

# Wastewater Treatment Facilities: Biorefinery for Biofuels

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# Objectives

- Transform the daily disposal of industrial and domestic sewage into large quantities of lipids using microorganisms
- Integrate the lipid, sugar, and thermochemical platforms for producing JP-Fuels, gasoline, biodiesel and chemicals



# Evaluation of BioDiesel Production Costs

## Soybean Oil

Note: Feedstock  
represent ~90%  
of total processing costs



**\$4.38/gal feedstock cost**

**\$0.35/gal processing cost**

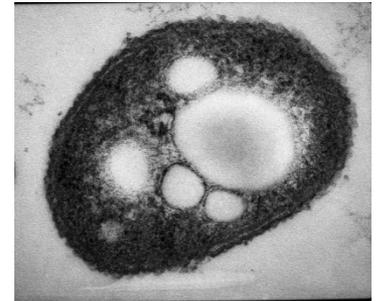
**\$0.10/gal transportation cost**

**= \$4.83/gal total cost**



# Sustainability and Logistical Barriers to Biofuels

- **The US Consumes approximately 300,000,000,000 gal/yr**
  - 165,000,000,000 gal/yr gasoline
  - 75,000,000,000 gal/yr diesel
- **Ethanol would account for 2%**
- **Soy Bean oil accounts for 0.33%**
- **When populations need that for food fuel will lose**
- **Not Sustainable.**
- **Single-cell oil produced in a wastewater treatment facility will not have a food market**
- **Wastewater treatment will always occur and scales with population.**



# The Oleaginous Microorganisms

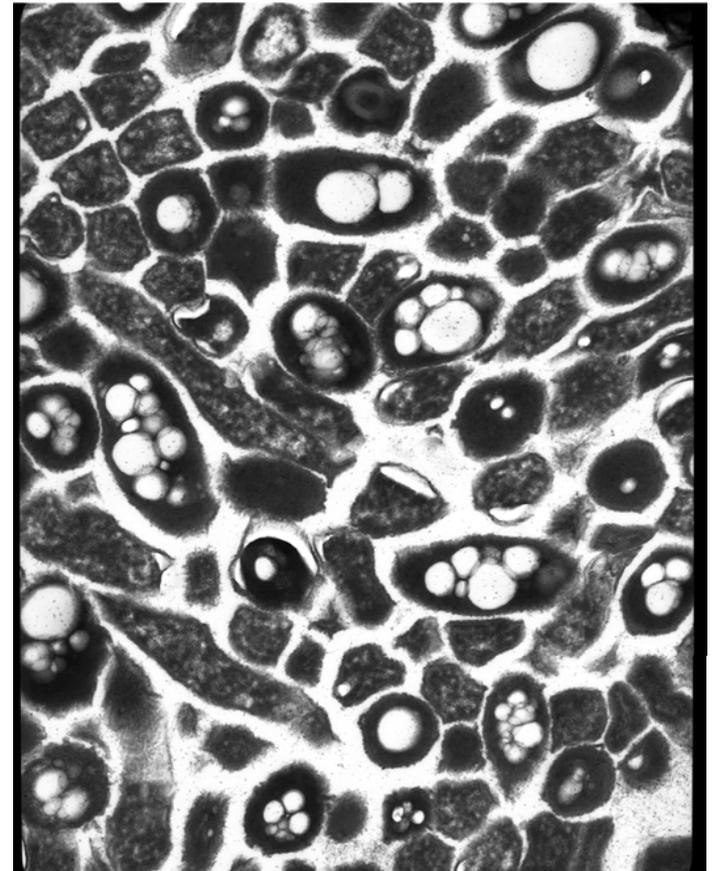
*Candida bobicola*, *Yarrowia lipolytica*, *Lipomyces lipofer*, *Lipomyces starkeyi*, *Trichosporon pullulans*, *Rhodotorula graminis*, *Mortierella alpina*, *Cryptococcus terricolus*, *Rhodococcus fascians*, *Rhodococcus erythropolis*, *Nocardia corallina*, *Nocardia asteroides*, *Nocardia globerula*, *Nocardia restricta*, *Dietzia maris*, *Acinetobacter lwoffii*, *Acinetobacter sp. strain HO1-N*, *Acinetobacter sp. strain 211*, *Pseudomonas aeruginosa strain 44T1*, *Gordonia amarae*, *Rhodococcus opacus*, etc



1. Granger, L-M; et al. *Biotech. & Bioengineering*, 1993, 42, 1151-1156
2. Zheng, S; et al. *Bioresource Tech.*, 2005, 96, 1522-1524
3. Xue, F; et al. *Process Biochem.*, 2006, 41, 1699-1702

# COMPARISON OF OIL PRODUCTION FROM FEEDSTOCKS

Sample	% Oil	Time (D)
Soybean	19	105
Canola	33	255
Camelina	36	255
Turnip	36	255
Flax	39	240
Tallow	46	365
Castor	55	195
<i>H. matrinialis</i>	23	2.5
<i>R. glutinis</i>	16-60	2.5
<i>R. opacus</i>	60-80	2.0



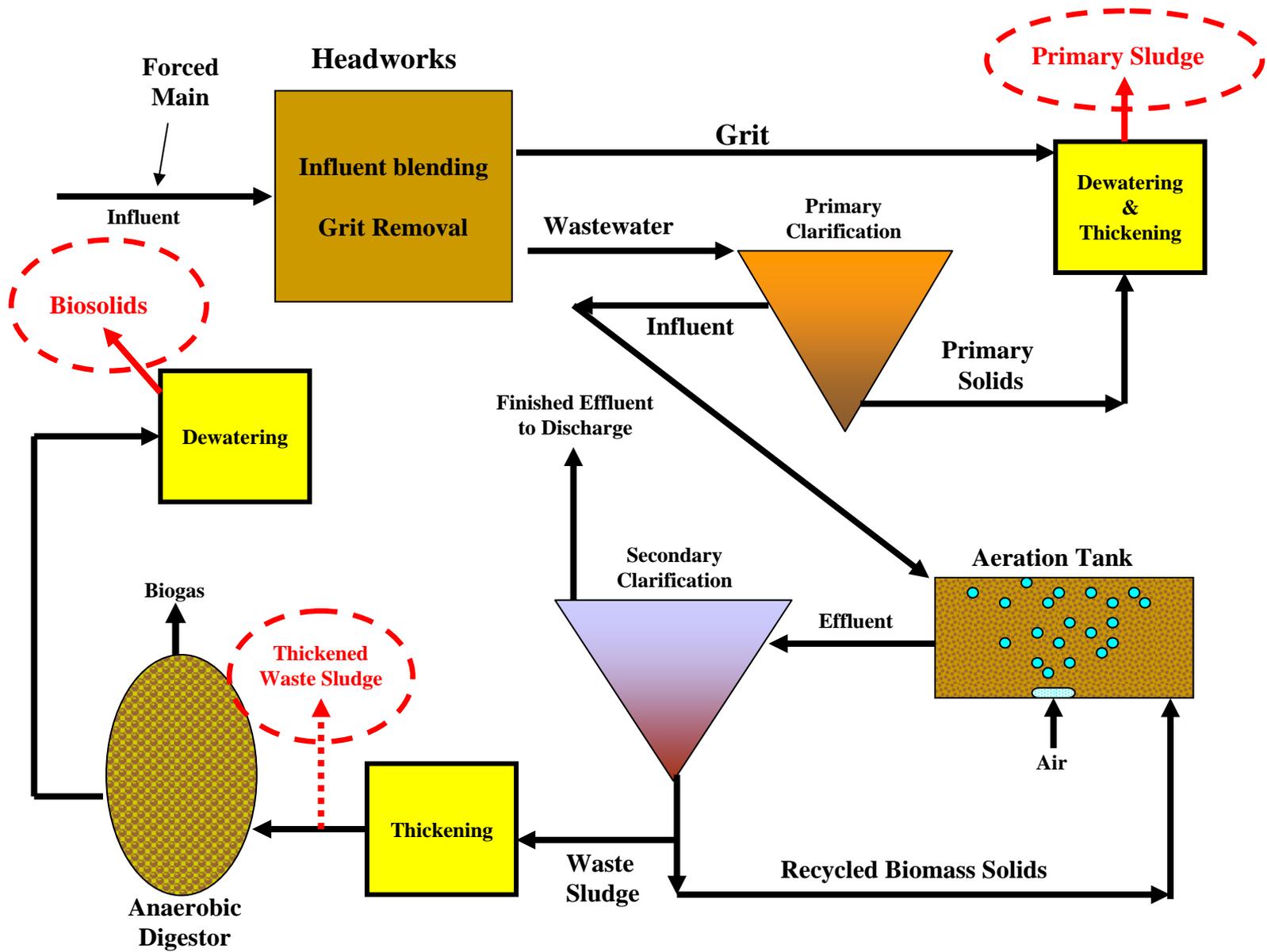
# WASTEWATER AS CARBON SOURCE

- **Wastewater contains:**
  - Carbon
  - Nitrogen
  - Phosphorous
  - Water
  - Micronutrients
- **Approximately 30 million gallons\* of wastewater are produced daily in Tuscaloosa**
  - Residential waste
  - Industrial waste

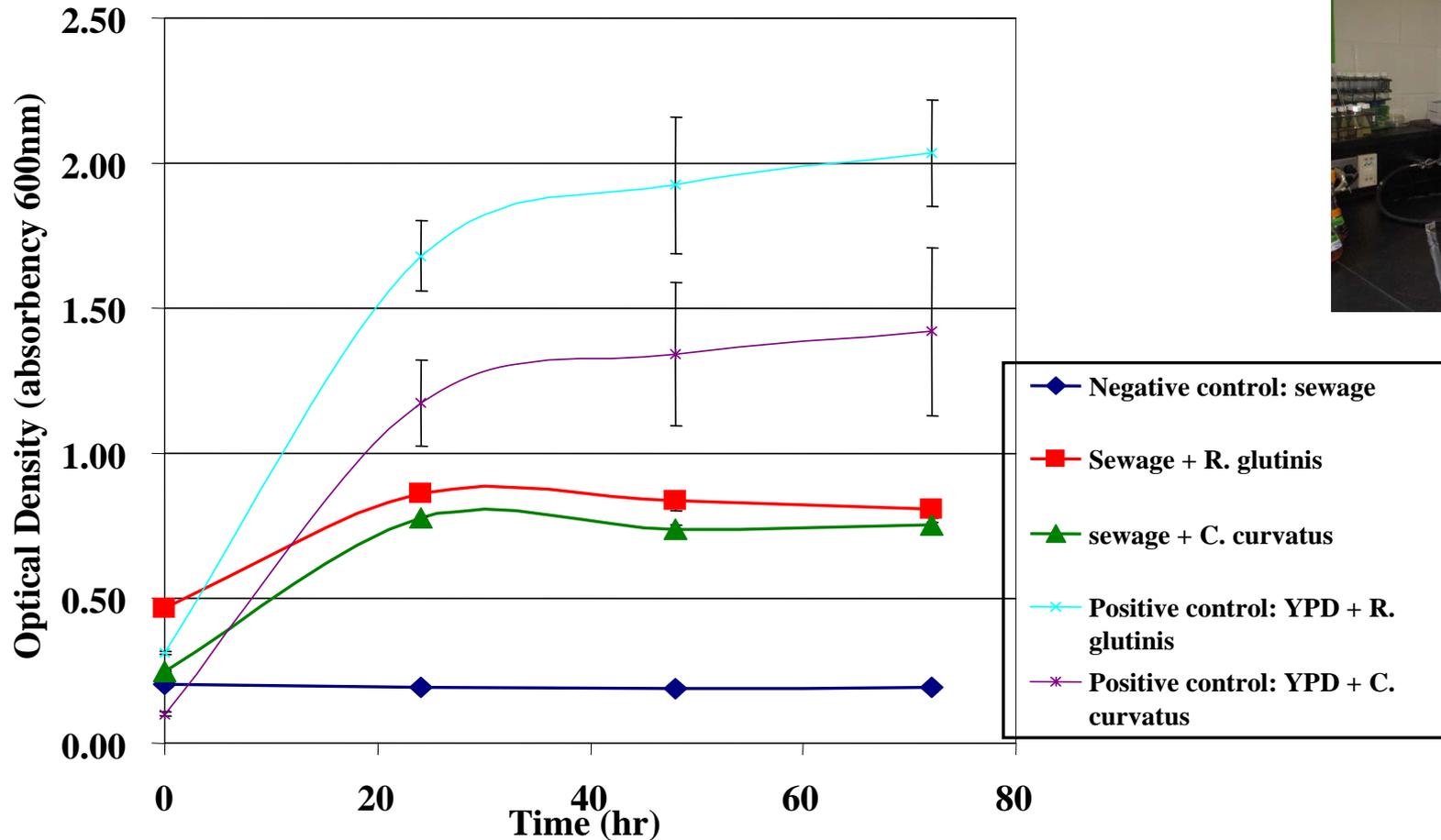


# 35 Billion Gallons of Municipal Wastewater Generated Daily

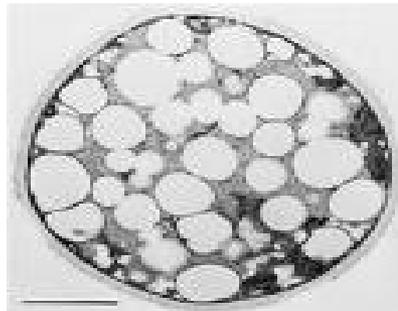
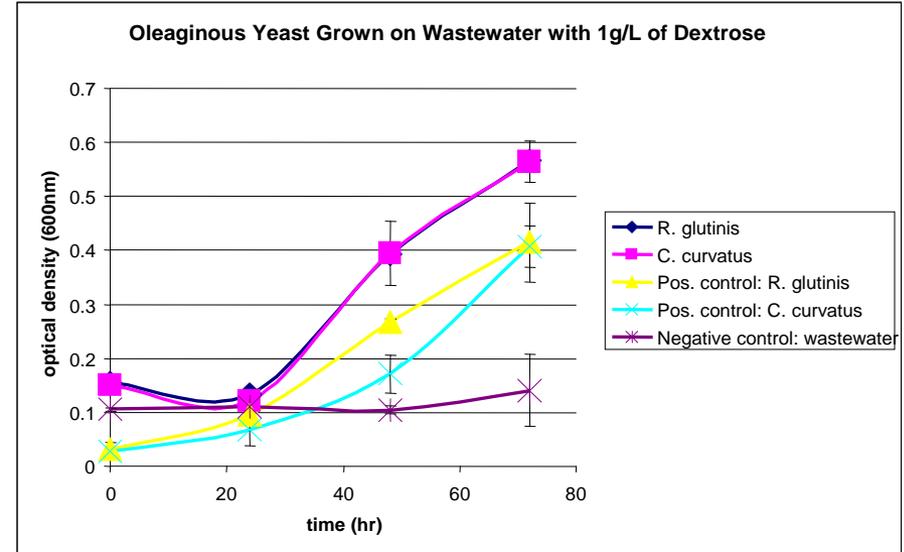
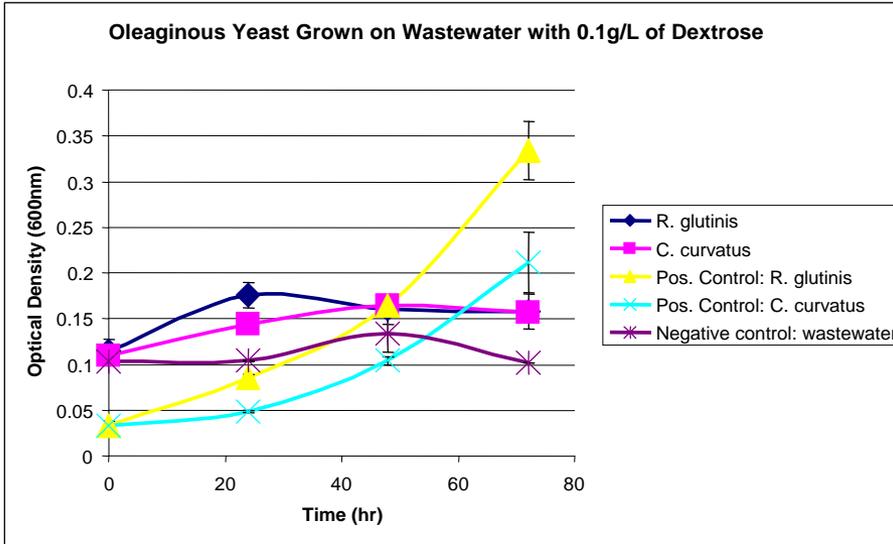




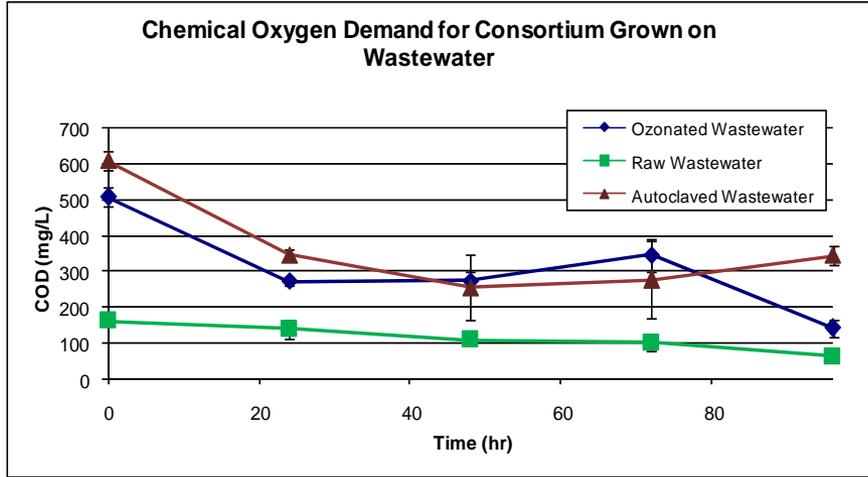
# Growth of *R. glutinis* and *C. curvatus* on Sewage from YPD



# COMPARISON OF GROWTH CURVES

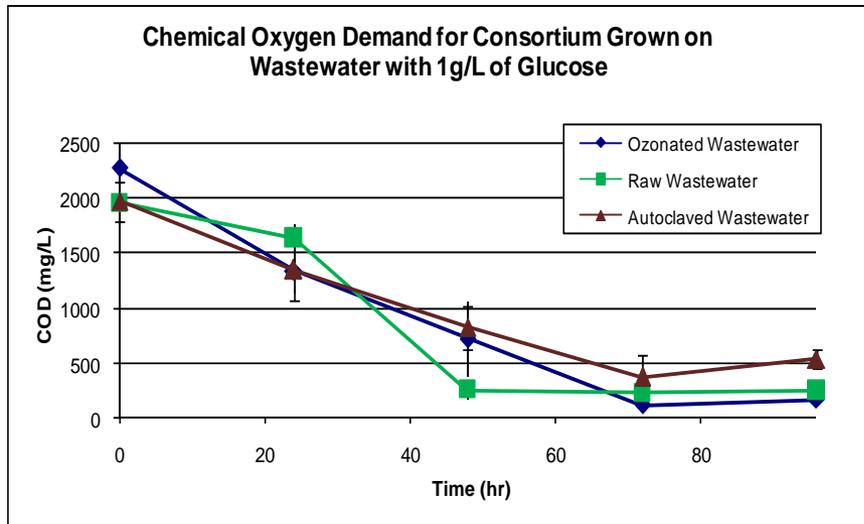


# CHEMICAL OXYGEN DEMAND



Sugar	Sample	% Reduction
0	CO	72.26
	RW	61.25
	CA	43.49

10% Oil Content



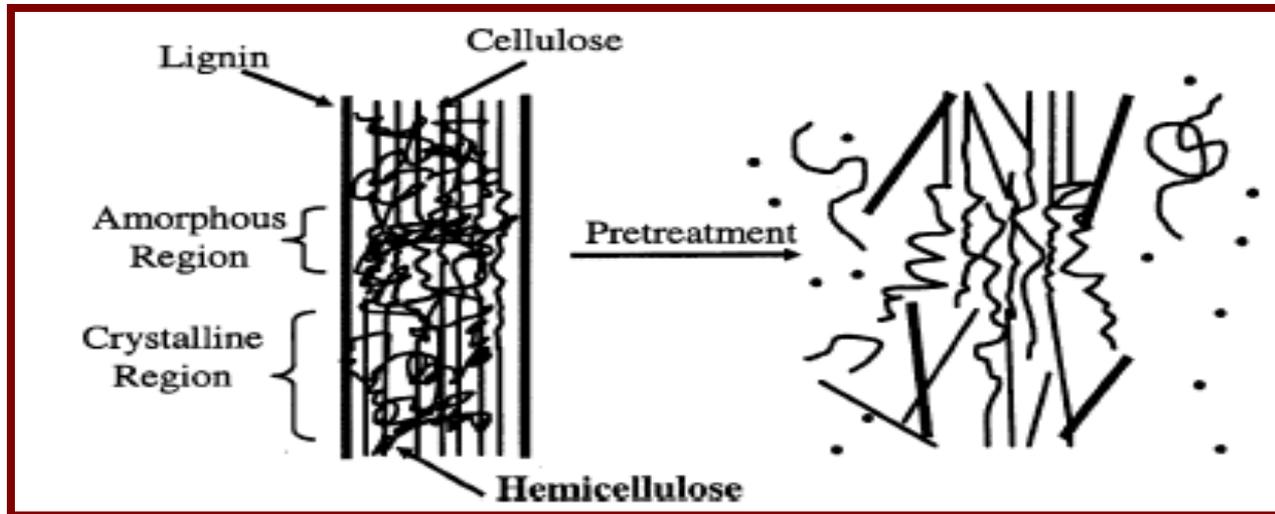
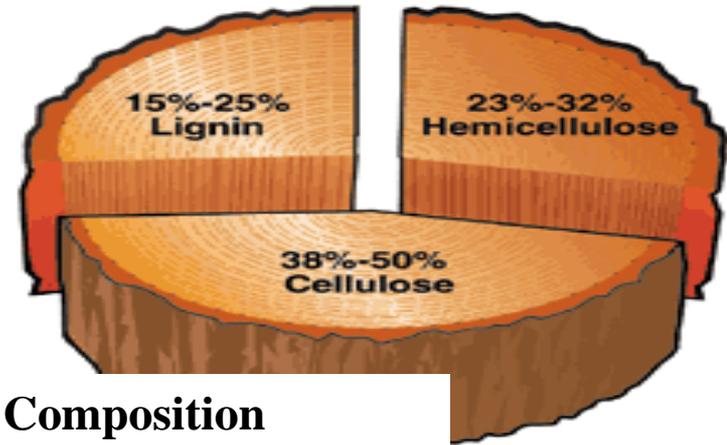
Sugar	Sample	% Reduction
1g/L	CO	92.70
	RW	87.27
	CA	72.83

# Carbon Sources Supporting Growth of Oleaginous Microorganisms

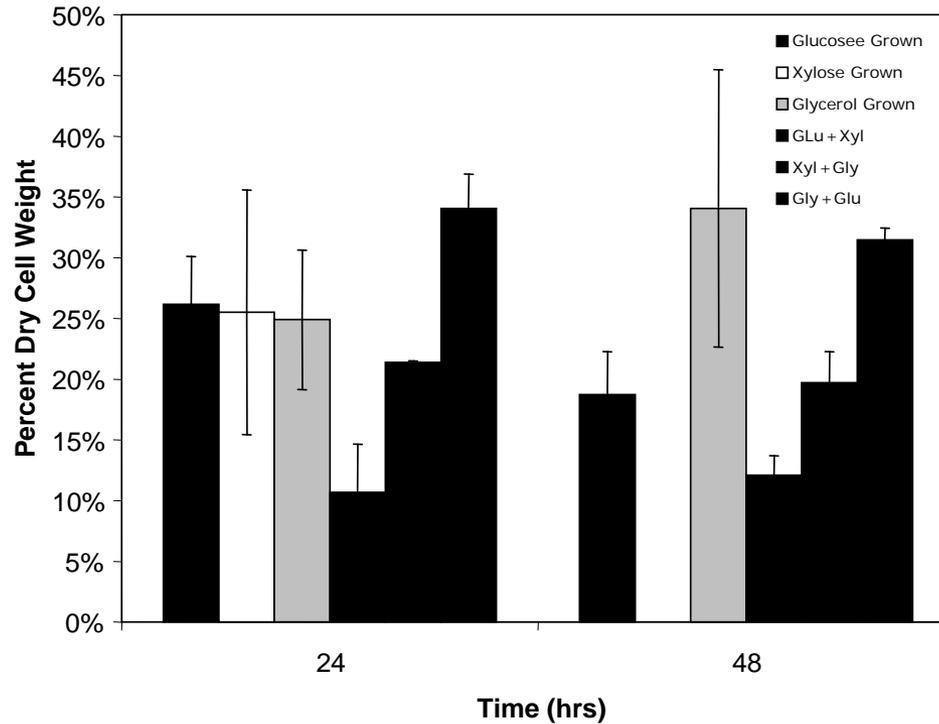
- **Sugars**
  - **Glucose**
  - **Xylose**
  - **Ribose**
  - **N-acetyl glucosamine**
- **Glycerol**
- **Flour**



# Lignocellulosic Biomass

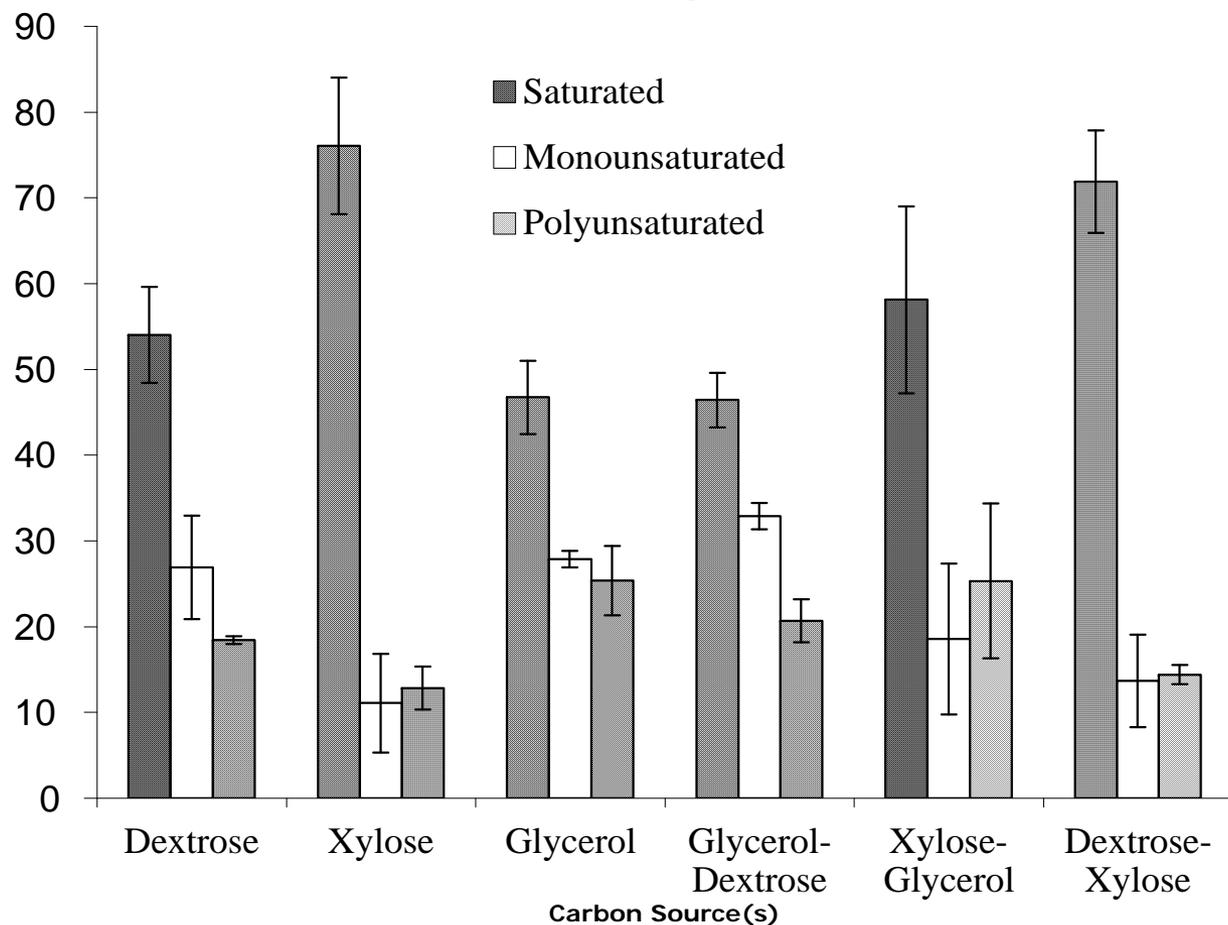


# Conversion of Lignocellulosic Biomass to Microbial Oil



# The Effect of Carbon Source on the Saturation of Fatty Acids Produced by *R. glutinis*

## 48 h

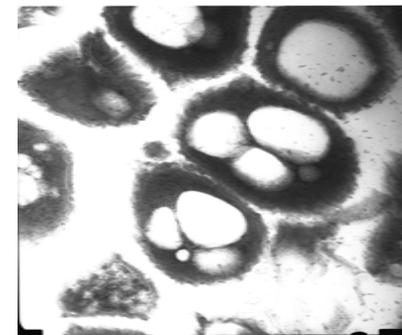


# Conversion of Lignocellulosic Biomass to Microbial Oil

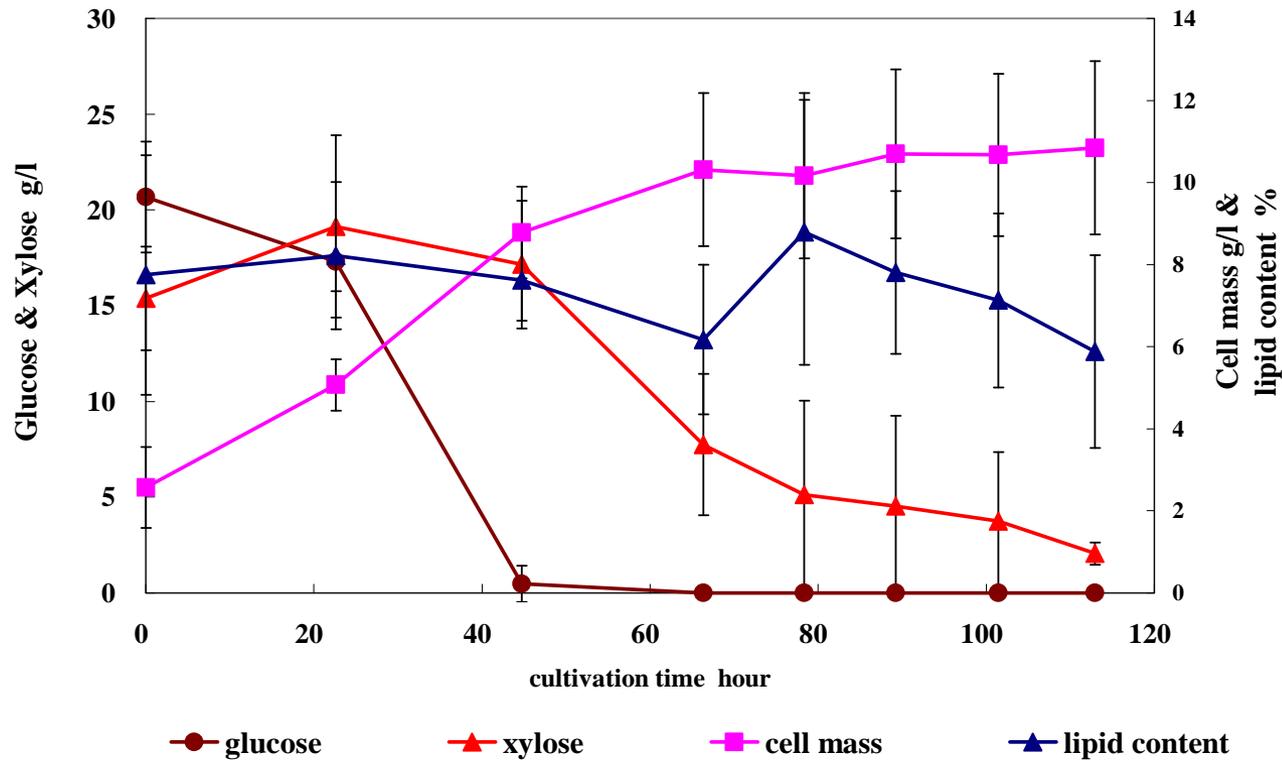
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<b>Sugar</b>	<b>Cell mass concentration in culture mg/ml</b>	<b>Total Lipid production. % dry wt.</b>
<b>Xylose</b>	<b>4.14</b>	<b>30</b>
<b>Dextrose</b>	<b>3.99</b>	<b>35</b>
<b>Artificial acid hydrolysate</b>	<b>4.12</b>	<b>22</b>
<b>Switch Grass Hydrolysates</b>	<b>2.63</b>	<b>26</b>

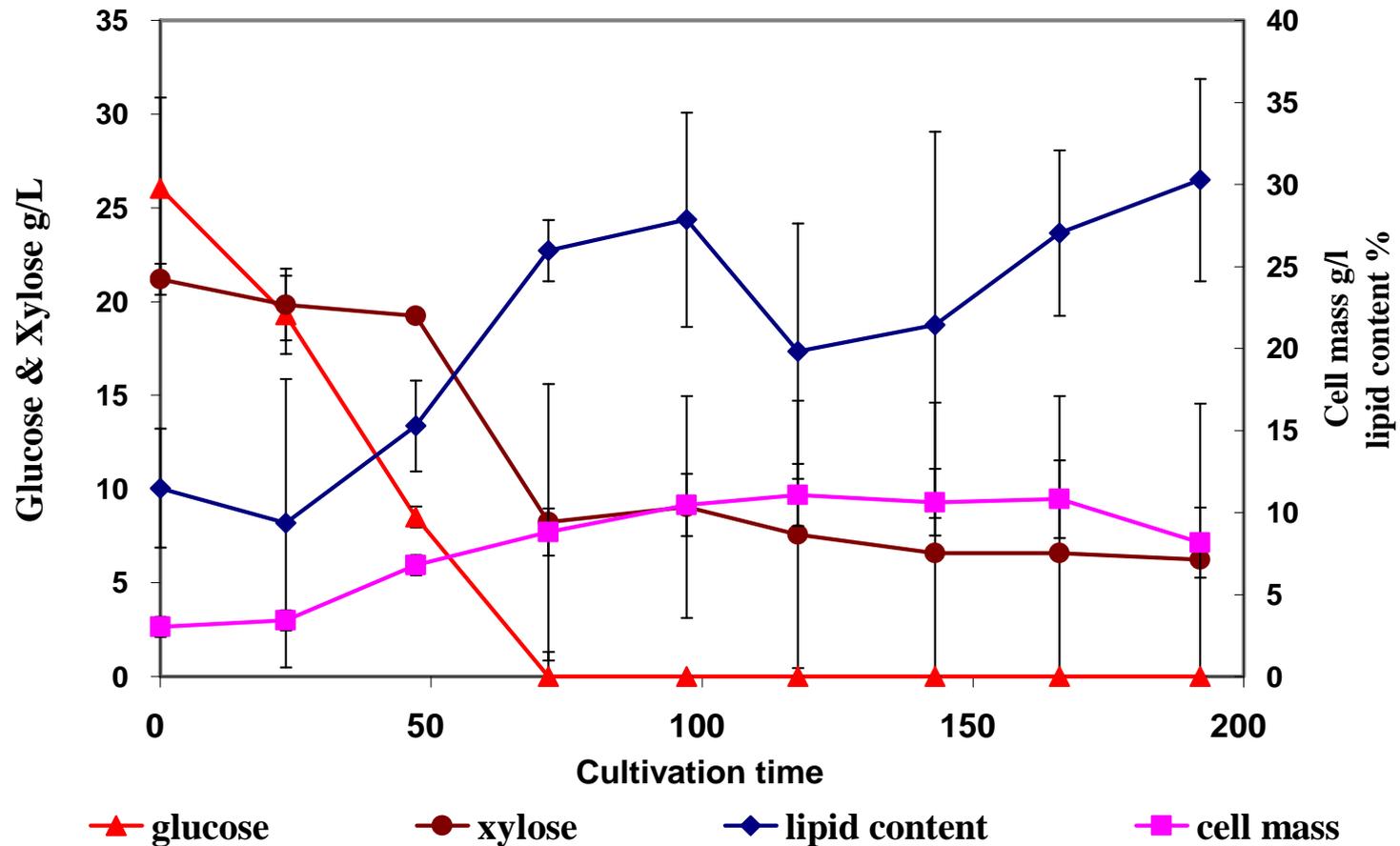
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# Conversion of 30 g/L Acid Hydrolysate by Oleaginous Microbes



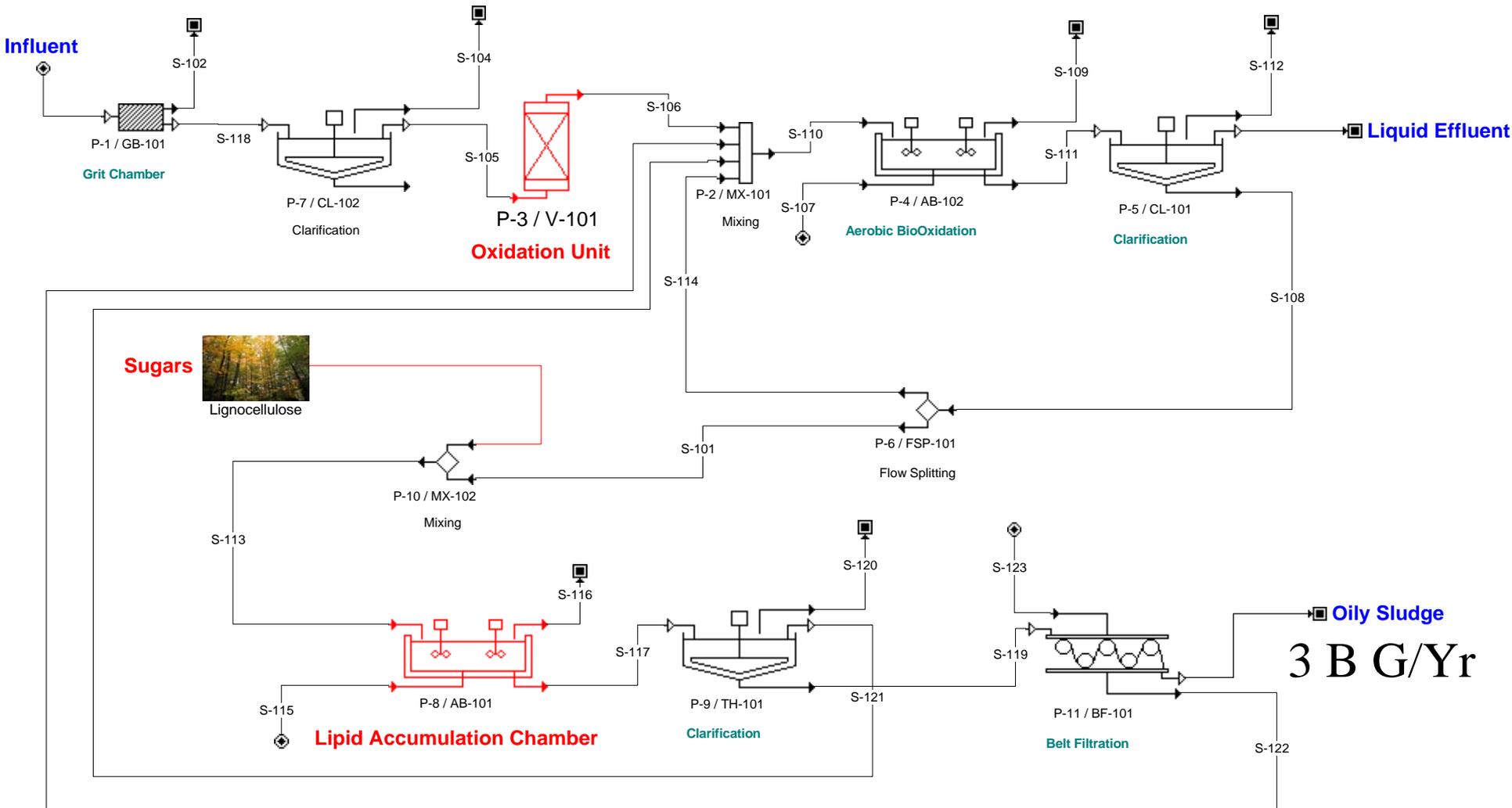
# Conversion of 45 g/L Acid Hydrolysate by Oleaginous Microbes



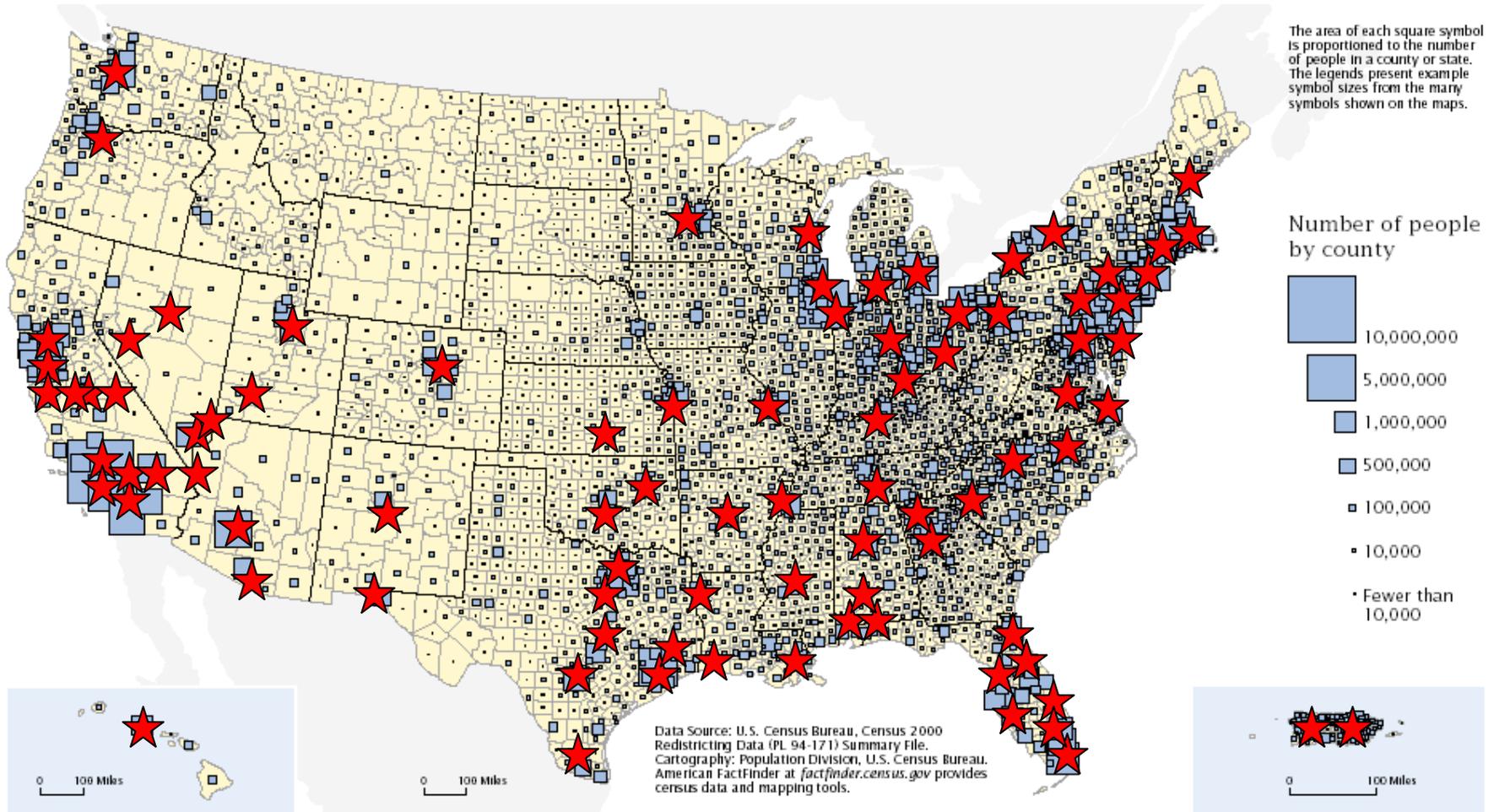
# Fatty Acid Composition

<b>Fatty Acid</b>	<b>Yeast</b>	<b>Rape</b>	<b>Canola</b>	<b>Tallow</b>	<b>Soy</b>
<b>Caproic (6:0)</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>
<b>Myristic (14:0)</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>3.0</b>	<b>0.0</b>
<b>Palmitic (16:0)</b>	<b>14.0</b>	<b>2.2</b>	<b>4.0</b>	<b>23.3</b>	<b>9.9</b>
<b>Stearic (18:0)</b>	<b>7.0</b>	<b>0.9</b>	<b>2.4</b>	<b>17.9</b>	<b>3.6</b>
<b>Oleic (18:1)</b>	<b>32.0</b>	<b>12.6</b>	<b>65.0</b>	<b>38.0</b>	<b>19.1</b>
<b>Linoleic (18:2)</b>	<b>20.0</b>	<b>12.1</b>	<b>17.3</b>	<b>0.0</b>	<b>55.6</b>
<b>Linolenic (18:3)</b>	<b>3.0</b>	<b>8.0</b>	<b>7.8</b>	<b>0.0</b>	<b>10.2</b>
<b>Eicosatrienoic (20:3)</b>	<b>8.0</b>	<b>7.4</b>	<b>1.3</b>	<b>0.0</b>	<b>0.2</b>
<b>Behenic (22:0)</b>	<b>0.0</b>	<b>0.7</b>	<b>0.4</b>	<b>0.0</b>	<b>0.3</b>
<b>Erucic (22:1)</b>	<b>0.0</b>	<b>49.9</b>	<b>0.1</b>	<b>0.0</b>	<b>0.0</b>

# Envisioned Process: The New Biorefineries



# Biorefinery Locations



# ACKNOWLEDGEMENTS

- Department of Energy
- Environmental Protection Agency
- MS/AL Sea Grant
- 23 students



**Thank You**

