“Trees into Fat”
Microbial Treatment of Lignin: Generation of Bacterial Bio-Oils

Tyrone Wells, Jr
School of Chemistry & Biochemistry
Institute of Paper Science Technology
Trees into Fat

BIOCONVERSION

Using biological systems to convert cheap starting materials into more valuable compounds
Trees into Fat: Overview

- **Background**
  - Benefits of Optimizing the use of Lignin
  - *Rhodococcus opacus* (*R. opacus*)
  - Bioconversion Process (β-ketoadipate Pathway)

- **Experimental**

- **Results of Cell Growth and Lipid Generation**
  - Model Compounds
  - Ethanol Organosolv (EOL)
    - Characterization of bio-oils

- **Future Work**
  - Pyrolysis Oils
Background

- Lignin
  - Monolignols
    - \( p \)-Coumaryl (H)
    - Coniferyl (G)
    - Sinapyl alcohol (S)
  - 3D polyaromatic macromolecule
    - Recalcitrant
  - Significantly less applications

Optimizing the Use of Lignin

- **Lignin**
  - 98% burnt as a non-optimized fuel

- **Lignin → Bio-oil**
  - Transesterification into biodiesel
  - Leverage domestic supplies of limited energy resources
  - Reduce dependence on foreign oil

Rhodococcus opacus

- Oleaginous
  - >20% of cell dry weight in oil

- Two Strains
  - DSM1069
  - PD630

- High affinity towards the digestion of aromatics

- β-ketoadipate pathway

Experimental

- **Materials**
  - Model Compounds, PHA and VanA
  - Ultrasonicated EOL (UE)
  - Glucose

- **Methods**
  - Petri Dish (Solid Media)
  - Liquid Media
  - UV-Vis measurements
Preliminary Growth

- Liquid Media, PD630
  - Calculated via OD at 600 nm, 1 w/V%
Preliminary Growth

- Liquid Media, PD630
  - Calculated via OD at 600 nm, 1 w/V%, 0.5 w/V%
Preliminary Growth

- Liquid Media, DSM1069
  - Calculated via OD at 600 nm, 1 w/V%
Preliminary Growth

- Liquid Media, DSM1069
  - Calculated via OD at 600 nm, 1 w/V%, 0.5 w/V%
### Progress

- **DSM1069 and PD630 Lipid Yields, 1 w/v%**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Carbon source</th>
<th>$t_{\text{max}}$ [h]</th>
<th>$Y_{\text{lipid}}$ [g/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSM 1069</td>
<td>glucose</td>
<td>12</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>PHA</td>
<td>12</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>VanA</td>
<td>24</td>
<td>0.02</td>
</tr>
<tr>
<td>PD630</td>
<td>glucose</td>
<td>12</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>PHA</td>
<td>36</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>VanA</td>
<td>48</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Various types of lipids within bio-oil

Comparison of FAME compositions at maximum specific yields and productivities

- Linoleic acid: C18:2
- Stearic acid: C18:0
- Palmitic acid: C16:0
- Myristate acid: C14:0
- C-oleic acid: C18:1
- C-palmitoleate acid: C16:1
- Linoleate
- C-oleate
- 10-me-stearate
- Stearate
- C-heptadecenoate
- 10-me-heptadecanoate
- Heptadecanoate
- t-palmitoleate
- C-palmitoleate
- Palmitate
- Pentadecanoate
- Myristate

strain-substrate-time [h] till maximum productivity (and yield)
GC/MS data
- Very long unsaturated chains of fatty acids
- Numerous bio-oil applications
- Triacylglycerols

Future studies
- Continue work with EOL under optimized fermentation environments
Future Work: Pyrolysis

Lignin → Heavy Oil Fraction

600°C, 1 hr
500 ml/min N₂ Gas Flow

Lignin → Light Oil Fraction

Future Work: Pyrolysis

- Heavy Oil Fraction
  - Non-water soluble
  - Hydrophobic globules
  - No viable growth

Lignin -> Heavy Oil Fraction

600°C, 1 hr
500 ml/min N₂ Gas Flow
EOL Pyrolysis Oil Adaptation

- Fermentation Growth of DSM1069 on Kraft Pyrolysis Oil
  - 68 h and at 0.3 w/V% substrate concentration
Rhodococcus strains on Kraft lignin pyrolysis oil at 0.5 w/V%
Pyrolysis

- **Light Oil Fraction**
  - Water soluble, pH 7
  - Viable growth on lignin oil
    - Bark
    - Tannin
    - Cellulose
    - Whole cell oils

600°C, 1 hr
500 ml/min N₂ Gas Flow
Preliminary growth on Pyrolysis Oils

72 hr
Oleaginous bacterial strains were successfully adapted to monolignol models and EOL.

Both strains converted carbon sources into bio-oils composed of long unsaturated chains of fatty acids apt for biodiesel applications.

Alternative fuel platform.
Thank you

- Acknowledgement
  - Matyas Kosa
  - Arthur Ragauskas
  - DOE Biorefinery Project
Fermentation Growth with DSM1069
- Calculated via CFU, 1 w/V%
Preliminary Growth
Bioconversion Process: Step 2

- Rhodococcus metabolizes aromatics into lipids
Rhodococcus metabolizes aromatics into lipids
β-ketoacidate Pathway
Transesterification

Bio-Oil + alcohol $\rightarrow$ Biodiesel

$\text{NaOH}$ (catalyst) + glycerol

Methyl ester

Bio-Oil: $\text{CH}_2-\text{C}-\text{O}-\text{R}_1$

$\text{CH}_2-\text{C}-\text{O}-\text{R}_2$

$\text{CH}_2-\text{C}-\text{O}-\text{R}_3$

Biodiesel: $\text{CH}_3-\text{C}-\text{O}-\text{R}_2$

$\text{CH}_3-\text{C}-\text{O}-\text{R}_2$

$\text{CH}_3-\text{C}-\text{O}-\text{R}_2$

Glycerol: $\text{CH}_2-\text{OH}$

$\text{CH}-\text{OH}$

$\text{CH}_2-\text{OH}$

$\text{CH}_2-\text{OH}$
Lifecycle Inventory of Biodiesel and Petroleum Diesel for Use in an Urban Bus

Renewable Biodiesel
Energy Yield* - Life Cycle Basis

<table>
<thead>
<tr>
<th></th>
<th>Energy Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gasoline</td>
<td>0.74</td>
</tr>
<tr>
<td>Diesel</td>
<td>0.83</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.34</td>
</tr>
<tr>
<td>Ethanol (Cellulosic Crop)</td>
<td>2.62</td>
</tr>
<tr>
<td>Biodiesel</td>
<td>3.20</td>
</tr>
</tbody>
</table>
Transesterification

\[
\begin{align*}
\text{Triglyceride} & \rightarrow \\
\text{Diglyceride} & \rightarrow \\
\text{Monoglyceride} & \rightarrow \\
\end{align*}
\]
Bioconversion Process

Lignin → Bio-Oil

lipid, TAG-triacylglycerol
## Progress

<table>
<thead>
<tr>
<th>Strain</th>
<th>Carbon source</th>
<th>$Y_{lipid}$ $t_{max}$ [h]</th>
<th>$dc_{lipid}/dt$ $t_{max}$ [h]</th>
<th>$[g/l\cdot h]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSM 1069</td>
<td>glucose</td>
<td>12</td>
<td>0.06</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>PHA</td>
<td>12</td>
<td>0.10</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>VanA</td>
<td>24</td>
<td>0.02</td>
<td>24</td>
</tr>
<tr>
<td>PD630</td>
<td>glucose</td>
<td>12</td>
<td>0.10</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>PHA</td>
<td>36</td>
<td>0.09</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>VanA</td>
<td>48</td>
<td>0.04</td>
<td>48</td>
</tr>
</tbody>
</table>

4-hydroxybenzoic acid (PHA)

Vanillic acid (VanA)