Microbial composition and metabolic potential development in microbial fuel cells during wastewater treatment

Olga Tsoy
Institute for Information Transmission Problems (Kharkevich Institute),
Bolshoy Karetny per. 19, build.1, Moscow 127051 Russia
Okinawa Institute of Science and Technology,
1919-1 Tancha, Onna-son, Kunigami-gun, Okinawa, Japan 904-0495
tsoy.v.olga@gmail.com

Larisa Kiseleva
Okinawa Institute of Science and Technology,
1919-1 Tancha, Onna-son, Kunigami-gun, Okinawa, Japan 904-0495
larisa.kiseleva@oist.jp

Igor Goryanin
Okinawa Institute of Science and Technology,
1919-1 Tancha, Onna-son, Kunigami-gun, Okinawa, Japan 904-0495
goryanin@oist.jp

Microbial fuel cells (MFCs) is a promising technique for a sustainable electricity generation and might also be used for wastewater treatment, powering remote and isolated (for example, underwater) devices or as biosensors. MFCs use microorganisms as catalysts for organic matter oxidation and electrons production. These microorganisms are able to transfer electrons (directly or through shuttles) outside of the cell to the anode. This process is called extracellular electron transfer (EET). Electrons then flow to the cathode where they combine with protons from the anode and oxygen from air to produce water. The first described EET-capable bacteria were *Shewanella* and *Geobacter* [1, 2]; and recently many other microorganisms were reported to produce electricity in MFCs.

Here we analyzed the development of microbial composition and functional potential in two MFCs used for wastewater treatment in Mizuho distillery (Okinawa, Japan) using MiSeq next-generation sequencing.

MFCs were inoculated by an anaerobic sludge and fed by wastewaters from Mizuho distillery during two years. DNA was extracted and sequenced from 9 samples of the inoculum (used

as a reference), the anode and MFC fluid in three months and six months after the inoculation. Additionally RNA was extracted and sequenced from 7 samples of the anode and MFC fluid in three months and six months after the inoculation.

The previous study [data not published] showed that the microbial community in MFC operating in subtropical region (Okinawa, Japan) is highly diverse and contain a significant fraction of not characterized bacteria. To estimate the biodiversity in MFC samples we used Metaxa 2 for small rRNA prediction. This tool can also predict the taxonomy and the amount of rRNA. We observed a significant amount of methanogenic archaea (as in previous study). Other major phyla in MFC are Deltaproteobacteria, Chloroflexi, Firmicutes and Bacteroidetes. Deltaproteobacteria (including *Geobacter*) might be the main current producers, while Firmicutes and Bacteroidetes might play role in organic matter fermentation.

Metagenomic assembly was performed using SPAdes 3.0.0 assembler followed by open reading frames (ORFs) calling using Prodigal. Predicted ORFs were annotated using HMMsearch 3.0 against PFAM-A database for functional potential analysis. The analysis showed that one of the most abundant PFAM families in MFC is Fer4 (and similar) that contains proteins with iron-sulfur clusters involved in redox-processes and electron transfer.

Raw DNA reads were further mapped to ORFs generating DNA-RPKM (Reads Per Kilobase per Million mapped reads) values. More than 60% of raw DNA reads were mapped. Metatranscriptomic data were filtered from rRNA and mapped to ORFs to generate mRNA-RPKM value. More than 50% of raw RNA reads were mapped. To analyze specific metabolic pathways KEGG Automated Annotation Server (KAAS) was used to assign KEGG Orthology to ORFs. KEGG orthologous group expression was estimated in all samples as mRNA-RPKM/DNA-RPKM ratio called MRPKM value as described in [3]. MRPKM values were generated and compared between samples for metabolic pathways that might be important for MFC operation such as EET, methanogenesis, organic matter oxidation, inorganic reduction, electron shuttles biosynthesis.

As MFCs have been operating for about two years already we are planning to perform the

similar analysis for DNA and RNA samples of the anode and the fluid in one year and a half after the inoculation time point.

We thank S. Naumenko, I. Goryanin and S. Garushiants for valuable discussions, D. Simpson for providing samples and assistance in MFC operation and R. Koyanagi for conducting next-generation sequencing.

References:

1. D. Lovley (2012) Electromicrobiology, *Annu. Rev. Microbiol.,* **66:**391-409.

2. L. Shi et al. (2007) Respiration of metal (hydr)oxides by Shewanella and Geobacter: a key role for multihaem c-type cytochromes., *Mol. Microbiol,* **65:**12–20.

3. S. Ishii et al. (2013) A novel metatranscriptomic approach to identify gene expression dynamics during extracellular electron transfer, *Nat. commun.,* **4:**1601.