

## II.E.3 Photobiological H<sub>2</sub> Production Systems: Creation of Designer Alga for Efficient and Robust Production of H<sub>2</sub> from Water

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### Introduction

Algal (e.g., *Chlamydomonas reinhardtii*) photosynthetic hydrogen (H<sub>2</sub>) production from water has tremendous potential as a clean and renewable energy resource. However, a number of technical issues must be addressed before algal H<sub>2</sub> production can become practical. This R&D project is to overcome a major technical barrier, “Rate of Hydrogen Production,” identified in the DOE *Hydrogen, Fuel Cells and Infrastructure Technologies Program Multi-Year Research, Development and Demonstration Plan* for “Molecular Genetics of Organisms for Photobiological Hydrogen Production.” This technical barrier consists of four physiological obstacles that seriously limit the absorbed light energy to hydrogen efficiency. The four physiological obstacles that currently challenge researchers and investors in the field of photosynthetic H<sub>2</sub> production are (1) restriction of photosynthetic H<sub>2</sub> production by accumulation of a proton gradient, (2) competitive inhibition of photosynthetic H<sub>2</sub> production by CO<sub>2</sub>, (3) requirement for bicarbonate binding at photosystem II (PSII) for efficient photosynthetic activity, and (4) competitive drainage of electrons by O<sub>2</sub> in algal H<sub>2</sub> production. In order for the photobiological H<sub>2</sub> production system to work, all of these four problems must be solved. The reason the solar-to-hydrogen energy conversion efficiency in a wild-type alga is so low (less than 0.1%) is because the wild-type organism does not possess the designer proton channel to solve the four proton-gradient-related problems (1-4) that seriously limit the rate of H<sub>2</sub> production as mentioned above.

In this project, we will overcome these four technical problems by creating an efficient, robust algal H<sub>2</sub> production system through a novel approach recently developed at Oak Ridge National Laboratory (ORNL). In this approach, a “designer alga” will be created by genetic insertion of hydrogenase promoter-programmed polypeptide proton channels into algal thylakoid membranes. This approach will allow us to simultaneously solve the four physiological problems because all four are related to proton gradient. The success of this work will have a significant impact (a tenfold improvement) on technology development in the field of renewable hydrogen research.

### Approach

We have recently developed a systematic approach to create a “super” photosynthetic organism—a designer alga specifically designed for the production of molecular hydrogen through photosynthetic water

### Objectives

- Develop advanced renewable photobiological hydrogen production technologies through creation of a designer alga by genetic insertion of a proton channel into an algal thylakoid membrane.
- By 2015, demonstrate an engineering-scale biological system that produces hydrogen at a plant-gate cost of \$10/kg projected to commercial scale.

### Technical Barriers

This project addresses the following technical barriers from the Biological Hydrogen Production section (3.1.4.2.5) of the Hydrogen, Fuel Cells and Infrastructure Technologies Program Multi-Year Research, Development and Demonstration Plan:

(Y) Rate of Hydrogen Production

### Technical Targets

This project is overcoming the four proton-related major physiological problems that constitute technical barrier Y in photobiological H<sub>2</sub> production by genetic insertion of a proton channel into an algal thylakoid membrane. This project is the key to achieving the following DOE 2010 and 2015 photolytic biological hydrogen production technical targets:

Characteristics	Units	Current status	2010 target	2015 target
Efficiency of Incident Light Energy to Hydrogen from Water	%	0.1	2	5

splitting (ORNL Invention Disclosure ID 0981) [1]. This designer alga will be able to overcome the four proton gradient-related physiological problems that currently challenge researchers and investors in the field of photosynthetic H<sub>2</sub> production. The key element of our proposed approach is creation of a designer alga for efficient, robust production of H<sub>2</sub> through genetic insertion of a programmable polypeptide proton channel into the thylakoid membrane. We propose to accomplish the genetic insertion of programmable thylakoid-membrane proton channels by transformation of a host alga with a designer polypeptide proton-channel gene linked with a hydrogenase promoter. The envisioned super alga that can be created by the proposed work should be able to perform autotrophic photosynthesis using ambient-air CO<sub>2</sub> as the carbon source and grow normally under aerobic conditions, such as in an open pond. When the algal culture is grown and ready for H<sub>2</sub> production, the proton-channel gene will then be expressed simultaneously with the induction of the hydrogenase enzyme under anaerobic conditions because of the use of the hydrogenase promoter. The expression of the proton-channel gene should produce polypeptide proton channels in the algal thylakoid membrane, thus dissipating the proton gradient across the thylakoid membrane without adenosine triphosphate (ATP) formation to enhance H<sub>2</sub> production. The use of polypeptide proton channels in algal thylakoid membrane can provide four advantages for H<sub>2</sub> photoevolution: (1) the accumulation of a proton gradient that impedes the photosynthetic electron transport from water to Fd/hydrogenase will be prevented; (2) the competitive inhibition of photosynthetic H<sub>2</sub> production by CO<sub>2</sub> (Calvin cycle activity) will be eliminated; (3) the requirement for bicarbonate binding at PSII for efficient photosynthetic activity will be satisfied; and (4) the newly discovered O<sub>2</sub>-sensitive pathway (drainage of electrons by O<sub>2</sub>) that competes with the H<sub>2</sub>-production pathway for photosynthetically generated electrons can be avoided by the dissipation of the proton gradient with the switchable proton channel.

This is a progressive approach of bioinformatics-assisted molecular designing, gene synthesis, and transformation with experimental verification and characterization to create and optimize the envisioned proton-channel designer alga with iterative improvement until achieving the desired results for enhanced photobiological H<sub>2</sub> production in support of the DOE/EERE Program mission. The envisioned switchable proton-channel designer alga is a major technology component that is required for the photobiological H<sub>2</sub>-production technology system to work. The reason the solar-to-hydrogen energy conversion efficiency in a wild-type alga is so low (less than 0.1%) is because the wild-type organism does not possess the designer proton channel to solve the four proton-gradient-

related problems (1-4) that seriously limit the rate of H<sub>2</sub> production as mentioned above. A successful switchable proton-channel designer alga must have a good proton-conducting channel that can be selectively (switchably) expressed only under H<sub>2</sub>-producing conditions with targeted insertion to the algal thylakoid membrane at the right amount. The switchability is envisioned to be conferred through innovative application of an anaerobic promoter such as the hydrogenase promoter. The targeted insertion will be accomplished through use of the proper thylakoid transit peptide deoxyribonucleic acid (DNA). Proton channels will be designed with computational analysis of certain natural polypeptide channels such as melittin. The amount of the proton channel expression will be controlled by the copy number of the designer gene and by tuning its promoter strength through application of bioinformatics techniques.

We anticipate that the first set of designer proton-channel genes may work partially or not work at all. This is also where the progressive approach will be applied to ensure the success of the project by generating new knowledge to iteratively improve the desired designer algae for enhanced photobiological H<sub>2</sub> production as we will use a number of experimental tools to characterize the genetic transfer and expression of the designer proton-channel genes and to determine: what is working, what is not working, and why. DNA (PCR) assays will be employed to verify the genetic transfer of the designer genes into the alga's nuclear genome. Assays of message ribonucleic acid (mRNA), protein, and algal H<sub>2</sub> production will be employed to characterize the expression of the designer proton-channel genes for enhanced photobiological H<sub>2</sub> production. When necessary, techniques of liquid nuclear magnetic resonance will also be employed to determine the nanometer structures of the expressed designer proton channels. These structure-function characterization data will be fed back into the computational molecular/gene design by comparing them with the computationally predicted structure and function for further improvement until the desired results are achieved for significant (more than 10-times) improvement of the absorbed light energy to hydrogen conversion efficiency in support of the DOE/EERE Photobiological H<sub>2</sub> Program.

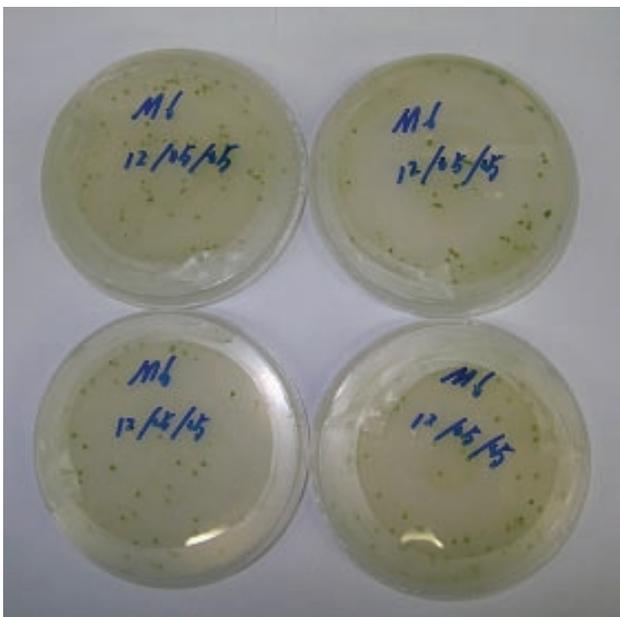
### FY 2006 Progress

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

We successfully completed the FY 2005 milestones on designing and synthesizing for the first set of proton-channel genes for this project within FY 2005. Briefly, our design approach using computer simulation focused on the pore size (e.g. nm required for proton conductivity) and the molecular stability of the

designer proton channel. Using this approach with the bioinformatics tools, we have been able to design the first set of genes (3 synthetic genes) that could encode the switchable proton channels in *Chlamydomonas reinhardtii*. Also, in collaboration with GENEART USA and Bioclone Inc., we recently synthesized this set of designer genes.

The following describes the work that was accomplished using a small amount of the FY 2005 funding that was carried over into FY 2006. We recently made a significant technical progress—the first set of designer proton-channel genes have now been delivered into the host alga *Chlamydomonas* using the gene-transformation technique of electroporation in our lab. Many transformants have now been generated (Figure 1). Theoretically, these transformants are expected to



**FIGURE 1.** We have now successfully delivered the first set of synthetic genes (DNA) into our *Chlamydomonas* host cells by use of electroporation. Many colonies of designer proton-channel transformants have now successfully been obtained. Each of green dots shown in this photograph represents an algal colony grown from a single transformed cell that contains the designer proton-channel DNA construct.

contain the envisioned proton-channel designer alga that could provide significant impact (10-fold improvement) on technology development in the field of renewable photobiological H<sub>2</sub> production. Next, we need to screen/characterize these transformants to identify and optimize the desired proton-channel designer alga with iterative improvement through our progressive-feedback approach of computer-assisted molecular design, designer-gene expression, and experimental characterization/verification until the desired result for enhanced photobiological H<sub>2</sub> production in support of the DOE/EERE Program mission is achieved.

## Conclusions and Future Directions

This project has the potential for a major breakthrough to improve photobiological H<sub>2</sub> production by a factor of more than 10 through creation/identification of the first switchable proton-channel designer alga within FY 2007-2008.

## FY 2006 Publications/Presentations

1. James W. Lee. Creation of Designer Alga for Enhanced Photosynthetic H<sub>2</sub> Production from Water, presented at the FuelCellSouth Partners Forum, February 23, 2006, Oak Ridge, Tennessee.

## Special Recognitions & Awards/Patents Issued

1. FuelCellSouth 2006 Crystal Flame Innovation Award, presented by the FuelCellSouth Partners Forum with award ceremony held April 25, 2006 at the Columbia Metropolitan Convention Center, South Carolina, in recognition of the designer-alga H<sub>2</sub>-production research accomplishment.

## References

1. J. W. Lee, "Designer proton-channel transgenic algae for photobiological hydrogen production," U.S. Patent Application pending; reported first in ORNL Invention Disclosure 0981, 2001.
2. J. W. Lee and E. Greenbaum, "A New Oxygen Sensitivity in Photosynthetic H<sub>2</sub> Production," *Applied Biochemistry and Biotechnology*, **105-108**, 303-313 (2002).