U.S. Department of Energy
Hydrogen Program

A Prospectus for Biological H_2 Production

The Hydrogen Economy

- **The hydrogen economy pertains to a world fundamentally different from the one we now know. Hydrogen as a fuel, and a source of electricity, could be produced in sufficient amounts domestically, cleanly, and cost-effectively from a variety of resources, including such renewables as sunlight, biomass and water.**
- **Hydrogen-powered fuel cells and engines would be as common as the gasoline and diesel engines of the late 20th century—they would power cars, trucks, buses, and other vehicles, as well as homes, offices and factories.**
- **There are many challenges to building a hydrogen economy. It's not a vision that will be realized tomorrow—but it is achievable, and together with its partners, the Department of Energy is working to make it happen.**

Goals of the DOE Biological H_2-Production Program

- **Use rational design, strain development, and strain optimization in unicellular green algae, cyanobacteria, photosynthetic, and dark fermentative bacteria to facilitate efficient production of H_2 from renewable resources.**
- **Investigate the feasibility of integrating different photo-biological H_2 processes and fermentative H_2 production in order to maximize solar spectral utilization, efficiently recycle biomass and fermentation products, and improve the economics of the process.**
- **Identify and develop cost-effective photobioreactor components such as transparent, durable hydrogen-impermeable materials.**
- **By 2015, verify the feasibility of these technologies to be competitive in the long term.**

Biological
Catalysis

Hydrogen

Hydrogen biotechnology seeks to convert and store the energy of Sunlight as renewable Hydrogen.

Biological catalysts for the generation of H_2 are found in microorganisms such as unicellular green algae, cyanobacteria, photosynthetic bacteria, and in some forms of dark fermentative bacteria.

Prospects for Photobiological Generation of Renewable Hydrogen

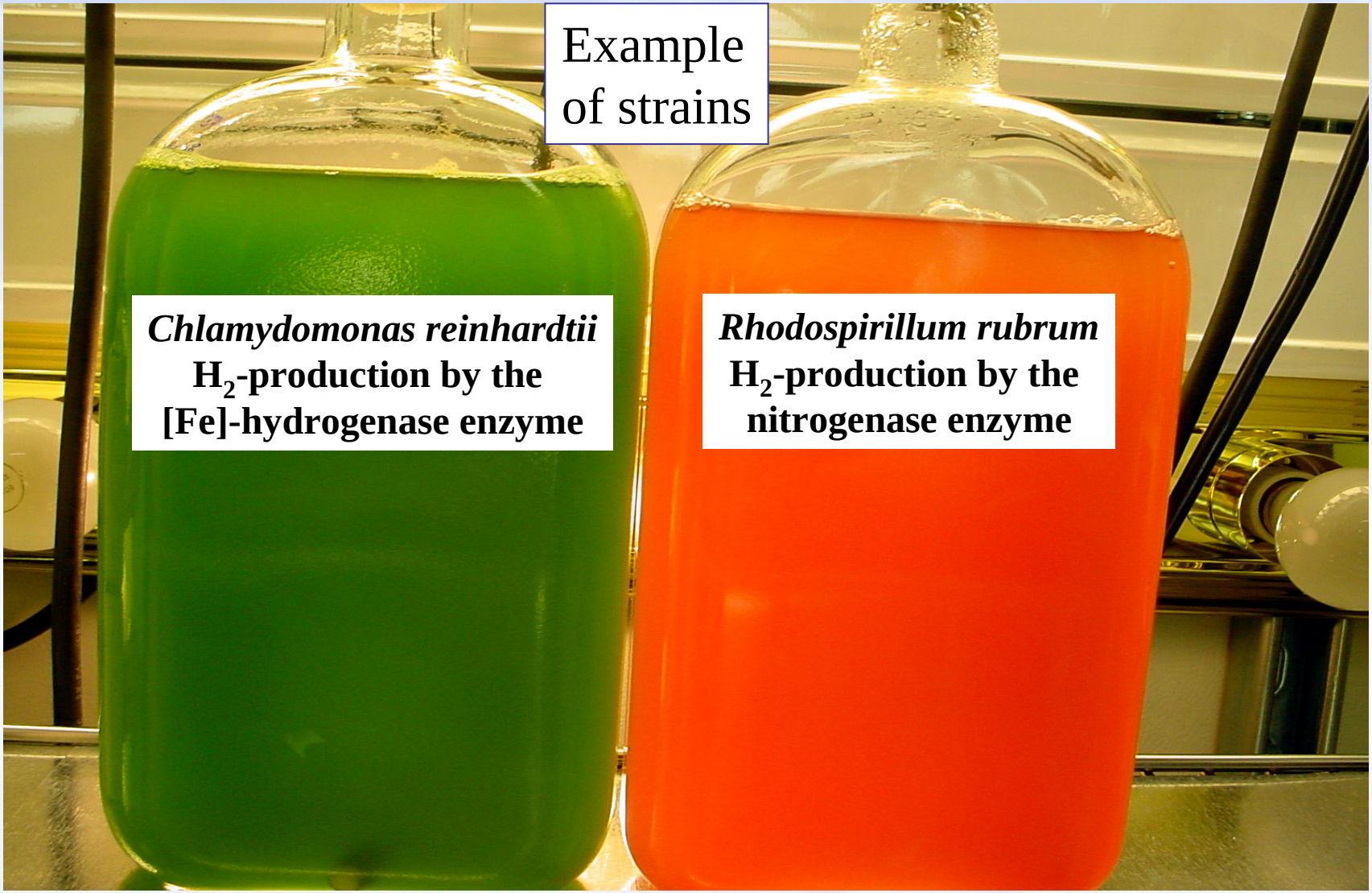
- **Average solar insolation in the US : 4.2 kWh m^{-2} d^{-1}**
- **Photosynthetic solar conversion efficiency : 0.42 kWh m^{-2} d^{-1}**
- **To displace all of the gasoline consumed in the United States, an area of 5,500 square miles would be needed, which is equivalent to 0.15% of the land area of the U.S.**

Sunlight as a source of energy is Clean and Unlimited. But it is also diffuse, averaging 4.2 kWh per square meter per day in the US. Assuming a 10% solar conversion efficiency to hydrogen, it was estimated that about 5,500 square miles of surface area would be needed for the harvesting and conversion of the enough sunlight to displace all of the gasoline consumed in the United States.

Technical Challenges

- **The primary technical challenge is to lower costs of production in order to make BioHydrogen a commercially viable primary energy carrier.**
- **Hydrogen production pathways in a variety of unicellular green algae, cyanobacteria, photosynthetic bacteria, and dark fermentative bacteria need to be optimized.**
- **A number of specific barriers to the cost effective production of BioHydrogen need to be overcome.**
- **These barriers are described in the technical plan section of the Multi-Year Research, Development and Demonstration Plan for DOE's Hydrogen, Fuel Cells & Infrastructure Technologies Program.**

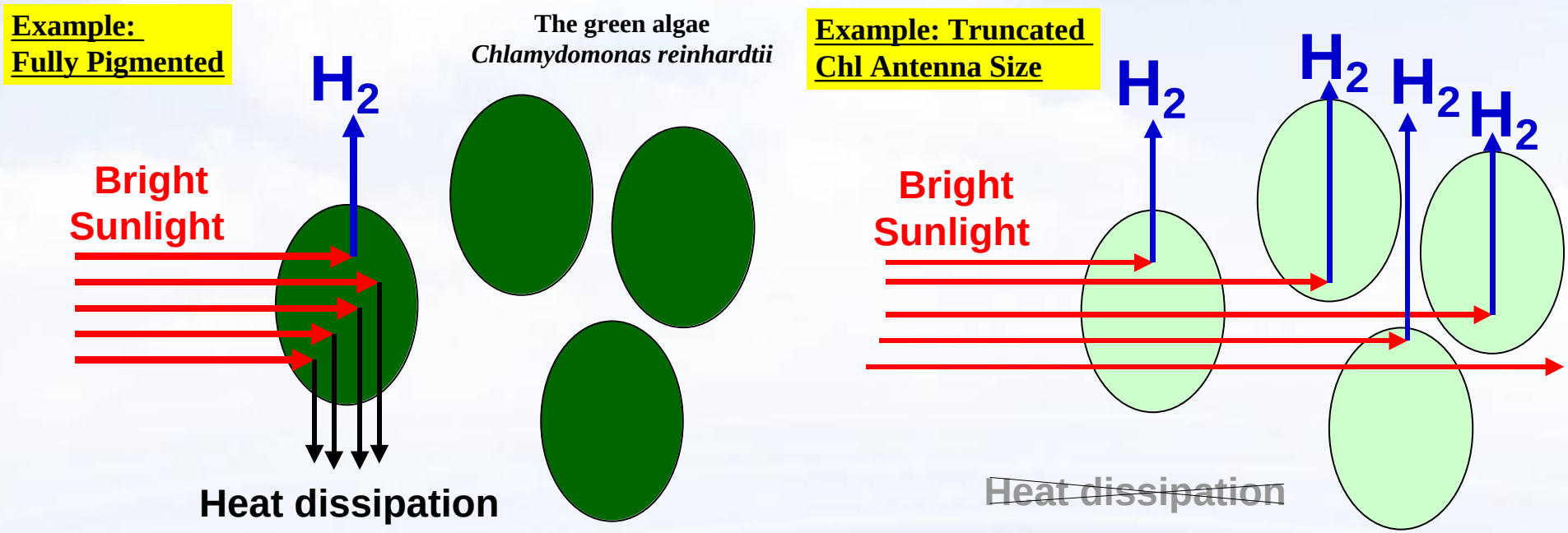
Example of strains



H_2-producing green algae **H_2-producing photosynthetic bacteria**

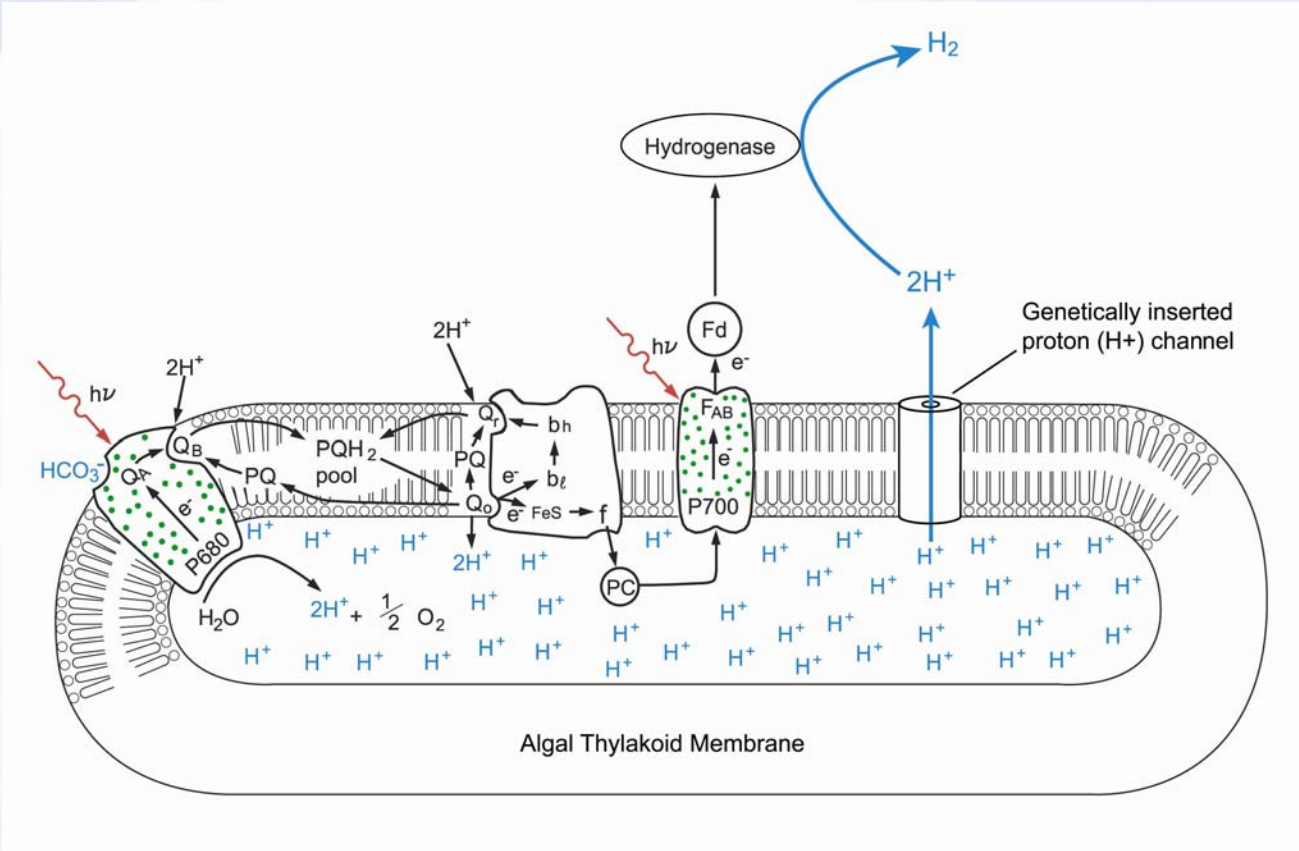
Green algae and photosynthetic bacteria could operate with a solar energy conversion efficiency to H_2 as high as ~10% and ~6%, respectively, provided that specific barriers are overcome.

Addressing Barrier X: Low sunlight utilization efficiency due to a large chlorophyll antenna size



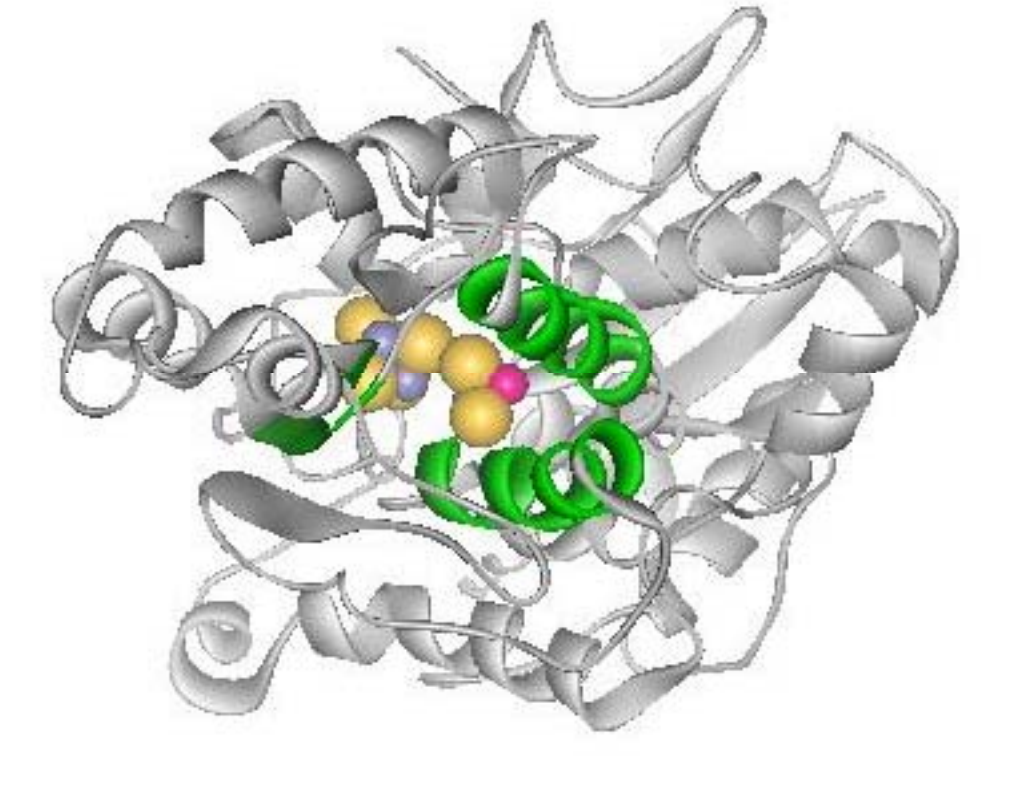
Schematic presentation of the fate of absorbed sunlight in fully pigmented (left) and truncated Chl antenna size algae (right). Fully pigmented cells at the surface of the culture over-absorb incoming sunlight (i.e., they absorb more than can be utilized by photosynthesis), and 'heat dissipate' most of it. This is alleviated by the truncated, or smaller Chl antenna size of the photosystems. The research seeks to develop green algae with a "truncated light-harvesting chlorophyll antenna", which produce more H_2 per bioreactor surface area.

Addressing Barrier Y: Slow rate of H_2 production due to non-dissipation of proton gradient across thylakoid membranes



The rate of photobiological H_2 production from water is limited by proton accumulation inside the algal thylakoids. This barrier is being eliminated upon a genetic insertion of proton channels into the algal thylakoid membranes. The proper application of such proton channels across thylakoid membranes could substantially enhance photobiological H_2 production. Moreover, it would also alleviate other competitive processes, such as inhibition of H_2 production by electron flow to CO_2.

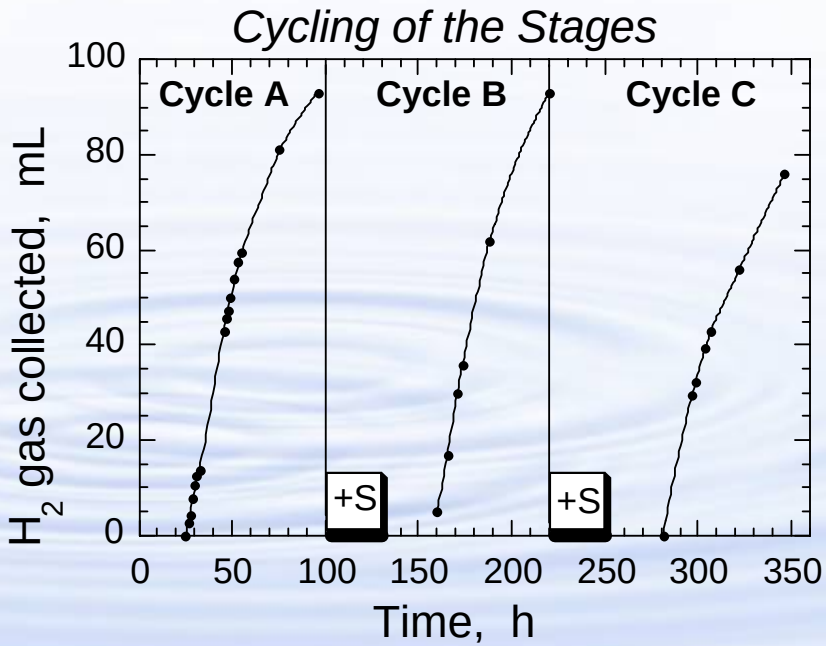
Addressing Barrier Z: (I) Discontinuity of H_2 photo-production due to co-generation of O_2, an inhibitor of the [Fe]-hydrogenase



Structural model of the *Chlamydomonas reinhardtii* [Fe]-hydrogenase. The O_2-sensitive catalytic site cluster is identified by yellow, red and purple space-filled atoms. The alpha helices, shown in green, line one of two hydrophobic gas pathways, which allows gas diffusion between the active site and the surface of the protein. The viewer is looking straight down one such pathway. The research seeks to engineer the gas pathways to prevent O_2 from reaching the catalytic site, but not H_2 from diffusing out of the protein.

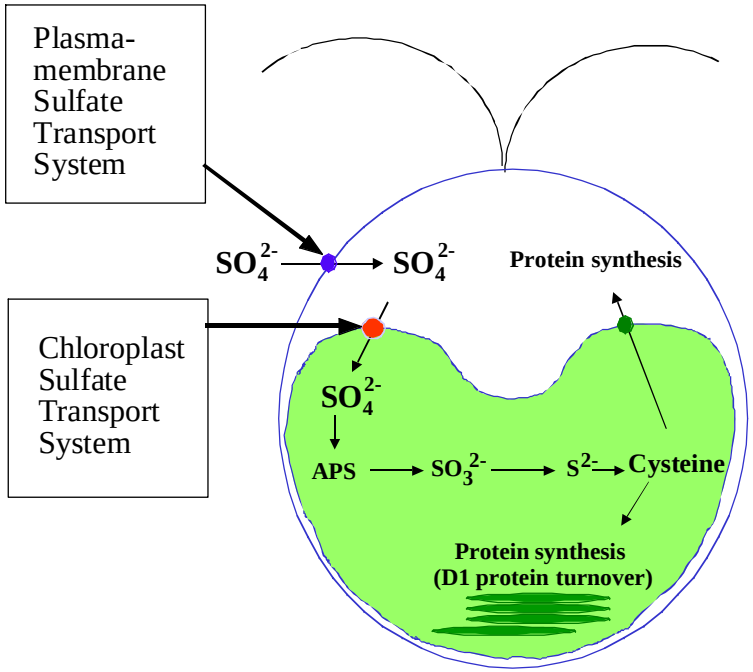
Addressing Barrier Z: (II) Discontinuity of H_2 photo-production due to co-generation of O_2, an inhibitor of the [Fe]-hydrogenase

An approach to bypass the O_2-sensitivity is to temporally separate normal photosynthesis from H_2 production. This was successfully implemented upon sulfur nutrient deprivation of the algae, which acted as a metabolic switch, causing anaerobiosis and inducing H_2 photo-production by the cells in a process that could be sustained for 3-4 days (see figure below). This breakthrough lead to the design of molecular biological approaches to limit sulfur nutrient availability to the chloroplast and to extend anaerobiosis of the culture, thus, genetically bypassing Barrier Z (see next slide).



Cycling between S-deprivation and S-replete conditions switches a green algal culture between H_2-production and normal photosynthesis, respectively.

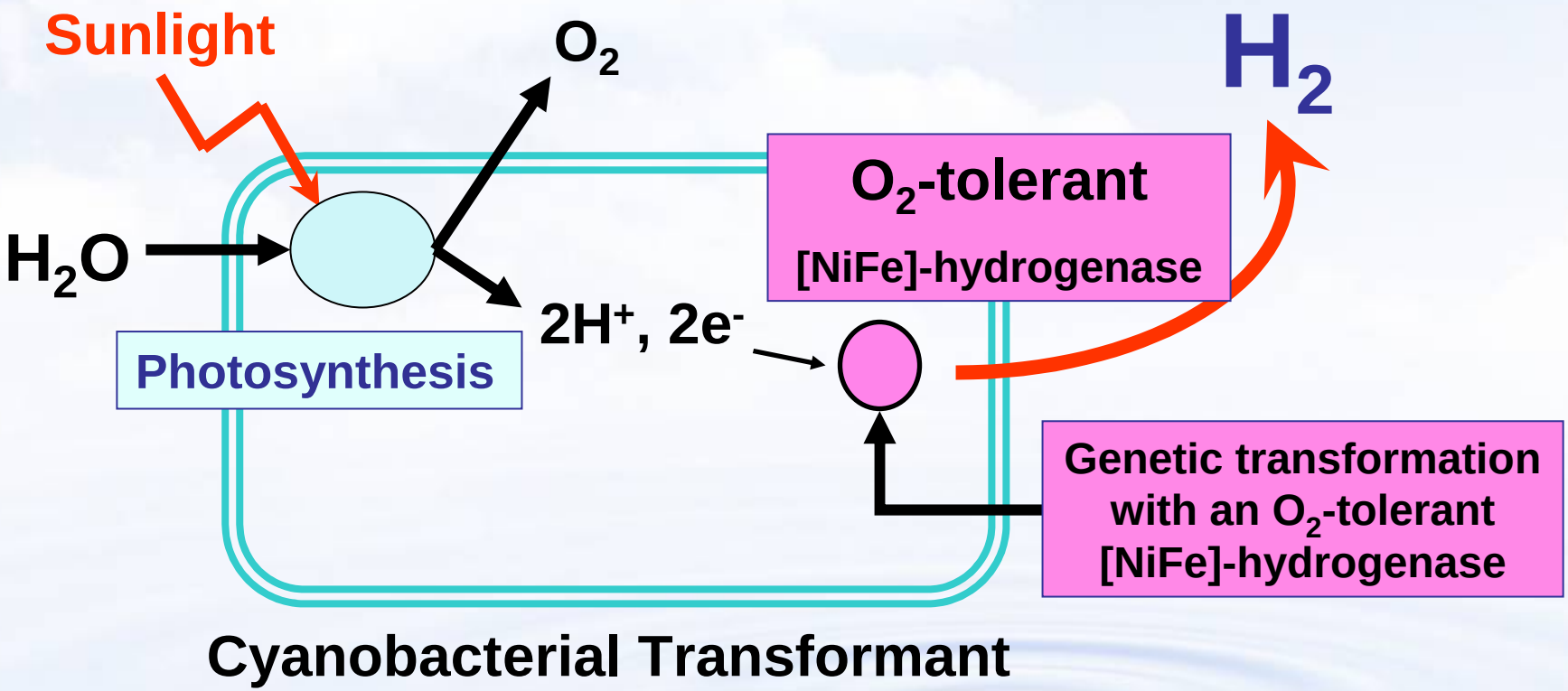
Addressing Barrier Z: (III) Discontinuity of H_2 photo-production due to co-generation of O_2, an inhibitor of the [Fe]-hydrogenase



Chlamydomonas reinhardtii

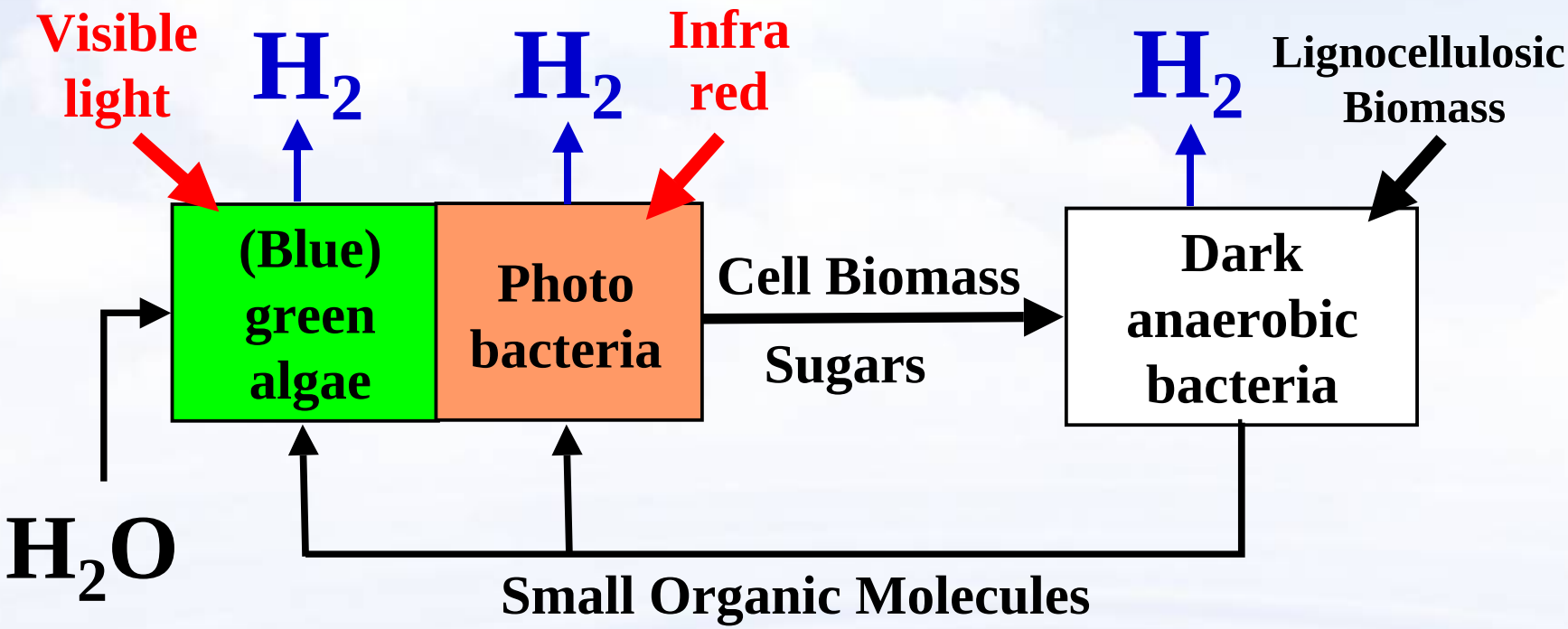
Sulfate uptake, assimilation, and cysteine biosynthesis by the chloroplast in the green alga, *Chlamydomonas reinhardtii,* is required for oxygenic photosynthesis. Sulfate anions are transported from the environment into the chloroplast through the "plasma membrane" and a "chloroplast sulfate transport system." The research seeks to genetically interfere with the function of the chloroplast sulfate transporter in order to impede oxygen evolution and to generate green algae in which photosynthesis is less active than cellular respiration. Such strains would be sulfur-limited, perform oxygenic photosynthesis under anaerobic conditions and constitutively produce H_2.

Addressing Barrier Z: (IV) Discontinuity of H_2 photo-production due to co-generation of O_2, an inhibitor of the [Fe]-hydrogenase



An O_2-tolerant [NiFe]-hydrogenase has been identified from the photosynthetic bacteria, *Rubrivivax gelatinosus* and *Thiocapsa roseopersicina*. This O_2-tolerant [NiFe]-hydrogenase will be genetically expressed in a cyanobacterium for continuous photo-production of H_2 and O_2 from water.

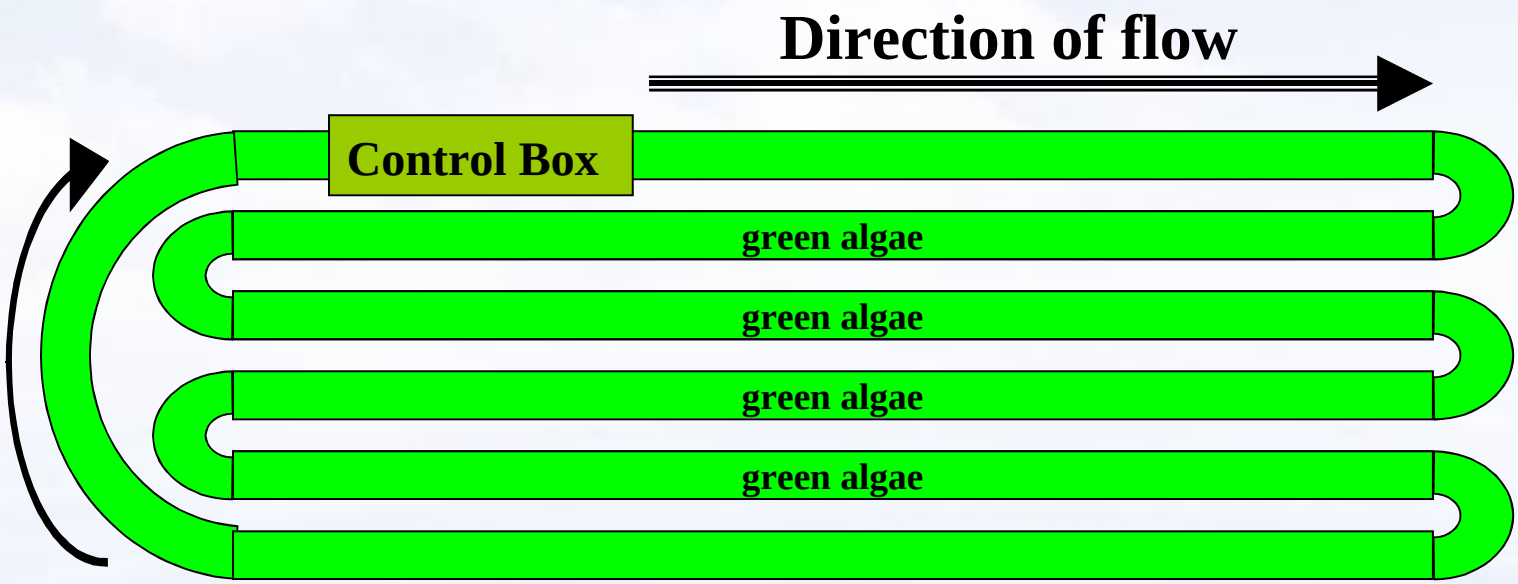
Integrated Biological H_2 Production



Illustrative Scenario: Green algae, cyanobacteria, and photosynthetic bacteria are co-cultured anaerobically in a photoreactor, and dark anaerobic bacteria in a fermentor. Feedstock for the dark anaerobic bacteria is derived from the cell biomass/sugars of the algae, cyanobacteria and photosynthetic bacteria. Additional feedstock for the dark anaerobic bacteria is derived from lignocellulosic products. The small organic molecule by-products of the dark, anaerobic, bacterial fermentation are subsequently utilized as feedstock for the algae, cyanobacteria and photosynthetic bacteria. The research seeks to implement specific aspects of this Integrated Biological H_2 Production System.

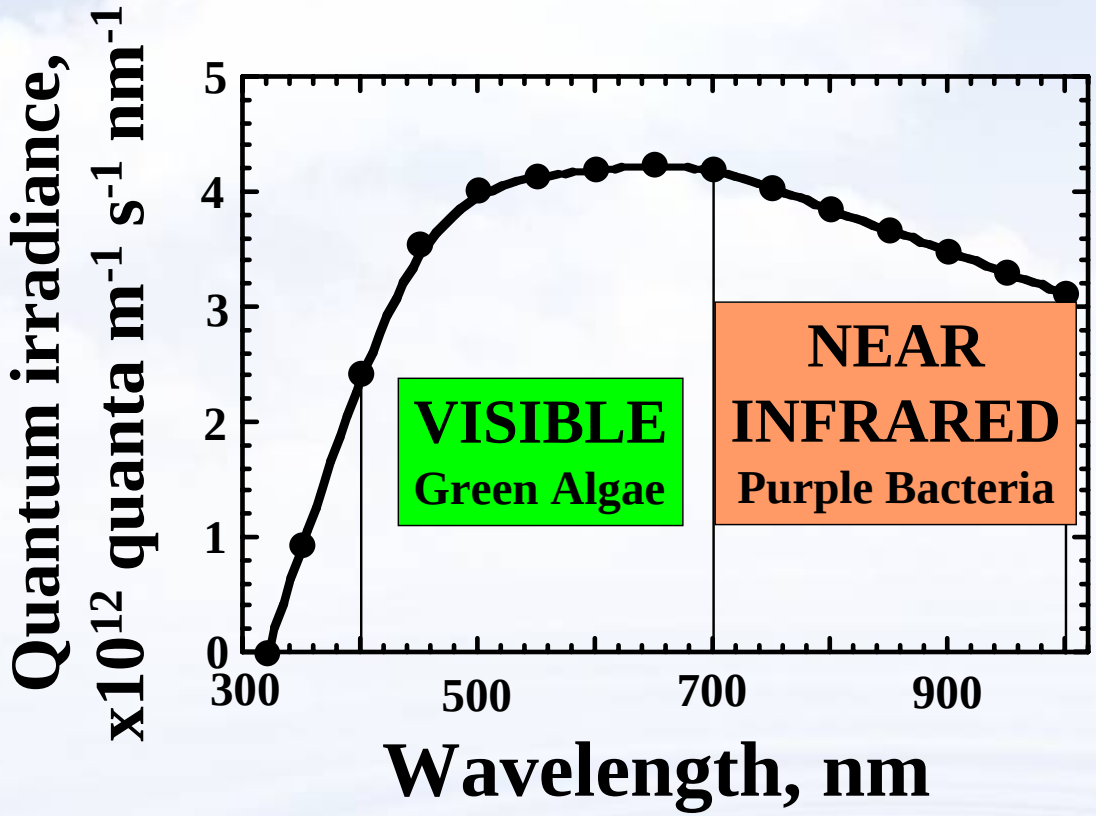
Addressing Barrier AA: Systems engineering for cost-effective photobiological H_2 production

Tubular Racetrack Photoreactor Design



Example of an enclosed, tubular racetrack, low-cost photobioreactor for photosynthetic microorganism growth, H_2-production, and H_2-gas harvesting. Depending on the optical properties of the cells (e.g. Barrier X), the tube diameter would be 6-12 inches wide. Other reactor configurations are possible, depending on climatic and local conditions.

Addressing Barrier AF: Limitation due to the high nitrogen/carbon (N/C) ratio in photosynthetic bacteria



To extend the absorption spectrum of H_2-photoproduction to the infrared region (700-900 nm), anoxygenic photosynthetic bacteria would be included to work in tandem with green algae and cyanobacteria. Hydrogen in photosynthetic bacteria, e.g. *Rhodospirillum rubrum*, is generated by the nitrogenase enzyme. This enzyme is expressed only under conditions of inorganic nitrogen limitation (low N/C ratio). To maximize H_2-production activity in photosynthetic bacteria, it is important to alleviate the positive suppression of gene expression by inorganic nitrogen in the medium. The research seeks to apply molecular engineering techniques to achieve constitutive expression of the nitrogenase enzyme under high N/C ratios in the medium.

Addressing Barrier AI: The fermentation hydrogen molar yield (mol H_2/mol substrate) is too low due to various biological limitations

Chemical maximum: $\text{Glucose} + 6H_2O \rightarrow 6CO_2 + 12H_2$

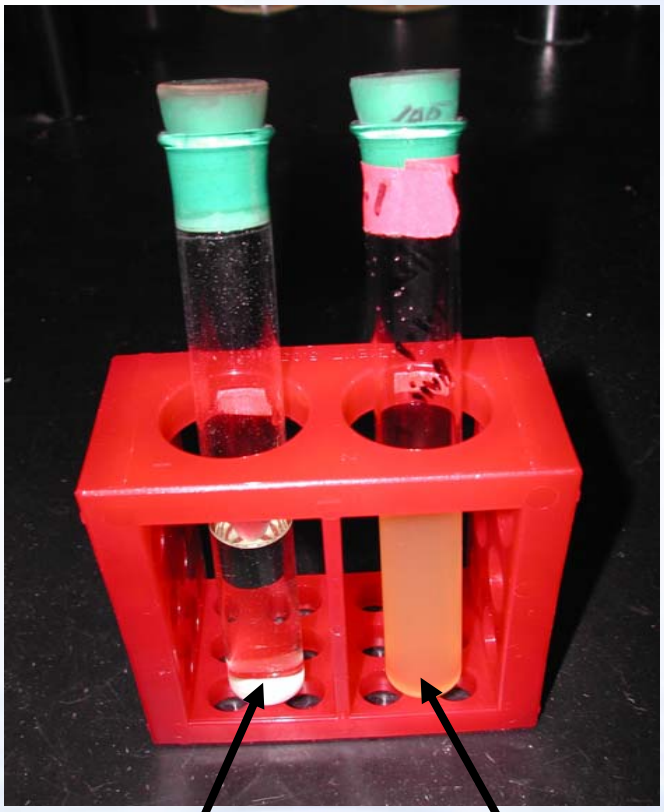
Biological maximum: $\text{Glucose} + 2H_2O \rightarrow 2\text{Acetate} + 2CO_2 + 4H_2$

Biological minimum: $\text{Glucose} \rightarrow \text{Butyrate} + 2CO_2 + 2H_2$

There is enough energy in glucose to produce 12 mol of H_2, yet biologically the maximal molar yield is 4. Most laboratories, however, reported an even lower H_2 molar yield around 2. The simultaneous production of waste organic acids and solvents lower the H_2 molar yield. One effective strategy is to perform metabolic engineering to re-direct microbial pathways preferentially toward H_2 production. New pathways must also be discovered to harness all of the energy stored in sugar substrates.

Addressing Barrier AK: Glucose feedstock is a major cost driver for economic H_2 production via fermentation

- **<u>Challenge</u>: glucose is too expensive to support economic H_2 production.**
- **<u>Solution</u>: identify microbes that can produce H_2 from glucose-rich cellulose and hemicellulose, both of which are major constituents of abundant lignocellulosic biomass.**



Crystalline cellulose pellet in the absence of cellulose digesting bacteria.

Break-down of cellulose and concomitant H_2-production in the presence of bacteria.

Recent Progress at NREL, UC Berkeley, ORNL, Venter Institute

- **Barrier X: Truncated chlorophyll antenna size strains in green algae were developed.**
- **Barrier Y: A thylakoid-spanning artificial proton channel was designed.**
- **Barrier Z-I: [Fe]-hydrogenase O_2-diffusion barriers were identified.**
- **Barrier Z-II: Parameters affecting continuity of H_2 production were identified.**
- **Barrier Z-III: Chloroplast sulfate permease genes were repressed to lower sulfate uptake and the Photosynthesis/Respiration ratio.**
- **Barrier Z-IV: Strains with O_2-tolerant [NiFe]-hydrogenase were identified.**
- **Barrier AA: A tubular photo-bioreactor was tested.**
- **Barrier AF: A high N/C ratio nitrogenase de-repressed photosynthetic bacterium strain was obtained.**
- **Barrier AI: A model microbe was selected for pathway engineering.**
- **Barrier AK: Microbes producing H_2 from cellulose and hemicellulose were screened and identified.**

Promise of Biological Hydrogen Production

Biological H_2 production holds the promise of generating a renewable fuel from nature's most plentiful resources, sunlight, water and biomass. The process would have a positive impact on climate change, environmental pollution and the question of energy supply and demand. Current projections of fossil fuel shortfalls require the development of energy sources that are clean, renewable and environmentally friendly. In addition to energy, scaled-up application of biological H_2 production may yield substantial amounts of high value bio-products, potentially useful in the food and synthetic chemistry industries.

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- Maness P-C, Smolinski S, Dillon AC, Heben MJ, and Weaver PF (2002) Characterization of the oxygen tolerance of a hydrogenase linked to a carbon monoxide oxidation pathway in *Rubrivivax gelatinosus*. Appl. Environ. Microbiol. 68: 2633-2636.

Selected Links to BioHydrogen R&D

- http://www.eere.energy.gov/hydrogenandfuelcells/
- http://www.nrel.gov/
- http://www.nrel.gov/basic_sciences/basicframe.html
- http://www.nrel.gov/docs/fy04osti/35593.pdf
- http://www.ihec2005.org
- http://pmb.berkeley.edu/newPMB/faculty/melis/melis.shtml
- http://gcep.stanford.edu/research/factsheets/biohydrogen_generation.html
- http://www.elsevier.com/wps/find/bookdescription.cws_home/702663/description
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- http://www.nordicenergy.net/index.cfm?pid=14-2883,135-2883,180-2883&tid=180-2883