GCEP Technical Progress Report April 2013

Project: Synthesis of Biofuels on Bioelectrodes

Alfred M. Spormann, Professor, Departments of Chemical Engineering, and of Civil and Environmental Engineering, Stanford University

Ann A. Lefnefsky, graduate student, Departments Civil and Environmental Engineering, Stanford University
Holly Sewell, graduate student, Departments Civil and Environmental Engineering, Stanford University
Joerg Deutzmann, postdoctoral researcher, Departments Civil and Environmental Engineering, Stanford University

Abstract
The proposed research explores the opportunities and bottlenecks of a novel approach for carbon-neutral synthesis of energy-dense transportation fuel from electrical energy (electrofuels). The technology is based on microbial CO2 fixation and biofuel production at cathodes in modified fuel cells by naturally occurring and genetically modified microbes. This approach takes advantage of known microbial enzymes, pathways, and organisms, but requires the engineering of novel pathways and communities for the production of biofuels as well as the engineering of the cathodic process. Biofuels encompasses a broadly defined class of relatively reduced gaseous or liquid organic molecules, and includes methane, ethane, long chain alcohols, oils, fatty acid esters, and isoprenes. While chemically diverse, they are biosynthetically typically derived from acetyl-CoA or related small molecule intermediates, with the exception of methane. We focus on synthesis of acetate, fatty acids, and surrogate compounds enable to study uptake of cathodic electons. However, because of the choice of experimental system including the specific microorganisms, the platform can be adopted to drive the autotrophic synthesis of isoprenes, methane,and other hydrocarbons that can be easily separated from the reactor and represent energy-dense biofuels.

Background
Petroleum and other fossil hydrocarbons are primarily used as energy source for liquid (transportation) fuels as well as raw material for organic syntheses of commodity and fine chemicals. These uses represent the largest contribution to a net release of CO_2 and global warming. Development of novel and alternative energy technologies to reduce or eliminate net CO_2 release as well as to sustainably produce fuels and other organic compounds from electricity and CO_2 are urgently needed but often limited by their incompatibility with the current liquid hydrocarbon-based infrastructure (e.g. H_2 or electricity) in storage, transport, and use. Currently, solar and wind energy are the most promising sources for renewable energy, and, similarly as nuclear energy, produce electricity as the primary energy form.

In the absence of better electricity and distribution technologies (e.g. battery), new approaches are needed to connect electrical energy to the infrastructure advantages of hydrocarbon fuels. This proposal explores ideas and proposes to test the bottlenecks of a new technology linking electricity to synthesis of fuels and other useful chemicals at cathodes using microorganisms.

Results

We approached the synthesis of biofuels on biocathodes at three levels:

1. Mediator-dependent cathodic electron uptake in Shewanella oneidensis MR-1

Redox-active mediator compounds play a major role in bioelectrochemical processes as they can be continuously reduced by the cathode to shuttle electrons to redox active membrane-bound proteins of the cell.

The model organism *Shewanella oneidensis MR-1* and the electrochemical mediator methyl viologen were used to address the following two questions.

1. Can mediator-dependent cathodic electron transfer be utilized to form biofuels or biofuel precursors?
2. Can mediator-dependent cathodic electrons be used to bias pyruvate fermentation products to more reduced compounds.

To address the first question, cell suspensions of *S. oneidensis MR-1* were placed in an H-cell electrochemical reactor with electrochemically reduced methyl viologen provided as the sole electron donor and either fumarate, hydrogen ions, or bicarbonate (dissolved CO_2) provided as an electron acceptor. The formation of succinate from fumarate, and formate from bicarbonate were recorded and monitored by HPLC. The formation of hydrogen gas was observed using a Hydrogen Analyzer from Peak Laboratories. The corresponding utilization of cathodic electrons was observed using a potentiostat. (Figure 1).

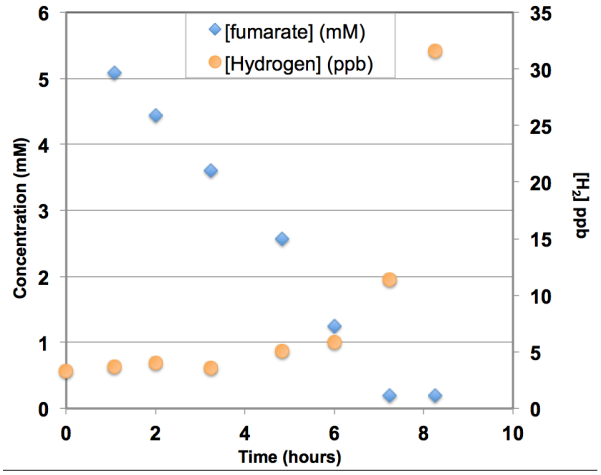

Figure 1: In the absence of fumarate (blue), hydrogen gas (orange) is produced.

The second question was addressed by providing pyruvate to cell suspensions of *S. oneidensis* in electrochemical cells with reduced methyl viologen. As can be seen by Figure 2, the proportion of lactate, the most reduced product of pyruvate fermentation increases with the addition of cathodic current and further increased by the addition of reduced methyl viologen. Additionally, the fraction of unidentified carbon is increased with the addition of electrostimilation. In unstimulated (pyruvate, but no applied cathodic potential), this undetermined fraction is most likely CO_2, however, when a cathodic potential is applied, two unknown compounds are observed using HPLC. Further work is being done to identify these unknowns using mass spec or NMR.

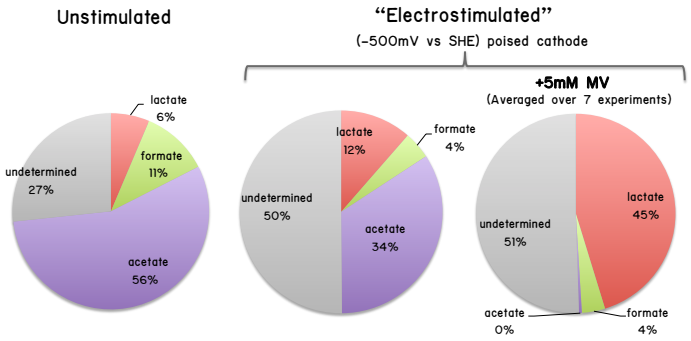

Figure 2: Carbon Balance of fermentation products
During normal pyruvate fermentation (unstimulated), the majority product is acetate. However, with the addition of a poised cathode and reduced methyl viologen, the majority product becomes lactate.

<u>2. Fe(II)-oxidizers as microbial platform for synthesis of biofuels on biocathodes</u>
One type of chemolithoautoroph being investigated as an electrochemical catalyst is iron oxidizers. These microbes respire Fe^{2+} and O_2 for energy and fix CO_2 using the Calvin Cycle.

In order to explore the use of Fe(II) oxidizers as a potential production platform, a cell suspension was inoculated into a reactor at pH 1.8 with a graphite electrode poised at +0.30 V vs. SHE. The current profile from this experiment is shown below in Figure 3.

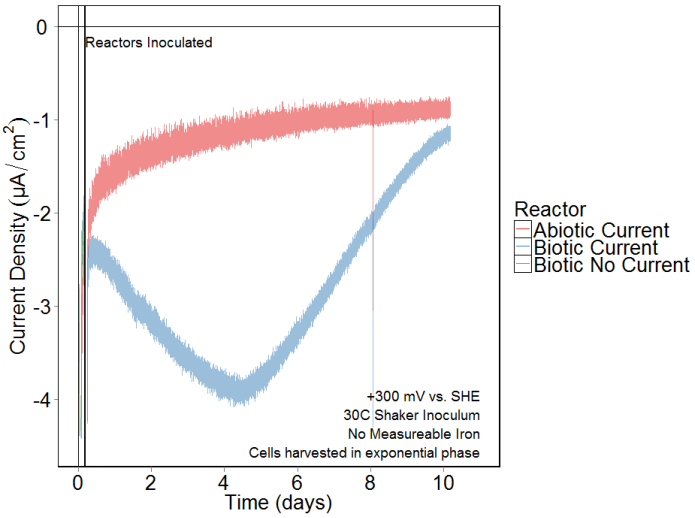

Figure 3: Current profile of Fe(II) oxidizer with less than 100 uM total Fe.

Another experiment was performed inoculating 1 mM Fe^{2+} into a reactor under the same conditions. The current profile from this experiment is shown in Figure 4.

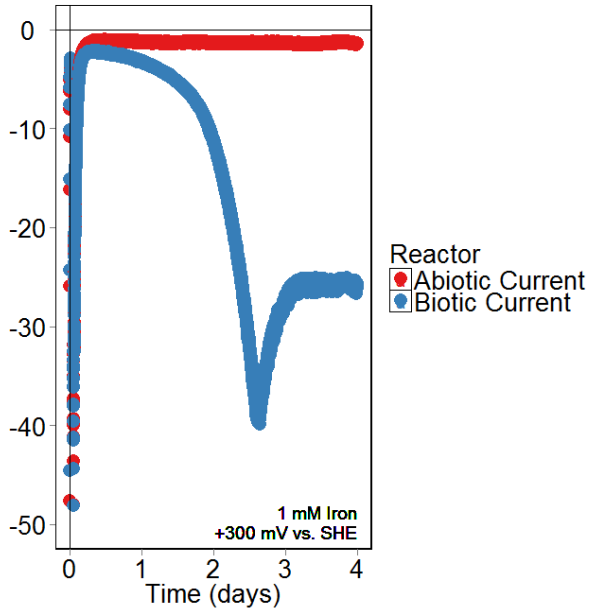

Figure 4: Current profile of Fe(II) oxidizer with 1 mM total Fe.

There was sustainable current consumption at the end of this experiment showing the cells can consume electrons from the cathode through a soluble iron shuttle.
Future work will focus on optimizing electron uptake and on genetically engineering the downstream pathway for production of reduced compounds.

3. Microbial electrosynthesis of acetate

Homoacetogenic microorganisms are capable of catalyzing the chemically challenging step of forming a multi-carbon compound (such as acetate) from the one carbon compound CO_2. Thus, CO_2 fixation is a catabolic process in these organisms, promising an effective and fast turnover of CO_2 into multi-carbon compounds like acetate with minimal losses due to low biomass formation. The homoacetogen *Sporomusa ovata* used for first experiments can grow on hydrogen + CO_2, but was also shown to grow on a graphite cathode (Nevin *et al.*, 2011).
Preliminary experiments showed that *Sporomusa ovata* is producing acetate on a graphite cathode (graphite granule bed with graphite rod as current collector) in our electrode setup. A Cathode potential of -400 to -500 mV resulted in accumulation of acetate in the culture medium, which was absent in no current controls or abiotic controls set to the same potential (Fig. 5). The mechanism of electron uptake from the cathode by the cells is not known, but hydrogen cannot be excluded as intermediate. Comparative experiments using *S. ovata* and *Acetobacterium woodii* (a homoacetogen shown not to accept electrons from electrode)(Nevin *et al.*, 2011) might help to elucidate these questions.

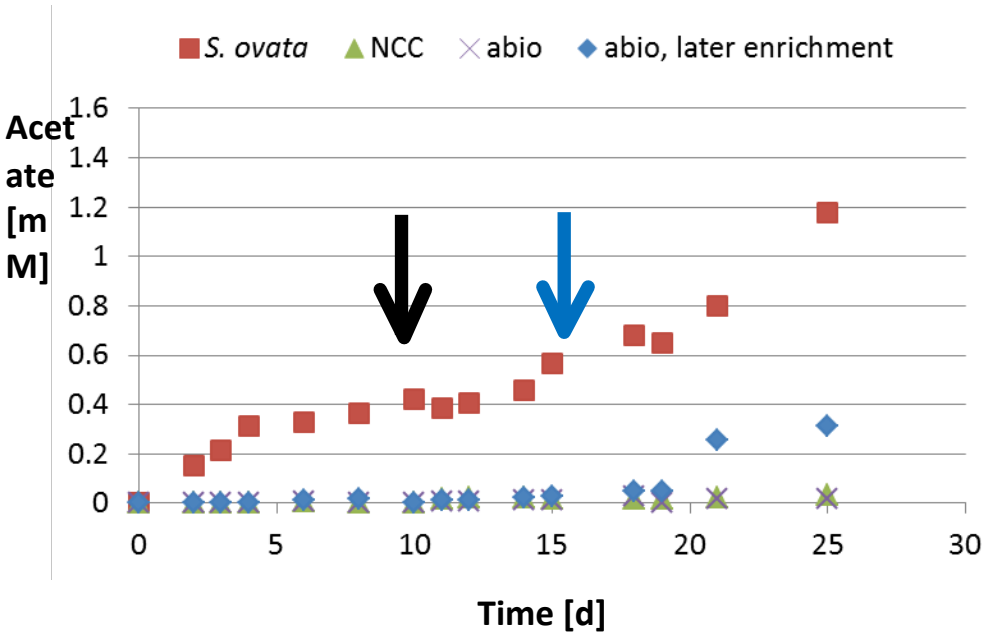

Figure 5. Acetate accumulation in the cathode chamber of an H-Type electrosynthesis cell at a cathode potential of -400 mV vs. SHE. Black arrow: decreased cathode potential to -500 mV. Blue arrow: Inoculation of enrichment culture. NCC: no current control, abio: abiotic control.

Another project started only recently is the isolation of novel organisms capable of more effective electrosynthesis. Until now, pure cultures tested for electrosynthesis were not isolated in this respect, but can accept electrons from a cathode by coincidence. Enrichments using a cathode and CO_2 as only energy and carbon source will select for the fastest and most effective electrosynthetic organisms. A first enrichment was inoculated in an H-cell type electrosynthesis cell and produced acetate after a few days of incubation (Fig. 1) .

References

[1] Gattrell, M., Gupta, N., & Co, a. (2006). A review of the aqueous electrochemical reduction of CO2 to hydrocarbons at copper. Journal of Electroanalytical Chemistry, 594(1), 1–19.

[2] Nevin, K. P., Hensley, S. A., Franks, A. E., Summers, Z. M., Ou, J., Woodard, T. L., Snoeyenbos-West, O. L. & Lovley, D. R. (2011). Electrosynthesis of Organic Compounds from Carbon Dioxide Catalyzed by a Diversity of Acetogenic Microorganisms. Applied and Environmental Microbiology.