Progress Report
GCEP Project : "Synthesis of Biofuels on Bioelectrodes"

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This report summarizes the accomplishments of the past 6 months when the project started.

Introduction and objectives

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 CO_2 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. The technology is scalable and could be employed decentralized. The potentially game-changing attributes of this approach reside in several key opportunities: (i) it preserves the advantage of current types of internal combustion engines and energy-dense transportation fuels, and its wide use in light vehicles, (ii) it makes use of the existing electrical energy distribution system (grid), and (iii) it can be used as an energy buffer and storage platform for fluctuating availability of electrical power. The fundamental contribution of this research for basic science is in the advanced understanding of microbial interactions and electron transport pathways to a cell from electrically conducting surfaces, such as metal-oxide-minerals or electrodes. This research will substantially advance our basic understanding of microbial redox processes, hydrogenases and other low potential redox enzymes, and of microbial community engineering.

Science background

Microbial life is inherently coupled to redox chemistry and typically involves transfer of electrons (or reducing equivalents) from soluble electron donors to electron acceptors that are extracted and returned to a cell's external environment. However, some microorganisms are capable of transferring cellular electrons to insoluble compounds, in particular to iron-oxide mineral surfaces, such as in hematite or goethite. These dissimilatory metal-reducing microorganisms, such as *Geobacter* or *Shewanella*, mediate such electron transfer through an ill-defined network of c-type cytochromes that eventually mediates electron transport to the insoluble mineral surface. This mechanism

evolved under geological constrains multiple times independently, and is today the key microbial feature in microbial fuel cells (MFC). In MFCs the solid anodic electrode serves as electron acceptor, whereas the cathode is oxidized typically by molecular oxygen. In this way, MFCs are being used successfully to convert inexpensive organic waste or biomass into electricity.

However, very recently a much underappreciated and understudied microbial reaction at the cathode is gaining significant interest for production of electrofuels. Rather than transferring electrons to high potential electrodes from low potential organic matter, microbes can also access and uptake low potential electrons directly or indirectly from a cathode. Those electrons are used to drive catabolic processes as electron donor. For example, solid phase microbial iron oxidation with nitrate has been observed in anaerobic systems. Strycharz et al reported that cathodic electrodes served as direct electron donors for microbially catalyzed reductive dehalogenation, enabling *Geobacter sulfureducens* to grow with fumarate as electron acceptor on cathodic electrons. More recently, Logan and coworkers (2009) reported methanogenesis at a low potential cathode, where most likely cathodic electrons were used directly by *Methanobacterium palustre* without cathodic H_2 as intermediate. These data collective provide strong evidence that cathodic, low potential electrons can be used by several microorganisms, and that the resulting catabolic reactions can support growth of the microbes and an associated microbial ecosystem. As cathodic electrons support autotrophic growth, this implies that biofuels could be synthesized from CO_2 and electricity electrons by metabolic engineering novel autotrophic pathways for biofuel synthesis in addition to, or rather than, cell mass synthesis. Such CO_2-based biofuels can serve a very critical function in a new energy economy: As electrical energy can currently not be stored well in light vehicles, conversion of electricity into biofuels to be used as transportation fuel can be employed in a decentralized system for local production of transportation fuel. Such scenario would take maximal advantage of the current (and expensive to change) fuel and car infrastructure, and bring important innovation in liquid transportation fuel technology enabling to convert solar wind and nuclear electric energy carbon-neutral into useful transportation fuel.

Results

This initial period of the project consisted of purchasing the equipment and developing the laboratory infrastructure necessary to conduct the proposed experiment. Progress was made on the following specific aspects of the project:

1. Culturing of potential electrogenic microorganisms

Currently, several microorganisms are cultured and maintained for the use in the bio-electrochemical reactor. They include homoacetogens such as *Sporomusa sphaeroides, Sporomusa ovata, Clostridium ljungdahlii* and *Moorella thermoaceticum,* as well as other promising candidate organisms. All culturing was done under strict anaerobic conditions in mineral medium and optimized for the conditions in the bio-electrochemical cells.

2. Experiments in the bio-electrochemical reactor

The mechanism of external electron transport (EET) between a microorganism and an electrode occurs either indirectly or directly. Direct EET means transfer of electrons between microorganisms and electrodes, such as via direct membrane interaction through a network of c-type cytochromes and other outer membrane proteines or electrically conductive filaments called "nanowires," which for the most part are still speculative. Indirect EET occurs by means of mediators, such as quinones or flavins, which "shuttle" electrons between the microorganism and electrode. As stated in the proposal, we will be examining the mechanisms by which microorganisms utilize cathodic electrons, both directly and indirectly.

2.1 Direct cathodic electron flow into microbial cells

So far, direct electron transfer has been observed in *Geobacter species Anaeromyxobacter dehalogenas* and *Sporomusa ovata*. A very recent publication showed that several homoacetogens are able to consume electrons from an electrode and are able to couple this to reduction of CO_2 to produce acetate. The possibility to generate multicarbon organic compounds by microbial electrosynthesis is very promising and is the main purpose of this project. A variety of microorganisms were used in the bioelectrochemical reactor to verify the previously observed results and to investigate direct electron flow into the cell. The next experiments the elucidation of the actual electron transport mechanism into the cell will be investigated.

2.2 Mediator shuttled electron flow into microbial cells

Direct electron transport into bacteria requires the attachment of cells to the cathode, i.e. biofilm growth on the electrode. For using microbial electrosynthesis for large scale applications, this might pose a bottleneck as the surface area of the electrode is limited and the bulk solution can not be utilized for the process. Another way of electron transport that could be advantageous is the use of electron shuttles that can transport electrons to the cell from the cathode. Several mediators have been studied in the literature and some organisms such as *Shewanella* species are known to produce their own mediator to interact with other cells.

To test if this approach is practicable for microbial synthesis, several initial experiments were conducted to investigate mediator shuttled electron flow into microbial cells. Initial results show that strains were able to uptake electrons from reduced electron shuttles with coulombic efficiency was almost 100%. Biological controls in which the electrodes were not poised did neither show any product formation nor current consumption, confirming that the electrons were derived exclusively from the cathode. Additional controls without shuttle also showed no activity, indicating that no direct electron transfer from the electrode occurred and the presence of the electron shuttle was necessary. Follow-up experiments will concentrate on keeping the cells alive and growing in the reactor to be able to design a self-sustainable system. For these experiments, the effect of different mediator concentrations on the growth rates and yields will be determined which currently is investigated.