ALGAL HYDROGEN PHOTOPRODUCTION

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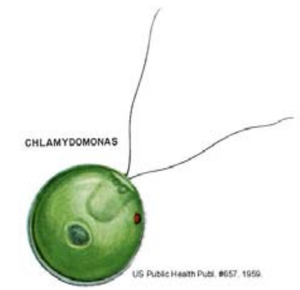


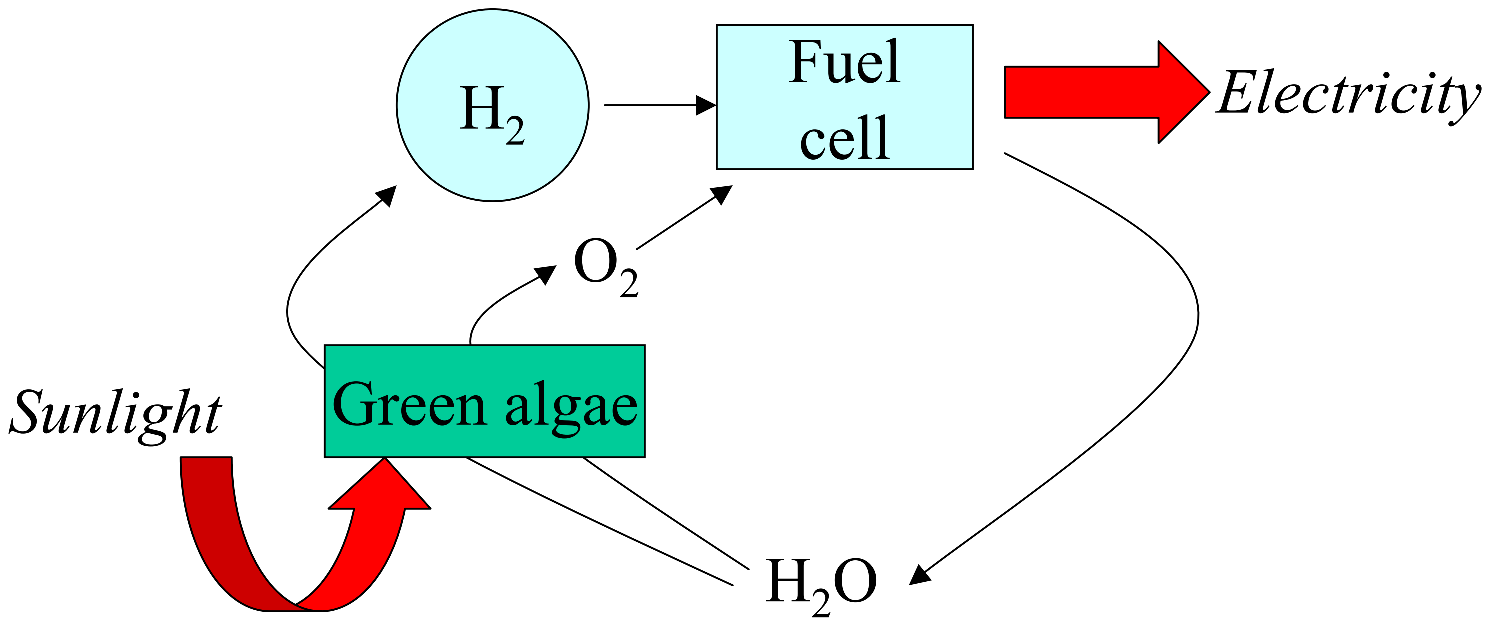
Relevance/Objectives

Develop Advanced, Renewable, Photolytic H_2-Generation Technologies Based on Algal Water-Splitting Processes

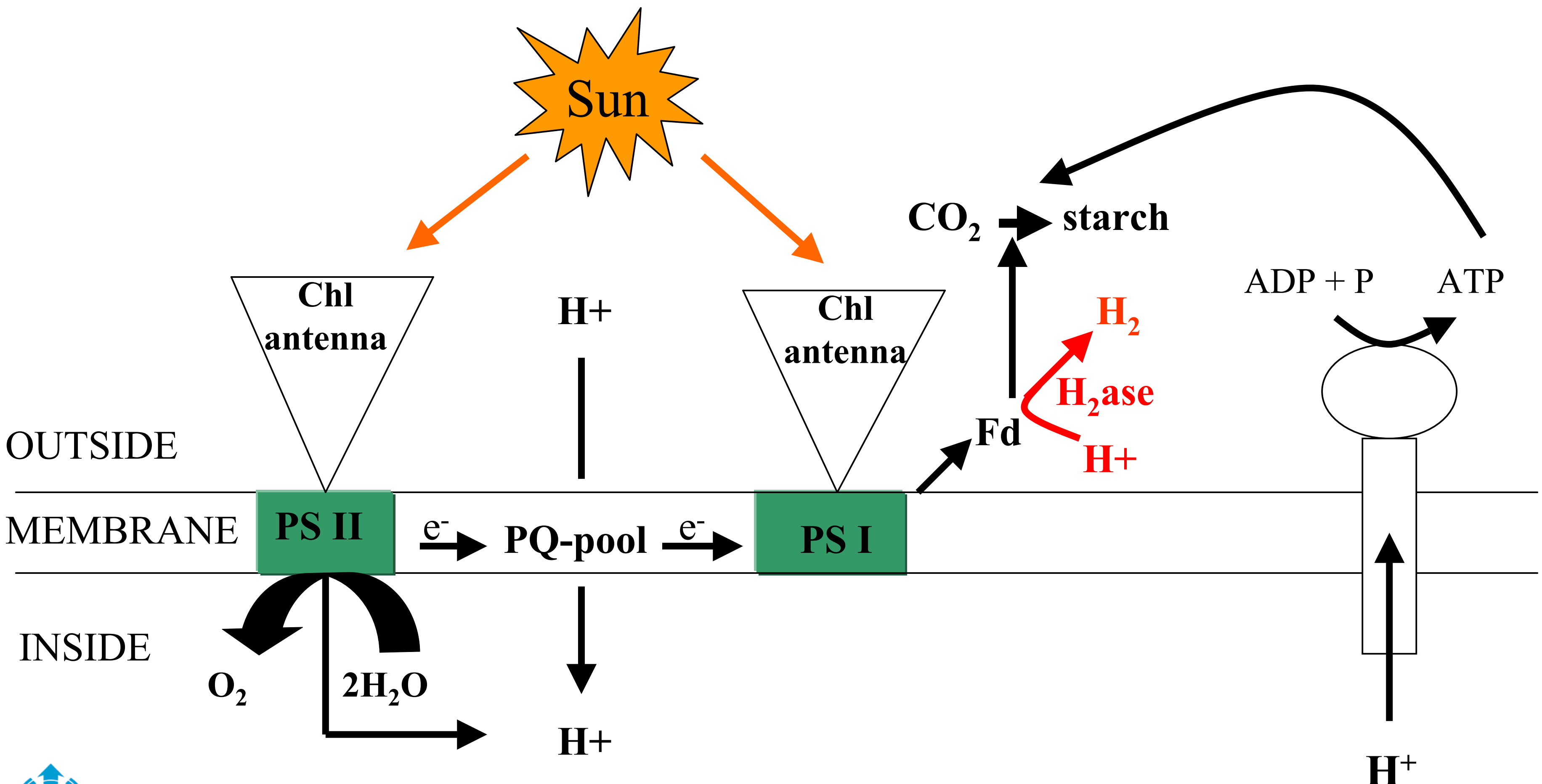
- Meet the technical challenges associated with the continuity of algal H_2-production under aerobic conditions (Technical Barrier M);

- Contribute to reaching the mid-term target of continuous H_2 photoproduction at a utilization efficiency of 20% and a cost of $30/kg by 2010 (Table 4.1.6).

Renewable Hydrogen Production/Utilization



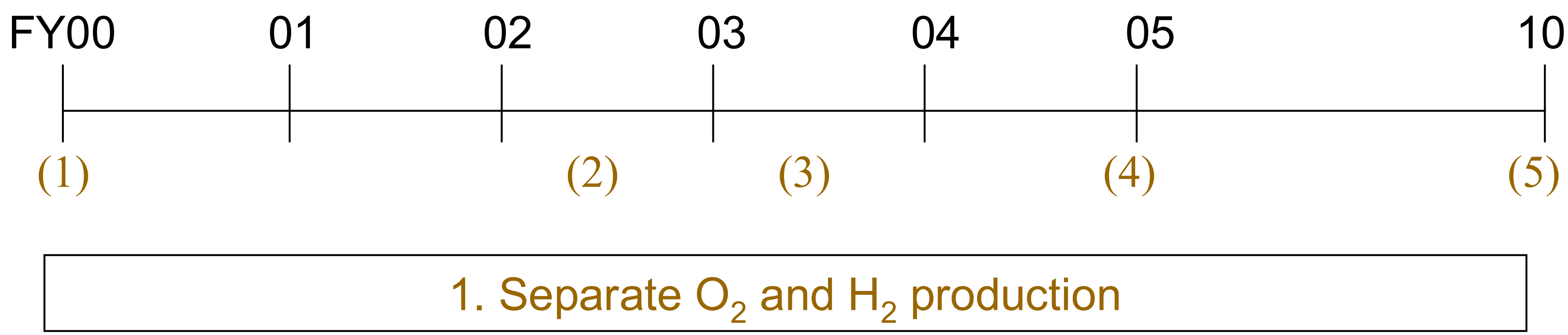
Biochemical Pathways (light reactions)



Approaches to Generate an O_2-Tolerant, Algal H_2-Producing System

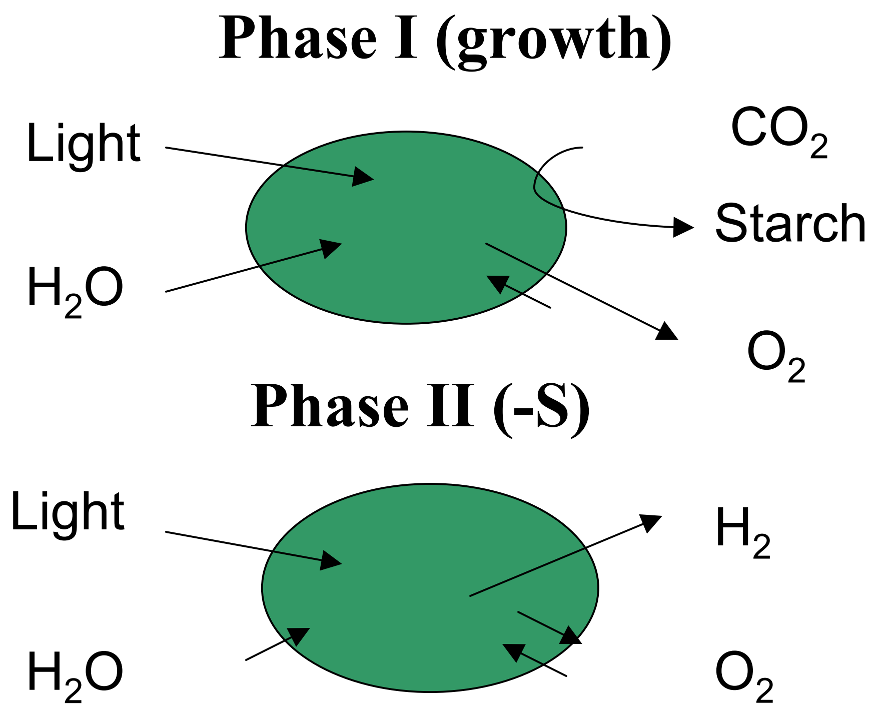
1. Separate O_2 and H_2-production either temporally or physically;
2. Engineer a hydrogenase that functions in the presence of O_2.

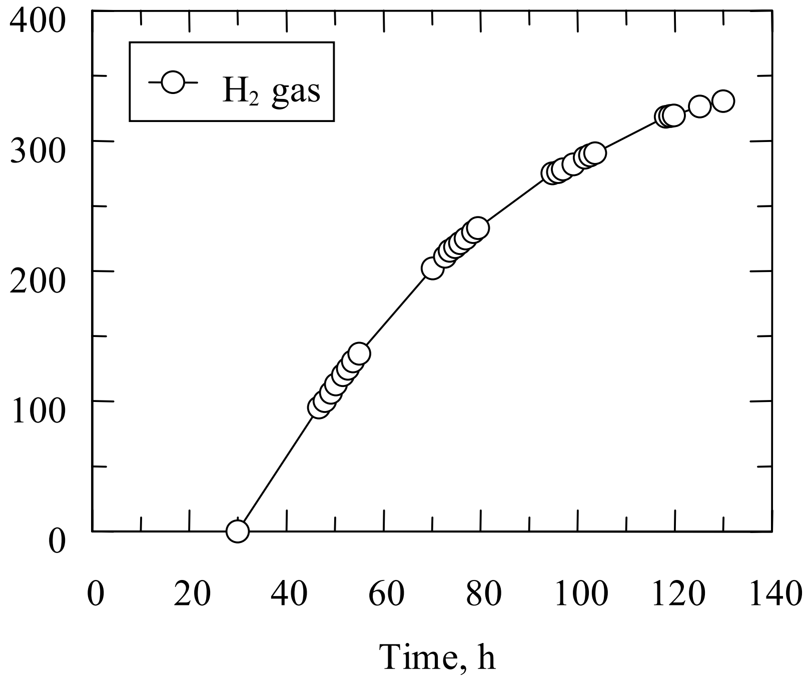
Significant Past Results and Future Milestones FY00-FY10



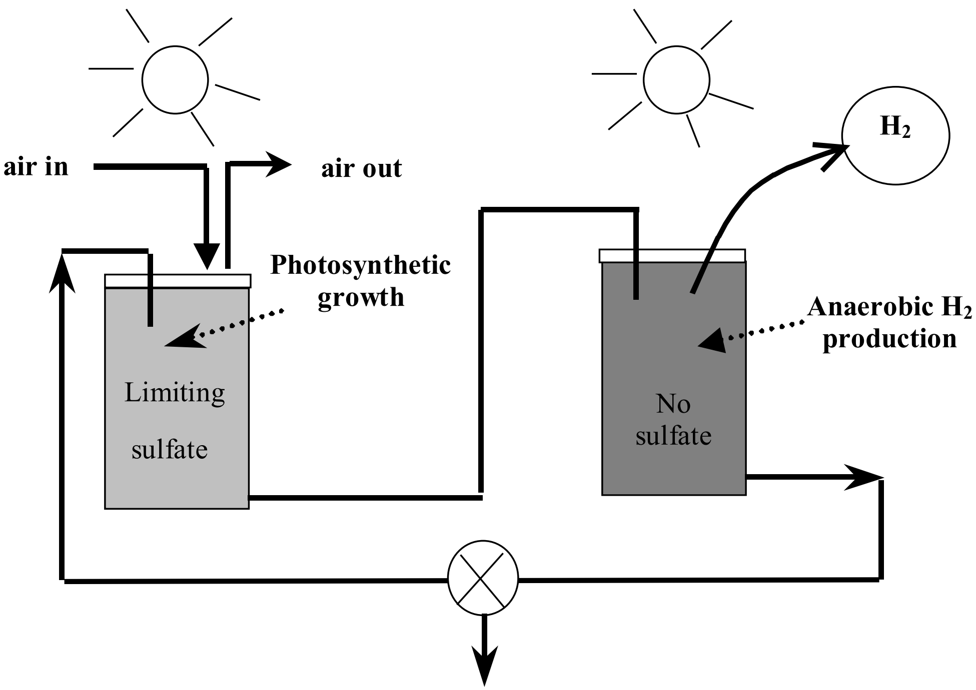
(1) 96 hours H_2-production per batch operation cycle;
(2) 240 hours of continuous H_2-production at sustained rates;
(3) 480 hours of continuous H_2-production (Milestone) but at decreased end rates;
(4) 500 hours of continuous H_2-production at sustained rates;
(5) 1500 hours of continuous H_2-production at sustained rates.

1. Temporal Separation of O_2 and H_2 Production (batch system)

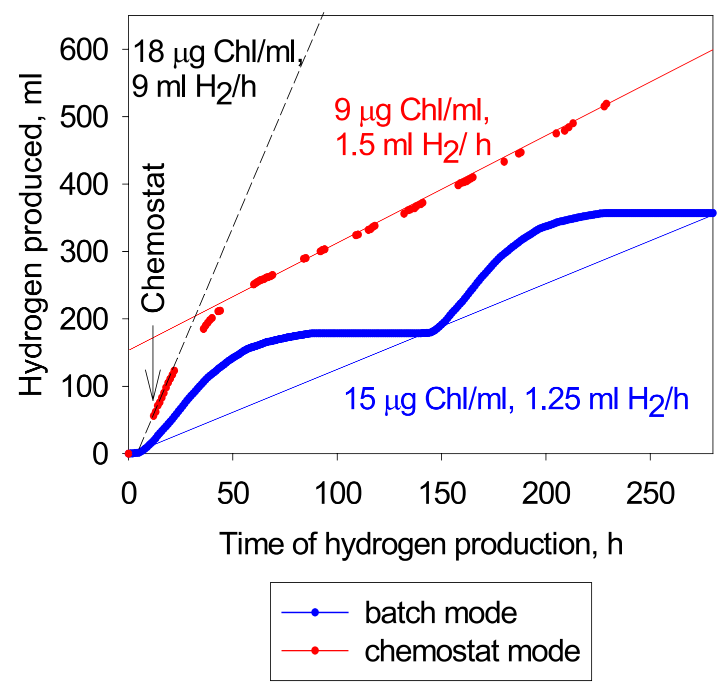




Physical Separation of O_2 and H_2 Production (continuous system)



- biomass
- co-products (dyes, protein, antioxidants, nutritional supplements, pharmaceuticals, etc.)
- fermentation products (acetate, formate)



NREL

This represents a major break-through!

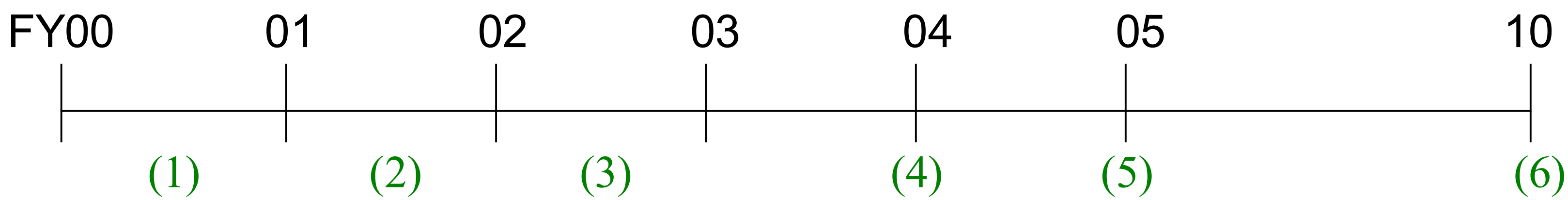
Effects of Research Progress on Projected H_2 Cost

Continuous photoproduction of pure H_2 at a rate of 1.5 ml H_2 gas/liter culture

- Current system hydrogen cost: \$200/kg, down from \$760/kg in FY00;
- Projected hydrogen cost: \$2.34/kg (land-based system), < \$1.40/kg (ocean-based system).

NREL

Significant Past Results and Future Milestones FY00-FY10

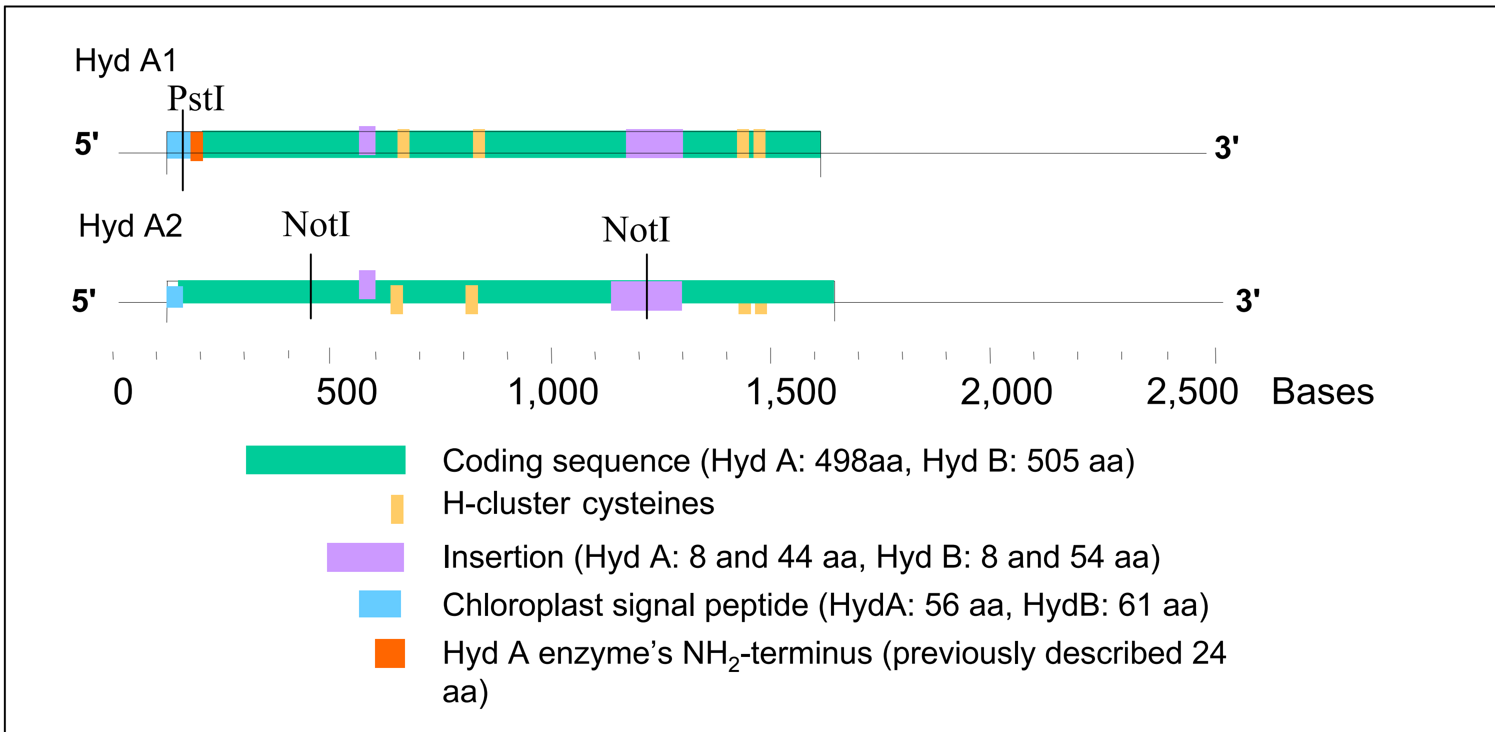


2. Engineer the hydrogenase enzyme

(1) Cloned and sequenced the catalytic site of 2 algal hydrogenases;
(2) Cloned and sequenced 2 algal hydrogenase genes;
(3) Generated our first mutant with improved O_2 tolerance (0.1% to 1% O_2);
(4) Generate 3 new mutants tolerant to O_2;
(5) Achieve 10% utilization efficiency in a theoretically integrated organism (with U.C. Berkeley and ORNL);
(6) Achieve 20% utilization efficiency in a physically integrated organism (with U.C. Berkeley and ORNL).

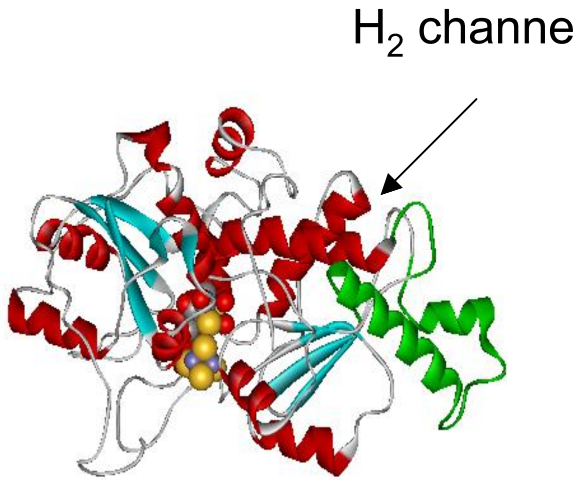
2. Engineering the Hydrogenase for O_2-Tolerance

Cloning of two algal hydrogenases

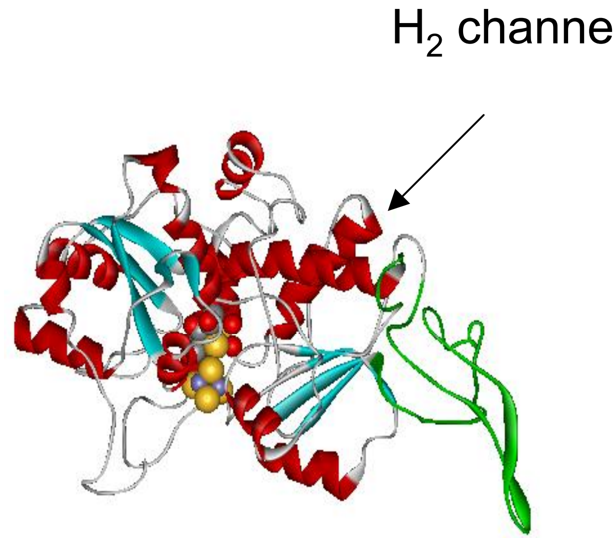


Engineering the Hydrogenase for O_2-tolerance

Structural modeling of the two proteins



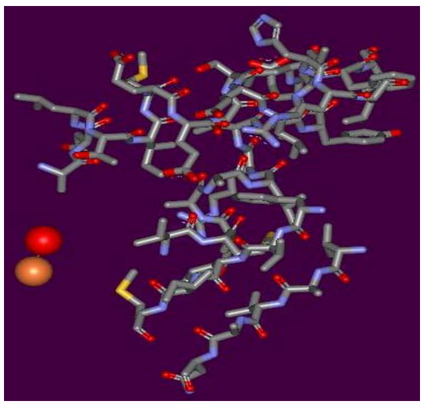
C. reinhardtii HydA1



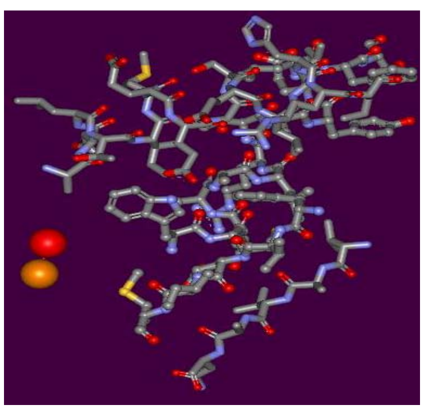
C. reinhardtii HydA2

Engineering the Hydrogenase for O_2-tolerance

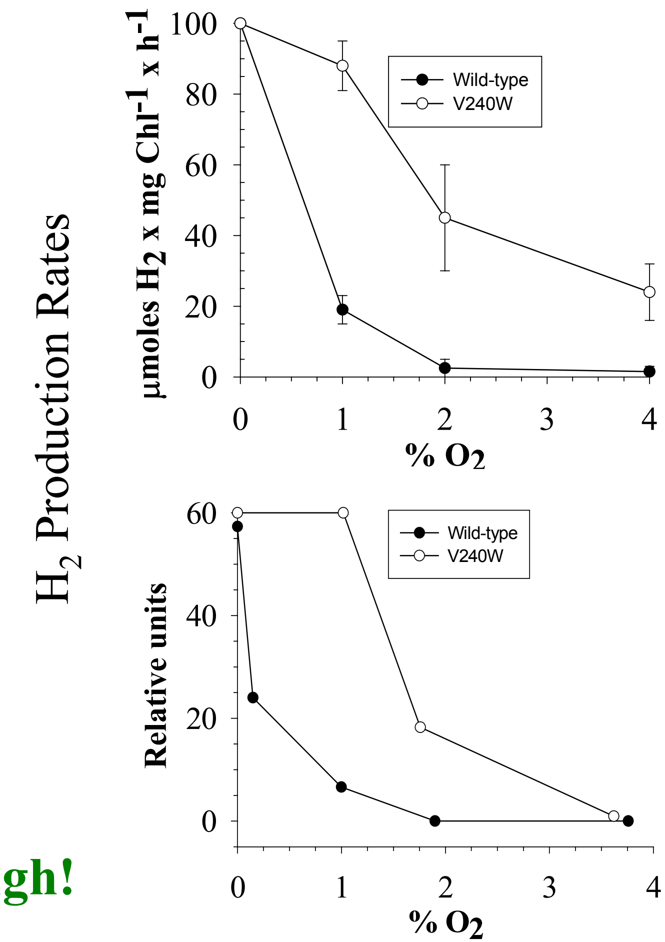
Generation of a H_2-channel mutant



HydA1



V240W



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Interactions with Others

- Papers: 7 published or in press; 3 submitted. Patents: 1 issued, 2 submitted.
- A. Melis (UC Berkeley) and J. Lee (ORNL) – Team is coordinating efforts to generate a small chlorophyll antenna mutant with non-limited rates of electron transport and an O_2-tolerant hydrogenase.
- A. Rubin (Moscow State University) and A. Tsygankov (Russian Academy of Sciences, Pushchino, Russia) – collaborating on physiological and engineering studies of algal H_2 production;
- T. Happe (U. Bochum, Germany) – collaborated on the cloning of the algal hydrogenases;
- D. Ahmann (Colorado School of Mines) – using gene shuffling techniques to generate O_2-tolerant mutants;
- R. Schulz (Christian Albrechts University, Kiel, Germany) –studying the H_2-photoproduction capability of different green algal species;
- W. Jacoby (University of Missouri) – examining photobioreactor engineering questions.

Plans and Future Milestones for FY04

- **Separate O_2 and H_2 production:**

  - maximize cell density in the continuous system to increase the volume of H_2 gas collected (May 2004);

  - improve the efficiency of H_2 photoproduction using immobilized algal cultures (July 2004).

- **Engineer the algal hydrogenases:**

  - Generate and test double mutants of the gas channel (August 2004)

  - Isolate algal hydrogenases for structural studies (September 2004)

Response to Last Year's Panel

- *"Little evidence of practicality"; "not likely to be a source of reasonably price hydrogen"; "mentions secret engineering/ economic study regarding practical (allegedly) application"; "it is difficult to know if biological hydrogen production will ever be a large scale source of hydrogen"; "see no useful purpose for this project".*

  Economic analyses done for DOE by the NREL Analysis Team (Nov. 2002) indicate that, currently, the two-stage system is not economical. However, when operated with a small antenna size mutant (U.C. Berkeley), at the maximum theoretical electron transport rates (ORNL), and at optimized productivity (NREL), **estimated prices of H_2 are about $2.34/kg**.

- *"Results are not encouraging"; "the goal is not within sight and presumably unattainable in green algae"; "the holy grail is beyond reach".*

  Our **results this year serve as definitive proof that it is indeed possible to engineer an O_2-tolerant hydrogenase** by modifying O_2 access to the catalytic site along the gas channel. This is a longer-term approach, and our milestone for the generation of an ideal organism is 2010.