Growth and Diversity of H₂-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
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.

Microbiology’s Scarred Revolutionary

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
Eukaryotes represent four of “Five Kingdoms” but just one Domain.
Global estimates of biomass carbon\(^1\) in Petagrams (\(10^{15}\) g) includes surface and subsurface soils & ocean sediments

<table>
<thead>
<tr>
<th></th>
<th>Plants &amp; algae</th>
<th>Prokaryotes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terrestrial</td>
<td>560</td>
<td>22-215</td>
</tr>
<tr>
<td>Marine</td>
<td>2</td>
<td>305</td>
</tr>
</tbody>
</table>

\(^1\)Biomass C includes live protoplasm, cell walls, and structural materials
Evolutionary lineages with species known to produce $\text{H}_2$

Some anaerobic fungi & protists contain “hydrogenosomes”, $\text{H}_2$-producing organelles evolved from bacterial symbionts

Hydrogenases are a diverse family of enzymes that catalyze the reversible reaction $2\text{H}^+ + 2\text{e}^- \leftrightarrow \text{H}_2$

$\text{H}^+$ and $\text{e}^-$ passage through the Cpl hydrogenase of *Clostridium pasteurianum*
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)

Photosynthesis

Biomass

INDIRECT

Hydrogenase
Prokaryotic $\text{H}_2$-generation

Anaerobic photosynthesis

Photosynthetic bacteria
Genera in betaproteobacteria include *Rhodobacter*, *Rhodospirillum*

Mechanisms:
- Direct biophotolysis (electrons from $\text{H}_2\text{O}$)
- Indirect $\text{H}_2$ from fermentation (electrons from reduced carbon)
- $\text{H}_2$ production by nitrogenase during $\text{N}_2$ fixation

Key advantage:
Availability of solar radiation
No $\text{O}_2$ to inhibit hydrogenase

Key drawback:
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$^+$

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

Cyanobacteria

Activation energy

$H_2 + \frac{1}{2} O_2$

Energy of formation
$\Delta G = 242 \text{ kJ/mol}$

$H_2O$

Anaerobic photosynthetic bacteria

Activation energy

$C_6H_{12}O_6 \rightarrow 12 H_2 + 6 CO_2$
$\Delta G = -3 \text{ kJ/mol}$

$4H_2 + 2 \text{ Acetate}$
$\Delta G = -46 \text{ kJ/mol}$

Anaerobic bacteria
Early experiments to create “hybrid” H₂-producing systems from chloroplasts and hydrogenase enzymes from fermenting Clostridium bacteria
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 H₂-generation

**N₂ fixation**

Free-living N₂ fixers (cyanobacteria, photosynthetic bacteria, *Azotobacter* spp.)

Symbiotic N₂ fixers (e.g., *Rhizobium, Frankia* spp.)

Mechanism:

H₂ is byproduct of nitrogenase enzyme

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**Key advantage:**

Use organic wastes as electron donors

Create artificial conditions in low-cost bioreactors

**Key drawback:**

Need for induction of nitrogenase

Nitrogenase’s O₂ sensitivity

Relatively low H₂ yields
If $\text{H}_2$ production by prokaryotes is so prevalent, why doesn’t $\text{H}_2$ build up in the atmosphere?

Prokaryotes’ ability to take up and oxidize $\text{H}_2$ is probably even more widespread than the ability to produce $\text{H}_2$.

$\text{H}_2$-oxidizing bacteria are diverse and widespread. “Syntrophs” consume $\text{H}_2$ 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 H₂.

( 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 $\text{H}_2$-uptake by soil and microbial biomass content

\[ Y = 0.000664X + 0.162 \]
\[ n = 38 \quad r = 0.904 \]
If we prevent microbes from consuming $\text{H}_2$, how much do we produce in experimental systems?

<table>
<thead>
<tr>
<th>Photochemical production</th>
<th>mmol $\text{H}_2$/g-hr</th>
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<tbody>
<tr>
<td>Oxygenic photosynthetic bacteria</td>
<td>0.4-1.3</td>
</tr>
<tr>
<td>Anoxygenic photosynthetic bacteria</td>
<td>3-10</td>
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<table>
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<th>Dark fermentation production</th>
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<tr>
<td>Spore-forming anaerobic bacteria</td>
<td>7-25</td>
</tr>
<tr>
<td>Nonspore-forming, facultative anaerobic bacteria</td>
<td>10-17</td>
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How feasible is “Industrial Revolution-Style” H₂ 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?

Biologically based energy systems:
- Broad distribution of energy production sites
- Less distance between points of energy production and use
- Energy production tightly linked to consumption
Questions?

Report from the American Academy of Microbiology
November 2006