

## GCEP Technical Progress Report April 2012

### Project: Capturing Electrical Current via Mechanisms Used for Interspecies Electron Transfer to Produce Methane

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#### **Abstract**

The proposed microbial electromethanogenesis cell (MEMC) process will allow for the cost effective production of methane from CO<sub>2</sub> produced, for example, from gas/coal powered power plants or anaerobic digestion of bio-waste. The MEMC technology used will allow for production of CH<sub>4</sub> with high efficiency relative to the electrical input. The technology is currently at the concept stage, with the scientific feasibility proven. However, the underlying science fundamentals such as the molecular and microbiological mechanisms of electron transfer from cathodes to cells and of the stability of the cathodic microbial communities are only insufficiently understood. This lack of understanding presents the most significant bottleneck that needs to be solved before such technology can be deployed. The proposed research will advance both the science and the technology of this process, and the outcome should enable commercialization of this technology. To this end we examined the capacity of pure cultures of methanogens, in particular of *Methanothermobacter marburgensis*, for cathodic electron uptake using electron shuttles.

#### **Introduction**

Methane, in form of natural compressed gas (NCG), is used already widely as a transportation fuel, and the gas storage and delivery technologies as well as the extensive knowledge of engineering and using NCG engines makes the proposed technology easy to integrate into the existing infrastructure. Development of novel and alternative energy technologies to reduce or eliminate net CO<sub>2</sub> release as generated during use of conventional petroleum hydrocarbons are urgently needed but often limited by their incompatibility with the current liquid hydrocarbon-based infrastructure in terms of 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 technologies (e.g. battery), new approaches are needed to convert electrical energy into an energy form that is taking advantage of the infrastructure of hydrocarbon fuels. This project investigates the science

basis for a novel, deployable technology

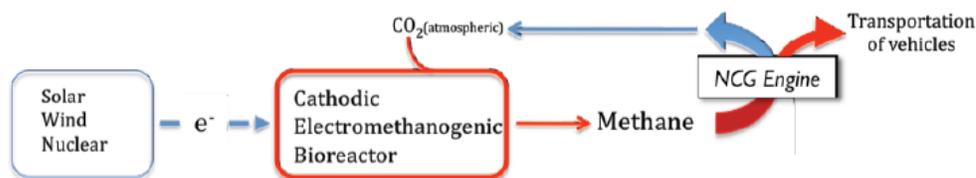


Figure 1 Basic impact of proposed technology innovation on change in energy economy.

for efficient conversion of electricity plus CO<sub>2</sub> into methane as the most simple transportation fuel in a process that is carbon dioxide-neutral (Figure 1). Although the proof or principle for this technology was recently provided by Logan and his group, this proposal is a high-risk-high-reward project, because the underlying microbiology and molecular mechanisms as well as their complexity are unknown.

## Background

About 1 billion tons of methane are annually formed by a unique group of microorganisms collectively called ‘methanogens’ in anoxic environments. Methanogens are archaea with a highly niche specialized energy metabolism of methane formation from chemically simple C1 and C2 compounds or H<sub>2</sub> plus CO<sub>2</sub>. Their metabolism is strictly anaerobic, and traces of molecular oxygen can irreversibly inhibit methanogenesis under reducing conditions. The pathways of methanogenesis, i.e., the reduction of CO<sub>2</sub> to CH<sub>4</sub> or the disproportionation of acetate, has been studied in great physiological and biochemical detail, confirmed by genetic analyzes, and is mediated by hydrogenotrophic or acetoclastic methanogens, respectively. Uptake of H<sub>2</sub> by methanogens is mediated by a class of membrane-associated Ni-Fe and Fe-hydrogenases. In natural environments this molecular hydrogen is formed from decomposing organic matter as mediated by primary and secondary fermenting bacteria that use H<sup>+</sup> as electron acceptor. Associated with this process of H<sub>2</sub> formation is also the formation of the C1 and C2 substrates of methanogens. Thus, interspecies electron transfer in form of H<sub>2</sub> or reduced C1 or C2 compounds is one known mechanism of metabolic interactions by methanogens. While key aspects of the physiology, biochemistry, and ecology of methanogenic and methanotrophic microbes are well understood, the mechanistic basis of the metabolic interactions of methanogens with other essential members of the microbial community as well as aerobic methanotrophic microbes is unclear. However, the key elements constituting the mechanism of direct electron transfer from a cathode to methanogenic cell may be found in the electron transfer networks operating in natural methanogenic communities and may provide the key understanding for a rational engineering of MEMCs. Recent findings suggest that modes of electron transfer other than interspecies H<sub>2</sub> transfer may exist in anaerobic communities associated with methane metabolism; these modes may be critical for cathodic electron uptake in electromethanogenesis. For example, anaerobic methane oxidation under sulfidogenic conditions involves a reverse methanogenic process of a *Methanosarcinales*- or *Methanomicrobiales*-like microbe and *Desulfosarcina/Desulfococcus*-related sulfate-reducing bacteria, where the former oxidize methane, and the latter reduce sulfate to H<sub>2</sub>S. Despite intense research, however, the form

of electron transfer from the methanogen to the sulfate-reducing bacterium, nor the chemical compound associated with electron transfer (if such exists) is unknown, suggesting the existence of other modes of electron transfer associated with methane metabolism. In another example of novel microbial interspecies/extracellular electron transfer, transfer of electron out of the cells of Fe(III)- or Mn(IV)mineral-reducing microorganisms has been postulated to be associated with electrically conducting nanowire structures, mediating a contact between a cell and a mineral for electron transfer. These examples above illustrate the existence of novel, so far ill-understood mechanisms of extracellular and interspecies electron transfer acting in strictly anoxic microbial communities, which might be pervasive in such communities but which may also form the molecular basis for electrical interaction of methanogens with cathodes, and, thus, for the process of microbial electromethanogenesis to be explored here. In the past period we examined metabolism of cathodic electrons introduced by mediators to *Methanothermobacter marburgensis*, a well studied H<sub>2</sub>-consuming methanogen.

## Results

### *Methanothermobacter marburgensis* – indirect electron transfer via mediators

As methanogens promise to be ideal biocatalysts to generate methane with cathodic electrons, *Methanothermobacter marburgensis*, a well characterized methanogen, was chosen for experiments in the bioelectrochemical reactor. However, although the cells were able to generate methane in cell suspension with hydrogen as electron donor, no methane production was observed in the reactors when hydrogen was replaced by the electrode (-700mV vs Ag/AgCl) and reduced methyl viologen (500uM). As it is known that methyl viologen can be toxic to microorganisms, we exposed *Methanothermobacter* cell suspensions to different methyl viologen concentrations in the range of 0-500uM and subsequently monitored the methane production with hydrogen as electron donor via gas chromatography. Fig. 2A shows that whereas significant amounts of methane were produced without methyl viologen, a significantly reduced methane formation rate was observed even at the lowest tested concentration of 50uM. At 100uM and above, no methane was produced anymore.

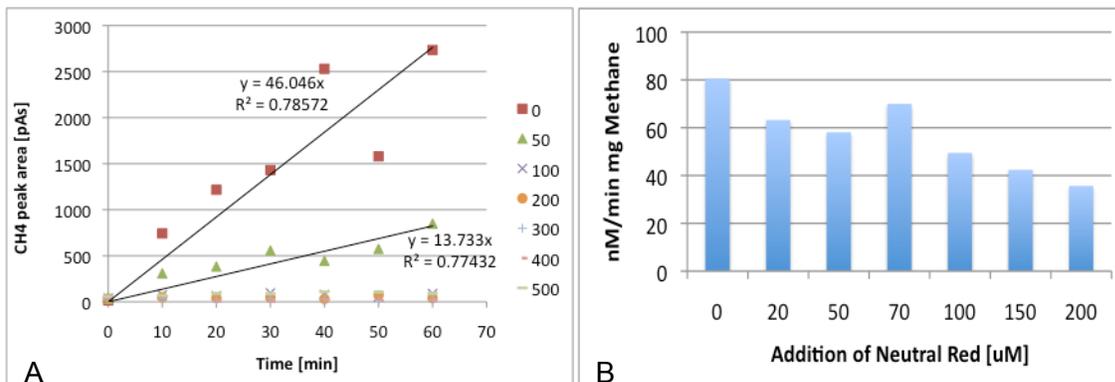


Fig. 2: Methane production in *Methanothermobacter marburgensis* cell suspensions with

hydrogen as electron donor and A) addition of 0-500 uM methyl viologen and B) addition of 0-200uM neutral red as electron shuttle compound

However, when 50uM methyl viologen were applied in the bioelectrochemical reactors, no methane production was observed. Presumably, the low concentration of MV was not enough to shuttle enough electrons to the cells to produce methane. As increasing the MV concentration was not an option, the same experiments were performed with neutral red as electron shuttle, which has a redox potential of app. -330mV. Cell suspensions of *Methanothermobacter marburgensis* were significantly less sensitive to neutral red compared to methyl viologen (Fig. 3B) although with 200uM the methane formation was also decreased by 50%.

Based on these experiments, we most likely will not be able to proceed with this methanogen using methyl viologen as electron shuttle. Experiments with neutral red are ongoing to see if this shuttle can be successfully applied in the bioelectrochemical reactors. membrane cytochromes that potentially might be important in external electron transfer (Fig. 4).

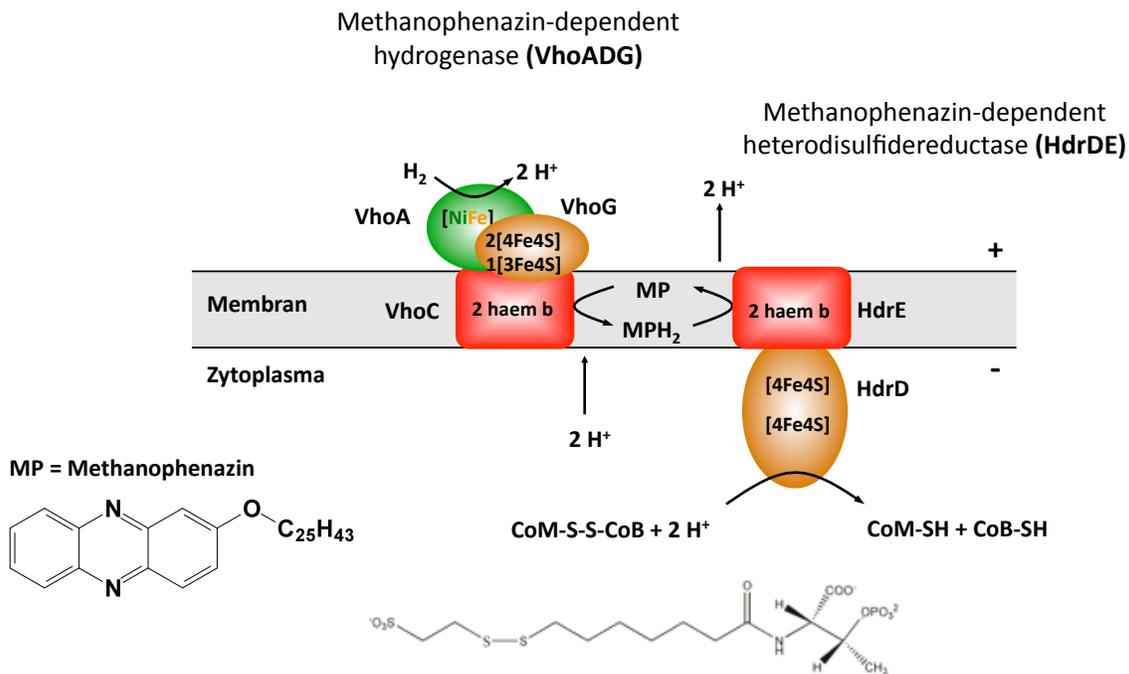


Fig. 4: Electron flow in methanogens with cytochromes

## **Progress**

Our experiments showed that *Methanothermobacter marburgensis* is not a good experimental platform for electromethanogenesis via methyl viologen as electron shuttle due to toxicity. These studies enabled us to set up a useful and rapid experimental system to test more pure cultures of methanogens with different shuttles as well as defined mixed consortia of methanogens with syntrophs.

## **Future Plans**

In the next steps, we will investigate other methanogens that might be less sensitive to methyl viologen including *Methanosarcina barkeri*, which has the additional advantage that in contrast to *Methanothermobacter marburgensis*, this strain contains outer membrane cytochromes that potentially might be important in external electron transfer.

## **Publications and Patents**

There are no publications and patents at this point.

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