Non-photosynthetic Biological CO₂ Fixation

Developing a Research Agenda for Utilization of Gaseous Carbon Waste Streams
National Academies
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Sources of electrons for biological CO₂ fixation

### Photosynthetic CO₂ Fixation

<table>
<thead>
<tr>
<th>Sun</th>
<th>CO₂</th>
<th>Biomass</th>
<th>Hydrolysis</th>
<th>Sugars</th>
<th>Fermentation</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solar</td>
<td>CO₂</td>
<td>Photosynthesis</td>
<td>1% efficiency</td>
<td>E. coli</td>
<td>Products</td>
<td></td>
</tr>
<tr>
<td>Solar</td>
<td>CO₂</td>
<td>Photosynthetic fermentation</td>
<td>Algae, cyanobacteria</td>
<td>S. cerevisiae</td>
<td>++</td>
<td></td>
</tr>
</tbody>
</table>

### Non-photosynthetic CO₂ Fixation

<table>
<thead>
<tr>
<th>Sun</th>
<th>CO₂</th>
<th>Electricity</th>
<th>H₂O</th>
<th>H₂</th>
<th>CO₂</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solar</td>
<td>Photovoltaics</td>
<td>Electricity</td>
<td>Electrolysis</td>
<td>H₂</td>
<td>Gas Fermentation</td>
<td>Acetogens, Knallgas Bacteria</td>
</tr>
<tr>
<td>Waste Industrial Gases</td>
<td>CO₂</td>
<td>Electricity</td>
<td>Electrolysis</td>
<td>CO, CO₂, H₂</td>
<td>Gas Fermentation</td>
<td></td>
</tr>
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<td>Photovoltaics</td>
<td>Electricity</td>
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<td>CO₂</td>
<td>CO</td>
<td>HCOOH</td>
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<td>Acetogens</td>
<td></td>
</tr>
</tbody>
</table>

Technological Maturity:
- ++++ Sun Solar Products
- ++ Sun Solar Products
- + Sun Solar Products
- + Sun Solar Products
- + Waste Industrial Gases Products
- + Sun Solar Products
- + Sun Solar Products
Advantages of biological gas fermentation

• Biology provides specificity, avoiding the need to separate and sell a wide range of products

• “Dirty” gas streams poison traditional catalysts, but not syngas-fermenting microbes

• Feedstock flexibility

• Comparatively low CapEx, allows monetization of smaller point sources

Major Players in Non-photosynthetic CO₂ Fixation

**Acetogens**

4H₂ + 2CO₂ → CH₃COOH + 2H₂O (ΔG°' = -95 kJ/mol)

- **Anaerobic microbes**
  - High electron efficiency of CO₂ fixation: ~92%
  - Relatively lower rates: 6.2 g Acetate L⁻¹ hr⁻¹, μ = 0.05 hr⁻¹
  - Low titer: ~30 g L⁻¹

**Knallgas Bacteria**

2H₂ + O₂ → 2H₂O (ΔG°' = -477 kJ/mol)

- **Aerobic microbes**
  - High rates: 1.6 g PHB L⁻¹ hr⁻¹, μ = 0.42 hr⁻¹
  - Lower efficiency of CO₂ fixation pathway: ~27%
  - High titer: 62 g L⁻¹ (intracellular)

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Acetogenic CO₂ fixation with the Wood-Ljungdahl Pathway

- Feedstock flexibility
- High electron efficiency
- Commercial interest
- Drivers: Preliminary genetic tools, native ethanol production
Technical Challenge: H₂/CO Mass Transfer

- Efficient feedstock utilization requires high mass transfer rates to deliver substrate to microbe
- CO and H₂ poorly soluble
- Reactor design for high mass transfer rate benefits from well-established literature and industrial practice

Technical Challenge: Genetic Tools and Metabolic Understanding

Genetic Tools

• Plasmids and transformation procedures (Kopke, 2010)
• Gene knockouts (Leang, 2013)
• CRISPR-cas9 knockouts (Huang, 2016)
• Temperature-sensitive replicons (Molitor, 2016)
• CRISPRi (Woolston, 2018)

Metabolic Understanding

• Discovery of Flavin-based electron bifurcation (Thauer, 2008)
• First GSM of acetogen (Nagarajan, 2013)
• Transcriptomics (2013-2016)
• Proteomics (Richter, 2016)

Technologies for metabolic engineering in acetogens are new, but developing rapidly.
Technical Challenge: Low ATP Yield Constrains Portfolio of Target Molecules

\[
\begin{align*}
\text{CO}_2 & \quad \text{CO}_2 \\
\text{WL} & \\
\text{CH}_3\text{-THF} & \quad \text{CO} \\
\text{Acetyl-CoA} & \quad \text{Product} \\
\text{pta} & \quad \text{ack} \\
\text{ATP} & \\
\text{Acetate} & \\
\end{align*}
\]

\[
\begin{align*}
4\text{H}_2 + 2\text{CO}_2 & \rightarrow \text{CH}_3\text{COOH} + 2\text{H}_2\text{O} \quad (\Delta G^0' = -95 \text{ kJ/mol})
\end{align*}
\]
Overcoming ATP limitation in gas fermentation

Separate metabolic capabilities of multiple strains

Two-stage bioreactor system optimizes division of labor, and intracellular product
overcomes challenge with dilute product stream

Overcoming ATP limitation in gas fermentation

Bolster ATP production through additional supplements

No supplement, $\mu = 0.04 \text{ hr}^{-1}$, $Y = 0.11$

Supplemented with 15 mM nitrate, $\mu = 0.08 \text{ hr}^{-1}$, $Y = 0.48$

$4\text{H}_2 + 2\text{CO}_2 \rightarrow \text{Acetate} + 2\text{H}_2\text{O}$

$4\text{H}_2 + \text{NO}_3^- + 2\text{H}^+ \rightarrow \text{NH}_4^+$

Nitrate respiration improves growth and yield
Overcoming ATP limitation in gas fermentation

Bolster ATP production through additional supplements

Arginine eliminates autotrophic acetate production in *C. autoethanogenum*


Arginine $\rightarrow$ Ornithine
$\rightarrow$ NH$_4^+$, CO$_2$

“Mixotrophy”
Co-feeding of fructose enhances CO$_2$ fixation

~4.5 ADP $\rightarrow$ ~4.5 ATP
Fructose $\rightarrow$ 3 Acetate

Jones et al. and Papoutsakis. 2016. *Nat Comm.* DOI: 10.1038/ncomms12800

Eventual solution will be decided by process economic considerations
Major Players in Non-photosynthetic CO₂ Fixation

**Acetogens**

\[ 4\text{H}_2 + 2\text{CO}_2 \rightarrow \text{CH}_3\text{COOH} + 2\text{H}_2\text{O} \quad (\Delta G^0' = -95 \text{ kJ/mol}) \]

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**Knallgas Bacteria**

\[ 2\text{H}_2 + \text{O}_2 \rightarrow 2\text{H}_2\text{O} \quad (\Delta G^0' = -477 \text{ kJ/mol}) \]

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Knallgas Bacteria Technical Challenges

- Explosive Gas Mixtures: Maintaining $O_2$ below explosion limit leads to drop in productivity
- Use Calvin Cycle for $CO_2$ fixation: Lower efficiency

<table>
<thead>
<tr>
<th>Energetic requirements of carbon fixation for acetyl-CoA production</th>
</tr>
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<tbody>
<tr>
<td>Pathway</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Reductive pentose phosphate</td>
</tr>
<tr>
<td>WL pathway</td>
</tr>
<tr>
<td>3HP/4HB cycle</td>
</tr>
<tr>
<td>Reductive TCA cycle</td>
</tr>
</tbody>
</table>

$^a$ Though one molecule of ATP is consumed in the WL pathway, some energy is conserved in the form of membrane gradients for ATP production. Thus, not quite one mole of ATP is consumed for every mole of acetyl-CoA that is produced.

$^b$ In organisms with the WL pathway, H$_2$ cannot be directly oxidized to form additional ATP as shown in Eq. (2). Instead, a fermentation product (i.e. acetate or ethanol) is needed to recover the ATP deficit from acetyl-CoA production.
New pathways for improved CO$_2$ fixation performance

Synthetic biology is allowing us to design new (non-natural) CO$_2$ fixation pathways


**CETCH**

- De Novo designed pathway for CO$_2$ fixation
- *in vitro* rate similar to those measured in CBB pathway
Microbial Electrosynthesis for CO$_2$ Fixation

Anaerobic microbes (acetogens) grown on cathode; accept electrons directly

Bypasses need for “intermediate” electron carrier

Technical Challenges

- Lack of mechanistic understanding of microbe-cathode interface
- Scale-up. Best rates $\sim$0.13 g Acetate L$^{-1}$ hr$^{-1}$

Compare to 6.2 g Acetate L$^{-1}$ hr$^{-1}$

Aryal et. al. *Green Chem.*, 2017, 19, 5748
Summary

• Non-photosynthetic CO$_2$ fixation using H$_2$, CO is an attractive technology for converting CO$_2$ to products with high specificity, bypassing some of the challenges of photosynthesis

• Major remaining technical challenges are overcoming energy limitations to improve rate and access higher-value chemicals, and dilute product streams

Questions?