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Strain improvement and mediator selection for microbial fuel cell by genome scale in silico model

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Abstract

Special attention is recently being paid to microbial fuel cells (MFCs) as promising research and practical application in the biological production of electricity from wastewater at low cost. In this work, the genome scale *in silico* model of *E. coli* has been employed for establishing the strain improvement strategy for MFC under the integrated framework. Initially, the possible candidate genes for the knockout analysis within the whole network can be identified by comparing the central metabolism of *E. coli* with four organisms which are known to be the efficient producers of electrons. It is followed by *in silico* analysis for strain improvement, thus rendering it possible to identify gene targets for achieving the enhanced production of electron. Finally, of various mediators available, including Neutral Red, Methylene Blue, Meldola's Blue, Safranine-T, 2-Hydroxy-1,4-nepthoquinone and Thionine, the best mediator was evaluated on the basis of their performance to transfer electron into the anode of MFC efficiently. Thus, the present framework supporting both biochemical and electrochemical systems predicts the optimal environmental and/or genetic condition for developing high performance MFC throughout strain improvement and mediator selection.

Keywords: Microbial fuel cells; *Escherichia coli*; Mediator; Constraints-based flux analysis; Gene knockout analysis

1. Introduction

'How can human civilization sustain without natural petroleum reserve?' is the prime question of the day to answer. In this regard, the central task is to formulate alternate energy sources or mechanisms or devices to combat the energy crisis. Furthermore, optimized production of energy from these devices as well as their efficient operation will certainly boost this process. Towards this end, emerging technology, microbial fuel cell (MFC), is considered as a bio-electrochemical transducer which can generate electricity from biodegradable compounds [1-3]. In this process electrons are generated by the microorganism and then supplied to the anode either through membrane bound compounds (e.g., *G. sulfurreducens*) or through soluble mediators or transporters, as added from outside (e.g., *E. coli*). However, MFC can perform better in the absence of natural electron acceptors (e.g., oxygen or nitrates) in the anode compartment as those are the lowest energy sink for electrons. Over and above, this phenomenon is also confirmed from the positive standard redox potentials of oxygen and nitrate.

A scan through the available literature and published works, both experimental and theoretical, explores the main focus of these works which is to determine the proper environmental and genetic conditions for increasing the production of electricity. Furthermore, a few other works also investigate the performance of MFC for different individual mediators Therefore, the performed works of MFC are highly fragmented and they cannot provide the holistic picture of MFC system for determining the optimum condition for maximizing the production of electricity considering all possible factors involved. Thus, developing a comprehensive framework, covering all the necessary aspects, is a dire necessity to construct high performance MFC. In this regard, the present framework allows us to identify the genetic condition for the enhanced electron production as well as to select the best mediator by resorting to *in silico* modeling and analysis technique.

2. Methodology

In the current work, *E. coli* was considered as the preferable microorganism for MFC [4]. The main objectives of this work are: i) to enhance the production rate of electron and ii) to choose the best mediator for efficient electron transport. To accomplish both objectives, initially a gene deletion analysis is employed to find out gene or set of genes that maximize the production rate of electron. Subsequently, some additional reactions are incorporated into the current *in silico* model to describe the process of mediator-driven electron transport.

Finally, the best mediator can be identified for achieving high performance MFC on the basis of redox potential difference between electron donor and acceptor.

2.1. Genetic manipulation for the enhanced electron production

The central metabolism of *E. coli* is compared with those of *G. sulfurreducens, R. ferrireducens, P. aeruginosa and D. desulfuricans* which are known as efficient producers of electron. Note that among these four organisms *P. aeruginosa* and *R. ferrireducens* have been observed to have 65% energy efficiency [5]. This comparison allows us to identify 21 gene candidates which are uniquely found in *E. coli* (Table 1). Finally, the gene knockout combinations among the candidates are identified as the possible gene targets for overproducing the electron.

Table 1: Unique genes in the central metabolism of *E. coli* compared to four other microbes

Metabolism	Unique genes in *E. coli*
Glycolysis	*aceE, agp, eno, fba, ald, fba, fsaAB, glgA, glgC, glgP, pgm, gpm, pps*
Pentose phosphate pathway	*gnd, talAB, tktAB, zwf*
TCA cycle	*citDEF, frdABCD, sucA, mgo*

2.2. Strain improvement by the addition of transport reactions

The current genome-scale *in silico* model of *E. coli* was extended to consider MFC by adding mediator-driven transport reactions and its oxidative phosphorylation with NADH, NADPH and Ubiquinol-8 (Q_8H_2) which act as the electron donors to the mediator [1] as follows:

$$\mathrm{Med_{ex}} \rightarrow \mathrm{Med} \tag{1}$$

$$\mathrm{Med + NADH \rightarrow NAD^+ + H^+_{ex} + Med_{ex} + 2e^-_{ex}} \tag{2}$$

$$\mathrm{Med + NADPH \rightarrow NADP^+ + H^+_{ex} + Med_{ex} + 2e^-_{ex}} \tag{3}$$

$$\mathrm{Med + Q_8H_2 \rightarrow Q_8 + 2H^+_{ex} + Med_{ex} + 2e^-_{ex}} \tag{4}$$

2.3. Identification of best possible mediator

Six mediators are considered as candidates in the current work; they include methylene blue (MB), thionine (Th), meldola's Blue (MelB), neutral red (NR), safranine-T (SafT) and 2-hydroxy-1,4-nepthoquinone (HNQ) which are commonly used for MFC system [6]. The criterion considered for selecting the best mediator is the difference of the standard redox potential between the electron donor (e.g. NADH or NADPH) and electron acceptor (mediator). In this regard, the higher is the potential difference, the more negative is the gibbs free energy difference and the more spontaneous is the transfer of electron from the electron donor to the mediator (as $\Delta G^0 = -n \times F \times \Delta E^0$). Thus, the best possible mediator can be identified by incorporating specific constraints into the current model as follows:

$$Max \sum_j r(j) \times v(j) \tag{5}$$

Subject to:

$$\sum_j S(i,j) \times v(j) = 0 \tag{6}$$

$$b(e) = \sum_j Se(e,j) \times v(j) \tag{7}$$

$$product \le \sum_j \sum_{\Delta G} r(j) \times v(j) \times \Delta G_k(j, \Delta G) \tag{8}$$

where

n=No of electrons exchanged
F= Faraday's constant, 96485 Coulomb/mol
ΔE^0= Standard redox potential difference between electron donor and acceptor
ΔG^0=Standard gibbs free energy difference
S(i,j)= Stoichiometric coefficient of intermediate i in reaction j
Se(e,j)= Stoichiometric coefficient of extracellular metabolite e in reaction j
r(j)= A parameter which is equal to 1 for three electron transport reactions and 0 for other reactions.
v(j)= Flux of the reaction j.
b(e)= Net transport flux for the extracellular metabolite e
k= Set of mediators, NR, MB, MelB, HNQ, SafT and Th
ΔG= Set of difference of gibbs Free Energy, ΔG_{NADH},ΔG_{NADPH} and ΔG_{Q8H2}

$\Delta G_k(j, \Delta G)$ = Gibbs free energy difference for mediator k for the electron transport reaction j

3. Results & discussion

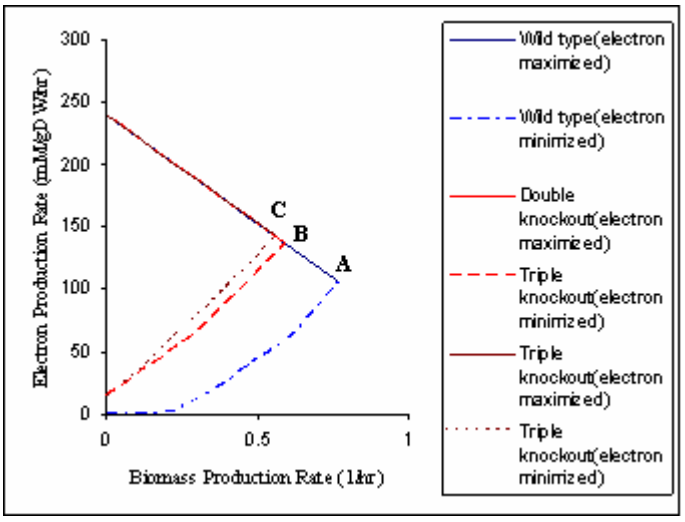

Fig. 1: Electron production envelopes under anaerobic condition as a function of biomass production rate for wild strain, double mutated and triple mutated strain of *E. coli*

The envelopes in Fig. 1 are obtained by finding the maximum and minimum production rate of electron at a specific level of biomass production keeping the glucose uptake rate constant at 10 mM/gDW/hr. Points A, B and C denote the production rates of electron at the maximum production rate of biomass. In case of double gene knockout analysis, among 210 combinations ($_{12}C_2$) of previously identified 21 candidate genes, point B represents the deletion of a specific combination, namely [*eno, zwf*], which can yield the production rate of electron, maximum among the 210 combinations, at the maximum production rate of biomass. Likewise, point C represents the desired combination, [*eno, aceE, zwf*] from 1330 combinations (21_{C_3}) for triple gene knockout analysis. However, the knockout analysis is usually confined upto triple gene due to practical reasons, Thus, on the basis of the developed framework, point C i.e. knockout of [*eno, aceE, zwf*] yields the production rate of electron which is maximum among all the possible combinations (of both double and triple gene knockout) when biomass production rate is maximized. However, enhanced production of electron comes at the cost of the reduced production of biomass. Among the six mediators considered in the current work thrionine is the best on the basis of the proposed methodology for the mutated *E. coli* strain (after triple gene knockout). The preference for choosing a single mediator for MFC goes like: Th>MB> MelB>HNQ>SafT>NR.

In case of double gene knockout, the removal of *eno* and *zwf* eliminates enolase (EC 4.2.1.11) and glucose 6-phosphate dehydrogenase (EC 1.1.1.49), which, in turn shifts the glycolytic flux toward the 3-phosphoglycerate branching point into the serine biosynthesis pathway and prevents the shifting of glucose-6-phosphate flux to the pentose phosphate pathway respectively. However, effect of deletion of the first gene is two-fold: i) reduced production of ATP, acetyl-CoA and CoA through TCA cycle which decreases the biomass production, ii) decreased production of phosphoenolpyruvate (pep) and pyruvate (pyr) which can be used as precursors for synthesis of various compounds including essential organic amino acids. In case of triple gene knock out, the additional gene *aceE* (corresponding enzyme: pyruvate dehydrogenase [EC 1.2.4.1]) only reinforces the effects of *zwf* deletion by preventing the conversion between pyruvate and acetyl-CoA. On the other hand, selection of thionine as best possible mediator can be predicted by observing its most positive redox potential (0.064 V) which ensures the highest difference of gibbs free energy.

4. Concluding remarks and future work

The developed framework covers almost all the major aspects for constructing MFC in an efficient way. Furthermore, the following initiatives are taken to make the current framework more comprehensive and general: i) a completely new algorithm is being developed to explore the candidates for gene deletion from the total set of genes present in *E. coli*, ii) permeability, a key factor for determining the choice of mediator, is incorporated in the current model which is found to vary depending on the molecular properties and redox states, iii) the surface reactions at the cathode and anode are also added to represent the complete picture of MFC system. Subsequently, a dynamic model for MFC is also being developed which can reveal time dependent behavior of the performance (Current or Power) of MFC. Therefore, it can be concluded that the on-going work ensuring the synergistic combination of the steady state model with the dynamic model can provide a deep insight for revealing various key aspects of MFC system.

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