Biological Organisms and Renewable H_2 Production

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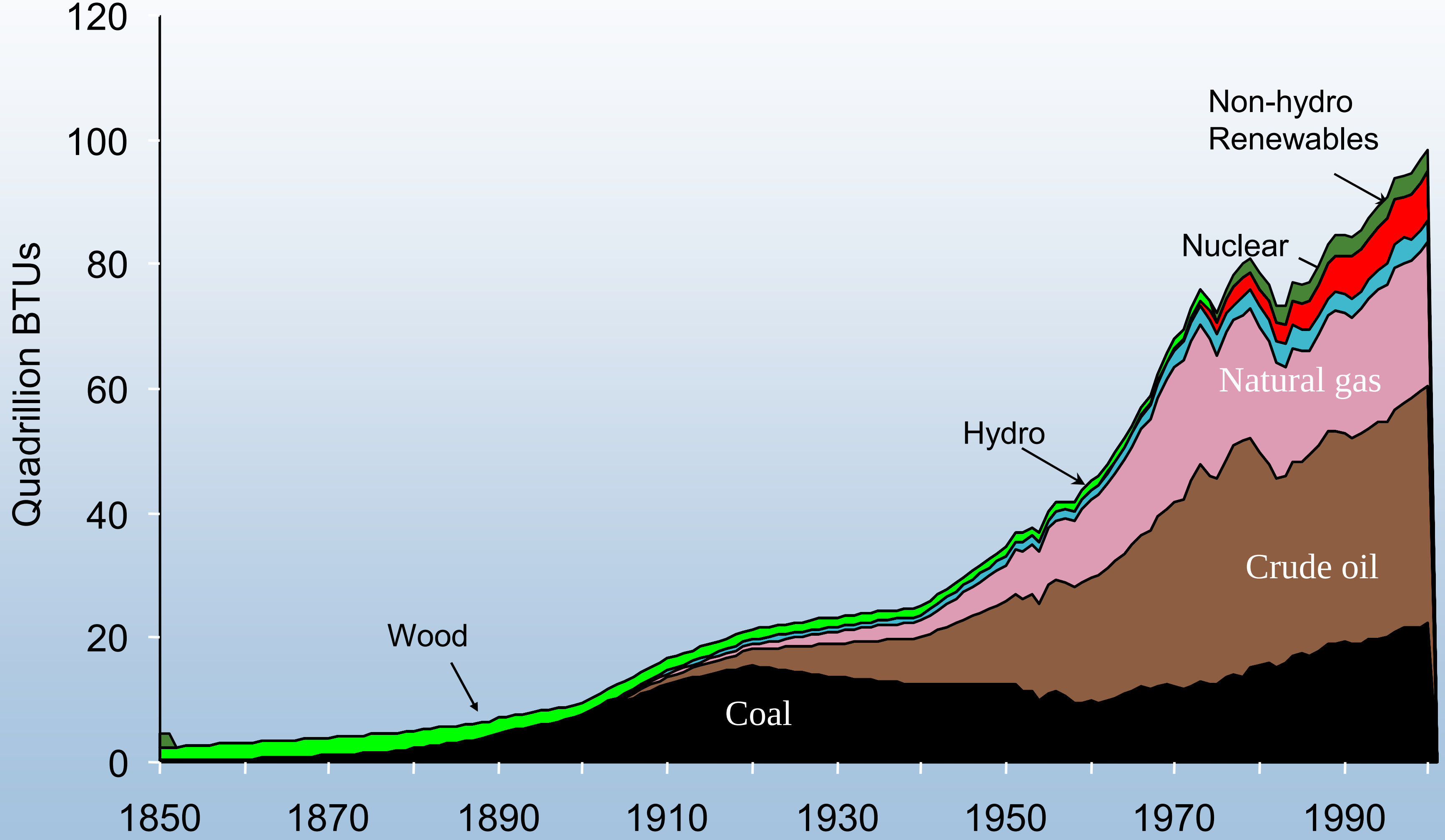
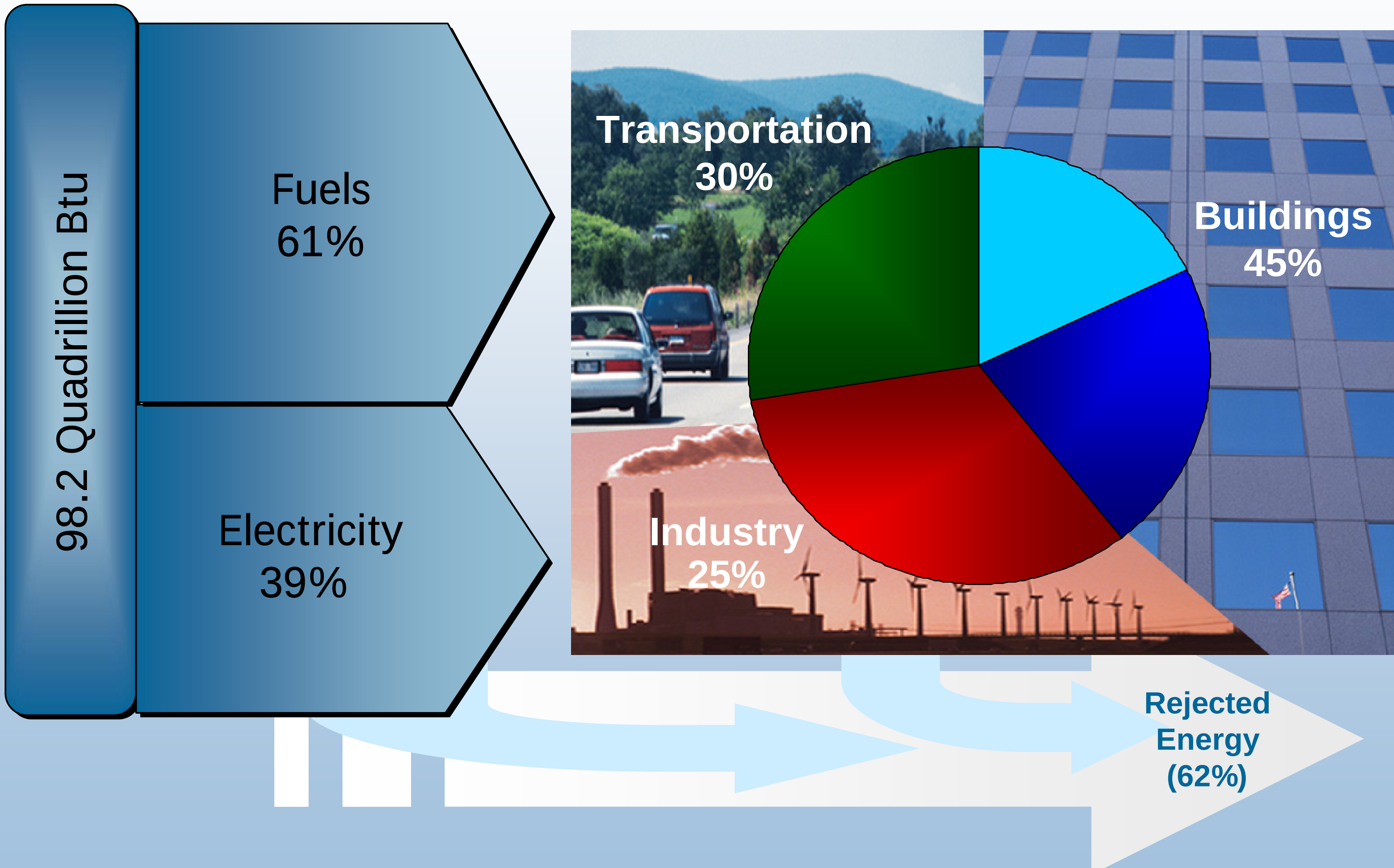
U.S. Energy Consumption

by source - 1850-2000

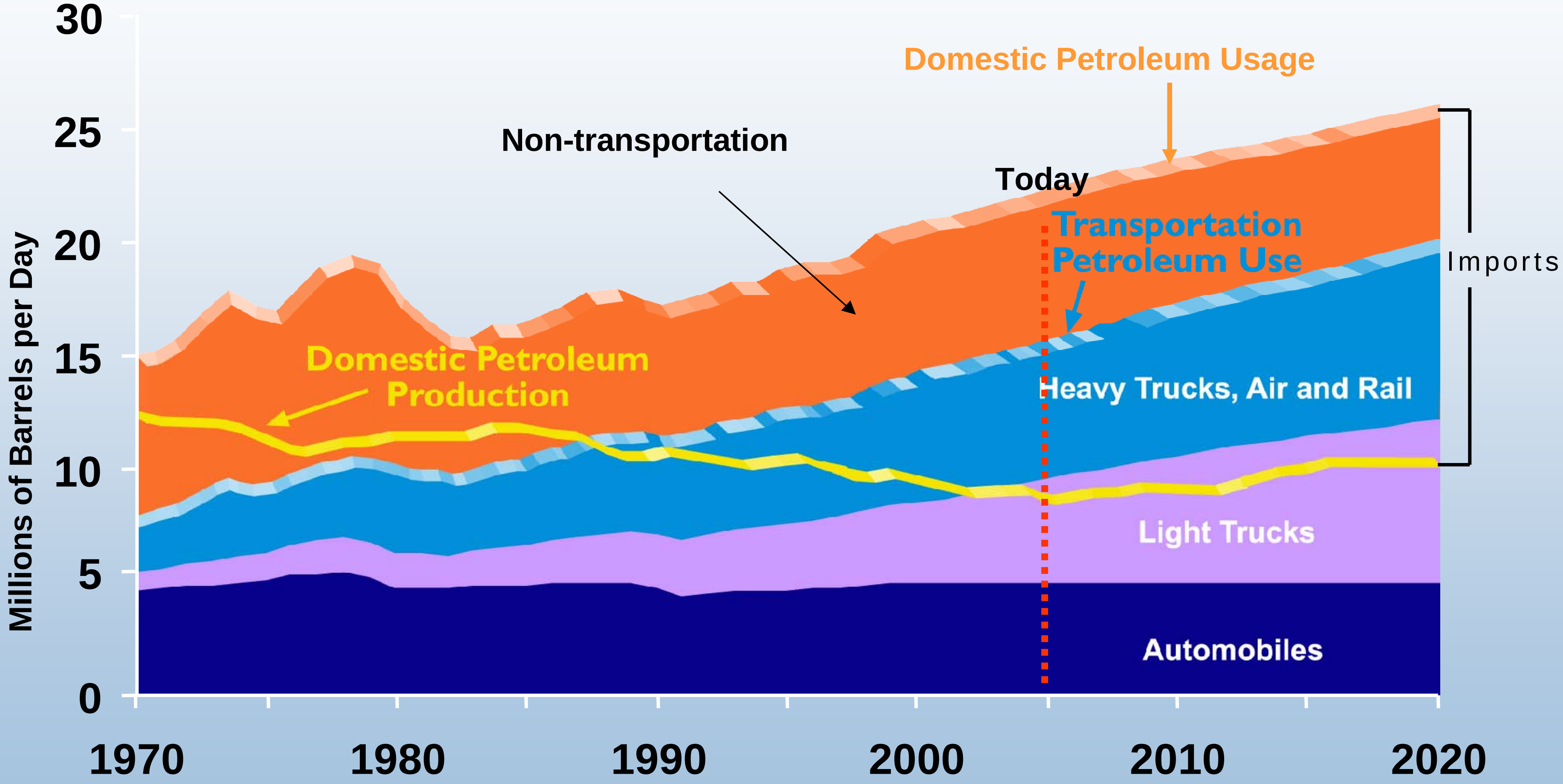


Source: 1850-1949, Energy Perspectives: A Presentation of Major Energy and Energy-Related Data, U.S. Department of the Interior, 1975; 1950-2000, Annual Energy Review 2000, Table 1.3.

U.S. Energy Flows



Dependence on Foreign Oil
U.S. Use of Petroleum



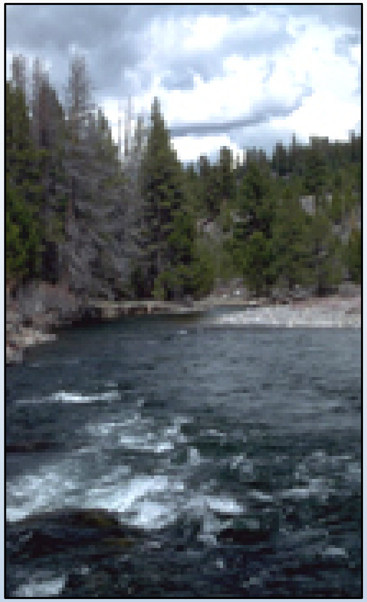
Actual: Annual Energy Review 2000 Tbls 1.2, 5.1 and 5.12
Forecast: Annual Energy Outlook 2002 Tbls 7 and 11
Split between Autos and Lt Truck: Transportation Energy Data Book Edition 21 Tbl 2.6
Updated October 2002

NREL National Renewable Energy Laboratory

Energy Challenges are Enormous

Energy Security and Reliability

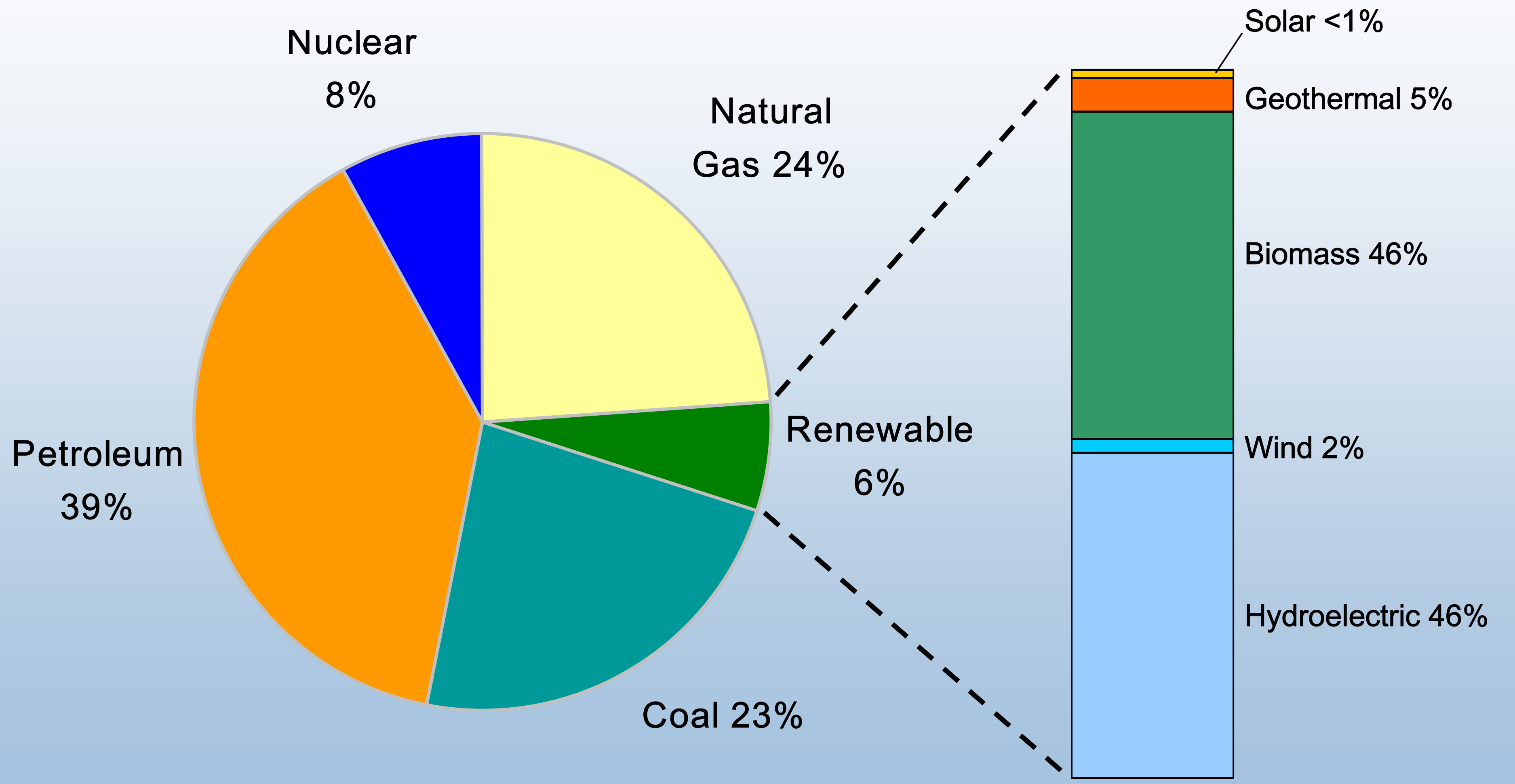
Economic Growth



Environmental Impact

Natural Disasters

The Role of Renewables in the U.S. Energy Consumption- 2003



Source: AEO 2004 tables (released in December 2003) based on US energy consumption. Overall breakdown Table A1 (Total Energy Supply and Disposition), and Renewable breakdown Table A18 (Renewable Energy, Consumption by Section and Source).

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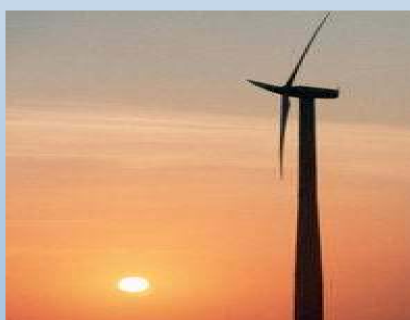
Technology-based Solutions:

There is no single nor simple answer

- Energy efficiency
- Renewable energy
- Non-polluting transportation fuels
- Separation and capture of CO_2 from fossil fuels
- Next generation of nuclear fission and fusion technology
- Transition to smart, resilient, distributed energy systems coupled with pollution-free energy carriers, e.g. hydrogen and electricity



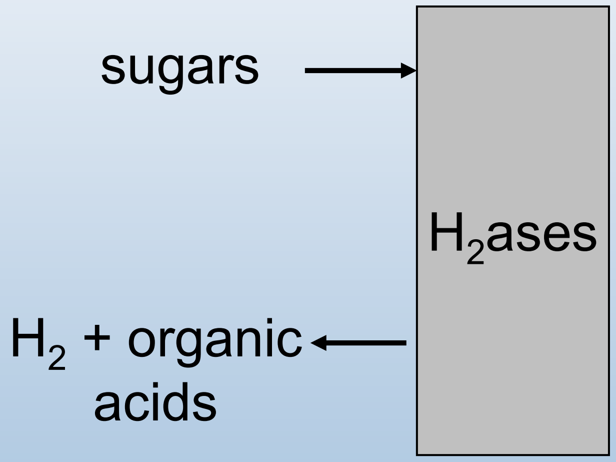


NREL & Renewable Hydrogen Production

- Photoelectrochemical hydrogen production from water
- Photobiological hydrogen production by algae and cyanobacteria
- Fermentation
- Hydrogen production from biomass
- Solar thermochemical hydrogen production
- Co-production of electricity and hydrogen from renewable technologies, e.g., wind - electrolysis

Biological Modes of H_2 Production (I)

Dark fermentative bacteria

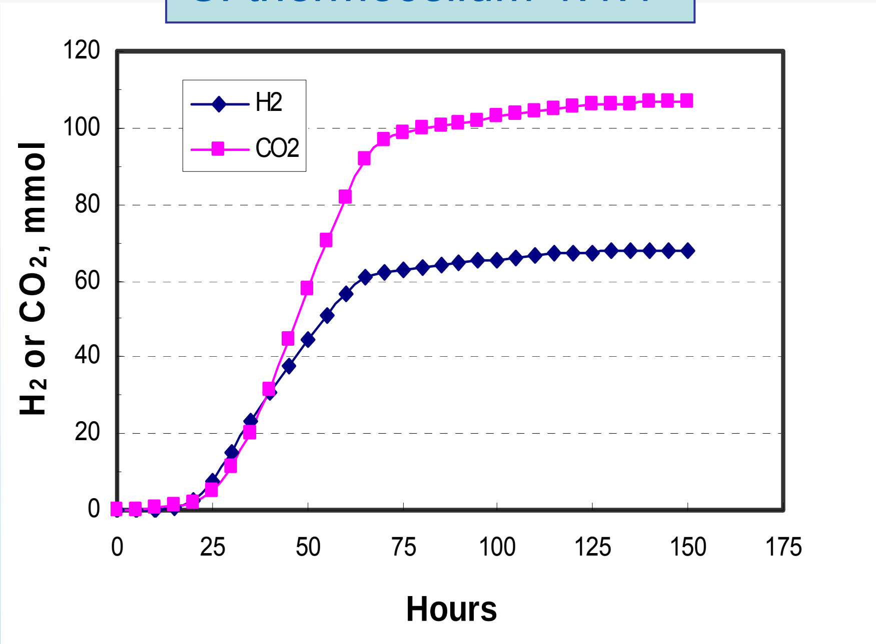


- No energy input required
- Maximum theoretical yield: $4H_2$/sugar with known organisms and technology
- Current yield 1-2 H_2/sugar
- Price of feedstock is too high (unless one uses wastewater remediation)
- Product inhibition

H_2 via Cellulose Fermentation

Strains	Rate of H_2 Production
ATCC	**1018**
1.1.1	**595**
YS	477
7.10.4	447
7.12.1	407
7.7.10	35
7.8.3	Traces
6.3.2	Traces
7.9.4	Traces

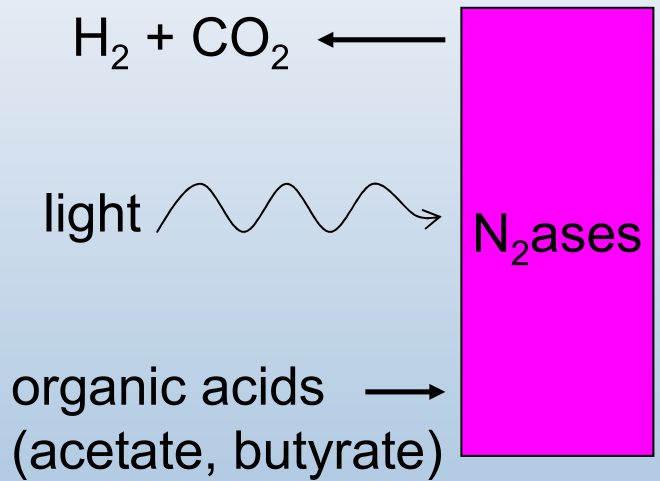
C. thermocellum 1.1.1



- Best H_2 producers from cellulose (Avicel) were identified
- **Using cellulose *in lieu* of glucose will address the feedstock cost barrier**

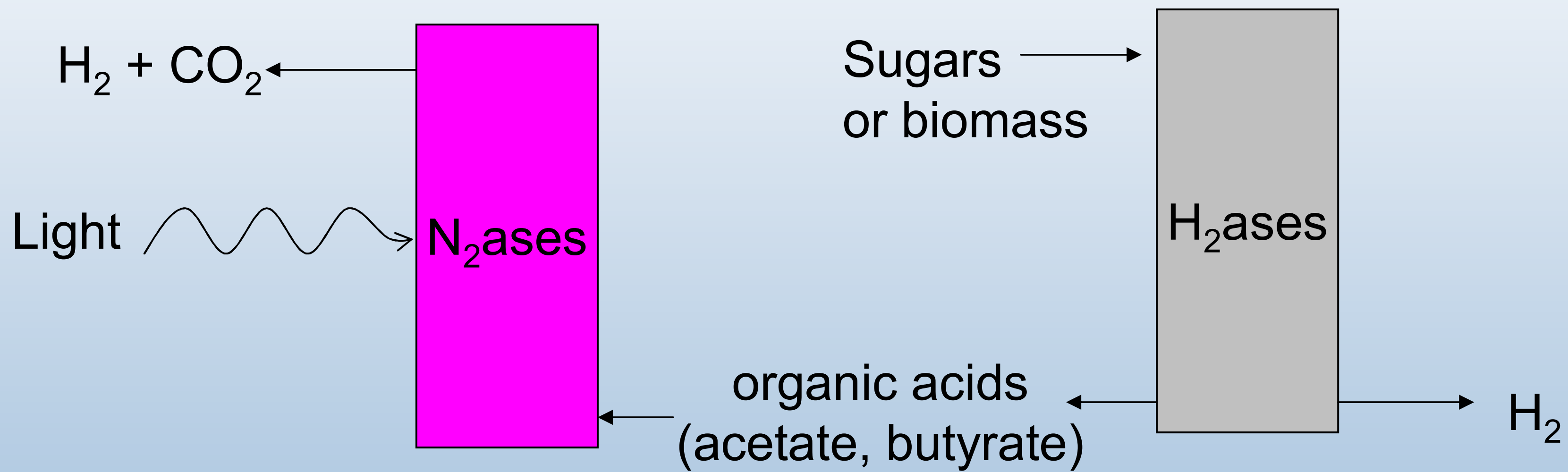
Biological Modes of H_2 Production (II)

Anoxygenic (no O_2 by-product) photosynthetic bacteria



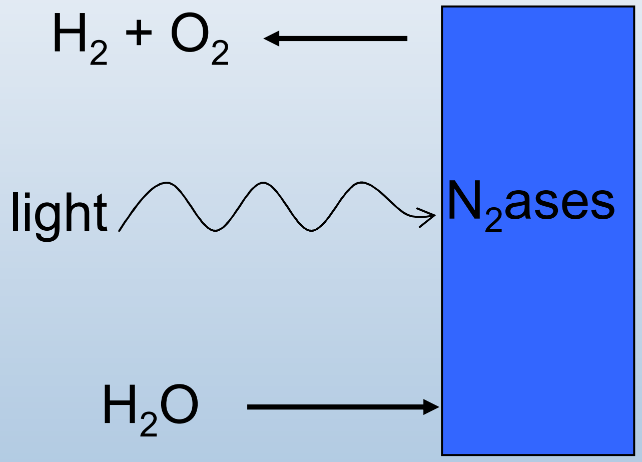
- Sunlight energy input required
- Maximum theoretical light conversion efficiency of <6% (low), since nitrogenases are energy-requiring enzymes
- High production rates
- Feedstock can be products of fermentation (possibility for integrated systems)

Integrated Fermentative/Photobiological system



Biological Modes of H_2 Production (III)

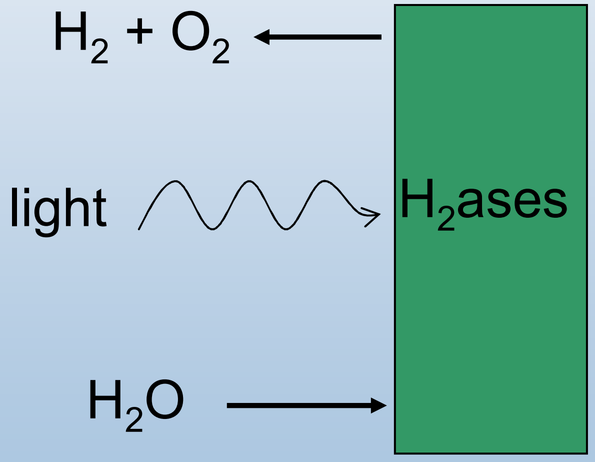
Specialized oxygenic (O_2 by-product) photosynthetic cyanobacteria



- Sunlight energy input required
- Maximum theoretical light conversion efficiency of <4% (nitrogenase is an energy-requiring enzyme)
- Feedstock is water
- No O_2 inhibition due to temporal or spatial separation between O_2 and H_2-evolution reactions

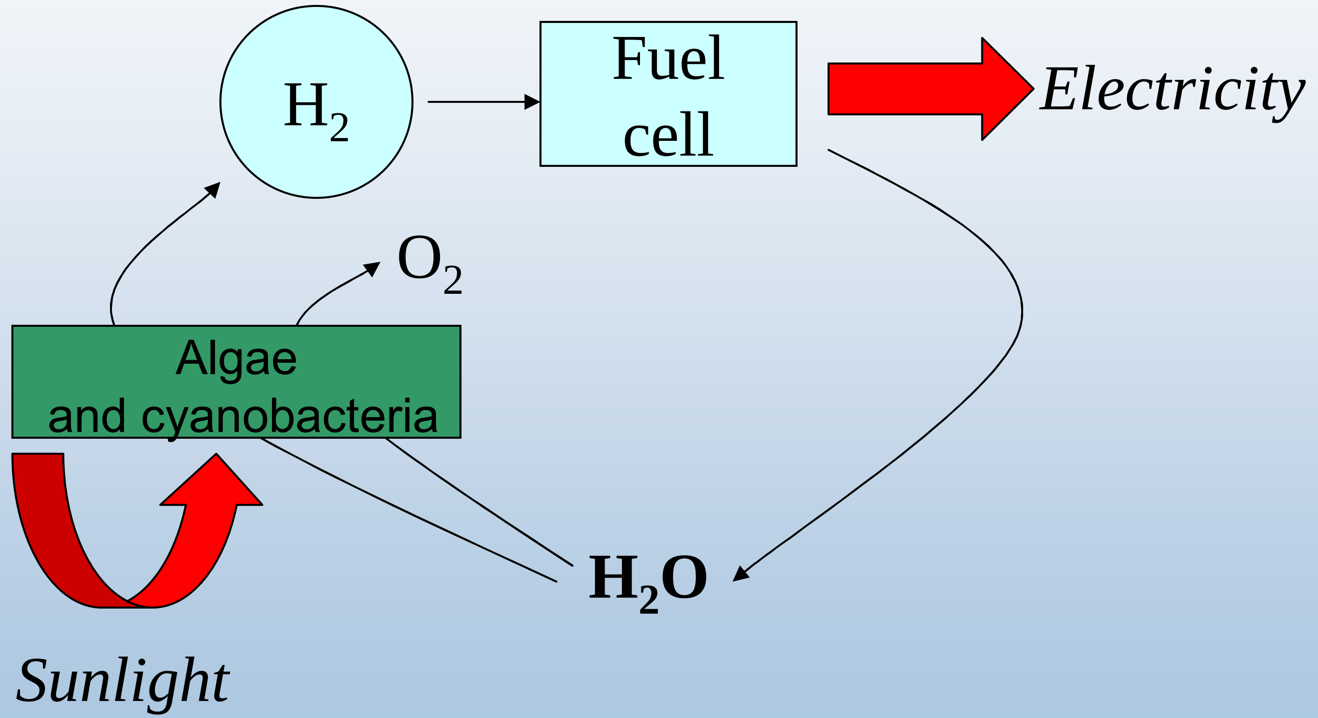
Biological Modes of H_2 Production (IV)

Oxygenic (O_2 by-product) photosynthetic green algae and specialized cyanobacteria



- Sunlight energy input is required
- Maximum theoretical light conversion efficiency is 10-13%
- Feedstock is water
- O_2 inhibits H_2 production

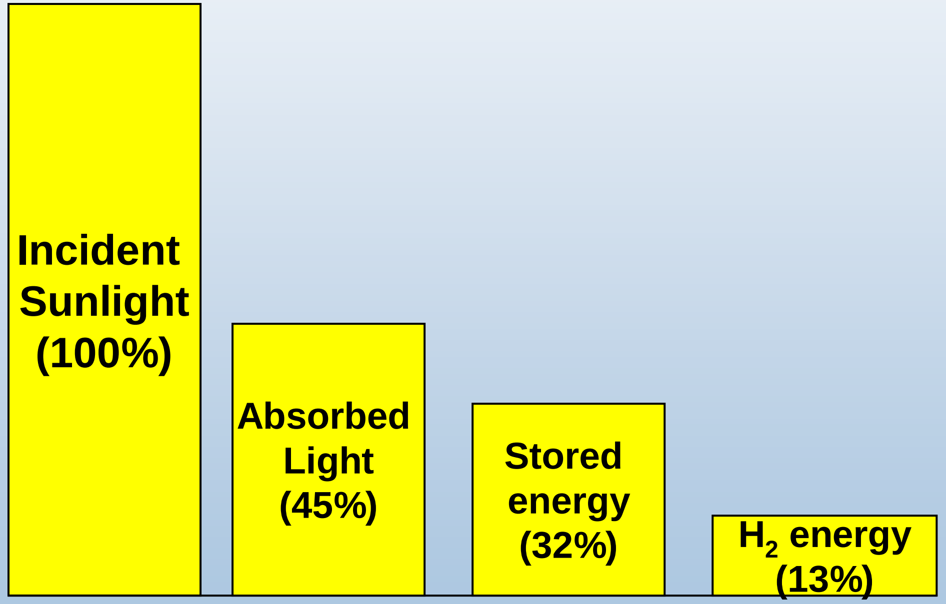
Vision



H_2
Fuel cell
Electricity
O_2
Algae and cyanobacteria
H_2O
Sunlight

Potential of Photobiological H_2 Production

- Light absorbed by the organism's pigments: 45% of incident solar energy;
- Conversion of light into reductants and oxidants: 32% of incident solar energy;
- Light utilization for H_2 and O_2 production: 13% of incident solar energy



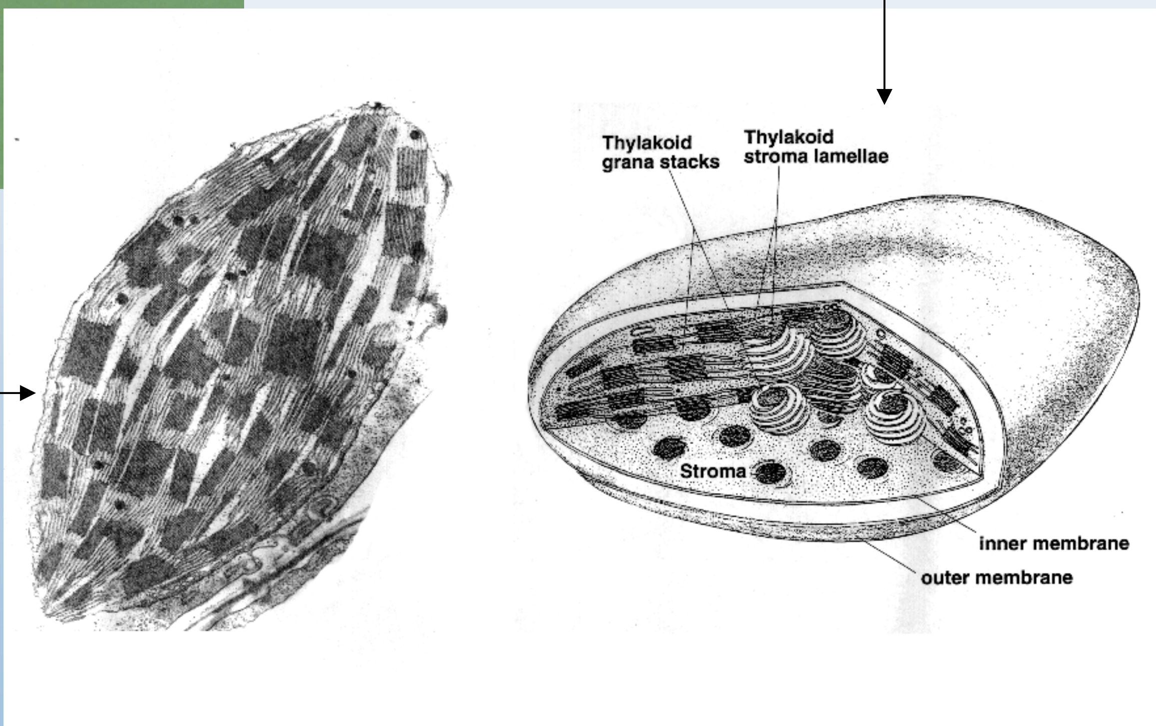
Potential of Photobiological H_2 Production from Water

- Amount of land area required to supply H_2 for the U.S. transportation needs (236 million cars): 0.12% of total land area, in the U.S. Southwest, or 100 km x 100 kilometers or about 4500 square miles.

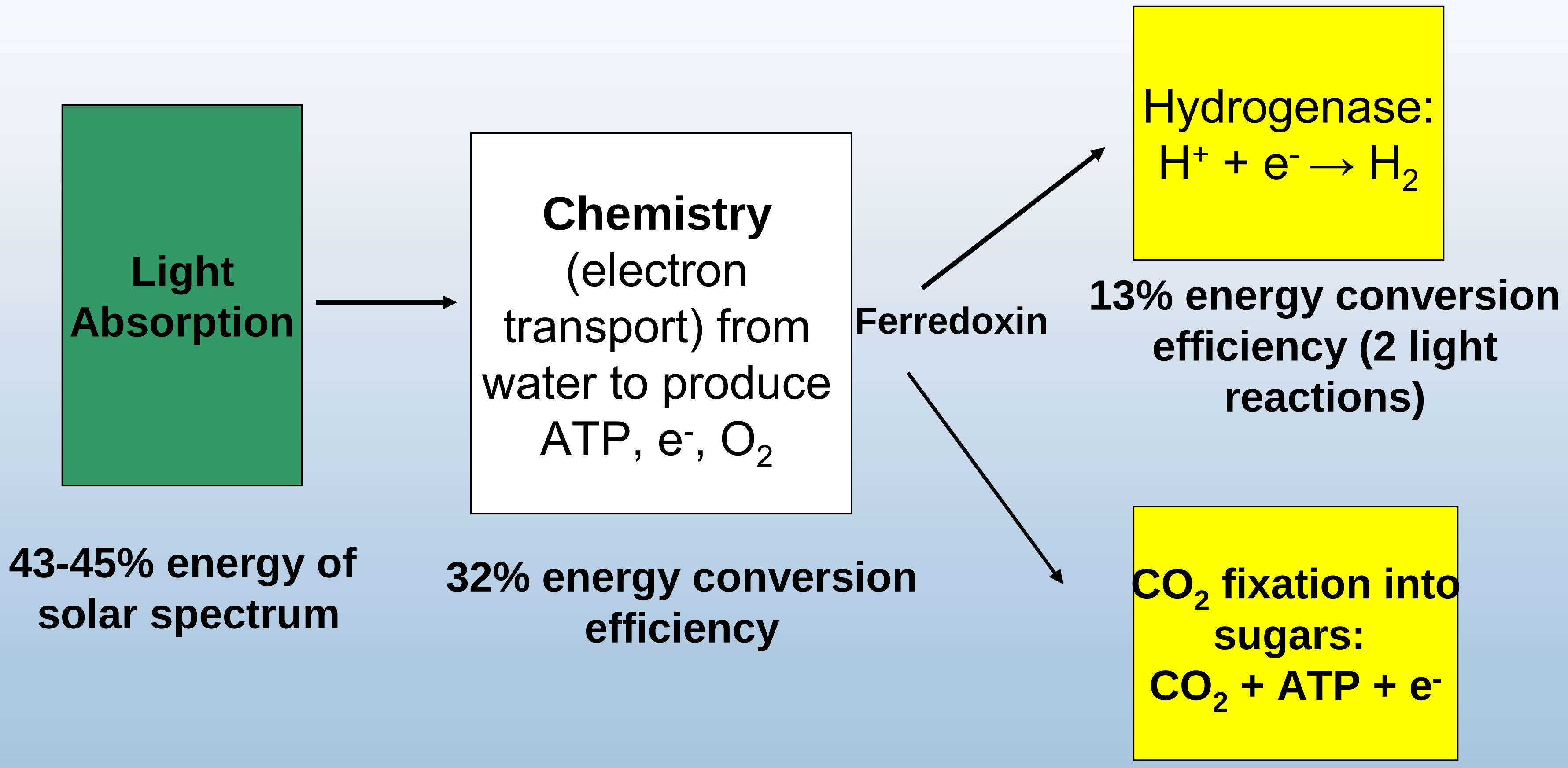
Algal Machinery for H_2 Photo-production

Stacked and non-stacked vesicles made of lipophyllic membranes defining a lumen region (inside)

Chloroplast (intracellular organelle housing the photosynthetic machinery)



Mechanism of Photobiological H_2 Production

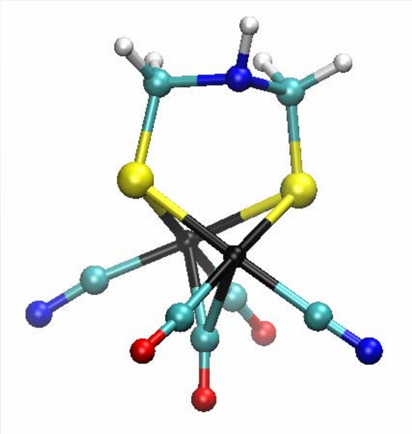


Technical Challenges and Approaches for Photobiological H_2 Production from Water (Algae and Cyanobacteria)

- The hydrogenases are sensitive to O_2 and are neither synthesized nor active in the presence of even small amounts of O_2 (NREL).
- Competition between the hydrogenase and the CO_2 fixation pathway favors the latter.
- Electron transport from H_2O to the hydrogenase enzyme is limited by the non consumption of ATP.
- Solar conversion efficiency is severely hindered by the presence of large arrays of chlorophyll in the algae (UCB).

O_2-tolerance of Hydrogenases (NREL)

First Approach: Engineer an algal [FeFe]-hydrogenase that is resistant to O_2 inactivation;



Advantages: potential 10% light conversion efficiency
Disadvantage: produces mixture of H_2 and O_2

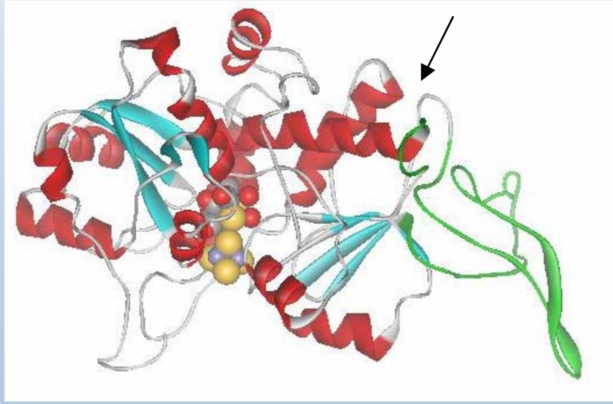
Second approach: Use the UCB/NREL sulfur-switch to induce culture anaerobiosis and subsequent H_2-production activity in algae.

Advantages: produces pure H_2
Disadvantages: maximum light conversion efficiency << 10%

Engineering an O_2-tolerant Hydrogenase

- The catalytic center is located in the middle of the enzyme structure; electrons are delivered to it directly by the electron donor ferredoxin (Paul King).

“H_2 channel”



Algal *(Chlamydomonas reinhardtii)* HydA2 hydrogenase (based on homology modeling

We proposed that O_2 inactivation could be prevented by limiting O_2 access to the catalytic site of the enzyme.

Engineering an O_2-tolerant Hydrogenase

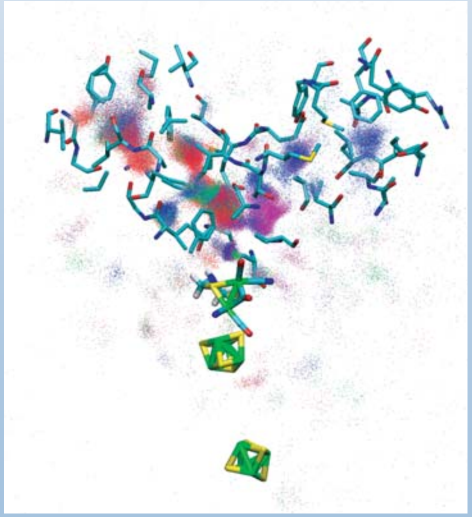
[FeFe]-hydrogenases from different organisms were co-expressed in *E. coli* . The O_2-tolerance of the *Clostridium* enzymes is at least two orders of magnitude higher than that of the algal enzymes. All the computational modeling and mutagenesis work can now be done with the *C. pasteurianum*, whose X-ray structure is known.

Organism	Protein Name	Subunit Composition	Activity (nMol H_2/ ml/min)	Specific Activity (nMol H_2/ mg/min)	Half-life after exposure to air
Chlamydomonas reinhardtii	HydA1	Monomeric	42	212	< 1 sec
Chlamydomonas reinhardtii	HydA2	Monomeric	12	708	< 1 sec
Clostridium acetobutylicum	CaI	Monomeric	28	2894	415±115
	CaII	Monomeric	3	682	
Clostridium pasteurianum	CpI	Monomeric	20	ND	120-300
Shewanella oneidensis	HydAB	Dimeric	14	850	

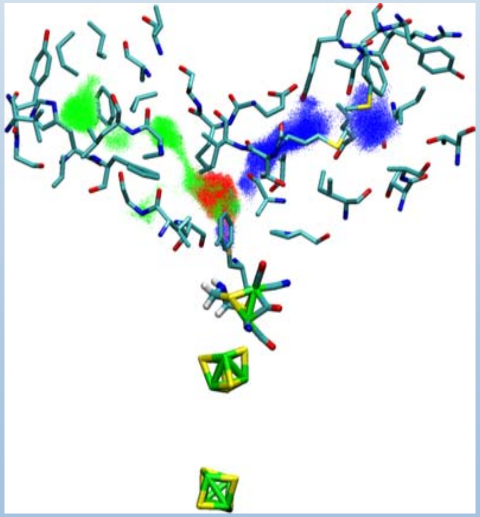
200X increase in O_2 tolerance

Engineering an O_2-tolerant Hydrogenase

- The H_2 gas produced at the catalytic site diffuses out through multiple pathways; O_2 gas, which inhibits enzyme activity, diffuses in through 2 very well-defined pathways (Paul King, Kwiseon Kim, Jordi Cohen and Klaus Schulten).



H_2 pathways

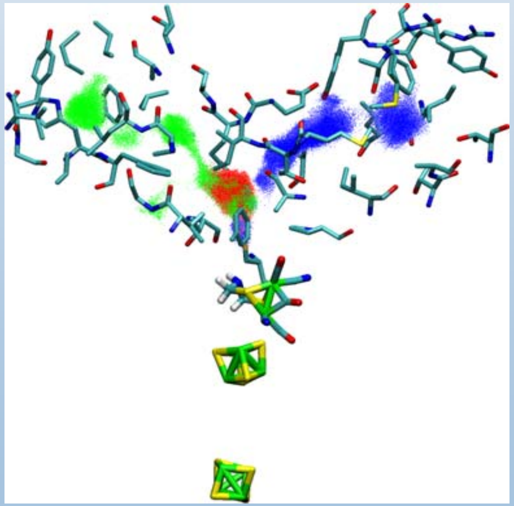


O_2 pathways

Engineering an O_2-tolerant Hydrogenase

Introduction of large amino acid residues in the cavity next to the catalytic site (red) resulted in non-assembly of an active enzyme. Mutations done along pathway A (green) resulted in small (< 20%) increase in O_2 tolerance. Other mutations yield inactive or unassembled hydrogenase. Future approach: introduce residues that compact the enzyme structure.

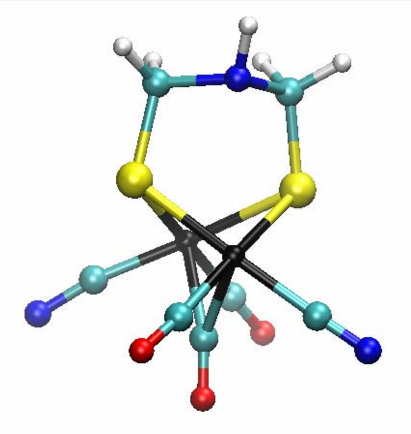
Pathway A



Pathway B

O_2-tolerance of Hydrogenases (NREL)

First Approach: Engineer an algal [FeFe]-hydrogenase that is resistant to O_2 inactivation;



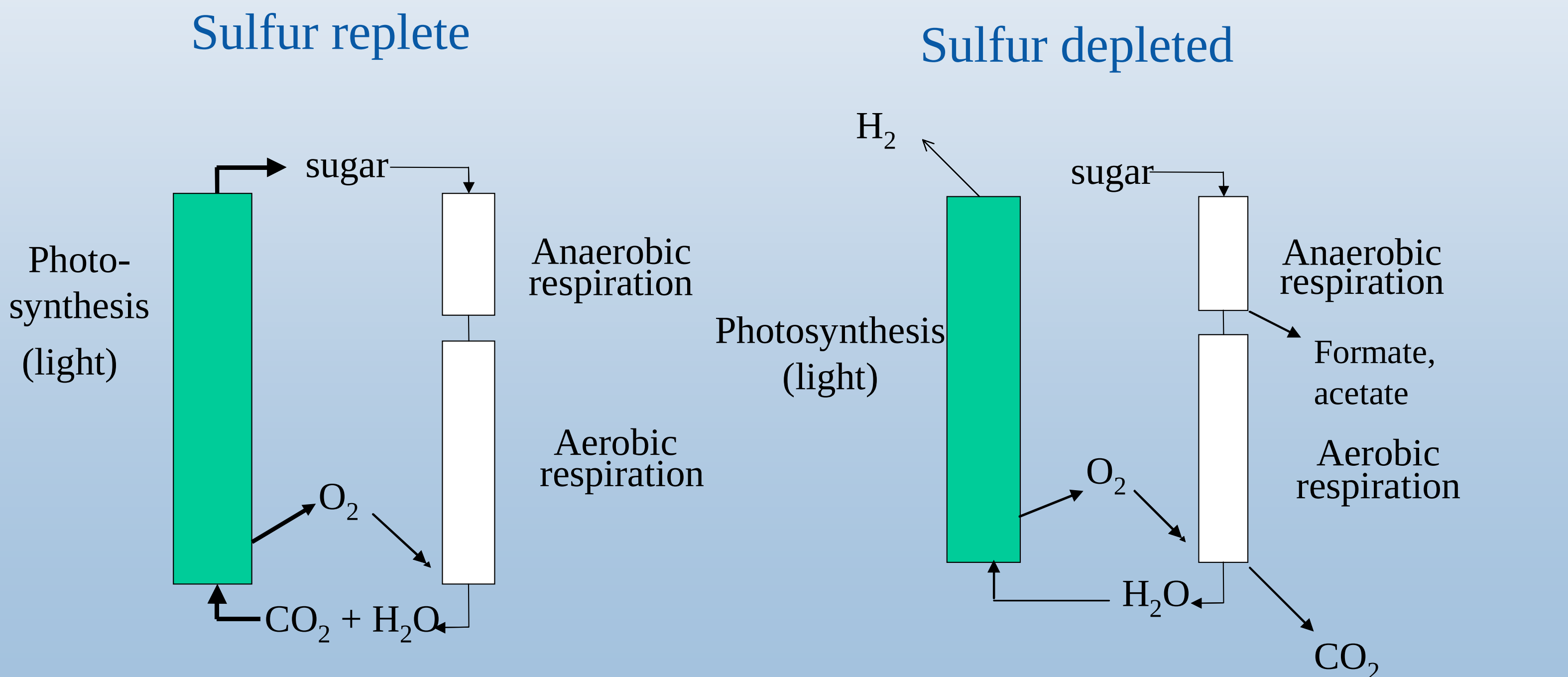
Advantages: potential 10% light conversion efficiency
Disadvantage: produces mixture of H_2 and O_2

Second approach: Use the UCB/NREL sulfur-switch to induce culture anaerobiosis and subsequent H_2-production activity in algae.

Advantages: produces pure H_2
Disadvantages: maximum light conversion efficiency << 10%

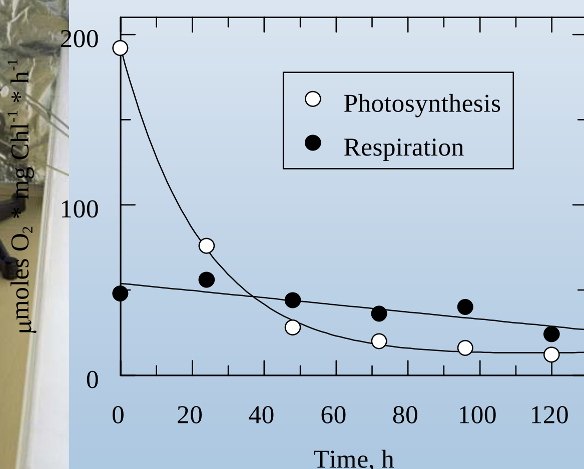
Sulfur Switch to Prevent Algal Hydrogenase Inactivation by O_2

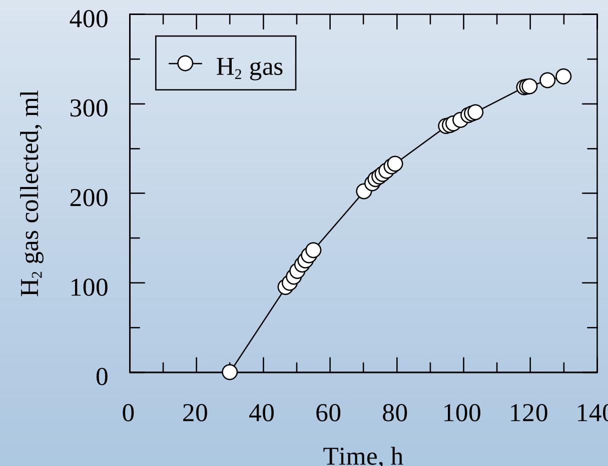
Sulfur deprivation decreases photosynthetic O_2 evolution, shuts off the CO_2 fixation into sugar and induces hydrogenase activity in *Chlamydomonas* (NREL and UCB, 2000).



Sulfur Switch to Prevent Algal Hydrogenase Inactivation by O_2

Batch system: Sulfur-deprived cultures gradually inactivate photosynthesis and become anaerobic (1-2 days). They then photoproduce H_2 for a total of 3-4 days. Cycles of +S and –S can be repeated for at least 3 times.

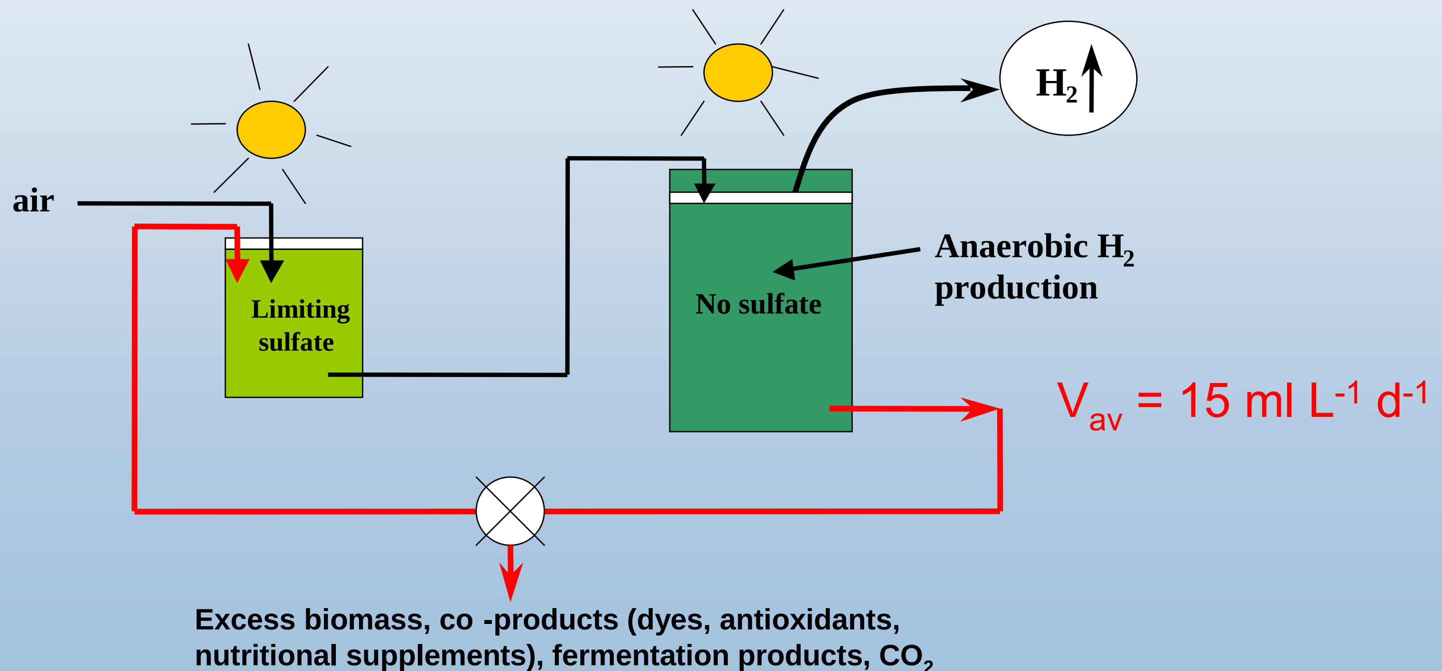




V_{av} = 30-60 ml L^{-1} d^{-1}

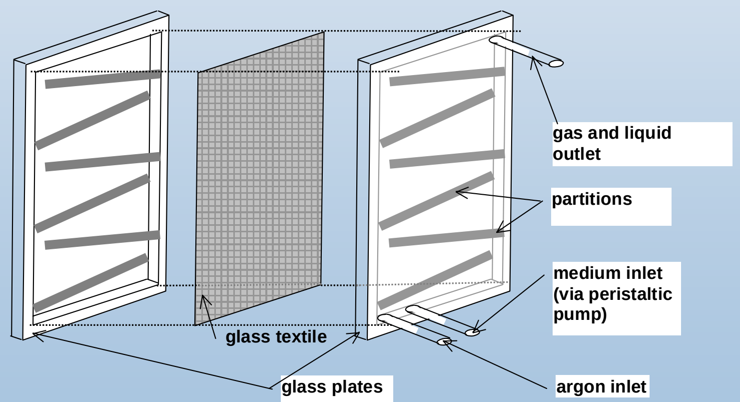
Sulfur Switch to Prevent Algal Hydrogenase Inactivation by O_2

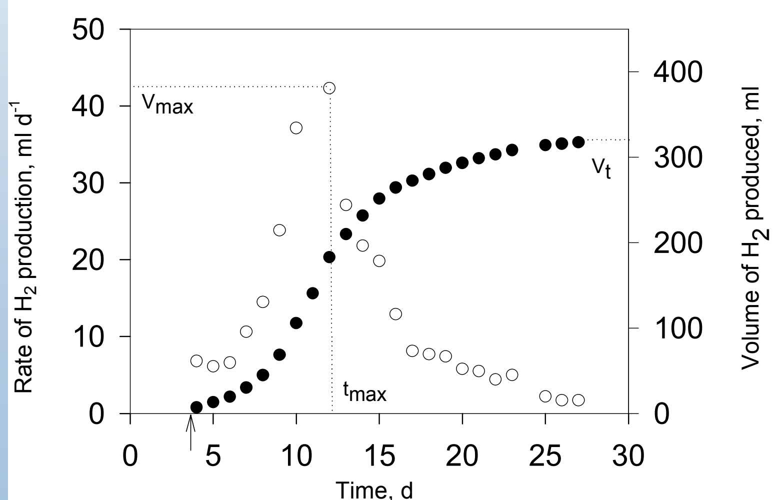
Continuous system: cultivation under limiting S concentrations in the first photobioreactor serves as a reservoir for algal cells that become competent in H_2 production when transferred to a 2nd photobioreactor where H_2 production is occurring.



Sulfur Switch to Prevent Algal Hydrogenase Inactivation by O_2

Batch system with immobilized cultures: *Chlamydomonas* cells immobilized on fiberglass can photoproduce H_2 at higher rates per cell volume than suspension cultures. H_2 production lasts 7 X longer than with suspension cultures.

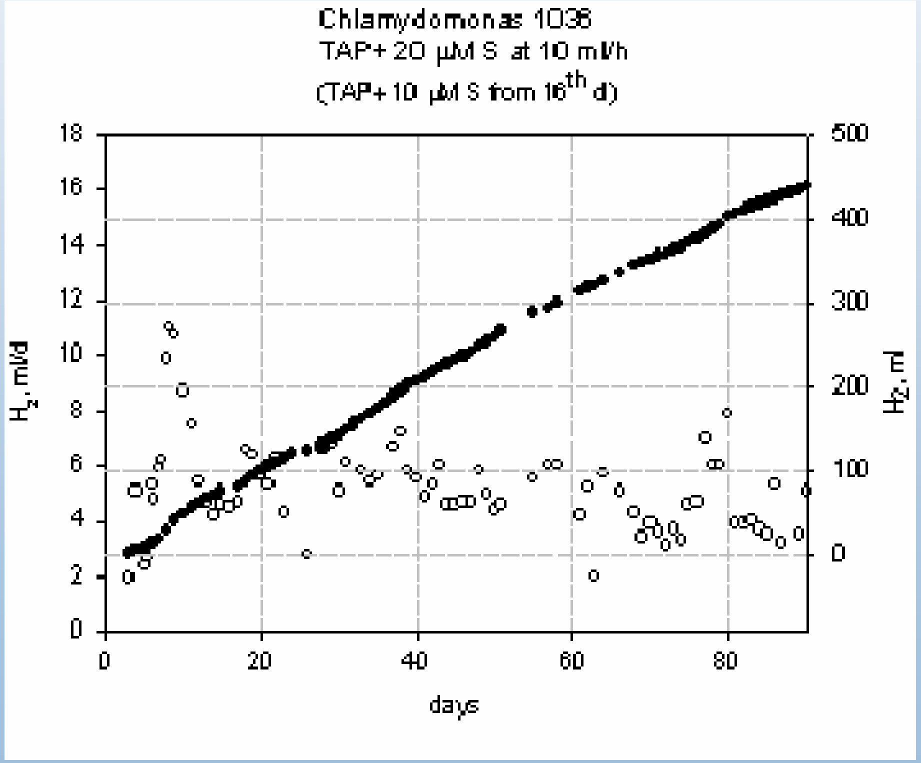




V_{av} = 917 ml L_{matrix}^{-1} d^{-1}; cyclic not investigated yet.

Sulfur Switch to Prevent Algal Hydrogenase Inactivation by O_2

Continuous system with immobilized cultures: *Chlamydomonas* cells were continuously cultivated in the presence of limiting sulfate. The cultures photoproduced H_2 for a total uninterrupted period of 90 days.



V_{av} = 283 ml L_{matrix}^{-1} d^{-1}

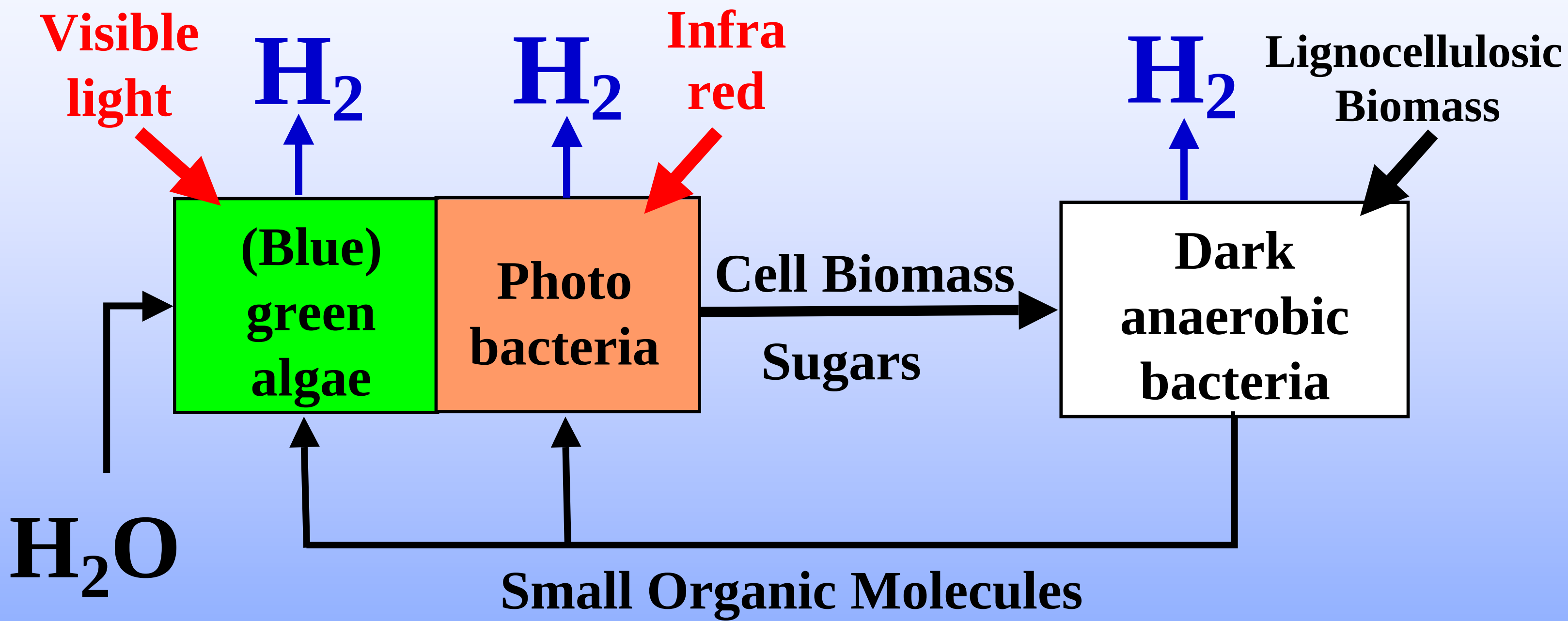
Sulfur Switch to Prevent Algal Hydrogenase Inactivation by O_2

Summary

Cultivation Mode	V_{av} (ml L^{-1} d^{-1})	Estimated cost under current laboratory conditions
Batch (one cycle) (1) suspension (2) immobilized	 60 917	 N/A N/A
Continuous (1) suspension (2) immobilized	 15 283	 $250/kg N/A
Cycles (1) suspension (2) immobilized	 21 N/A	 $720/kg N/A

Estimated cost of optimized system: $2/kg.

Integrated Biological System



- The integrated system seeks to maximize both light and feedstock utilization

Team and Collaborators

NREL: Chris Chang, Kwiseon Kim, Paul King, Pin Ching Maness, Mike Seibert, Sharon Smolinski, Drazenka Svedruzic, Jianping Yu

Collaborators: Matt Posewitz (Colorado School of Mines,); Sergey Kosouvov and Anatoly Tsygankov (Pushchino, Russia); Klaus Schulten (Beckman Institute, University of Illinois); Michael Flickinger (University of Minnesota); Vekalet Tek (Florida International University)