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Workshop:

Metabolomic Research
using Bio-electrochemical Systems

John M. Pisciotta
April 27, 2015
Metabolomics and System Biology
Philadelphia, PA
Workshop Overview

- Types of BESs & their Set up
- Reactor Configurations
- Electrode Types & Materials
- HPLC analysis of cell metabolites
- Bioelectrochemical techniques (CA, LSV)
- Cell & Metabolite Sampling
- Unique Opportunities for Metabolomics
- Considerations
- Summary / Demos Units
Bioelectrochemical Systems: Types

- **Microbial Fuel Cells (MFCs)**: exo-electrogenic bacteria on anode breakdown chemicals and donate useful electrons through a circuit. (Air cathode) = Electricity

- **Microbial Electrolysis Cells**: exo-electrogenic bacteria on anode breakdown chemicals and donate useful electrons through a circuit (Anaerobic cathode + voltage boost = Hydrogen Gas)

- **Microbial Electrosynthetic Cells**: use electrotrophic bacteria on the MEC cathode to accept electrons to store input electrical energy as chemical bonds.

- **Enzymatic BESs**: use redox active enzymes attached to electrodes to carry out specific reactions.
BES History

• **1911**: M.C. Potter, at the University of Durham discovered that bacteria could produce direct electric current.

• **1934**: Cohen develops 35 V microbial ½ cell.

• **1999**: BH Kim discovered microbes do not require external mediators to generate current.

• **2014**: hundreds of new BES article every year!
O2 = Terminal Electron Acceptor (TEA) in Aerobic Systems

Aerobic respiration allows full use of the energy in food/fuel, it is little wonder this mode of existence predominates today.

Aerobic Electron Transport Chain

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**Diagram Description**

- **I** represents the entry of electrons from NADH.
- **II** shows the transfer of electrons from FADH to NAD+.
- **III** involves the oxidation of NAD+ to NADH.
- **IV** depicts the final electron acceptor, O2, which gets reduced to H2O.

The process continues with the transport of protons (H+) across the mitochondrial membrane, generating ATP through oxidative phosphorylation.
BES = Bioelectrochemically Controlled Combustion

- Iron reducing bacteria

Luef et al., 2013. ISME Journal
MFC electron flow

Diagram showing an MFC (Methanogenic Fuel Cell) with electron flow from anode to cathode. The diagram includes:
- Anode
- Cathode
- Growth medium
- Rhodoferax ferrireducens bacterium
- Fe^{2+} and Fe^{3+}
- Anaerobic and aerobic conditions
- Porous membrane
- Oxygen (O_2) reaction at the cathode
- Oxygen (O_2) and Proton (2H^+) production at the anode
- Water (H_2O) formation at the cathode
Understanding the Engineering

Microbes consume organic waste and transfer high potential electrons through an electrical device positioned between microbes and the system’s cathode TEA (O2).

Not just any Microbe can donate Electrons to MFC anode:

- **EXOELECTROGENS**
  (ex. Geobacter sp.)
Electron Transfer Mechanisms from exo-electrogen to anode

1) Direct electron transfer: *Geobacter sulfurreducens*

2) Soluble Mediators: *Shewanella oneidensis*

Metabolomic methods can help ID mediators
Other BESs use Enzymes:
Metabolomic methods can determine product formation rates

Zebda et al., 2011. Nature Communications
Some BESs use Enzymes:

- Difficulty in purifying enzymes
- Difficulty in adhering to electrode
- Difficulty in sustaining enzyme activity (just catalysts)

- Can not self repair or regenerate.
- Can not spread
- Can not be genetically programmed

Zebda et al., 2011. Nature Communications
BES Configurations

• Single Chamber
  Simple & inexpensive
  Amenable to High throughput screening

• Dual Chamber
  Complex & Expensive
  Separation of Anolyte from Catholyte (PEM)
  Preferred for metabolomic studies
Ohm’s law used to calculate current

\[ I = \frac{V}{R} \]
**Single Chamber Customization: Spec Tube MFC**

- **Resistor**
- **Cathode**
- **Anode** (Isolation Loop)
- **Standard Test tube w/ “Fuel”**

**Diagram Descriptions:***
- **Screw cap clamps wire between Cathode’s inner Pt surface and Glass top of test tube**
- **Paint-on Anode insulator Prevents short circuit, creates Water tight seal w/ Cathode PTFE layer**
Electrogenic Activity
(Current determined by V drop across resistor)
Dual Chamber “H-type” Reactor

- Headspace
- PEM
- Reference
- Working electrode
- Counter electrode

Diagram showing a dual chamber reactor with components labeled as Potentiostat, Headspace, PEM, Reference, Working electrode, and Counter electrode.
Potentiostat

- Electronic hardware required to control a three electrode cell and run most electroanalytical experiments.
- Serves to maintain electric potential of the working electrode at a constant level versus a reference electrode by adjusting current at an auxiliary electrode.
Electrodes & Electrode Materials used on other Type of BESs

- **Photosynthetic** Microbial Fuel Cells
  (UMD / Baskakov)

- **Dark Electrotrophic** Systems
  (Penn State / Logan)

- Experimental Considerations for optimizing *Metabolomic analysis* using BESs
Electrogenic and Electrotrophic Microbial Fuel Cells
Electrogenic Activity of Cyanobacteria

Idea: Convert sunlight to electricity using photosynthetic organisms on an anode and H2O as electron source.

(A living solar panel)

PI: Ilia Baskakov,
U. Maryland Center For Marine Biotechnology
Photosynthetic Microbial Fuel Cell
-Illuminated biofilm donates electrons to anode (B). 
For details see references 5, 6.

Single Chamber Cyanobacterial Fuel Cell
Carbon paint anode / carbon cloth Pt cathode
Rapid Light-Dependent Rise in Voltage

Photosynthetic Biofilm
Increasing light boosts electrogenic response (polyaniline coating boosts voltage)

Zou, et al. 2010, Bioelectrochemistry
Conductive Polymer coated electrodes allow Greater Power

-Carbon Paint Base-

- ▲ Untreated
- ● Poly Pyrrole
- ○ Poly Aniline
Poly Pyrrole nanostructure also affects –Power–

Photosynthetic Biofilm

![Graph showing power density vs. current density](image)

- Fibrular Poly P
- Poly P

Zou, et al. 2010, Bioelectrochemistry
Likely through Decreasing Resistance

Photosynthetic Biofilm

EIS Analysis

- Untreated
- Poly Pyrrole, granular
- Poly Pyrrole, fibrular
Electrochemical impedance spectroscopy (EIS)

- Allows analysis of the internal resistance of BESs, electrodes & materials, catalyst coatings, biofilms and reactions on the anodes and the cathodes.

- Requires potentiostat
Non-phototrophic bacteria: *Sediminibacterium* (a2), *Prosthecobacter* (a3, a4) and *Methylococcus* (a7) detected.

Pisciotta et al., 2010, PLoS One
Scenedesmus microalgae growing on PMFC carbon nanofiber electrodes

Zou et al., 2009
Electrogenic fingerprints.

Pisciotta et al., 2011. AMB
What is the Biological Basis?
Electrogenic activity peaks under strong light, suggesting possible function: photo-protection.
Can metabolite production be influenced by Bioelectrochemical Interfacing?
Metabolomic HPLC analysis

Photo-protective Carotenoids less prominent in electrically-connected (thin line) versus Disconnected (thick line) PMFCs.

Pisciotta et. al., 2011. AMB
DARK
Electrotrophic BES.
Electrochemical Methods to ID effective e- uptake from Biocathodes

1) Linear Sweep Voltammetry (LSV)
   - *Short term* electron uptake (i.e. reduced overpotential)

2) Cyclic Voltammetry (CV)
   Reversibility of electron exchange / mediator characterization

3) Chronoamperometry (CA)
   - *Long term* electron uptake by monitoring Negative Current over time

4) Metabolomic Identification of Product (ex. GC of gas phase)
Electrotrophic CO$_2$ fixation using Biocathodes

• **Idea:** Enrich and isolate bacteria able to accept electrons from a cathode for reduction of CO$_2$ into fuel.

  *the “electrotrophs”*
Sources Tested:

• Chesapeake Bay Sediment

• Baltimore Harbor Sediment

* -200 mV vs Ag/AgCl reference electrode (aka “Set”)

MFC establishment conditions

Unpoised

-200 mV*
Sediment MFC anode to MEC cathode by *Inversion Method*.

A) MFC anodes in anaerobic sediment establish electrogenic biofilm.

B) MFC anode then inverted to form functional MEC biocathode.

(Pisciotta. et al., 2012. AEM)
Linear Sweep Voltammetry (LSV) indicates highest short term electron uptake by harbor biocathode.
Chronoamperometry
Development of negative current
(-650 mV vs Ag/AgCl)

20:80 (CO₂:N₂)
50 mM PBS,
10 ppt NaCl
No substrate
Sustained current uptake Vs. sterile control.
Metabolomic GC Analysis

CO\textsubscript{2} depletion from cathode chamber headspace after 1 week at -650 mV or -750 mV

Week 1, -550 mV (no changes detected)
Week 2, -650 mV
Week 3, -750 mV

All Reactors
Degassed w/ 20:80 CO\textsubscript{2}/N\textsubscript{2}
Prior to each weekly trial
Biogas production and electron consumption.
Electrotrophic growth is apparent.

Bay, -650 mV (note Bay’s lack of gas bubbles).

Harbor, -650 mV.

Follow up objective 1)
Transfer to sterile MECs - Is direct perpetuation possible?

Task 2.2.3 – Milestone 47

Delayed Growth was Apparent
Mass Spectrometric Analysis of Cell Metabolites

1) Targeted Analysis

2) Metabolite Profiling

3) Global Metabolomic Analysis
Microbial Metabolomic (Lipidomic) Spectrum Profiling

Monoacylglycerides  Diacylglycerides  Triacylglycerides

Phospholipids

Note: DSG = 631
TSG = 897
MS/MS Identification vs Standard

MS/MS Confirmation of MSG @ 365

Glycerol Backbone

Note loss of H2O (m/z 18)
From glycerol results in 81 and 63 m/z peaks

Unfragmented
MSG C18:0
Drugs Induce Distinct Metabolic Response Patterns

Chloroquine vs. Control

Artemisinin vs. Control

Pyrimethamine vs. Cont.

1 microM drug
3hr time
Isolated trophozoites

*Data From Two Independent Experiments

John Pisciotta,* Abhai Tripathi,* Joel Shuman,† Vladimir Shulaev‡ & David J. Sullivan, Jr,*
Issues concerning Metabolomic comparisons:

- Extraction method standardization
- Large library of metabolite standards needed
- Timing and degree of BES exposure
- Normalization of cell / enzyme number to electrode surface area
- Hybrid Systems -

Anode respiration to boost Photosynthetic metabolite production

Enhanced waste to fuel conversion with a bioelectrochemically controlled autotrophic bioreactor. Pisciotta et al., 2013. Blusens Industrial Report III
Concluding Remarks:

• Metabolomic researchers interested in redox active proteins can gain fresh insight by using BES systems.

• The MFC research community also has much to gain from an improved understanding of Metabolomics.

• Stable Isotope studies may eventually help determine how voltage influences electrically directed metabolism in exo-electrogens and electrotrophs
Acknowledgments

- **JHU Sullivan Lab**: Metabolomic GC-MS/MS training using Malaria.

- **UMD Baskakov Lab**: Metabolomic analysis of pMFC using HPLC / PDA detector.

- **PSU Logan Lab**: Electrochemical Biocathode analysis

- **West Chester University**: Hybrid Designs (MEC-PBR)
Central Dogma of Molecular Biology, 1958

DNA → Genomics

mRNA → Transcriptomics

Protein → Proteomics

Metabolites > Metabolomics

UV Stress >> Thiamine dimers >> Tyrosinase mRNA 2x >> Melanin 7x

Eller et al., Nature. 1994
Why Metabolomics?

- It sometimes provides best (or only) insight into pathways involved in response to a stress.

  A genetically hard-wired metabolic transcriptome in *Plasmodium falciparum* fails to mount protective responses to lethal antifolates.

Ganesan et al., 2008. PLoS Pathogens
Parent product ion MS/MS of synthetic PG
Q-1 1ppm PG scan m/z 110-990 (M –H)–

Sn1 16:0, Sn2 18:2

Q-3 product ion scan of m/z 747 scanned m/z 110-990
Note 50X > sensitivity

SIM additional 5x > sensitivity ~ 250X
16s Clone Library Chesapeake Bay

- Mesorhizobium: 24
- Rhodococcus: 22
- Azospirillum: 19
- Gemmata obscuriglobus: 10
- Sphingopyxis alaskensis: 10
- Labrenzia aggregata: 3
- Endosymbiont of Tevnia: 3
- Marine actinobacterium: 3
- Remainder: 3

(n = 97)
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