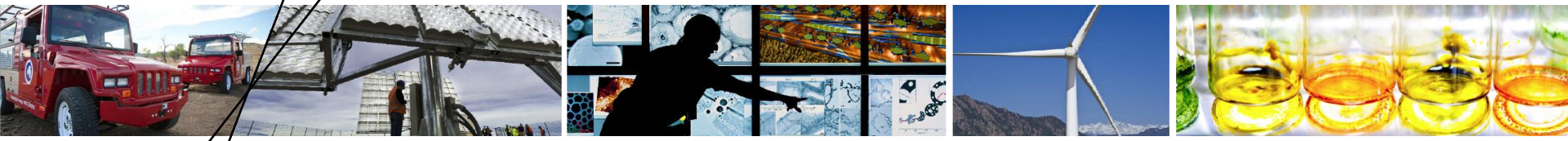
National Renewable Energy Laboratory

Keith Wipke, FCHT Laboratory Program Manager
Richard V. Greene, Biosciences Center Director



Biological Hydrogen Production Workshop

September 24, 2013

The Hydrogen Program at NREL: A Brief Overview

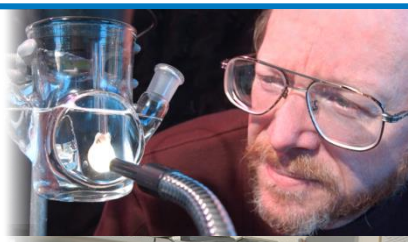
"Integration is the Word"

NREL Fuel Cell & Hydrogen Technologies Program

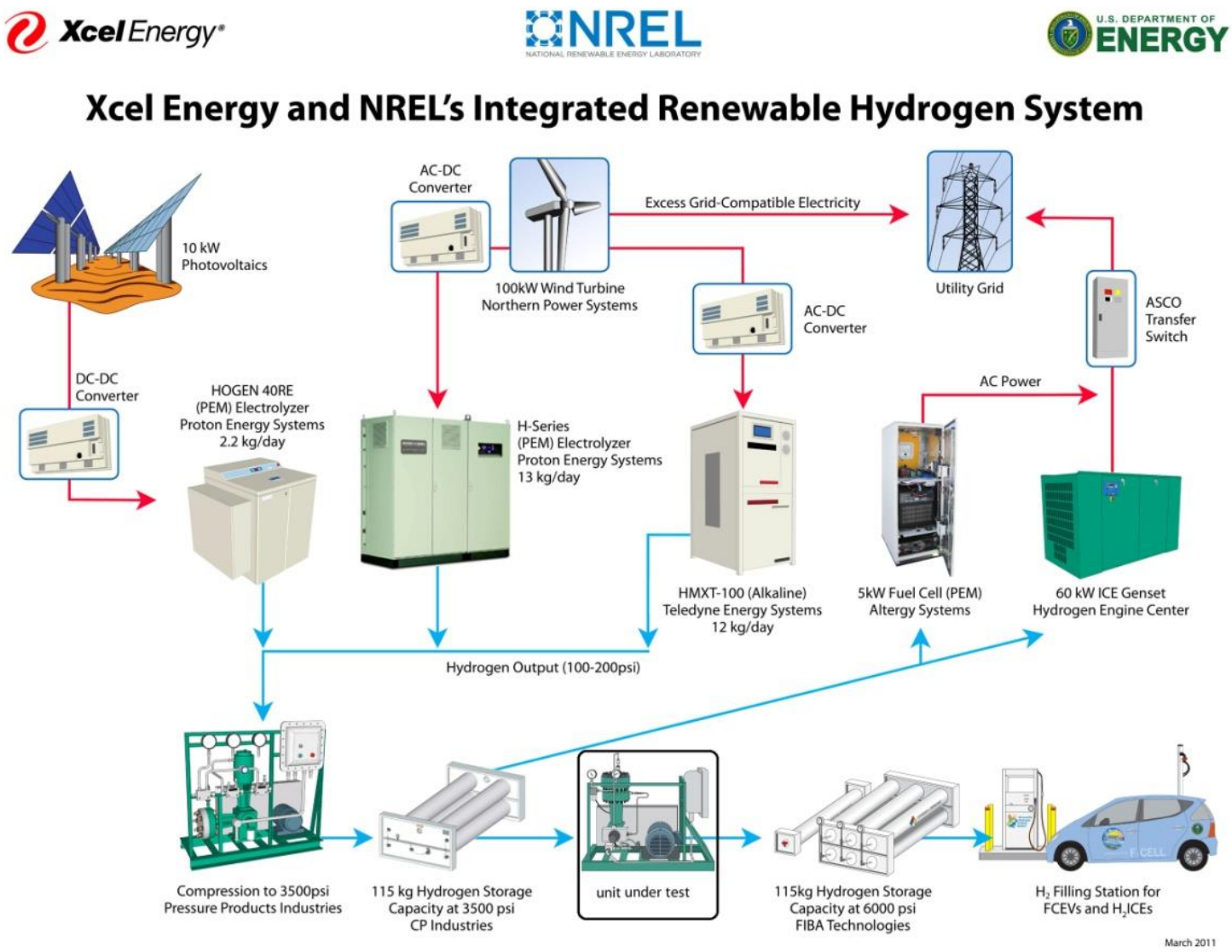
- Renewable Hydrogen Production
- Hydrogen Delivery
- Hydrogen Storage
- Fuel Cell Manufacturing R&D
- Fuel Cells
- Technology Validation
- Codes, & Standards
- Analysis
- Market Transformation
- Education







Hydrogen Production from Renewable Sources at NREL

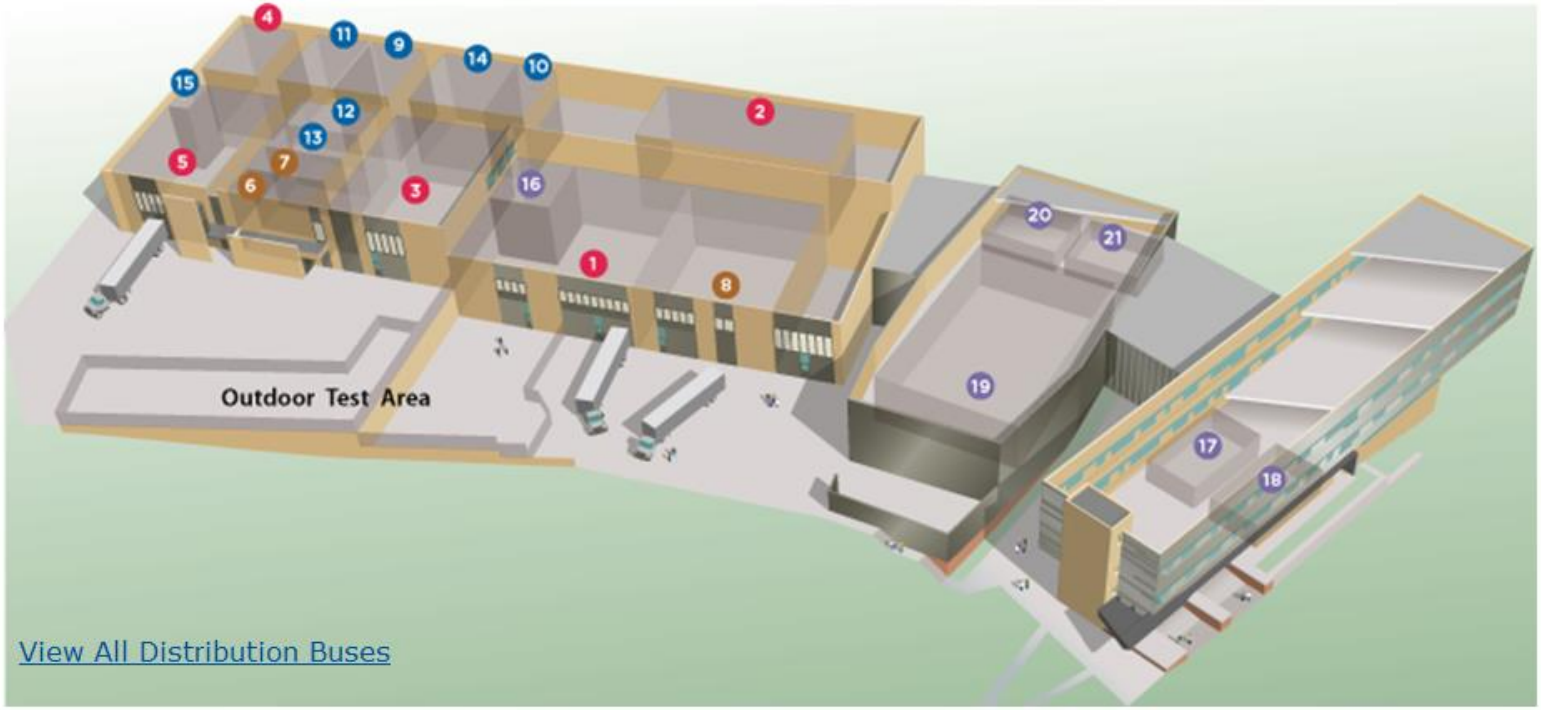


Refueling at NREL's Hydrogen Station

ESIF: West Elevation

ESIF: Northwest Elevation

Major ESIF Laboratories/Capabilities



Electricity Laboratories

1. Power Systems Integration
2. Smart Power
3. Energy Storage
4. Electrical Characterization
5. Energy Systems Integration

Research Electrical Distribution Bus (REDB) – AC and DC

Thermal Laboratories

6. Thermal Systems
7. Thermal Storage Materials
8. Optical Characterization and Thermal Systems

Thermal Distribution Bus

Fuel Laboratories

9. Energy Systems Fabrication
10. Manufacturing
11. Materials Characterization
12. Electrochemical
13. Energy Systems Sensor
14. Fuel Cell Development
15. High-Pressure Testing

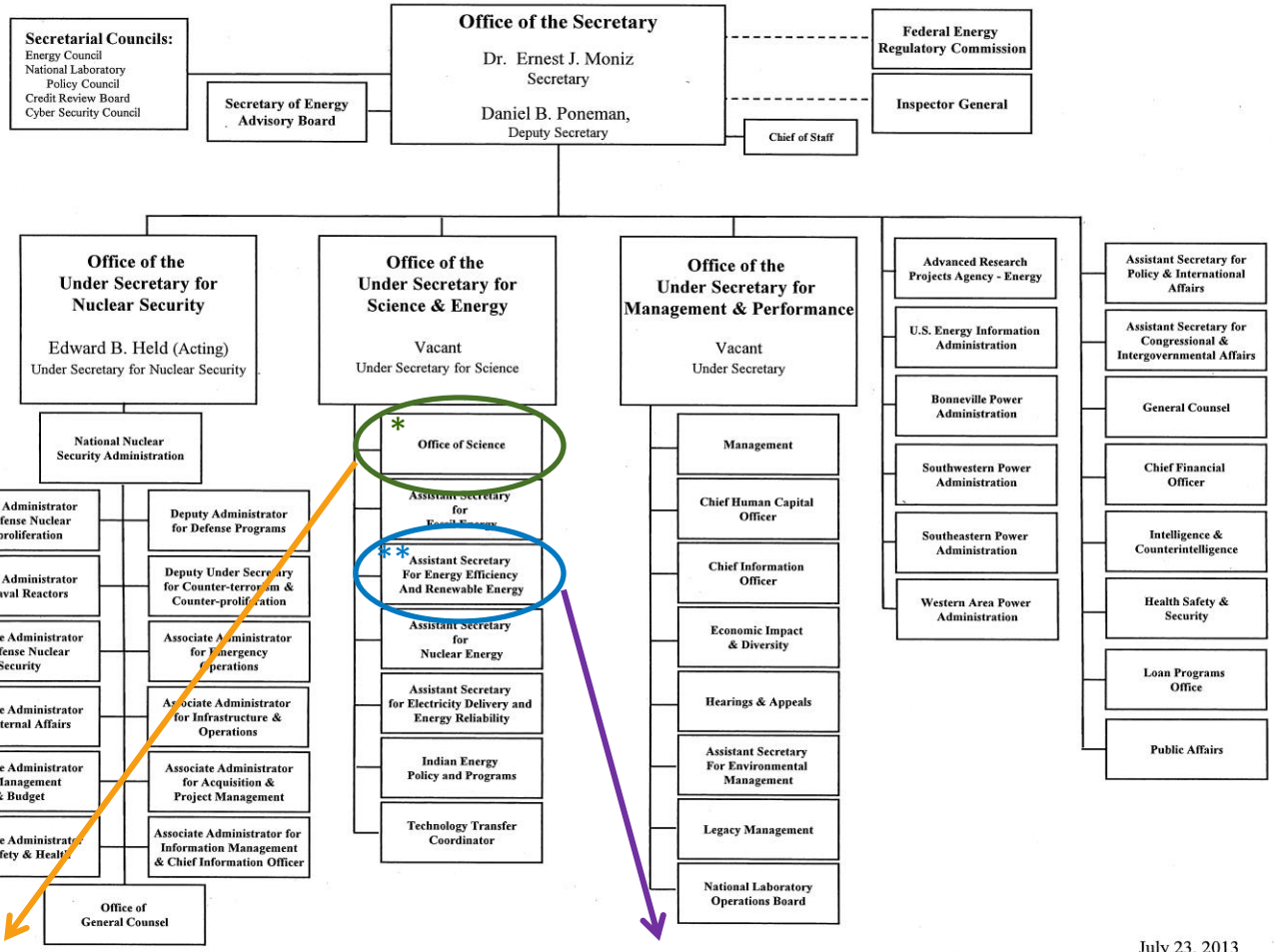
Fuel Distribution Bus

Data, Analysis, and Visualization

16. ESIF Control Room
17. Visualization Room
18. Secure Data Center
19. High Performance Computing

Supervisory Control and Data Acquisition (SCADA) System

DEPARTMENT OF ENERGY



July 23, 2013

* Office of Basic Energy Sciences
Office of Biological & Environmental Research

** Fuel Cell Technologies Office

Integrating Basic Science & Translational R&D to Understand and Develop Photobiological Algal Systems for Producing Hydrogen at NREL

Translational Research & Development (EERE:FCT)

Goal: Develop algal systems (enzymes or organisms) capable of sustained H_2 photoproduction under aerobic conditions

(a) Developed chemochromic sensors for detection of H_2 by isolated algal colonies
(b) Identified and cloned the algal *HYDA1* and *HYDA2* [FeFe]-hydrogenases genes
(c) Introduced *HYDA1*, *HYDA2* and their maturation genes into *E. coli* for mass-production of the respective enzymes
(d) Developed computational models of gas diffusion in clostridial [FeFe]-hydrogenase; generated and tested mutants for O_2 tolerance (no positive transformants with high hydrogenase activity have been identified, yet).
(e) Shifted R&D direction towards the introduction of the clostridial hydrogenase (higher O_2 tolerance) gene into Chlamydomonas, using an algal strain with no native hydrogenase activity. Demonstrated expression of Ca1 in Chlamydomonas and H2 photoproduction.
(f) Performed computational modelling of the interaction between ferredoxins and hydrogenases.
(g) Expressed clostridial hydrogenase in double knock-out algal strain and demonstrated H2 production in vivo

Basic Research (SC:BES/BER)

Goal: understand structure, function and transcriptional regulation of hydrogenases

(a) Identified the maturation proteins responsible for assembly of algal hydrogenases using chemochromic sensors.
(b) Expressed bacterial [FeFe]-hydrogenases in *E. coli* and found that clostridial hydrogenases have higher tolerance to O_2 inactivation.
(c) Generated an algal strain with no native hydrogenase background activity.
(d) Generated a collection of cyanobacterial Hox operon mutants.
(e) Demonstrate higher reductant flux in vitro towards H_2 production with fused Fd/H2ase
(f) Developed high throughput high-sensitivity biological sensor for single colony H_2 production

PHOTOBIOLOGY: Improving Algal Photosynthetic Hydrogen Production – O_2 tolerance

Scientific Achievement

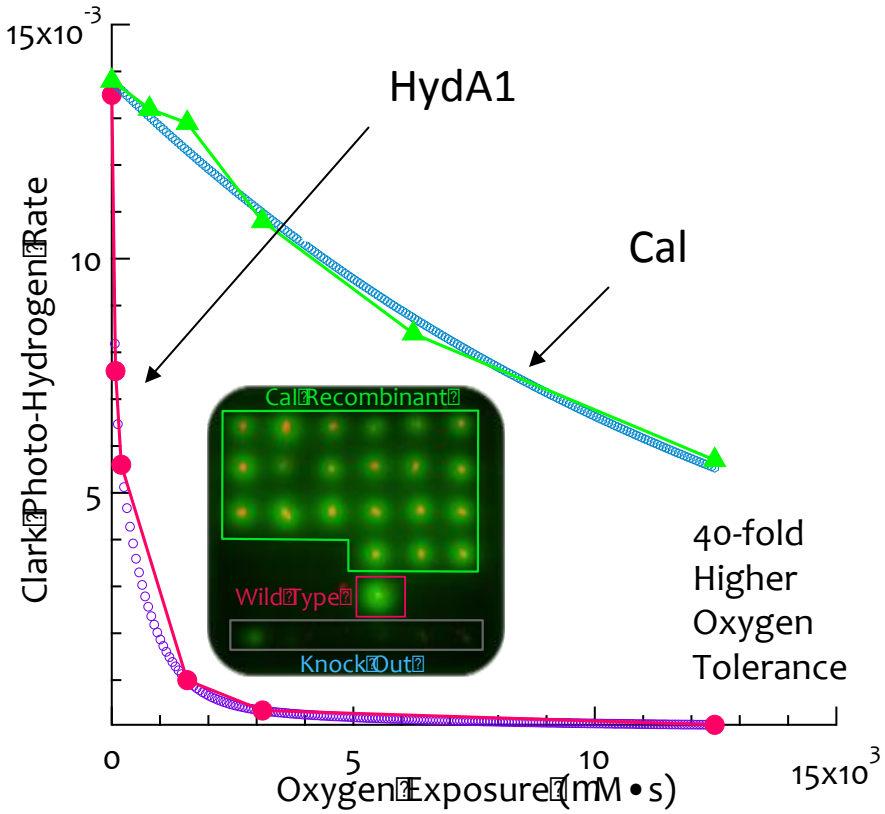
A more oxygen-tolerant Clostridial hydrogenase expressed in *Chlamydomonas* catalyzed photo-hydrogen production

Significance and Impact

Oxygen sensitivity is a major limitation to the use of photosynthetic microbes for solar hydrogen

Research Details

- Photosynthetic water-splitting utilizes sunlight energy to split water.
- Green algae can link water-splitting to hydrogen production using hydrogenases, but only for short periods due to high sensitivity to oxygen.
- Bacterial hydrogenases **(CaI)** showing higher oxygen tolerance were expressed in a hydrogenase deficient algal mutant (*Posewitz, CSM*)
- Under photosynthetic conditions, the **CaI** cells showed 40-fold higher tolerance to oxygen.
- This is an essential step towards engineering green algae for efficient photo-production of hydrogen from water splitting.



GFP Screening and Oxygen Inactivation Kinetics of Photo-Hydrogen Production: Under illumination, photosynthesis produces hydrogen detected as a GFP halo that identified **CaI** expressing cells. Exposure of anaerobic cells to oxygen inactivates native algal hydrogenase (HydA1, red trace) at a faster rate than bacterial hydrogenase (CaI, green trace).

Seth Noone, Kath Ratcliff, Reanna Davis, Matt Wecker, Jon Meuser, Matthew C. Posewitz, Paul W. King and Maria L. Ghirardi

FERMENTATION: Developed Genetic Tools in *Clostridium thermocellum* for Improved Hydrogen Production from Cellulose

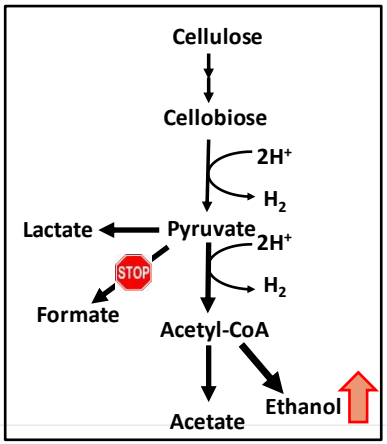
Fuel Cell Technologies Office

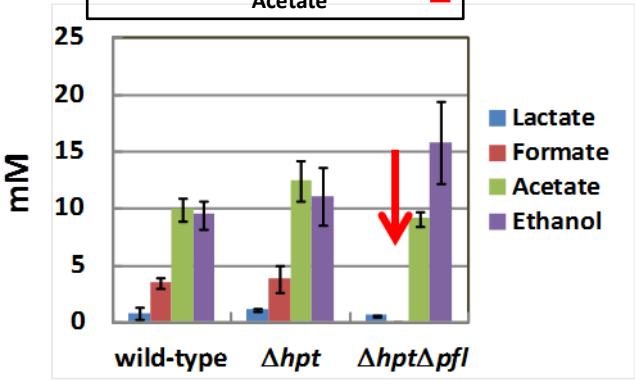
Scientific Achievement

NREL has developed proprietary genetic tools to stably manipulate the genome of *Clostridium thermocellum* for improved hydrogen production.

Significance and Impact

***C. thermocellum* exhibits one of the highest rates of cellulose hydrolysis. This in-house capability enables us to engineer its metabolic pathways to tailor the production of desirable biofuels and biochemicals including hydrogen.**



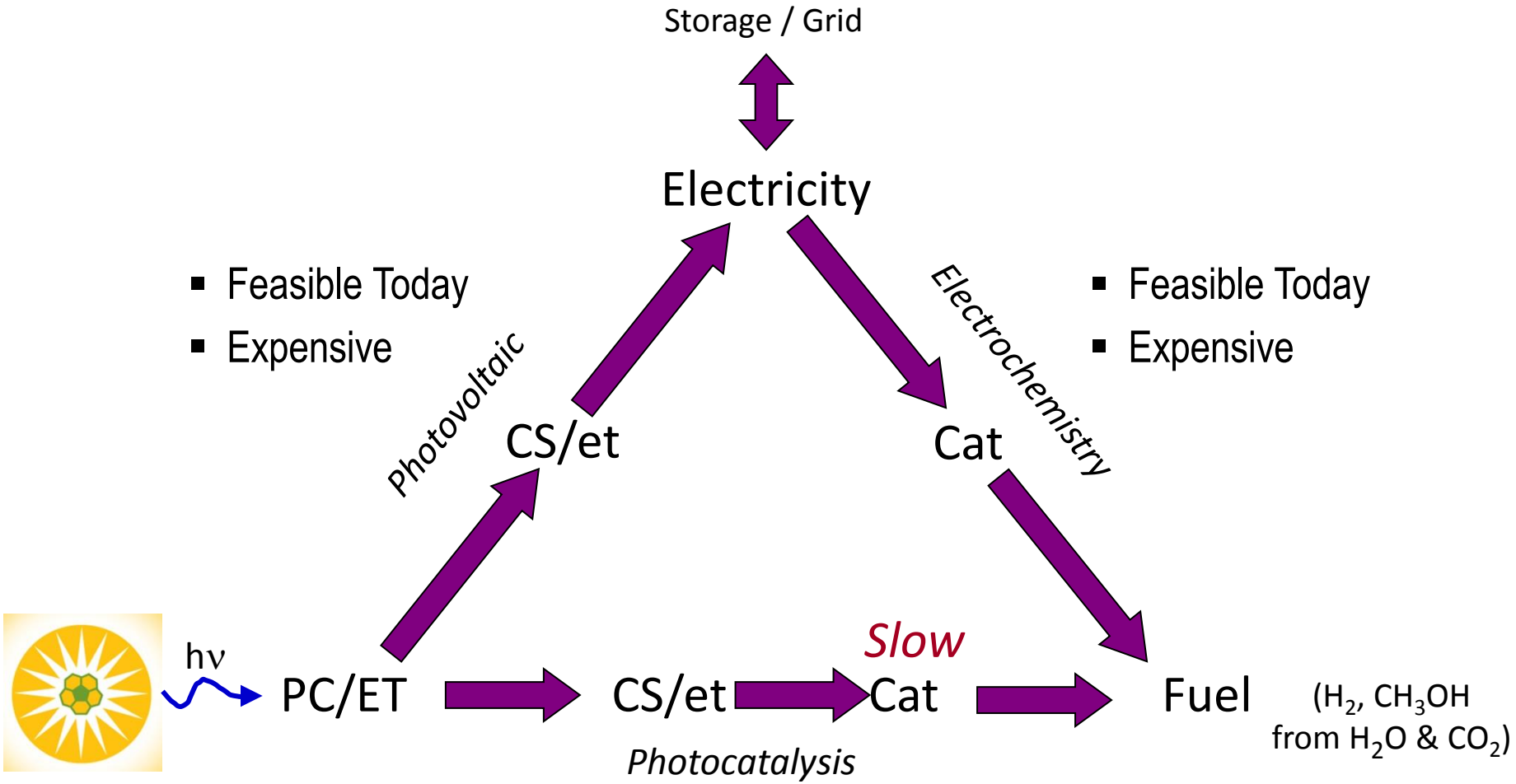


Research Details

- *Clostridium thermocellum* combines cellulose hydrolysis with H_2 production, hence is a model microbe for consolidated bioprocessing (CBP).
- Yet the competing metabolic pathways (top figure) lower the yield of H_2 from cellulose, a technical barrier as to its techno-economic feasibility.
- We have developed genetic tools and obtained mutants lacking the competing pyruvate-to-formate reaction, as evidenced by a lack of formate production in the mutant (red arrow, lower figure).
- The mutant exhibited a 50% increase in the specific activity of H_2 production and up to 60% increase in ethanol production.
- Improving H_2 yield and total H_2 output via additional genetic engineering forms the thrust of this research I building an H_2 economy.

Pin-Ching Maness, Katherine Chou, & Lauren Magnusson

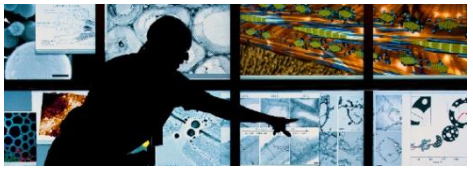
Strong NREL Capacity in Artificial Photosynthesis (Analogous to Natural Systems)



Control is often more important than power (or efficiency).

Photosynthesis did not evolve to make us biofuels nor necessarily to be the most efficient. It evolved because it lets organisms survive.

NREL
NATIONAL RENEWABLE ENERGY LABORATORY

Thank You!

www.nrel.gov