Fermentative Hydrogen Production: Strain Selection and Reactor Operation

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Fermentative production of H\textsubscript{2} from sugars

• Full oxidation of glucose to H\textsubscript{2}:
  \[ \text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{H}_2\text{O} \rightarrow 12 \text{H}_2 + 6 \text{CO}_2 \]

• Known pathways:
  \[ \text{C}_6\text{H}_{12}\text{O}_6 + 2 \text{H}_2\text{O} \rightarrow 4 \text{H}_2 + 2 \text{C}_2\text{H}_4\text{O}_2 + 2 \text{CO}_2 \]
  \[ \text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2 \text{H}_2 + \text{C}_4\text{H}_8\text{O}_2 + 2 \text{CO}_2 \]

• Typical yield < 2 mol H\textsubscript{2}/mol glucose
Central Metabolism of Clostridium acetobutylicum 824

Glucose → 2 Pyruvate → 2 Acetate

2 CO₂ → 2 Acetyl-CoA → Acetoacetyl-CoA → Butyryl-CoA → Butyrate

2 NAD⁺ → 2 NADH

2 H⁺ → Fd_{ox} → Fd_{red} → NADH → NAD⁺

2 Ethanol → 2 Acetate

Acetone → 4 e⁻ → Butanol → 4 e⁻ → Butyrate

4 e⁻ → H₂ → NADH → NAD⁺
Strategies to Increase Fermentative H$_2$ Yields

- Reactor configurations
  - Continuous gas release in batch systems
  - Chemostat reactors

- Uncouple hydrogen consumption (methanogenesis/homoacetogenesis)

- Strain selection
Continuous gas release system (respirometer)
Continuous gas release in batch cultures

43% increased H₂ production

H₂ production is higher in CSTR than in batch

**Batch Tests:** Overall conversion efficiency of 26% (8.7%)

**CSTR Tests:** Overall conversion efficiency of 44% (15%)

**Reasons:** H₂ loss due to acetogenesis, shifts in community structure
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• Strain selection
Uncoupling Hydrogen Consumption

- Anaerobic food chain involves interspecies $\text{H}_2$ transfer

- $\text{H}_2$ production requires preventing:
  - Hydrogenotrophic methanogenesis
  - Homoacetogenesis
  - $\text{H}_2$-oxidizing propionate/ethanol production

Limiting Methanogenic $H_2$ Consumption

- Heat treatment of inoculum: kills non-spore forming bacteria such as methanogens that are hydrogen consumers

- Low pH (~6): limits methanogen growth

- Short SRT: methanogens grow too slowly

H₂ production maximized with heat treatment to kill non spore formers and using a low pH

HT = heat treated
NHT = non heat treated

Oh et al. (2003) *Environ. Sci. Technol*
Limiting Homoacetogenic H$_2$ Consumption

- Homoacetogenesis:

  \[ 4 \text{H}_2 + 2 \text{CO}_2 \rightarrow \text{CH}_3\text{COOH} + 2 \text{H}_2\text{O} \]

Solution:
- low P$_{H2}$ with continuous gas release
- CO$_2$ scavenging with KOH

Gas production

H2 Yield (mol/mol):
- 2.0 w/ scavenging
- 1.4 w/o

H2, %

CO2, %

Time, hr
Fermentation products

No CO₂ scavenging

With CO₂ scavenging
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Central Metabolism of *Clostridium acetobutylicum* 824

H₂ yield from megaplasmid-deficient strain M5?

Production decreased in megaplasmid-deficient strains
Respirometric Results

Oh et al, In Preparation
Hydrogen Yield Results

<table>
<thead>
<tr>
<th>Yield (mol H₂/mol)</th>
<th>2.4</th>
<th>1.6</th>
<th>1.0</th>
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<tbody>
<tr>
<td>Time (h)</td>
<td>0</td>
<td>100</td>
<td>200</td>
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<tr>
<td>Hydrogen (%)</td>
<td>0</td>
<td>5</td>
<td>10</td>
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</tbody>
</table>

- M5
- ATCC 824
- Mixed culture
Comparison of H₂ yield for Clostridium spp.

Clostridium species: acetobutylicum, cellulolyticum, cellobioparum, celerecrescens, populetii, phytofermentans

Summary

Fermentative hydrogen yields can be increased by:

- Continuous release of $H_2$
- Continuous flow system
- Reducing $H_2$ consumption by methanogens and acetogens
- Using strains that divert more electrons to proton reduction than other products
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