

# Enzyme Catalysis for Biomass Based Diesel Fuels

The Center for BioEnergy Sustainability (CBES)  
At Oak Ridge National Laboratory (ORNL)  
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“People don’t buy what you do, they buy why you do it.” –Simon Sinek



# Outline



Why use an enzymatic approach to biodiesel?

- Benefit over chemical catalysts
- Sustainability

Overview of enzymatic biodiesel

- Enzymes for commercial production
- Transesterification
- Esterification

Enzyme Reuse

Economics of Enzymatic Biodiesel



# Quick Definitions



**TAG** = Triglycerides (fat/oil)

**DAG** = Diglycerides

**FFA** = Free fatty acids

**FAME (or ME)** = Fatty Acid Methyl Esters (biodiesel made using methanol)

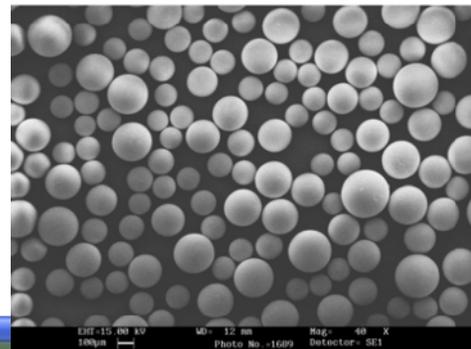
**FAEE** = Fatty Acid Ethyl Esters (biodiesel made using ethanol)

## Immobilized vs. Liquid Enzymes

**CALB** = **C**andida **A**ntarctica **L**ipase **B**

**Novozym 435** = CALB immobilized on a plastic support (.5mm beads)

**TL** = **T**hermomyces **L**anuginosa lipase



# Advantages of Enzymes for Biodiesel



## Benefits of Enzymatic Processing

- No soap formed during the reaction
- High quality glycerin
- Very little excess methanol
- Can esterify and transesterify
- No caustic chemicals required
- Reduces or eliminates post processing (water wash, ion exchange, etc.)



	Traditional acid esterification	FAeSTER enzymatic esterification
Capital Cost	high	low
Catalyst Cost	low	high
Methanol Distillation Variable Cost	high	low
Footprint	large	small
Safety	uses hazardous /toxic chemicals	no hazardous/toxic chemicals

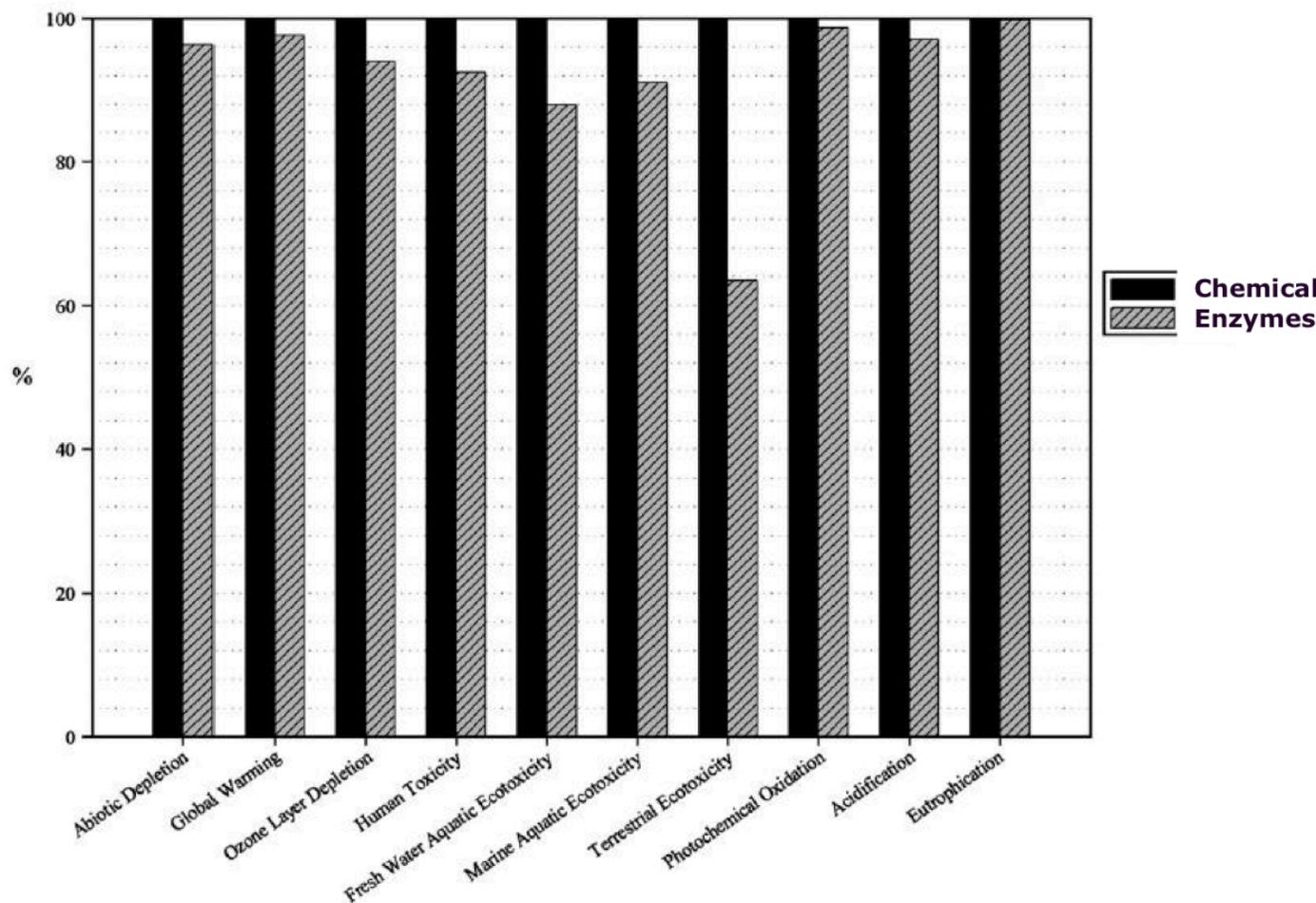
# Sustainability Profile

- **National Research Council's Committee on Water Implications of Biofuels Production: 1 gallon of wastewater: gallon of biodiesel produced. (Oct. 2007)**
- **DOE funded Piedmont research for reduction of industrial waste water**
- **2007, Harding et al, LCA analysis between enzymatic & chemical catalysis for biodiesel**
- **Flammable and hazardous substance exposure reduction**



LCA results from Harding et al 2007 shows better environmental performance of enzymes as catalyst than chemical catalyst

no



Improved performance linked to lower energy requirements for heating in the process

Terrestrial ecotoxicity levels are reduced by 40% with the removal of mineral acid from the process

Fig. 4. LCA results – chemical vs. biological catalysts (biodiesel production by alkali catalysis assuming 94% methanol recovery (case 1) is compared to production using lipase as a biocatalyst (case 2)).

# Hurdles to Enzymes for Biodiesel



- 15 years of research
- Hundreds of articles
- Many different enzymes, reaction conditions, etc.
- Overall conclusion in most cases – too expensive

Problems with enzymatic production	Solutions from the literature, other companies
Very slow reaction times	Use enzymes with faster reaction rates for transesterification (like TL-IM), use cosolvents, multi-stage glycerol removal
Unable to achieve actual ASTM and EU specifications	Almost no work done here – researchers report conversion to esters, generally ignore commercial ASTM specifications like acid number and bound glycerin.
High catalyst cost	Reuse the enzyme for 60 – 100 batches. No reuse data which is even close to commercially viable
Untested on very low quality feedstocks with impurities and other “real world” feedstocks	Tested waste fish oils, yellow greases, animal fats, mostly in relatively short batch reuse systems
Enzyme deactivation due to the alcohol	Use ethanol and other higher alcohols and/or cosolvents to reduce the impact
Enzyme deactivation due to glycerol	Add cosolvents, multi-stage glycerol removal, use different carriers

# Background



## **Early Work (1987 - 1995)**

- Studied cosolvents like iso-octane, hexane, diesel fuel.
- Evaluated aqueous, non aqueous, and solvent-free systems, different types of alcohols, impact of water
- Studied a wide variety of liquid enzymes
- M. Haas & T. Foglia (USDA), M. Mittelbach (Graz), and many others
- No real biodiesel production, so these were interesting but not practical

## **Resurgence of interest (1999 - 2008)**

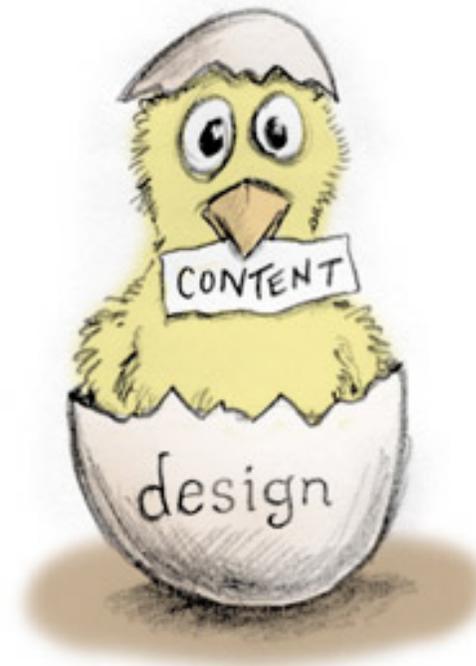
- Better immobilized enzymes
- Solvent free systems, good enzyme reuse
- First large scale production plant in China using cosolvents

## **Current Developments**

- Novozymes developing enzymes, immobilization techniques, lowering costs
- Piedmont Biofuels and other US producers with commercial installations
- Others: Spain, Israel, S. Korea, Taiwan
- Renewed interest from universities and private biodiesel producers for lab and pilot sized reactors

# Why Now?

- Chicken or the egg problem - enzyme development vs. market development.
- Confluence of events:
  - Biodiesel industry is growing and more secure
  - Drive for lower cost feedstocks, presence of high FFA virgin oils
  - Demand for increased fuel quality
  - Competition for fats/oil from other biomass based diesel will push efficiency
  - Industry recognition of problems with soaps, low quality glycerin difficulty/expense of esterification
- Causes process development:
  - Commercial enzyme reuse trials
  - Lower cost enzyme production

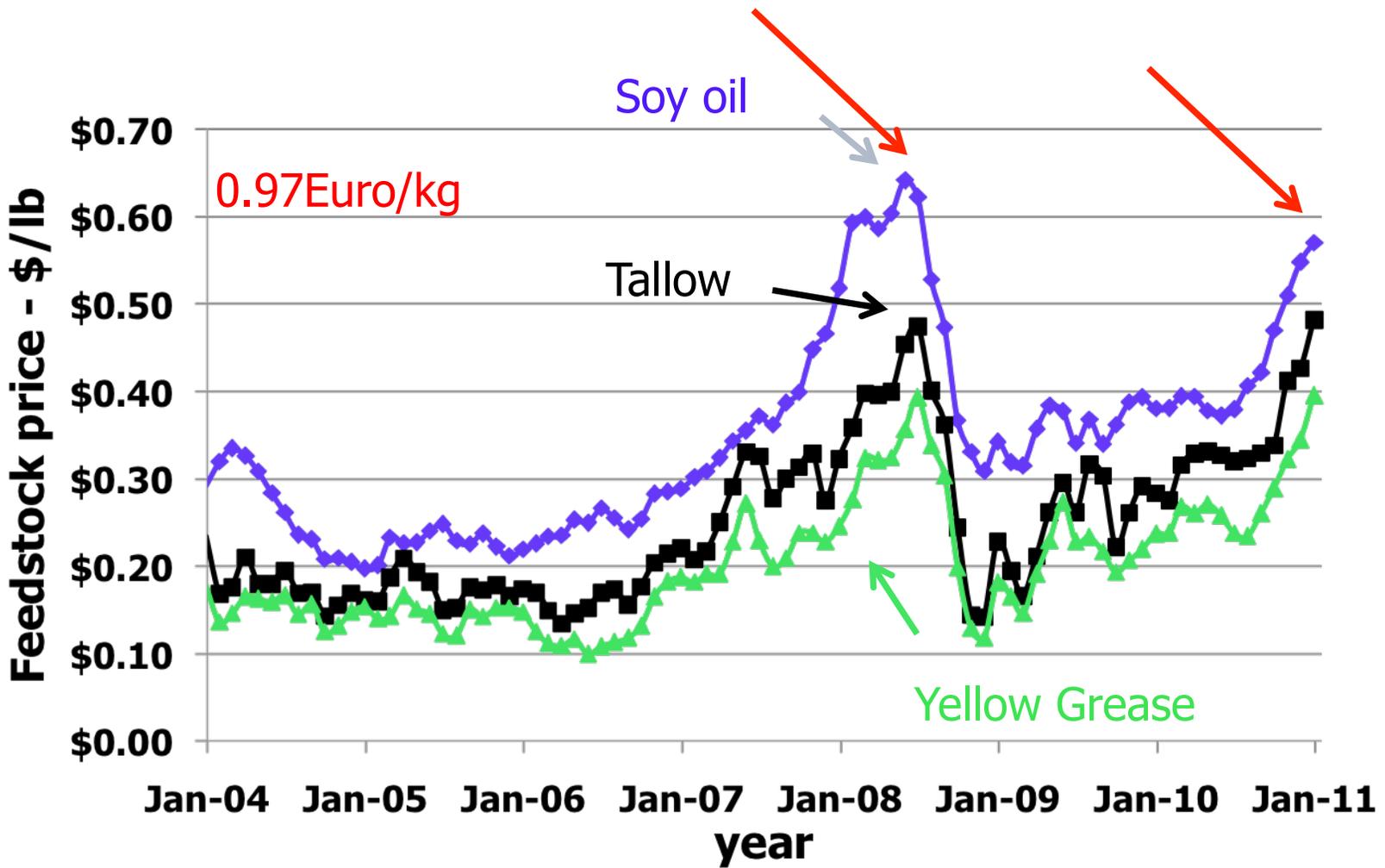


<http://blog.abovethefolddesign.com/2010/12/14/design-vs-content/>

→ Commercial Viability!

# Access to affordable feedstocks

Feedstock prices rising for the 2<sup>nd</sup> time; now in accordance to petrol market



# Reusability



## Enzymes used for biodiesel production lose activity by:

High heat (>50C or >122F)  
Excess alcohol

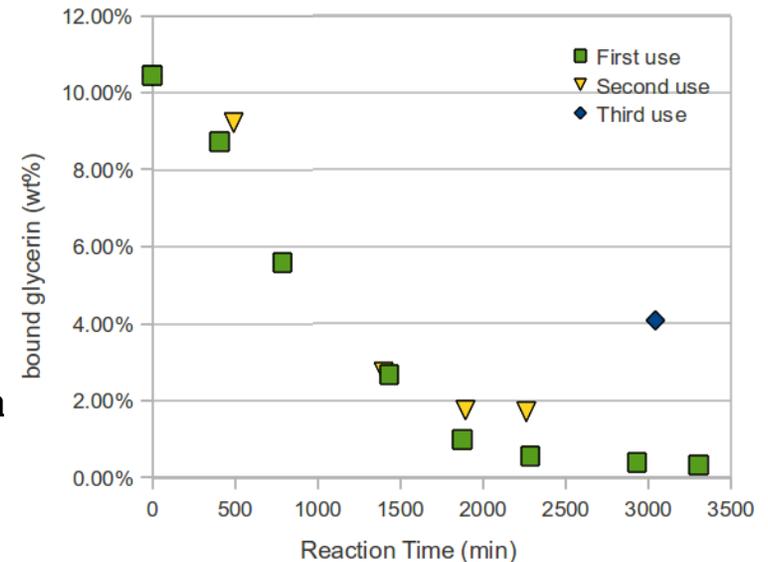
## In addition, Immobilized enzymes can lose activity by:

Any alcohol out of phase  
Large excess of alcohol in phase (~5% or more)  
Glycerol out of phase  
High shear can cause immobilized enzymes to come off of the carrier  
Certain polar contaminants  
Mineral acids

## Temporary vs. Permanent activity loss

Temporary: physical blocking of active sites  
Permanent: denaturing due to excess methanol

3 Enzyme Reuses, immobilized TL-HC  
Similar reaction conditions



**Reusability of enzymes is the key to commercial viability!**

# Summary Articles

- Haas, 2002 (review of early literature)
- Fjerbaek, 2009
- Ranganathan, 2007
- Basic components of reviews
  - enzyme type
  - liquid vs immobilized
  - support type
  - reaction conditions
  - conversion
  - number of reuses
  - tolerance to water, methanol

**Table 1.** Biodiesel production with various lipases.

Lipase	Oil/fat	Alcohol	Yield	Form	Conditions and observations	References	
<i>Pseudomonas fluorescens</i> <sup>a</sup>	Soybean oil	Methanol	90%	Free	35°C, 3:1 molar alcohol added in three steps, 90 h, 150 rpm	Kaieda et al. (2001)	
<i>Pseudomonas cepacia</i> <sup>b</sup>			>80%	Free			
<i>Candida rugosa</i>			90%	Free			
<i>Pseudomonas fluorescens</i> <sup>a</sup>	Sunflower oil	Methanol	>95% (24 h, molar ratio 4.5:1)	Imm.	40°C, 200 rpm, 3:1 molar alcohol added in three steps, 10 wt% enzyme based on oil weight, 30 h	Soumanou and Bornscheuer (2003)	
<i>Rhizomucor miehei</i> <sup>c</sup>			>80%	Imm.			
<i>Thermomyces lanuginosa</i> <sup>d</sup>			>60%	Imm.			
<i>Candida antarctica</i> <sup>e</sup>	Sunflower oil	Methanol	93.2% (1-propanol)	Imm.	40°C, 3:1 molar ratio of alcohol added in four steps, 10 wt% enzyme based on oil weight, 24 h	Deng et al. (2005)	
<i>Rhizomucor miehei</i> <sup>c</sup>			Ethanol	79.1% (96% EtOH)			Imm.
<i>Thermomyces lanuginosa</i> <sup>d</sup>			1-Propanol	89.8% (methanol)			Imm.
<i>Thermomyces lanuginosa</i> <sup>f</sup>			2-Propanol	72.8% (1-propanol)			Imm.
<i>Pseudomonas cepacia</i> <sup>g</sup>			1-Butanol	88.4% (96% EtOH)			Imm.
<i>Pseudomonas fluorescens</i> <sup>a</sup>	Mahua oil	Isobutanol	45.3% (96% EtOH)	Imm.	40°C, 200 rpm, 4:1 molar ratio of alcohol to oil, 10 wt% enzyme based on oil weight; lipases were pH-tuned	Kumari et al. (2007)	
<i>Pseudomonas cepacia</i> <sup>b</sup>			Ethanol	96% (6 h)			Imm.
				92% (2.5 h)			CLEA <sup>m</sup>
			99% (2.5 h)	PCMC <sup>n</sup>			
<i>Porcine pancreatic lipase</i> <sup>h</sup>	Babassu oil	Butanol	95%	Imm.	40–50°C, 150 rpm, 10:1 molar alcohol to oil, 20 wt% of total substrate enzyme	Paula et al. (2007)	
<i>Pseudomonas cepacia</i> <sup>l</sup>	Jatropha oil	Ethanol	98%	Imm.	50°C, 200 rpm, 4:1 molar ratio of alcohol to oil, 5 wt% water based on enzyme weight, 10 wt% enzyme based on oil weight, 8 h	Shah and Gupta (2007)	
<i>Candida antarctica</i> <sup>j</sup>	Tallow	Methanol	74%	Imm.	30°C, 200 rpm, 3-step addition of 3:1 molar alcohol to tallow, 10 wt% enzyme based on oil weight, 72 h	Lee et al. (2002)	
<i>Candida sp.</i> 99-125	Rapeseed oil	Methanol	83% (36 h, 5 wt% enzyme, BSTR)	Imm.	40°C, 180 rpm, alcohol molar ratio 3:1 added in three steps. Solvent: <i>n</i> -Hexane for salad oil, otherwise petroleum ether	Deng et al. (2003); Nie et al. (2006); Tan et al. (2006)	
	Salad oil		95% (30 h, 20 wt% enzyme, BSTR)	Imm.			
	Waste oil		92% (22 h, three PBRs in series)	Imm.			
<i>Pseudomonas cepacia</i> <sup>k</sup> mixed with <i>Candida antarctica</i> <sup>e</sup>	Vegetable oil, unspecified	Ethanol	96% (30 h, 15 wt% enzyme, BSTR)	Imm.	35°C, 200 rpm, 4:1 molar ratio of alcohol, 5 wt% enzyme based on oil weight, respectively; addition of CA after 1 h, 24 h	Wu et al. (1999)	
	Restaurant grease		95%	Imm.			
<i>Rhizoptus oryzae</i> mixed with <i>Candida rugosa</i> <sup>l</sup>	Soybean oil	Methanol	>99%	Imm.	45°C, 200 rpm, 4.5:1 molar ratio of alcohol added in 10 steps, 30 wt% enzyme based on substrate, 10 wt% water, imm. on silica gel, 1 wt% RO and 1 wt% CR, 21 h	Lee et al. (2006)	
<i>Thermomyces lanuginosa</i> (TL) <sup>d</sup> mixed with <i>Candida antarctica</i> (CA) <sup>e</sup>	Rapeseed and waste oil	Methanol	95%	Imm.	35°C, 130 rpm, 4:1 molar ratio alcohol to oil, <i>tert</i> -butanol to oil volume ratio 1:1, 3 wt% TL and 1 wt% CA, 12 h	Li et al. (2006)	

Fjerbaek et al.: Biodiesel Production Using Enzymatic Transesterification. 2009.

# Watanabe and Shimada (2001, 2005)



**No cosolvent**

**Use multi-stage methanol addition  
to avoid deactivation**

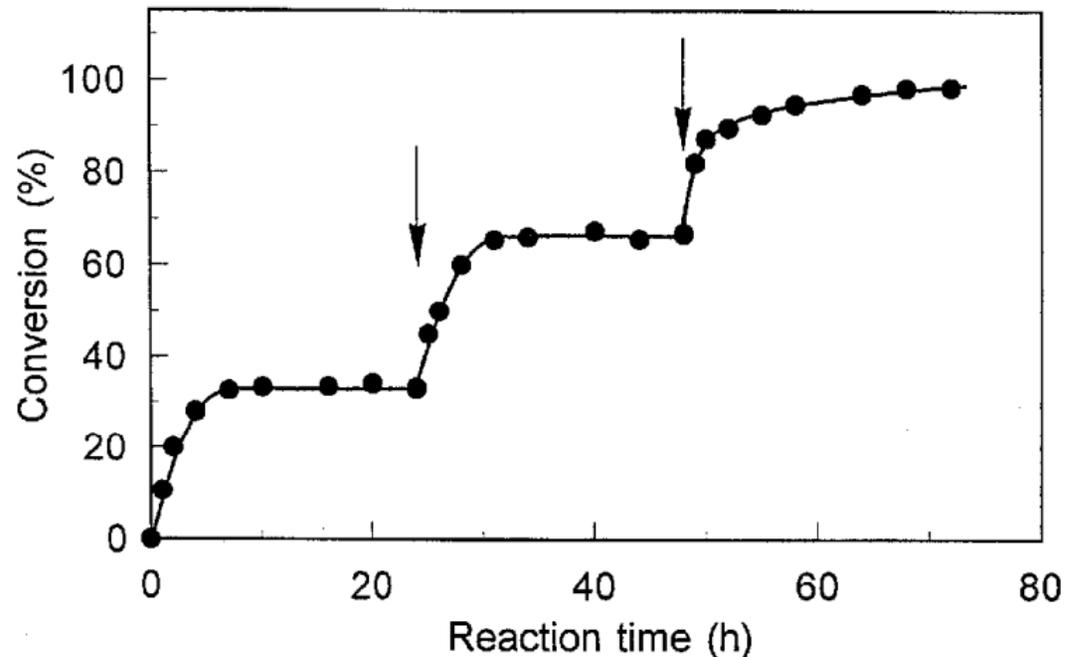
**Tested batch and continuous**

## **Advantages**

- No cosolvents
- Minimize methanol use
- Excellent conversion (98%+)
- Good enzyme reuse

## **Disadvantages**

- Still has long reaction times
- Still had high FFA at end of process
- Enzyme reuse still not commercially viable



**FIG. 4.** Time course of methanolysis of vegetable oil. A reaction mixture of 28.95 g oil, 1.05 g methanol (the molar ratio of methanol to the oil, 1:1), and 1.2 g immobilized *Candida* lipase was shaken at 30°C. After 24- and 48-h reactions, 1.05 g methanol was added as indicated with arrows.

# Novozymes (2009)



## Longevity trials: Esterification

### Reaction Conditions

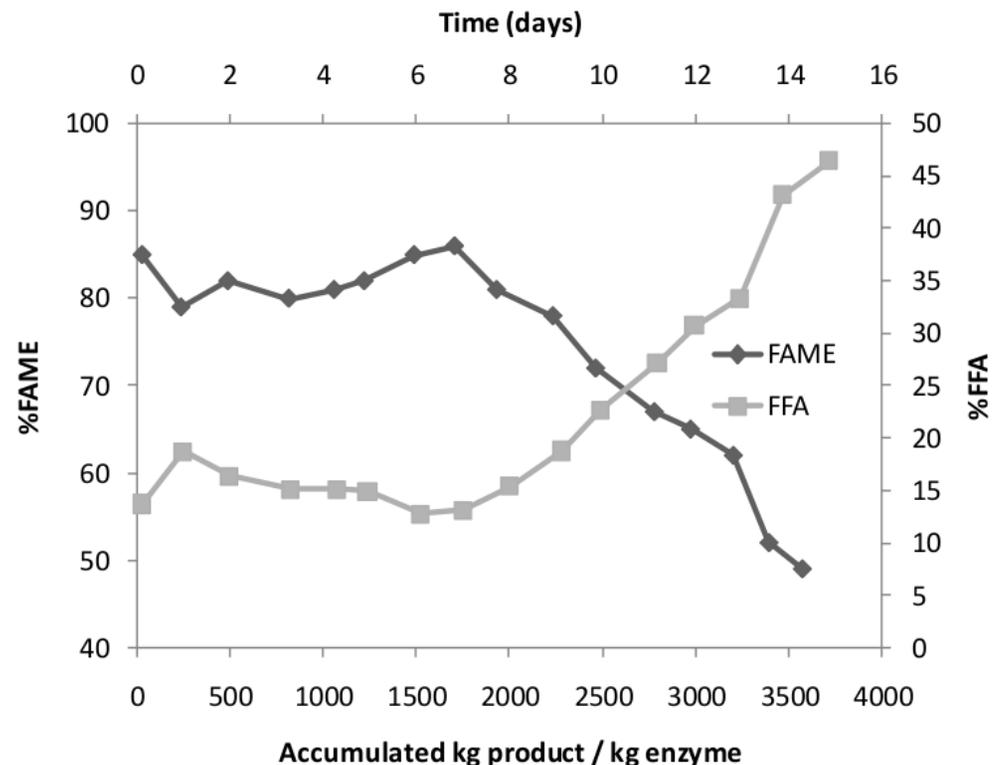
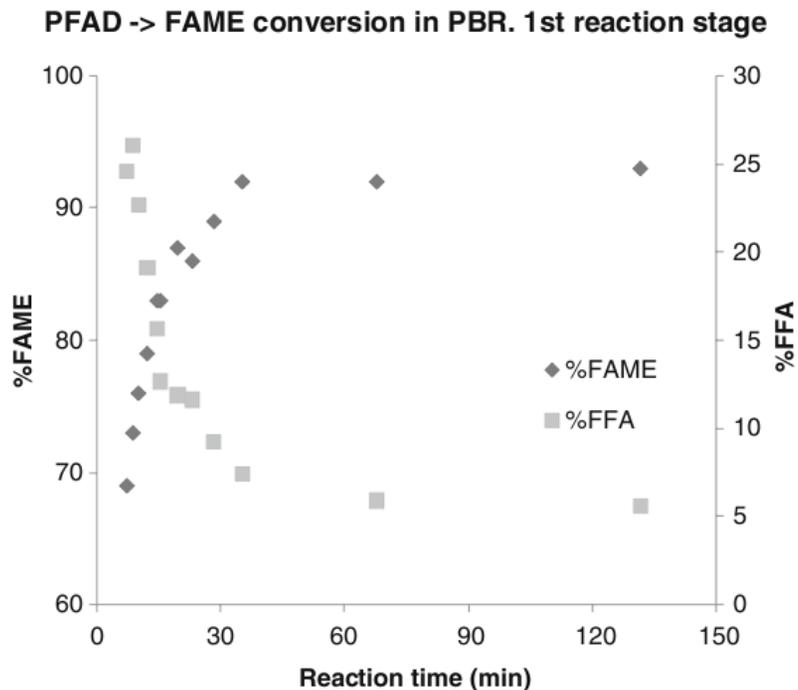
- 20% by volume methanol, 2 stage reaction, 45C,
- Majority of reaction finished in 60 minutes
- Blended FAME, MeOH, and PFAD to address the high melting point

## Still has deactivation

### Large excess methanol use (~2:1)

### Still high FFA content (3 - 5%) after 2<sup>nd</sup> stage

Novozym 435 lifetime in PFAD -> FAME reaction.  
1st reaction stage at constant flowrate



# Enzymatic approach to Waste Reduction: PBI



- Began investigation of enzyme catalysis for biodiesel, 2009
- Focus on esterification first
- 2010--- Lab working on Pilot scale
- 2011--- Pilot moving to Commercial
- 2012--- Commercial Integration
- Validation for multi-feedstock production scheme
- First to demonstrate enzyme biodiesel to meet full ASTM specification.
- Economic analysis & Enzyme reuse



# Commercial Lipase Comparison



	Conversion with NO Water	
	Transesterification	Esterification
CAL-B	Moderate	<b>Very Fast</b>
TL-IM	Slow	Fast

	Conversion with Some Water	
	Transesterification	Esterification
CAL-B	Slow or not at all	<b>Very Fast</b>
TL-IM	<b>Very Fast</b>	Varies with concentration

*Candida Antarctica Lipase B, Thermomyces Lanuginosa*

# Enzymes For Biodiesel Production



**Commercial  
formulations:  
Callera series**



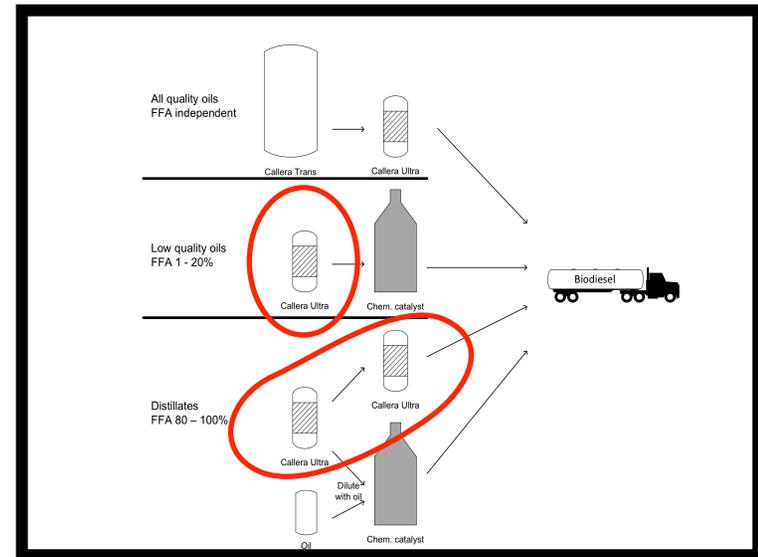
# How can you use this process?

Enzymatic Esterification :

A. Pretreatment for existing chemical plants

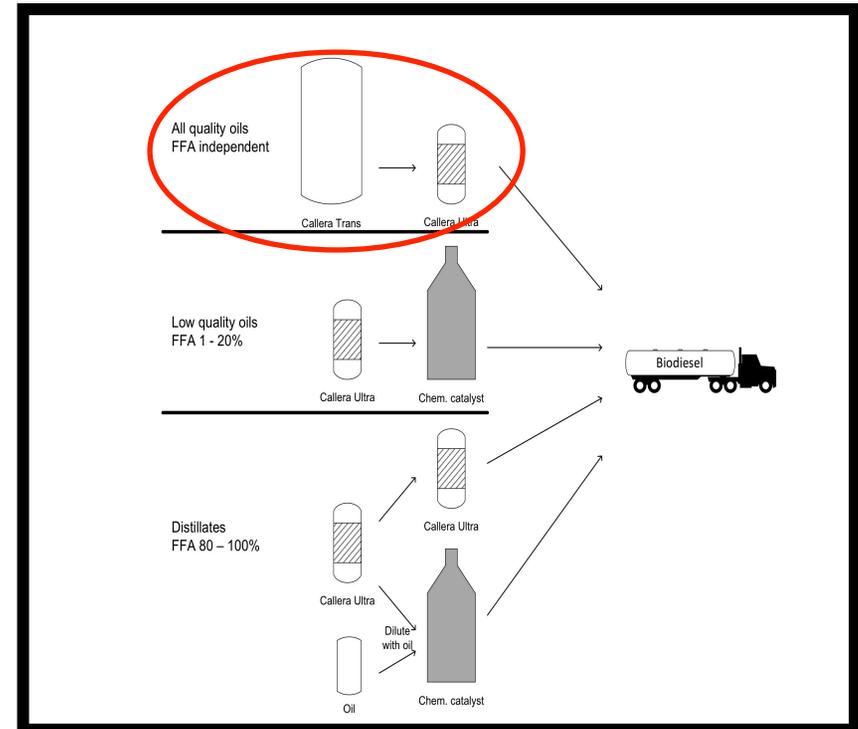
B. Full conversion

Esterification for High FFA feedstocks



## Enzymatic Polishing:

- **TRANSESTERIFICATION** followed by the **FAeSTER** esterification **PROCESS**
- **100% enzyme-based process** in 2 steps
- **Enzymes: Liquid TL** followed by **CALB**

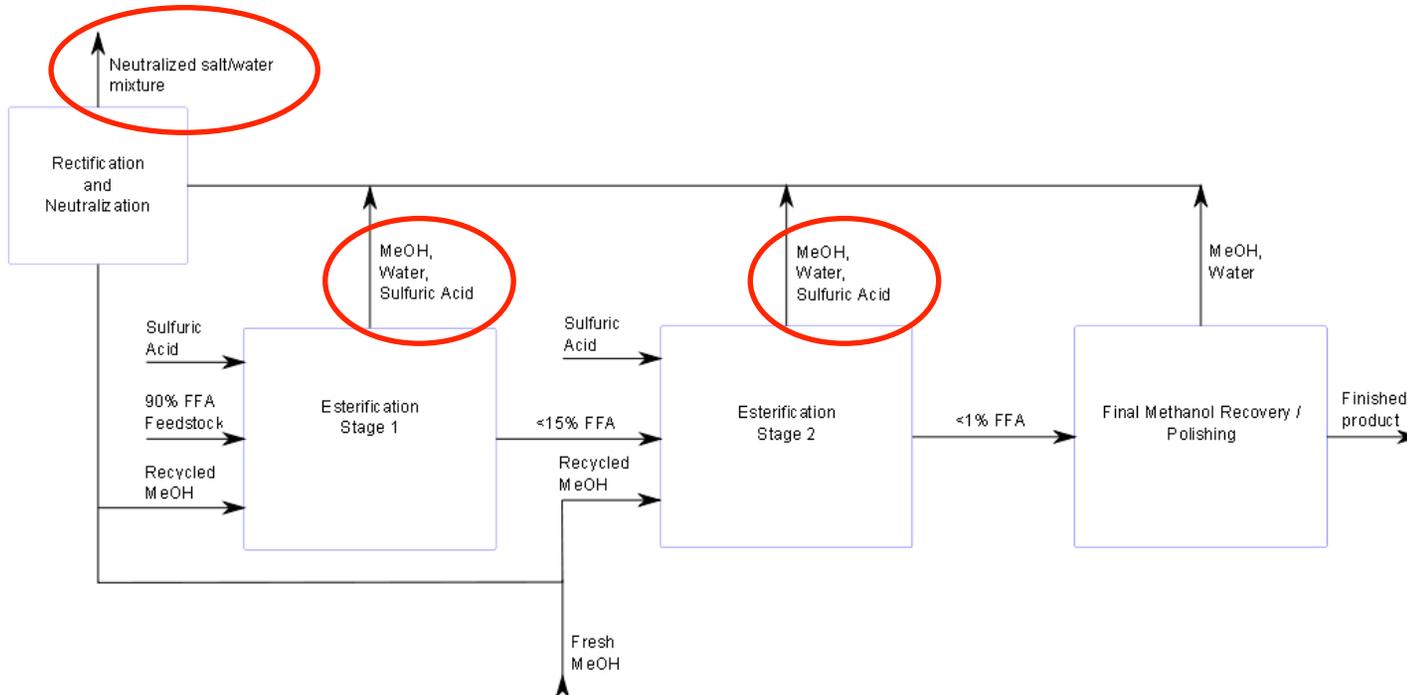




# Esterification



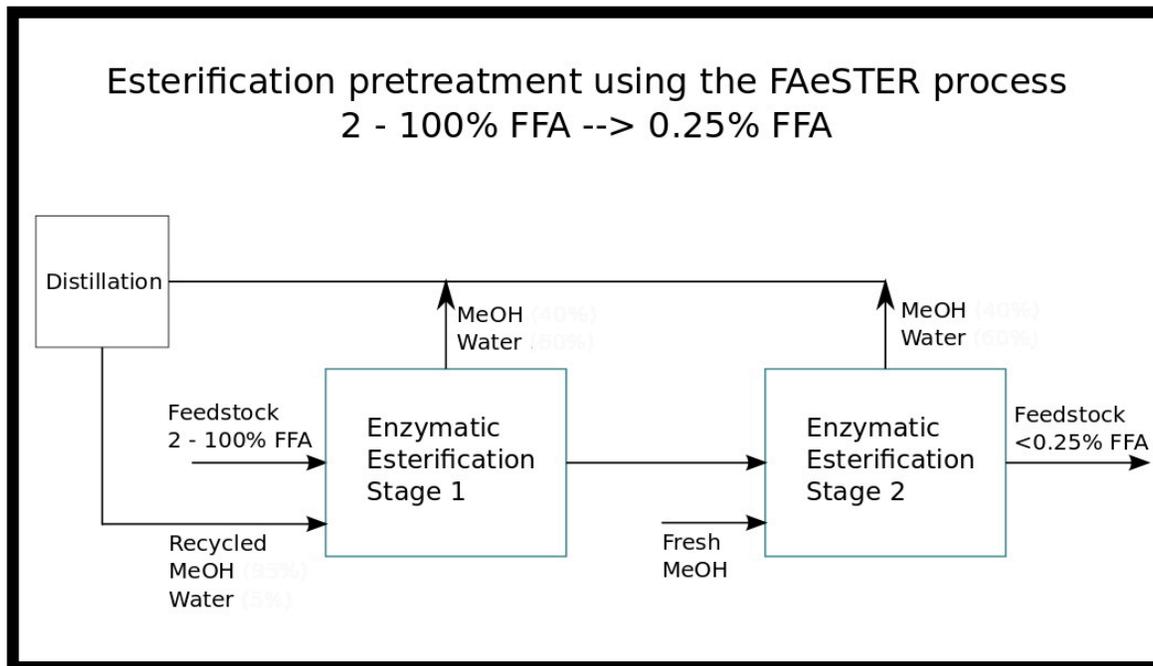
## Traditional Esterification P&ID 100% FFA --> Biodiesel 2 stage process



Hurdles to the producer:

- Excess Alcohol Ratios
- Acid Waste Streams
- Reaction Times
- Capital Infrastructure

# FAeSTER Process

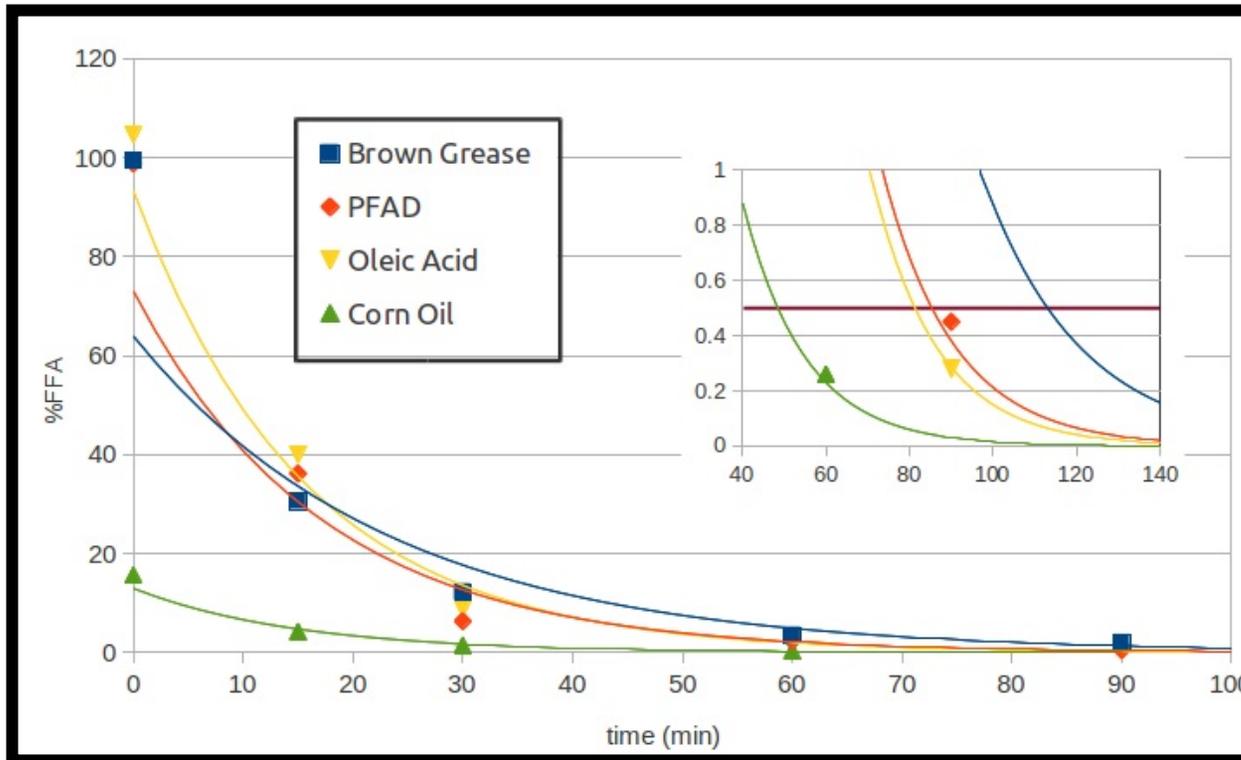


**FAeSTER Process:**  
**Fatty Acid**  
**esterification**

- Esterification using Callera Ultra/L Immobilized CALB or Liquid CALB
- Replaces traditional sulfuric acid technology
- Incoming Feedstocks: 2-100% FFA
- Patent Pending, continuous process
- Low temperature process
- Maintains water balance
- 6-12 times less methanol than acid esterification
- No acidic methanol sidestream

# FAeSTER as Feedstock Pretreatment

Esterification occurs quickly within 30 minutes



Achieves acid value specification by 90 minutes

Feedstock	Time to <0.50% FFA
Brown Grease	123
PFAD	90
Oleic Acid	82
Corn Oil	51

# Transesterification



# Transesterification using TL-IM

- Enzyme used: TL-IM for glyceride conversion
- Feedstock: soy and yellow grease (5 %FFA)
- Quantity of methanol used (15.5% by volume)
- Weight % of enzyme (0.5% - 5%)
- Reaction time (600 min - 2000min)
- Re-focus efforts on liquid TL
- Glycerol agglomeration

# Transesterification via Liquid Enzyme

# M4

Discovering the Power of Nature

- Quantity of methanol use (1.3 - 1.7:1 molar ratio or 14.8 - 19.4 weight%)
- Weight % of enzyme (0.5% - 2.0%)
- Reaction time (4h-24h) (improvement from 10h-33h)
- Temperature 35-45C/95-113F
- Enzyme reuse up to 10 times, 90-95% conversion
- Separate, redose with alcohol & re-react
- FFA formation, water & glycerol in enzyme solution
- FFA can be polished by FAeSTER process or Biofame process
- Commercial scale validation for both soy oil and yellow grease
- Ultrafiltration may assist enzyme re-use
- Can re-use of wet methanol
- No need to dry feedstock



# 100% Enzyme-Based Fuel meets ASTM D6751



2010: ASTM D6751  
achieved using only  
immobilized  
enzymes

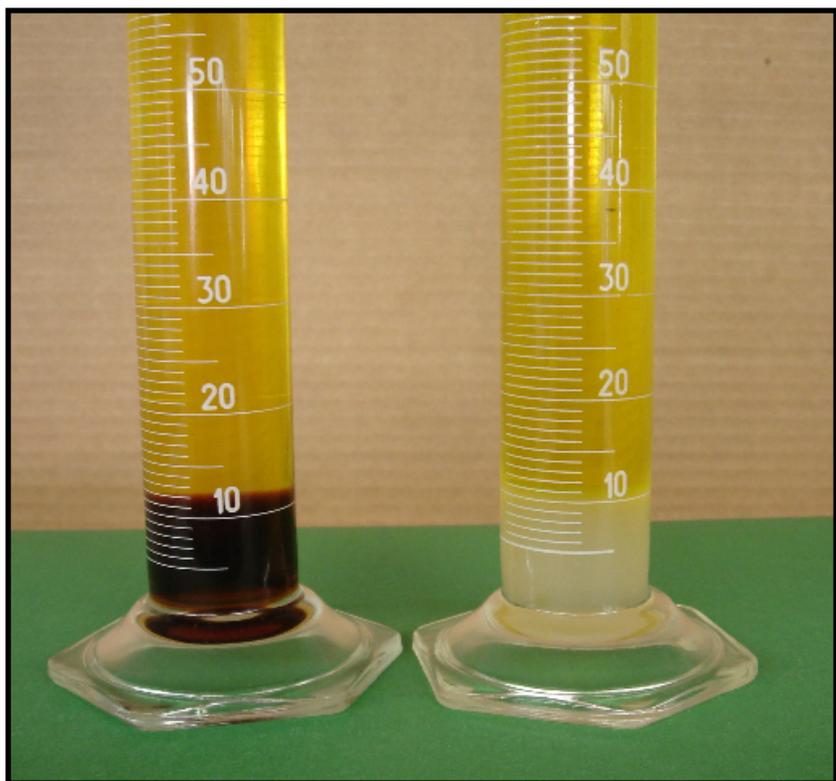
2011: ASTM D6751  
achieved using  
Liquid TL & CALB

2012 and beyond:  
Commercial Scale  
Integration

## Analysis from Piedmont's FAeSTER enzymatic process

Property	Method	Limits	Result
Bound Glycerin	D - 6584	0.24 max.	0.164% mass
Monoglycerides	D - 6584		0.307% mass
Diglycerides	D - 6584		0.085% mass
Triglycerides	D - 6584		0.011% mass
Moisture (Karl Fisher)	D - 6304	Report	204 ppm
Sulfated Ash	D - 874	0.02 max.	0.002% mass
Soap Content	Cc - 17 - 95	Report	Non-Detectable
Water & Sediment	D - 2709	0.05 max.	0% Volume
Kinematic Viscosity 40C	D - 445	1.9 - 6.0	4.65 Cst
Sulfur - Non Petroleum	D - 4294	15 max.	2.8 ppm
Cloud Point	D - 2500	Report	0C
Cold Soak (Filtration Test)	D - 6217	360 max.	97 seconds
Oxidation Stability	EN 14538	3 min.	>6 hrs
Copper Strip Corrosion	D - 130	No. 3 max.	1a
Flash Point	D - 93	130 min.	160C
Visual Appearance	D - 4176	Pass/Fail	Pass
Calcium/Magnesium & Sodium/Potassium	EN 14538	5 max.	Non-Detectable PPB
Acid Number	D - 664	0.50 max.	0.22 mg/KOH/g
Phosphorus Content	D - 4951	0.001 max.	Non-Detectable PPB
Distillation Temp, AET, 90% Recovered	D - 1160	360 max.	340C
Carbon Residue	D - 4530	0.050 max.	Non-Detectable

# Glycerol from Enzymatic Process



Chemical FAME/glycerol vs. FAME/enzymatic glycerol  
YELLOW GREASE FEEDSTOCK



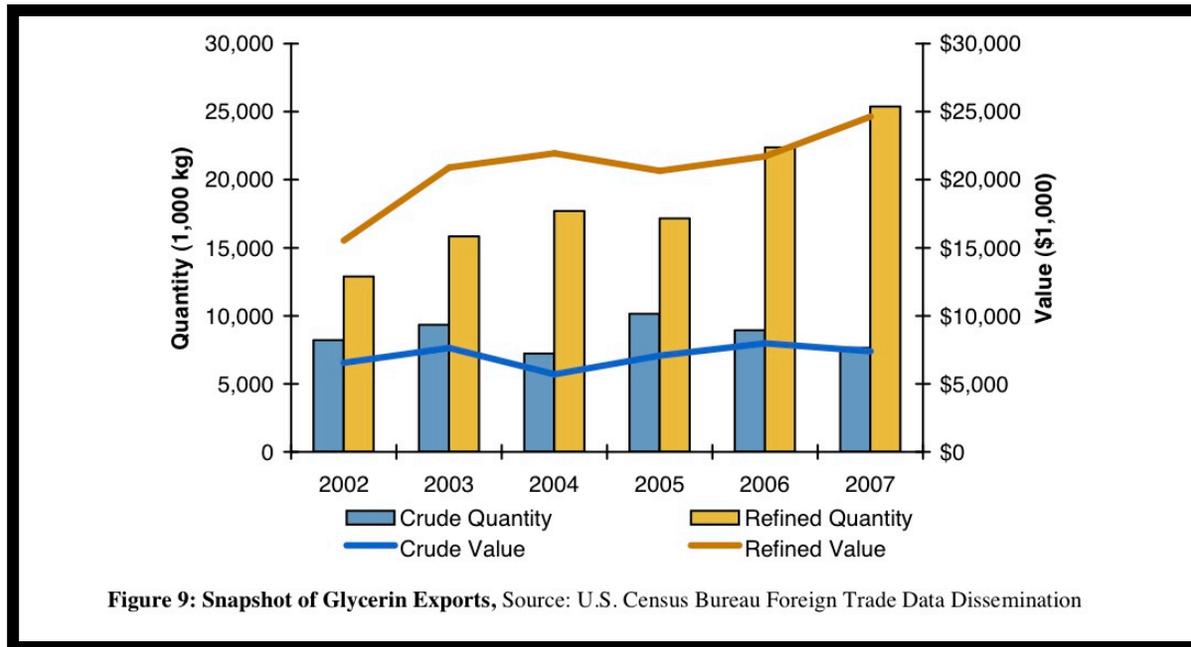
Chemical glycerol vs. enzymatic glycerol

- High purity Glycerol 99.6%
- Economic Impact to the producer:
- 40-50 cents/lb.

Test	Unit	Method	Enzymatic Glycerol	Chemical Glycerol
Glycerol Content	% mass	AOCS Ea 6-94	97.55	55.78
Moisture	ppm	ASTM D6304	20500	22900
Ash	% mass	IUPAC 3.A.4	0	12.87
Methanol	% vol.	EN 14110	0.14	0.45
MONG	% mass	IUPAC 3.A.6	0.4	29.17

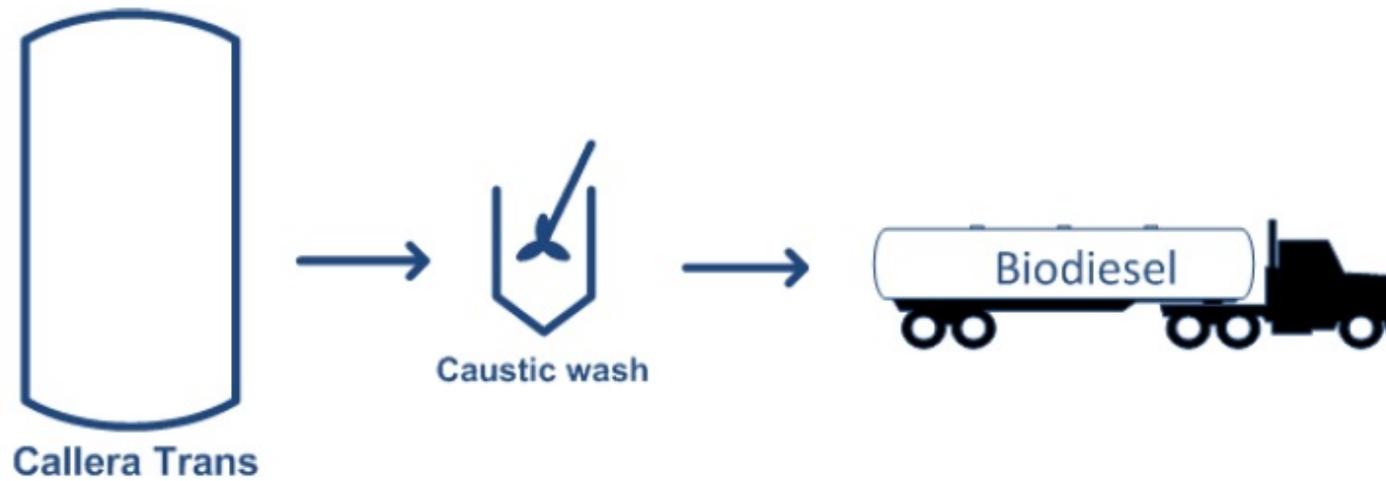
# Glycerol Co-product Value M4

Discovering the Power of Nature



<b>Crude</b>	<b>Mixture of methanol (1/3), glycerin (1/3), and FFA/bio (1/3) plus some metals.</b>
<b>Refined</b>	<b>70 – 80 % glycerin, salts (7 - 10%)</b>
<b>Technical</b>	<b>95 % glycerin or higher</b>
<b>USP</b>	<b>99 % glycerin or higher, plus certification for USP grade</b>

# BioFAME process





# Enzyme Re-use & Economics

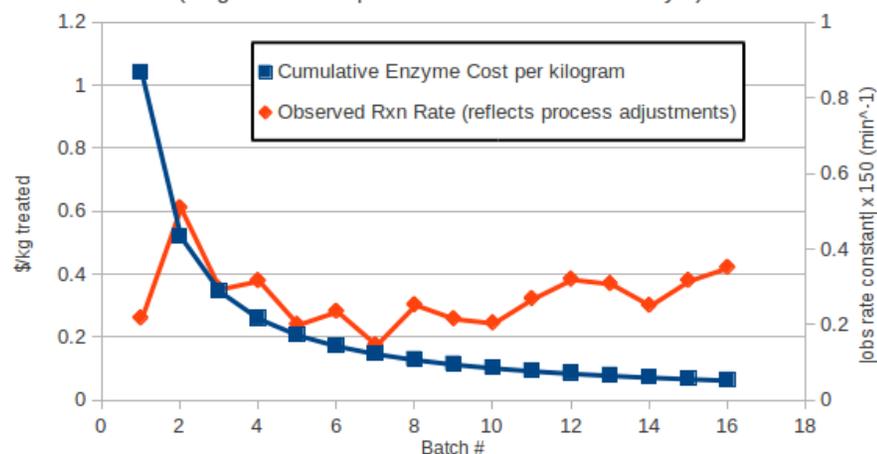


## Enzyme Life & economics

- Productivity of the enzyme vs. price- how many times can I reuse it?
- Immobilized CalB lipase can process minimum 10T oil/kg enzyme (Acid distillate, yellow grease, brown grease).
- Piedmont life-trial data shows CalB : \$0.15/gallon
- The liquid formulations are less expensive and are cost effective with only a few re-uses.

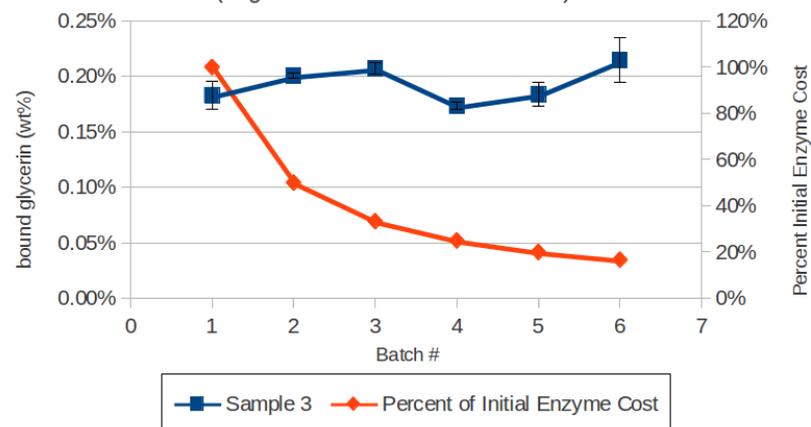
# Enzyme Reuse

Pilot Scale CALB Longevity Trials: Rate Variation and Enzyme Cost  
(35 gal feedstock pretreatment w/ 0.25wt% catalyst)



## Esterification with CALB

Reuse Economics for Liquid TL Enzyme for Transesterification  
(40g batches at 35C w/ 1.0wt% TL)



## Transesterification with Liquid TL



# CAPITAL COST savings

Acid esterification \$3.36 million

FAeSTER process <\$1million



Process Comparison: Acid Esterification and FAeSTER Process			
		High FFA Feedstock (90%+)	
		Acid esterification (sulfuric)	FAeSTER Enzymatic Esterification
		Continuous, two stage esterification using sulfuric acid, 40:1 MeOH:FFA first stage, 20:1 second stage	Continuous three stage esterification using FAeSTER process
System Information	Capacity (gal / year)	3,000,000	3,000,000
	Methanol rectification (gal / year)*	<b>9,305,744</b>	<b>74,274</b>
	Final % FFA	<0.25% FFA	<0.25% FFA
	Continuous / Batch	Semi-continuous	Continuous
	Operational days per year	300 days, 24 hours/day	300 days, 24 hours/day
	Flow Rate (gpm)	35	7
Capital Cost Comparison	Capital Costs – New plant construction		
	Methanol rectification	\$2,193,500	\$150,000
	Boiler	\$220,000	\$25,000
	Esterification equipment	\$661,250	
	Total (includes automation, feedstock drying, assembly, lab equipment, other system components, etc.)	<b>\$3,461,921</b>	
	Capital Costs – Bolt-on to existing plant		
	Methanol rectification	\$2,193,500	
	Boiler	\$220,000	
	Esterification equipment	\$661,250	
	Total (includes automation, feedstock drying, assembly, etc.)	<b>\$3,461,921</b>	<b>&lt;\$1,000,000</b>
FAeSTER Advantages	No additional methanol rectification requirements for existing plants, low capital and operating costs, minimal equipment footprint, no acidic methanol, continuous process easily adjustable to any FFA level, same equipment used for full enzymatic biodiesel production process		

# VARIABLE COSTS Savings

Acid esterification \$1.56/gallon

FAeSTER process \$0.34/gallon

## Process Comparison: Acid Esterification and FAeSTER Process



		<b>High FFA Feedstock (90%+)</b>	
		<b>Acid Esterification</b> Two stage esterification using sulfuric acid	<b>FAeSTER Process</b> Continuous three stage esterification using Novozym 435
<b>System Information</b>	Capacity (gal / year) Methanol rectification (gal / year)* Final % FFA Continuous / Batch Operational days per year Flow Rate (gpm)	3,000,000 <b>9,305,744</b> <0.25 % FFA Semi-continuous 300 days, 24 hours/day 35	3,000,000 <b>74,274</b> <0.25 % FFA Continuous 300 days, 24 hours/day 7
<b>Variable Cost Comparison</b>	Variable costs per gallon Methanol Rectification (heat, cooling) Catalyst Cost Total variable costs excluding feedstock and dep. and amort. (includes heat, electricity, methanol consumed, water disposal)	\$1.34 \$0.04 <b>\$1.56</b>	\$0.01 \$0.16 <b>\$0.34</b>
<b>FAeSTER Advantages</b>	<b>No additional methanol rectification requirements for existing plants, low capital and operating costs, minimal equipment footprint, no acidic methanol, continuous process easily adjustable to any FFA level, same equipment used for full enzymatic biodiesel production process</b>		

# Summary



- Enzyme catalyst based biodiesel production is commercially viable
- Meets ASTM requirements
- **REDUCED ENERGY CONSUMPTION:** Low temperature process, 40-45C, lower methanol dosage, re-use of wet methanol, feedstock does not need water removal, eliminate waste water & associated treatment
- Eliminates need for acids and caustic catalysts
- Applicable to all grades of feedstock
- Positive enzyme re-usability
- Competitive Variable and Capital costs to the producer





**Thank you.**

**Rachel Burton**  
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