Growth and Diversity of H_2-Producing Bacteria

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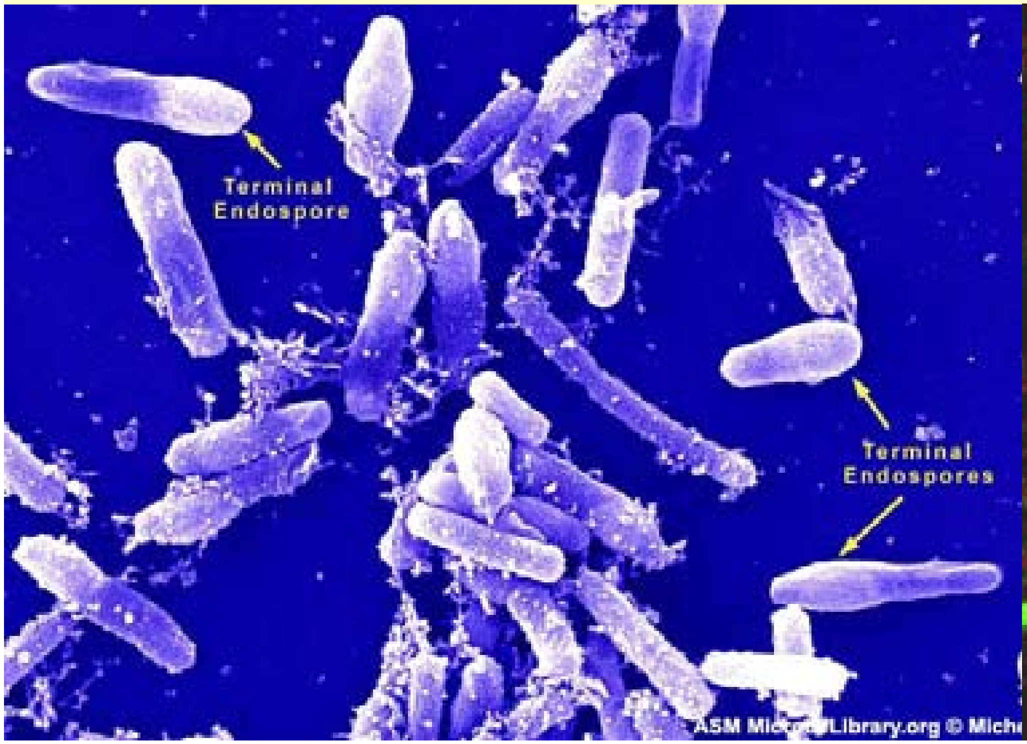
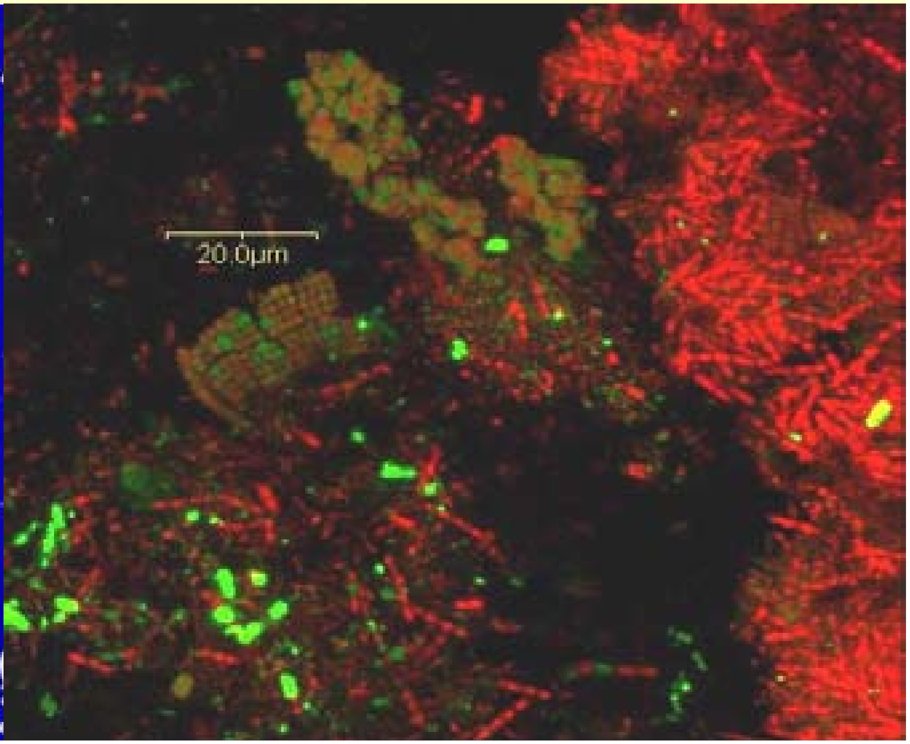
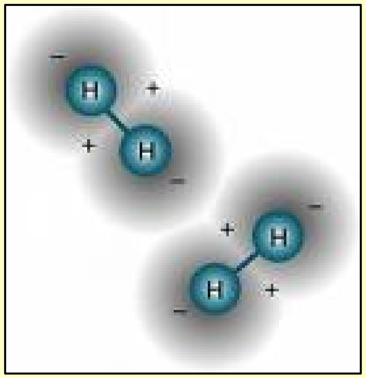






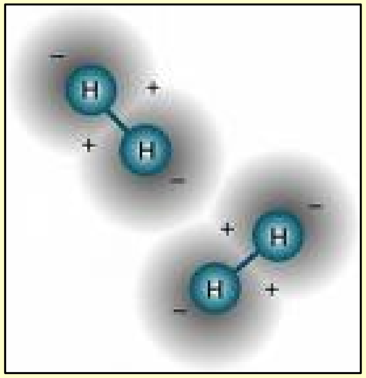
Early Archaean landscape

3.5 billion years ago

- **Abundance and diversity of H_2-producing microorganisms**

- **Known biochemical pathways for biohydrogen production**

- **Microbial insights which might help us develop a human-scale hydrogen economy**



www.sciencemag.org • SCIENCE • VOL. 276 • 2 MAY 1997

MICROBIAL BIOLOGY

Microbiology's Scarred Revolutionary

Carl Woese revised the tree of life and started a new age in microbial biology by recognizing a third domain of life—but he paid the price for his radical ideas

Carl Woese at Univ of Illinois, Champaign-Urbana

With DNA sequencing films revealing a universal tree of life

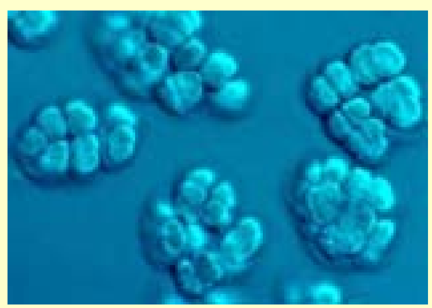
Five Kingdoms

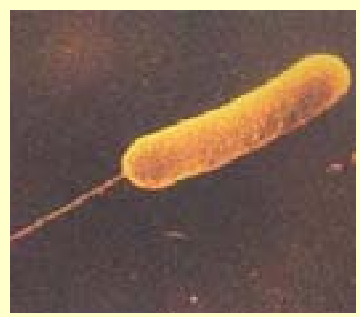
Animals

Plants

Fungi

Protists





Monera

Five Kingdoms

Eukaryotes

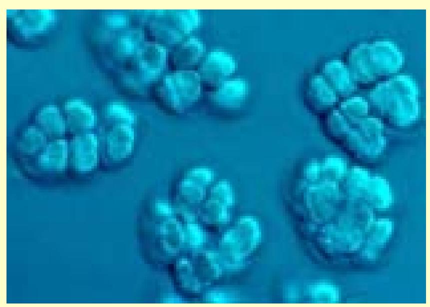
Animals

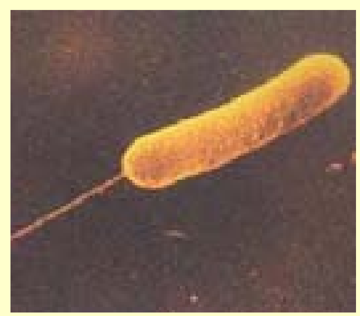
Plants

Fungi

Protists

Prokaryotes



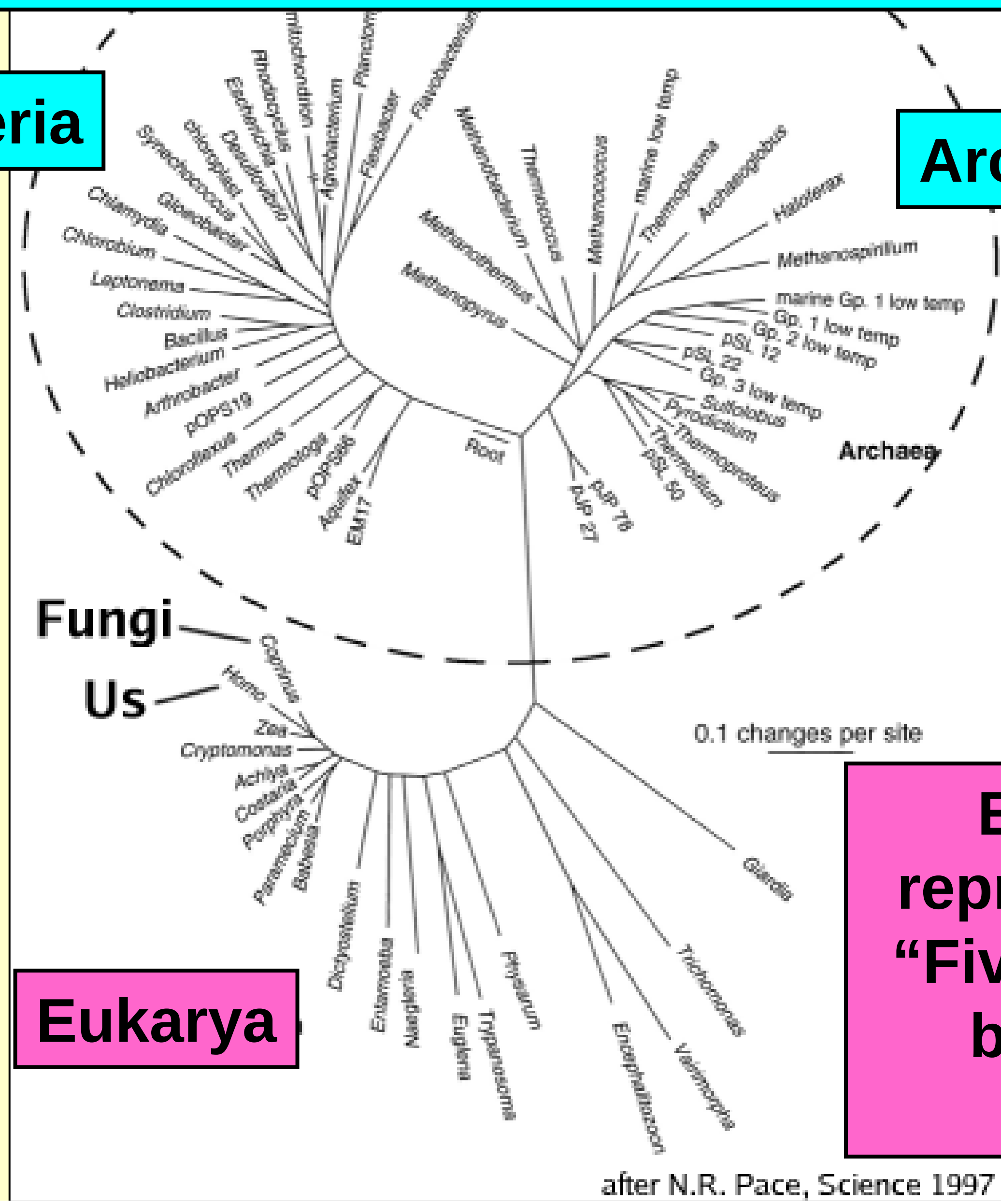


Monera

Prokaryotes make up two Domains in the Universal Tree of Life

Bacteria

Archaea



Eukarya

Eukaryotes represent four of “Five Kingdoms” but just one Domain

Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 6578–6583, June 1998

Perspective

Prokaryotes: The unseen majority

William B. Whitman†, David C. Coleman‡, and William J. Wiebe§*

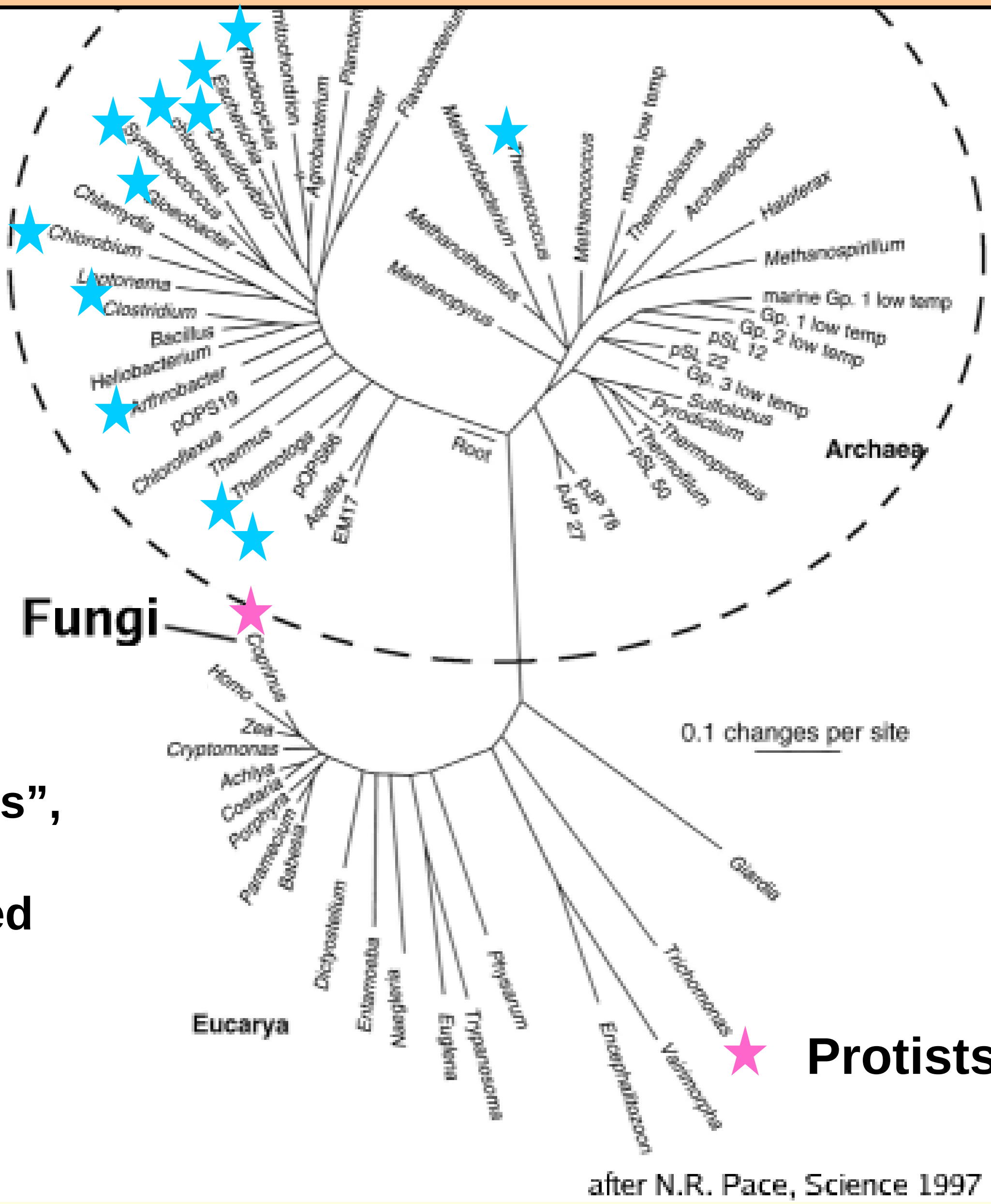
*Departments of *Microbiology, ‡Ecology, and §Marine Sciences, University of Georgia, Athens GA 30602*

Global estimates of biomass carbon[1] in Petagrams (10^{15} g) includes surface and subsurface soils & ocean sediments

	Plants & algae	Prokaryotes
Terrestrial	**560**	**22-215**
Marine	**2**	**305**

[1]Biomass C includes live protoplasm, cell walls, and structural materials

Evolutionary lineages with species known to produce H_2

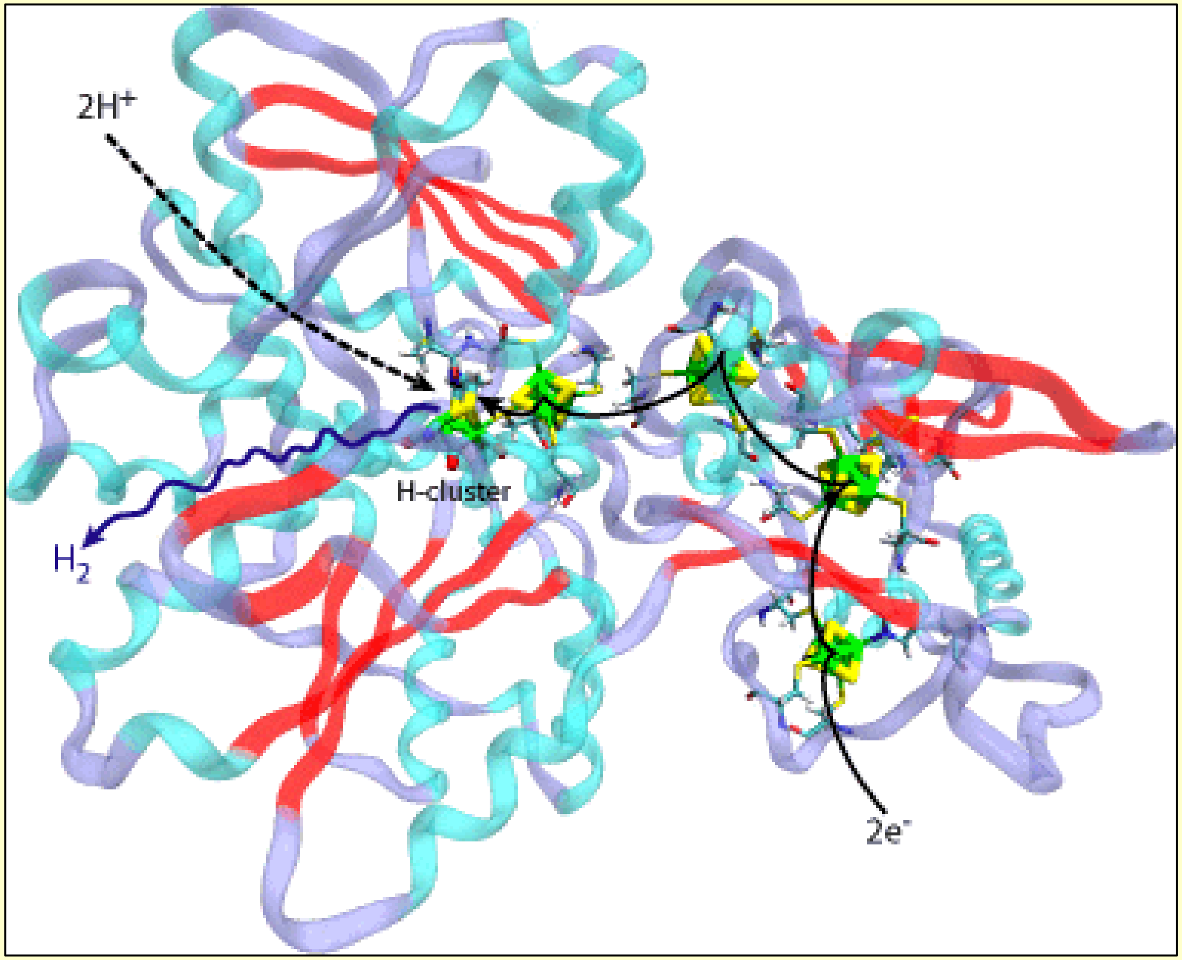


Some anaerobic fungi & protists contain “hydrogenosomes”, H_2-producing organelles evolved from bacterial symbionts

Akhmanova et al. Nature 1998

Hydrogenases are a diverse family of enzymes that catalyze the reversible reaction $2H^+ + 2e^- \leftrightarrow H_2$

H^+ and e^- passage through the CpI hydrogenase of *Clostridium pasteurianum*



Theoretical & Computational Biophysics Group
Univ of Illinois-Champaign Urbana

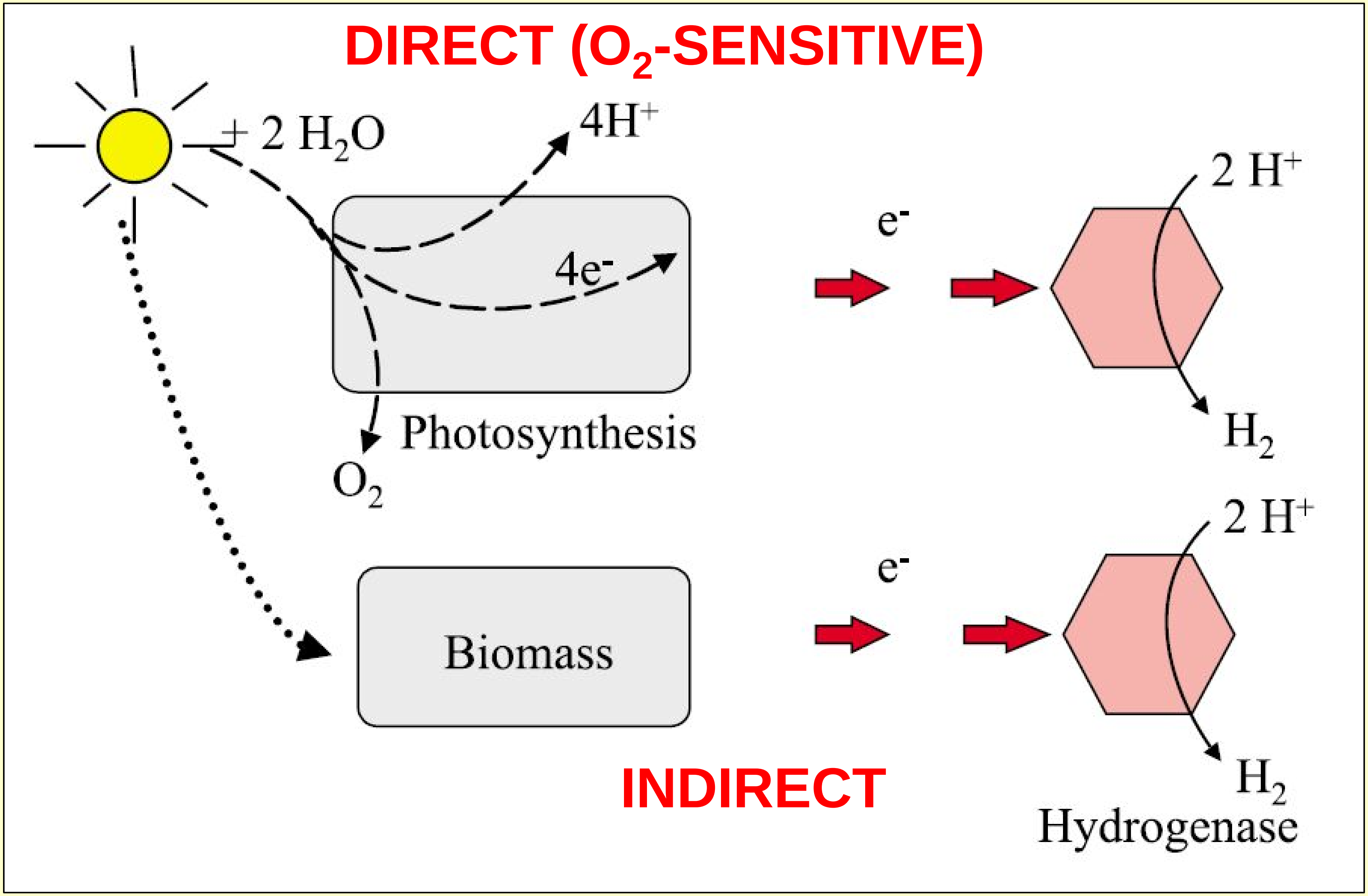
Prokaryotic H_2-generation

Oxygenic photosynthesis

Cyanobacteria (formerly called "blue-green algae")
Genera include *Anabaena, Nostoc, Synechocystis*
Mechanisms:
- Direct biophotolysis
- Indirect H_2 generation from reduced carbon

Key Advantage:
Availability of solar radiation

Key Drawback:
O_2 sensitivity of hydrogenase
Low efficiency of solar energy capture


DIRECT (O_2-SENSITIVE)
+ 2 H_2O
$4H^+$
$4e^-$
O_2
Photosynthesis
e^-
2 H^+
H_2
Biomass
e^-
2 H^+
H_2
INDIRECT
Hydrogenase

Prokaryotic H_2-generation

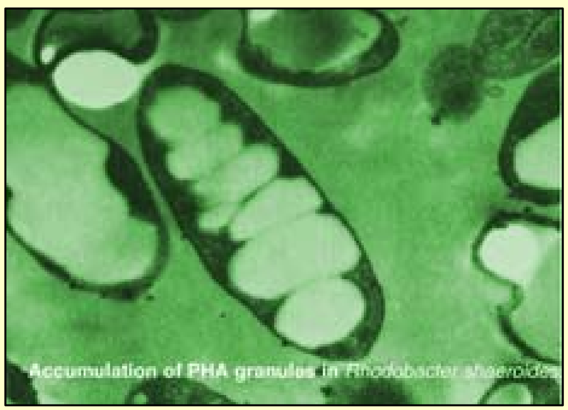
<u>Anaerobic photosynthesis</u>

Photosynthetic bacteria

Genera in betaproteobacteria include Rhodobacter, Rhodospirillum

Mechanisms:

- **Direct biophotolysis (electrons from H_2O)**
- **Indirect H_2 from fermentation (electrons from reduced carbon)**
- **H_2 production by nitrogenase during N_2 fixation**



<u>Key advantage:</u>

Availability of solar radiation

No O_2 to inhibit hydrogenase

<u>Key drawback:</u>

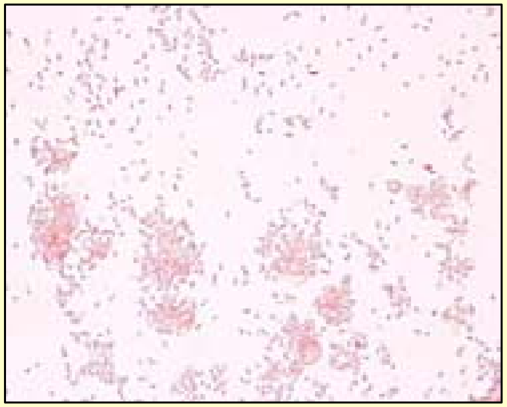
Low efficiency of solar energy capture

High cost of photobioreactors

Prokaryotic H_2-generation

Dark-fermentation

Anaerobes and facultative anaerobes
Sporeforming genera (Clostridium)
Non-sporeforming genera (Enterobacter, Citrobacter)
Mechanisms:
- Electrons derived from reduced carbon to generate reductant
- Reductant is oxidized and electrons transferred to H^+

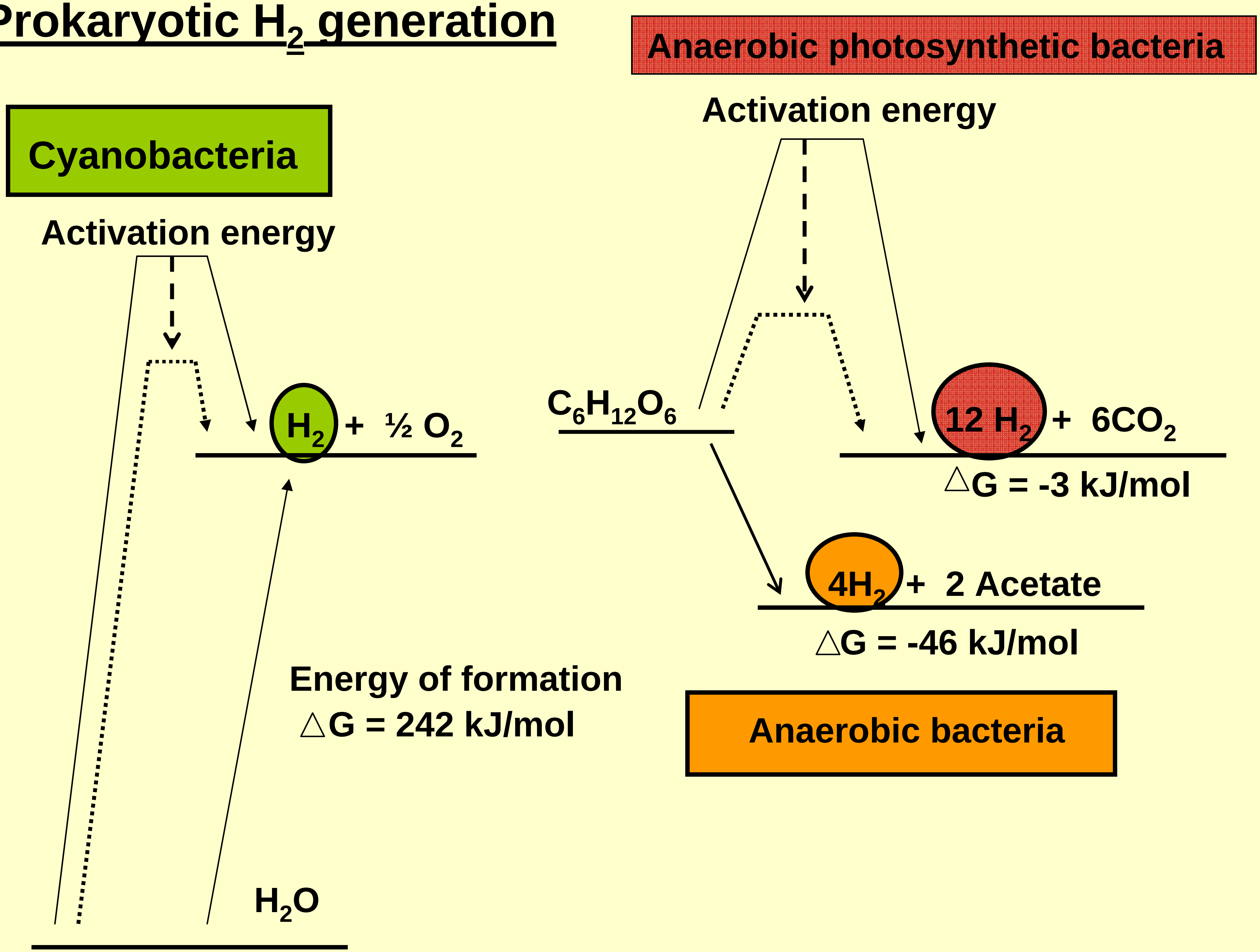


Pure culture of Clostridium isolate from PSU soil

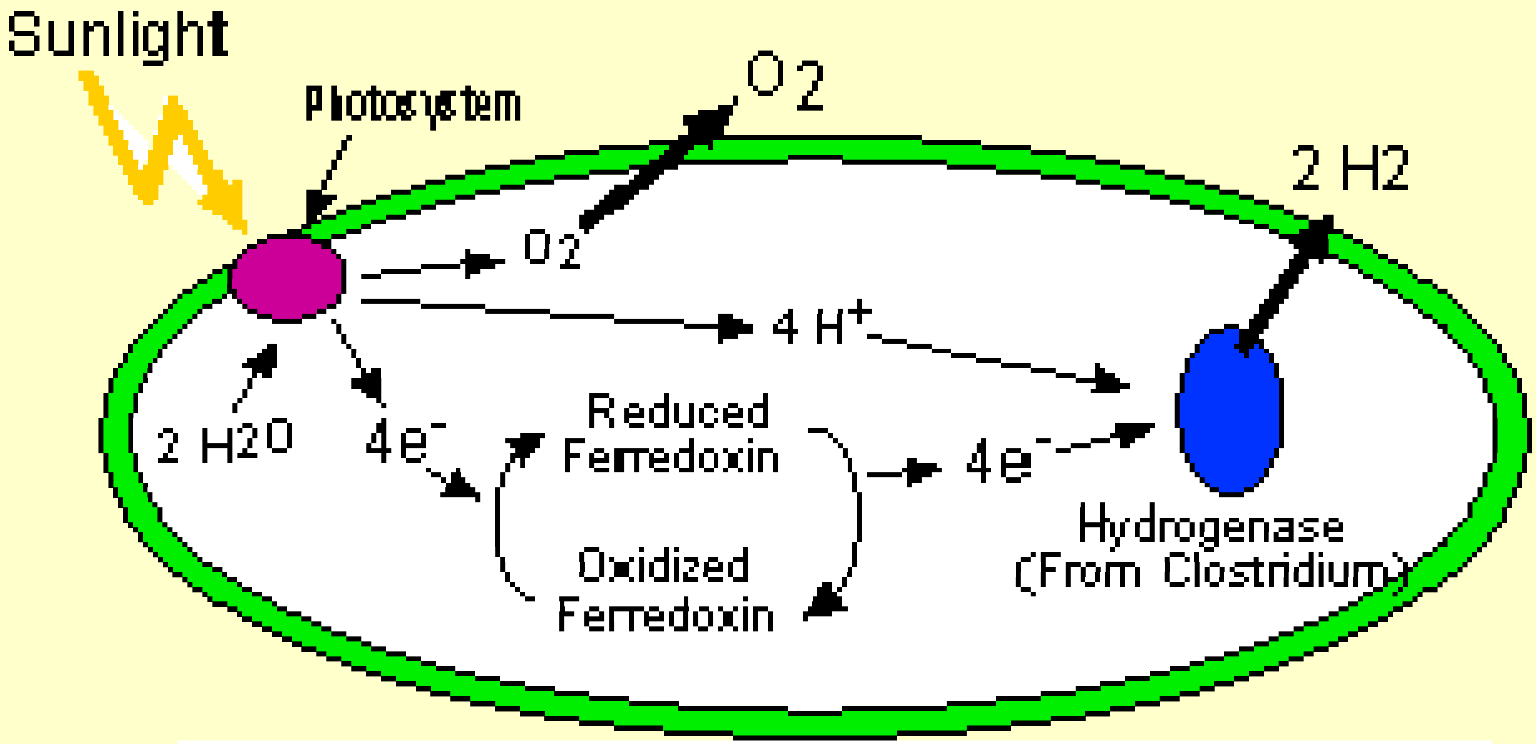
Key advantage:
Use renewable wastes as electron donors
Lower cost bioreactors

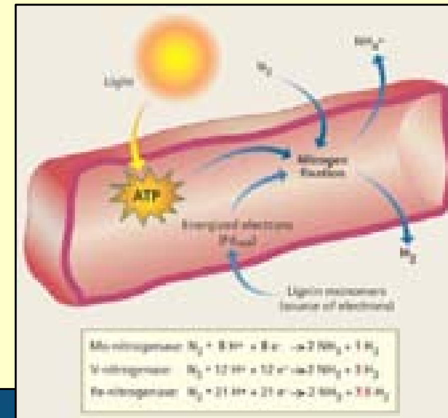
Key drawback:
Low molar H_2 yields from organic wastes compared to methane generation

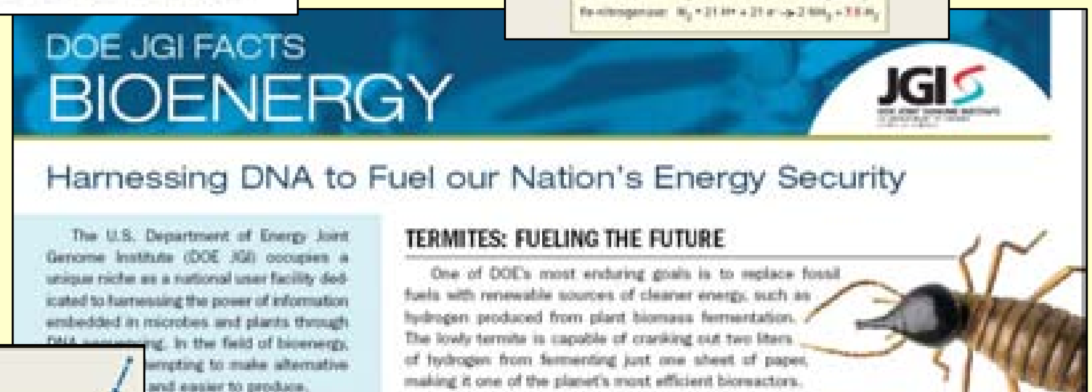
Prokaryotic H_2 generation



Early experiments to create "hybrid" H_2-producing systems from chloroplasts and hydrogenase enzymes from fermenting *Clostridium* bacteria





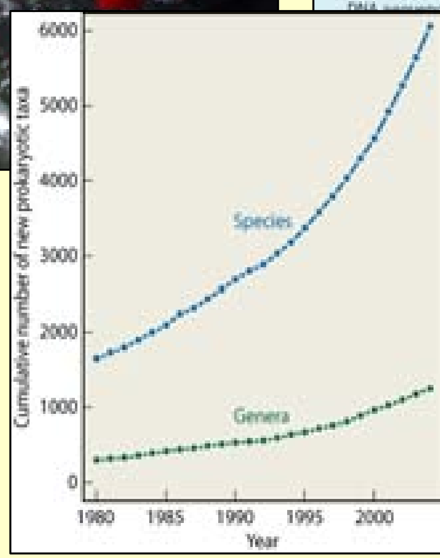

DOE JGI FACTS
BIOENERGY

Harnessing DNA to Fuel our Nation's Energy Security

The U.S. Department of Energy Joint Genome Institute (DOE JGI) occupies a unique niche as a national user facility dedicated to harnessing the power of information embedded in microbes and plants through DNA sequencing. In the field of bioenergy, ... empting to make alternative ... and easier to produce.

TERMITES: FUELING THE FUTURE

One of DOE's most enduring goals is to replace fossil fuels with renewable sources of cleaner energy, such as hydrogen produced from plant biomass fermentation. The lowly termite is capable of cranking out two liters of hydrogen from fermenting just one sheet of paper, making it one of the planet's most efficient bioreactors.



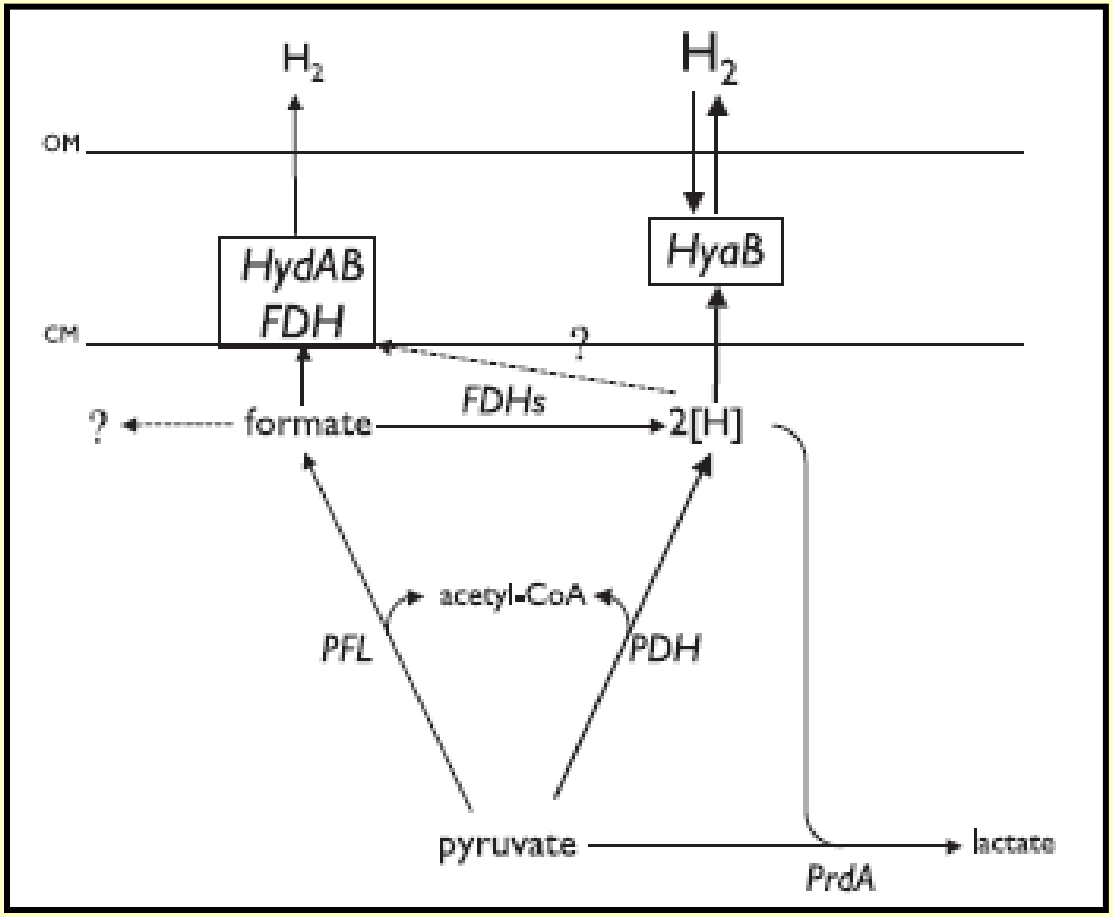
Only about 1% of extant prokaryotic species have been studied.

Need for continued discovery of new species, enzymes, pathways for energy production.

Other mechanisms for prokaryotic H_2 generation

Pyruvate conversion during stationary phase

H_2 metabolism of *Shewanella oneidensis* MR-1



Meshulam-Simon et al. 2007. *Appl Environ Microbiol*

Other mechanisms for prokaryotic H_2-generation

N_2 fixation

Free-living N_2 fixers (cyanobacteria, photosynthetic bacteria, *Azotobacter* spp.)

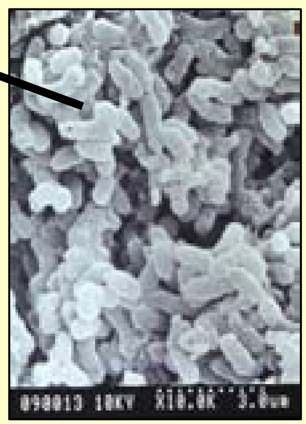
Symbiotic N_2 fixers (e.g., *Rhizobium, Frankia* spp.)

Mechanism:

H_2 is byproduct of nitrogenase enzyme

N_2-fixing nodules on legume roots

Bacteroids in nodules



Key advantage:

Use organic wastes as electron donors
Create artificial conditions in low-cost bioreactors

Key drawback:

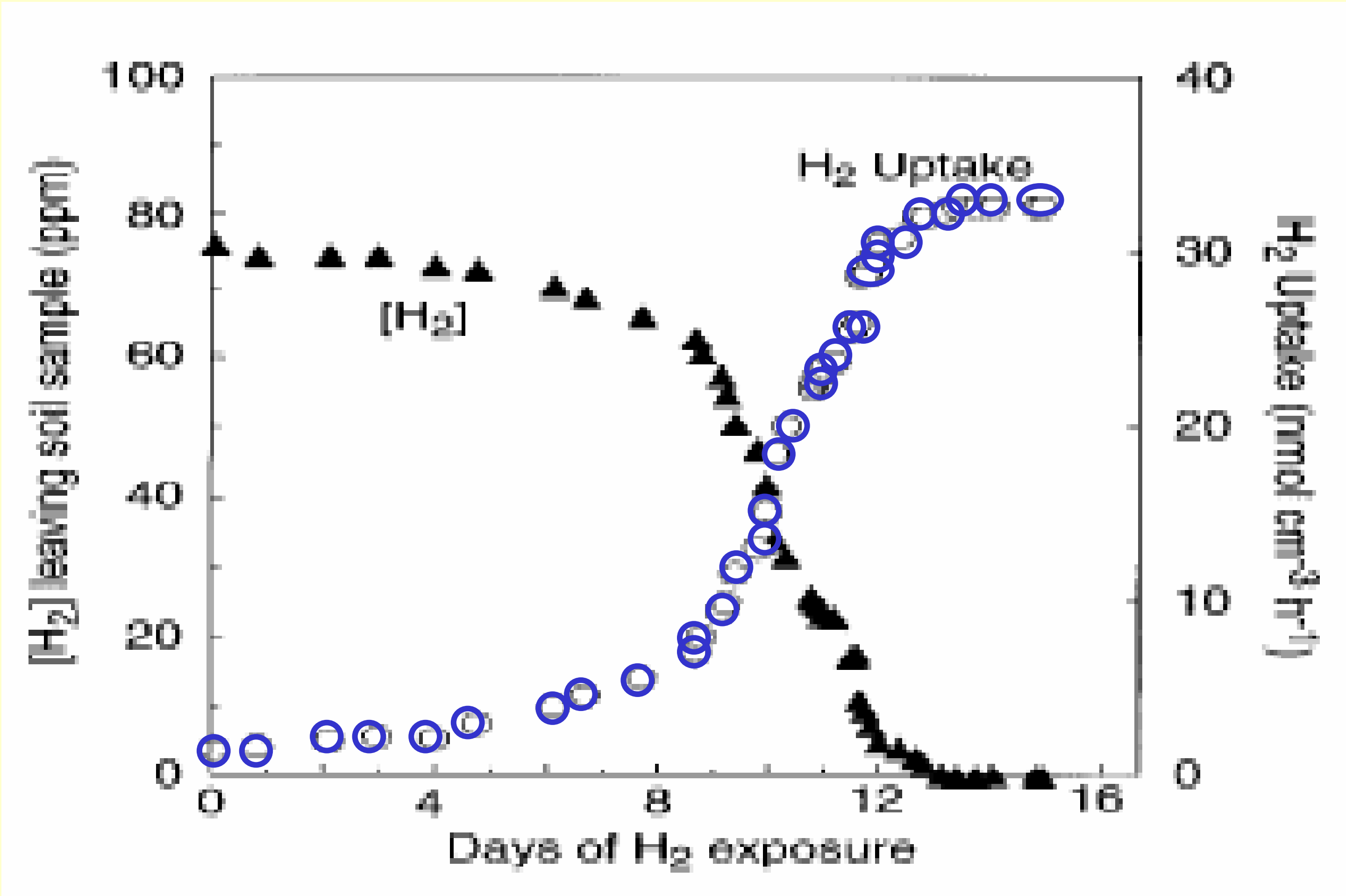
Need for induction of nitrogenase
Nitrogenase's O_2 sensitivity
Relatively low H_2 yields

If H_2 production by prokaryotes is so prevalent, why doesn't H_2 build up in the atmosphere?

Prokaryotes' ability to take up and oxidize H_2 is probably even more widespread than the ability to produce H_2

H_2-oxidizing bacteria are diverse and widespread "syntrophs" consume H_2 as fast as it's produced

H_2 is readily consumed by microbial biomass when injected into soil (33 nmol H_2 cm^{-3} hr^{-1})



Dong et al. 2001

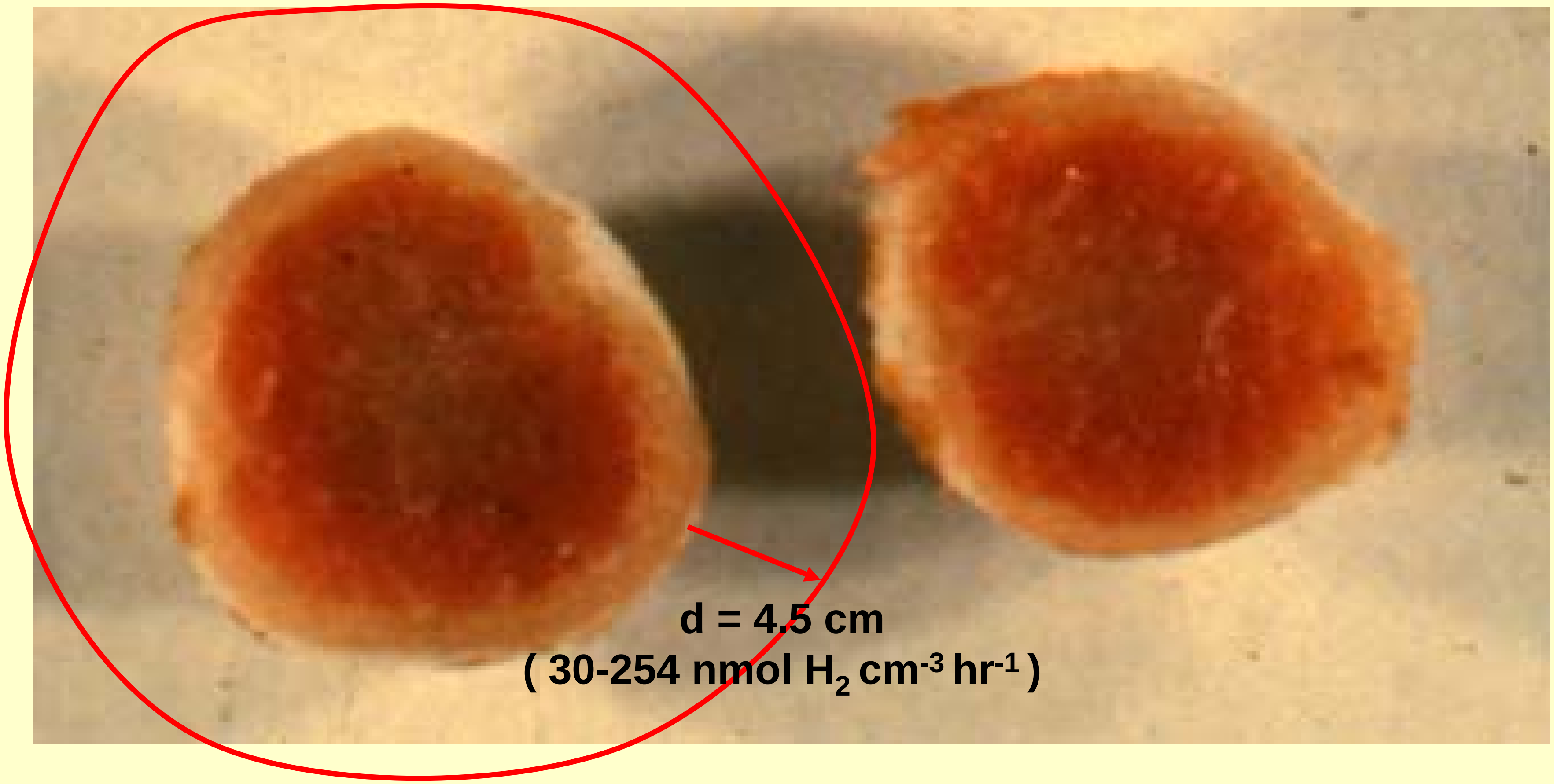
Hypothesis: Legumes "fertilize" soils not only with fixed N but also with H_2.

(Dong et al., 2003)

(Invention disclosure for patent application: http://www.wipo.int)

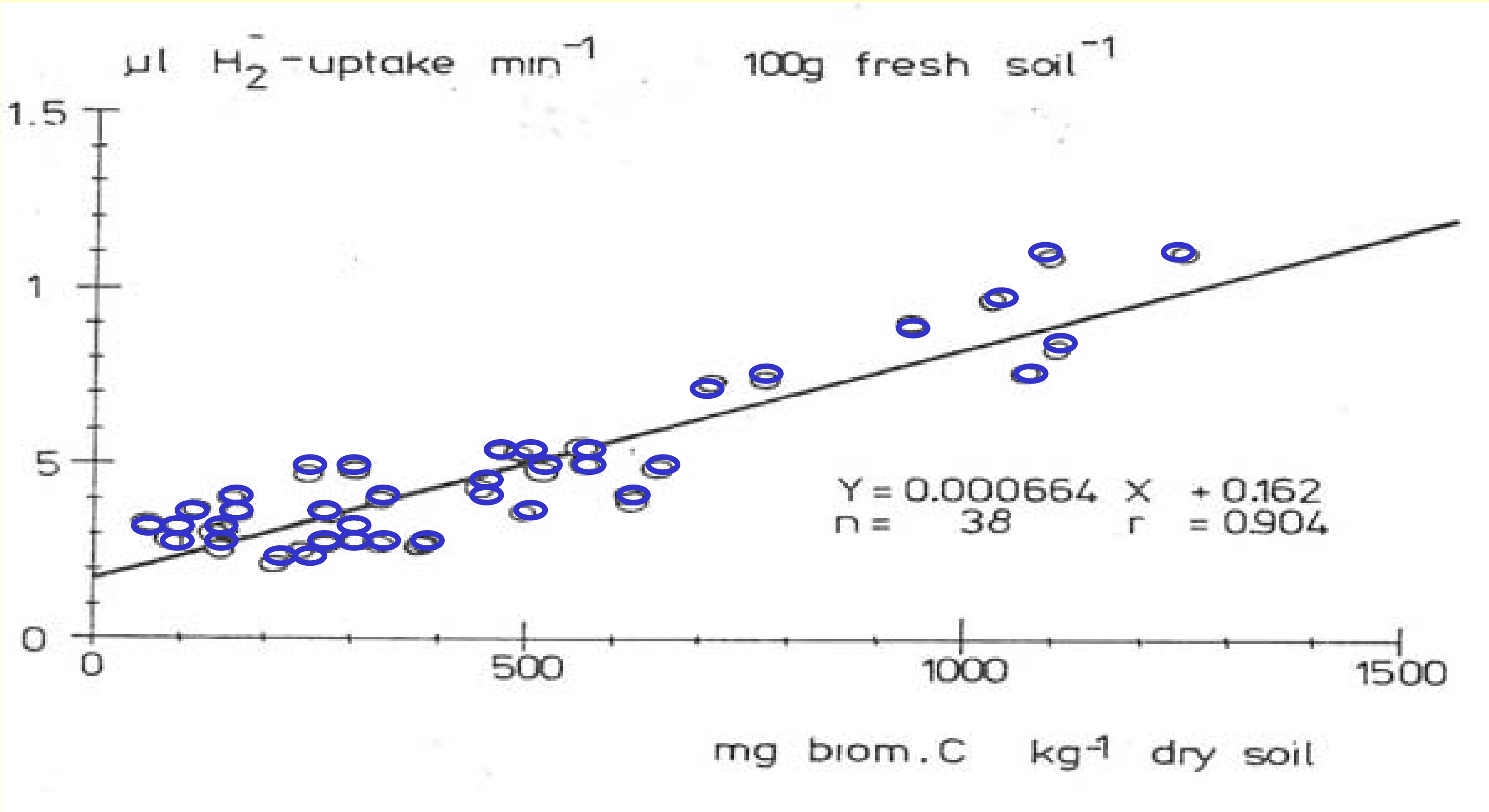
Some rhizobia lack "uptake hydrogenases" and cannot recycle H_2 from nitrogenase

H_2 leaking from these nodules is consumed within 5 cm of the nodule surface



Favre et al 1983

Relationship between the rate of H_2-uptake by soil and microbial biomass content



Popelier et al., 1985

If we prevent microbes from consuming H_2, how much do we produce in experimental systems?

Photochemical production	**mmol H_2/g-hr**
Oxygenic photosynthetic bacteria	**0.4-1.3**
Anoxygenic photosynthetic bacteria	**3-10**
Dark fermentation production	
Spore-forming anaerobic bacteria	**7-25**
Nonspore-forming, facultative anaerobic bacteria	**10-17**

From Tanisho, *BioHydrogen*, 1998

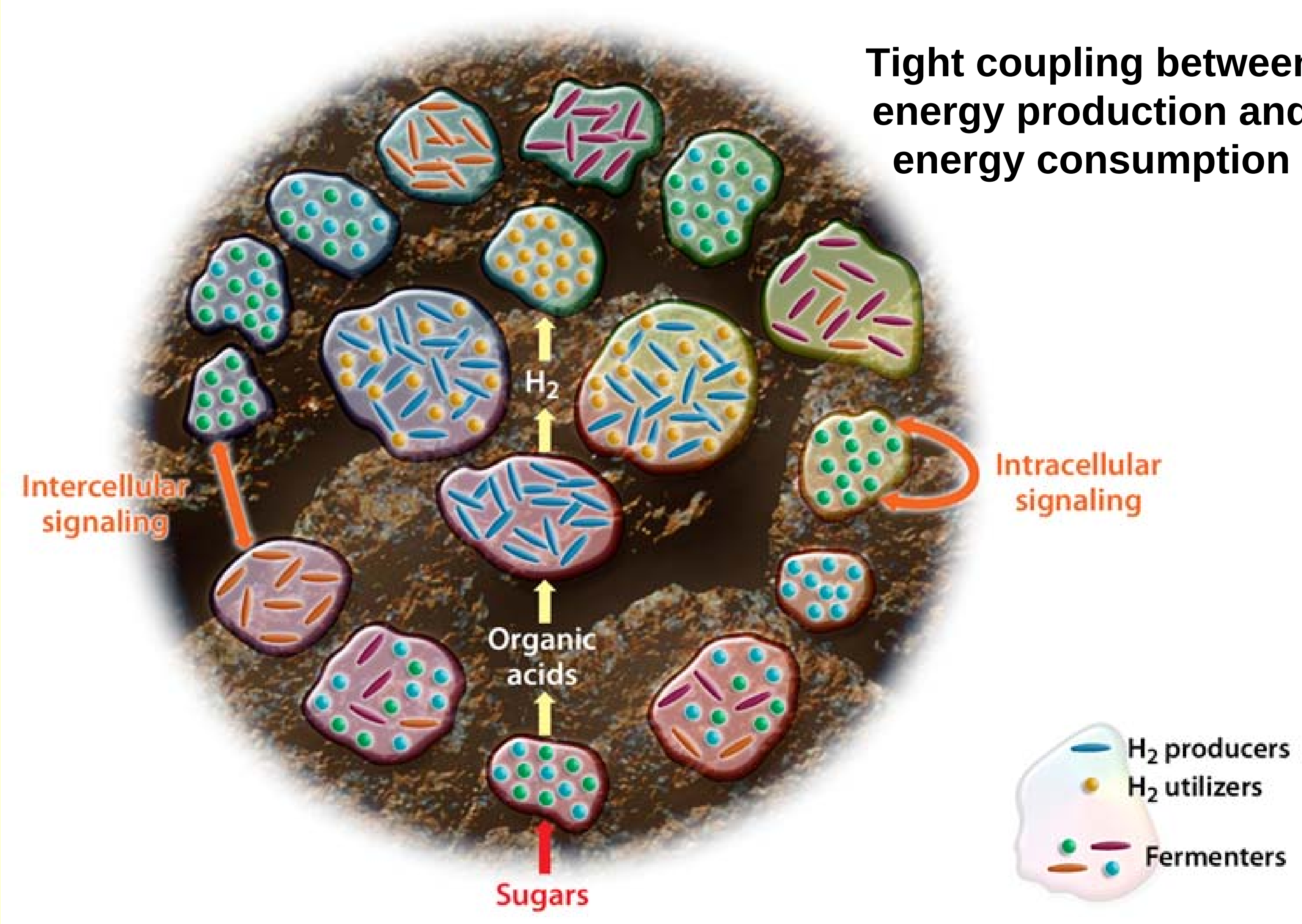
How feasible is "Industrial Revolution-Style" H_2 production?

Large-scale production and storage
Utility-based dissemination

What would a "Biological Revolution" energy system look like?

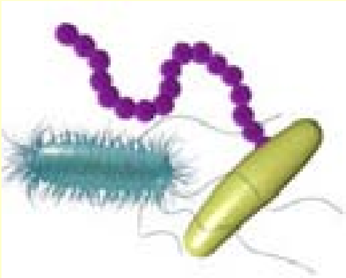
Prokaryotic Life Exists Predominantly in Biofilms

Tight coupling between energy production and energy consumption



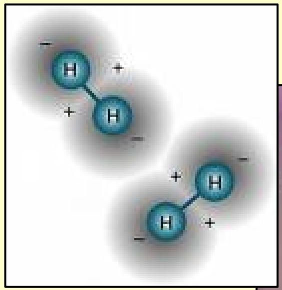
Source: USDOE Genomes to Life

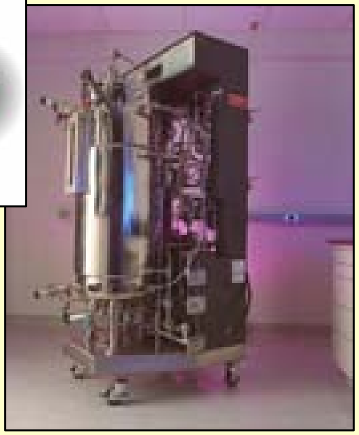
Can humans learn how to be “syntrophs” to microbial energy partners?



<u>Biologically based energy systems:</u>

- Broad distribution of energy production sites
- Less distance between points of energy production and use
- Energy production tightly linked to consumption





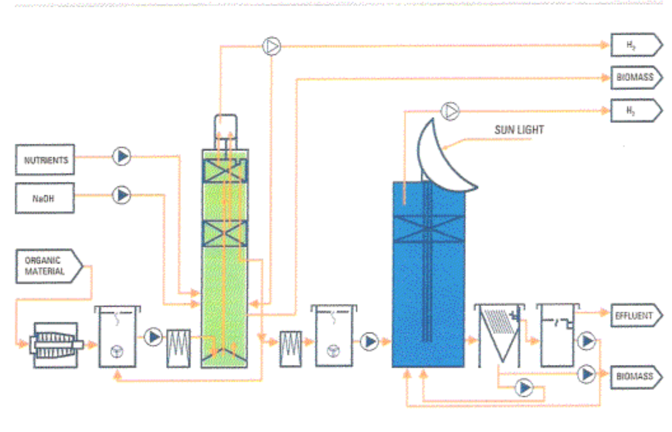
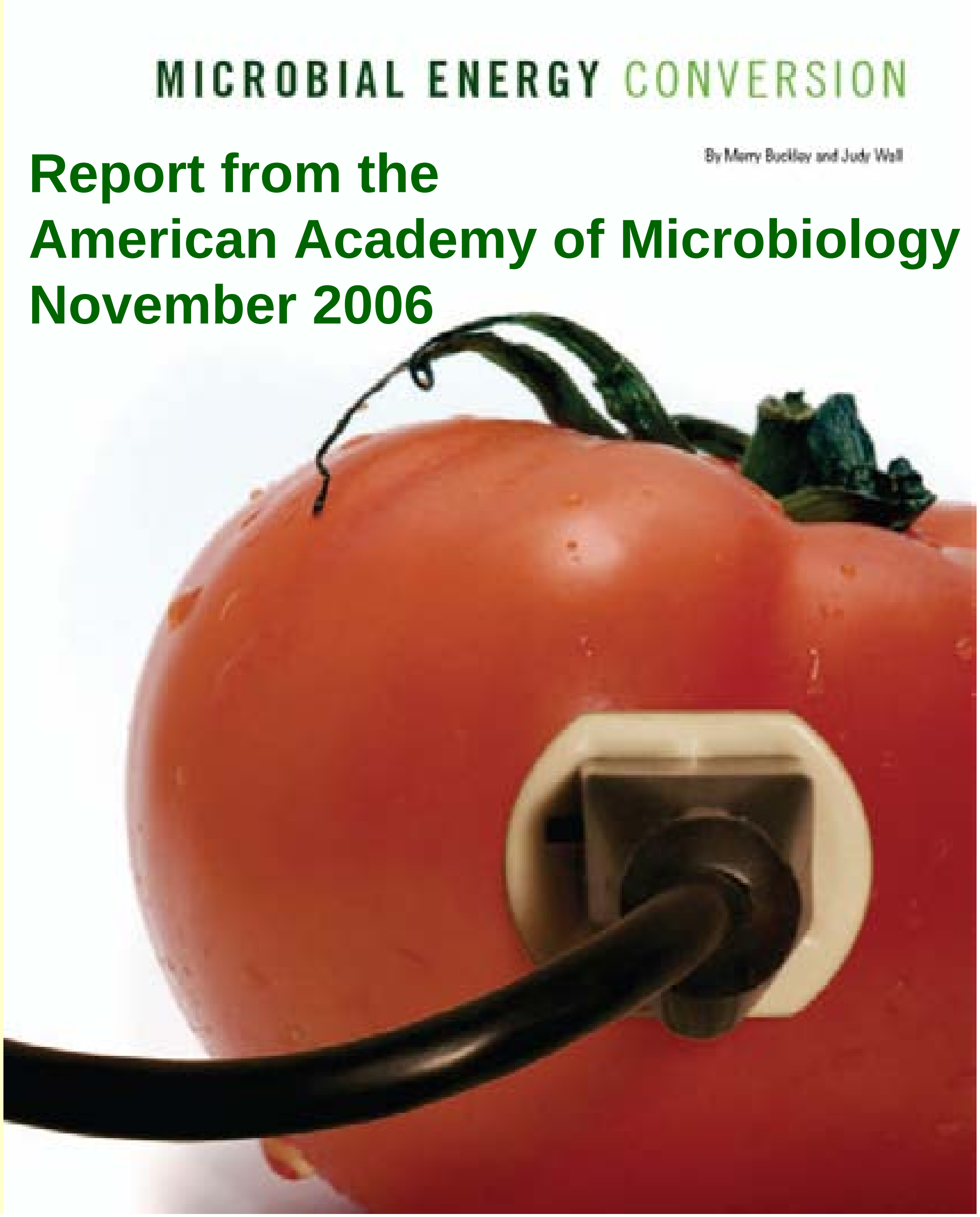


Figure 8. First design of a bioprocess for the conversion of biomass to hydrogen in a thermo-bioreactor, followed by a photo-bioreactor (adapted by ATO from the conceptual design by Paques Biosystems B.V.).

Questions?

Report from the
American Academy of Microbiology
November 2006



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SOCIETY FOR
MICROBIOLOGY