Bioresource Technology 152 (2014) 220–224

Contents lists available at ScienceDirect

Bioresource Technology

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

Enhancement of bioelectricity generation by manipulation of the electron shuttles synthesis pathway in microbial fuel cells

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HIGHLIGHTS

- The *phzM* overexpression strain showed higher electrochemical activity.
- Overexpressing *phzM* decreased the charge transfer resistance of MFC.
- The electron shuttle (pyocyanin) concentration increased upon *phzM* overexpression.
- Genetic modification is efficient to improve the electricity power output of MFC.

ARTICLE INFO

Article history:
Received 22 September 2013
Received in revised form 24 October 2013
Accepted 28 October 2013
Available online 5 November 2013

Keywords:
Microbial fuel cells
Pyocyanin (PYO)
Electron shuttles
phzM
Pseudomonas aeruginosa

ABSTRACT

Microbial fuel cells (MFCs) are promising for generating bioenergy and treating organic waste simultaneously. However, low extracellular electron transfer (EET) efficiency between electrogens and anodes remains one of the major bottlenecks in practical applications of MFCs. In this paper, pyocyanin (PYO) synthesis pathway was manipulated to improve the EET efficiency in *Pseudomonas aeruginosa*-inoculated MFCs. By overexpression of *phzM* (methyltransferase encoding gene), the maximum power density of *P. aeruginosa-phzM*-inoculated MFC was enhanced to 166.68 μW/cm^2, which was four folds of the original strain. In addition, the *phzM* overexpression strain exhibited an increase of 1.6 folds in PYO production and about a onefold decrease in the total internal resistance than the original strain, which should underlie the enhancement of the EET efficiency and the electricity power output (EPT). On the basis of these results, the manipulation of electron shuttles synthesis pathways could be an efficient approach to improve the EPT of MFCs.

1. Introduction

Microbial fuel cells (MFCs) are renewable devices that can convert chemical energy into electricity by a number of electrochemically active bacteria (EAB), in which EAB catalyze the oxidation of a series of carbon source including organic substrate or even pollutants in wastewater and transfer the generated electron to anodes (Grant, 2003; Kim et al., 2004; Lewis and Nocera, 2006; Logan, 2009; Logan and Regan, 2006; Lovley, 2008; Rabaey and Verstraete, 2005). Two strategies of EET mechanisms were employed by EAB: direct electron transfer through outer membrane redox active proteins (*c*-type cytochromes) or conductive nano-wire, and indirect mediated by exogenous or endogenous electron shuttles (i.e., flavin, riboflavin) (Chaudhuri and Lovley, 2003; Inoue et al., 2010; Shi et al., 2009). The electron shuttles, usually series of redox compounds, are secondary metabolites secreted by EAB, and different bacteria may adopt different electron shuttles under different conditions (Rabaey et al., 2005). A diverse range of microorganisms have been used in MFC systems, such as *Geobacter*, *Shewanella*, *Pseudomonas* and *Escherichia coli*, etc., among which *Pseudomonas aeruginosa*, an opportunistic pathogen, gets more and more attention since it can produce an endogenous mediator such as PYO and/or phenazine-1-carboxamide (PCA), that avoid the drawbacks of high cost, short lifetime and toxicity to the microorganisms of exogenous mediators (Rabaey et al., 2005).

Previous studies showed that PYO, a phenazine based soluble redox metabolite, that can function as mediator to transfer

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http://dx.doi.org/10.1016/j.biortech.2013.10.086

electrons between bacteria and anode in MFCs (Liu et al., 2012; Rabaey et al., 2005; Zhang et al., 2012), is produced from chorismic acid via the phenazine pathway. *P. aeruginosa* PAO1 contains a complex phenazine biosynthetic pathway consisting of two seven-gene homologous operons, designated *phzA1B1C1D1E1F1G1* and *phzA2B2C2D2E2F2G2*, responsible for synthesis of phenazine-1-carboxylic acid (PCA) (Mavrodi et al., 2001; Yong and Zhong, 2013). Subsequent conversion of PCA to PYO is mediated by two phenazine-modifying genes, *phzM* and *phzS* (Greenhagen et al., 2008; Mavrodi et al., 2001, 1998), which encode phenazine-specific *S*-adenosylmethionine (SAM)-dependent methyltransferase (involved in the conversion of PCA to 5-methylphenazine-1-carboxylic acid betaine, MCAB) and NADH (or NADPH)-dependent flavoprotein monooxygenase (involved in the hydroxylative decarboxylation of MCAB to PYO), respectively (Gohain et al., 2006a,b). Parsons revealed that *phzM* alone has no detectable activity toward PCA (Parsons et al., 2007). Expression of *phzS* alone, nevertheless, can only catalyzed PCA to 1-hydroxyphenazine (1-OH-PHZ) in PYO-nonproducing *Pseudomonas fluorescens* (Mavrodi et al., 2001). In addition, isolation of either PhzM and PhzS results in no PYO formation suggesting that both of *phzM* and *phzS* are required for the production of PYO and an at least transient physical interaction between PhzM and PhzS (Parsons et al., 2007).

To date, although the *P. aeruginosa*-inoculated MFCs involve an efficient "direct" electron transfer process by the electron shuttle (PYO), the EET efficiency electricity between electrogens and anodes, the energy conversion efficiency, and the power density are still too low to be the main bottleneck for practical applications of the MFCs. One rational strategy to enhance the EET efficiency could be achieved via engineered bacteria conducted by direct or indirectly manipulating the metabolic engineering of EAB (Kouzuma et al., 2010). For example, overexpression of QS system could increase PYO production and resulted in an increase of about 1.6 times of the maximum current of the *rhl* overexpressed strain over the wild-type strain (Yong et al., 2011). This result implied that manipulating electron transfer pathways could be an efficient approach to improve EPT of MFCs.

Since *phzM* is the key enzyme of PYO biosynthetic pathway in *P. aeruginosa*, which catalyze the conversion of PCA to MCAB, plays an important role in the electricity production of the *P. aeruginosa*-inoculatd MFCs. This paper is concerned with the enhancement of bioelectricity generation by manipulation the electron shuttles synthesis pathway in microbial fuel cells (MFCs) as low extracellular electron transfer (EET) efficiency between electrogens and anodes has been one of the major bottlenecks in practical applications of MFCs. The pyocyanin (PYO) synthesis pathway was manipulated to improve the EET efficiency in *P. aeruginosa*-inoculated MFCs. Results showed that overexpression *phzM* in the wild-type *P. aeruginosa* PAO1 enable the strain produce more PYO, and strikingly, led to a higher current output than the original strain, which suggested that manipulation of the electron shuttles synthesis pathways might provide a efficient strategy to improve the EPT of bioelectrochemical devices.

2. Methods

2.1. Bacterial strains, plasmids, media and growth conditions

The bacterial strains and plasmids used in this study are listed in Table S1. The *phzM* overexpression strain (designated as *P. aeruginosa-phzM*) was constructed by transforming recombinant plasmid pBBR1MCS-5-*phzM* into *P. aeruginosa* PAO1 (Kovach et al., 1995). *P. aeruginosa* and *E. coli* strains were routinely grown in Luria–Bertani (LB) medium at 37 °C with shaking at 200 rpm. When needed, the growth medium was supplemented with antibiotics at following concentrations: for *E. coli*, 100 μg/mL of ampicillin and 10 μg/mL of gentamicin; for *P.aeruginosa*, 100 μg/mL of gentamicin. DNA manipulations and nucleotide sequencing, plasmids construction and transformation were described in the Supplementary Materials.

2.2. Analysis of enzymatic activities

PCA and PYO standard were purchased from Sigma (Sigma Chemical Co., MI, USA). Conversion of PCA to PYO by *phzM* and *phzS* was monitored discontinuously at 313 nm. Kinetic analyses were conducted according to the reported method (Greenhagen et al., 2008; Parsons et al., 2007).

2.3. Air-cathode MFC set-up

A single-chamber MFC (5.5 cm (width) × 5.5 cm (length) × 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. 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 1 g/L glucose as a carbon source and electron donor. PBS 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 MFCs circuit, and the potential of the MFCs was recorded by a digital multimeter (ZX94A). All other electrochemical measurements were conducted using an Autolab PGSTAT302N electrochemical working station (Metrohm Instruments, Switzerland).

For the MFCs inoculation, 0.6 mL of overnight *P. aeruginosa* cultures were inoculated in 30 mL LB broth and incubated with shaking at 37 °C until the optical density (OD_{600}) reached about 3.7. Then 1.34 mM IPTG was added as the inducer for *phzM* expression. After 10 h cultivation subsequent, the *P. aeruginosa* cultures were mixed with 20 mL 4 g/L glucose and 5 mL 50 mM PBS solution, which were transferred into the chamber of the MFCs and purged with nitrogen gas for 30 min to remove the dissolved oxygen. The chamber was continuously purged with nitrogen gas to ensure the strictly anaerobic condition during MFCs operations.

2.4. Electrochemical measurements

Polarization curve was measured by varying the external resistance from 30 to 11,110 Ω, and the data were recorded at 10 min interval. The Electrochemical parameters, such as 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 described by Wen et al. (2011). Cyclic voltammograms (CV) were measured on a three-electrode configuration with a reference electrode (Ag/AgCl) on an Autolab PGSTAT302N electrochemical workstation. The setting parameters of DPV were: scan region, 0.4 to −0.6 V: scan rate, 100 mV/s, and the second circle of the cyclic voltage curve was chosen to analyze the oxidation–reduction quality of the air-cathode MFCs.

2.5. LC–MS analysis of PYO

For LC–MS analysis, 20 mL of anode culture suspension from both *P. aeruginosa* PAO1 and *P. aeruginosa* inoculated MFCs were centrifuged (8000 rpm for 10 min), and the supernatants were extracted with 3 mL chloroform, which was pooled and evaporated to dry at room temperature. The dried-PYO samples were resuspended in 1 mL acetonitrile and vortexed vigorously for 2 min,