DEVELOPMENT OF EFFICIENT AND ROBUST ALGAL H_2-PRODUCTION SYSTEMS

Part A: Creation of Designer Alga for Efficient and Robust Production of H_2

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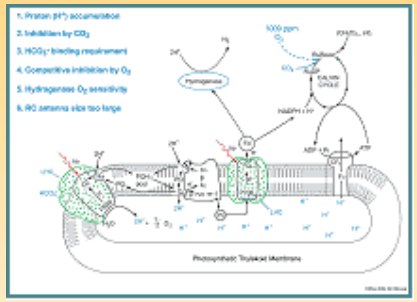
Abstract

Algal photosynthetic hydrogen (H_2) production is a potentially clean energy resource. However, there are a number of technical issues that must be addressed before algal H_2 production can become practical. In this paper, we will discuss the following six physiological problems that currently challenge researchers and investors in the field of photosynthetic H_2 production.

These problems are: *(1)* restriction of photosynthetic H_2 production by accumulation of a proton gradient, *(2)* competitive inhibition of photosynthetic H_2 production by CO_2, *(3)* requirement for bicarbonate binding at photosystem II (PSII) for efficient photosynthetic activity, *(4)* competitive inhibition by O_2, *(5)* classic O_2 sensitivity of the hydrogenase enzyme, and *(6)* light-saturation phenomenon due to large antenna size.

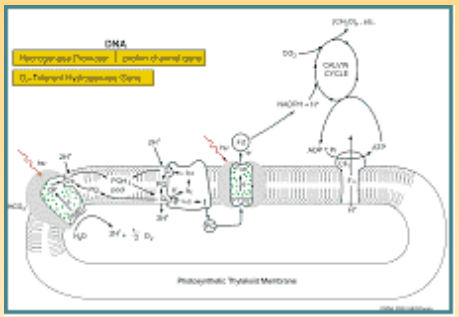
Potential solutions to overcome these roadblocks to photosynthetic H_2 production will also be presented. The solutions are based on a novel approach that has recently been developed at Oak Ridge National Laboratory (2001 ORNL Invention Disclosure). In this approach, a "designer alga" for efficient and robust H_2 production will be created by genetic insertion of hydrogenase promoter–programmed polypeptide proton channels in photosynthetic thylakoid membranes. This designer alga will be integrated also with the benefits of an O_2-tolerant hydrogenase that will be created by NREL and a smaller chlorophyll antenna size that will be created by UC Berkeley. Therefore, this ORNL effort is complementary with those of NREL and UC Berkeley, and will contribute jointly with the sister NREL and UC projects to achieving a common goal of effective photobiological H_2 production. By use of this approach, we will be able to simultaneously solve the six physiological problems for efficient and robust production of H_2 through photosynthetic water splitting.



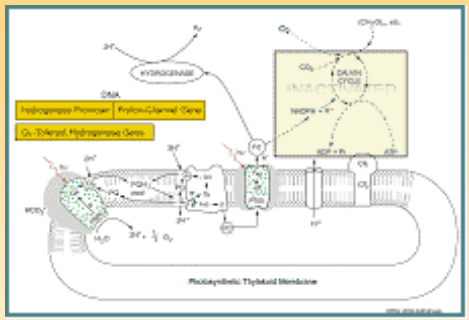
Objective: Overcoming nation's roadblocks to photosynthetic H_2 production by creation of designer alga

To meet DOE H_2 Program goal ($10/MMBtu), our proposed work will solve the six problems in algal H_2 production

Approach: Creation of designer alga for efficient and robust production of H_2 through genetic insertion of a proton channel into thylakoid membrane



*ORNL-invented concept: designer alga performing normal photosynthesis under **aerobic** conditions*



*Solution: designer alga becomes an efficient and robust H_2-production system under **anaerobic** conditions*

Project Timeline: If DOE can provide funding support of this 3.0 FTE effort, the project objective can be achieved within 4 years with the following milestones.

Path Forward—Milestones

Creation of designer alga for efficient and robust production of H_2

[3.0 FTE effort by Lee, Mets, Xu, Evans, Zhou, and a postdoctor]

	Year 1	Year 2	Year 3	Year 4
Year 1—Design and construction of DNA sequence coding for polypeptide proton channel	→			
Year 2—Genetic transfer of hydrogenase promoter-linked polypeptide proton-channel DNA into DS521		→		
Year 3—Characterization and optimization of the polypeptide proton-channel gene expression			→	
Year 4—Demonstration of efficient and robust production of H_2 in designer alga (ready for next phase: scale up and commercialization)				→

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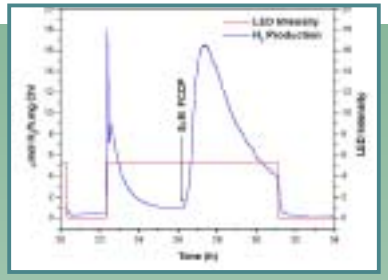
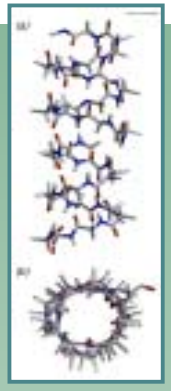
Accomplishment/progress: This is a new project. Because of the Congress and DOE budget situation under the "Continuing Resolution," this project has not received any funding until last month (April 2003). It was last month that DOE H_2 Program office authorized $50K for J. Lee:
(1) to attend this H_2 Review meeting, and
(2) to make preparation for a full start of the project on October 1, 2003. So, the following are the results of our preliminary studies.



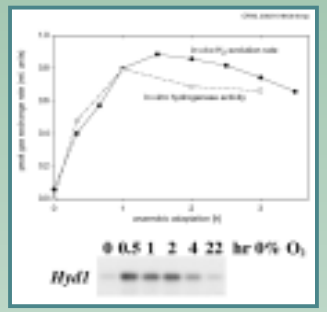
Proof of principle demonstrated by proton uncoupler FCCP experiments in wild-type algal H_2 production with 1000 ppm O_2



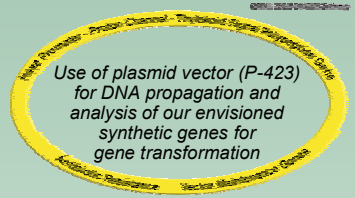
A preliminary design of polypeptide proton channel achieved by computer simulations at ORNL

The transformants will be screened and cultured for a number of assays to test for the predicted features of the designer alga

Our customer-designed state-of-the-art OLIS Photospectrometer system can be used to measure the activity of our envisioned polypeptide-proton channels in the designer alga at ORNL



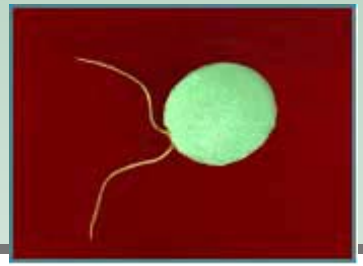
Anaerobic hydrogenase-induction studies demonstrated the potential of Hyd1 Promoter to serve as our envisioned genetic switch



DNA analyzers at ORNL

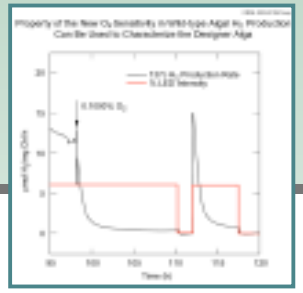
Our dual-reactor-flow detection system can be used for both H_2-production and recyclable-growth assays

DNA map of the hydrogenase gene (Hyd1) cloned by one of us (L. Mets) and ready for construction of our envisioned genetic switch



We can deliver the genes (DNA) into our Chlamydomonas host cells by use of a biolistic gene gun (PDS-1000/He system)

Microarray equipment for mRNA assays at ORNL



Property of our newly discovered O_2 sensitivity in wild-type (C. reinhardtii 137c) algal H_2 production can be used as a reference to test the designer alga

Significant interactions or collaborations with others: This is an multi-disciplinary R&D team with scientist from three ORNL divisions and University of Chicago. We also collaborate with the teams of NREL and UC Berkeley in a complementary way to achieve the common objective for the DOE Photobiological H_2 Program.

Plans and future milestone: Presented in Project Timeline.

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