Biological Systems for Hydrogen Photoproduction

DOE Hydrogen Program

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NREL

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Team members:
Mike Seibert, Paul King, Sergey Kosourov, Christine English, Sharon Smolinski

Project ID# PDP 26

Overview

DOE Hydrogen Program

Timeline

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

Barriers

- Barriers addressed
  - Production Barrier: Continuity of H_2 production.

Parameters	Current Status	2013 Target	Maximum Potential
Duration of continuous photoproduction	90 days (-S, anaerobic) 6 days (-S, aerobic, immobilized)	10 min (aerobic)	12 hours (aerobic)
O_2 tolerance (half-life in air)	~ 4 min after exposure to air (clostridial enzyme)		

Budget

- Funding received in FY08: $2,000K
- Funding allocated for FY09: $0

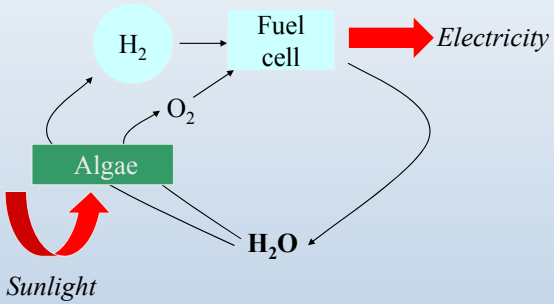
Partners

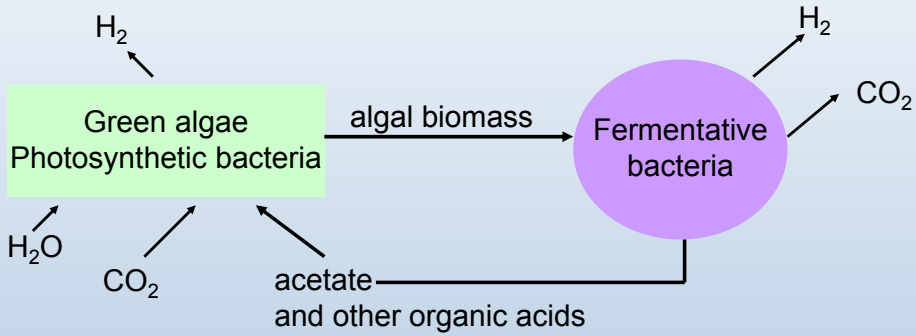
- **Dr. Anatoly Tsygankov, Russian Academy of Sciences, Pushchino, Russia.**
- **Dr. Michael Flickinger, North Carolina State University.**
- **Dr. Klaus Schulten, The Beckman Institute, University of Illinois.**
- **Dr. Mace Golden, GoldenBioenergy, CO**

Objectives

DOE Hydrogen Program

Develop and optimize aerobic photobiological systems for the production of H_2 from water (subtask 1); utilize the sulfur-deprivation platform to address biochemical and engineering issues related to photobiological H_2 production (subtask 2); integrate photobiological with fermentative organisms to more efficiently utilize the solar spectrum and the substrates/products from each reaction (subtask 3).





FY2007/08:

Subtask 1: Engineer a H_2-producing catalyst ([FeFe]-hydrogenase) that has an extended half-life, following exposure to O_2.

Subtask 2: Demonstrate improvements in the light conversion properties of a H_2-producing anaerobic algal system by immobilizing the cultures at high cell density on a thin-film matrix;

Subtask 3: Test the ability of H_2-producing, fermentative organisms to consume algal biomass; optimize photosynthetic bacterial H_2 production.

Milestones

	Milestones	Completion Date	
3.3.1.	Participate in DOE's annual program review	06/08	completed
3.3.2	Generate and test 5 new mutants for O_2 tolerance	01/08	completed
3.3.3	Determine the light conversion efficiency of stacked photobioreactors vs. single photobioreactors	03/08	completed
3.3.4	Test the performance of immobilized ATPase mutants	06/08	completed
3.3.5	Test the performance of immobilized antenna mutant	10/08	
3.3.6	Introduce and test the expression of a clostridial hydrogenase gene in *Chlamydomonas*	08/08	
3.3.7	Design and test a new DNA construct for E. coli expression of hydrogenases	12/08	

Plan and Approach

DOE Hydrogen Program

Subtak 1:

1. Apply site-directed mutagenesis to a bacterial hydrogenase; express mutant enzymes in *E. coli* and test mutants for increased tolerance to O_2;
2. Initiate random mutagenesis/higher-throughput approaches for generation of hydrogenases with increased tolerance to O_2; express mutant enzymes in *E. coli* and test mutants for increased tolerance to O_2;
3. Develop DNA plasmids to express heterologous wild-type or mutated hydrogenases in *Chlamydomonas reinhardtii* in order to test O_2-tolerance in the alga.

Subtask 2:

1. Determine the rates and light conversion efficiency of H_2 production by *C. reinhardtii* cultures immobilized in alginate films;
2. Test the performance of truncated antenna and proton channel mutants under the same conditions;

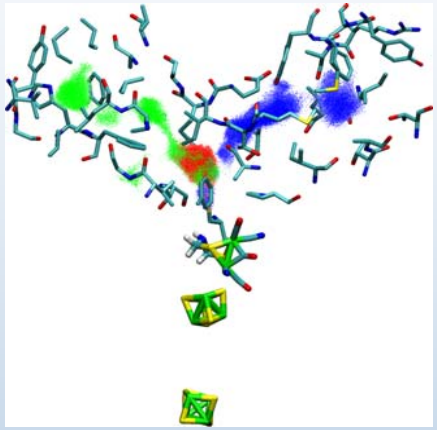
Subtask 3: the continuation of this subtask will be determined by the results of a techno-economic analysis to be performed in FY08-09

1. Test the advantages of using single or stacked reactors of green algae and photosynthetic bacteria for increased H_2 production per illuminated area;
2. Determine whether a H_2-producing, fermentative consortium can utilize spent algal and photosynthetic bacteria biomass;
3. Find a consortium of thermophilic organisms that is able to ferment organic wastes, produce organic acids to support photosynthetic bacteria growth, and produce H_2 at high efficiency.

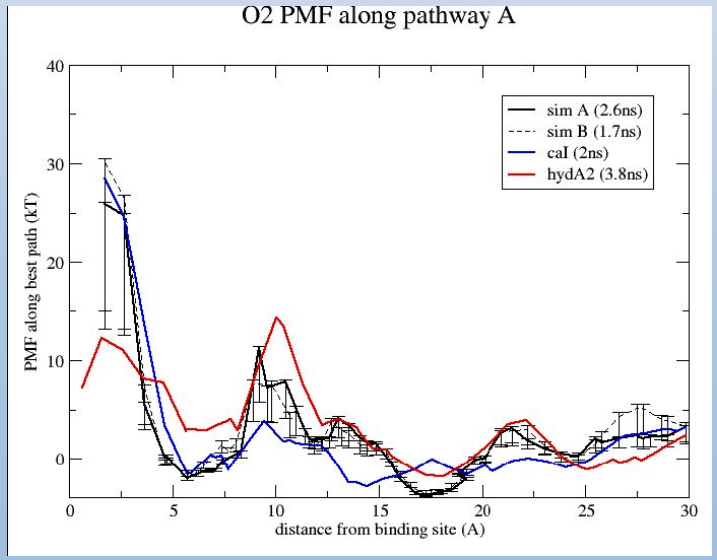
Technical Accomplishments

DOE Hydrogen Program

Subtask 1. Site-directed mutagenesis (C. English P. King, and M. Seibert)



Two pathways for O_2 diffusion from the surface of the hydrogenase protein to its catalytic site were previously identified by computational simulations (molecular dynamics and solvent accessibility). Single and double mutants along the two pathways were generated to sterically hinder O_2 diffusion to the catalytic site. All mutants tested showed loss of activity and increased sensitivity to O_2.

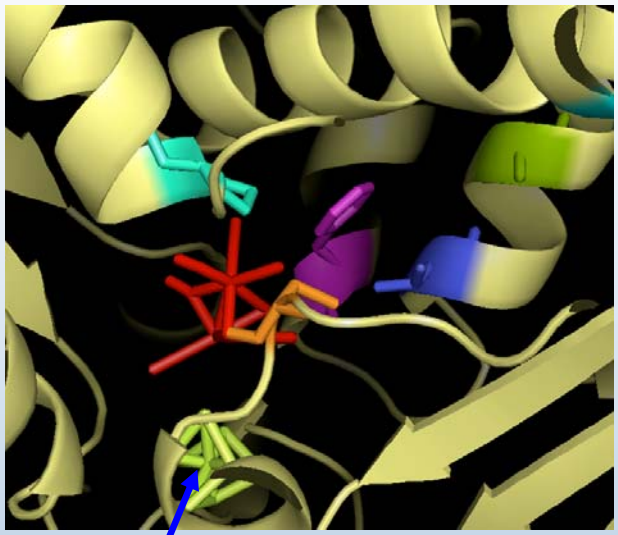


Computational simulations of the mean potential energy required to move a molecule of O_2 from the surface of the protein (right side of the graph) to the catalytic cluster (left side of the graph) suggested the presence of a high energy barrier a few Å from the cluster, at the junction of the two gas pathways (see red region in the top figure). We attempted to engineer hydrogenases in which this barrier was increased by substitution with larger residues.

Technical Accomplishments

DOE Hydrogen Program

Subtask 1. continued



H-cluster

Amino acid residues line a cavity that is likely to form the high-energy barrier described above in WT strains (see graph). One of the residues was mutated to a much larger residue. The mutated enzyme was expressed in *E. coli,* and its activity was measured in membrane-free cell extracts. For this work, we prepared enzyme without the addition of chemical reductant. Both wild-type and mutant disclosed more than one level of O_2 tolerance, characteristic of a distribution of redox states of the enzyme. These distribution of states differ for the mutant compared to wild-type, affecting the measured rate of O_2 inactivation. It is not immediately clear how this new discovery could impact the HFC&IT project.

These new results underscore the complexity of the hydrogenase system with respect to factors that control O_2 tolerance (redox state of the enzyme, O_2 diffusion, nature of the ligands to the catalytic center), and point out the need for further research to deconvolute the influence of these factors on the measurement of O_2 tolerance of the enzyme.

Technical Accomplishments

DOE Hydrogen Program

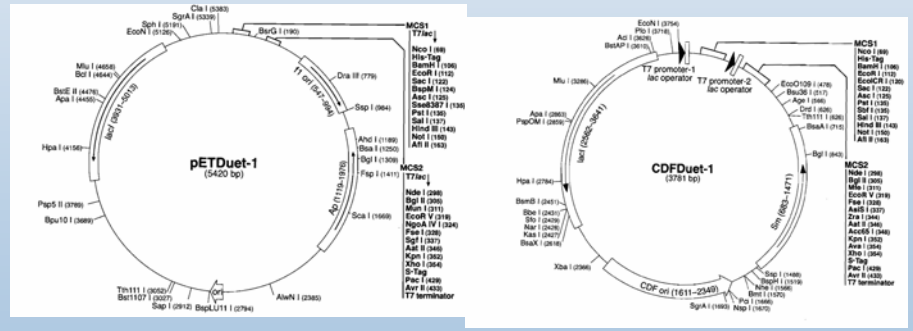
Subtask 1. Random mutagenesis/higher throughput assay (P. King, M. Golden)

Rationale: to improve the probability of finding O_2-tolerant mutants

Mutagenesis

Error-prone PCR amplification of a partial gene sequence: introduces random errors

Expression in *E. coli*



Screening

An automated gas chromatograph assay will be developed to screen for O_2-tolerant mutants

The *hydDA* gene (encoding for the structural hydrogenase protein from *C. acetobutylicum*) and the gene encoding for one of its assembly proteins, *hydE*, will be incorporated into the multiple cloning site (MCS) of the pETDuet-1 plasmid (left), and the *hydF* and *hydG* genes, encoding for the other two assembly proteins will be incorporated into the MCS of the CDFDuet-1 plasmid (right); the *hya E. coli* promoter will be used instead of the T7 promoter to allow anaerobic induction of all heterologous genes.

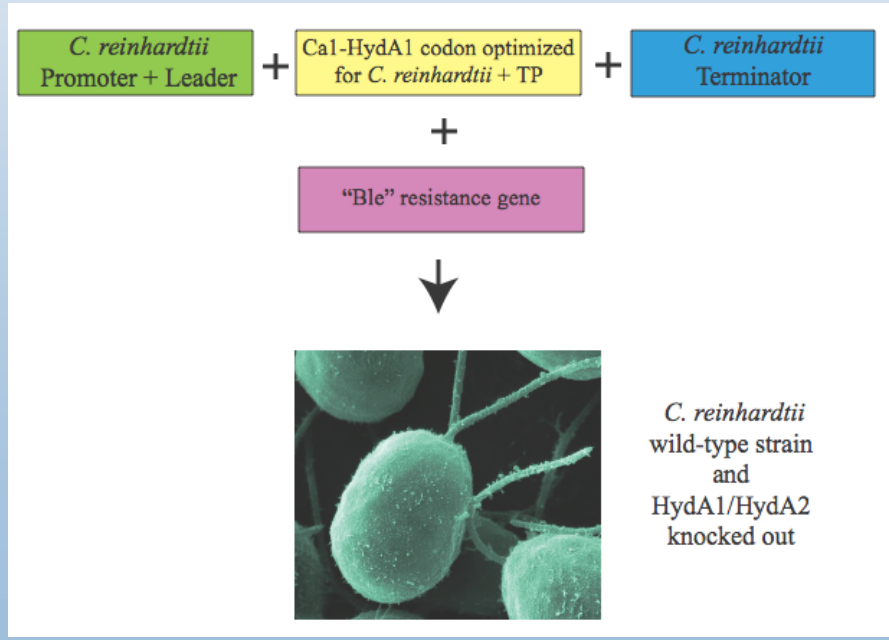
Technical Accomplishments

DOE Hydrogen Program

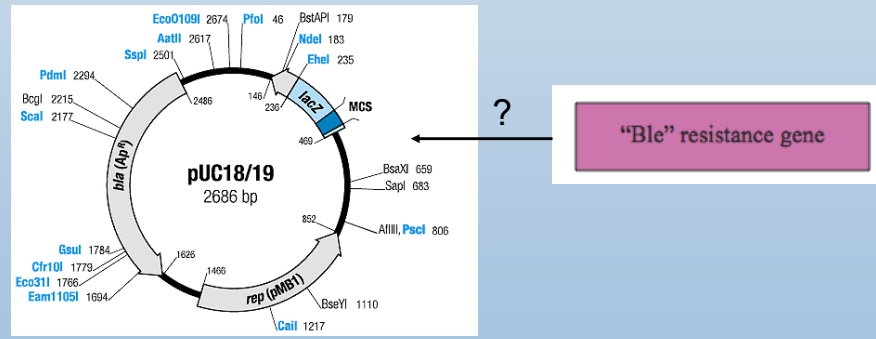
Subtask 1. Expression of clostridial hydrogenase in *C. reinhardtii* (P. King, C. English)

Rationale: the clostridial [FeFe]-hydrogenase is highly homologous to the algal hydrogenase, and it is ~ 400X more tolerant to O_2

Protocol



Current status:the DNA construct is ready; we are now attempting to clone in *ble* resistance gene.

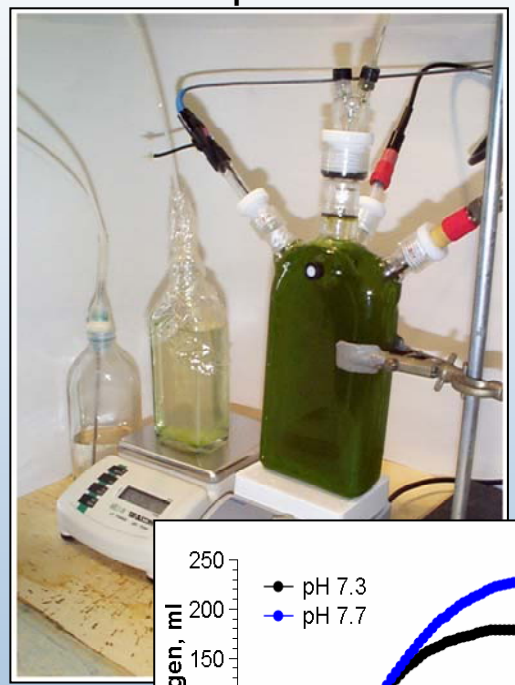


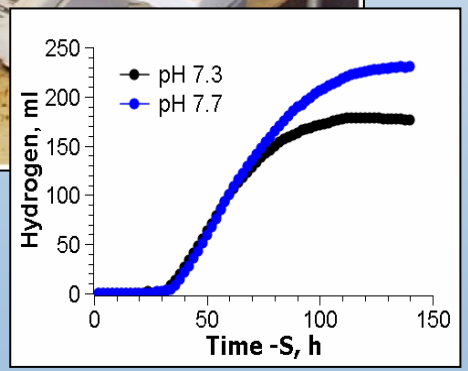
Technical Accomplishments

DOE Hydrogen Program

Subtask 2. Light-conversion efficiency of alginate-immobilized algae (S. Kosourov, M. Flickinger, M. Seibert)

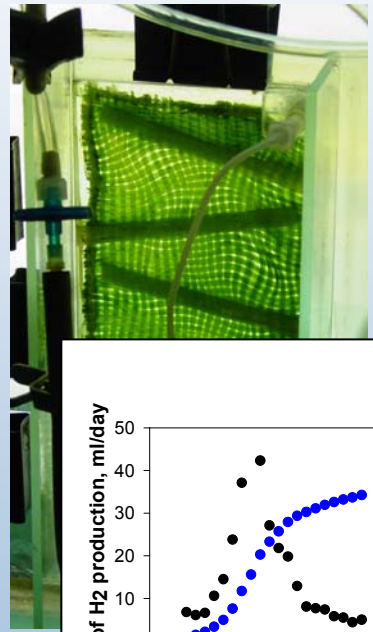
Cell suspensions

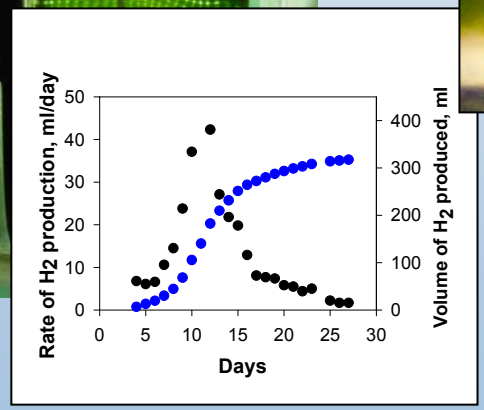




Low cell-density, short duration

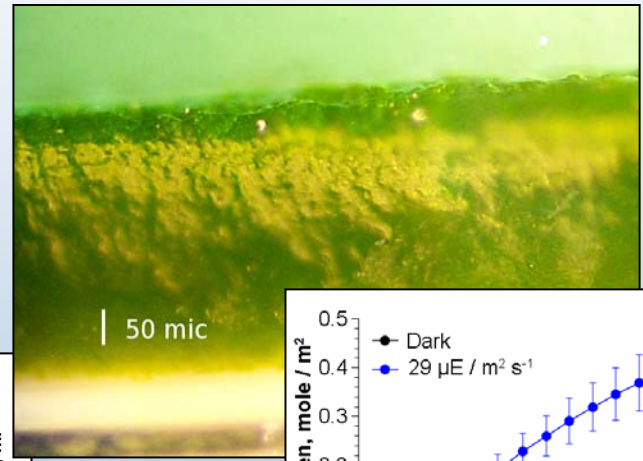
Immobilized onto glass fibers

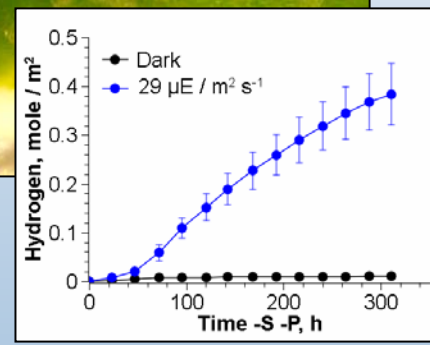




Higher cell density, longer duration
Non-degradable matrix

Immobilized into alginate films





High cell density
Long duration
Degradable matrix

Technical Accomplishments

DOE Hydrogen Program

Subtask 2. continued

	Light Intensity ($\mu E \cdot m^{-2} \cdot s^{-1}$)	Maximum specific rate of H_2 production ($\mu moles \cdot mg\ Chl^{-1} \cdot h^{-1}$)	Average specific rate of H_2 production ($\mu moles \cdot mg\ Chl^{-1} \cdot h^{-1}$)	Maximum light conversion efficiency into H_2 (%)	Average light conversion efficiency into H_2 (%)
Suspension	2x 150 (two illuminated surfaces)	9.44	3.79	0.53	0.21
Glass fibers	120	6.4	2.37	0.36	n.a.
Alginate films	29	4.71	2.43	2.17	1.28

- Funded by the U.S. AFOSR

Cell biomass in formulation, g	Average total Chl concentration, μg per 6 cm^2 screen	The total yield of H_2 in an atmosphere of argon, mol m^{-2} (A)	The total yield of H_2 in an atmosphere of air, mol m^{-2} (B)	Hydrogen production in (B) as a % of (A)
0.125	43 +/- 3	0.06 +/- 0.01	0.04 +/- 0.01	67
0.25	90 +/- 12	0.14 +/- 0.05	0.08 +/- 0.02	57
0.5	188 +/- 20	0.26 +/- 0.06	0.14 +/- 0.02	54
1	278 +/- 44	0.30 +/- 0.02	0.14 +/- 0.02	47
2	402 +/- 38	0.27 +/-.03	0.02 +/- 0.01	7

Substantially increased light conversion efficiencies

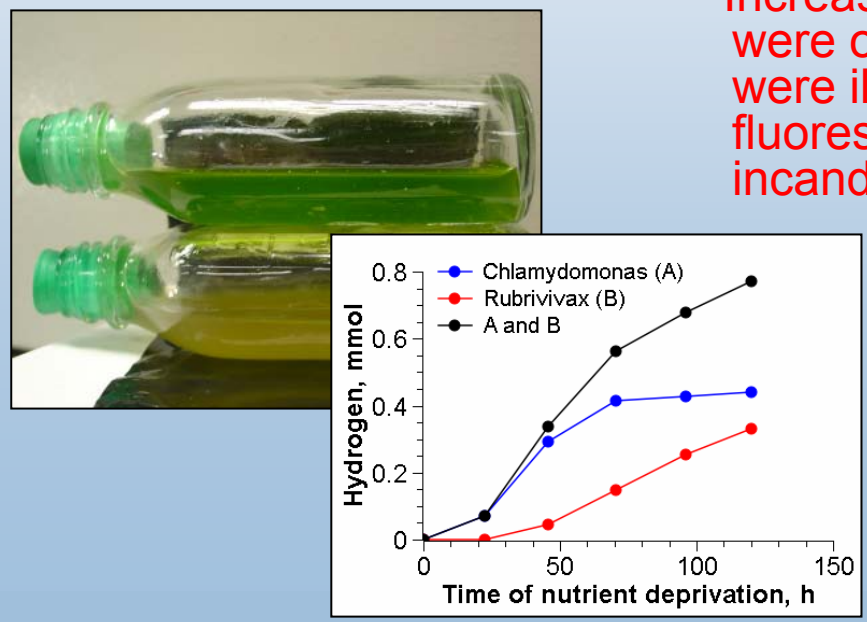
} Tolerance to O_2 depends on the amount of immobilized biomass

Technical Accomplishments

DOE Hydrogen Program

Subtask 3. Stacked photobioreactors of green algae and photosynthetic bacteria (S. Kosourov, P.C. Maness, M. Seibert)

Rationale: Green algae absorb visible light (400-700 nm);photosynthetic bacteria have absorption peaks in the infra-red (750-900 nm); by stacking two photobioreactors one is able to extend the portion of the absorbed spectrum and thus increase light conversion efficiency to H_2.

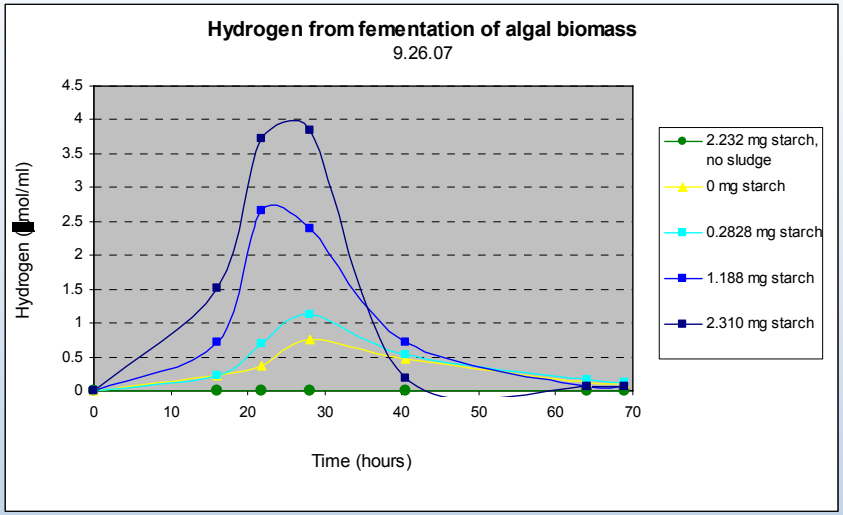


Increased rates of H_2 photoproduction were observed when the stacked cultures were illuminated with a combination of fluorescent (below 700 nm) and incandescent (including infra-red) lamps.

Technical Accomplishments

DOE Hydrogen Program

Subtask 3. Fermentative H_2-production from algal biomass (S. Smolinski, P.C. Maness, S. Kosourov)



The amount of starch present in algal biomass harvested at the end of sulfur-deprivation experiments was determined, and different amounts of biomass were added to a fermentor. Fermentative H_2 production was demonstrated and the total amount of H_2 produced was a function of the amount of biomass added.

The high molar yield of H_2/glucose observed (see last column of the table) was probably due to the capability of the fermentative organism to degrade other biomass components, such as proteins and/or lipids.

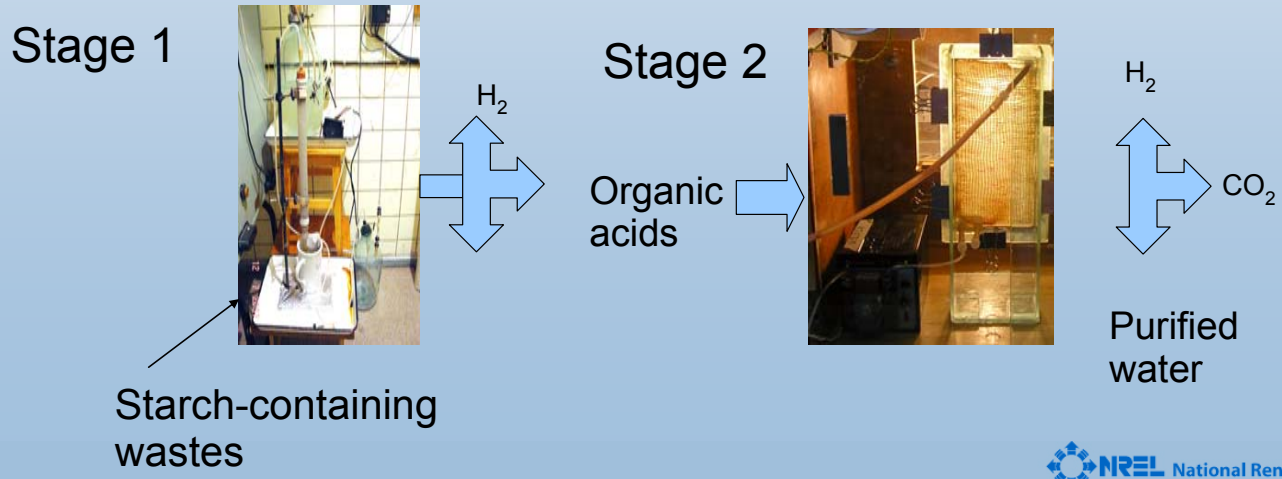
Algal biomass available (mg DW)	Algal protein available (mg)	Algal starch available (mg glucose)	Algal starch consumed (mg glucose)	Maximum H_2 accumulated in headspace (μmol)	μmole H_2/μmol glucose consumed
23.0	7.64	0.28	0.21	5.11	4.29
68.9	22.9	1.19	1.18	26.51	4.04
137.8	45.7	2.31	2.15	43.65	3.67

Technical Accomplishments

DOE Hydrogen Program

Subtask 3. H_2-production by photosynthetic bacteria (A. Tsygankov, M. Seibert)

- Samples were taken from a variety of sites in Russia, and the ability of fermentative consortia from each sample to simultaneously ferment various starch-containing wastes and produce H_2 was tested (stage 1, below). Conditions were optimized for H_2 production at 37C;
- The inhibitory effects of organic acids on photosynthetic bacterial growth and H_2 production is being studied in order to better define the type of photosynthetic bacteria that will be used in stage 2 (below);
- A quick procedure for immobilization of photosynthetic bacteria on glass fibers for stage 2 was devised.



DOE Hydrogen Program

Summary

- **Subtask 1:** (1) We discovered the existence of different states of the clostridial enzyme with differences in O_2 sensitivity and whose distribution varies between wild-type and mutants; a procedure for random mutagenesis/high throughput screening is being developed; DNA constructs for expression of clostridial hydrogenases in *C. reinhardtii* are being made.
- **Subtask 2:** (1) High light conversion to H_2 efficiencies have been demonstrated when sulfur-deprived algae are immobilized into alginate films; (2) the film was shown to protect the cultures from inactivation by atmospheric O_2 and partial rates of H_2-production were measured even under aerobic conditions.
- **Subtask 3:** (1) Increased rates of H_2 photoproduction were observed when stacked cultures of green algae and photosynthetic bacteria were illuminated with a combination of fluorescent (below 700 nm) and incandescent (including infra-red) lamps; (2) fermentative H_2-production was detected when algal biomass was used as the substrate; (3) high yields of H_2/glucose suggest that other cell components (besides glucose) are also being fermented.

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