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OMICS International has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

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.**

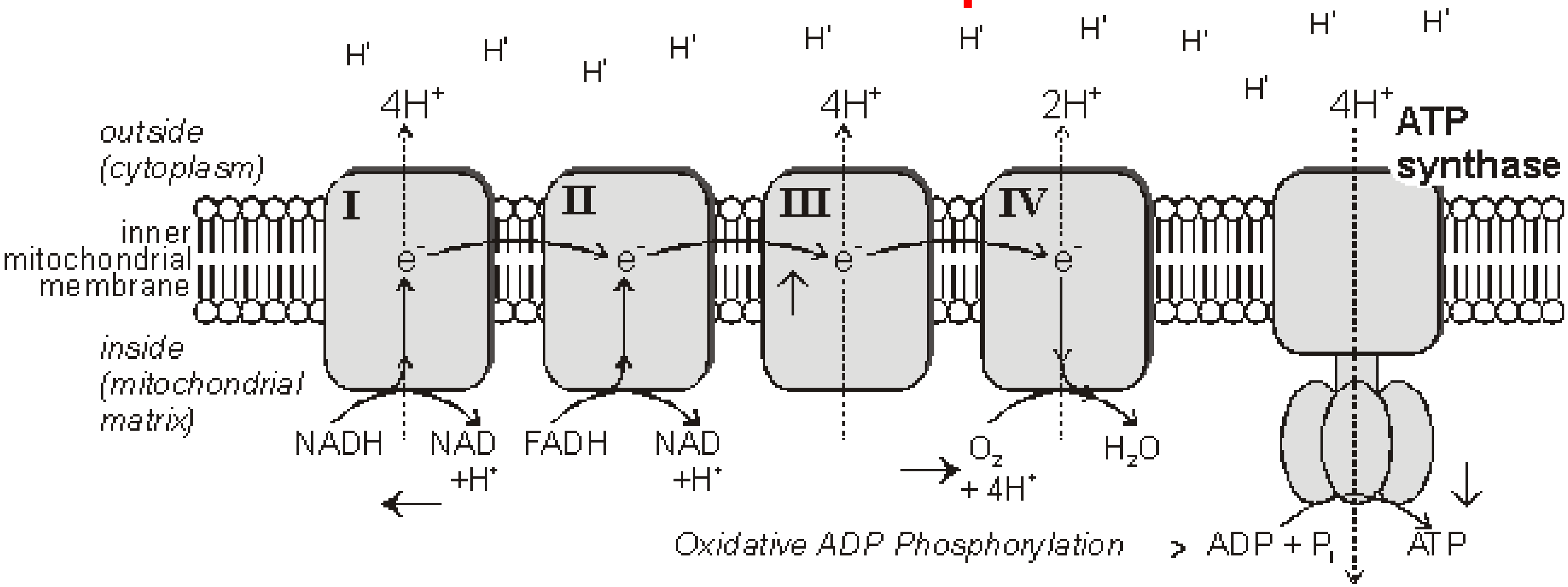
BES History

- **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 <u>not</u> require external mediators to generate current.
- **2014:** hundreds of new BES article every year!

O2 = Terminal Electron Acceptor (TEA) in Aerobic Systems

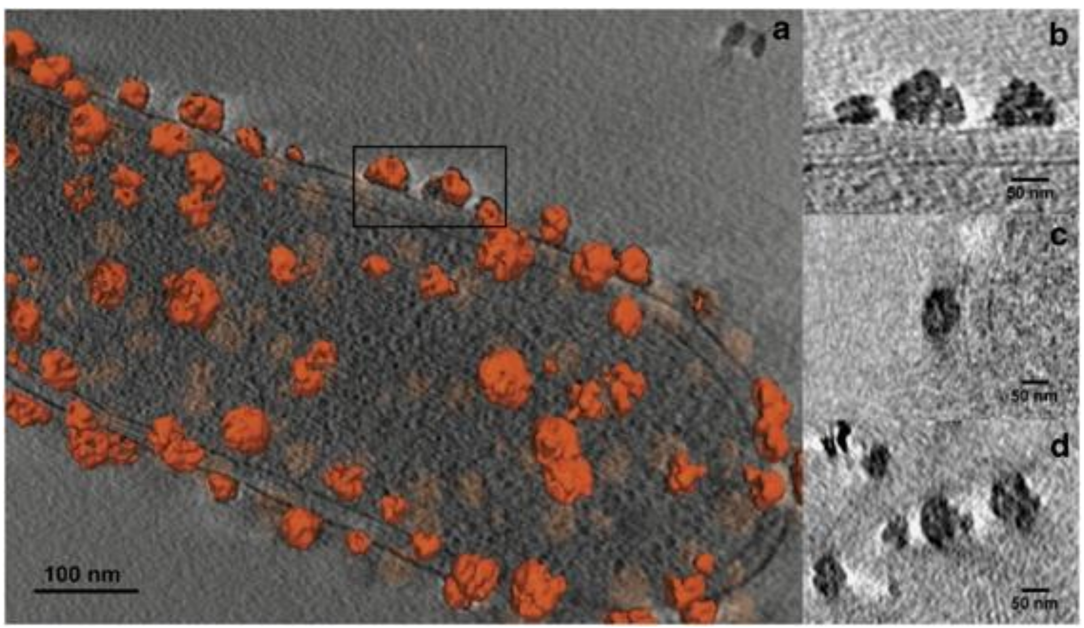
Aerobic respiration allows full use
of the energy in food/fuel, it is little wonder
This mode of existence predominates today

Aerobic Electron Transport Chain

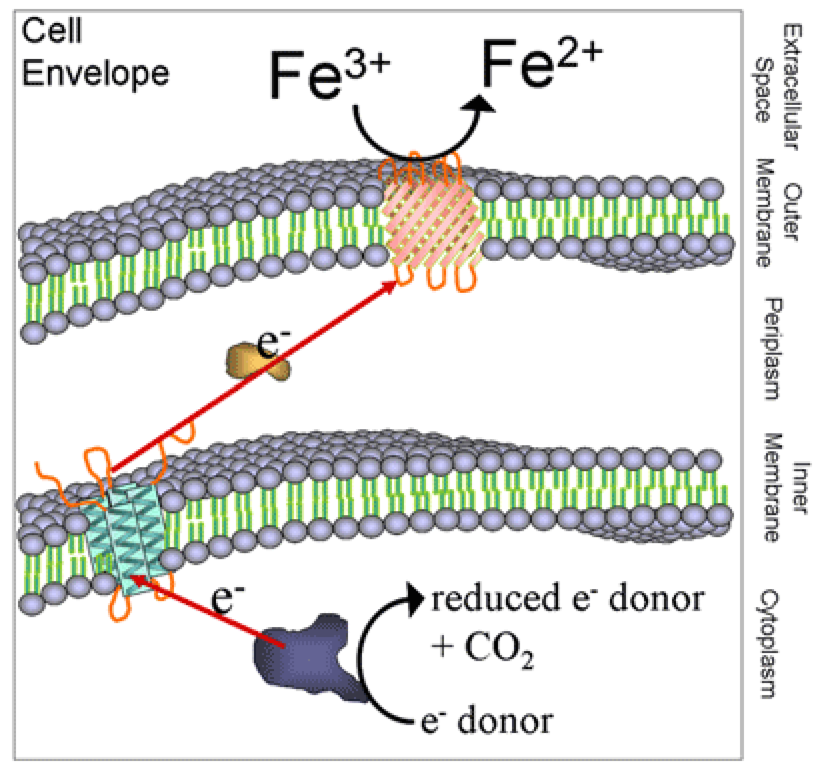


BES = Bioelectrochemically Controlled Combustion

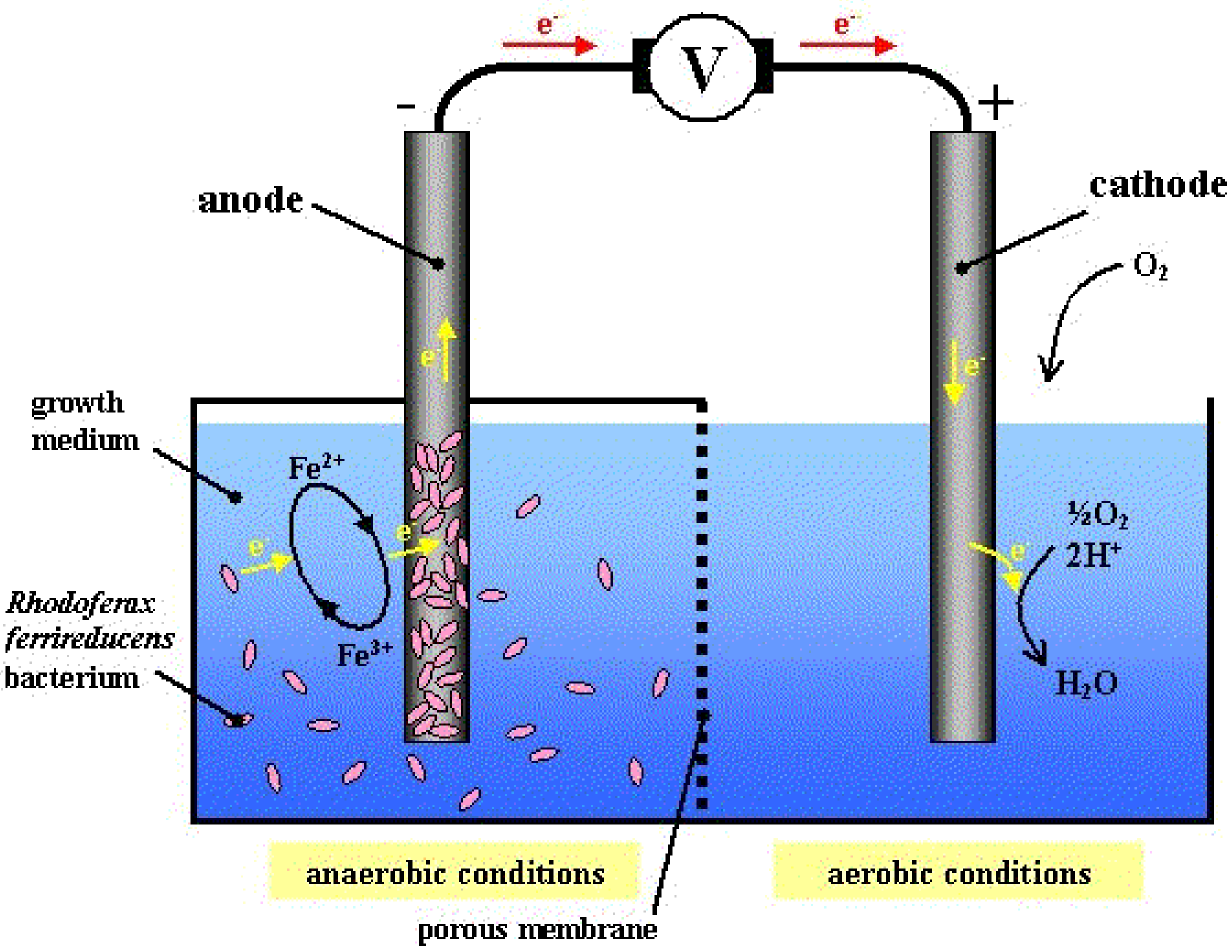
- **Iron reducing bacteria**



Luef et al., 2013. ISME Journal

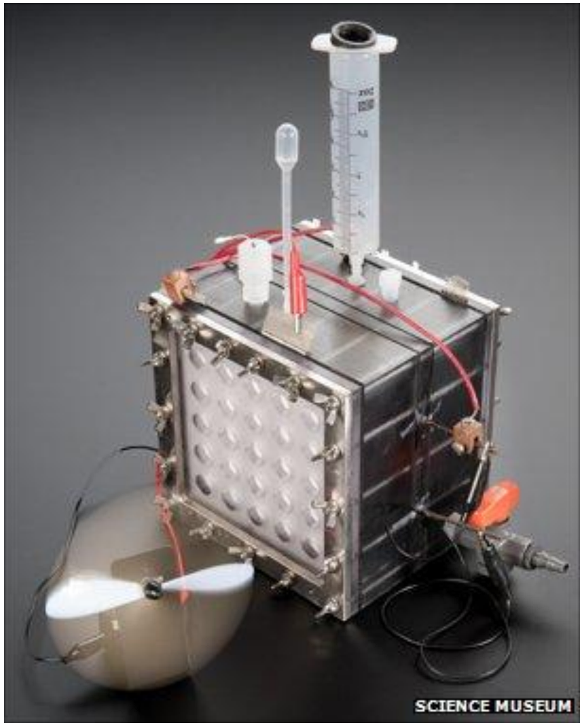


MFC electron flow



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:

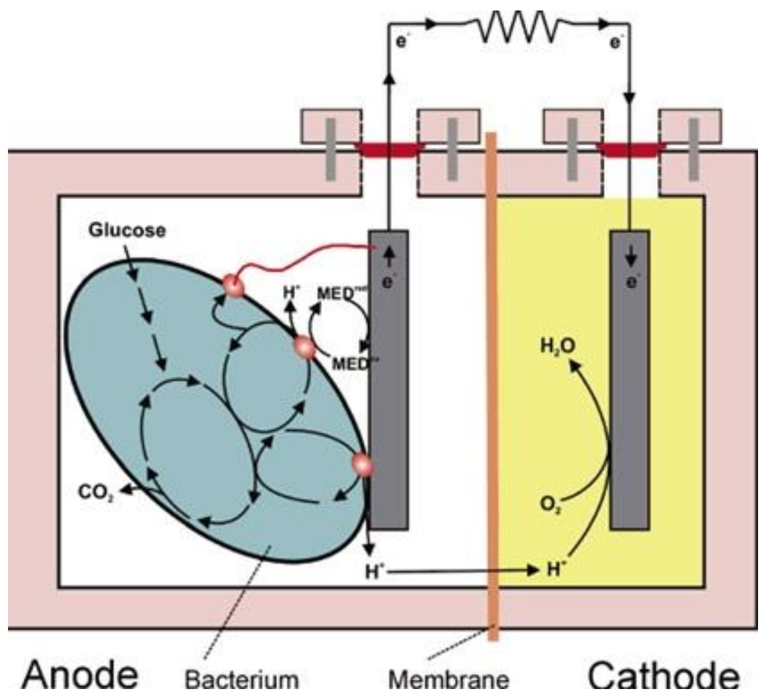
-EXOELECTROGENS

(ex. Geobacter sp.)

Electron Transfer Mechanisms from exo-electrogen to anode

1) *Direct* electron transfer: *Geobacter sulfurreducens*

2) Soluble Mediators: *Shewanella oneidensis*

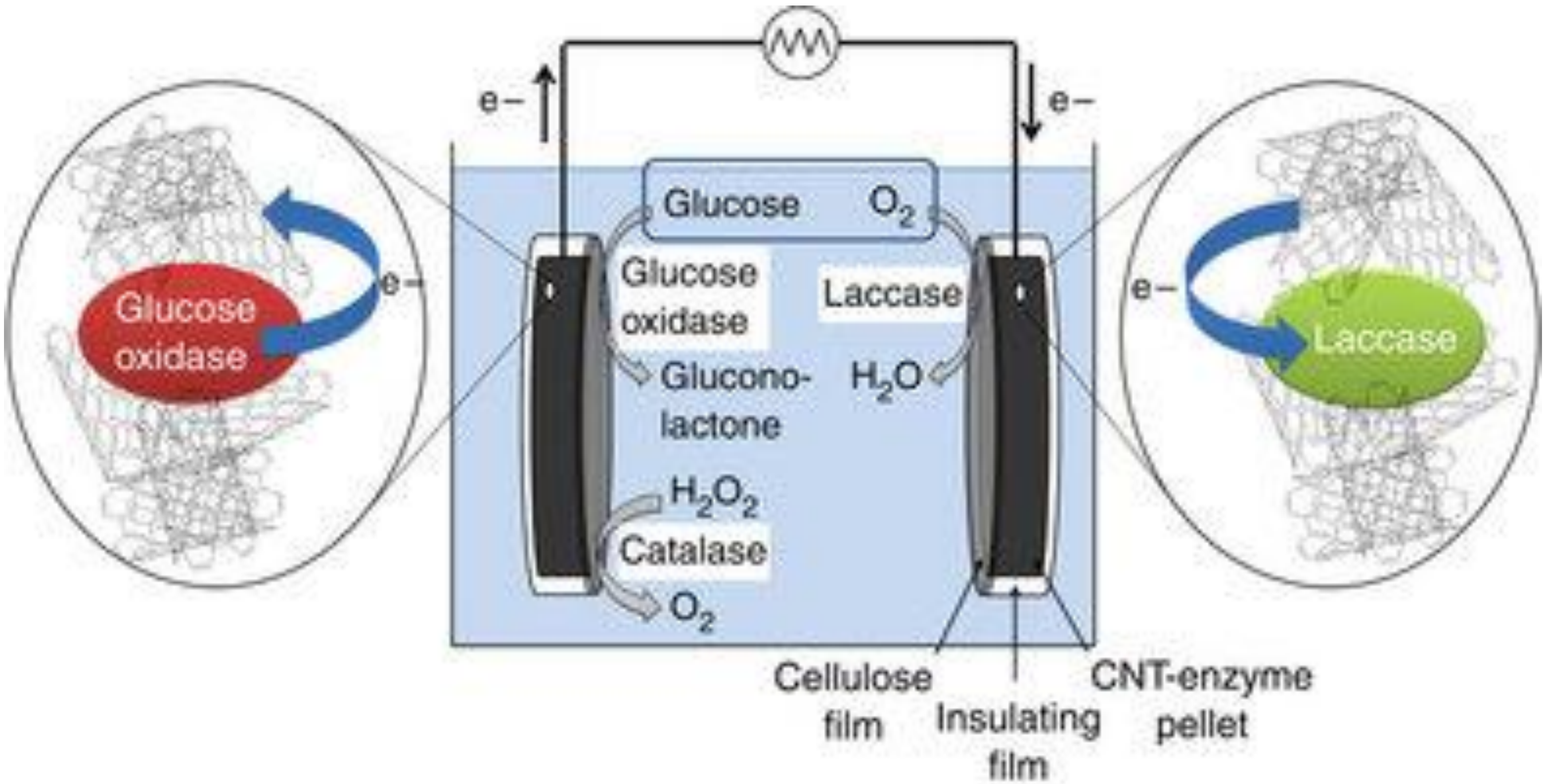


Metabolomic methods can help ID mediators

Image Source
Seminar only

Other BESs use Enzymes:

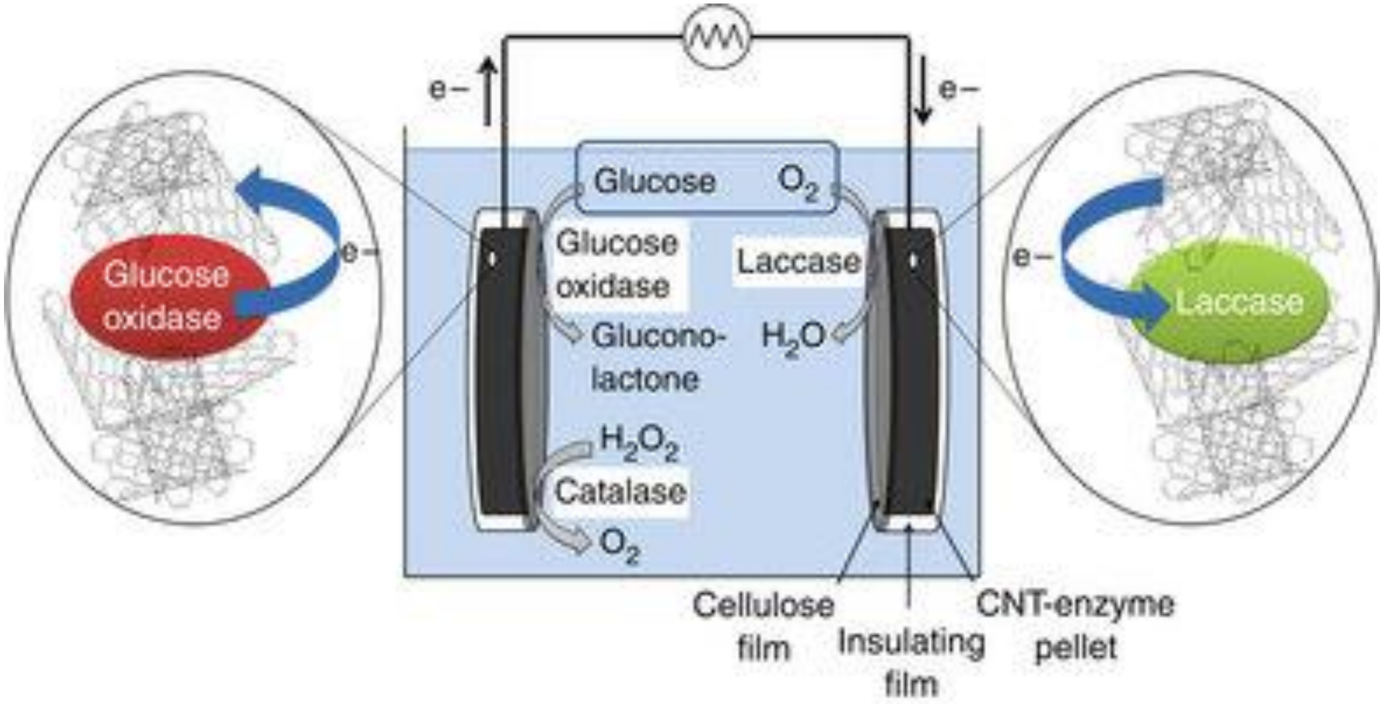
Metabolomic methods can determine product formation rates



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)



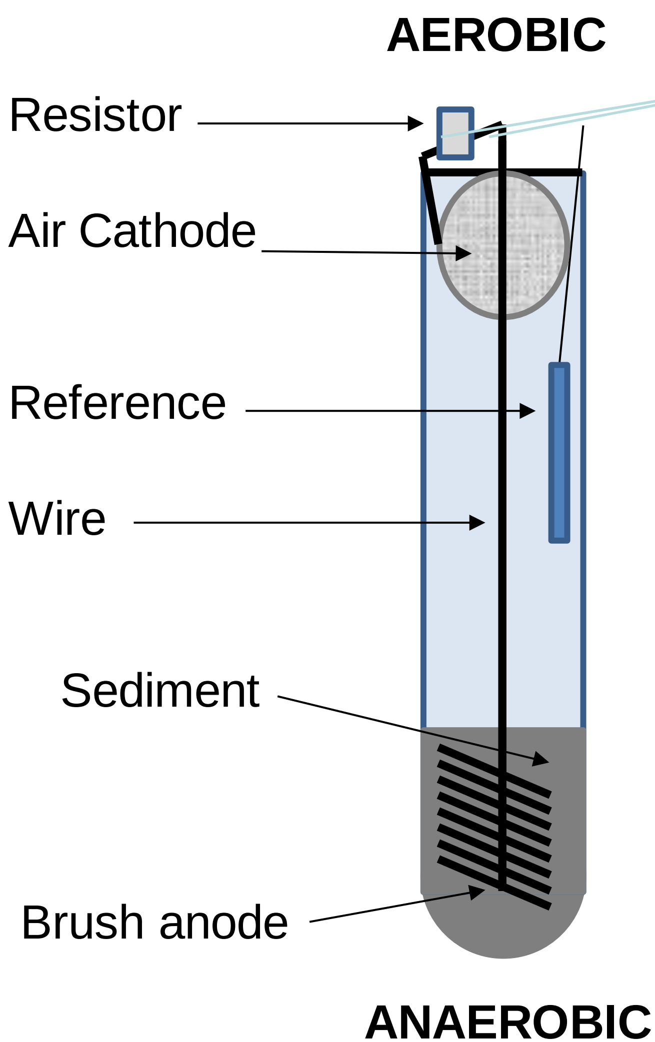
-Can not self repair or regenerate.
-Can not spread
-Can not be genetically programmed

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

Single Chamber MFC



Ohm's law used to
Calculate current
I = V/R

Single Chamber Customization: **Spec Tube MFC**

Resistor

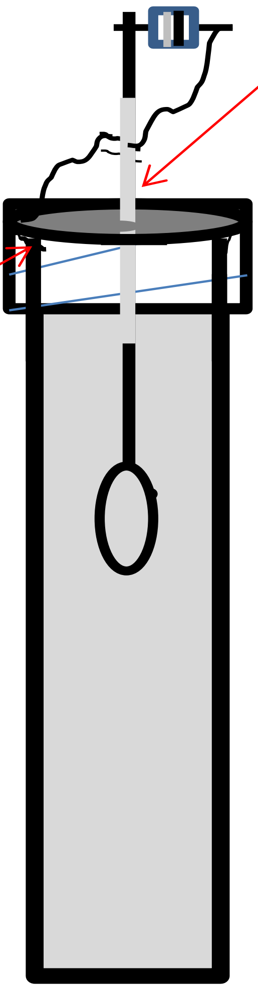
Cathode

Anode

(Isolation Loop)

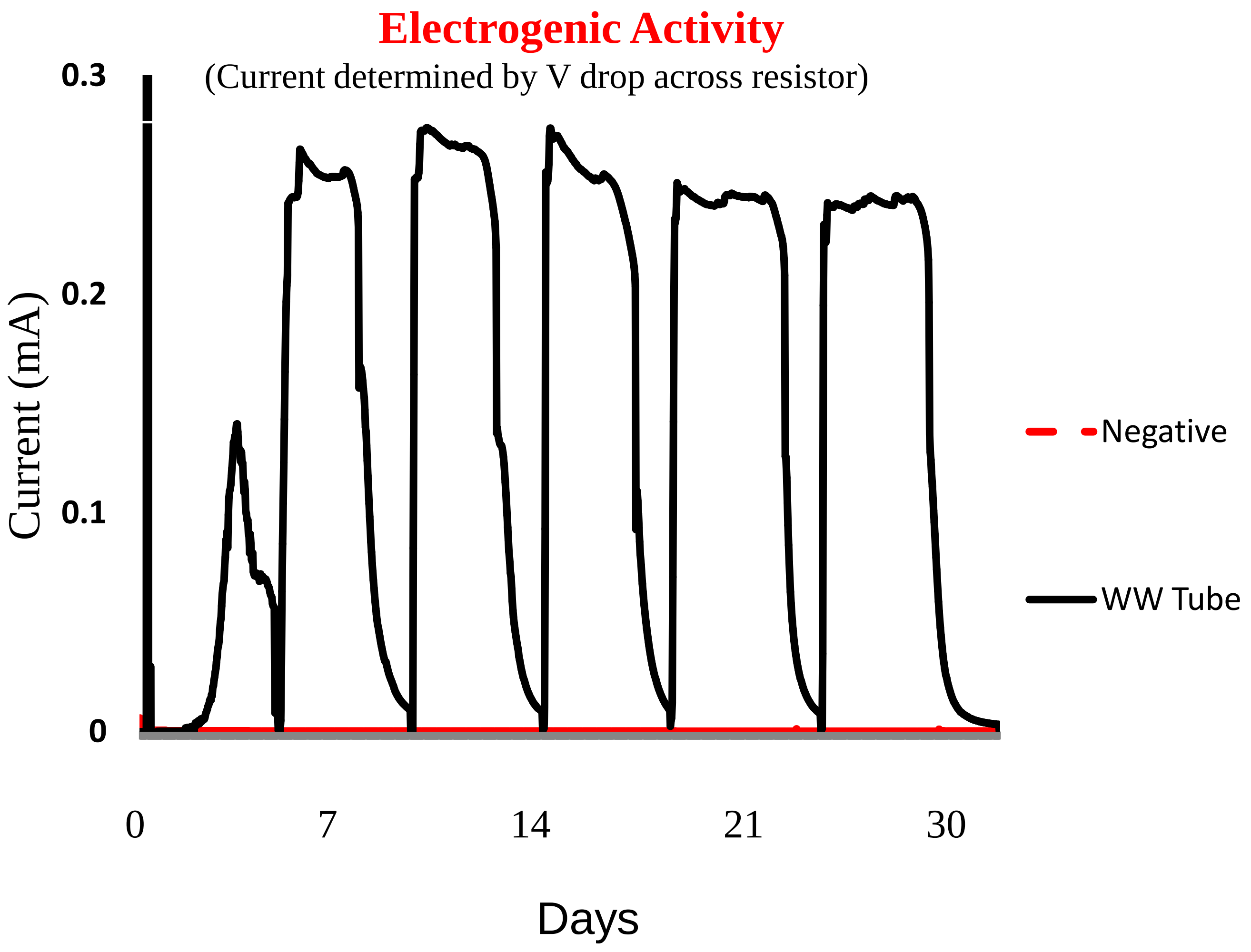
Standard Test tube w/ "Fuel"

Screw cap clamps wire between Cathode's inner Pt surface and Glass top of test tube

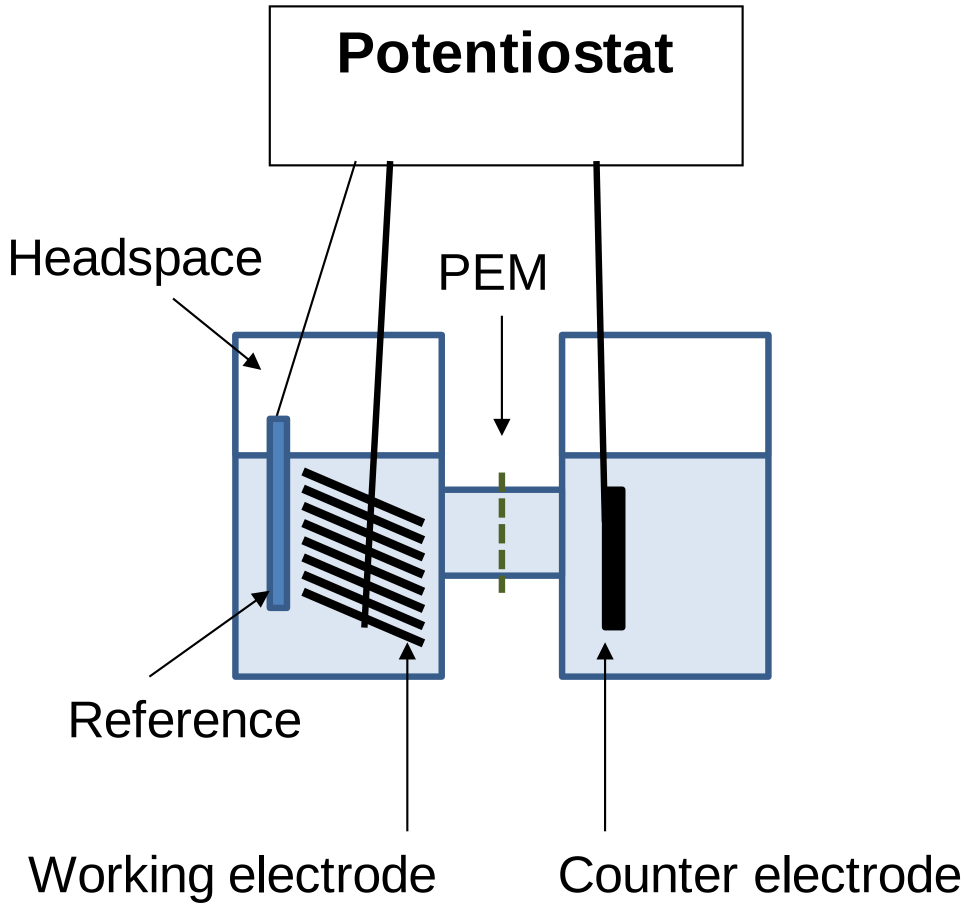


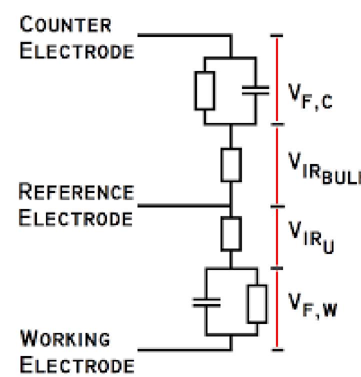
Paint-on Anode insulator Prevents short circuit, creates Water tight seal w/ Cathode PTFE layer

Spec Tube MFC



Dual Chamber “H-type” Reactor





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

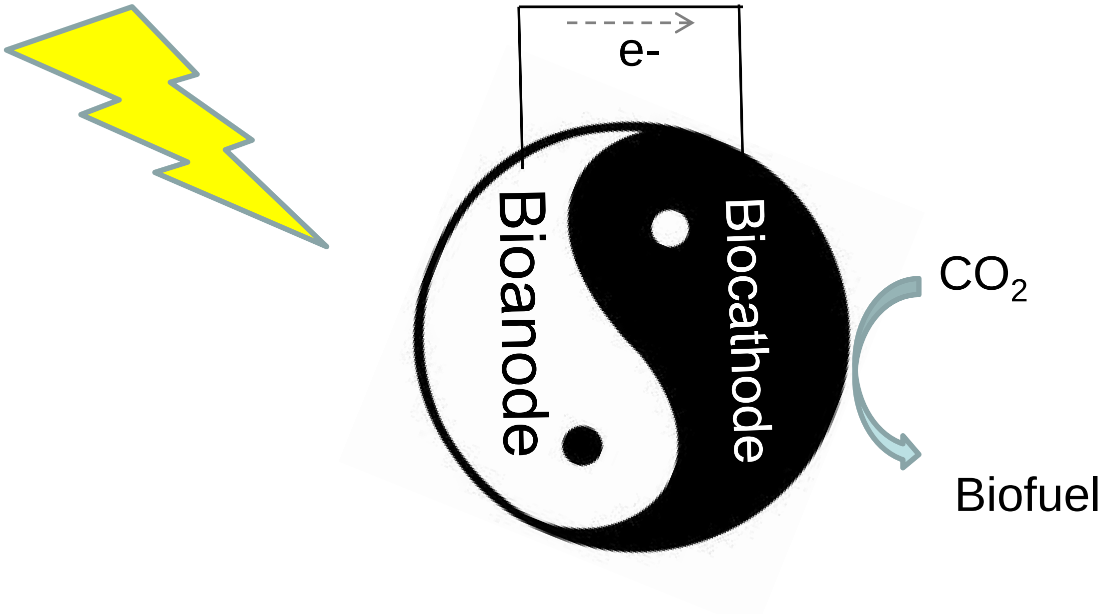
  (UMD / Baskakov)

- **Dark Electrotrophic** Systems

  (Penn State / Logan)

- Experimental Considerations for optimizing Metabolomic analysis using BESs

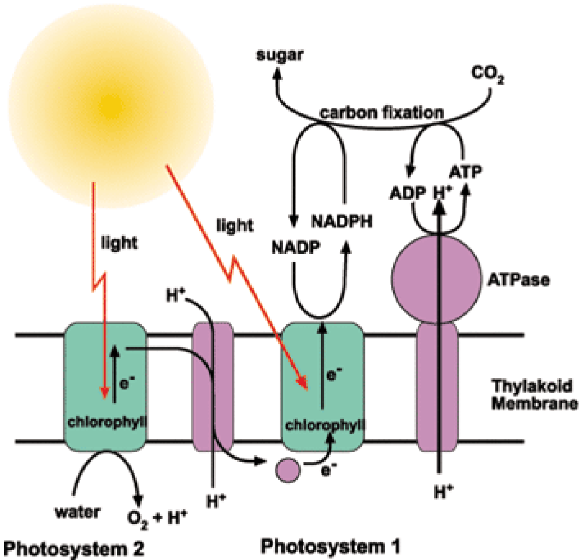
Electrogenic and Electrotrophic Microbial Fuel Cells



Electrogenic Activity of Cyanobacteria

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

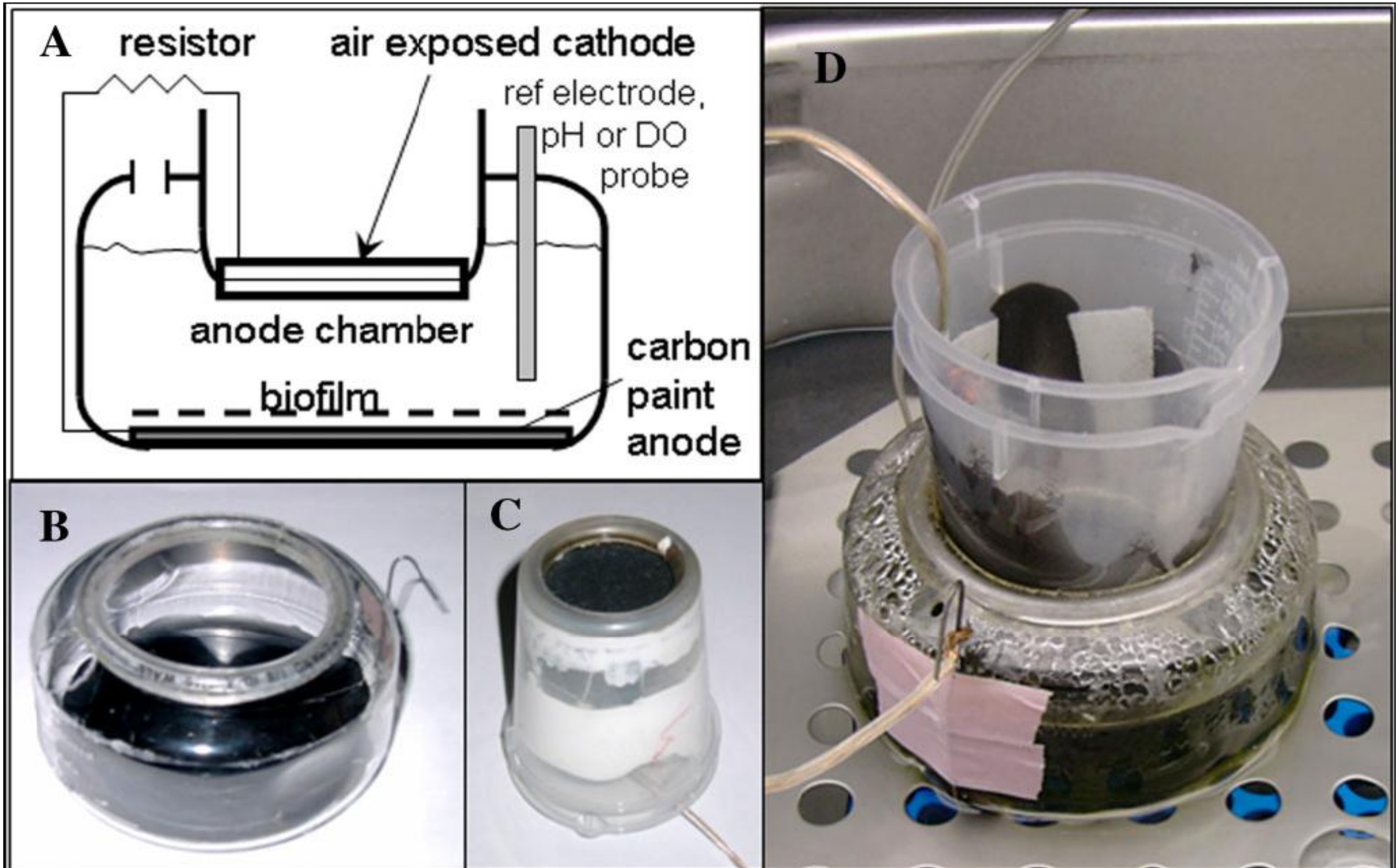
(***A living solar panel***)



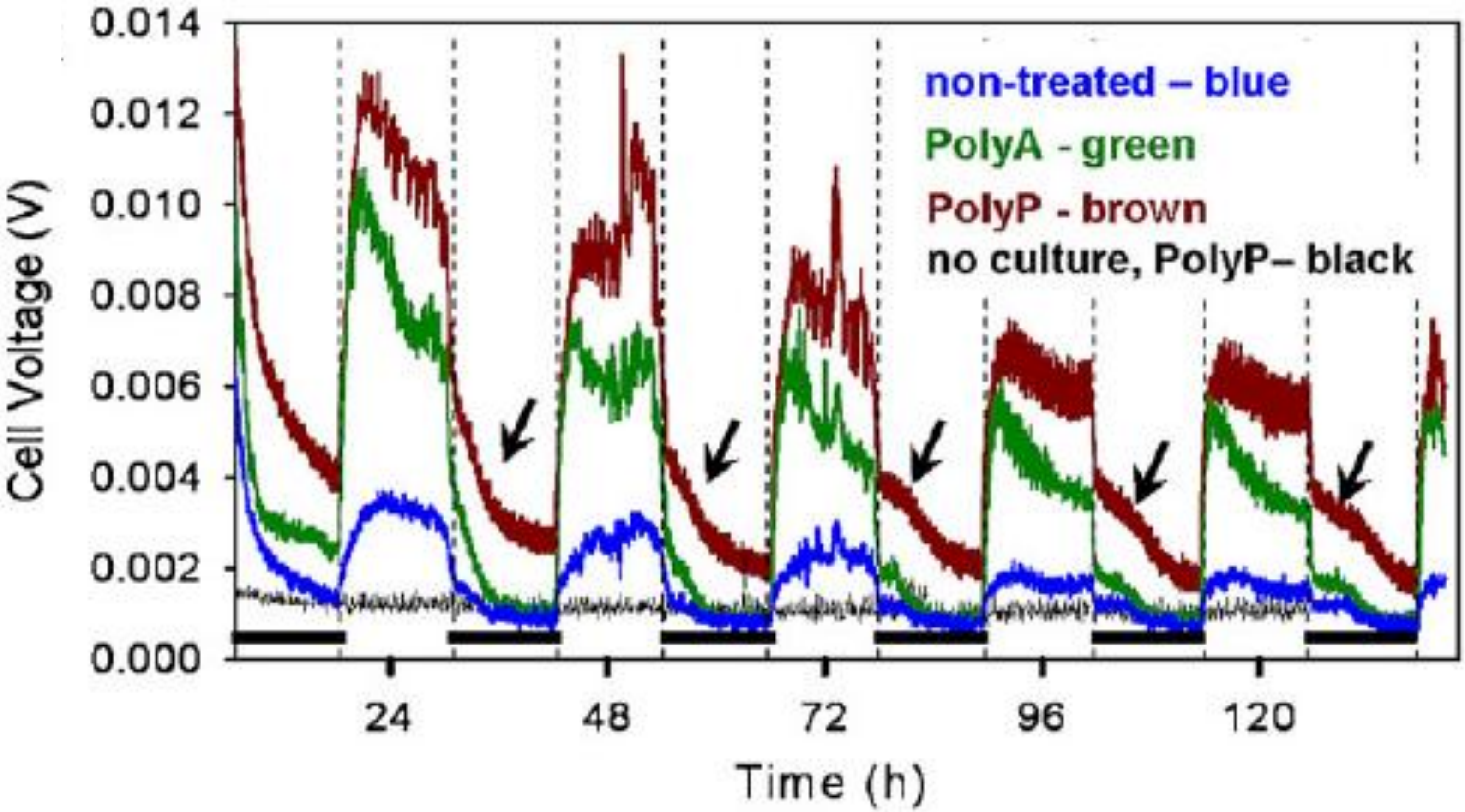
PI: Ilia Baskakov,
U. Maryland Center For Marine Biotechnology

Single Chamber Cyanobacterial Fuel Cell

Carbon paint anode / carbon cloth Pt cathode

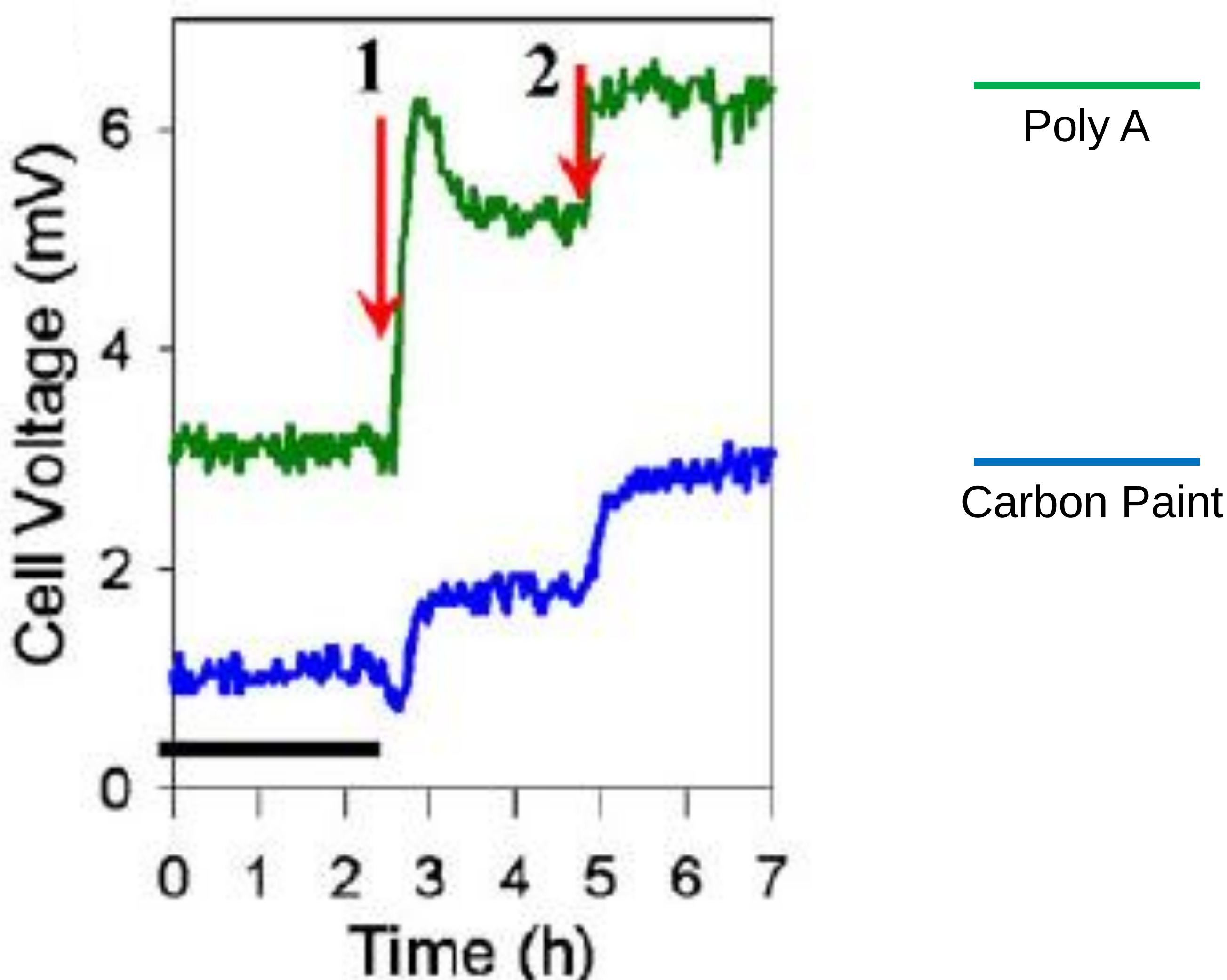


Rapid Light-Dependent Rise in Voltage

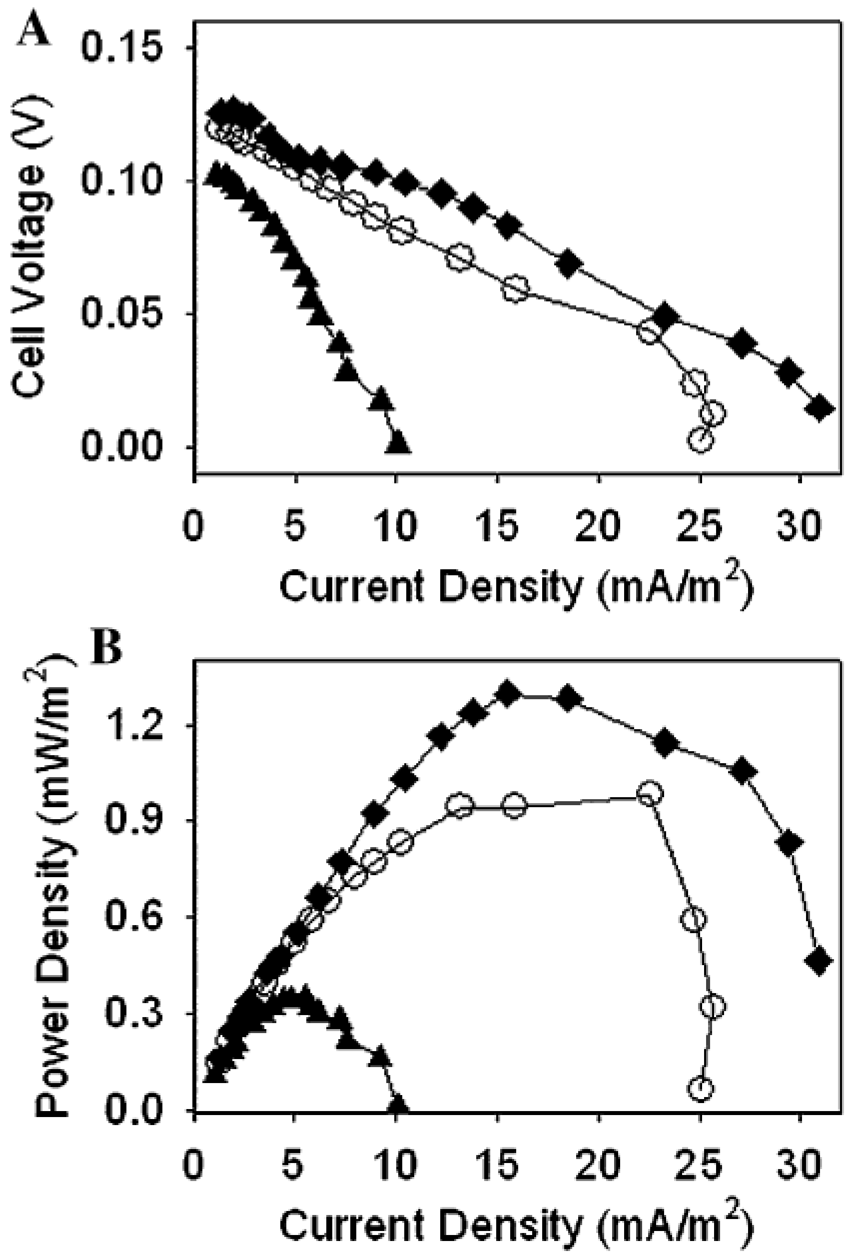


Photosynthetic Biofilm

Increasing light boosts electrogenic response (*polyaniline coating boosts voltage*)



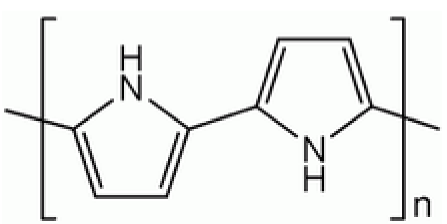
Zou, et al. 2010, Bioelectrochemistry



Conductive Polymer coated electrodes allow Greater Power

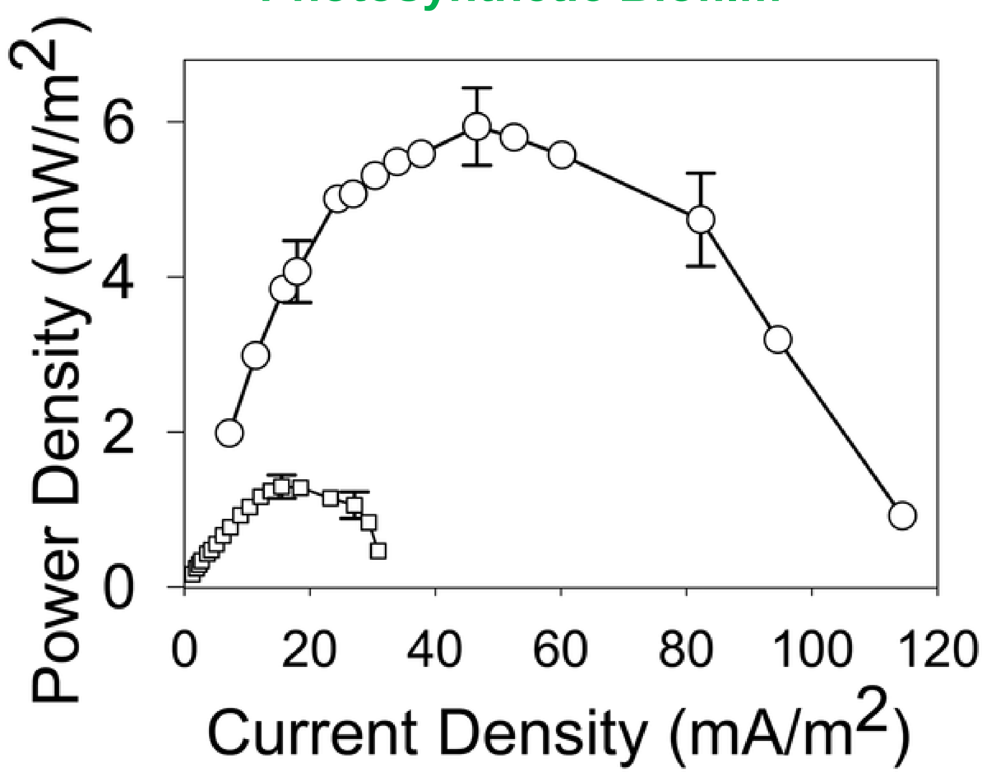
-Carbon Paint Base-

▲ Untreated
◆ Poly Pyrrole
○ Poly Aniline

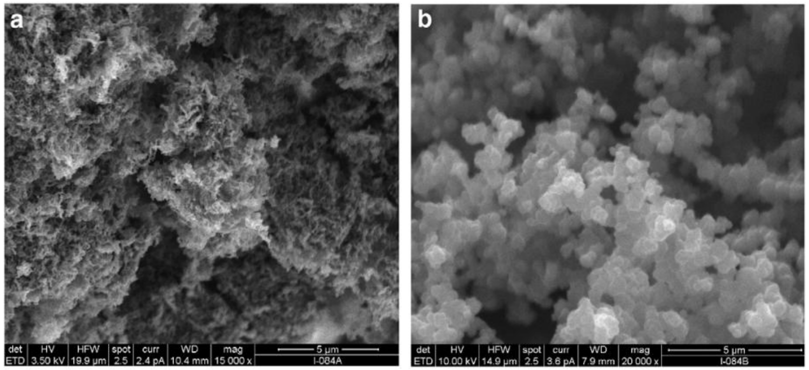


Poly Pyrrole **<u>nanostructure</u>** also affects –**Power-**

Photosynthetic Biofilm



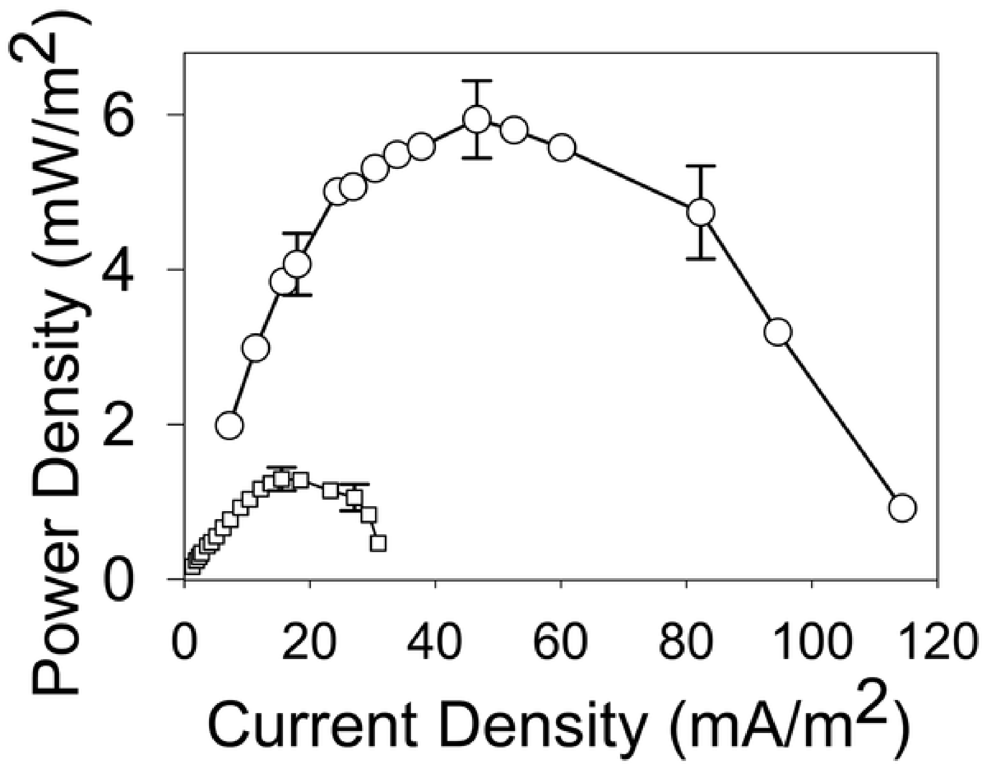
○ Fibrular Poly P
□ Poly P



Fibrular Granular

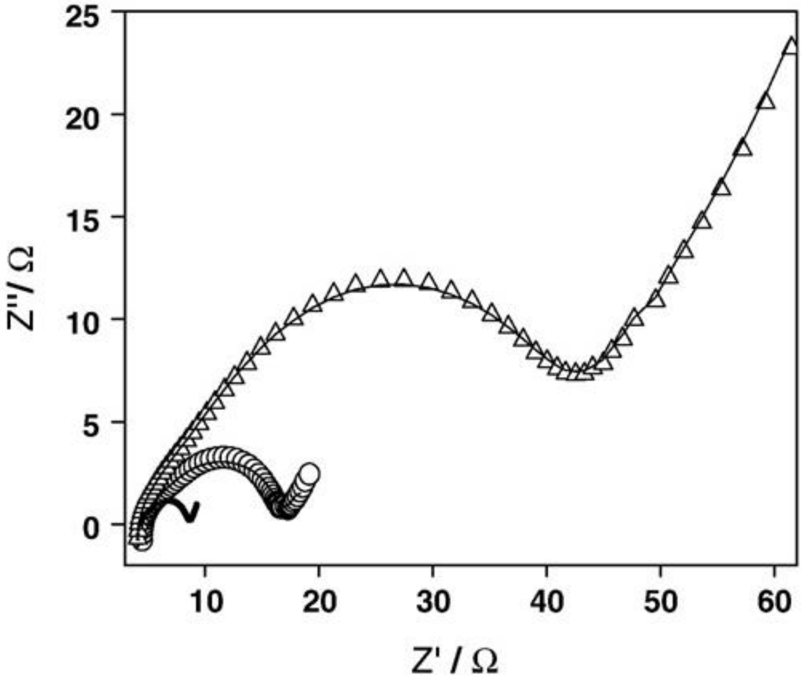
Zou, et al. 2010, Bioelectrochemistry

Likely through Decreasing Resistance



Photosynthetic Biofilm

EIS Analysis



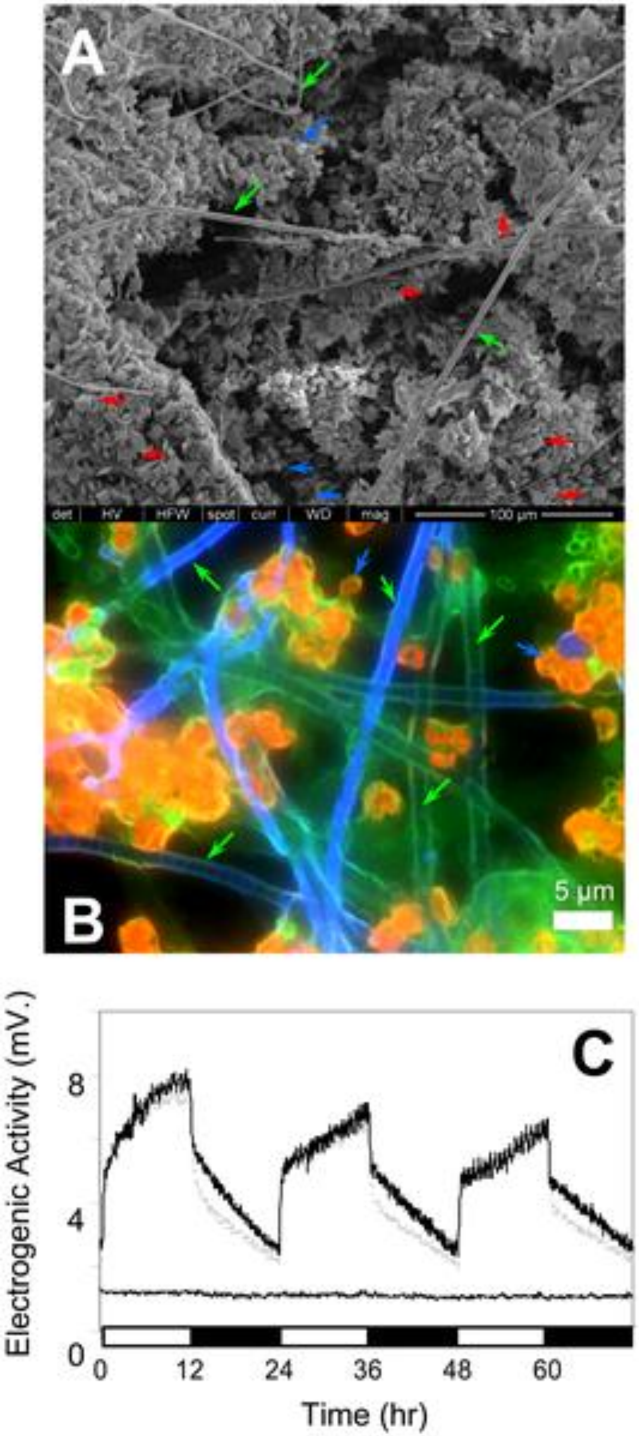
△ Untreated
○ Poly Pyrrole, granular
- Poly Pyrrole, fibrular

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 potetiostat

Species Composition of Photosynthetic biofilm

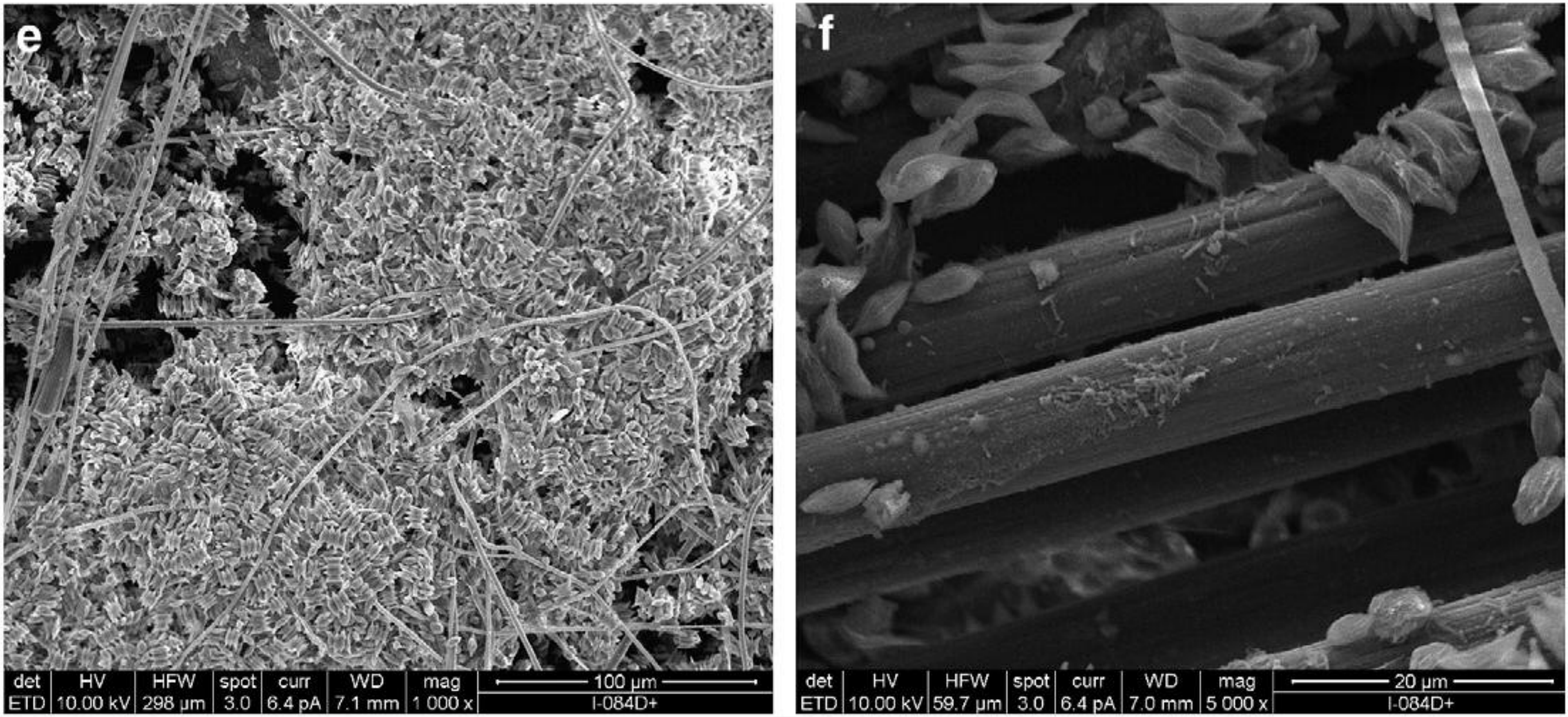


Gene	Closest Phylotype[1]	Identity, %
16S	*Pseudanabaena*	96%
16S	*Phormidium*	95%
16S	*Leptolyngbya*	95%
16S	*Prosthecobacter*	95%
16S	*Sediminibacterium*	93%
16S	*Methylococcus*	83%
23S	*Scenedesmus*	99%
23S	*Pseudanabaena**	92%
23S	*Cyanothece*	91%
23S	*Leptolyngbya**	88%

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

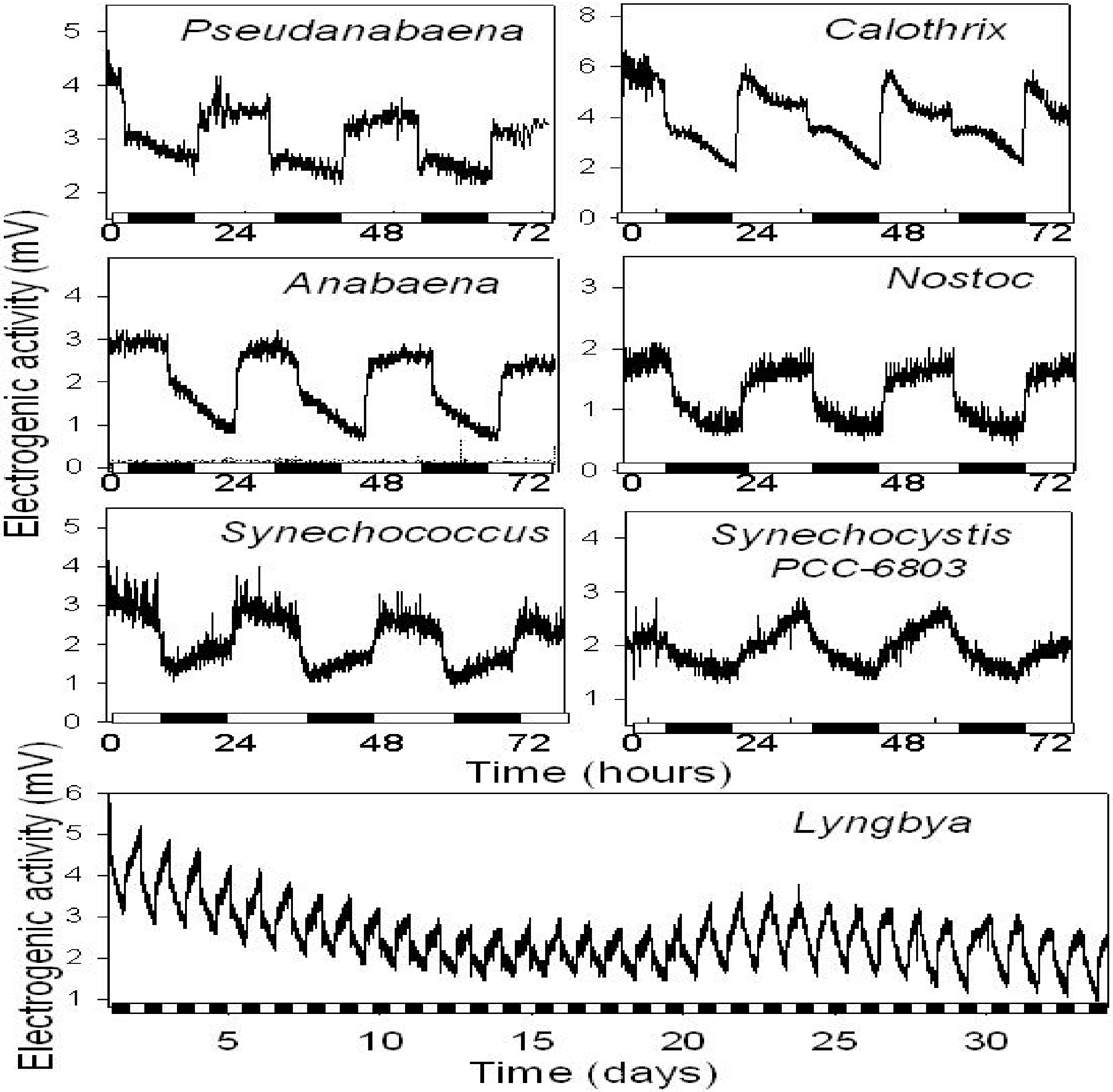
Pisciotta et al., 2010, PLoS One

Scenedesmus microalgae growing on PMFC carbon nanofiber electrodes



Zou et al., 2009

Electrogenic fingerprints.



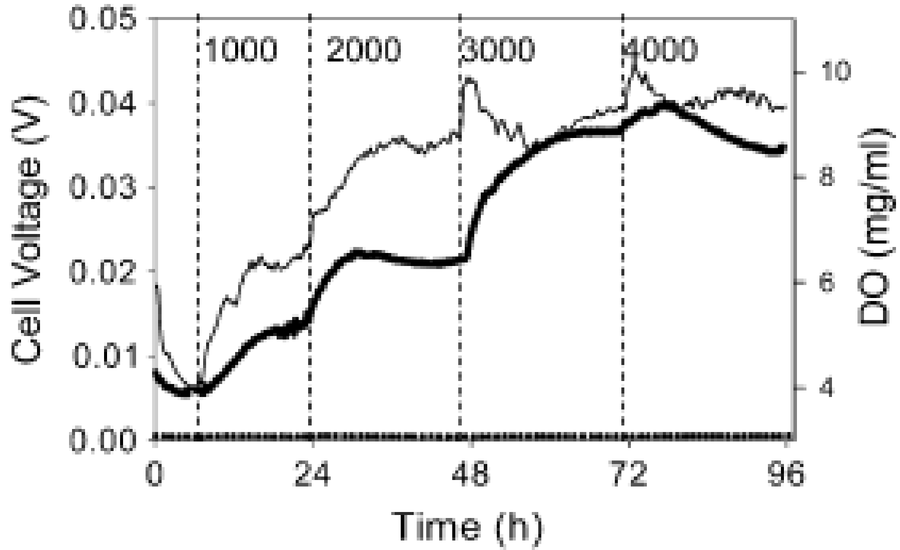
Pisciotta et. al., 2011. AMB

What is the Biological Basis?

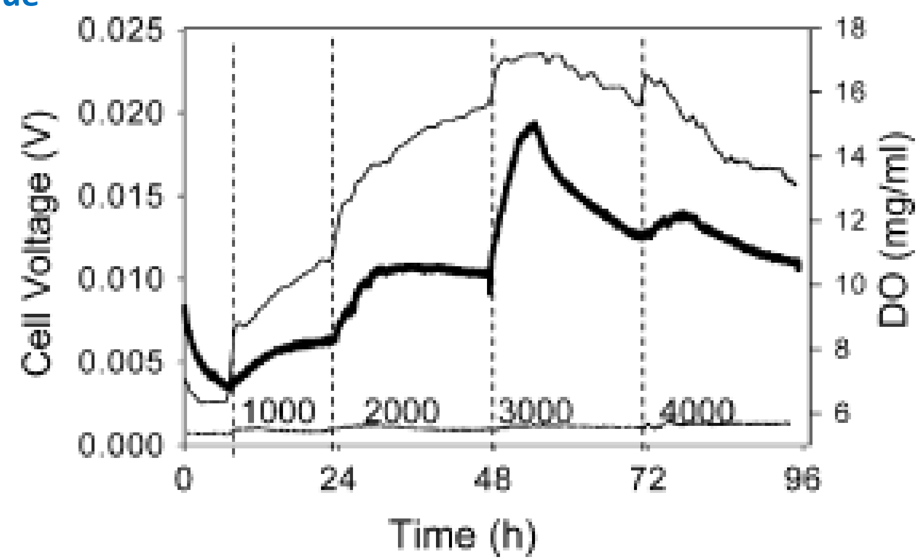
Electrogenic activity peaks under strong light,

Suggesting possible function: photo-protection

Red



Blue

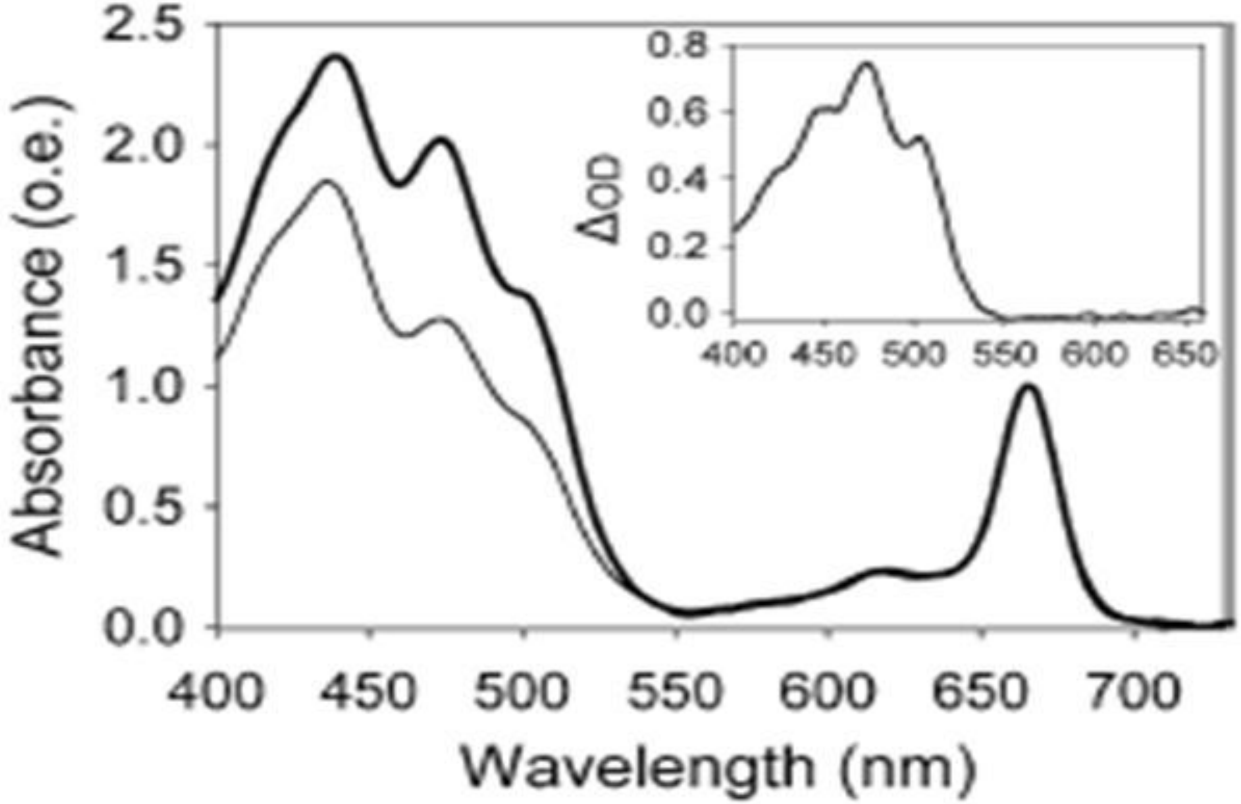


DO Thin line
E.A. Thick line

Can metabolite production be influenced by Bioelectrochemical Interfacing?

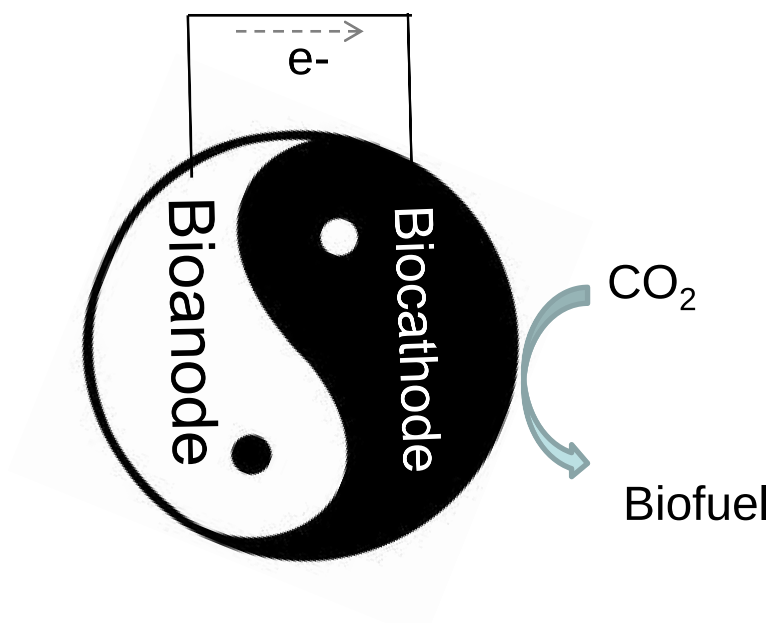
Metabolomic HPLC analysis

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



Pisciotta et. al., 2011. AMB

DARK
Electrotrophic BES.



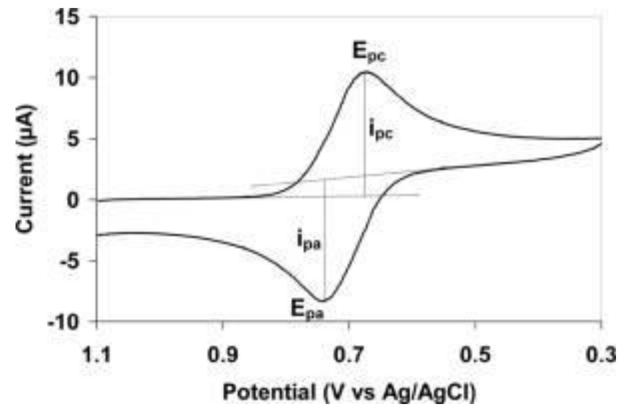
Electrochemical Methods to ID effective e- uptake from Biocathodes

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.*

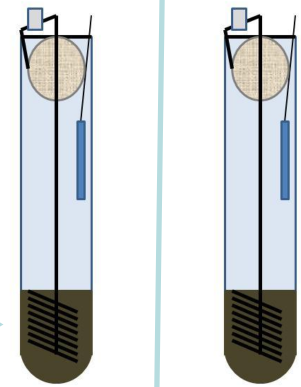
the "electrotrophs"

MFC establishment conditions

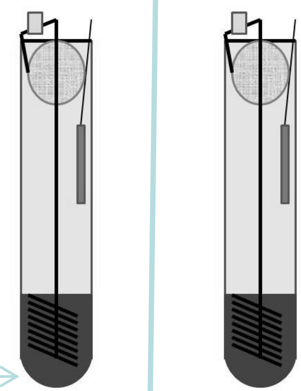
Sources Tested:

Unpoised | -200 mV*

- **Chesapeake Bay Sediment**

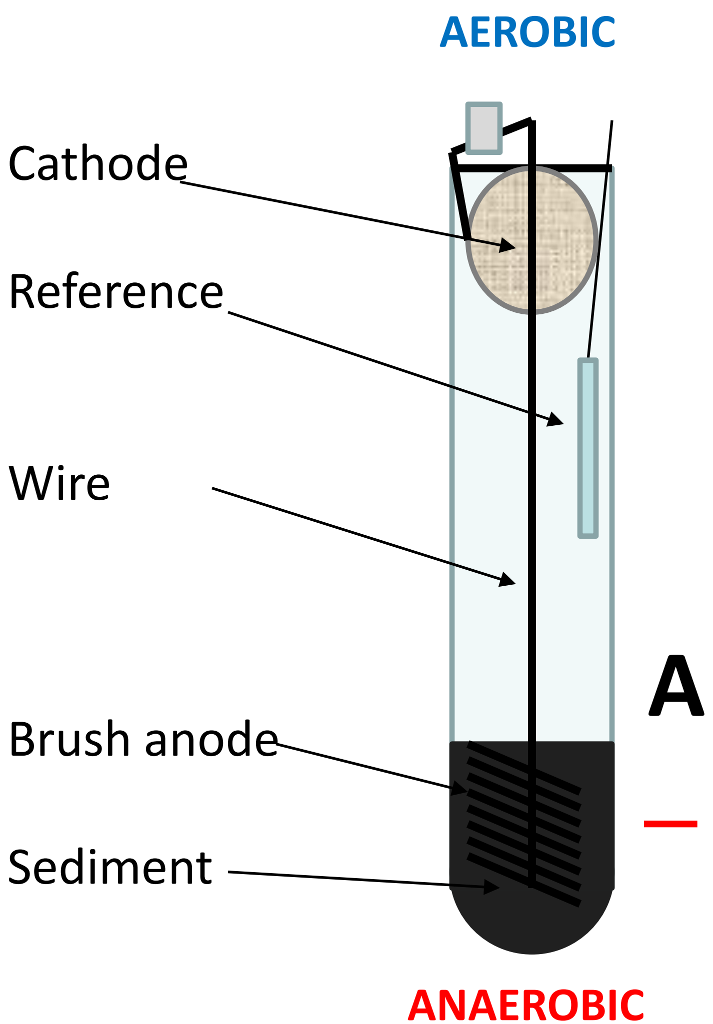


- **Baltimore Harbor Sediment**



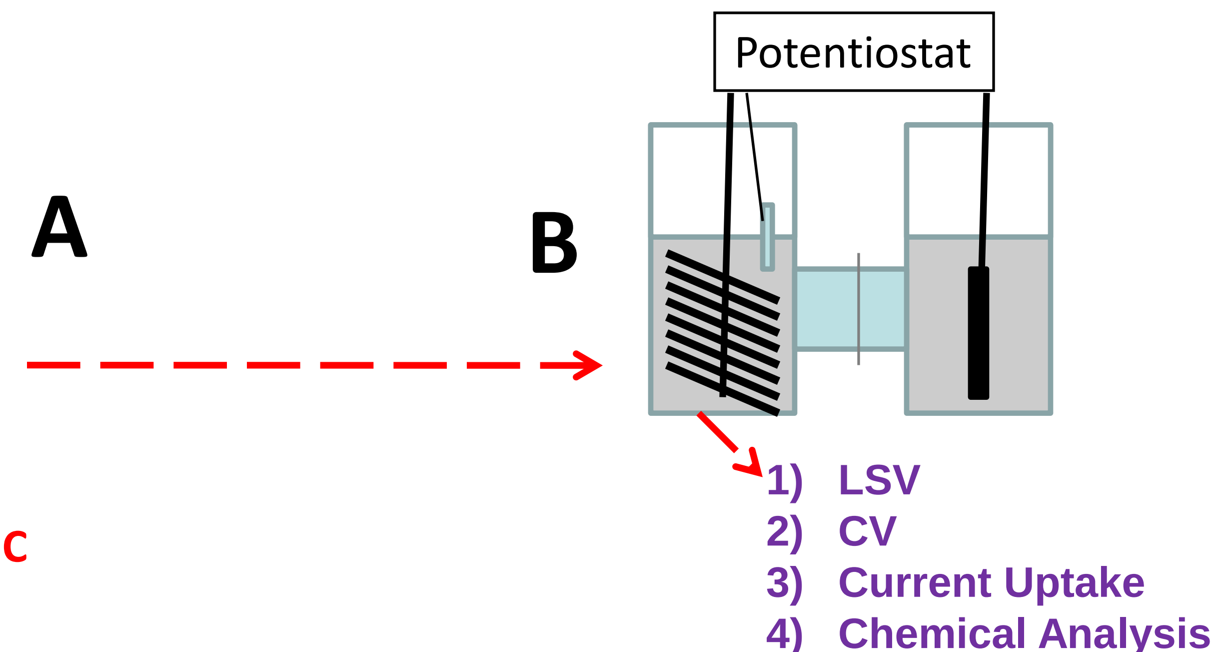
* -200 mV vs Ag/AgCl reference electrode (aka “Set”)

Sediment MFC anode to MEC cathode by *Inversion Method*.



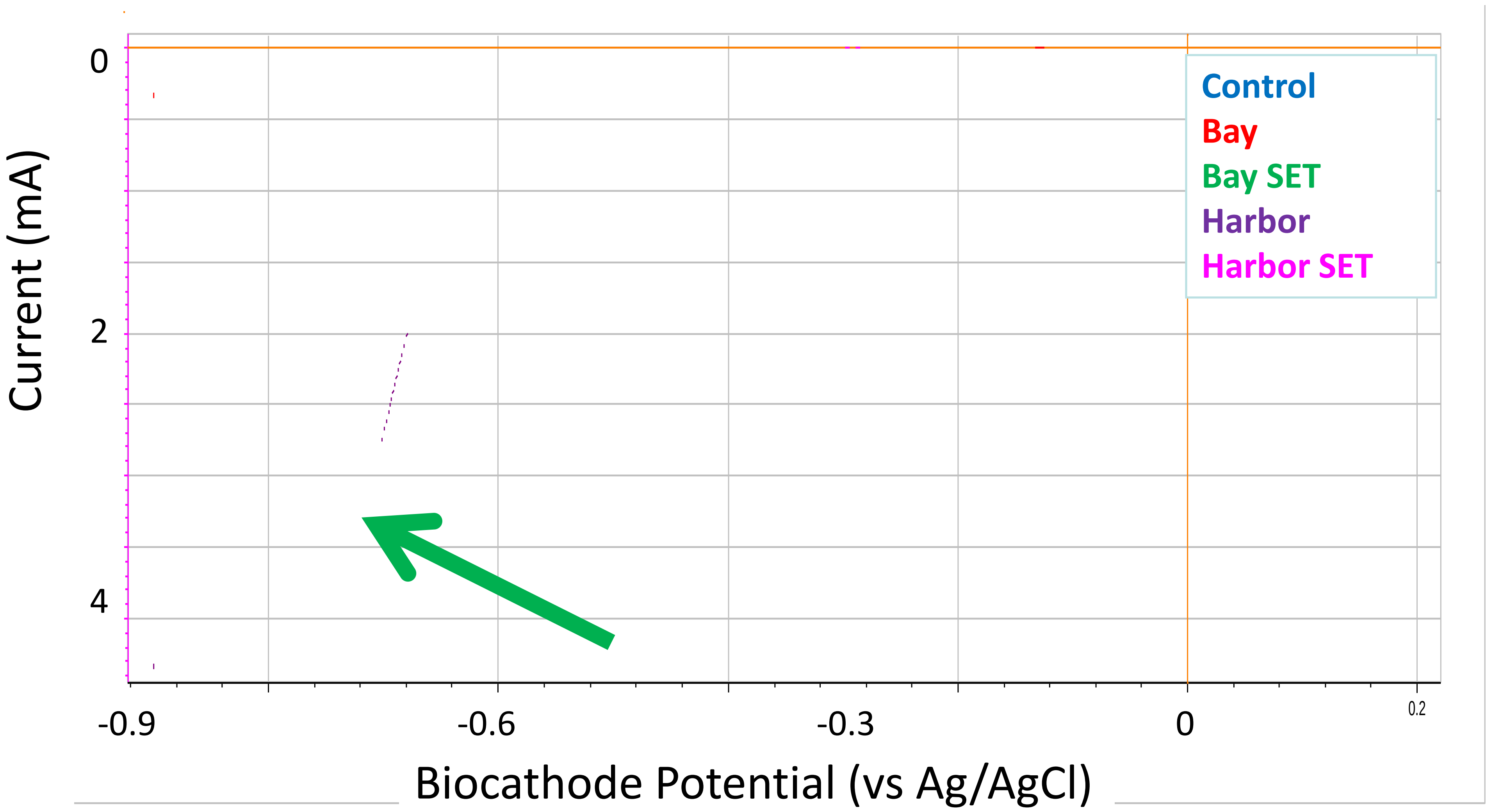
A) MFC anodes in anaerobic sediment **establish electrogenic biofilm.**

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

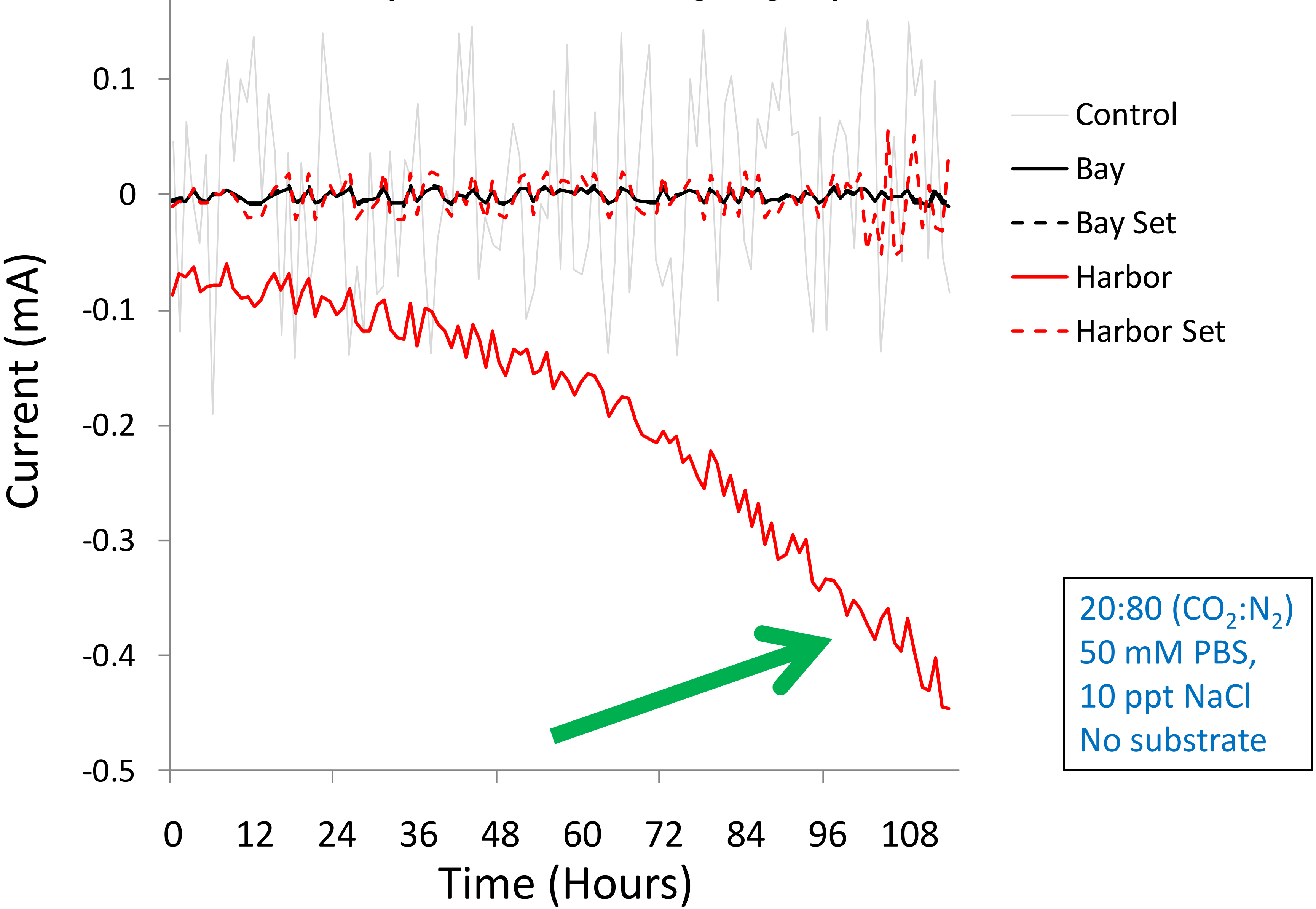


(Pisciotta. et al., 2012. AEM)

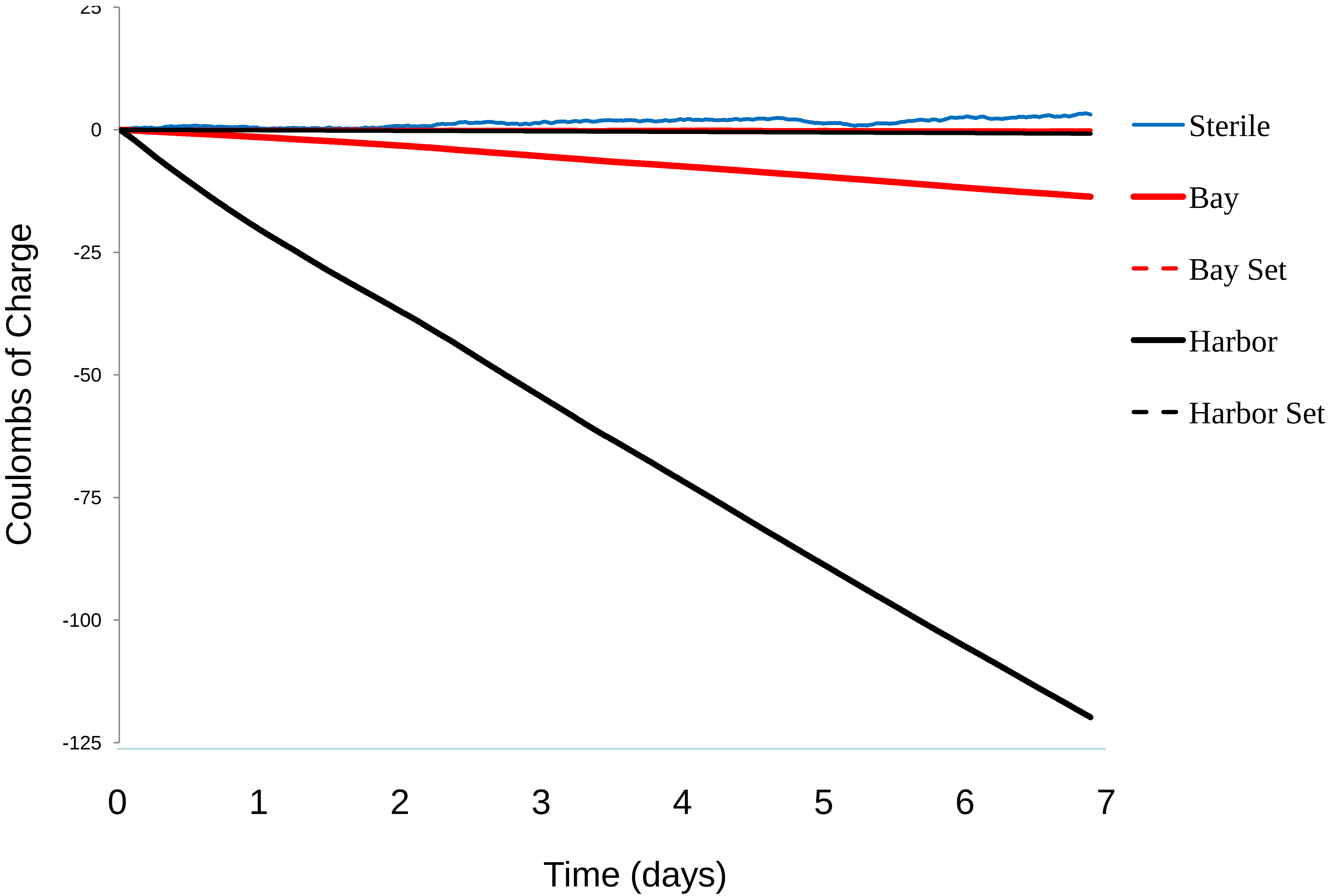
Linear Sweep Voltammetry (LSV) indicates highest *short term* electron uptake by harbor biocathode.



Chronoamperometery
Development of negative current
(-650 mV vs Ag/AgCl)

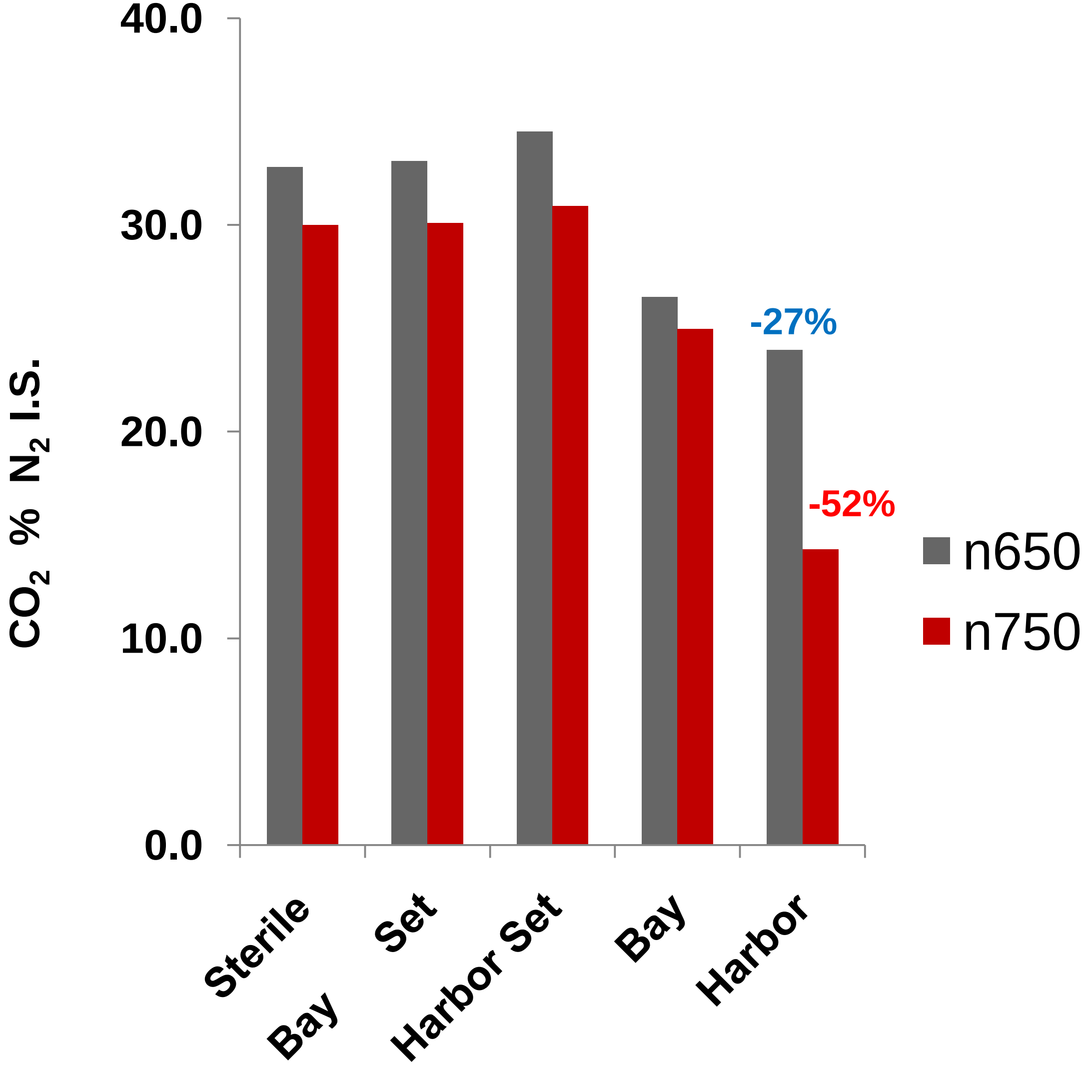


Sustained current uptake *Vs. sterile control*.



Metabolomic GC Analysis

CO_2 depletion from cathode chamber headspace after 1 week at **-650 mV** or **-750 mV**



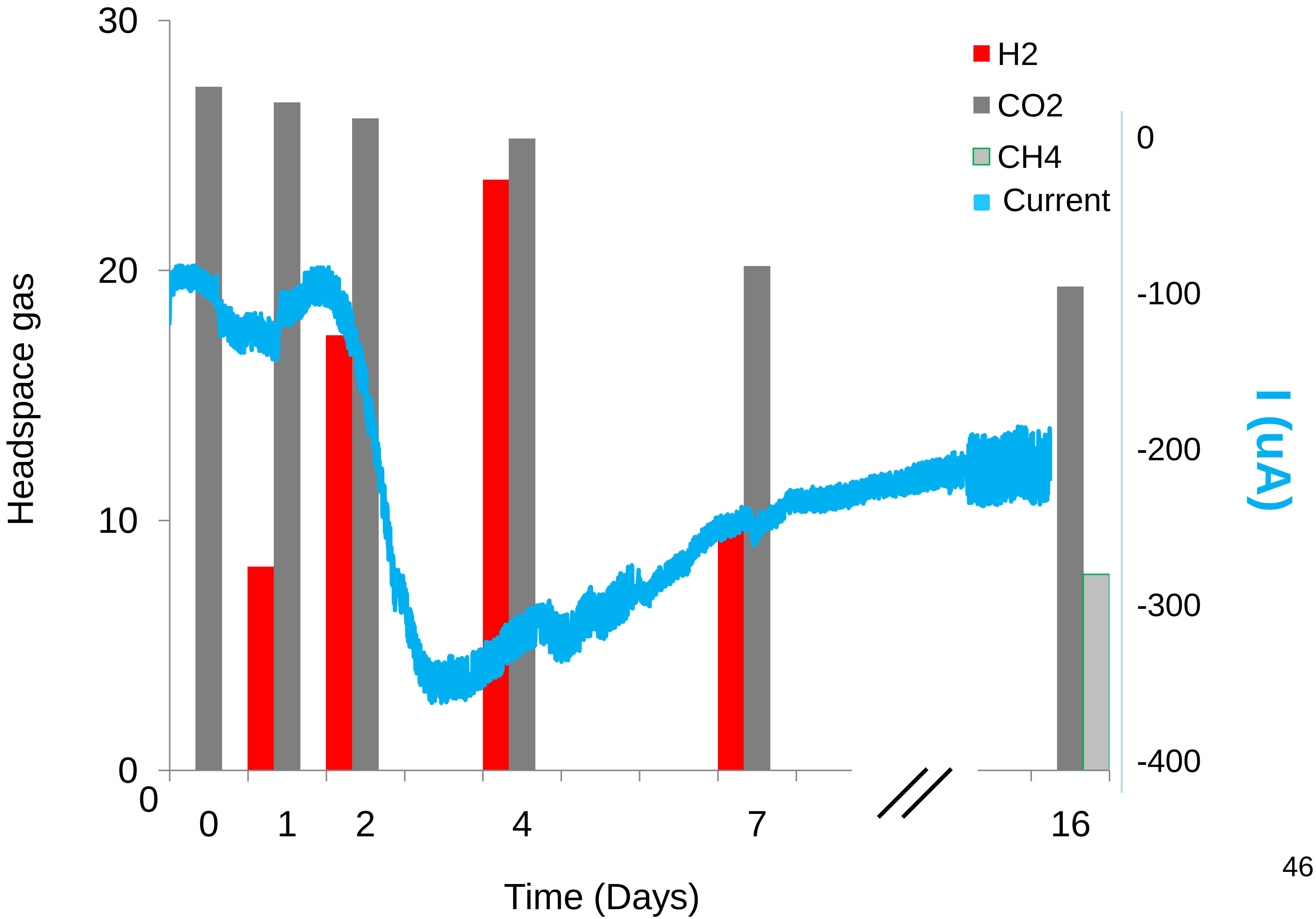
Week 1, -550 mV
(no changes detected)

Week 2, -650 mV

Week 3, -750 mV

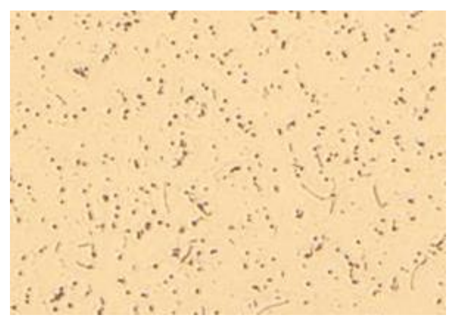
All Reactors
Degassed w/ 20:80 CO2/N2
Prior to each weekly trial

Biogas production and electron consumption.



Delayed Growth was Apparent

-Is direct perpetuation possible?



Mass Spectrometric Analysis of Cell Metabolites

1) Targeted Analysis

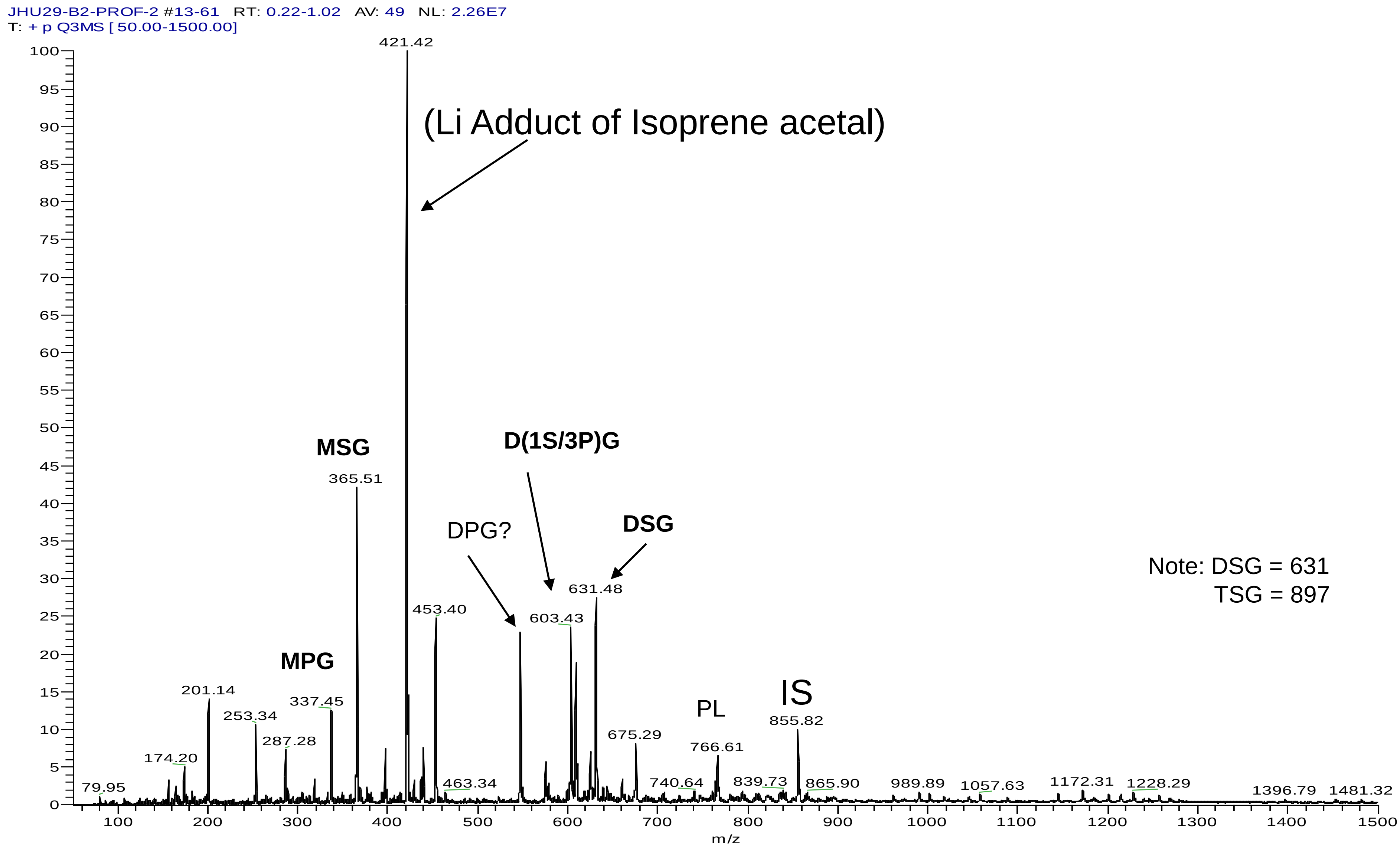
2) Metabolite Profiling

3) Global Metabolomic Analysis

Microbial Metabolomic (Lipidomic) Spectrum Profiling

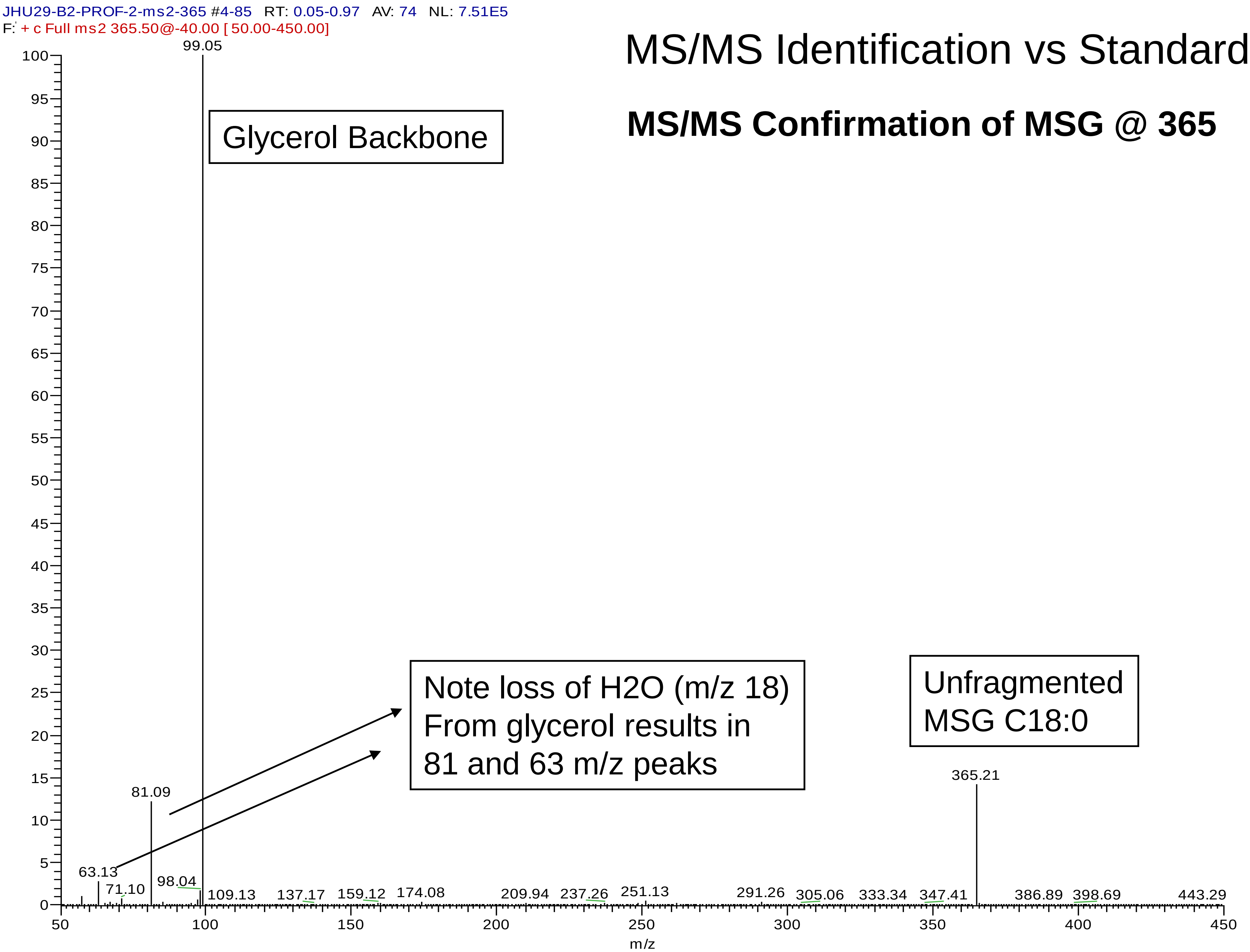
Monoacylglycerides | Diacylglycerides | Triacylglycerides

Phospholipids

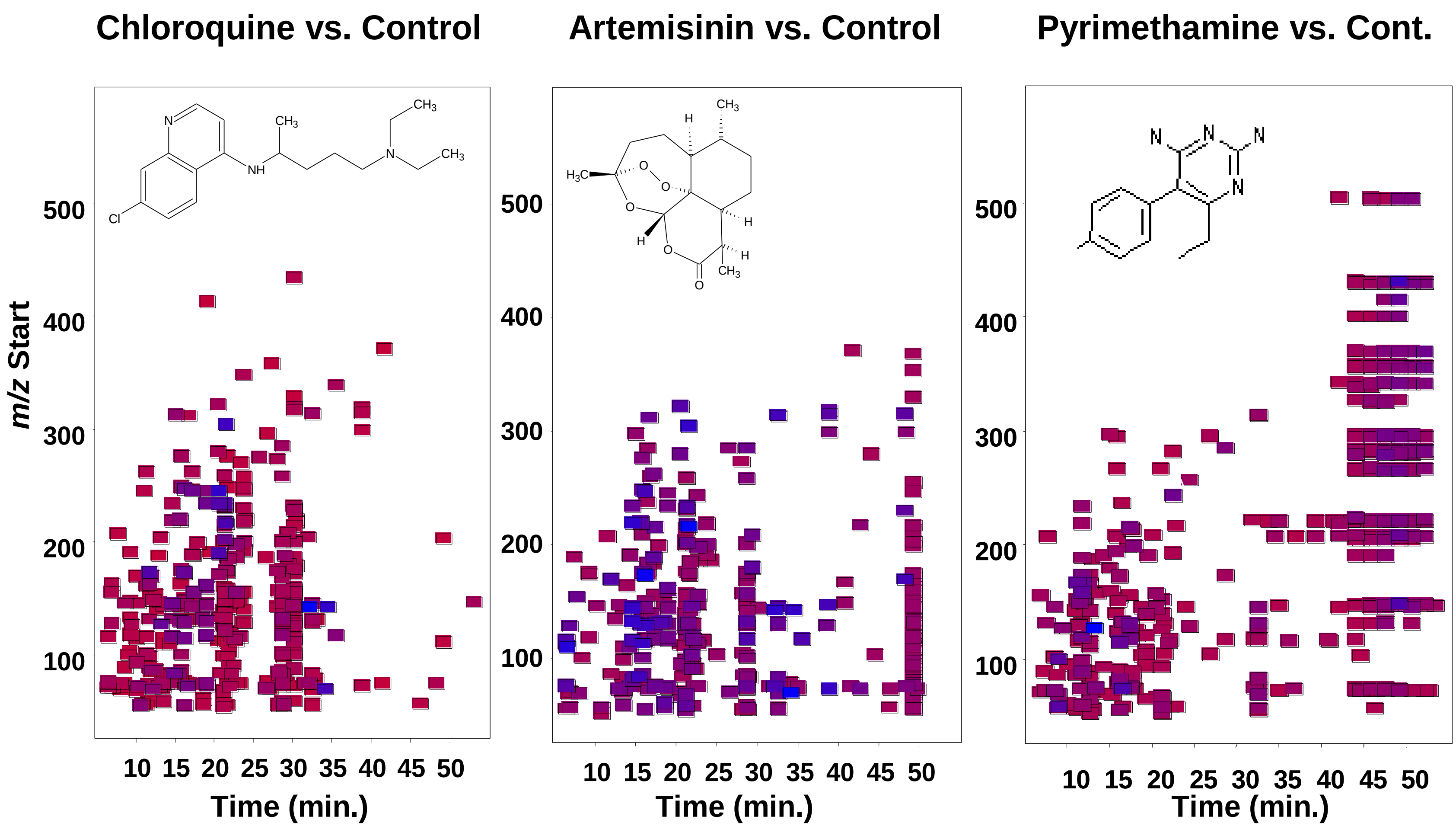


MS/MS Identification vs Standard

MS/MS Confirmation of MSG @ 365



Drugs Induce Distinct Metabolic Response Patterns



*Data From Two Independent Experiments

1 microM drug
3hr time
Isolated trophozoites

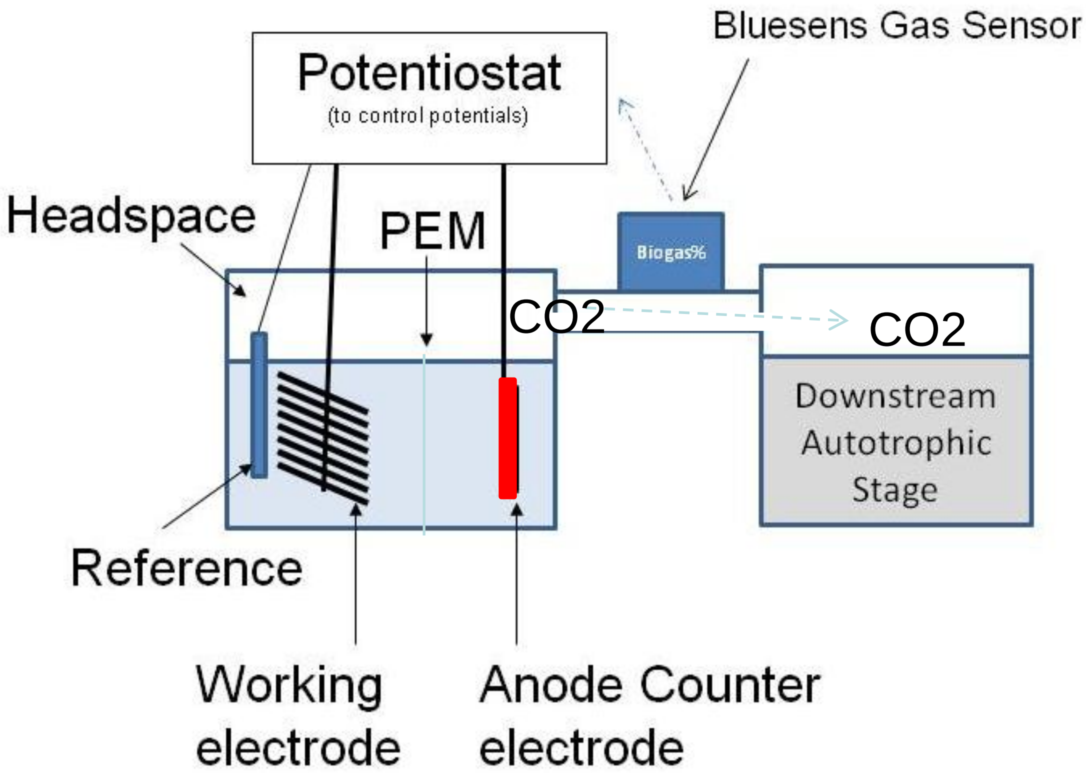
John Pisciotta,* Abhai Tripathi,* Joel Shuman,‡ Vladimir Shulaev‡ & David J. Sullivan Jr*

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**: Electochemical Biocathode analysis

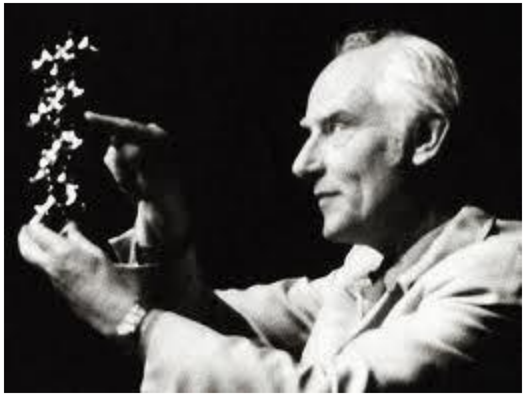
- West Chester University: Hybrid Designs (MEC-PBR)

Central Dogma of Molecular Biology, 1958

DNA **Genomics**

mRNA Transcriptomics

Protein Proteomics



Francis Crick

1962 Nobel Prize
w. Crick and Wilkins

Metabolites > *Metabolomics*

UV Stress >> Thiamine dimers >> **Tyrosinase mRNA 2x** >> Melanin 7x
Eller et al., Nature. 1994

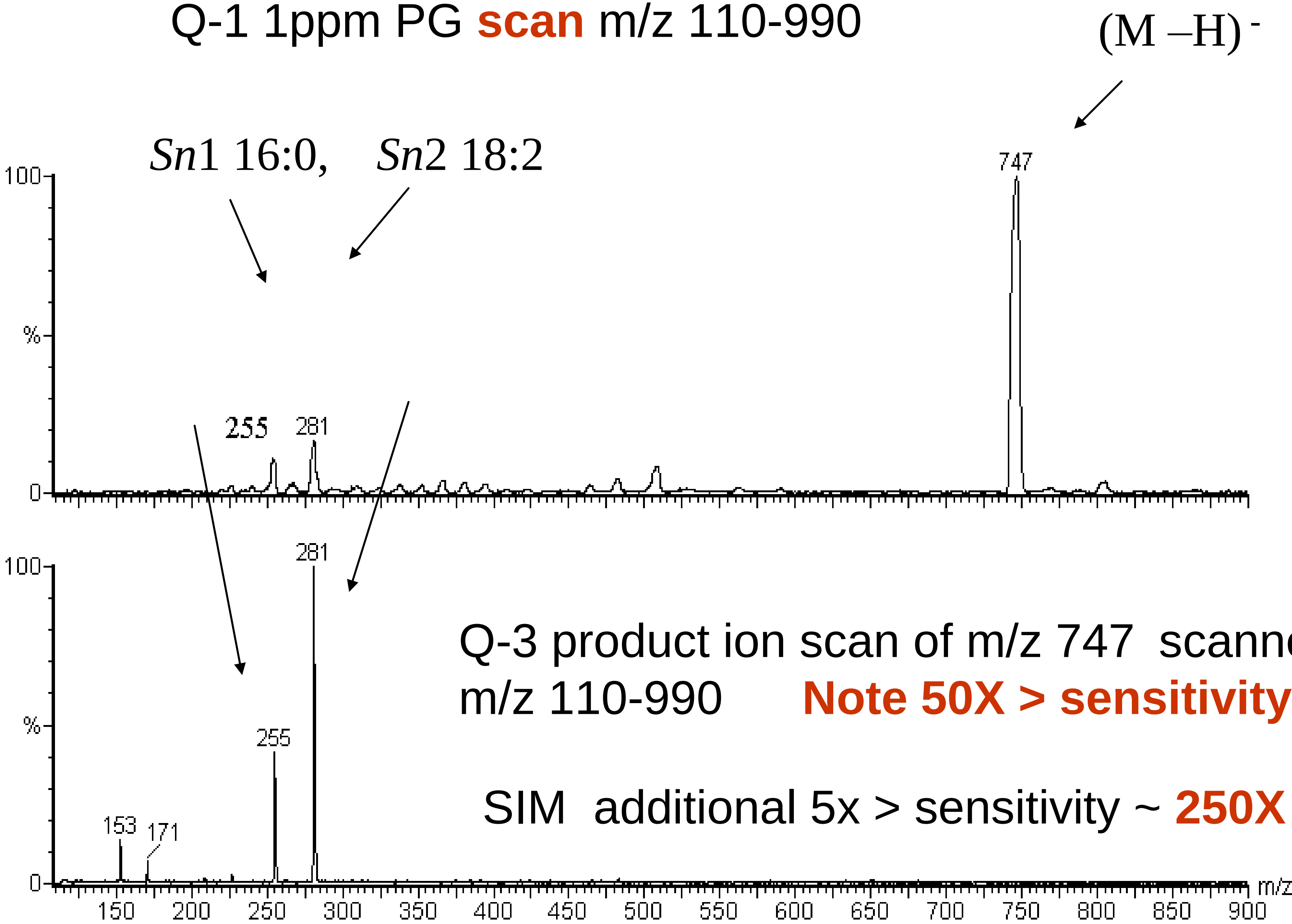
Why Metabolomics?

- 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

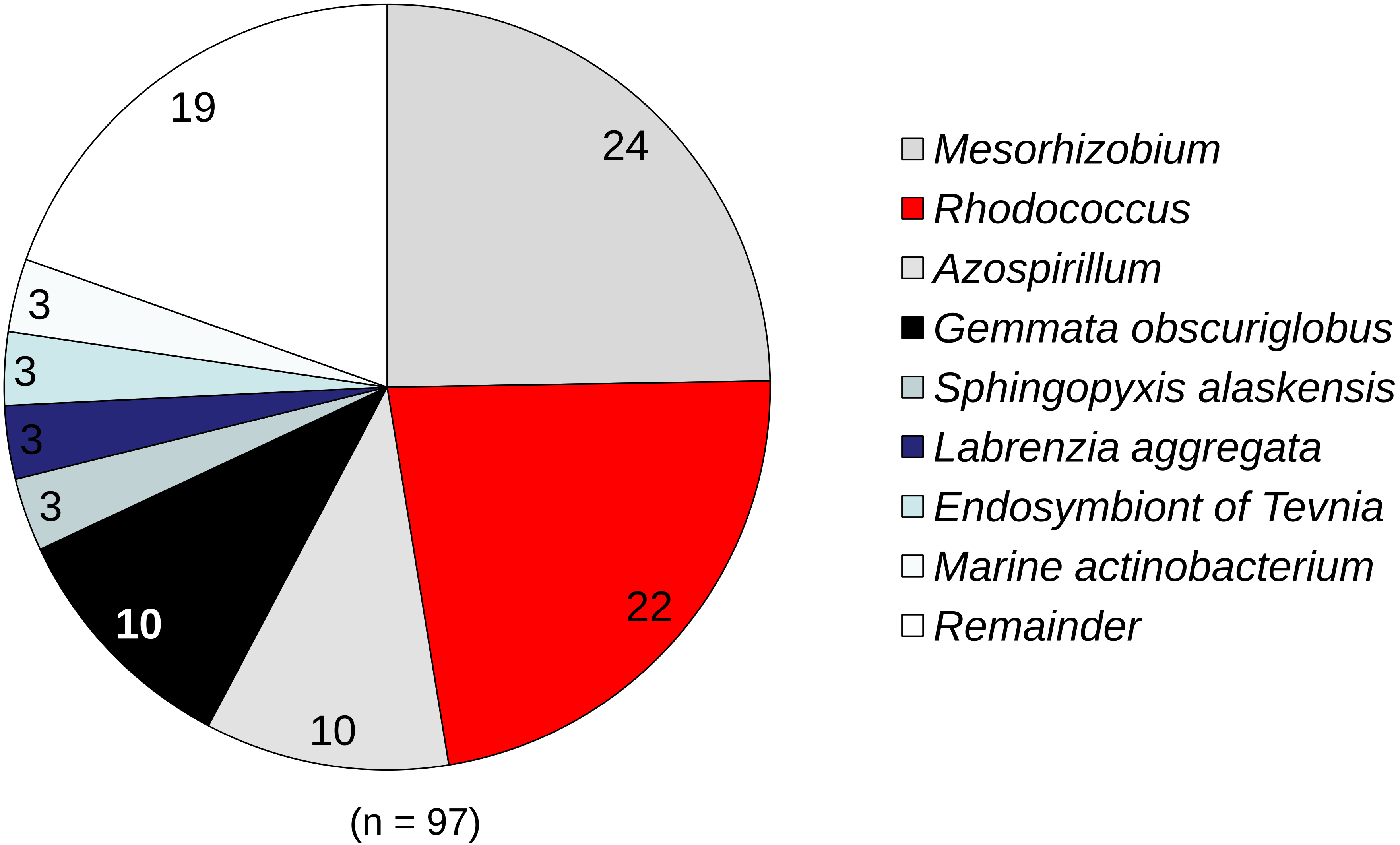
Parent product ion MS/MS of synthetic PG
Q-1 1ppm PG **scan** m/z 110-990



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



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