine the effect on intracellular NAD(H/$^+$) availability and NADH/NAD^+ levels in vivo. Thus the electricity production performance of *P. aeruginosa*-inoculated MFC was investigated. Results showed that the overexpression of *nadE* augmented the levels of NAD^+ and NADH and enhanced pyocyanin (PYO) production, both of which resulted in improved bioelectricity generation and efficiency of electron transfer. Our study provides a promising approach for enhancing the electricity production performance of MFCs.

2. Materials and methods

2.1. Bacterial strains, plasmids, and culture conditions

The bacterial strains and plasmids used in this study are listed in Table S1. Unless indicated otherwise, *P. aeruginosa* and *E. coli* strains were grown at 37 °C with shaking at 200 rpm in Luria-Bertani (LB) medium. When needed, antibiotics were added at the following concentrations: ampicillin, 100 μg/mL for *E. coli*; gentamicin, 10 μg/mL for *E. coli*, and 50 μg/mL for *P. aeruginosa*. Liquid cultures of *P. aeruginosa* containing pBBR1MCS-5 (vector control) or recombinant pBBR1MCS-5-*nadE* were grown with gentamicin to measure *nadE* gene expression and quantify NAD^+, NADH, and PYO.

DNA manipulations, nucleotide sequencing, plasmid construction (Orkin, 1990), and plasmid transformation (Farinha and Kropinski, 1990) were performed as described in Supplementary materials (SM).

2.2. Expression analysis

Fifty-milliliter cultures of recombinant *P. aeruginosa* in LB medium supplemented with 50 μg/mL gentamicin were grown at 37 °C to OD_{600}=0.43 (Optimized by Central Composite Design (CCD), Section 8 in SM). IPTG (0.69 mM, Optimized by CCD, Section 8 in SM) was then added to induce the expression of recombinant *nadE*. Cultures were further grown at 30 °C for an additional 10 h (Optimized by CCD, Section 8 in SM), harvested by centrifugation, and used to determine enzyme activity.

2.3. Enzymatic assay

NAD synthetase activity was assayed in a coupled reaction system with NAD synthetase and alcohol dehydrogenase (Ozment et al., 1999). One unit of enzyme activity was defined as the amount of enzyme that converts 1 μmol deamide-NAD to NAD per minute at 37 °C.

2.4. Air-cathode MFC assembly

A single-chamber MFC (with dimensions of 5.5 cm (length) × 5.5 cm (width) × 6 cm (height)) was used in this study (Fig. S1). Each reactor had one port at the top for the addition and sampling of solutions, as well as for gassing. A Pt-loaded carbon cloth (7 cm^2) was used as the electrode material with an untreated anode and a treated cathode. The anodic medium used was the bacterial culture supplemented with 10 g/L glucose as a carbon source and electron donor. PBS (50 mM, pH 7.0) was used as buffer solution to adjust the pH of anodic electrolyte. For current generation measurement, a 1000 Ω external resistor was connected to the MFC circuit, and the potential of the MFC was recorded by a digital multimeter (ZX94A). All other electrochemical measurements were conducted using an Autolab PGSTAT302N electrochemical working station (Metrohm Instruments, Switzerland).

2.5. Electrochemical analysis

Cyclic voltammograms were measured on a three-electrode configuration with a reference electrode (Ag/AgCl) on an Autolab PGSTAT302N electrochemical workstation. If components are oxidized/reduced during this potential sweep over the culture, current peaks will appear on the voltammogram, which has a peak on both the upper and lower curve. CV scan rate was 10 mV/s, and the potentials were originally in the range of −400 to 400 mV. Cyclic voltage curves were obtained over a voltage of 0.1 V, and the second circle of the cyclic voltage curve was chosen to analyze the oxidation–reduction quality of the air-cathode MFCs.

A polarization curve was obtained by varying the external resistance over a range of 1–9000 Ω, and data were recorded at 10 min interval at the steady stage of MFCs. Current density and power density were normalized to the MFC volume or electrode surface area (24 mL or 7 cm^{-2}). The anode and cathode potentials were measured using an Ag/AgCl electrode as the reference.

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