Biological Systems for Hydrogen Photoproduction

DOE Hydrogen Program

2007 Hydrogen Program Review

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NREL

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Project ID#PD9

Overview

DOE Hydrogen Program

Timeline

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

Barriers

- Barriers addressed
  - Production Barrier Z: Continuity of H_2 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 (*Clostridium* hydrogenase *in vitro*)	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

DOE Hydrogen Program

- Develop and optimize anaerobic and aerobic photobiological systems for the production of H_2 from water; integrate photobiological with fermentative organisms to more efficiently utilize the solar spectrum and the substrates/products from each reaction.

- **FY2006/07:**

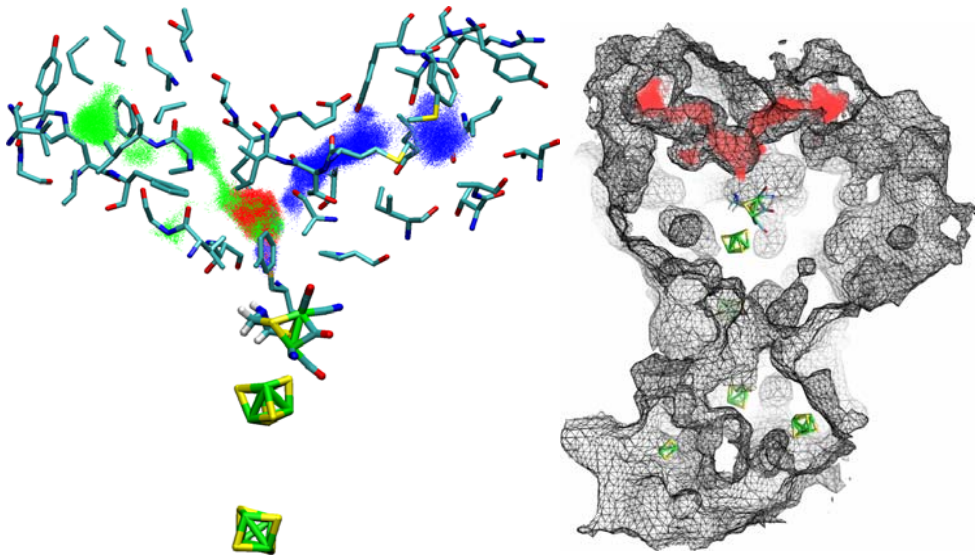
Subtask 1: Engineer a H_2-producing catalyst ([FeFe]-hydrogenase) that prevents O_2 from inactivating the enzyme's catalytic site under aerobic conditions.

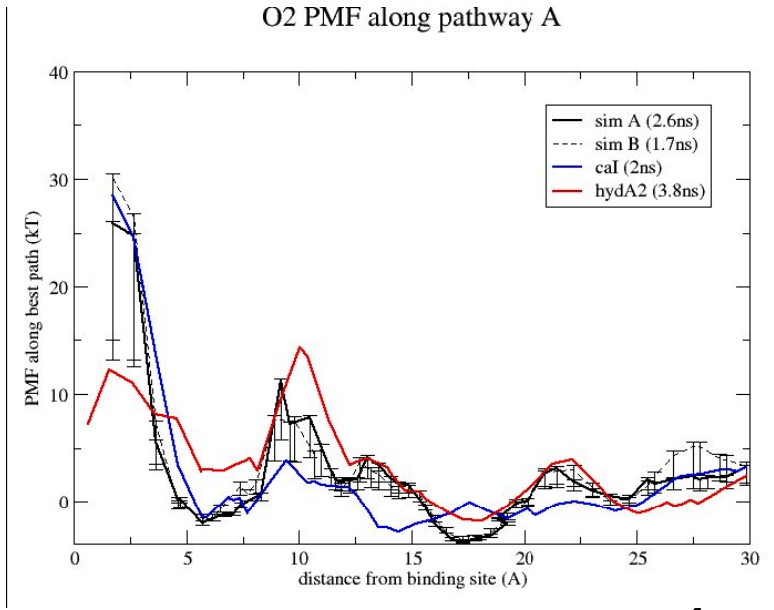
Subtask 2: Improve the light conversion properties of a H_2-producing anaerobic algal system by immobilizing the cells on a flat matrix.

Subtask 3: Test the ability of H_2-producing, fermentative organisms to consume algal biomass and to produce extra substrate (acetate) required for high yields of algal or photosynthetic bacterial H_2-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

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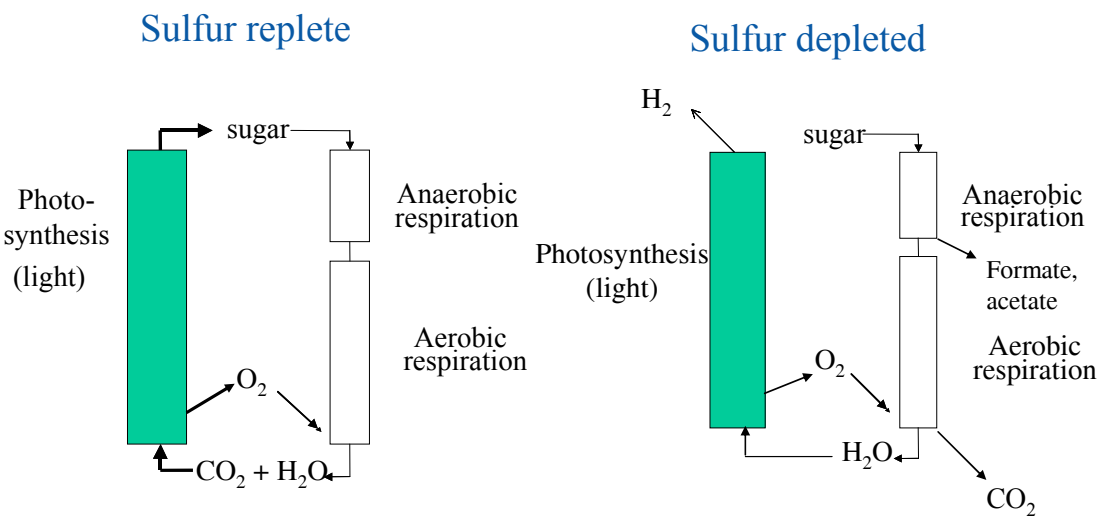
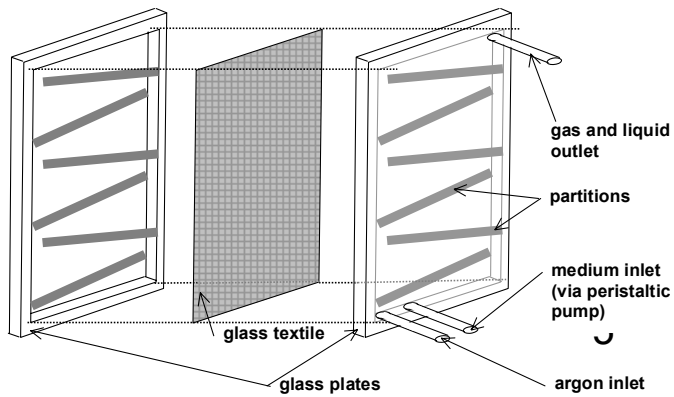
- Subtask 2: Investigate different types of cell immobilization matrices compatible with H_2-producing, sulfur-deprived algal cells; test their ability to prolong the H_2-production phase and to increase the light conversion efficiency of the cultures.





Approaches

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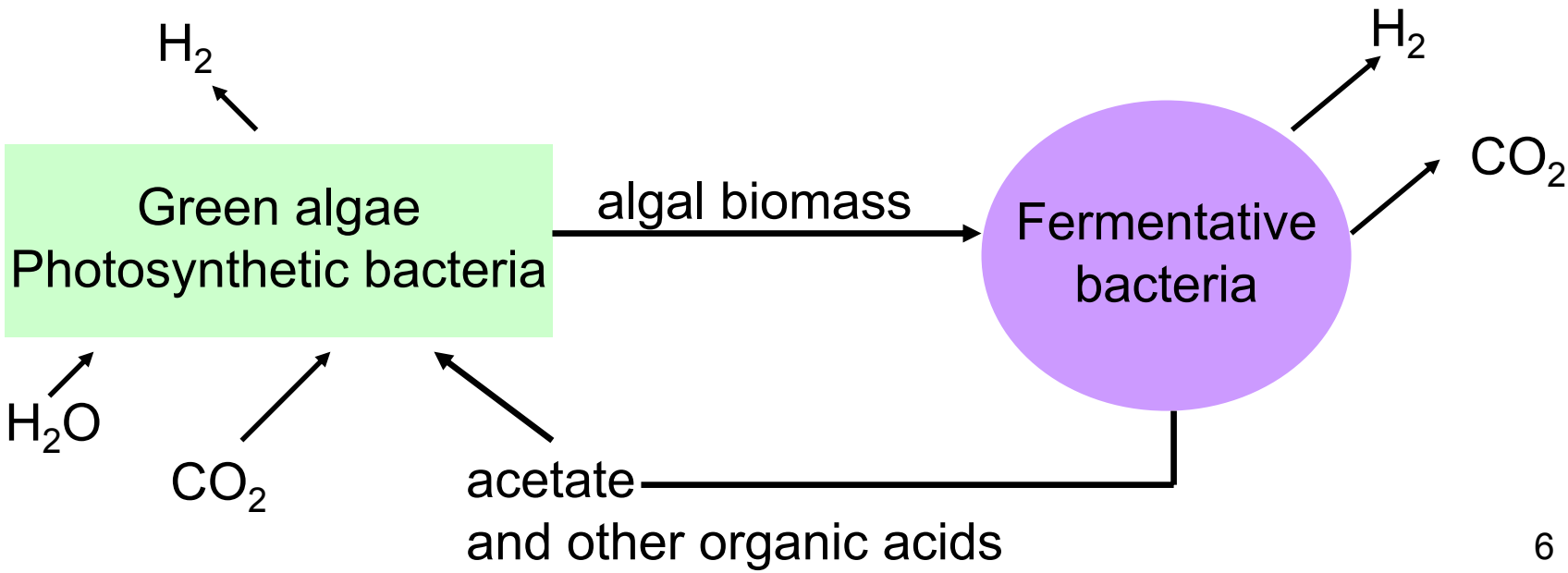
- Subtask 3: Test whether a consortium of H_2-producing fermentative bacteria is capable of metabolizing algal or bacterial biomass; test whether photosynthetic bacteria and sulfur-deprived algae are capable of photoproducing H_2 using added organic acids generated in the fermentative reactor.



Technical Accomplishments/ Progress/Results

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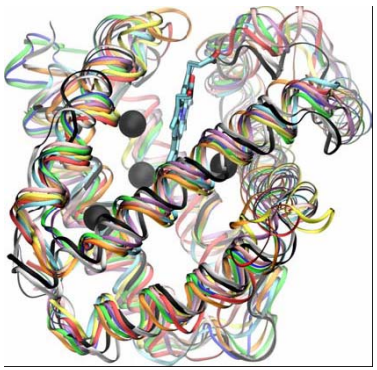
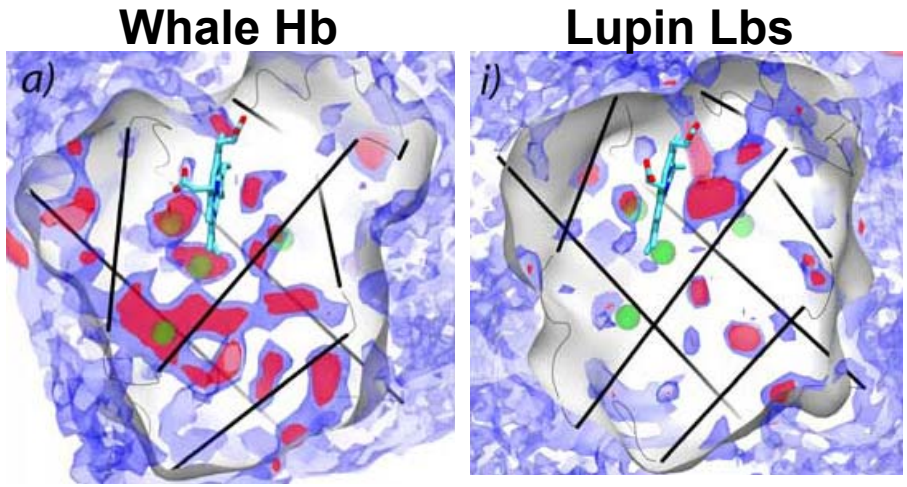
- **Subtask 1:** Substituting larger, bulkier residues for smaller residues in an effort to restrict gas accessibility into the hydrogenase's catalytic site has been partially successful; however, in some cases modified enzymes showed reduced activities and/or increased O_2 sensitivities;
  A new computational dynamics study on the X-ray structures of members of the globin family presents evidence that predicting O_2 diffusion through homologous globins does not correlate with the protein's tertiary structure, rather O_2 pathways correlate better with the occurrence of specific amino acid residues (see below).



(Cohen and Schulten, submitted)



Technical Accomplishments/ Progress/Results

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- 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 H_2-production activity in the presence of O_2 compared to suspension cultures.

Technical Accomplishments/ Progress/Results

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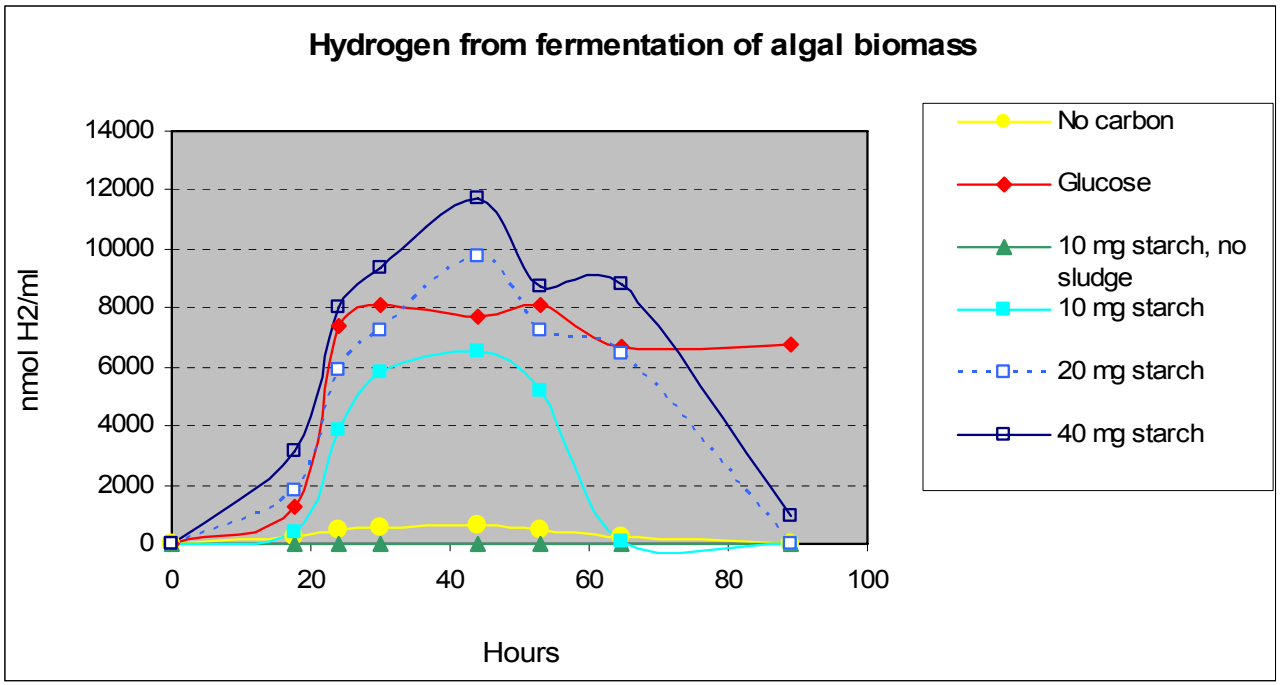
- Subtask 3:

  Significant H_2 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

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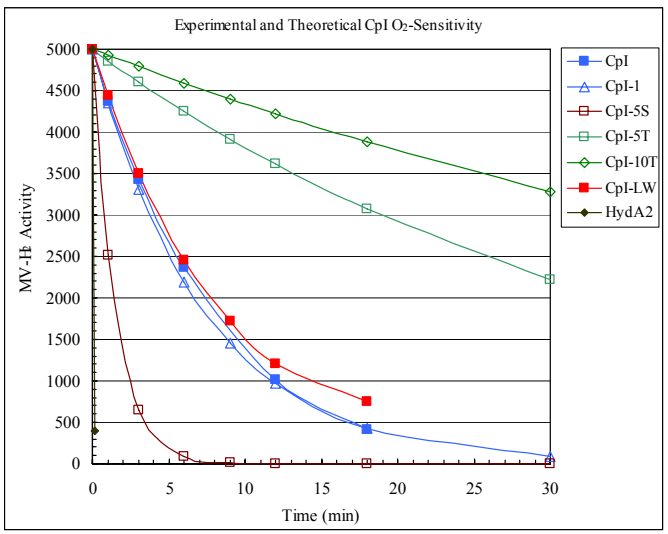
- Subtask 1:

(a) Shift the mutagenesis work towards newly identified targets;

(b) Attempt to express the more O_2-tolerant clostridial hydrogenase in *C. reinhardtii*;

(c) Initiate a random mutagenesis approach, using one of the high-throughput screening techniques currently under development.



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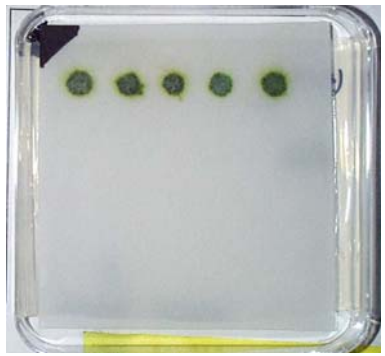

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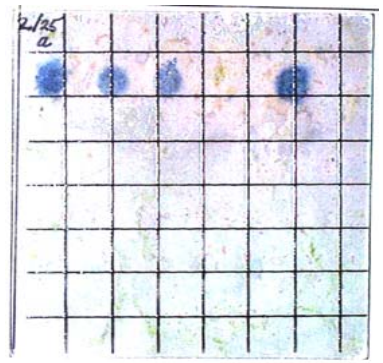

Future Work

DOE Hydrogen Program

- Subtask 2:

(a) Continue to investigate new matrices, environmental parameters and photobioreactor designs for immobilization of algal cultures for H_2 photoproduction;

(b) Initiate tests on the H_2-production performance of sulfur-deprived, truncated-antenna mutants (see poster # PDP 33 from U.C. Berkeley) in suspension and under immobilized conditions.

Future Work

DOE Hydrogen Program

- Subtask 3:

(a) Study the H_2 yield from the fermentation of sulfur-deprived algal biomass;

(b) Test various pretreatments of algal biomass to increase its H_2 fermentability;

(c) Immobilize photosynthetic bacteria and determine their ability to produce H_2 using the waste effluent from a fermentative bioreactor;

(d) Test an integrated system with actual agricultural/industrial waste streams.

Summary

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- **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 O_2 accessibility of the hydrogenase catalytic site.
- **Subtask 2:** The incident light conversion efficiency of H_2-producing algae immobilized on a matrix was increased to 0.93% from 0.12% (in suspension cultures), and significant rates of H_2 production were detected in the presence of O_2 in the headspace.
- **Subtask 3:** A consortium of fermentative organisms is able to produce H_2 by fermenting sulfate-replete algal biomass.