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Investigations to the Use of Lipases for Biodiesel Production

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Outline

- **Importance of sustainable biodiesel production**
- **Chemical vs. enzymatic transesterification**
- **Lab scale optimization of an enzymatic biodiesel process**
  - **Testing of suitable enzymes**
  - **Optimization of transesterification process**
- **Alternative enzymatic biodiesel process**
  - **Use of methyl acetate**
  - **Separation of the reaction products**

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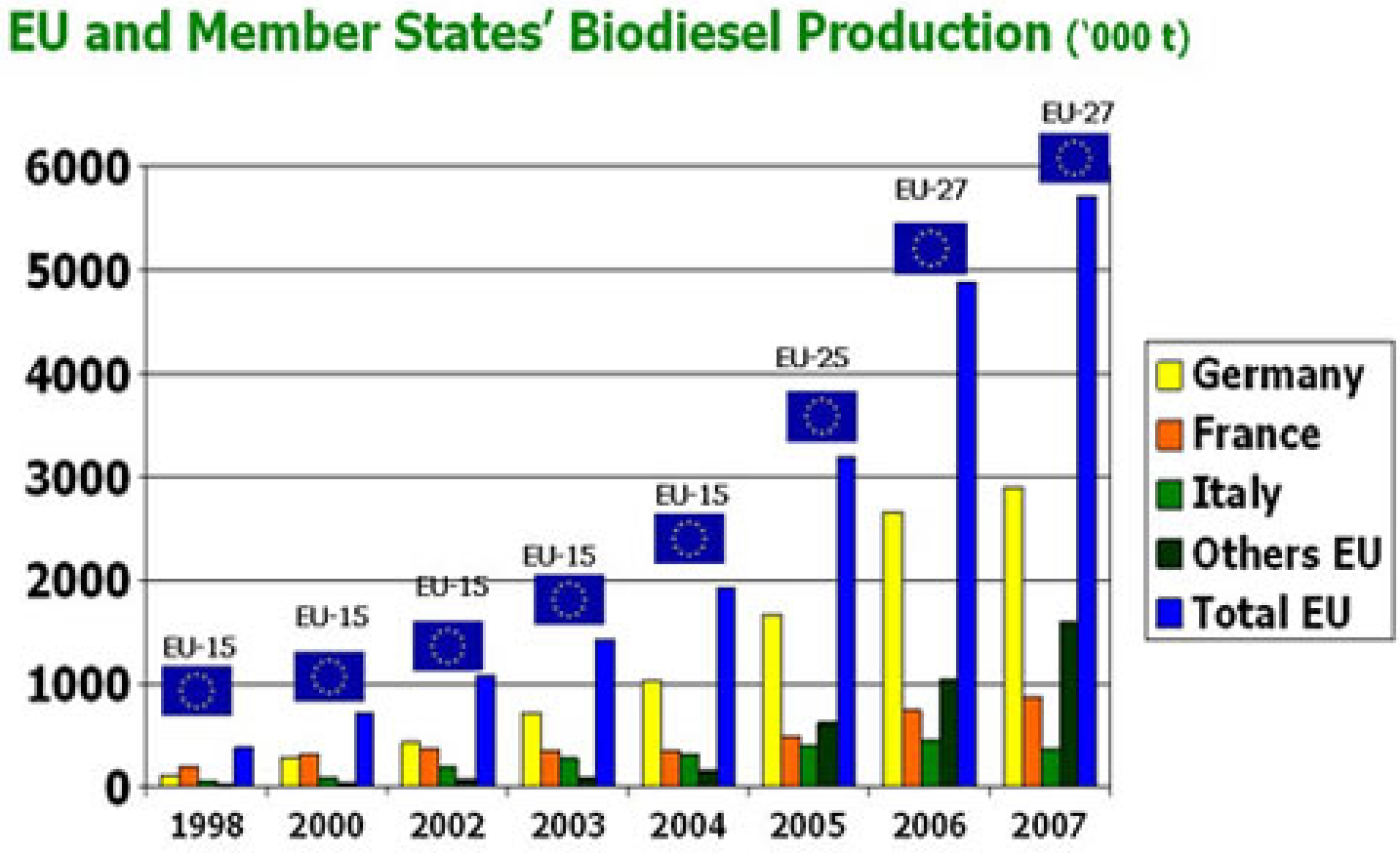
Biodiesel Production in EU



Source: http://www.ebb-eu.org/stats.php#

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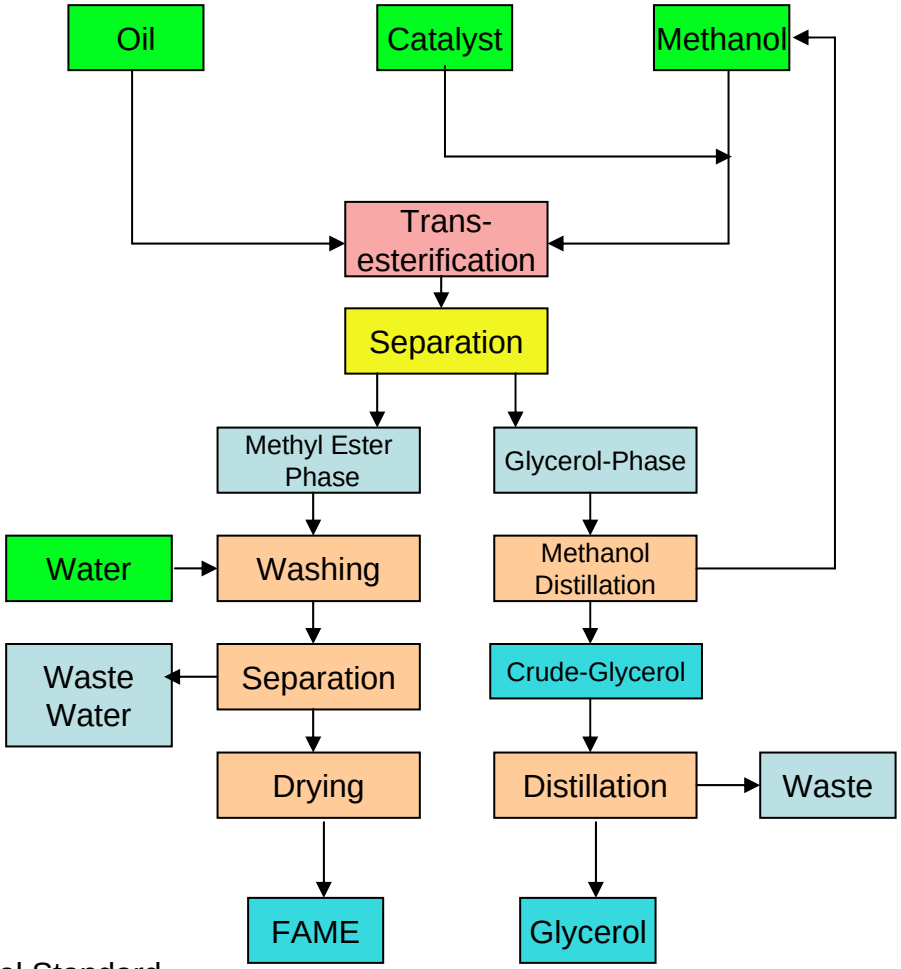
Conventional Biodiesel Process



EU: EN 14214
USA: ASTM D 6751
Australia: Australian Biodiesel Standard

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Lipase Catalyzed Biodiesel Process

Advantages and Disadvantages

Lipases: *Candida antarctica* B-lipase (CALB) “Novozym 435”; *Thermomyces lanuginosa* lipase (TLL) “Lipozyme TL IM”; *Rhizomucor miehei* lipase (RML) “Lipozyme RM IM”

Advantages

- sustainable process
- feedstock flexibility (compatible with variations in the feedstock quality; high ffa contents feasible)
- fewer process steps
- higher glycerol quality (no salts)
- improved phase separation (no emulsification from soaps)
- reduced energy consumption and wastewater volumes

Disadvantages

- high enzyme price
- lower reaction rate
- destabilization of enzymes by short-chain alcohols

Source: Nielsen et. al., Eur. J. Lipid Sci. Technol. 110 (2008)

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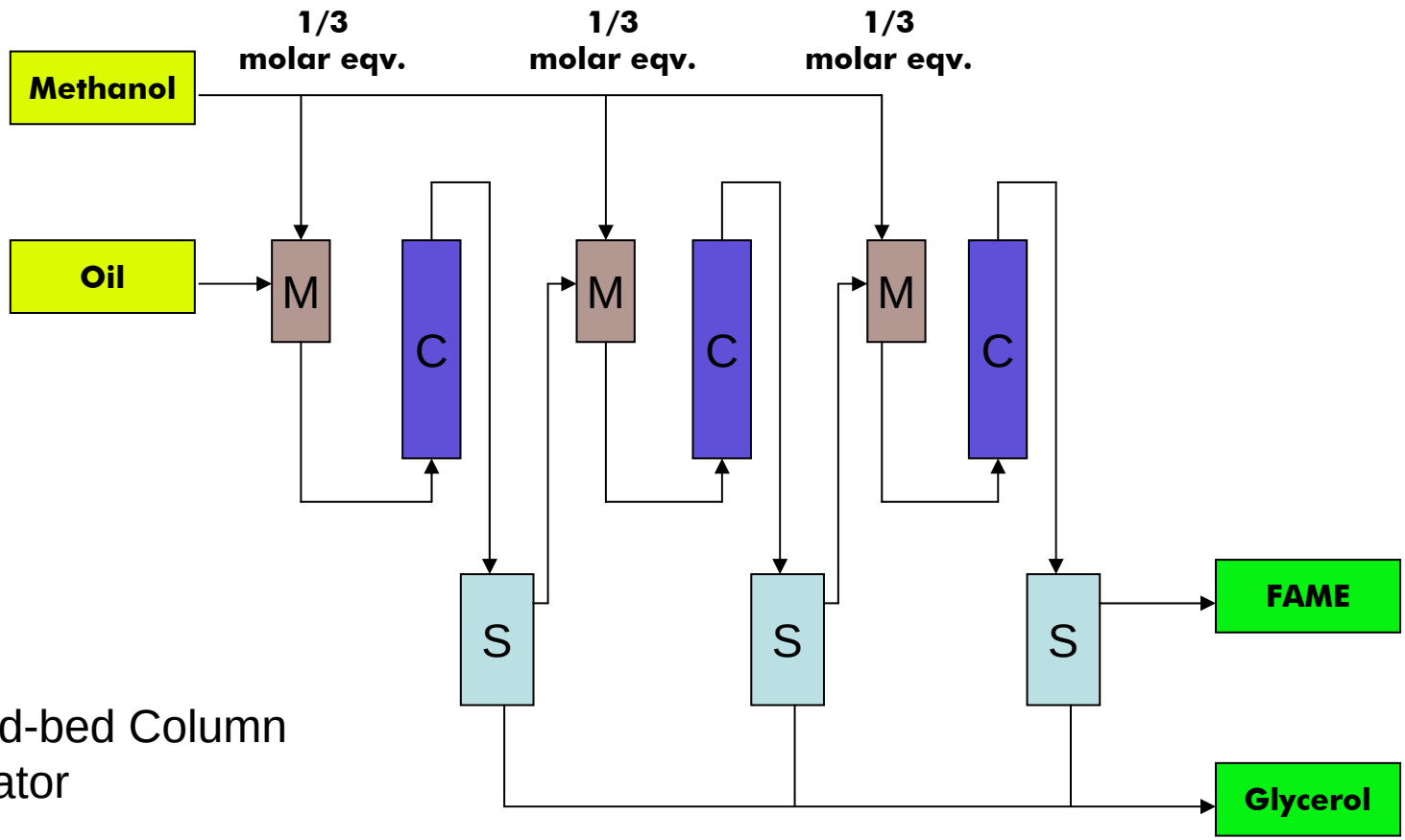
Lipase Catalyzed Biodiesel Process

Process Scheme



M Mixer
C Packed-bed Column
S Separator

Source: Shimada et. al., J. Mol. Catal. B Enzym. 17 (2002)

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Batch Evaluation Tests

Experimental Set-up

Materials and Methods:
refined rapeseed oil
methanol
enzymes: Novozym 435 (Novozymes); Lipozyme TL IM (Novozymes); CALB immo (c-Lecta)

Equipment:
eppendorf tube (2 ml)
water bath
vortexer
centrifuge

Process parameters:
oil amount: 0,6 g
enzyme amount: 10 %
reaction time: 72 h
3 steps for methanol addition (3 x 24 h)
temperature: varied
oil/methanol: varied

Analytics:
methyl ester yield by GC (DIN EN 14103)

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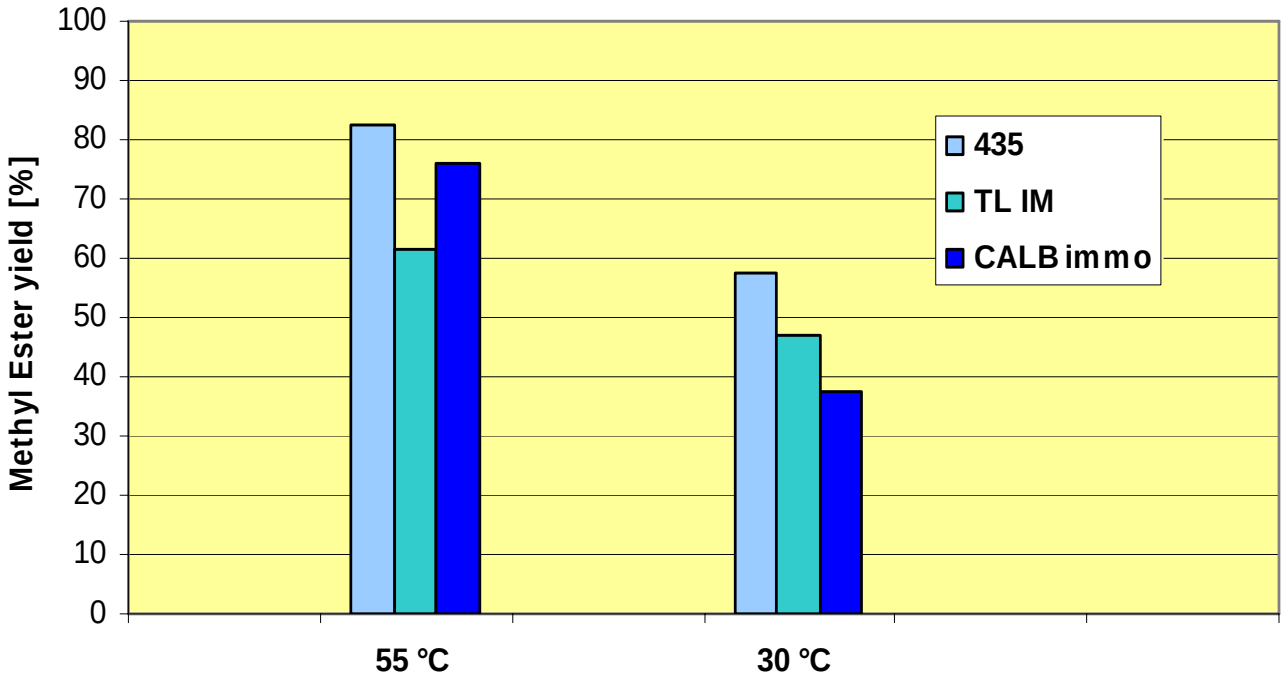
Batch Evaluation Tests

Comparison of Different Enzymes at Various Temperatures



Process parameters: enzyme amount: 10%; 3 methanol addition steps; reaction time: 72h; molar oil methanol ratio: 1:4

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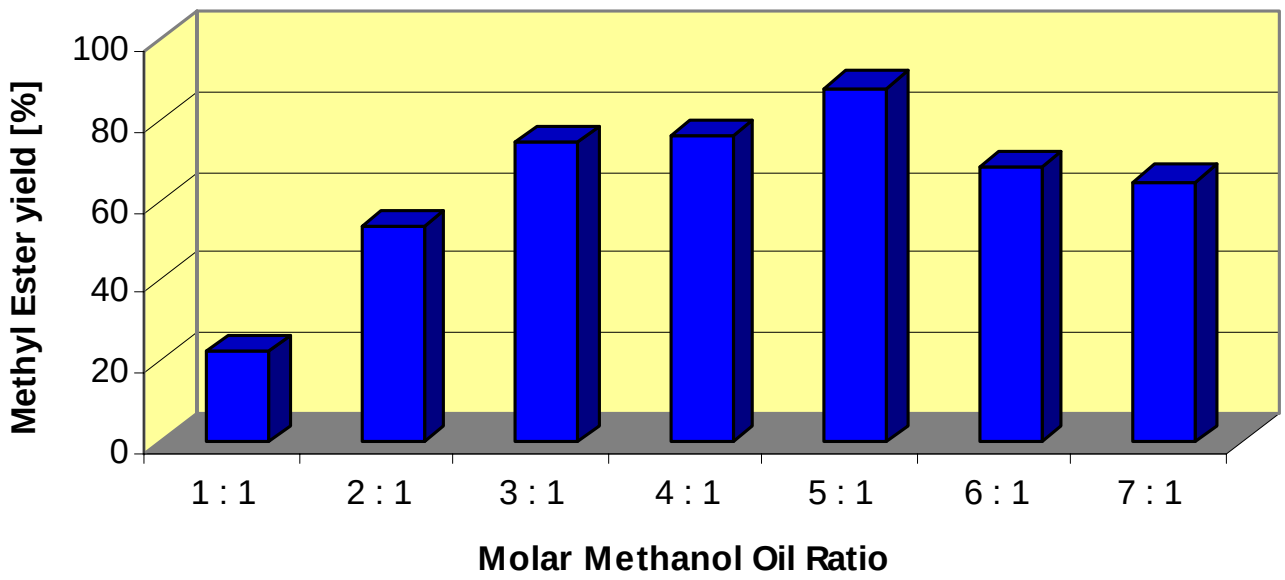
Batch Evaluation Tests

Optimization of Molar Methanol / Oil Ration (Used Enzyme: CALB immo)



Process parameters: enzyme amount: 10%; 3 methanol addition steps; reaction time: 72h; reaction temperature: 55°C

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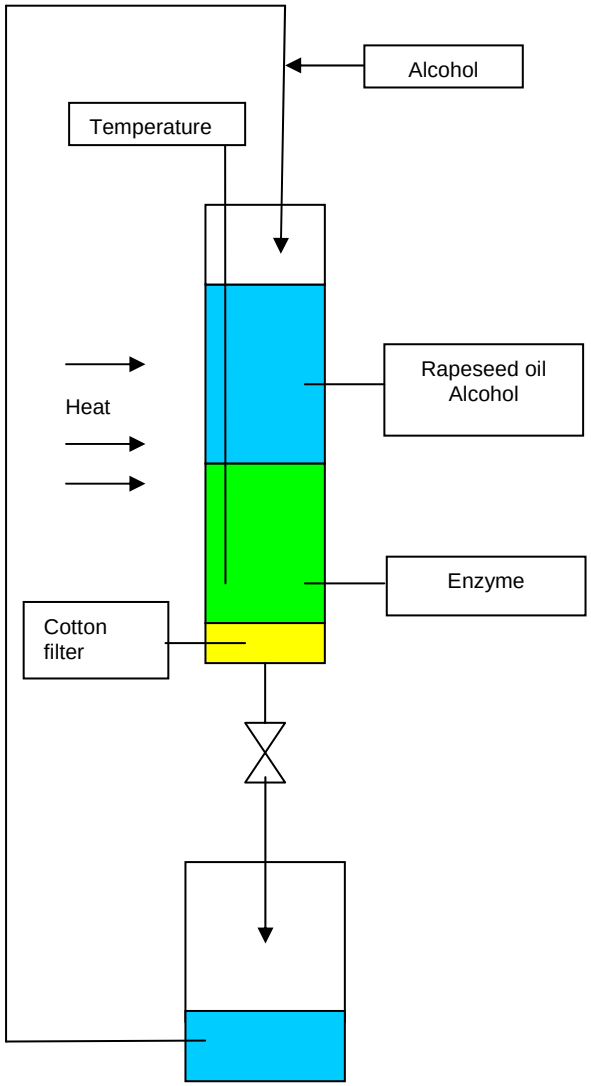
Continuous Tests

Experimental Set-up



Materials and Methods:
refined rapeseed oil
methanol, ethanol
CALB immo (c-Lecta)

Equipment:
packed-bed column

Process parameters:
oil amount: 100 g
temperature: varied (30°C / 55 °C)
oil / methanol (ethanol) ratio: 1:4
enzyme amount: 10 %
reaction time 8 h per step
1 to 5 steps for alcohol addition

Analytics:
methyl ester yield by GC (DIN EN 14103)

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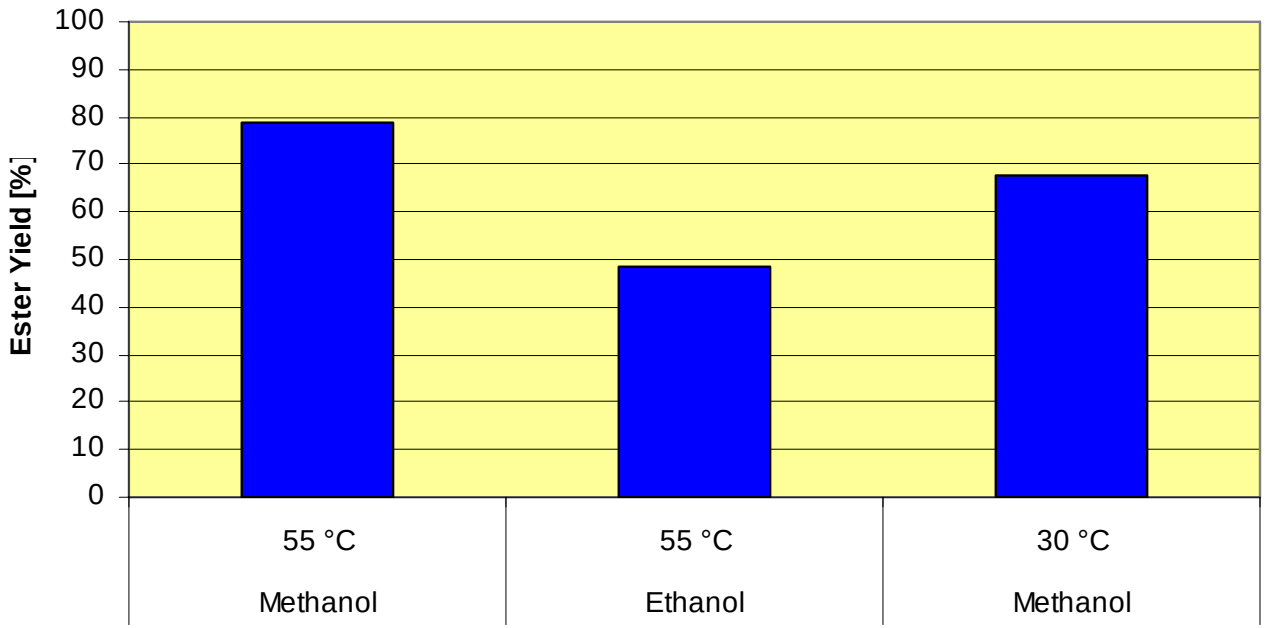
Continuous Tests

Ester Yield (Enzyme: CALB immo)



Process parameters: oil amount: 100g; enzyme amount: 10%; 5 methanol / ethanol addition steps; flow rate: 8h per 1 step; molar oil methanol ratio: 1:4

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First Conclusions

A simple experimental set-up is suitable to get information about enzyme properties and process behavior.

The methyl and ethyl ester yields got from packed-bed column experiments are lower than described in the literature, particularly the ethyl ester yields were unsatisfied.

CALB immo has similar transesterification properties like Novozym 435.

There are some further investigations planned:

- optimization of the transesterification behavior
- application of other oils and fats (particularly with higher acid values)
- enzyme stability tests
- scale up

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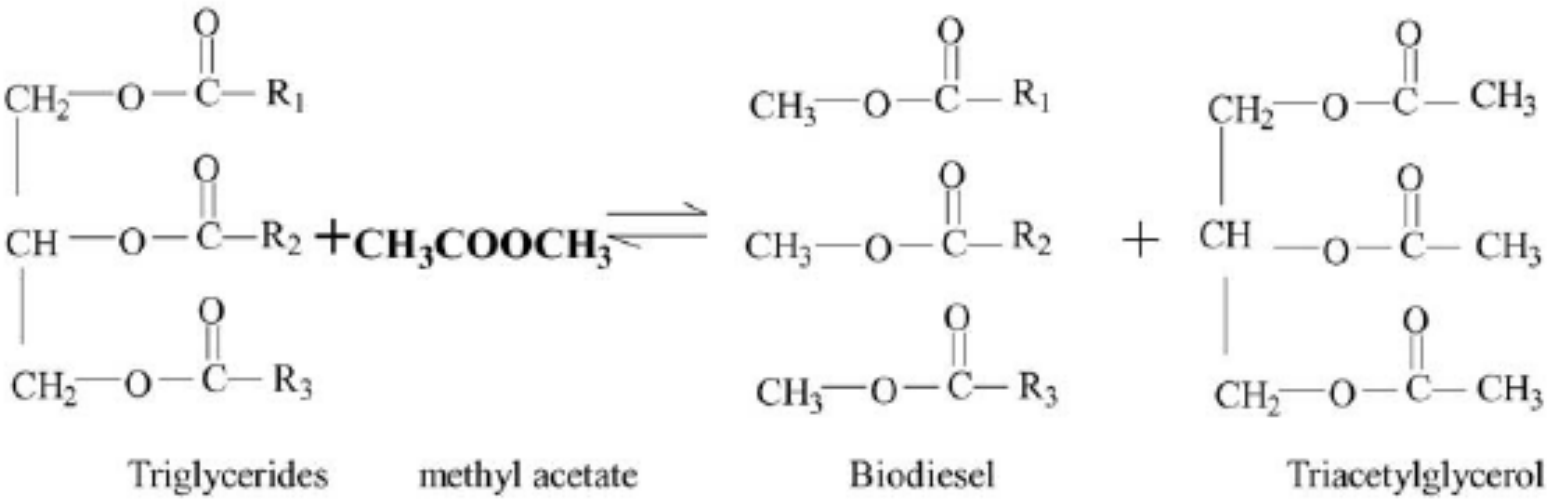
Alternative Process

Use of Methyl Acetate

Ester-ester interchange



Source: Xu et. al., Biotechnology Letters 25 (2003)

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Triacetin

Wikipedia:

The triglyceride **1,2,3-triacetoxypropane** is more generally known as **triacetin** and **glycerin triacetate**. It is the triester of glycerol and acetic acid.

It is an artificial chemical compound, commonly used as a food additive, for instance as a solvent in flavourings, and for its humectant function, with E number **E1518** and Australian approval code A1518. Triacetin is also a component of casting liquor with TG.

Triacetin can also be used as a fuel additive as an antiknock agent which can reduce engine knocking in gasoline, and to improve cold and viscosity properties of biodiesel.

In a 1994 report released by five top cigarette companies, triacetin was listed as one of the 599 cigarette additives. The triacetin is applied to the filter as a plasticizer. …

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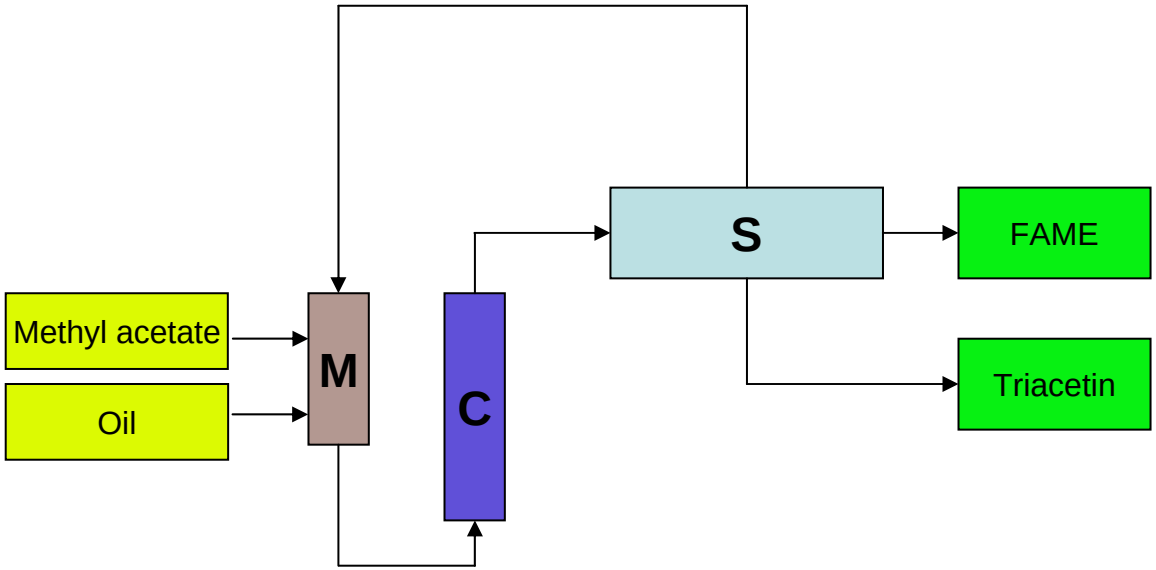
Process Scheme



M Mixer
C Packed-bed Column
S Separating Step

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Optimization of Transesterification

Experimental set-up

Materials and Methods:
refined rapeseed oil
methyl acatate
Novozym 435 (Novozymes)

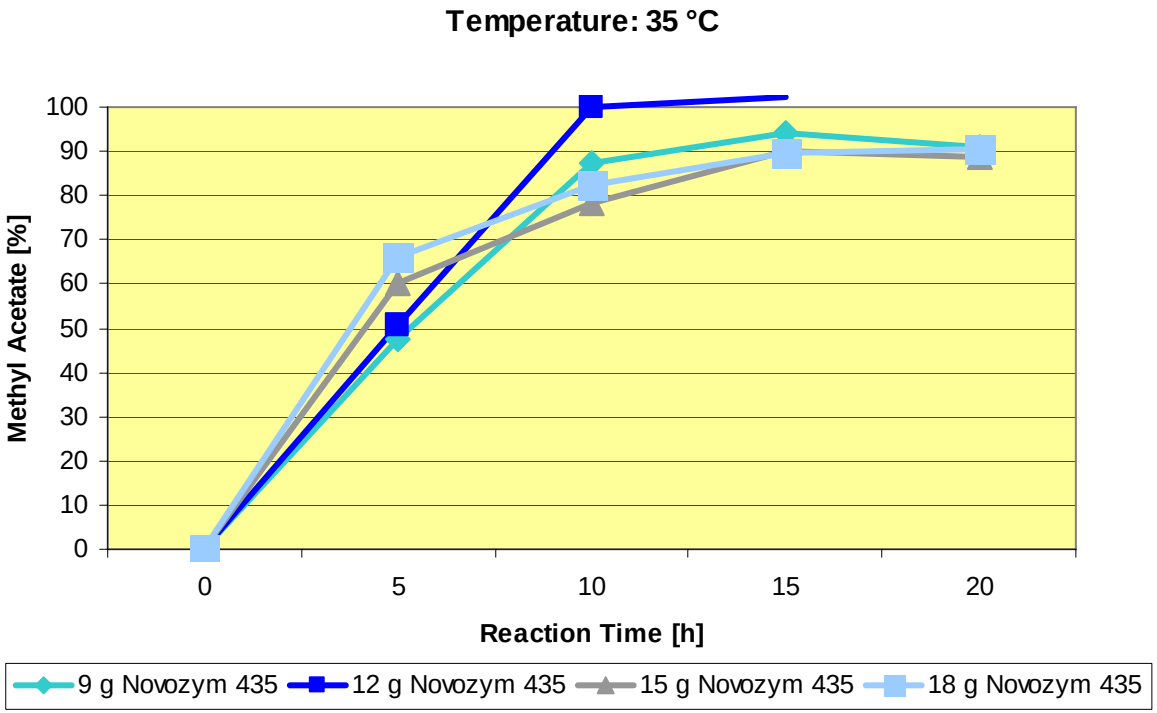
Equipment:
Packed-bed column

Process parameters:
oil amount: 36,5 g
methyl acetate amount: 36 g
molar oil / methyl acetate ratio: 1:12
temperature: 35°C / 45°C / 55°C
enzyme amount in the column: 3 g / 6 g / 9 g
reaction time: up to 4 runs; each 5 h

Analytics:
methyl ester yield by GC (DIN EN 14103)

Optimization of Transesterification

Methyl Ester Yield at 35 °C (2)



Process parameters: oil amount: 36,5 g; methyl acetate amount: 36 g; molar oil / methyl acetate ratio: 1:12; reaction time: 3 runs, each 5 h

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Membrane Filtration Tests

Membrane Stability

Tested membranes:
Ceramic – UF – membranes
Carbon – UF – membranes
Polymer – UF - membranes
Polymer – NF / RO - membranes

Tested in:
Methyl acetate
Rapeseed methyl ester (RME)
Mixtures from both

Determination method:
Visible changes of membrane over time

Results:
Polymer, PVC, Plexiglass membranes are unsuitable
Ceramic and carbon membranes are suitable

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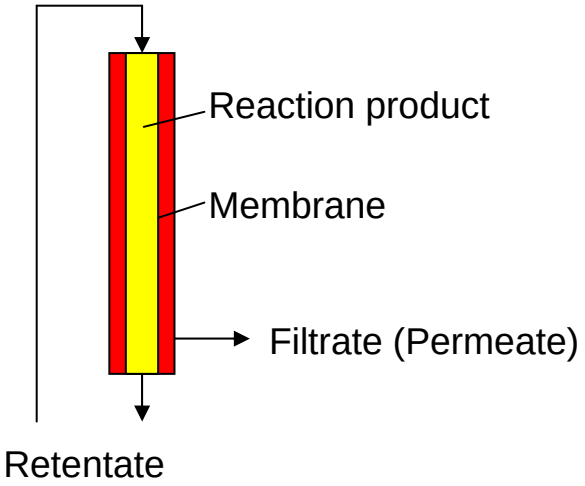
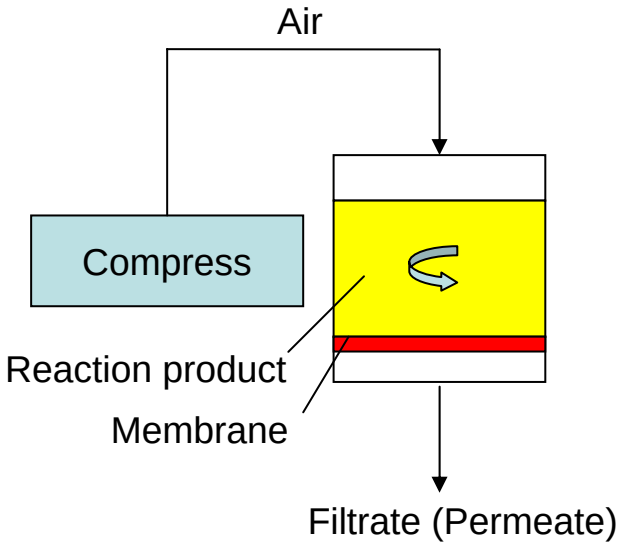
Membrane Filtration Tests

Experimental Set-up (1)





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Membrane Filtration Tests

Experimental Set-up (2)

Used membranes:
Ceramic membrane with cut-off 1 kDa; 3 kDa
Ceramic pipe membrane with cut-off 0,05 µm
Carbon membrane with cut-off 100 kDa

Test mixtures (Model systems):
RME – Methyl acetate
Triacetin – Methyl acetate
RME – Triacetin
RME – Triacetin – Methyl acetate

Analytics:
Determination of composition by Refractometer
(only qualitive)

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Membrane Filtration Tests

Results with Model Systems

Mixtures of RME and methyl acetate (NF, UF):
Permeate: RMA
Retentate: Methyl acetate

Mixtures of triacetin and methyl acetate (NF, UF):
Permeate: Triacetin
Retentate: Methyl acetate

Mixtures of RME, methyl acetate and triacetin (NF, UF):
Permeate: Mixture of RME and triacetin
Retentate: Methyl acetate

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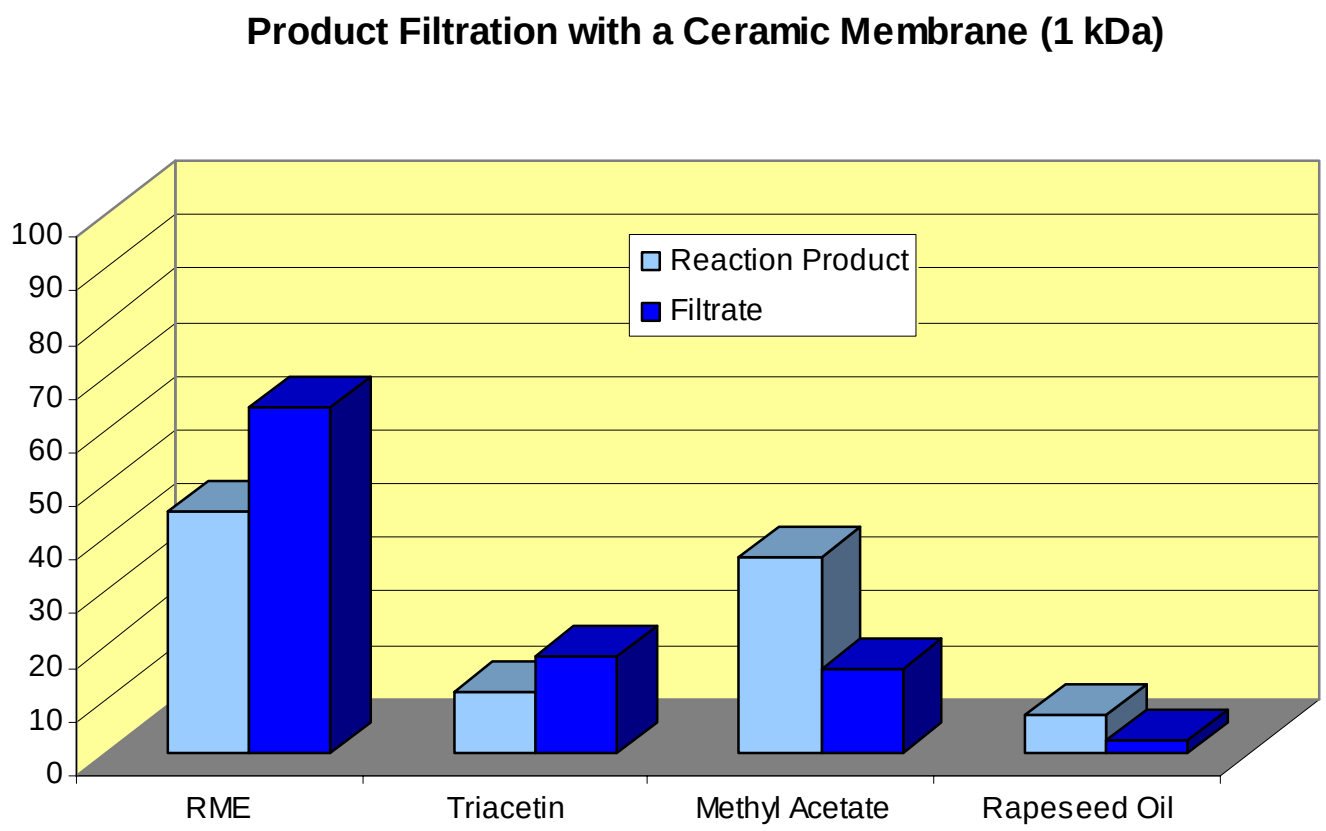
Membrane Filtration Tests

First Results with a Real Reaction Product



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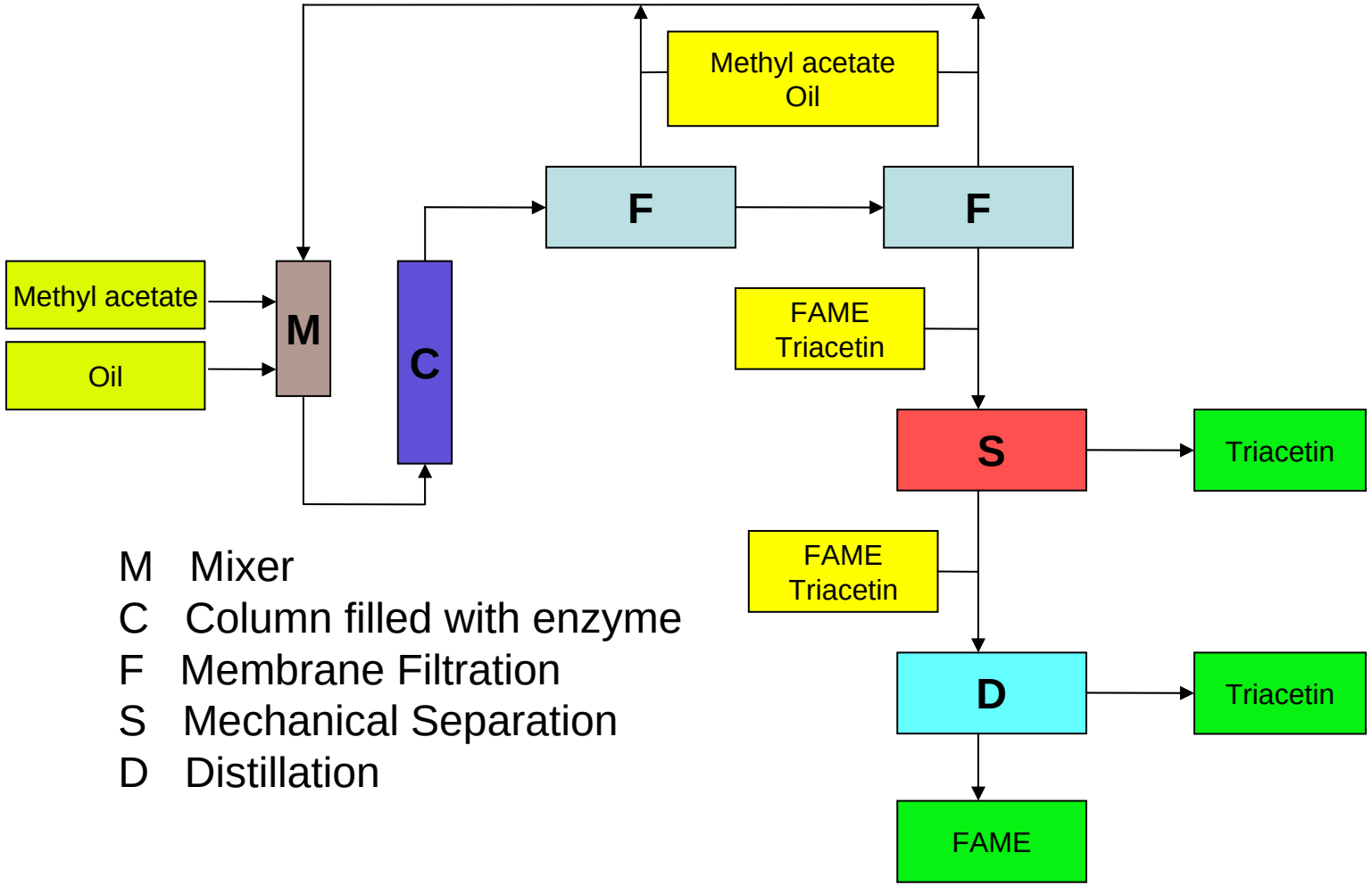
Advanced Process Scheme



M Mixer
C Column filled with enzyme
F Membrane Filtration
S Mechanical Separation
D Distillation

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Conclusions

The use of methyl acetate is a suitable alternative for FAME production.

The conversion reaction leads to high FAME yields.

The by-product triacetin can be used as biodiesel additive. Triacetin traces in the FAME could improve the fuel properties.

Besides enzyme stability, the design of the process steps to separate the reaction product is crucial regarding to the total process economy.

There are some further investigations planned:

- Optimization of the conversion process
- Enzyme stability
- Optimization of the separating processes
- Process design and economy

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Acknowledgements

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Thank You for Your Attention.

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