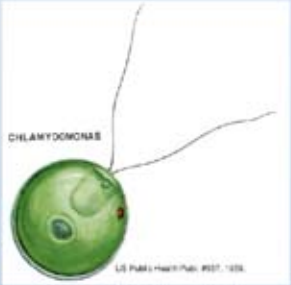
Biological Systems for Hydrogen Photoproduction

2004 DOE Hydrogen, Fuel Cells & Infrastructure Technologies Program Review

Maria L. Ghirardi, Pin-Ching Maness and Michael Seibert



National Renewable Energy Laboratory
May 24, 2004

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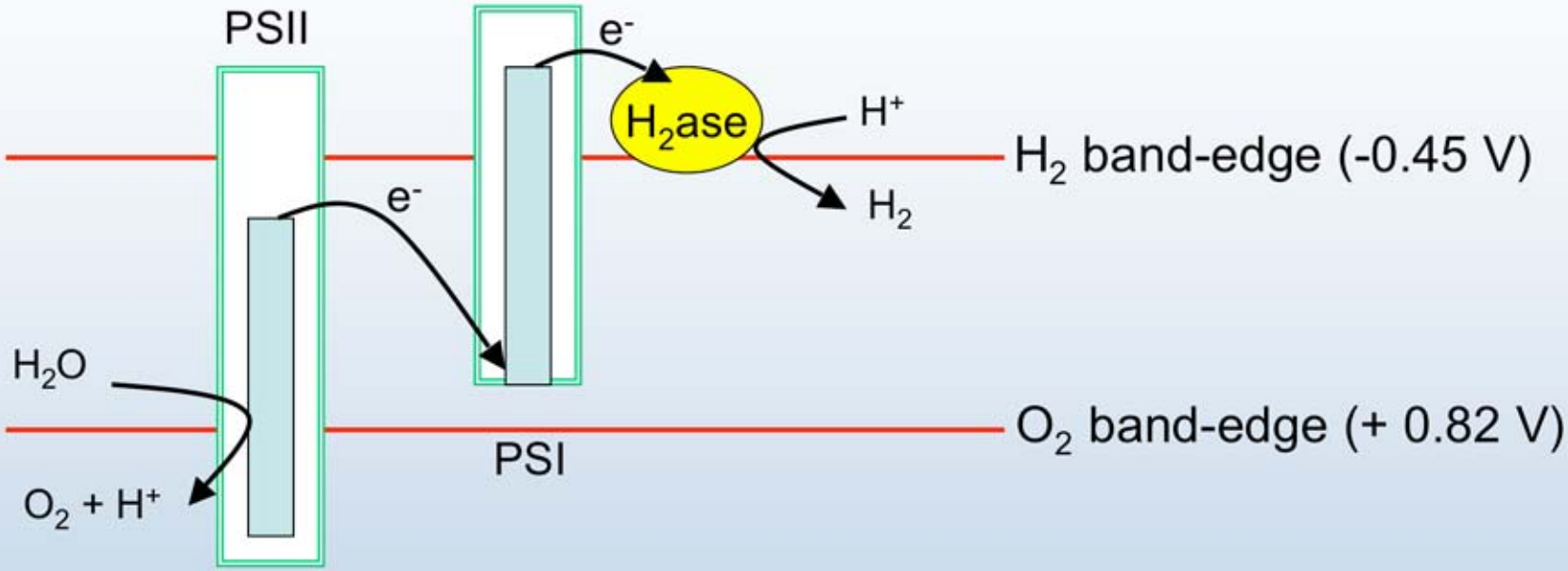
Project Goal and Budget

- Goal
  - Develop photolytic H_2-production technologies based on microbial H_2O-splitting processes.

- Budget
  - Total Funding in FY04: $710 K (3 tasks)
    - NREL's Share: $690 K
    - Subcontractor's Share: $20 K

Problem to be Addressed and Approaches



Subtask 1. Engineer an algal hydrogenase that is resistant to O_2 inactivation;
Subtask 2. Develop and optimize a physiological method to promote culture anaerobiosis and subsequent H_2-production activity in algae;
Subtask 3. Introduce a bacterial hydrogenase with increased O_2 resistance into a water-splitting photosynthetic cyanobacterial system.

Objectives for FY04

- Identify additional amino acid residues in the algal HydA1 hydrogenase as targets for site-directed mutagenesis aimed at increasing its resistance to O_2;
- Extend H_2 production in the continuous system by adjusting algal culture parameters; improve the efficiency of H_2 photoproduction using immobilized algal cultures;
- Initiate studies for expression of the O_2-resistant *Rubrivivax gelatinosus* bacterial hydrogenase in a photosynthetic cyanobacterium.

Technical Barriers and Targets

Photobiological Hydrogen Generation Barriers:

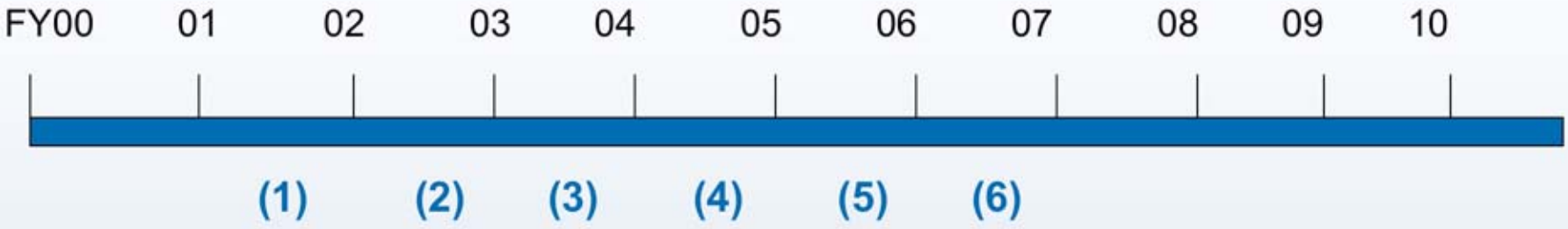
- **K.** Continuity of Photoproduction

DOE Technical Targets for Photobiological H_2 Production:

Parameters	2005	2010
Utilization efficiency of absorbed light	10%	20%
Conversion efficiency of absorbed light to H_2	0.5%	5%
Duration of continuous H_2 photoproduction	500 h	1500 h
Cost of low pressure H_2 at plant gate	$100/kg	$30/kg

Meeting the technical targets involves a collaboration between NREL, UCB and ORNL to address the 3 barriers identified by DOE (I, J and K).

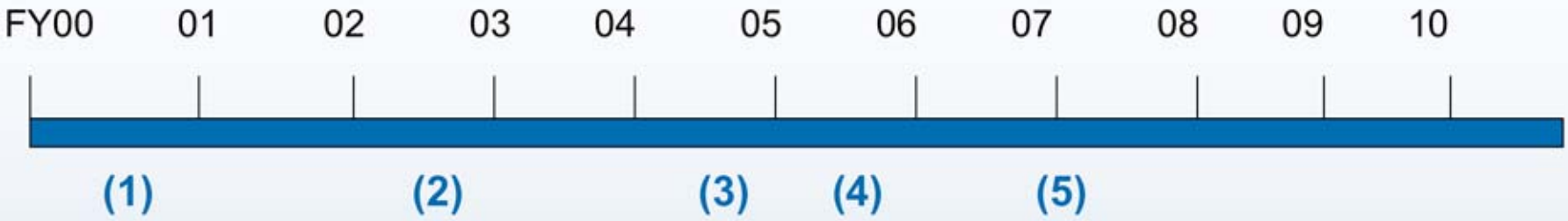
Project Timeline



Subtask 1

(1) Cloned and sequenced the algal hydrogenase *HydA1* gene;
(2) Discovered and sequenced a second algal hydrogenase, HydA2;
(3) Generated the first algal mutant with improved O_2 resistance;
(4) Published a landmark article on the expression of active algal hydrogenase in the bacterium *E. coli* (joint effort with an Office of Science project); completed simulations of O_2 diffusion in an Fe-hydrogenase and identified targets for mutagenesis;
(5) Will perform simulations of H_2 diffusion in an Fe-hydrogenase;
(6) Will generate and test additional hydrogenase mutants for improved O_2-resistance.

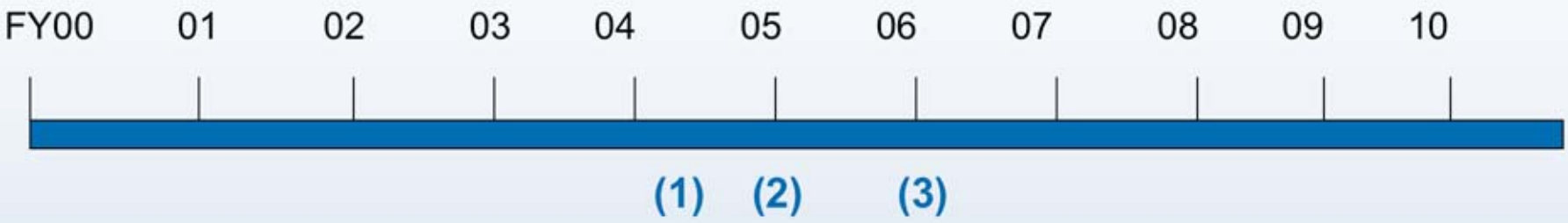
Project Timeline



Subtask 2

(1) Demonstrated 96 hours of H_2-production per batch operation cycle (with UCB);
(2) Maintained continuous H_2-production for 240 h at sustained rates;
(3) Achieved 6 months of continuous H_2-production at variable rates (*exceeding the 2010 technical target for duration of production*); immobilized sulfur-deprived algal cells for batch H_2 production;
(4) Will determine the feasibility of immobilized algal cells for cyclic H_2-production;
(5) Will develop a suspension or immobilized system to produce H_2 continuously at sustained and optimized rates.

Project Timeline



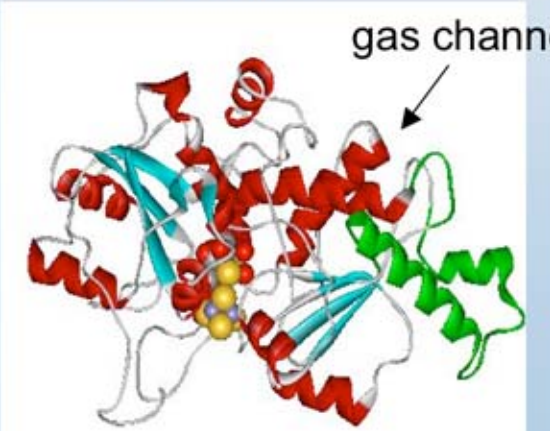
Subtask 3

(1) Started new subtask in January 2004;
(2) Will create a cyanobacterial hydrogenase knockout mutant;
(3) Will transfer a bacterial O_2-resistant hydrogenase into the above mutant.

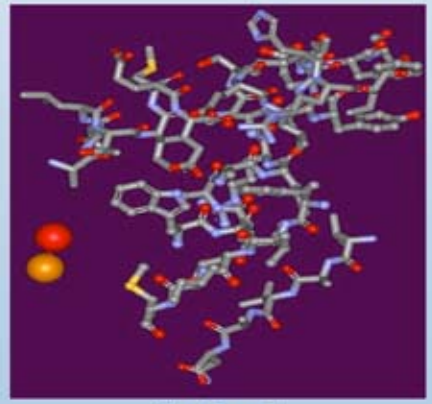
Technical Accomplishments/Progress
Subtask 1

Background:

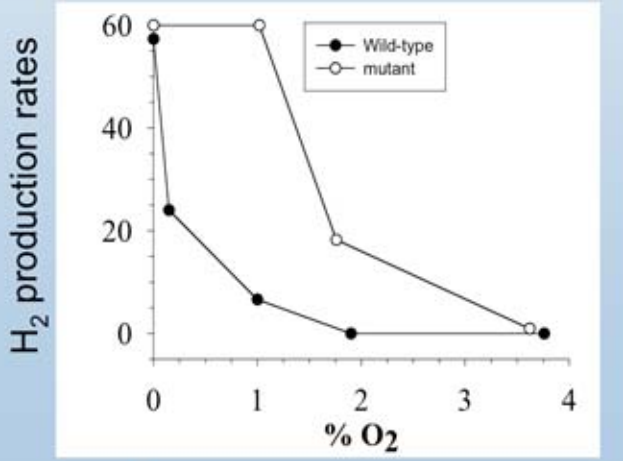
- Cloned two algal hydrogenase genes, *HydA1* and *HydA2;* key to current progress
- Modeled the two hydrogenase protein structures by homology to a bacterial Fe-hydrogenase that had been crystallized;
- Identified a gas channel as the putative O_2 access to the catalytic site;
- Modified a residue in the gas channel and observed increased O_2 resistance.



C. reinhardtii HydA1



Mutant

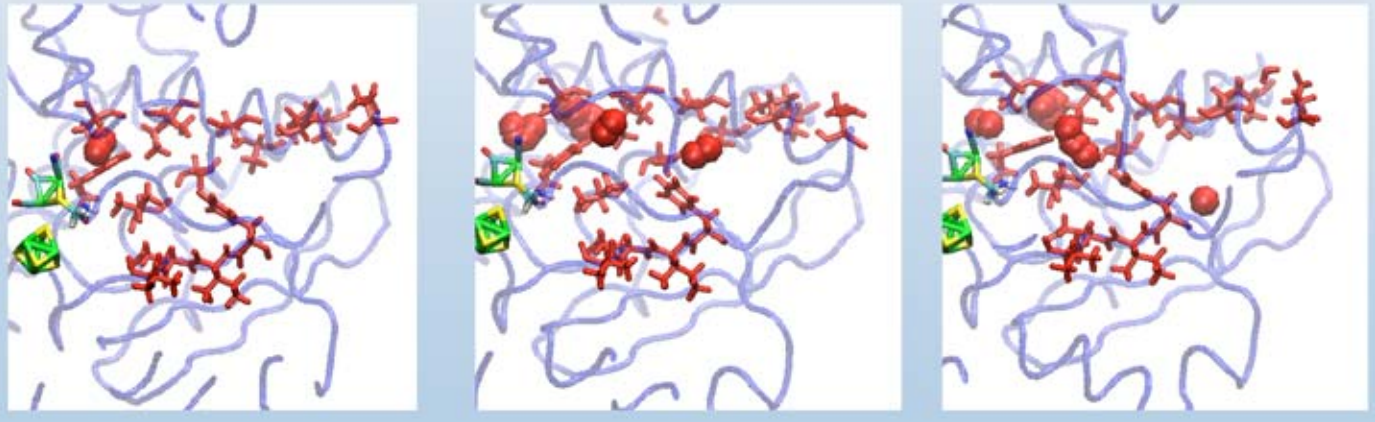


Technical Accomplishments/Progress
Subtask 1

FY04 Results

Molecular dynamics modeling of O_2 diffusion in the Fe-hydrogenase allowed us to identify future targets for site-directed mutagenesis (proprietary information) aimed at preventing O_2 inactivation of the algal enzyme.

Collaborators: K. Schulten, University of Illinois, and the NREL Computational Sciences Center.

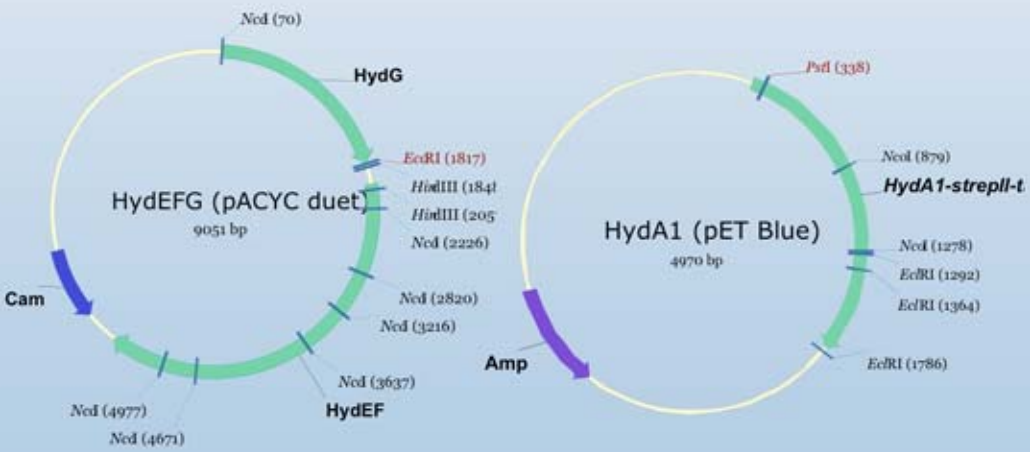


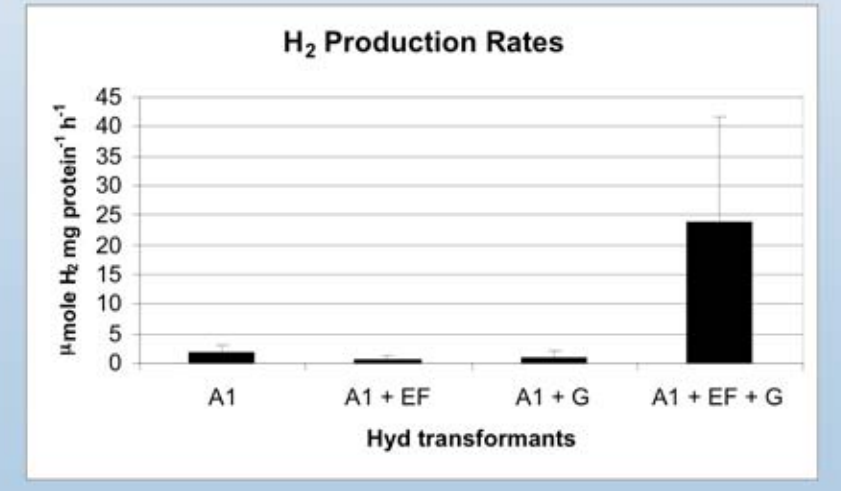
0 psec **236 psec** **443 ps**

Technical Accomplishments/Progress
Subtask 1

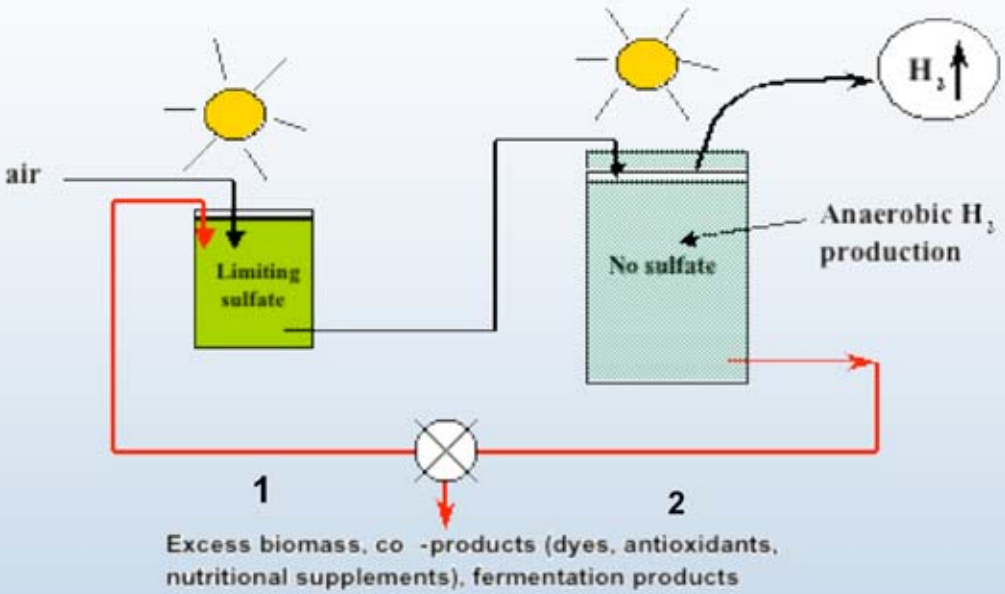
FY04 Results

We discovered how to produce an active algal [Fe]-hydrogenase in *E. coli* by co-expressing it with assembly genes identified under a DOE Office of Science project. This discovery allows us obtain large amounts of active recombinant algal (and other Fe-) hydrogenases, thereby accelerating our ability to generate and test site-directed mutants.





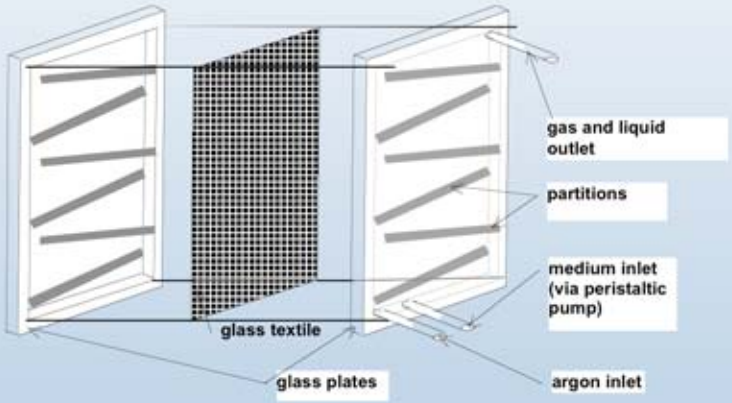
Technical Accomplishments/Progress
Subtask 2

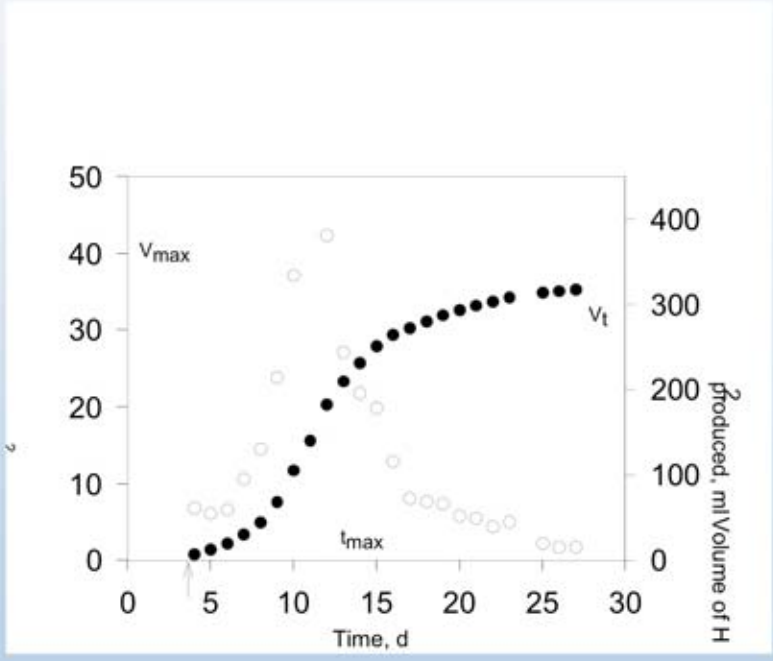


- We found that a light intensity of 50 $\mu E\ m^{-2}\ s^{-1}$ and a D_{1-2} dilution rate of 0.02 h^{-1} yielded the highest H_2-photoproduction rate at an input of 60 μM sulfate;
- The overall duration of H_2 production was extended to 6 months (DOE's continuity technical target for 2010 is 1500 hours or about 2 months).

Technical Accomplishments/Progress
Subtask 2

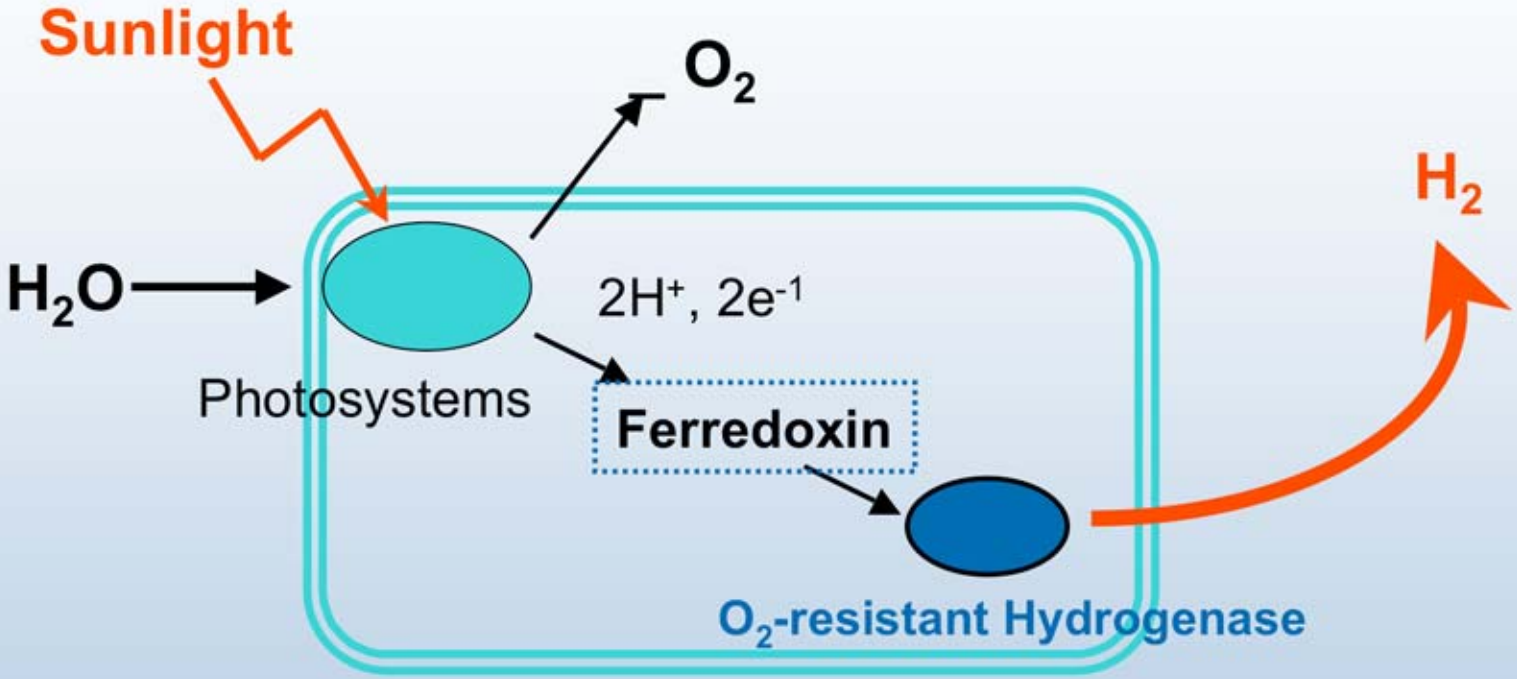
Algae were immobilized on fiberglass surfaces and sulfur-deprived. H_2 production was observed for a 6X longer period of time and at a 1.7X higher rate per liter of photobioreactor than batch suspension cultures.





Suspension cultures: 250 ml H_2 gas in 4 days at an average rate 2.5 ml L_{PhBR}^{-1} h^{-1}
Immobilized cultures: 380 ml H_2 gas in 25 days at an average rate 4.3 ml L_{PhBR}^{-1} h^{-1}

Technical Accomplishments/Progress
Subtask 3



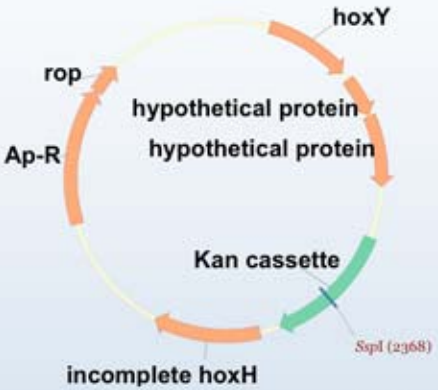
Cyanobacterial Recombinant

- A complementary approach to surmount the O_2-sensitivity issue

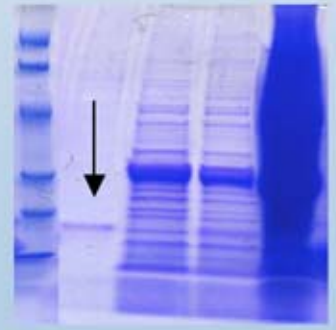
Technical Accomplishments/Progress
Subtask 3

- Constructed a plasmid to exchange the host native gene with an inactive hydrogenase gene to meet our FY04 milestone (create a hydrogenase knockout in the cyanobacterial host).

- Constructed an expression system in *E. coli* to mass produce cyanobacterial ferredoxin, the electron mediator between photosynthesis and hydrogenase. This will facilitate experiments to test the linkage of the two systems *in vitro* to complement the genetic work *in vivo* (milestone FY05).



pSMART LC-Amp containing Hox amplicon with mutated hoxH (hoxH::KanR)



Project Safety

- Safety Vulnerability Techniques
  - A hazard identification and control program is employed to identify possible failure modes and associated risks. Redundant engineering and procedural controls are used to ensure that acceptable levels of risk are not exceeded.
  - Hydrogen safety is being addressed *via* gas sensor monitoring, and potential H_2 and O_2 gas mixtures are prevented *via* active catalytic systems.
  - Gas pressure build up inside the bioreactors is prevented by appropriate venting.
  - Genetically modified organisms are physically contained and disposed of by heat or bleach treatment; NREL Radiation Safety Program guidelines for the utilization and disposal of radioactive materials are applied.
- Management of Change

  Safety techniques to address systems that simultaneously produce H_2 and O_2 are being evaluated and will be implemented in the future as mutant organisms become available.
- Lesson Learned

  Monitor the build-up of gas pressure inside bioreactors and ensure that any accumulated gas is continuously and properly vented.

Interactions and Collaborations

Collaborations:
Dr. A. Tsygankov, Russian Academy of Sciences, Pushchino, Russia

Non-Program funded interactions:
Dr. K. Schulten, Beckman Institute/ University of Illinois
NREL Computational Sciences Center
Institute of Biological Energy Alternatives (IBEA)

Publications:
4 manuscripts published, 3 in press, 5 submitted and 3 in preparation.

Patent applications submitted/granted:
1 application submitted.

Responses to Previous Year Reviewers' Comments

1. *"Encourage priority on immobilized algae cultures to go from +S to –S without centrifugation".* **The feasibility of using immobilized cultures for H_2 production was demonstrated this year. More cost-efficient immobilization matrices are one of the foci of next year's research.**

2. *"Is transfer of hydrogenase enzyme system from R. gelatinosus worth trying?"; "Focus on hydrogenase from Rubrivivax into cyanobacteria as planned".* **These encouraging comments have resulted in the funding of Subtask 3, a new subtask in FY2004**.

3. *"Very long term R&D and very basic"; "… may need to be funded out of basic R&D"; "Will this meet the President's goal?" "…may need to be funded out of the Office of Science as longer term objectives".* **There are clear benefits in using parallel applied and exploratory approaches to biological H_2 production, as evidenced by the results reported this year in Subtask 1 regarding the heterologous expression of the algal hydrogenase in the bacterium *E. coli*.**

Responses to Previous Year Reviewers' Comments (continued)

4. "Curiously schizophrenic – the H_2 production being measured "indirect biophotolysis" yet the engineering is all aimed at "direct"…Are the researchers clear on the distinction? Is the economist who gave the remarkable $ value?" "They will have very different costs"

Direct biophotolysis: H_2 from H_2O

(a) Simultaneous H_2 and O_2 production (Subtask 1)

(b) Spatially separated sulfur-deprived cultures (Subtask 2)

Indirect biophotolysis: H_2 from starch

Estimated costs of $2.34/kg (W. Amos, NREL) are based on an optimized spatially separated system

Future Work

Subtask 1

- Model H_2 gas diffusion to ensure that it will not be inhibited in current and future O_2-resistant mutants;
- Continue iterative process of (a) O_2-gas-diffusion computational simulations and (b) experimental generation and testing of the O_2-resistance of hydrogenase mutants expressed in *E. coli*.

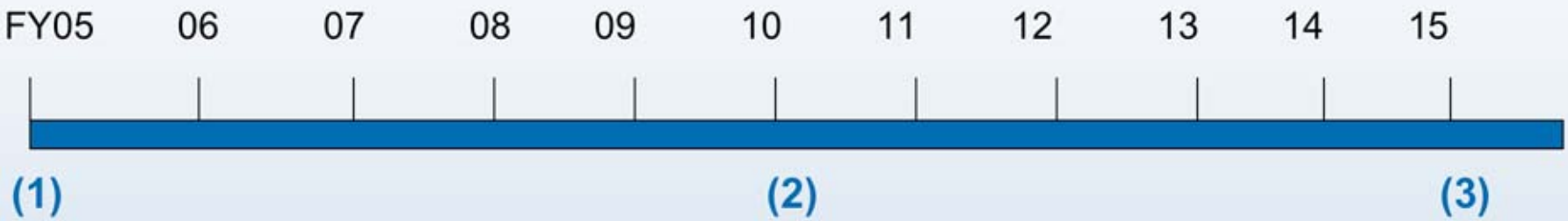
Subtask 2

- Continue optimization of H_2 production in the continuous system by investigating the effect of other culture parameters (e.g., limiting sulfate concentration and pH) ;
- Engineer a cyclic H_2-production system using fiberglass immobilized cultures;
- Initiate studies on the feasibility of low-cost matrices for cell immobilization (proprietary information);
- Perform an economic analysis of immobilized algal systems.

Subtask 3

- Construct a hydrogenase knockout mutant in *Synechocystis;*
- Link cyanobacterial photosynthesis to the oxygen-resistant bacterial hydrogenase, *in vitro* and ultimately *in vivo*.

Future Work (continued)



NREL, UCB and ORNL team consolidated goal:

(1) "Virtual" optimized organism; H_2 cost $100/kg;
(2) Combined triple mutant organism; H_2 cost $30/kg;
(3) Optimized organism; H_2 cost $2/kg.

Exploratory research will continue in parallel to address other related issues