BIODIESEL PRODUCTION BY ENZYMATIC TRANSESTERIFICATION OF OLIVE OIL

P.T. Vasudevan, Fernando Sanchez and Robert Coggon
University of New Hampshire
Chemical Engineering Department
View of Administrative Building
OUTLINE

- Introduction
- Objectives
- Results
  - Parametric studies
  - Feasibility studies
  - CoFoam Comparison
- Conclusions
INTRODUCTION

BIO DIESEL

- UNH has established a biodiesel group. Focus is on micro algae as this is seen as the best option of producing biodiesel in large quantities
  - Algae can yield 5000-20000 gallons/acre/year
  - Grows best off of waste streams

- Chemical processes for production are well known

- Transesterification of olive oil using lipase
# Enzymatic transesterification using different alcohols and lipases

<table>
<thead>
<tr>
<th>Oil</th>
<th>Alcohol</th>
<th>Lipase</th>
<th>Conv. (%)</th>
<th>Solvent</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapeseed</td>
<td>2-Ethyl-1-hexanol</td>
<td>C. rugosa</td>
<td>97</td>
<td>None</td>
<td>3</td>
</tr>
<tr>
<td>Mowrah, Mango, Kernel, Sal</td>
<td>C4-C18 alcohols</td>
<td>M. miehei (Lipozyme IM20)</td>
<td>86.8-99.2</td>
<td>None</td>
<td>3</td>
</tr>
<tr>
<td>Sunflower</td>
<td>Ethanol</td>
<td>M. miehei (Lipozyme)</td>
<td>83</td>
<td>None</td>
<td>3</td>
</tr>
<tr>
<td>Fish</td>
<td>Ethanol</td>
<td>C. antarctica</td>
<td>100</td>
<td>None</td>
<td>3</td>
</tr>
<tr>
<td>Recycled restaurant grease</td>
<td>Ethanol</td>
<td>P. cepacia (Lipase PS-30) + C. antarctica (Lipase SP435)</td>
<td>85.4</td>
<td>None</td>
<td>3</td>
</tr>
<tr>
<td>Tallow, Soybean, Rapeseed</td>
<td>Primary alcohols*</td>
<td>M. miehei (Lipozyme IM60)</td>
<td>94.8-98.5</td>
<td>Hexane</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Secondary alcohols*</td>
<td>C. antarctica (Lipase SP435)</td>
<td>61.2-83.8</td>
<td>Hexane</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>M. miehei (Lipozyme IM60)</td>
<td>19.4</td>
<td>None</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>M. miehei (Lipozyme IM60)</td>
<td>65.5</td>
<td>None</td>
<td>3</td>
</tr>
<tr>
<td>Sunflower</td>
<td>Methanol</td>
<td>P. fluorescens</td>
<td>3</td>
<td>None</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td></td>
<td>79</td>
<td>Pet ether</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>82</td>
<td>None</td>
<td>3</td>
</tr>
<tr>
<td>Palm kernel</td>
<td>Methanol</td>
<td>P. cepacia (Lipase PS-30)</td>
<td>15</td>
<td>None</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td></td>
<td>72</td>
<td>None</td>
<td>3</td>
</tr>
<tr>
<td>Canola</td>
<td>Methanol</td>
<td>Novozym 435</td>
<td>97.9</td>
<td>Water</td>
<td>1</td>
</tr>
<tr>
<td>Soybean</td>
<td>Methanol</td>
<td>P. cepacia Lipase</td>
<td>67</td>
<td>Water</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>Novozym 435</td>
<td>65</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lipozyme 1M</td>
<td>&lt;98</td>
<td>Hexane</td>
<td>2</td>
</tr>
</tbody>
</table>

\*Primary alcohols: Methanol, Ethanol

\*Secondary alcohols: 2-Ethyl-1-hexanol
Olive Oil Production and Consumption

[Graph showing the timeline of olive oil production and consumption from 1970 to 2000, with separate lines for World Production, EU Production, World Consumption, and EU Consumption.]
Production by Country

![Graph showing production by country from 1993 to 2003. The countries include Spain, Italy, Greece, Portugal, Turkey, Syria, Morocco, Libya, and others in Europe. The graph indicates fluctuations in production over the years.](image-url)
INTRODUCTION

TRANSESTERIFICATION REACTION:

\[
\begin{align*}
\text{Triglyceride} & \quad \text{Alcohol} \quad \text{Fatty acid esters} \quad \text{Glycerol} \\
\text{CH}_2 - \text{OOC} - R_1 & \quad \text{CH}_2 - \text{OOC} - R_2 + 3R'\text{OH} & \quad \text{Catalyst} & \quad \text{R}_1 - \text{COO} - R' \quad \text{CH}_2 - \text{OH} \\
\text{CH}_2 - \text{OOC} - R_3 & \quad & & \quad \text{R}_2 - \text{COO} - R' \quad \text{CH}_2 - \text{OH} \\
& & & \quad \text{R}_3 - \text{COO} - R' \quad \text{CH}_2 - \text{OH}
\end{align*}
\]
GOALS

- Ensure that enzyme retains activity
- Minimize enzyme denaturation
- Evaluate long-term performance of Novozym®435
- Investigate used-oil utilization
- Scale-up and kinetics
Materials and Methods

- Triglyceride: triolein
- **Catalyst:** Novozym®435, lipase B from *Candida antarctica* from Sigma Aldrich
- Hexane, methanol, isopropanol – HPLC grade
- Methyl oleate, triolein standards
- Reverse Phase C18 HPLC coupled to UV detector
- UV Absorbance detection at 230 nm
## Gradient HPLC Method

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Methanol (%)</th>
<th>Isopropanol (%)</th>
<th>Hexane (%)</th>
<th>Flow Rate (m³/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>2.5e-9</td>
</tr>
<tr>
<td>19</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>2.5e-9</td>
</tr>
<tr>
<td>20</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>8.33e-9</td>
</tr>
<tr>
<td>35</td>
<td>64</td>
<td>20</td>
<td>16</td>
<td>8.33e-9</td>
</tr>
<tr>
<td>46</td>
<td>64</td>
<td>20</td>
<td>16</td>
<td>8.33e-9</td>
</tr>
</tbody>
</table>
Experimental Set-Up

► Conversion studies were performed in a batch reactor

► Reactor consisted of a glass vial with a polybutyl terephthalate open-top cap and a PTFE-silicone septum.

► The vial measured 0.025 m in diameter by 0.057 m in height.

► Vial placed in a constant-temperature bath and contents were mixed by a stir bar.
Reaction Procedure

► Triolein conversion analyzed every 2.5 h for the first 10-12.5 h of reaction.

► Final sample drawn after 20-30 h to check for final conversion.
PARAMETRIC STUDIES

► Molar ratio of methanol to triolein
► Semi-batch (step-wise addition) vs batch operation
► Enzyme activity
► Mixing speed
► Reaction temperature
EFFECT OF MOLAR RATIO OF REACTANTS

► Stoichiometry requires 3:1 ratio for triolein to be converted

► Mixtures with more than 3:1 deactivate Novozym®435

► Compromise solution between reactant availability and enzyme denaturation
EFFECT OF MOLAR RATIO ON CONVERSION

40°C, 100 rpm, 500 U enzyme

Conversion [%]

Time [h]

3:1
4:1
6:1
8:1

Chemical Engineering
University of New Hampshire

Bioenergy 1: From Concept to Commercial Processes - Tomar, Portugal
Observation and Inference

► The initial rates (slopes of the curves during the first 2.5 h) at ratios 4:1, 6:1 and 8:1 are almost identical, but lower than the rate corresponding to a ratio of 3:1.
  - This indicates that the extent of inhibition of Novozym® 435 by methanol is limited and remains constant for methanol to triolein ratios greater than 4:1.

► Methanol in excess of the stoichiometric amounts leads to higher final conversions of triolein.
  - Attributed to the presence of other components in olive oil that also compete for methanol. Presence of other products (not identified) was revealed during analysis. Thus when methanol is present in excess, there is enough reactant available for almost complete conversion of triolein.
EFFECT OF MOLAR RATIO ON RATE
40°C, 100 rpm, 500 U enzyme

![Graph showing the effect of molar ratio on rate with data points at different ratios and corresponding Vo values.](image)
### Effect of methanol to triolein molar ratio

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Initial rate x $10^3$ (mg/U-s)</th>
<th>Final conversion (%)</th>
<th>Yield (g biodiesel/g oil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:1</td>
<td>1.4</td>
<td>91.8</td>
<td>0.41</td>
</tr>
<tr>
<td>6:1</td>
<td>1.3</td>
<td>80.3</td>
<td>0.36</td>
</tr>
<tr>
<td>4:1</td>
<td>1.5</td>
<td>60.2</td>
<td>0.27</td>
</tr>
<tr>
<td>3:1</td>
<td>2.4</td>
<td>54.6</td>
<td>0.25</td>
</tr>
</tbody>
</table>
SEMI-BATCH VS BATCH – EFFECT ON CONVERSION

![Graph showing the effect of different ratios on conversion over time.](image-url)

- **2*4:1**
- **4*2:1**
- **8:1**

Time [h]

Conversion [%]
EFFECT OF ENZYME ACTIVITY ON CONVERSION

60°C, 100 rpm, 8:1 ratio

Conversion [%] vs. Time [h]

- 3000 U
- 2000 U
- 1000 U
- 500 U
- 0 U
EFFECT OF ENZYME ACTIVITY ON RATE

![Graph showing the effect of enzyme activity on rate with Activity [U/mL oil] on the x-axis and Vo [%conv/h] on the y-axis. The graph shows a positive correlation as enzyme activity increases.]
EFFECT OF TEMPERATURE ON RATE

500 U enzyme, 100 rpm, 4:1 ratio

Temperature [°C] vs. Vo [%conv/h]

- Temperature range from 30°C to 70°C
- Vo values from 0% to 16% conversion per hour

Chemical Engineering
University of New Hampshire

Bioenergy 1: From Concept to Commercial Processes - Tomar, Portugal
### Effect of Temperature

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Initial rate x 10^3 (mg/U-s)</th>
<th>Final conversion (%)</th>
<th>Yield (g biodiesel/g oil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.23</td>
<td>31.7*</td>
<td>Not calculated</td>
</tr>
<tr>
<td>40</td>
<td>1.53</td>
<td>60.2</td>
<td>0.27</td>
</tr>
<tr>
<td>50</td>
<td>3.44</td>
<td>58.4</td>
<td>0.26</td>
</tr>
<tr>
<td>60</td>
<td>3.81</td>
<td>59.3</td>
<td>0.27</td>
</tr>
<tr>
<td>70</td>
<td>3.33</td>
<td>53.4</td>
<td>0.24</td>
</tr>
</tbody>
</table>
FEASIBILITY: REUTILIZATION OF ENZYME

► Major drawback compared to chemical catalysts: Cost

► This reaction: raw materials and energy costs are low
  enzymatic catalyst: main expense

► Glycerol has negative effects on lipase activity
FEASIBILITY: REUTILIZATION OF ENZYME
60°C, 1000 U enzyme, 8:1 ratio

![Graph showing relative activity of enzyme over batch number.]
## Effect of Enzyme Reutilization

<table>
<thead>
<tr>
<th>Batch</th>
<th>Relative Activity (%)</th>
<th>Yield (g biodiesel/g oil)</th>
<th>Productivity·$10^5$ (g biodiesel/U enzyme-h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>0.42</td>
<td>3.96</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>0.41</td>
<td>3.96</td>
</tr>
<tr>
<td>3</td>
<td>96</td>
<td>0.40</td>
<td>3.69</td>
</tr>
<tr>
<td>5</td>
<td>95</td>
<td>0.42</td>
<td>3.69</td>
</tr>
<tr>
<td>6</td>
<td>83</td>
<td>0.41</td>
<td>3.62</td>
</tr>
<tr>
<td>8</td>
<td>71</td>
<td>0.41</td>
<td>3.42</td>
</tr>
<tr>
<td>11</td>
<td>43</td>
<td>0.34</td>
<td>2.40</td>
</tr>
</tbody>
</table>
FEASIBILITY: SCALE-UP

► Next objective: to assess biodiesel production in larger reactor

► Physical conditions in large reactor rarely duplicate those in smaller one — heat transfer, diffusion... — overall reaction rate

► Change in controlling regime — results from small scale become unreliable to predict those in large scale
SCALE-UP: Conversion versus Time
60C, 100 rpm, 500 U enzyme, 8:1 ratio

Conversion [%]

Time [h]

- vial
- scale-up
SCALE-UP: Effect of Methanol Addition
60°C, 100 rpm, 500 U enzyme, 8:1 ratio

![Conversion vs Time Graph](graph.png)
FEASIBILITY: USED OIL
60°C, 100 rpm, 1000 U enzyme, 8:1 ratio

Conversion [%] vs Time [h]

- ▲ clean
- ○ used
Work in Progress

- New reactor design that has virtually eliminated loss of solvent
- Effect of other solvents on yield and conversion
- Effect of ultrasonic agitation on yield and conversion
- New analytical method has been developed that is reliable and cheaper than HPLC
Other Supports

- Developed an effective technique to immobilize enzymes on hydrophilic CoFoam.
- Hydrophilic polyurethane foam is cast on to reticulated foam.
- Works in the temperature range 4°C – 107°C.
- Hydrophilicity, pore size and foam density can be altered.
- Enzymes are covalently bound.
- Adds strength and there is no need to cut to small pieces to increase surface area.
Reticulated Foam
Coated Reticulated Foam

250 μM
Pressure drop (kPa) vs Flow rate (L/min)
Immobilization of Lipase

►► Video clip
Hydrolytic Activity

- Novozyme 435
- C. antarctica B Cofoam

Activity (U/g biocatalyst)

Triacetin, Tripropionin, Tributyrin, Olive oil
Synthetic Activity

- CoFoam
- Novozyme 435

![Graph showing synthetic activity over reaction time](image)

- Reaction time (min): 30, 60, 90, 120, 150, 180, 210, 240
- mmol methyl oleate / g catalyst: 0, 1, 2, 3, 4, 5, 6, 7
CONCLUSIONS

► Inhibition of Novozym®435 by methanol is reversible and remains constant for molar ratios greater than 4:1

► Methanol in excess of stoichiometric amounts leads to higher final conversions

► Step-wise methanolysis indicates some improvement

► At enzyme concentrations higher than 2000 U/mL oil, addition of catalyst barely affects reaction rates

► Mixing speed does NOT play a role within 50-400 rpm
CONCLUSIONS

- Initial reaction rate reaches a maximum at approx. 60°C
- 95% activity retained after 5 batches, >70% after 8
- Under similar conditions, results can be extrapolated to large scale and used oil within a margin of 10-15%
- First order kinetics with respect to oil concentration
Acknowledgements

- Hydrophillix, Inc.
- Dr. Francisco Plou, ICP for hydrolytic activity measurements
QUESTIONS