

Biological Systems for Hydrogen Photoproduction

2007 Hydrogen Program Review

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NREL

May 16, 2007

Project ID#PD9

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Overview

Timeline

- Project start date: FY00
- Project end date: continuing
- Percent complete: N/A

Barriers

- Barriers addressed
 - Production Barrier Z:
Continuity of H₂ production.

Parameters	Current Status	2010 Target	Maximum potential
Duration of continuous photoproduction	80 days (sulfur-deprived)	30 minutes (aerobic)	12 hours (aerobic)
O ₂ tolerance (half-life in air)	2-4 min (<i>Clostridium</i> hydrogenase <i>in vitro</i>)	10 minutes	12 hours

Budget

- Funding received in FY06
\$466K
- Funding for FY07
\$930K

Partners

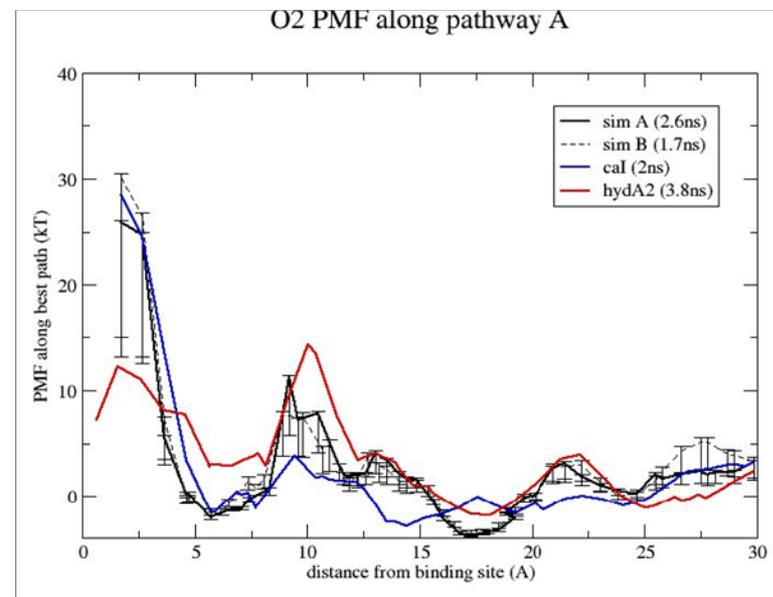
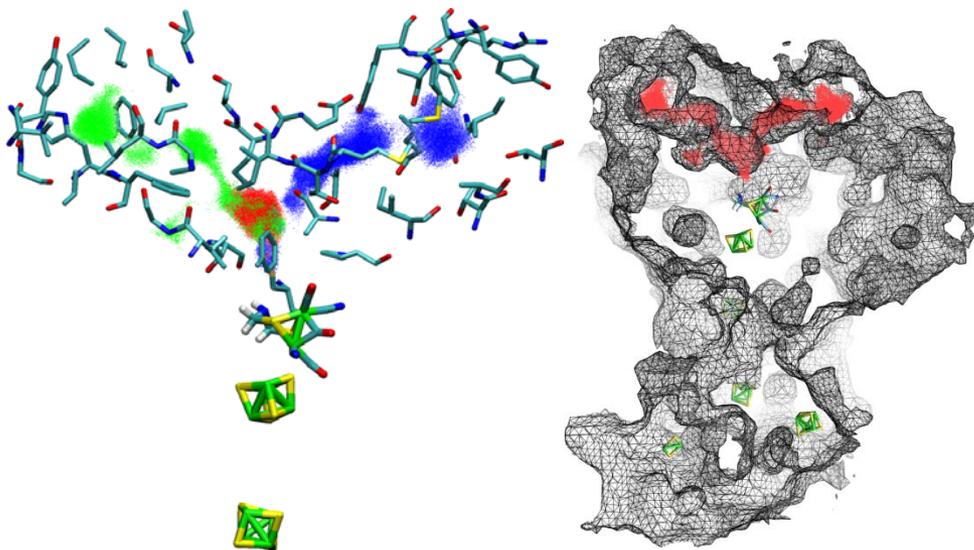
- Dr. Anatoly Tsygankov, Russian Academy of Sciences, Pushchino, Russia.
- Dr. Michael Flickinger, University of Minnesota.
- Dr. Klaus Schulten, The Beckman Institute, University of Illinois.

Objectives

- Develop and optimize anaerobic and aerobic photobiological systems for the production of H₂ from water; integrate photobiological with fermentative organisms to more efficiently utilize the solar spectrum and the substrates/products from each reaction.
- **FY2006/07:**
 - Subtask 1: Engineer a H₂-producing catalyst ([FeFe]-hydrogenase) that prevents O₂ from inactivating the enzyme's catalytic site under aerobic conditions.
 - Subtask 2: Improve the light conversion properties of a H₂-producing anaerobic algal system by immobilizing the cells on a flat matrix.
 - Subtask 3: Test the ability of H₂-producing, fermentative organisms to consume algal biomass and to produce extra substrate (acetate) required for high yields of algal or photosynthetic bacterial H₂-production in a second reactor.

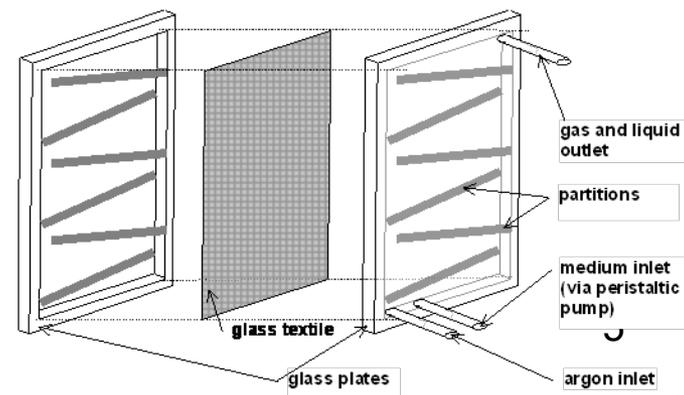
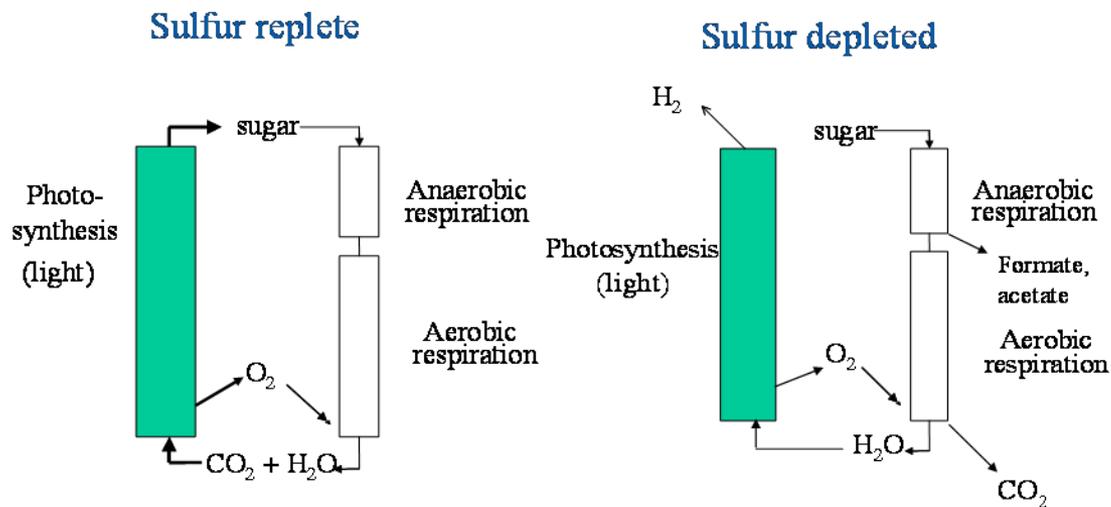
Approaches

- **Subtask 1:** Use computational simulation methods to identify pathways by which O_2 gas diffuses through the [FeFe]-hydrogenase protein structure to the catalytic site. With this knowledge, apply molecular engineering techniques to alter the hydrogenase structure to prevent O_2 from accessing its catalytic site. Either site-directed or random mutagenesis methods will be used.



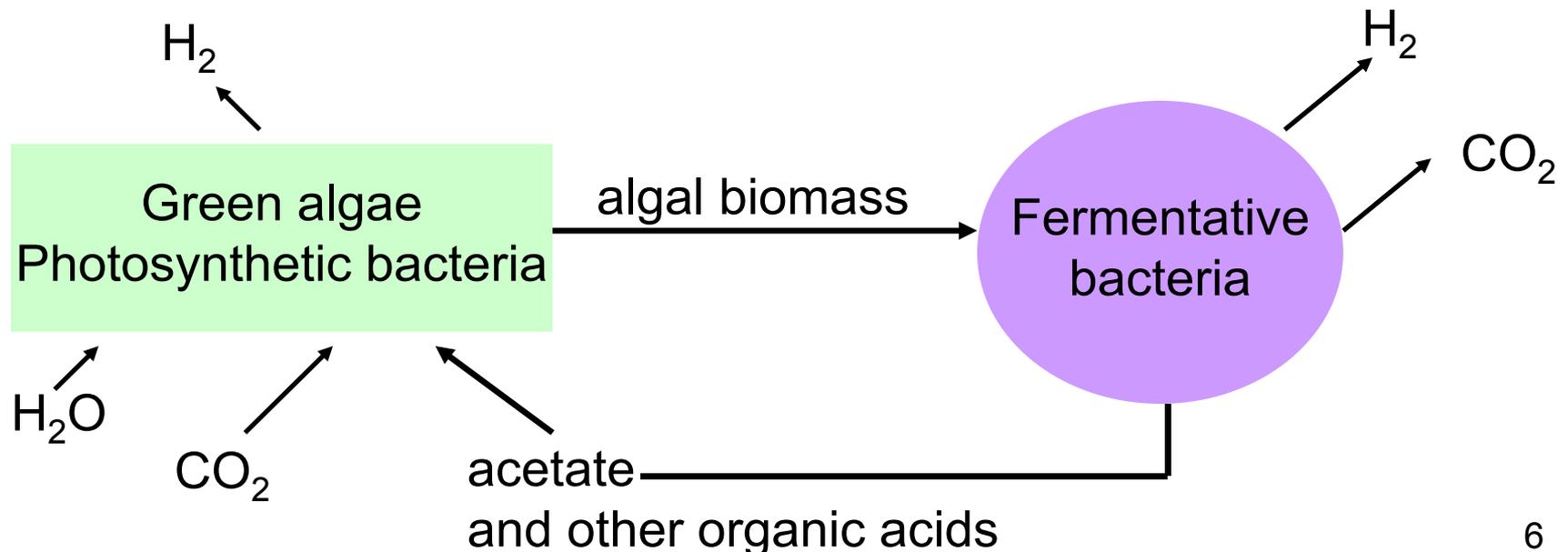
Approaches

- Subtask 2: Investigate different types of cell immobilization matrices compatible with H₂-producing, sulfur-deprived algal cells; test their ability to prolong the H₂-production phase and to increase the light conversion efficiency of the cultures.



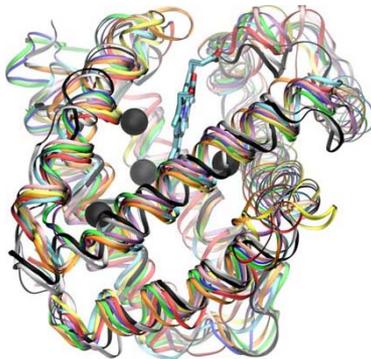
Approaches

- **Subtask 3:** Test whether a consortium of H₂-producing fermentative bacteria is capable of metabolizing algal or bacterial biomass; test whether photosynthetic bacteria and sulfur-deprived algae are capable of photoproducing H₂ using added organic acids generated in the fermentative reactor.

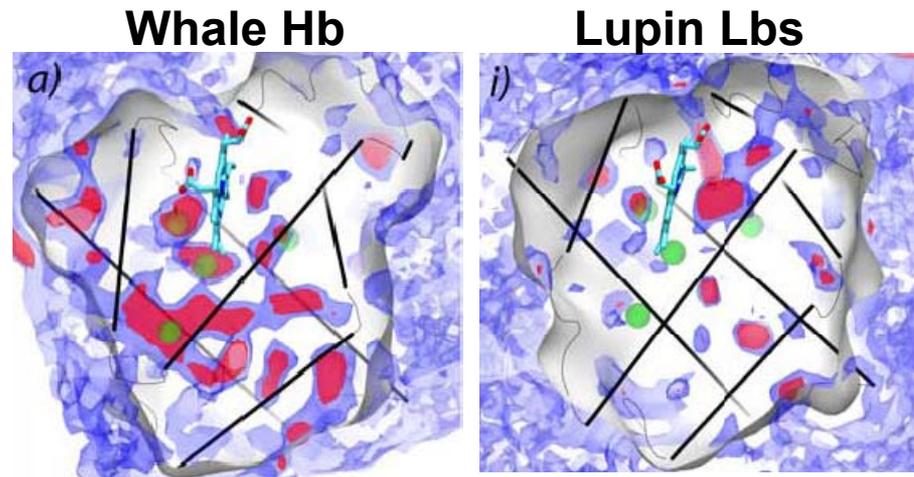


Technical Accomplishments/ Progress/Results

- **Subtask 1:** Substituting larger, bulkier residues for smaller residues in an effort to restrict gas accessibility into the hydrogenase's catalytic site has been partially successful; however, in some cases modified enzymes showed reduced activities and/or increased O₂ sensitivities;
A new computational dynamics study on the X-ray structures of members of the globin family presents evidence that predicting O₂ diffusion through homologous globins does not correlate with the protein's tertiary structure, rather O₂ pathways correlate better with the occurrence of specific amino acid residues (see below).



(Cohen and Schulten, submitted)



Technical Accomplishments/ Progress/Results

- **Subtask 2:** This subtask was not funded by DOE during 2006. In the absence of DOE funds, interim support from the U.S. Air Force Office of Scientific Research enabled progress to continue as follows:

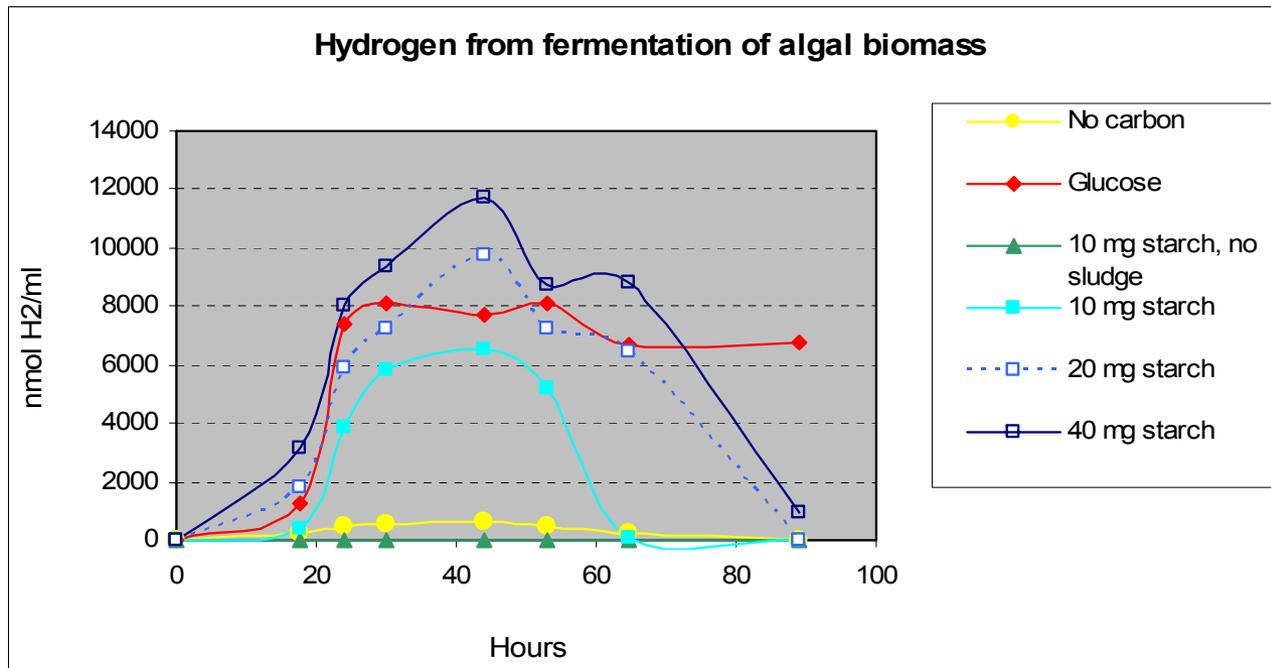
Parameters	Cell suspension	Immobilized cells (glass fibers)	Immobilized cells (alginate strips)
Illuminated reactor surface (cm)	2 x 261 cm ² = 522 cm ²	200 cm ²	6 cm ²
Rate of H ₂ production per reactor (ml/h and μmoles/h)	2.5 82.5	0.7 31.2	0.036 1.085
Energy of incident light per m ² per hour	308,106 J/m ²	92,448 J/m ²	44,683 J/m ²
Efficiency of incident light energy conversion into H ₂	0.12%	0.36%	0.93%

Sulfur-deprived *C. reinhardtii*, immobilized onto new substrate materials, has led to a significant increase in the stability of H₂-production activity in the presence of O₂ compared to suspension cultures.

Technical Accomplishments/ Progress/Results

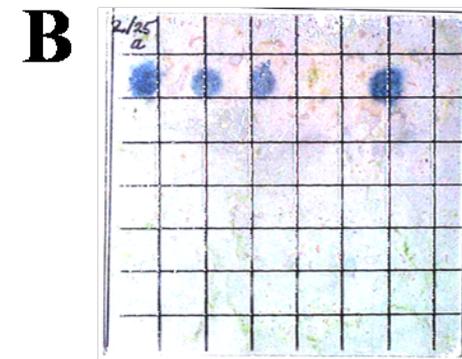
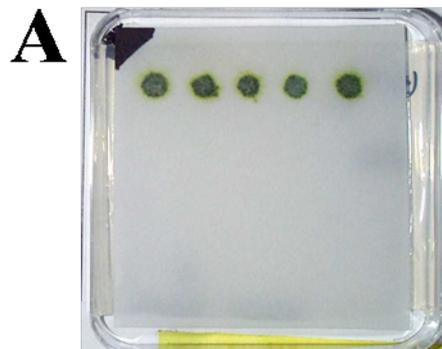
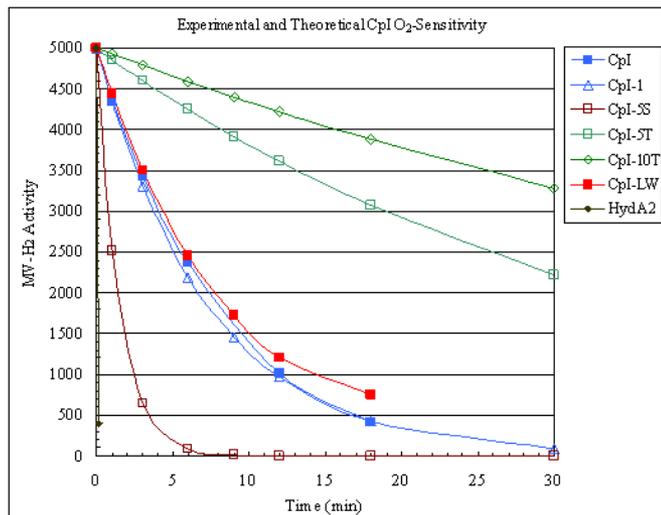
- Subtask 3:

Significant H₂ production was observed using a consortium of fermentative organisms from sewage sludge fed with algal biomass. The algae had not been sulfur-deprived.



Future Work

- Subtask 1:
 - (a) Shift the mutagenesis work towards newly identified targets;
 - (b) Attempt to express the more O₂-tolerant clostridial hydrogenase in *C. reinhardtii*;
 - (c) Initiate a random mutagenesis approach, using one of the high-throughput screening techniques currently under development.



Future Work

- **Subtask 2:**
 - (a) Continue to investigate new matrices, environmental parameters and photobioreactor designs for immobilization of algal cultures for H₂ photoproduction;
 - (b) Initiate tests on the H₂-production performance of sulfur-deprived, truncated-antenna mutants (see poster # PDP 33 from U.C. Berkeley) in suspension and under immobilized conditions.

Future Work

- **Subtask 3:**
 - (a) Study the H₂ yield from the fermentation of sulfur-deprived algal biomass;
 - (b) Test various pretreatments of algal biomass to increase its H₂ fermentability;
 - (c) Immobilize photosynthetic bacteria and determine their ability to produce H₂ using the waste effluent from a fermentative bioreactor;
 - (d) Test an integrated system with actual agricultural/industrial waste streams.

Summary

- **Subtask 1:** Recent computational simulations indicate that substituting smaller amino acid residues, identified in the gas pathways of gas-transport proteins, might have a more significant effect than our previous approach towards reducing the O₂ accessibility of the hydrogenase catalytic site.
- **Subtask 2:** The incident light conversion efficiency of H₂-producing algae immobilized on a matrix was increased to 0.93% from 0.12% (in suspension cultures), and significant rates of H₂ production were detected in the presence of O₂ in the headspace.
- **Subtask 3:** A consortium of fermentative organisms is able to produce H₂ by fermenting sulfate-replete algal biomass.