

# ENZYMATIC HYDROLYSIS OF SUNFLOWER OIL IN SC CO<sub>2</sub>

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Enzyme was combined with SCF to use the benefits from both: enzyme (higher reaction rates, better product purity, safer process, etc.) and SCF (higher reaction rates, better transport properties, etc.). Hydrolysis of sunflower oil in SC CO<sub>2</sub>, was catalyzed by the native lipase from *Aspergillus niger*. The reaction was performed in a high-pressure batch-stirred-tank reactor. Thermal stability of the lipase was studied in SC CO<sub>2</sub> at 300 bar. The temperature optimum was determined to be at 50°C. With further temperature increase a rapid lipase deactivation was observed.

Because hydrolysis could be performed only in the presence of higher water concentrations, and enzymatic activity is sensitive to the pH of the bulk solution, pH dependence of the hydrolysis in SC CO<sub>2</sub> was studied.

From Arrhenius diagram activation energy  $E_a$  (32.678 kJ/mol) was determined. Other thermodynamic properties like free energy change, Gibb's energy, enthalpy and entropy of formation were calculated, as well.

The hydrolysis of sunflower oil was also performed in high-pressure flat-shape membrane reactor. The biocatalyst was retained in the reactor by polymeric membranes. In the high-pressure membrane reactor one can avoid the negative effect of depressurization steps on enzyme activity, which are necessary in the high-pressure batch reactor if the biocatalyst should be reused. Therefore in the membrane reactor biocatalyst might be used for a long-term operation.

When nylon membranes were used in the reactor for long-term hydrolysis performance at 100 bar and 50°C, the conversion was kept constant for at least 15 h.

## INTRODUCTION

The use of enzymes in compressed gases and supercritical or near critical fluids has been proposed as a mean of improving the activity of enzyme in anhydrous environments [1].

Hydrolysis as a reaction for the production of fatty acids and glycerol as a major source of surfactants and detergents from fats and oils is an important industrial reaction. Fat splitting, with lipase as a catalyst, is advantageous compared to a conventional process due to low energy consumption, high product quality and safer process.

Commercial non-immobilized lipase from *Aspergillus niger* was used as biocatalyst for hydrolysis of sunflower oil in SC CO<sub>2</sub>. Combining enzymes with supercritical fluids makes the possibility to use the benefits from both: enzymes (higher reaction rates, better product purity,

safer process etc.) and supercritical fluids (higher reaction rates, better transport properties etc.).

For the use of enzymes as biocatalysts in dense gases they must possess a good stability and activity in these media. Therefore thermal stability of lipase from *Aspergillus niger* in SC CO<sub>2</sub> was determined.

Hydrolysis was performed in a high-pressure batch stirred tank reactor, with the aim to study the influence of reaction parameters on the yield and initial reaction rates.

Using enzymes in high-pressure batch system may cause changes in biocatalysts activity due to compression/expansion influence. Continuous reactors for SCFs have the advantage over batch reactors that they do not require depressurization to feed in the reactions or to recover the products. Therefore, a high-pressure continuous stirred tank membrane reactor, where nylon membrane was used as a separation unit, was designed. The hydrolysis of sunflower oil was also performed in membrane reactor, to compare between reactions in a batch and continuous system at supercritical conditions.

## **I - MATERIALS AND METHODS**

### **Chemicals**

Non-immobilized lipase from *Aspergillus niger* – Lipolase 100T – was kindly donated from NOVO Nordisk, Denmark. Carbon dioxide 4.5 was supplied by Messer MG Ruše, Slovenia. Sunflower oil was from Oljarica, Oil factory, Kranj, Slovenia (concentration of linoleic acid was 64.6% and amount of oleic acid was 21.1%). Phosphate buffers and other chemicals were from Merck, Germany.

### **Determination of lipase thermal stability**

Lipase preparation was exposed at different temperatures to SC CO<sub>2</sub> at 300 bar. Then after slow depressurization the lipase residual activity was measured.

### **Hydrolysis in a high-pressure batch stirred-tank reactor**

Hydrolysis was performed in a 78 mL autoclave with a magnetic stirrer (600rpm). The reaction mixture contained vegetable oil and an aqueous solution of phosphate buffer. To the emulsion enzyme preparation was added. Finally, gas (CO<sub>2</sub>) was pumped into the reactor up to the desired pressure. During the reaction, samples were taken from the reactor in a trap by using a sampling valve. The apparatus for high pressure hydrolysis was already presented in the literature [2]. The concentration of free fatty acids was determined by the volumetric method [3] and the concentration of free oleic was determined by the HPLC method [4].

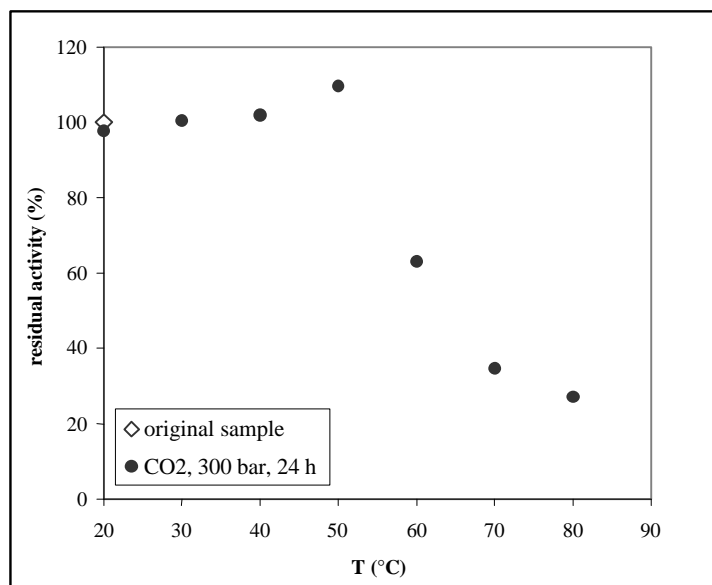
## **II-RESULTS AND DISCUSSION**

### **Lipase thermal stability**

Because temperature plays a great role in enzyme activation/deactivation, thermal stability of the native lipase from *Aspergillus niger* (Lipolase 100T) in SC CO<sub>2</sub> was studied. The biocatalyst was incubated in SC CO<sub>2</sub> at 300 bar and the temperature range between 20°C and 80°C. After 24 hours the pressure was reduced very slowly and residual activity of the lipase was determined. Between 20°C and 50°C a slightly increase in lipase activity was observed,

while with further temperature increase a rapid biocatalyst's deactivation appeared (**Figure 1**). Optimum temperature was determined to be 50°C.

Thermal activation/deactivation on the one hand and water distribution on the other hand, are the reasons for these changes in activity of the biocatalyst. Water was extracted from the enzyme microenvironment by the SC CO<sub>2</sub>. The higher was the temperature, the less water remained in the enzyme preparation. However, there could also exist another reason for the decreased activity at higher temperatures. A pH-induced inactivation, due to the increased solubility of CO<sub>2</sub> in the enzyme's micro-aqueous phase with temperature, could take place.



**Figure 1:** Thermal stability of Lipolase 100T after 24 h incubation in SC CO<sub>2</sub> at 300 bar.

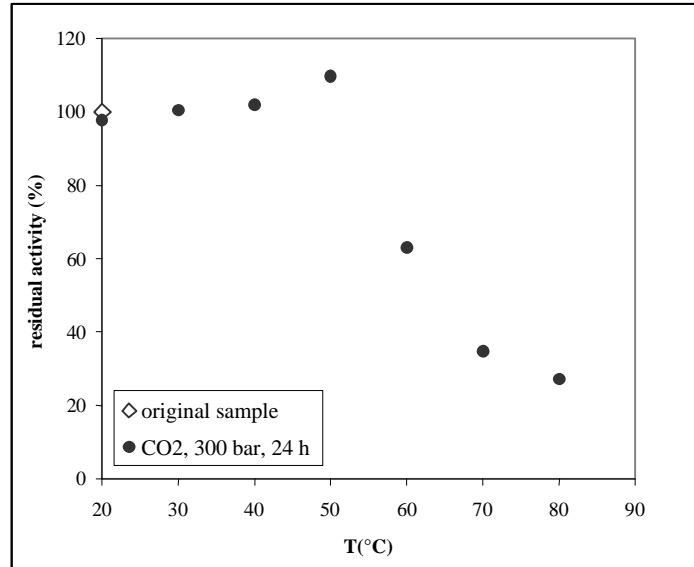
### Hydrolysis of sunflower oil in the high-pressure batch stirred reactor

Lipase-catalyzed hydrolysis of sunflower oil was performed in the high-pressure batch stirred tank reactor (HP BSTR) with the aim to study the influence of reaction parameters on the yield and initial reaction rates.

#### *Influence of temperature on the conversion*

Influence of temperature on the conversion was studied because, as mentioned above, temperature is one of the most important reaction parameters. With higher temperature reaction rate increases and enzyme activation/deactivation occurs. In supercritical fluids optimal temperature depends also on operational pressure.

Hydrolysis of sunflower oil was performed in SC CO<sub>2</sub> at different temperatures and 200 bar in the HP BSTR. Between 35°C and 50°C free fatty acid concentration in the product increased, while with further temperature rise it decreased. The results are shown in **Figure 2**.



**Figure 2:** Temperature influence on hydrolysis of sunflower oil in SC CO<sub>2</sub> at 200 bar. The reaction was catalyzed by Lipolase 100T and stirred with rotational speed of 600 rpm. Concentration of free fatty acids after 48 h is presented.

#### *Effect of pH on the conversion*

Lipase-catalyzed hydrolysis of sunflower oil in SC CO<sub>2</sub> was performed in the presence of phosphate buffer solutions of three different pH values: pH 6, pH 7 and pH 8. At 50°C and 200 bar optimum pH was found to be 7. At pH 7 the concentration of linoleic acid produced after 48 h was 0.586 g/g oil phase. At lower and higher pH values reaction rates were lower and therefore also lower amount of free fatty acids was produced. At pH values far removed from optimal pH the forces stabilizing the native protein conformation may be so disturbed that denaturation occurs [5]. At this point a significant decrease of reaction rates is expected.

#### *Thermodynamic properties*

From the slope of the straight lines of Arrhenius diagram (not shown) calculated activation energy ( $E_a$ ) was 32.678kJ/mol. Considering the transition-state theory, the Gibb's energy ( $\Delta G_f$ ), enthalpy ( $\Delta H_f$ ) and entropy ( $\Delta S_f$ ) of formation were calculated from equations

$$\Delta H_f = E_a - RT \quad (1)$$

where  $E_a$  is the activation energy and  $R$  is the gas constant.

$$\Delta S_f = R \cdot \ln\left(\frac{A \cdot N_A \cdot h}{RT}\right) - R \quad (2)$$

where  $N_A$  is Avogadro constant,  $A$  is frequency factor and  $h$  is Planck's constant. The Gibbs' energy of formation is given by

$$\Delta G_f = \Delta H_f - T\Delta S_f \quad (3)$$

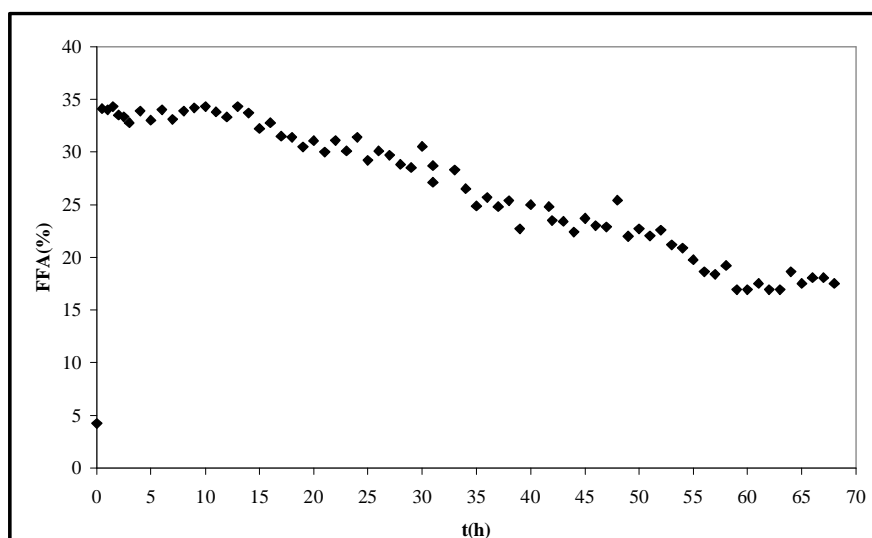
Enthalpy and entropy of formation or activation of chemical reaction provide valuable information about the nature of the transition state, and hence about the reaction mechanism. Values for each magnitude are present in **Table 1**.

**Table 1:** Thermodynamic values for hydrolysis of sunflower oil with Lipolase 100T in SC CO<sub>2</sub>.

	symbols	values
<b>enthalpy of formation</b>	$\Delta H_f$	29.734 kJ/mol $\pm$ 0.002 kJ/mol
<b>free energy of formation</b>	$\Delta G_f$	107.263 kJ/mol $\pm$ 0.002 kJ/mol
<b>entropy of formation</b>	$\Delta S_f$	- 0.219 kJ/mol K $\pm$ 0.002 kJ/mol K

If enthalpy of formation is large, large amount of stretching, squeezing or braking chemical bonds for the formation of the transition state is necessary. Large and negative  $\Delta S_f$  indicates that the formation of transition state requires the reacting molecules to adopt precise conformations and approach one another at a precise angle.

### Hydrolysis of sunflower oil in the high-pressure flat-shape membrane reactor



**Figure 3:** Free fatty acid concentration in the product during the long-term operation in high-pressure flat-shape membrane reactor.

To compare between reactions in high-pressure batch and continuous system, enzyme-catalyzed hydrolysis of sunflower oil was also performed in high-pressure flat-shape membrane reactor. Polysulfone and nylon membranes served as a separation unit to retain the biocatalyst in the reactor. In the high-pressure membrane reactor one can avoid the negative effect of depressurization steps on enzyme activity, which are necessary in the high-pressure batch reactor if the biocatalyst should be reused. Therefore in the membrane reactor biocatalyst might be used for a long-term operation.

When nylon membranes were used in the reactor for long-term hydrolysis performance at 100 bar and 50°C, the conversion was kept constant for 15 h. Hydrolysis of sunflower oil was catalysed by nonimmobilized lipase preparation from *Aspergillus niger*. The flow of CO<sub>2</sub> was constant (0.8 L/h).

The reactor continuously operated at the constant flow of both substrates (0.1ml/min) till the conversion decreased significantly as indicated on **Figure 3**. Probably the enzyme deactivation was the reason for the decrease in conversion. After 70 h the change in FFA concentration in the product was 50%.

## CONCLUSION

Lipase-catalyzed hydrolysis of sunflower oil in SC CO<sub>2</sub> was performed in two types of high-pressure reactors; high-pressure batch stirred-tank reactor and high-pressure flat-shape membrane reactor. From the comparison of the results it is obvious that in the high-pressure batch stirred-tank reactor the higher concentration of free fatty acids was obtained in the product than in the high-pressure flat-shape membrane reactor. On the other side, in the high-pressure batch reactor, if the enzyme should be reused, it must be exposed to many compression/expansion steps which could strongly affect the biocatalysts activity. In the high-pressure membrane reactor this influence could be avoided and the biocatalyst could be used for a longer period of time.

## REFERENCES :

- [1] KAMAT, S., CRITCHELY, G., BECKMAN, E.J., RUSSELL, A.J. *Biotechnol and Bioeng* 46, **1995**, p.610
- [2] KNEZ, Ž., RIŽNER, V., HABULIN, M., BAUMAN, D., *J Am Oil Chem Soc*, 72(11), **1995**, p.1345
- [3] LEITGEB, M., KNEZ, Ž., *J Am Oil Chem Soc*, 67(11), **1990**, p.775
- [4] KNEZ, Ž., HABULIN, M., *Biocatalysis*, 9(1-4), **1994**, p.115
- [5] BAILEY, J.E., OLLIS, D.F., *Biochemical engineering fundamentals*, 2<sup>nd</sup> Ed., McGraw-Hill Book Company, New York, **1986**.