Microbial Synthesis of Biodiesel

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Diesel (1892)

Petroleum-based Diesel

Plant-derived diesel (e.g. soybean, rapeseed, palm)
Diesel (1892)

“The fact that fat oils from vegetable sources can be used may seem insignificant to-day, but such oils may perhaps become in course of time of the same importance as some natural mineral oils and the tar products are now. ... In any case, they make it certain that motor-power can still be produced from the heat of the sun, which is always available for agricultural purposes, even when all our natural stores of solid and liquid fuels are exhausted.”

Biodiesel from Plant Oils
Biodiesel from Plant Oils

\[
\begin{align*}
\text{CH}_2\text{-OC(O)R} & \quad \text{base} \quad \text{CH}_2\text{OH} \\
\text{CH-OC(O)R} + 3 \text{R'O}H & \quad \rightarrow \quad 3 \text{R-COOR'} + \text{CHOH} \\
\text{CH}_2\text{-OC(O)R} & \quad \text{CH}_2\text{OH}
\end{align*}
\]

Vegetable Oil  Alcohol  Vegetable Oil Alkyl Ester  Glycerol
27-35 mm\(^2\)/s\(^*\)  4-5 mm\(^2\)/s\(^*\)

*Kinematic viscosity of diesel ~ 1.8-3.0 mm\(^2\)/sec
Problems with Plant Biodiesel

• food v/s fuel (100% soybean oil = ~5% diesel)

• oilseed supply is geographically restricted

• glycerol accumulation
Microbial Biodiesel

Acetyl-CoA $\xrightarrow{\text{ACC}}$ Malonyl-CoA $\xrightarrow{\text{FAS}}$ Fatty acyl-ACP
Microbial Biodiesel

Xylose → Glucose → Glycerol → Acetyl-CoA → Malonyl-CoA → Fatty acyl-ACP

Acetyl-CoA \( \rightarrow \) Malonyl-CoA \( \rightarrow \) Fatty acyl-ACP

\( \text{ACC} \rightarrow \text{Malonyl-CoA} \)

\( \text{FAS} \rightarrow \text{Fatty acyl-ACP} \)
Microbial Biodiesel

Xylose
Glucose
Glycerol

Acetyl-CoA $\xrightarrow{\text{ACC}}$ Malonyl-CoA $\xrightarrow{\text{FAS}}$ Fatty acyl-ACP

Acetyl-CoA $\xrightarrow{\text{CO}_2}$ Malonyl-CoA $\xrightarrow{\text{CO}_2}$ CO$_2$

Phospholipids
Microbial Biodiesel

Xylose → Acetyl-CoA → Malonyl-CoA → Fatty acyl-ACP

Glucose → Acetyl-CoA → Malonyl-CoA → Fatty acyl-ACP

Glycerol → Acetyl-CoA → Malonyl-CoA → Fatty acyl-ACP

Acetyl-CoA + Malonyl-CoA → Fatty acyl-ACP + CO₂

Fatty acyl-ACP → Triglycerides

Fatty acyl-ACP → Phospholipids

Phospholipids

Triglycerides
Microbial Biodiesel

- Xylose
- Glucose
- Glycerol

Acetyl-CoA → Malonyl-CoA → Fatty acyl-ACP

Acetyl-CoA + CO₂ → Malonyl-CoA
Malonyl-CoA + CO₂ → Fatty acyl-ACP

Fatty acid alkyl esters

Fatty aldehydes

Fatty alcohols

Triglycerides

Phospholipids
Near-term Specific Aims: *E. coli*

- Enhancing carbon flux through fatty acid biosynthesis
- Evaluating fatty acid productivity in scaleable fermentation process
- Converting fatty acids to fatty alcohols
Overproducing Fatty Acids in *E. coli*

- **Glycerol** → **Acetyl-CoA**
- **ACC** → **Malonyl-CoA**
- **β-Oxidation Pathway**
- **FAS**
- **TE**
- **Fatty Acyl-CoA**
- **Free Fatty Acid**
- **Glycerol-3-phosphate Acyl transferase**
- **Glycerol-3-phosphate**
- **Phospholipids**
Overproducing Fatty Acids in *E. coli*

![Graph showing fatty acid production in different strains of *E. coli*]

<table>
<thead>
<tr>
<th>Strain</th>
<th>12:1</th>
<th>12:0</th>
<th>14:1</th>
<th>14:0</th>
<th>16:1</th>
<th>16:0</th>
<th>18:1</th>
<th>18:0</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>N.D.</td>
<td>2</td>
<td>N.D.</td>
<td>7</td>
<td>N.D.</td>
<td>71</td>
<td>4</td>
<td>5</td>
<td>89</td>
</tr>
<tr>
<td>Medium chain TE</td>
<td>N.D.</td>
<td>22</td>
<td>39</td>
<td>39</td>
<td>22</td>
<td>61</td>
<td>12</td>
<td>7</td>
<td>202</td>
</tr>
<tr>
<td><em>fadD</em> null</td>
<td>4</td>
<td>25</td>
<td>72</td>
<td>135</td>
<td>120</td>
<td>133</td>
<td>66</td>
<td>19</td>
<td>574</td>
</tr>
<tr>
<td>Overexpress ACC</td>
<td>3</td>
<td>22</td>
<td>82</td>
<td>164</td>
<td>127</td>
<td>173</td>
<td>78</td>
<td>18</td>
<td>667</td>
</tr>
<tr>
<td><em>E. coli</em> TE</td>
<td>11</td>
<td>94</td>
<td>201</td>
<td>442</td>
<td>293</td>
<td>409</td>
<td>194</td>
<td>25</td>
<td>1669</td>
</tr>
</tbody>
</table>
Overproducing Fatty Acids in *E. coli*
Overproducing Fatty Acids in *E. coli*
Overproducing Fatty Acids in *E. coli*

\[ y = -2.4399 + 0.4225x \quad R = 0.99694 \]

\[ \text{Total Fatty Acids (umol/ml)} \]

\[ \text{Concentration of Glycerol (mg/ml)} \]

\[ \text{Time (Hour)} \]

\[ \text{Time (Hour)} \]
Overproducing Fatty Acids in *E. coli*

\[
y = -2.4399 + 0.4225x \quad R = 0.99694
\]

<table>
<thead>
<tr>
<th>Fatty Acid Type</th>
<th>C-14</th>
<th>C-16</th>
<th>C-18</th>
</tr>
</thead>
<tbody>
<tr>
<td>% as free fatty acids</td>
<td>100</td>
<td>35</td>
<td>33</td>
</tr>
</tbody>
</table>
Next Steps

• *Flux*: identify rate-limiting steps

• *Stoichiometry*: identify and eliminate wasteful bioconversions

• *Feedstocks*: use cheaper carbon sources

• *Product quality*: cetane #, cloud point, stability

• *Biofuel chemistry*: fatty acid alkyl esters, fatty alcohols
From Sunlight, CO$_2$ to Microbial Biodiesel?

\[
\text{Acetyl-CoA} \xrightarrow{\text{ACC}} \text{Malonyl-CoA} \xrightarrow{\text{FAS}} \text{Fatty acyl-ACP}
\]
From Sunlight, CO$_2$ to Microbial Biodiesel?

\[
\text{CO}_2 \rightarrow \text{Acetyl-CoA} \xrightarrow{\text{ACC}} \text{Malonyl-CoA} \xrightarrow{\text{FAS}} \text{Fatty acyl-ACP}
\]
From Sunlight, CO$_2$ to Microbial Biodiesel?

\[
\begin{align*}
\text{CO}_2 & \rightarrow \text{Acetyl-CoA} \rightarrow \text{Malonyl-CoA} \rightarrow \text{Fatty acyl-ACP} \\
\text{[H]} & \quad \text{ACC} & \quad \text{FAS} & \quad [\text{H}]
\end{align*}
\]