II.E.5 Hydrogen from Water in a Novel Recombinant Oxygen-Tolerant Cyanobacteria System

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Objective

- Develop an O_2-tolerant cyanobacterial system for sustained and continuous light-driven H_2 production from water.

Technical Barriers

This project addresses the following technical barrier from the Hydrogen Production section (3.1.4.2) of the Hydrogen, Fuel Cells and Infrastructure Technologies Program Multi-Year Research, Development and Demonstration Plan:

(Z) Continuity of Photoproduction

Technical Targets

Cyanobacteria's hydrogenases are highly O_2-sensitive. Thus, they cannot produce H_2 continuously. This project is performing genetic-engineering studies of cyanobacteria. The outcome of these studies will lead to a much more O_2-tolerant cyanobacteria system, which may be used for continuous photo-hydrogen production. Therefore, this project will facilitate efficient H_2 production from renewable sources and will address the DOE 2010 target for photolytic hydrogen production from water.

Approach

Cyanobacteria have the ability to split water photolytically into O_2 and H_2, but their hydrogenases are highly O_2-sensitive. In contrast, a few anoxygenic photosynthetic bacteria have O_2-tolerant H_2-evolving hydrogenases, but they can not use water as the electron donor. We are using the following two approaches to address this problem.

1. Transfer to and express known O_2-tolerant hydrogenases from anoxygenic photosynthetic bacteria in cyanobacteria (Venter Institute and NREL):
   - Determine if the O_2-tolerant hydrogenase can link to the host electronic carrier, ferredoxin
   - Further characterize the O_2-tolerance property of these hydrogenases
   - Transfer and express a known O_2-tolerant hydrogenase from *Thiocapsa roseopersicina* in cyanobacteria (*Synechocystis* and *Synechococcus*)
   - Transfer and express a known O_2-tolerant hydrogenase from *Rubrivivax gelatinosus* in cyanobacteria
2. Identify novel O_2-tolerant hydrogenases from Venter Institute's global ocean sampling project, and transfer them into cyanobacteria (Venter Institute):
   - Sample ocean waters, and sequence environmental samples using culture-independent shotgun sequencing approach
   - Construct environmental genomic databases
   - Build Hidden Markov Models (HMMs) and search putative hydrogenase sequences through the databases
   - Retrieve original DNA samples or library for cloning the genes of novel hydrogenases
   - Express the genes and screen for O_2-tolerant hydrogenase
   - Transfer the novel O_2-tolerant hydrogenase into cyanobacteria

FY 2006 Progress

This project did not receive funding in FY 2006. DOE plans to restart project funding in FY 2007.

Conclusions and Future Directions

- Heterologous expression of an active NiFe-hydrogenase likely will require the assistance of its native assembly and maturation proteins. We will begin to transfer the hydrogenase assembly genes along with its structural genes in FY 2007.

FY 2006 Publications/Presentations

- Maness presented U.S. biological H_2 research activities during the International Partnership of H_2 Economy Workshop in Seville, Spain (Oct. 24-26, 2005).
- Maness gave an oral presentation of cyanobacterial research during the 15th Western Photosynthesis Conference in Pacific Grove, CA (Jan. 5-8, 2006).
- Qing Xu, Gergely Maroti, Shibu Yooseph, Yingkai Tong, Hamilton O. Smith, and J. Craig Venter. *Identification and Analysis of NiFe-hydrogenases from the Sargasso Sea Microbes.* Poster presentation at Genomes, Medicine, and the Environment Conference, Hilton Head Island, SC (Oct. 16-18, 2006).