Investigations to the Use of Lipases for Biodiesel Production

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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
Biodiesel Production in EU

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

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

Flowchart:
- **Oil**
- **Catalyst**
- **Methanol**
- **Trans-esterification**
- **Separation**
  - **Methyl Ester Phase**
  - **Glycerol-Phase**
    - **Washing**
    - **Methanol Distillation**
    - **Crude-Glycerol**
    - **Waste Water**
    - **Separation**
    - **Drying**
    - **Distillation**
    - **FAME**
    - **Glycerol**
    - **Waste**
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

Lipase Catalyzed Biodiesel Process

Process Scheme

Methanol

1/3 molar eqv.

M  Mixer

1/3 molar eqv.

C  Packed-bed Column

1/3 molar eqv.

S  Separator

M  Mixer

Oil

FAME

Glycerol

**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)
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
Batch Evaluation Tests

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

![Bar chart showing methyl ester yield vs molar methanol/oil ratio]

- **Methyl Ester yield [%]**
- **Molar Methanol Oil Ratio**: 1 : 1 2 : 1 3 : 1 4 : 1 5 : 1 6 : 1 7 : 1

**Process parameters**: enzyme amount: 10%; 3 methanol addition steps; reaction time: 72h; reaction temperature: 55°C
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)
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
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
Ester-ester interchange

Triglycerides + methyl acetate → Biodiesel + Triacetylgllycerol

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. …
Process Scheme

M  Mixer
C  Packed-bed Column
S  Separating Step

- Methyl acetate
- Oil
- FAME
- Triacetin
Experimental set-up

Materials and Methods:
refined rapeseed oil
methyl acetate
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)

Temperature: 35 °C

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
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
Membrane Filtration Tests

Experimental Set-up (1)

Air

Compress

Reaction product
Membrane

Filtrate (Permeate)

Reaction product
Membrane

Filtrate (Permeate)

Retentate
**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 qualitative)
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
Membrane Filtration Tests

First Results with a Real Reaction Product

Product Filtration with a Ceramic Membrane (1 kDa)

- **RME**
  - Reaction Product: ~70%
  - Filtrate: ~10%
- **Triacetin**
  - Reaction Product: ~30%
  - Filtrate: ~10%
- **Methyl Acetate**
  - Reaction Product: ~30%
  - Filtrate: ~10%
- **Rapeseed Oil**
  - Reaction Product: ~10%
  - Filtrate: ~10%
Advanced Process Scheme

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

Methyl acetate
Oil

FAME
Triacetin

FAME
Triacetin

Triacetin

Triacetin

FAME
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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Thank You for Your Attention.

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