

# Novozymes Eversa<sup>®</sup> Transform 2.0

The freedom to choose a wide variety of feedstocks, to reduce operational costs, and to 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<sup>®</sup> Transform 2.0 works at 40°C, requiring less energy for cooling.

Lower methanol and rectification costs, due to lower dosage and the 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 for 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 the esterification of free fatty acids. The product contains a protein-engineered carboxylic ester hydrolase (EC 3.1.1.3) produced by the submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism.

## Process

The full process is illustrated in figure 1, and the process steps Feedstock pretreatment, 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 the 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%.

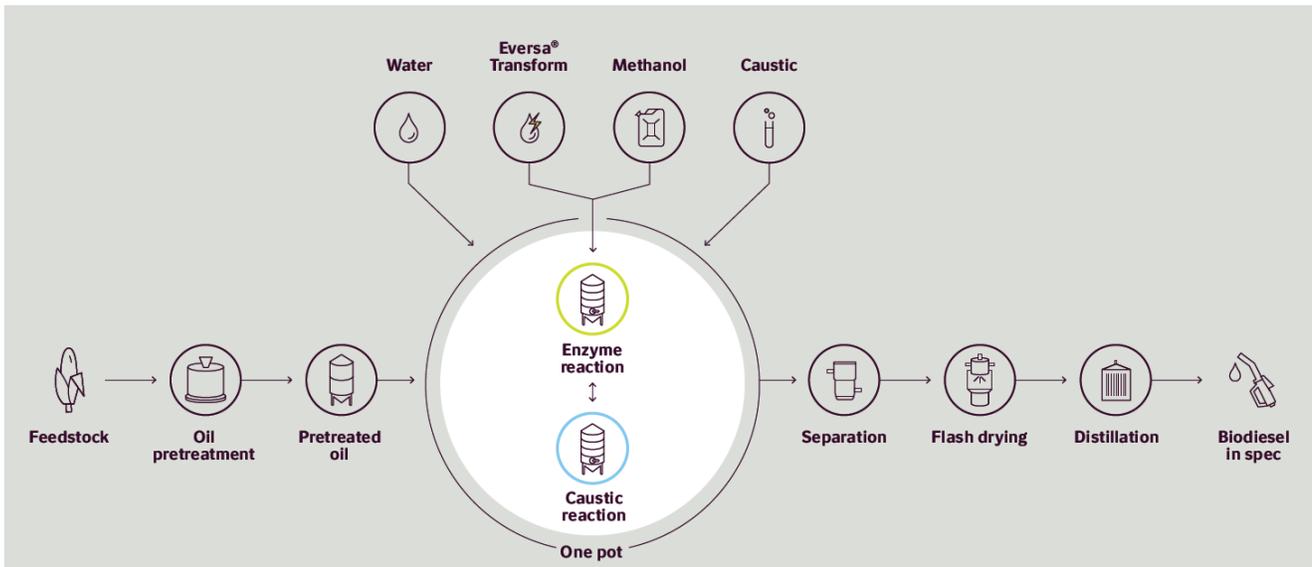


Fig. 1. The basic layout of the enzymatic process. Recommended conditions for the enzyme reactor: 0.3–0.4% Novozymes Eversa® Transform 2.0 enzyme, 1.2–1.5 equivalent MeOH, 40°C/104°F, 1.5–2% water and 36 to 48 hours reaction time

## Feedstock pretreatment

The enzyme process can handle oils with any FFA. However, the enzyme is sensitive toward 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 pretreatment process. The generally recommended pretreatment procedure for enzymatic biodiesel is exemplified here and described in detail in table 1 and the following paragraphs.

- Examples of well-documented feedstocks and pretreatment method. Please note: The method below should be regarded as a worst-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 microns 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 5,000 ppm
- 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 microns. It is recommended to measure turbidity (NTU-IR) in order to control for residual particles. 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 pretreated feed oil should not contain more than 5,000 ppm 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 a 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 pretreatment

## Enzymatic reaction

The enzymatic reaction takes place after proper feedstock pretreatment 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 methyl esters.

### Typical process conditions:

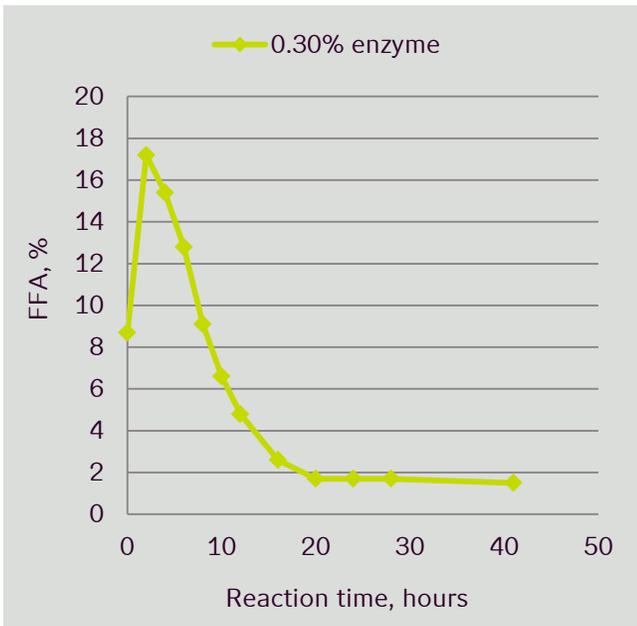
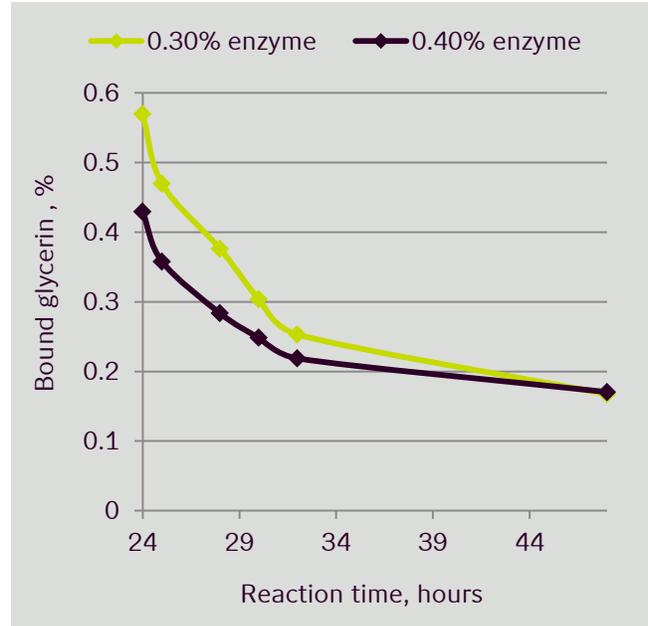
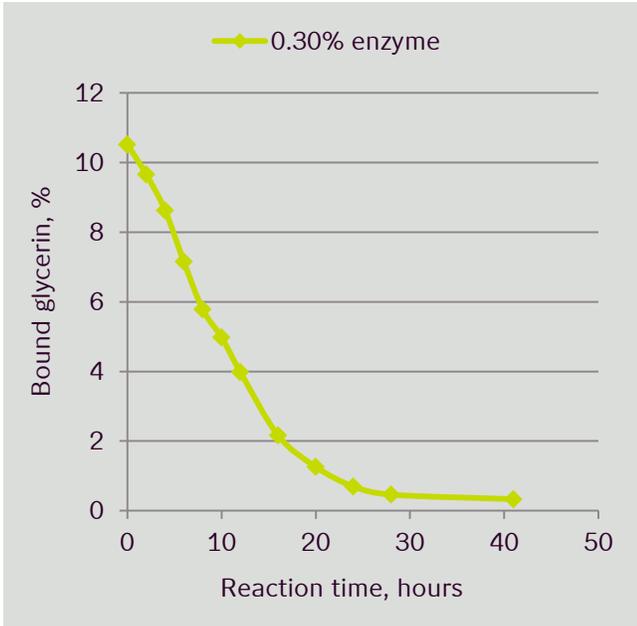
- Mixed fed-batch tank operation but can also be operated as CSTR
- Temperature: 40°C ± 2°C (104°F ± 4°F)
- Water: 1.5–2.0%
- Enzyme: 0.3–0.4%
- Methanol: 1.2–1.5 equiv.

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 the 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 a maximum of 40% at the end for reaction. Analysis is recommended to be done by FTMIR (Eurofins QTA).

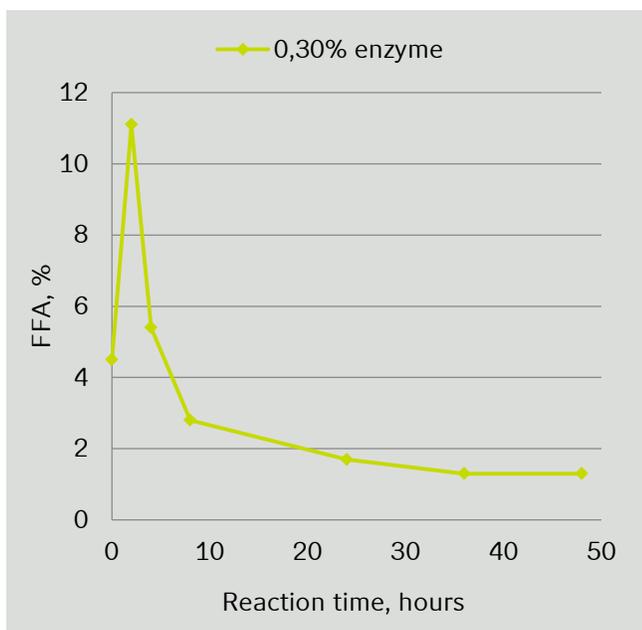
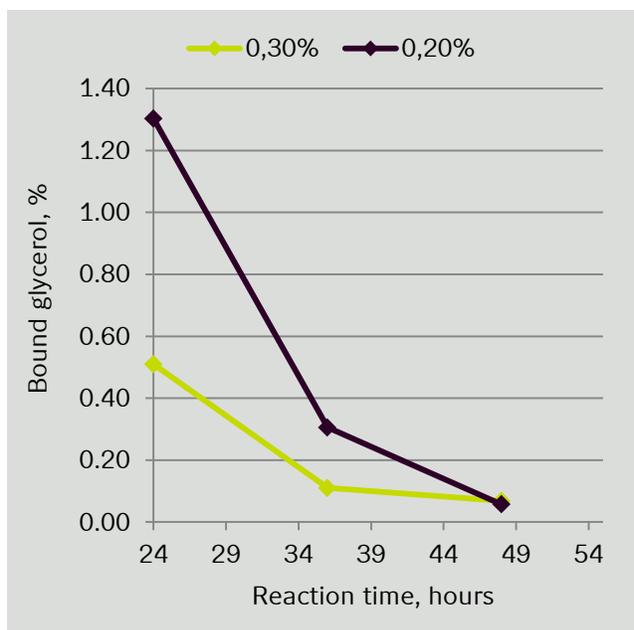
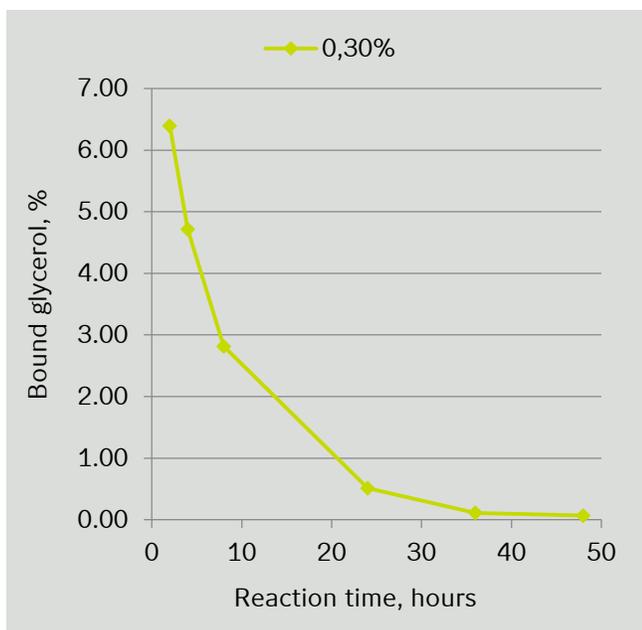
# Controlling the enzymatic reaction

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

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



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



	<b>UCO</b>	<b>CPO</b>
Water, %	2.0	2.0
Enzyme, %	0.30–0.40	0.30–0.40
Methanol, equiv.	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 the 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 equiv. NaOH to FFA, 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, these methods generally require investments in new unconventional technology.

Batch number	Before polishing					After polishing				
	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 that can be separated in order to:

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

Methanol and water are removed from the heavy phase by drying. The dry heavy phase is then acidulated followed by the separation of FFA/FAME fraction from the crude glycerin. It is recommended that the FFA/FAME fraction is recycled and processed separately in order to avoid buildup of impurities, which could inactivate the enzyme in the 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 glycerin	
Acidulation	Standard method for soap-stock splitting	Approx. 3–6% salts in recovered glycerin. Salt can be removed from glycerin by precipitation to get <1% salt in glycerin
Methanol/water separation	Standard method without final distillation stages	Wet (95%) methanol and water are reused

Table 5. Unit operations in processing side streams

## Safety, handling and storage

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

### 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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