H₂ Production by Oxygenic Phototrophs

Eric L. Hegg

Bioresour. Technol. 2011, 102, 8589-8604

Michigan State University
Great Lakes Bioenergy Research Center
Major Challenges to H₂ Photoproduction

- **Technical Challenges**
  - Mixture of H₂ and O₂; H₂ separation and storage
  - CO₂ addition and overall reactor design

- **Biological Challenges**
  - Poor efficiency of H₂ production
  - Poor heterologous expression of H₂-forming enzymes
  - Low quantum yields
  - Competition for reducing equivalents; poor electron coupling
  - Sensitivity of H₂-forming enzymes to O₂

---

M. Ghirardi, Abstract #1751, Honolulu PRiME 2012
Overcoming Low Efficiency: Improving ET

• Eliminate or down-regulate pathways competing for electron
  • Production of organic acids
  • Formation of NADPH/carbon fixation

Strategy depends on good genetics and an understanding of the metabolic pathways

• Identify endogenous electron transfer partner
  • Which ferredoxin or cytochrome?
Overcoming Low Efficiency: Improving ET

- Engineer improved coupling
  - Mutate docking site for enhanced binding

- Fuse H₂ase to ferredoxin

- Fuse H₂ase directly to PS-I

- Localize to a synthetic protein scaffold

*PNAS* 2011, 108, 9396-9401

*Photochem. Photobiol.* 2006, 82, 676-682
Overcoming $O_2$ Sensitivity

- **Utilize non-oxygenic photosynthesis**
  - Purple bacteria (*Rhodobacter sphaeroides*)
  - Selective light
  - Sulfur-deprivation

- **Engineer enzyme to be less $O_2$ sensitive**
  - Inhibit diffusion of $O_2$
  - Alter redox potentials

- **Separate $H_2$ and $O_2$ biosynthesis**
  - Temporal separation (e.g. $H_2$ produced from fermentation)
  - Spatial separation
  - Heterocyst forming bacteria
  - Expression of [FeFe]-$H_2$ase in *Anabaena* sp. PCC 7120
  - Mutations can increase heterocyst frequency
  - Other compartments? Carboxysomes?

*Wolk et al.*
Identifying New Organisms

- **Cyanothecae sp. ATCC 51142**
  - H₂ from nitrogenase
  - 465 μmol H₂ per mg chl per hr
  - Simultaneous light-driven H₂ and O₂ production
    - Over 100 hrs in presence of CO₂

- **Volvox carteri**
  - Multicellular green alga with differentiated cells
    - *C. reinhardtii* is most well-characterized relative
  - First multicellular eukaryote discovered to have H₂ metabolism

*Nat. Commun. 2010, 1:139*