Microbial Electrocatalysis:
Synthetic Biology Meets Energy and The Environment

Hao SONG, Professor
School of Chemical Engineering and Technology
Key Laboratory of Systems Bioengineering (MOE)
Tianjin University

2017 China-America Frontiers of Engineering Symposium (CAFOE)
June 22-24, 2017
Microbial Electrocatalysis (cell discharge/charge)

Fundamental mechanisms:
Microbial extracellular electron transfer

Microbial electrocatalysis

Microbial electrosynthesis (cells intake electrons from electrodes, converting to reducing equivalents)

Microbial consortia (methane fermentation, anaerobic digestion)

Cell Charge

Cell discharge

Cell-cell exchange of electrons

Bio-mining

Microbial fuel cells

Microbial electrolysis cells (H2)

Unbalanced fermentation (anaerobic respiration)

CO2

Chemicals

Microbial electrosynthesis (cells intake electrons from electrodes, converting to reducing equivalents)
Microbial cell discharge

**Microbial fuel cells** in wastewater treatment: degrade wastes and harvest electricity

**Microbial electrolysis cells** (H₂ production)

**Marine sediment fuel cell** (Military use: power distributed—sensors)

**Biomining**: Uranium, gold, etc.

**Gold**
Microbial cell charge

Microbial Electrosynthesis ——
A new and efficient biosynthesis approach

The efficiency of Electrosynthesis is much higher than Photosynthesis

Moorella thermoacetica

Electrofuel project, US DOE

Yang Peidong* et al., Science, 2016
Extracellular electron transfer is critical in Industrial/Environmental fermentation processes

**Interspecies Electron transfer**

Methanogenesis

Anaerobic Digestion

**Interspecies H₂ Transfer**

**Direct Interspecies Electron Transfer via Nanowires**

**Interspecies Electron Transfer Mediated by Electron Transport through Conductive Activated Carbon**

*Current Opinion in Biotechnology, 2013*
Key scientific issues about “Microbial Electrochemical Systems” (MES): Low efficiency of electron transfer between “cell-electrode interfaces”, which limits industrial applications of microbial electrocatalysis systems.

Engineering Strategies:
1) Engineering cells
2) Strengthen “cell-electrode interfaces”

Electroactive/Conductive Biofilm
Three Mechanisms of Extracellular electron transfer

a. Direct contact-based

b. Shuttle-mediated

c. Conductive pili-based (conductive matrix in the biofilm)
Reconstruct extracellular electron transfer pathways
Micro-, meso-, and macro-scale rational design approaches

In micro-scale level (single cell level): increase biosynthesis and transport of electron shuttle

In meso-scale level (biofilm): rational design graphene-cell 3D self-assembled conductive biofilm.

In macro-scale level: fermenter-exoelectrogen microbial consortia

E. coli
Shewanella

Solve the problem of limited spectrum of carbon sources of exoelectrogens (e.g., xylose)

Power increase ~10x

Inward electron transfer, ~15 folds

3D self-assembled conductive biofilm

Power increase ~25 folds

Inward electron transfer, ~74 folds

ACS Catalysis 2015, 5: 6937.
ACS Synthetic Biol. 2015, 4: 815.
ACS Synthetic Biol. 2017 in press
Biotechnology for Biofuels, 2017 in press
Bioresource Tech. 2013, 130: 763.

Biotech. & Bioeng. 2015, 112: 2051.
Development of Genomic regulation Tools in *Shewanella*

CRISPRi: transcriptional regulation of gene expression

sRNA: translational regulation of gene expression

CRISPRi–sRNA: Synergistic Transcriptional–Translational Regulation of genes expression

H. Song*, et al., ACS Synth Biol, 2017 in press
Rewire cell metabolism: (1) cofactor engineering, knock-out lactate biosynthesis

Knockout lactate synthesis

E. coli

NADH is the reducing power, releasable intracellular electron pool.
But, large amount of electrons were stored in lactate

**In micro-scale level (single cell level): Increase biosynthesis and transport of electron shuttle**

H. Song*, et al., *Electrochem Comm*, 2012
Rewire cell metabolism:
(2) Cofactor engineering, increase anaerobic TCA cycle

**E. coli**

Glucose → NAD⁺ → NADH → Pyruvate → Succinate → NAD⁺

PEP → NAD⁺ → NADH → Ethanol → CO₂

**TCA Cycle**

H₂ → Formate → Acetate → Lactate → ArcA/B

Electron transfer is increased efficiently

**Specific enzyme activity**

- **CS**
  - BL21: 150
  - arcA⁻: 300

- **OGDH**
  - BL21: 10
  - arcA⁻: 20

- **SDH**
  - BL21: 2
  - arcA⁻: 1

**Electron transfer is increased efficiently**

**Current density (µA/cm²)**

- BL21
- arcA⁻

**Cell voltage (mV)**

- BL21
- arcA⁻

**Power density (mW/m²)**

- BL21
- arcA⁻

_H. Song*, et al., ACS Catalysis, 2012_
Modular design approach in engineering NAD biosynthesis pathways: increase total NAD level

(A) Modular assembly of critical genes

(B) Enhanced extracellular electron transfer

3.4X increase

H. Song* et al., to be submitted

Engineered Shewanella

WT–Shewanella oneidensis
Rewire cell metabolism - cofactor NADH

(4) Regulate NADH/NAD\(^+\) ratio

Enhanced extracellular electron transfer

H. Song* et al., to be submitted
(5) Electron shuttle biosynthesis: Increase biosynthesis of riboflavin (vitamin B2)

Bacillus VB2 biosynthesis into Shewanella

VB2 biosynthesis in Shewanella increased 25 times

Output power increase ~10x

Microbial electrosynthesis ~15x

H. Song*, et al. ACS Synthetic Biology, 2015
Membrane Engineering:
Increase cell permeability, thus accelerate shuttle transport

Porin synthesis

Morphology and permeability change (AFM images)

Wild-type cell membrane

Engineered Cell membrane

Increase Electron Transfer Efficiency

(1) Genetic engineering of electroactive biofilm

Second messenger c-di-GMP regulates biofilm formation

In Meso-scale (multi-cellular)
Construction of conductive biofilm

Engineering conductive biofilm, Electron transfer efficiency increased 3X

H. Song*, et al., Biotech & Bioeng, 2015
**Self-assembled 3D electroactive biofilm**

*Shewanella* reduces graphene oxide, and self-assembly with rGO

**Electron transfer increase ~25 X**

Inward electron transfer increases ~74倍

(3) Artificial conductive pili

Artificial conductive pili

Graphene anode

S. oneidensis MR-1 cell

Lactate $\rightarrow CO_2$

Electronic transfer path

Cytochrome protein (OmcA)

Reductive Oxidative

Electron transfer path

3D Graphene/PANI electrode

PANI: 聚吡咯导电高分子

polypyrrole: conductive polymer

$\sim$3X increase in electron transfer

H. Song* et al., ACS Nano, 2012
Conductive artificial biofilm complex matrix

Graphite + conductive polymer + electrogen

Morphology of biofilm complex

8X increase in electron transfer than naturally occurring biofilm

Chem. Comm., 2011
Chem Comm., 2013
On Macro-scale level: Design microbial consortia

Genetic engineering single cells

- ACS Synthetic Biology, (2015)
- Chemical Communications, (2015)
- Biotech and Bioeng, (2013).
- Chemical Communications, (2013).
- Chemical Communications, (2011).

Existing key questions:
- Narrow spectrum of carbon sources.
- Intensive engineering of exoelectrogens may lead to lower viability of cells

Microbial consortia: Division of Labor

Carbon source

fermenters → Exoelectrogens

Other microbes

Electrodes
For example, we incorporate xylose utilization function into *Shewanella*, resulting in a low power output, $\sim 2\text{mW/m}^2$. 

H. Song et al., Biotechnology for Biofuels, 2017
(1) “Fermenter-exoelectrogen” microbial consortia: Expand the spectrum of carbon sources

Principle “Division of Labor”: fermenter + exoelectrogen

**E. coli**

- Xylose → Xyritol → Xylose → Xylose-5-P → Ribulose-5-P
- Glycerate-3-P → Succinate → Pyruvate → Formate
- Phosphoenol-pyruvate → Pyruvate → Lacatate → Ethanol → Acetate

**Riboflavin synthesis pathway**

- **E. coli** synthesize riboflavin, used by **Shewanella** as electron shuttle
- **Metabolites exchange**: E. coli → lactate/formate → Shewanella
Incorporate flavin biosynthesis pathway from *Bacillus* into *E. coli*, producing electron shuttle.

**WT E. coli - WT Shewanella**
2). Symbiotic *Saccharomyces-Shewanella* for microbial electrocatalysis

*Saccharomyces* (fermenter) – *Shewanella* (electrogen) consortium

**Engineered yeast:**
- Lactic acid biosynthesis
- No ethanol biosynthesis

**Engineered *Shewanella***:
- Lactate utilization
- Electro-active biofilm

Engineering lactate transporter in *Shewanella*

H. Song*, AIChE J. 2017
3). Three microbes’ consortia: *E. coli*-Bacillus-Shewanella

**Division of labor**

**Interactions between the 3 species**

**Achieve a highest coulombic efficiency**

<table>
<thead>
<tr>
<th></th>
<th>Coulombs of charge</th>
<th>Conversion efficiency ($\varepsilon_m$) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. oneidensis</em> + <em>E. coli</em>-Lac-Rib</td>
<td>188.4</td>
<td>31.6</td>
</tr>
<tr>
<td><em>S. oneidensis</em> + <em>E. coli</em>-Rib + <em>E. coli</em>-Lac</td>
<td>222.4</td>
<td>37.4</td>
</tr>
<tr>
<td><em>S. oneidensis</em> + <em>B. subtilis</em> + <em>E. coli</em>-Lac</td>
<td>331.2</td>
<td>55.7</td>
</tr>
</tbody>
</table>

Acknowledgements

✧ **Grants**
- 863 program
- 973 program
- NSFC

✧ **Team members**
- Dr. Yong Yangchun
- Dr. Yang Yun
- Dr. Liu Ting
- Dr. Yu Yangyang
- Dr. Cao Yingxiu
- Mr. Li Feng
- Ms. Hu Yidan
- Ms. Lin Tong
- Ms. Fang Lixia
- Ms. Ding Wenqi
- Ms. Chen Xiaoli
Thank you very much!