# Novozymes' Enzymatic Biodiesel Process

## *New product: Eversa® Transform 2.0*

**The freedom to choose a wide variety of feedstocks, reduce operational costs, and increase the safety and sustainability of biodiesel production**

Novozymes offers a simple enzymatic process based on a liquid enzyme to convert both glycerides and free fatty acids (FFA) into biodiesel. This enables the producer to optimize feedstock purchases without limitations imposed by the FFA content, resulting in lower raw material costs and higher yields. The glycerin by-product is relatively pure compared to the glycerin from chemical biodiesel.

Making the change from a chemical catalyst to the Novozymes enzymatic process allows producers to optimize their total process economy, increase return on investment, and create safer, more sustainable operations for both personnel and the environment.

### Benefits of the Novozymes' enzymatic biodiesel process

- **Feedstock flexibility**
  Novozymes' enzymatic process is equally effective in converting both FFA and glycerides, allowing full feedstock flexibility with respect to FFA.
- **Lower energy and methanol costs**
  Eversa® Transform 2.0 works at 40°C, requiring less energy for cooling.
  Lower methanol and rectification costs due to lower dosage and possibility of using wet methanol.
- **Lower capital costs than other technologies**
  Estimates from customers and engineering partners indicate the initial investment in equipment can be recovered in 1-3 years.
- **Greater return on investment, greater freedom from uncertainty**
  Lower costs for raw materials and operations create a better overall production economy, greater return on investment and more stable profits, making the business less dependent on government subsidies and policies. Improved process robustness and predictability, ensure product for the customer when they need it.

- **Greater safety and sustainability**
  Reduction of hazardous chemicals and by-products creates greater protection for both personnel and the environment.
  The enzyme process eliminates the need for sodium methoxide, one of the most hazardous chemicals in traditional biodiesel plants.
  The organic nature of enzymes and their mild process conditions do not generate toxic components as in some chemical biodiesel processes.
- **Trusted partner for the future**
  Novozymes' business depends on innovation and successful long-term industry partnerships. By choosing Novozymes as a business partner, you gain access to innovation and technical support now, and also years into the future.

## Customer support

Enzymatic biodiesel is an innovative technology based on the development of new enzymes as well as a novel application technology. The technology is supported by Novozymes Technical Service team and partners.

## Product

Eversa® Transform 2.0 is a liquid lipase product with high activity in the transesterification of glycerides and in esterification of free fatty acids. The product contains a carboxylic ester hydrolase (EC 3.1.1.3) produced by submerged fermentation.

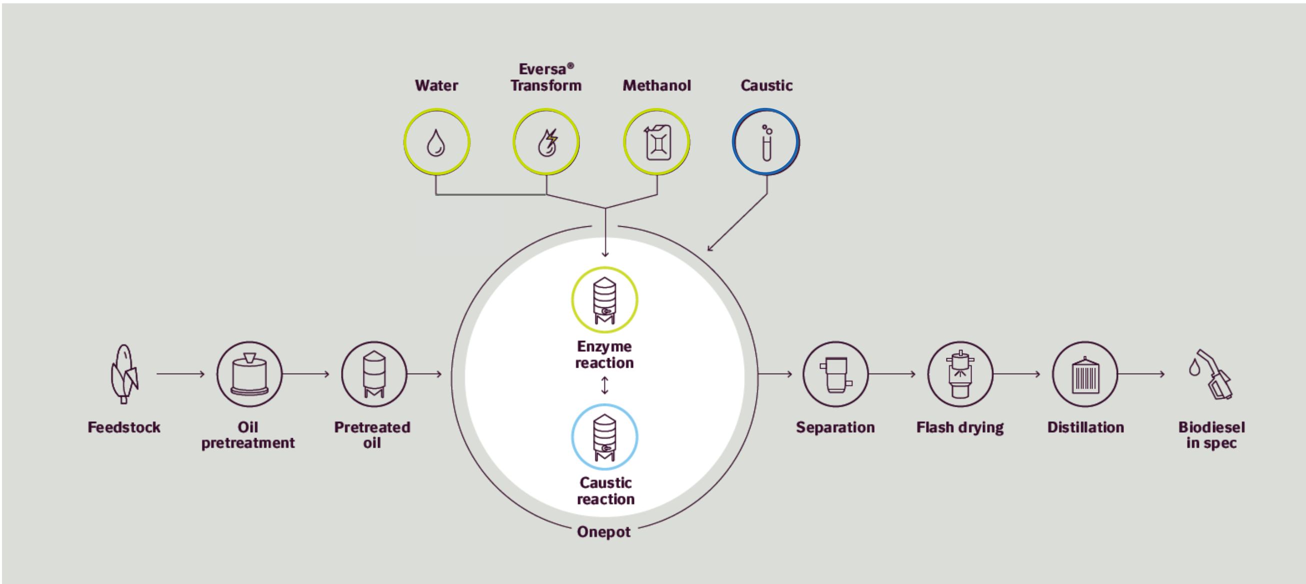
## Process

The full process is illustrated in figure 1 and the process steps Feedstock pre-treatment, Enzymatic reaction, Polishing and Side streams processing are described below.

The enzymatic production process uses a liquid lipase – Eversa® Transform 2.0 – that catalyzes the production of FAME from glycerides as well as FFA. The reaction is carried out in a mixed fed-batch tank operation or as a continuous stirred tank reactor setup (CSTR). By controlling the reaction conditions, bound glycerin is reduced below specification limit of 0.23%, and FFA is reduced to around 2% which is eliminated by a polishing step. A flow chart for the enzymatic process is shown in figure 1.

The enzymatic reaction combined with the polishing method can typically generate up to 96% biodiesel yield without any re-cycling of materials (yield calculated by weight of dry FAME phase relative to weight of feedstock). In cases where distillation is used, the overall yield without oil recycling is up to 93%.

# Novozymes Eversa® Transform 2.0




The basic layout of the enzymatic process.
**Recommended conditions for the enzyme reactor:** 0.3-0.4% Novozymes Eversa® Transform 2.0 enzyme, 1.4-2.0 equivalent MeOH, 40°C/104°F, 0.5-2% water and 30 to 36 hours reaction time.

Fig. 1. The basic layout of the enzymatic process. Recommended conditions for the enzyme reactor: 0.3-0.4% Novozymes Eversa® Transform 2.0 enzyme, 1.2-1.5 equivalent MeOH, 40°C/104°F, 1.5-2% water and 36 to 48 hours reaction time.

## Feedstock pre-treatment

The enzyme process can handle oils with any FFA. However, the enzyme is sensitive towards certain contaminants so the oil must be pretreated to remove any suspended matter and other harmful impurities. The enzymatic process requires careful checking of certain variables regarding feed oil quality in order to achieve an efficient and robust pre-treatment process. The generally recommended pre-treatment procedure for enzymatic biodiesel is exemplified here and described in detail in table 1 and following paragraphs below.

- Examples of well-documented feedstocks and pre-treatment method. NB! The method below should be regarded as a worse-case scenario. In some cases, the method can be simplified
    - Corn oil
    - Used cooking oil
    - Crude palm oil
- Settling at 60°C to separate free water and heavy particulate matter by decantation or centrifugation
- Filtration at 10 micron to remove residual solids
- Water wash with 5-10% water at 80-90°C to remove emulsifiers and water soluble contaminants
- Centrifugation to remove washing water and emulsifiers
- Flash drying to get water below 5000ppm
- Neutralization of mineral acids with aqueous NaOH

| Contaminant | Unit operation | Criteria and measures |
|---|---|---|
| Particles, free water | Settling, centrifugation, filtration | Separates free water and other heavy materials. Removes particles down to 10 micron. It is recommended to measurement turbidity (NTU-IR) in order to control for residual particles.<br>Typically, NTU < 10 is recommended. |
| Emulsifiers and soaps | Water washing | Washing with 5-10% water at 80°C will limit natural and synthetic emulsifiers to avoid enzyme inhibition and phase separation difficulties. Separate degumming might be necessary for oils very high in P content. Degumming procedure available from Novozymes upon request. |
| Moisture | Centrifugation, drying | The pre-treated feed oil should not contain more than 5000ppm moisture before mineral acid neutralization |
| Mineral acids | Neutralization with dilute aqueous NaOH | NaOH dosage is typically 50-100 ppm.) To secure efficient and uniform neutralization, the NaOH is added as a 4% solution and mixed into the oil using a dynamic mixer. It is highly recommended to perform mini batches in lab to determine the correct amount for each different oil. The mini-batch assay for mineral acid neutralization is available from Novozymes upon request. |

Table 1. Typical oil contaminants and how to handle them during pre-treatment

## Enzymatic reaction

The enzymatic reaction takes place after proper feedstock pre-treatment has been carried out (see section above).
The enzymatic process is a combined transesterification and esterification reaction as both glycerides and FFA are converted into methylesters.

**Typical process conditions:**

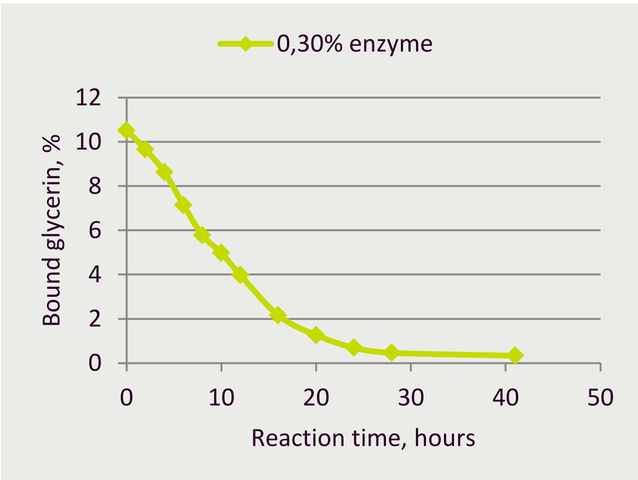
- Mixed fed-batch tank operation but can also be operated as CSTR
- Temperature 40ºC ± 2ºC (104ºF ± 4ºF)
- Water: 1.5-2.0%
- Enzyme: 0.3-0.4%
- Methanol: 1.2-1.5 eqv.

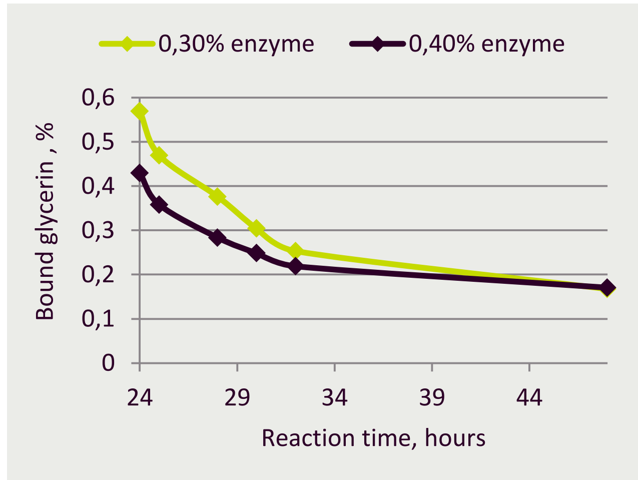
The methanol is added gradually during the first 20-24 hours of reaction. Ideally continuous addition should be used, but if this is not possible, divide the methanol into portions and add hourly. After 4-6 hours of reaction, it is important to start controlling the methanol concentration in the heavy phase. For feedstocks with FFA < 30%, methanol should be kept at 13-18% concentration in heavy phase during the enzymatic reaction of in total 36-48 hours. For feedstocks with FFA > 30%, the methanol concentration in the heavy phase is kept at 13-18% during the first 24 hours and then gradually methanol concentration is increased up to max 40% at the end for reaction. Analysis is recommended to be done by FTMIR (Eurofins QTA).

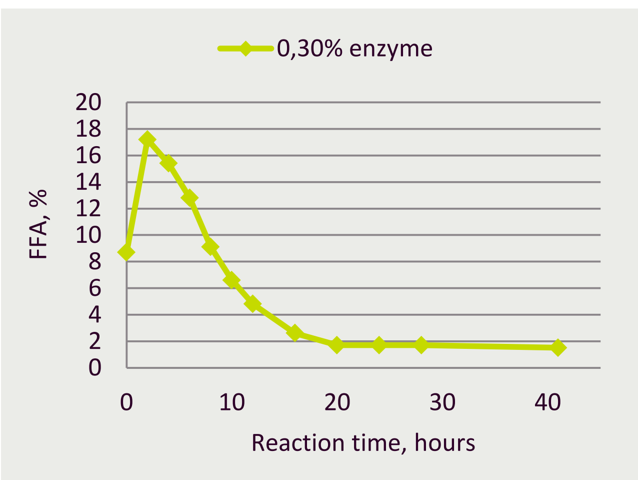
## Controlling the enzymatic reaction

During enzymatic reaction, bound glycerol and FFA are converted into methylesters. Typical reaction data are shown below.

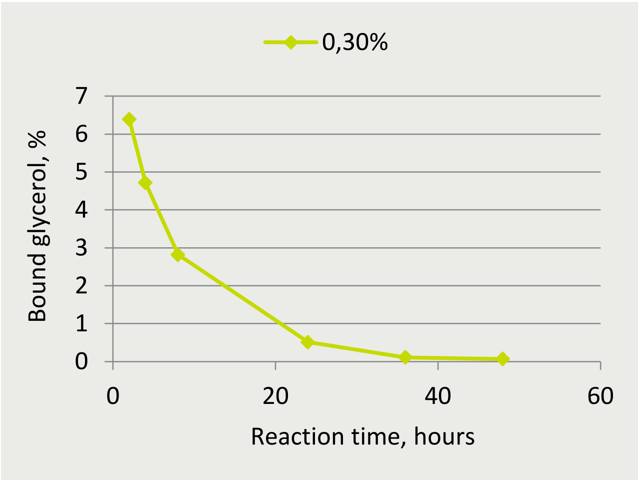
**Conversion of used cooking oil  (UCO) – see reaction in table 2 below**

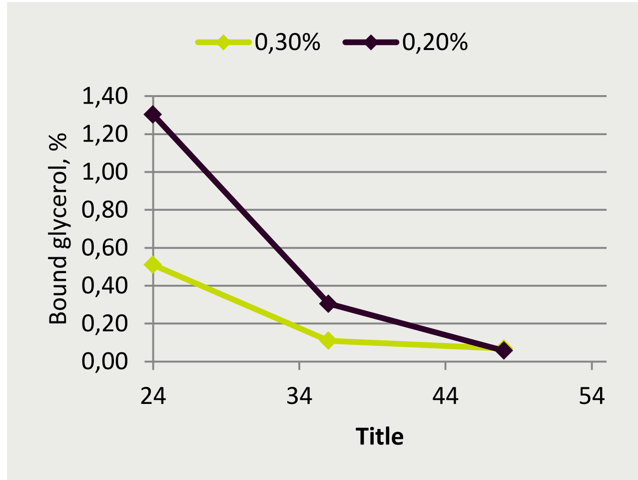


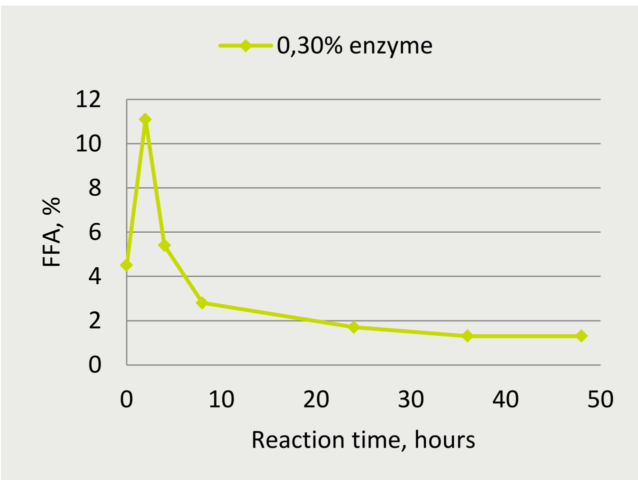



**Conversion of Crude palm oil (CPO) – see reaction conditions in table 2 below**




| | UCO | CPO |
|---|---|---|
| Water, % | 2.0 | 2.0 |
| Enzyme, % | 0.30 – 0.40 | 0.20 – 0.30 |
| Methanol, eqv. | 1.5 | 1.5 |
| Temperature | 104°F / 40°C | 104°F / 40°C |

Table 2. Reaction conditions used in examples above

## Polishing for FFA elimination

Before polishing, FFA should be around 3% or below. The standard recommended polishing method is called "the one pot method" which is a very simple and robust method. The method can be applied as a batch process, e.g. by using the enzymatic reactor, or alternatively, as a continuous process. The method is based on neutralization of FFA by mixing dilute caustic directly into the reaction mixture at 60°C/140°F. After the FFA neutralization step, the FAME phase can be easily separated from the soap / heavy phase at high FAME yields. The crude biodiesel is then water washed, dried and eventually distilled to reach B100 specifications.

Polishing steps to meet EN B100 specification
(acid number, MAG, DAG, TAG, bound glycerol, methyl ester >96.5)

| Step | Conditions | Comments |
|---|---|---|
| Heating | 60°C/131°F | Enzyme can only be used once |
| Neutralization | 1.05-1.15 eqv NaOH to FFA<br>1 hour reaction time | NaOH is added as 4-8% dilution in methanol or water. |
| Separation | By gravity, 3-4 hours | Soap goes in heavy phase. 3-400 pm is left in FAME phase after centrifugation |
| Washing | 2% water | Residual soap in FAME is washed out |
| Drying | Standard | |
| Distillation | Standard | Distillation step is important to reach < 0.2% DAG |

Table 3.

Although the one pot FFA polishing method is strongly recommended, alternative FFA polishing methods exist, e.g. enzymatic esterification, resin esterification and adsorption. However, generally these methods require investments in new unconventional technology.

| | Before polishing | | | | | After polishing | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Batch number | FFA | MG | DG | TG | BG | FFA | MG | DG | TG | BG |
| Batch # 1 | 1.4 | 0.51 | 0.22 | 0.02 | 0.17 | 0.10 | 0.54 | 0.24 | 0.03 | 0.18 |
| Batch # 2 | 1.5 | 0.58 | 0.37 | 0.13 | 0.22 | 0.10 | 0.62 | 0.41 | 0.11 | 0.23 |

Table 4. Data from one pot polishing trials with UCO

## Processing of side streams to FFA fraction, glycerol, methanol and water

The heavy phase side-stream contains a number of materials which can be separated in order to:

- Generate higher biodiesel yield by re-cycling the FFA fraction
- Get more value from the glycerol by-product
- Reuse of (wet) methanol and water
- Avoid / reduce waste

Methanol and water is removed from the heavy phase by drying. The dry heavy phase is then acidulated followed by separation of FFA/FAME fraction from the crude glycerin. It is recommended that the FFA/FAME fraction is re-cycled and processed separately in order to avoid build-up of impurities which could inactivate the enzyme in enzymatic reaction. The methanol/water phase is separated to wet (95%) methanol and water and then reused.

| Step | Conditions | Comments |
|---|---|---|
| Drying/distillation | Standard method to remove methanol and water from glycerine | |
| Acidulation | Standard method for soap-stock splitting | Approx. 3-6% salts in recovered glycerine. Salt can be removed from glycerine by precipitation to get <1% salt in glycerine |
| Methanol/water separation | Standard method without final distillation stages | Wet (95%) methanol and water is reused |

Table 5. Unit operations in processing side streams

## Safety, handling and storage

The product is produced by fermentation using a nonpathogenic, non-toxigenic microorganism known to be safe. The product is Generally Recognized as Safe (GRAS) for the intended use as a processing aid in the production of biodiesel and glycerol co-product. The product complies with the safety requirements of the US Federal Food, Drug and Cosmetic Act (FFDCA).

Safety, handling and storage guidelines are provided with all products.

# Economic advantages of Novozymes' enzymatic biodiesel process

The new lipase process is often economically superior to chemical process for high FFA feedstocks due to:

- **Feedstock flexibility**
- **Lower energy and methanol costs**
- **Higher yields or higher capacity**
- **Higher quality glycerin**

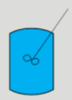
## Economical comparison – example 1

**Plant**

- Stand alone, 25.000 tons/year
- No methanol rectification
- Recirculation of FFA stream

**Enzymatic Process**

Corn oil and Acid oil



**Chemical Process**

Soy oil

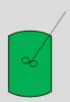
| Cost item | Cost difference ($/ton) |
|---|---|
| Feedstock | +150 |
| Catalyst | -10 |
| Methanol | 0 |
| Yield | 0 |
| Energy, steam, chemicals | +5 |
| Glycerol quality (credit) | 0 |
| | **Total +145 $/ton** |

Favourable for enzyme process: +
Favourable for chemical process: -

## Economical comparison – example 2

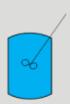
**Plant:**

- Bolt-on to Ethanol plant, 7.500 MT/year
- No methanol rectification
- FFA stream to distillers grade product



**Enzymatic**

Corn oil



**Chemical**

Corn oil

| Cost item | Cost difference ($/ton) |
|---|---|
| Feedstock | 0 |
| Catalyst | -10 |
| Methanol | +50 |
| Yield | +60 |
| Energy, steam, chemicals | +5 |
| Glycerol quality (credit) | 0 |
| | **Total +105 $/ton** |

Favourable for enzyme process: +
Favourable for chemical process: -

**About Novozymes**

Novozymes is the world leader in biological solutions. Together with customers, partners and the global community, we improve industrial performance while preserving the planet's resources and helping build better lives. As the world's largest provider of enzyme and microbial technologies, our bioinnovation enables higher agricultural yields, low-temperature washing, energy-efficient production, renewable fuel and many other benefits that we rely on today and in the future. We call it Rethink Tomorrow.

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