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# Biotechnological Utilisation of Fusel Oil for Biolubricant Production

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## 1. Introduction

Complete utilisation of by-products from agricultural and food industrial production is an important task both from economical and environmental aspects. There are numerous possibilities for manufacture of bioproducts by biotechnological processes, among them biofuels attracted the greatest attention (Watanabe et al., 2000). Industrial by-products can be, however, processed by several biotechnological methods, for example their utilisation as a food additive, which requires intensive research and development work (Filipini & Hogg, 1997, Demirbas, 2000).

Fusel oil is a by-product of distilleries, its average composition is 10% ethanol, 13% n-propanol, 15% i-butanol, 51% i-amyl-alcohol, 11% miscellaneous alcohols and water. Nowadays fusel oil is usually burned to cover the energy demand of the distilleries. Researches have been carried out to utilise it as an additive to improve octane number in gasoline or for production of natural flavours and lubricants (Özgülsün & Karaosmanoglu, 1999).

Esterification of fusel oil with oleic acid using sulphuric acid catalyst was studied by Turkish researchers and bio-lubricant—according to ASTM (American Society Testing and Materials) standard—was manufactured for industrial purposes. Pure, natural lubricants manufactured by environmental-safe processes, however, have gained more and more attention recently, since they do not contain toxic compounds and are biological degradable. The demands against a bio-lubricant are that it should provide maximal protection during its usage, do not pollute the environment and do not accumulate (Özgülsün et al., 2000).

Unfortunately the used lubricants are usually deposited in the environment, endangering our planet. To solve the problem lubricants should be manufactured from plant oil derivatives. There are several industrial application possibilities for fatty acid esters, as natural compounds. Oleic acid (cis-9-octadecenoic acid) is one of the most important fatty acids in nature, it can be obtained from plant oils (Bélafi-Bakó et al., 1994), its esters produced by enzyme catalysis can be applied as lubricant (Linko et al., 1998).

Modern enzymology has achieved improvements in the development and application of lipase as catalyst. New immobilisation techniques make possible to use enzymes in industrial processes in a similar way to the classical catalysis for heterogeneous reactions.

For example, esters produced from long-chain fatty acids (12–20 carbon atoms) and short-chain alcohols (3–8 carbon atoms) have been used increasingly in the food, detergent, cosmetic and pharmaceutical industries. Esters prepared from the reaction of long-chain acids with long-chain alcohols (12–20 carbon atoms) also have important applications as plasticizers and lubricants (Zaidi et al., 2002, Dossat et al., 2002). The direct effect of the ester group on the physical properties of a lubricant is to lower the volatility and raise the flash point.

Compared with conventional chemical synthesis from alcohols and carboxylic acids using mineral acids as a catalyst, the use of enzymes such as lipases to produce these high value-added fatty acid esters in solvent-free media may offer many significant advantages (Yadav & Lathi, 2003). These include the use of any hydrophobic substrate, higher selectivity, milder processing conditions and the ease of product isolation and enzyme reuse. The ecological properties of oleochemical esters have been intensively studied within the last couple of years. In general, their aquatic toxicity is very low or almost negligible. For the aquatic compartment the fish, daphnia, algae and bacteria are the most relevant test organisms and standardized test methods, such as laid down in the OECD methods 201–210 (Willing, 1999).

Esterification reactions by lipase in non-conventional media have been studied in our laboratory for long (Gubicza et al., 2003). Enzymatic esterification of fatty acids and ingredients of fusel oil was studied by (Gulati et al., 2003) using lipase from *Aspergillus terreus*. They found that in n-hexane solvent the alcohols were able to react with the fatty acids (miristic acid, palmitic acid, stearic acid), except oleic acid. Using other lipase preparations (*Candida antarctica*, *Candida rugosa*, *Rhizomucor miehei*, *Porcine pancreas*), however, made it possible the successful oleic acid esterification with similar low molecular weight alcohols. Description of the correct kinetics on the particular esterification reaction is even more difficult due to the various possible inhibition effects.

In our earlier work natural aroma esters were produced by enzymatic esterification in organic solvents and in solvent-free media (Gubiza, 2000). In this work the purpose was to find a utilisation of fusel oil, where bio-lubricants can be manufactured. The alcohol compounds of fusel oil were esterified with oleic acid using enzyme catalysis in non-conventional, solvent-free media (section 2) and in ionic liquid (section 4), moreover the kinetics of the reaction was described (section 3).

## 2. Esterification in solvent-free system

After determining the optimal working parameters in the solvent-free system (molar ratio, temperature, enzyme concentration, initial water content) the reactions were completed in integrated system, where pervaporation – and effective and mild membrane separation process (Garcia, 1999) - was connected to the bioreactor.

### 2.1 Experimental

The catalyst used was Novozyme 435, a commercial *Candida antarctica* lipase (E.C. 3.1.1.3. Triacylglycerol acylhydrolase) immobilized on a macroporous acrylic resin with a water content of 1-2 % w/w. The enzyme was provided as a gift by Novo Nordisk A/S (Denmark). The nominal activity of the catalytic preparation was approximately 7000 Propyl Laurate Units (PLU/g). One propyl laurate unit (PLU) is defined as the number of  $\mu\text{mol}$  of

n-propyl laurate obtained in the standard test corresponding to the esterification of lauric acid with n-propyl alcohol, after 15 min at atmospheric pressure.

The fusel oil was provided as a gift by Distillery Győr (Hungary). All the other chemicals used in analysis were of analytical grade and purchased from Reanal Ltd. (Hungary) and Sigma Chemical Co. (USA).

Two different procedures were used for ester production. Firstly, synthesis of esters was carried out in shaking flasks (150 rpm) containing 25 ml solution of several alcohols and oleic acid mixture with different molar ratios, different temperatures and various amounts of enzyme by using New Brunswick Scientific (USA) shaking incubator to study the esterification kinetics. The starting time of the reaction was the addition of the enzyme.

In the other procedure a 200 ml round flask reactor was thermostated and connected with a pervaporation unit using hydrophilic membrane for continuous removal of water produced. The reaction mixture was circulated through the pervaporation unit by a peristaltic pump. The vacuum pump, manometer and the cooled traps were connected to the pervaporation unit.

The laboratory scale pervaporation unit was purchased from Carbone Lorraine (Germany) and it was jacketed later. The membrane surface area was  $2.0 \times 10^{-2} \text{ m}^2$ . The membranes used for the pervaporation experiments (PERVAP 2201, PERVAP 2202, CMC-VP-43) were provided by GFT (Germany) and Celfa (Switzerland).

Aliquots of the reaction mixture were withdrawn periodically and the residual acid content was assayed by titrating against potassium hydroxide (0.1 M) using phenolphthalein as an indicator and ethanol as a quenching agent. The percentage esterification was calculated from the values obtained for the blank and the test samples. The fusel oil esters were confirmed by chromatographic analyses of the samples using a Hewlett Packard Model 5890 Series II GC equipped with a flame ionisation detector and a 30 m HP-FFAP capillary column. The percentage esterification calculated by both GC analysis (which showed product formation) and titrimetry (which showed acid consumption) were found to be in good agreement. The water content of the reaction mixture was measured by Mettler DL 35 automatic Karl-Fischer titrator

## 2.2 Results

The esterification reaction of oleic acid with the fusel oil fraction occurs as follows:



In this reversible reaction, the molar ratio of reactants, temperature, enzyme and removal of water from the reaction mixture are the variables affecting the conversion and the reaction rate.

### 2.2.1 Water content

Water level is critical for enzymes and affects enzyme action in various ways: by influencing enzyme structure via noncovalent bonding and disruption of hydrogen bonds; by facilitating reagent diffusion; and by influencing the reaction equilibrium. Too low water content usually reduces enzyme activity. High water content can also decrease reaction rates by aggregating enzyme particles and causing diffusional limitations. The optimal amount of water is often within a narrow range.

Optimal water content is not only important to preserve the catalytic activity of an enzyme, but also to achieve high reaction rates and yields, and stability of the enzyme. Water requirements for enzymes in organic media vary greatly; therefore each enzyme must be examined at various levels of hydration.

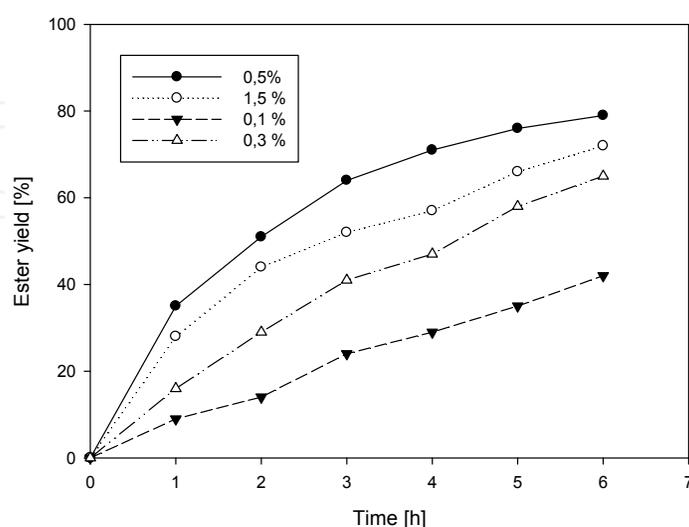


Fig. 1. Influence of water content on the synthesis of isoamyl oleate. Oleic acid, 1.84 mol/l; i-amyl-alcohol, 3.78 mol/l; temperature, 40 °C; Novozym 435, 0.5 %; speed, 150 rpm

The role of the water content in the reaction mixture was studied in the range of 0.1 - 1.5 %. As it can be seen in Figure 1 the ester yield as a function of the initial water content has a minimum. In very low water content (0.1 %) the amount of ester produced is small: in this case water present in the reaction mixture is not enough for the enzyme's optimal work. Increasing the water content the yield is growing, and a maximal value is reached at about 0.5 % initial water content. Then at higher and higher water contents the ester yields obtained are gradually decreased, which can be explained by the fact that at high water content the opposite reaction, the hydrolysis is favoured. Based on the results of these measurements, 0.5 % initial water content was used in the further experiments.

### 2.2.2 Effect of temperature

The effect of temperature was studied in a series of experiment in the range of 30 - 60 °C under the same conditions (initial water content, molar ratio, enzyme concentration). No thermal deactivation was observed up to 60 °C, and - as it is shown in Figure 2 - the concentration of the oleates produces increased in higher temperatures. This curve is typical of enzyme with high thermostability and which thermal denaturation, during the time of the assay, is negligible. After 12 hours reaction time (not shown) 92 % conversion was obtained in each case.

The activation energy of the enzymatic reaction was determined based on the well-known Arrhenius-equation. The logarithm of the reaction rate data were plotted as a function of reciprocal temperature and the activation energy was calculated from the slope of the regression line. It's value was 16.2 kJ/mol, which is similar to the value reported by (Garcia, et al., 1999) (11.7 kJ/mol), determined for the esterification of isopropyl alcohol and palmitic acid in solvent-free system by the same Novozyme 435 lipase preparation.

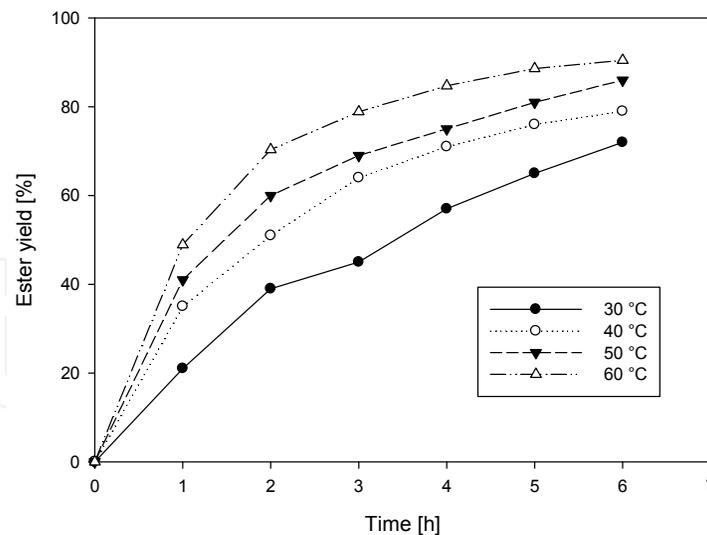


Fig. 2. Influence of reaction temperature on the synthesis of isoamyl-oleate. Oleic acid, 1.84 mol/l; i-amyl-alcohol, 3.78 mol/l; Novozym 435, 0.5 %; speed, 150 rpm.

### 2.2.3 Effect of the molar ratio

It is well-known, that acid/alcohol molar ratio is one of the most important parameters in enzymatic esterifications. Since the reaction is reversible, an increase in the amount of one of the reactants will result higher ester yields and as expected, this will shift the chemical equilibrium towards the product side. One way of shifting the reaction toward the synthesis is to increase the alcohol concentration. However, high alcohol concentration may slow down the reaction rates due to inhibition. Therefore, it is necessary to optimize the actual excess nucleophile concentration to be employed in a given reaction. In order to determine the effect of molar ratio, oleic acid was esterified at molar ratios of 1:1, 1:2, 1:5 and 2:1 oleic acid/iso-amyl alcohol under the following conditions: 40 °C temperature applying 0.5 % enzyme concentration, 0.5 % initial water content.

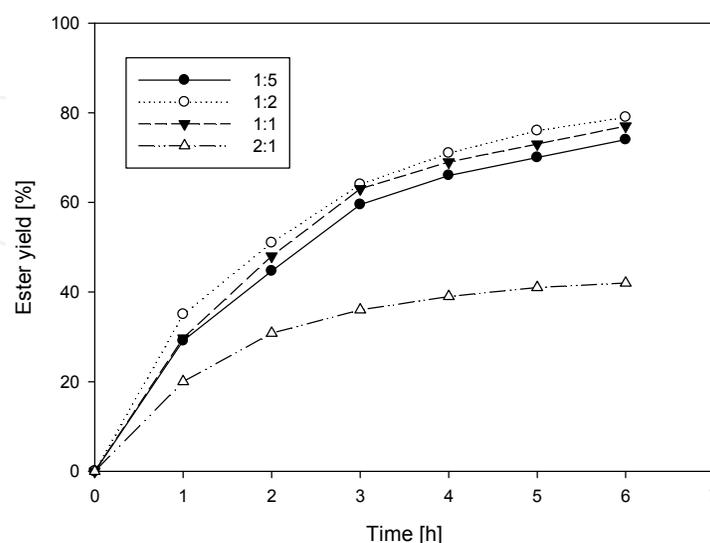


Fig. 3. Influence of the acid/alcohol molar ratio on the synthesis of isoamyl-oleate. Novozym 435, 0.5 %; temperature, 40 °C; speed, 150 rpm.

As expected, a higher ester conversion was obtained in a shorter period of time for 1:2 and 1:5 molar ratios compared to 1:1. The highest acid conversion was achieved in case of 1:2 molar ratio, as it can be seen from Figure 3, thus alcohol excess was used in the further experiments.

#### 2.2.4 The effect of chain length of alcohols in fusel oil on the esterification reaction

The main alcohol compounds of fusel oil, two with linear chains (ethanol, propanol) and three with a branch chains (isopropyl alcohol, isobutyl alcohol and isoamyl alcohol) were applied in the next serial of experiments to determine the effect of chain length. In the measurements the conditions were as follows: 1:2 acid/alcohol molar ratio, 0.5 % enzyme and 60 °C temperature.

The results shown in Figure 4, that the esterification rates of oleic acid with ethanol are smaller than with isoamyl alcohol, indicating some effect of the length and structure of the alcohol molecule. The difference between the isoamyl oleate and ethyl oleate was 33 %. Based on this result, it was assumed that esterification of oleic acid with model solution of fusel oil will be similar to the ester synthesis with i-amyl alcohol, since it is the main compound of fusel oil.

#### 2.2.5 Removal of excess water produced by pervaporation

The optimal parameters of the reaction was determined in the shaken flasks experiments: 0.5 % water concentration, 1:2 oleic acid-isoamyl alcohol molar ratio, 60 °C temperature. According to the analysis data without water removal, the reaction reaches equilibrium after 12 hours reaction time.

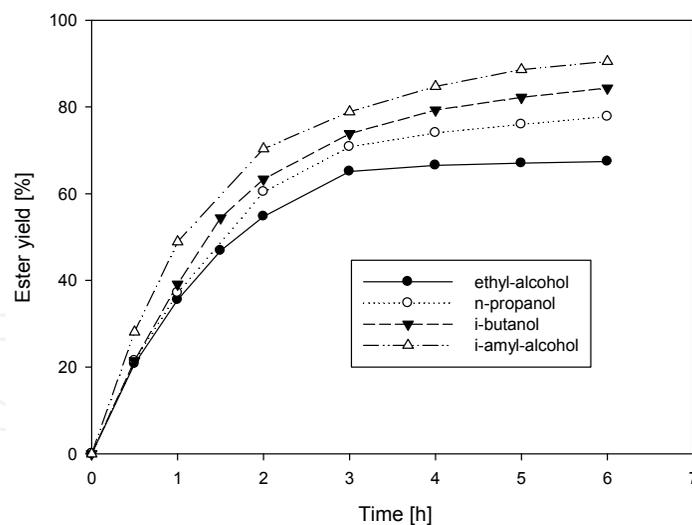


Fig. 4. Influence of chain length of alcohols. Acid/alcohol molar ratio, 1:2; Novozym 435, 0.5 %; temperature, 60 °C; speed, 150 rpm.

The esterification of oleic acid resulted in 92 % yield, while water produced as excess during the process and it accumulated as a second phase in the bottom of the flask. Since water excess has a strong inhibition effect on the enzyme, stops the further conversion of substrate, therefore it should be removed. Pervaporation seemed a suitable membrane separation technique for continuous water removal, helping to convert all the substrate. Thus an

integrated system was designed for the enzymatic reaction, where a thermostated bioreactor is combined with a pervaporation unit. The membrane unit consists of two stainless steel plates, the membrane is located between them. The permeate passed through the membrane was condensed in traps cooled by dry ice - acetone mixture. The permeate side was kept under 0.8 kPa vacuum pressure. During the procedure the feed stream was recirculated in liquid phase on the primary side of the membrane module, while the permeate was obtained in vapour phase. The reaction mixture was recirculated through a glass filter to keep the immobilised enzyme in the reactor. In the integrated system the bioreactor was thermostated at 60 °C temperature, and the experiments were carried out under the optimal conditions determined earlier. Samples were taken from the reactor and the analysis has shown that the water content was managed to keep at 0.5 %. In the integrated system 99.8 % conversion of the oleic acid was achieved.

Firstly hydrophilic pervaporation membranes were tested. It was turned out that the CMC-VP-43 type membrane has the highest flux (0.05 kg/m<sup>2</sup>h) and selectivity (58.94), so this membrane was used for the further experiments.

### 2.2.6 Tribological properties of the biolubricant

The tribological properties of the biolubricant manufactured were tested according to the IP and ASTM standards. The chemical, physical parameters of the product were given in Table 1. The thermal and oxidation stabilities were measured by the modified IP 8.6 method using an oxidative and thermal treating. The results obtained were compared to a commercial, synthetic, DB-32 dicarboxylic acid ester used as a reference lubricating oil (Table 2).

Properties		Biolubricant	DB-32
Density	g/cm <sup>3</sup>	0.864	0.925
ASTM colour	-	0	0
Flash point (Cleveland)	°C	211	204
Pour point	°C	-27	n.d.
Viscosity at 100 °C (KV <sub>100 °C</sub> )	mm <sup>2</sup> /s	2.28	2.7
Viscosity at 40 °C (KV <sub>40 °C</sub> )	mm <sup>2</sup> /s	6.39	9.03
Viscosity index (VI <sub>E</sub> )	-	207	148
Acid value	mg KOH/g	0.01	0.1
Iodine-bromine value	g I/100 g	68.4	n.d.

n.d.: no data.

Table 1. Properties of the biolubricant compared to the reference oil

As it can be seen from Table 1, the bioester produced is considered as a low flashpoint lubricant. Due to its low pour point, it can be applied also at low temperatures. Its viscosity

index is quite high, thus the viscosity of the ester is not highly influenced by temperature. The good conversion of oleic acid resulted in the low acid number, while the iodine-bromine number refers to the quite low unsaturation.

The data in Table 2 show that the stability of the measured ester based on thermal and oxidative treatment is higher than the reference oil, i.e. the increase in viscosity and acid number is lower, the change of its colour is acceptable. According to these data the biolubricant is suggested to use mainly at the high speed and low load regime of the tribological circumstances. In the mechanical industry it can be applied e.g. as a cooling lubricant compound for metalworking processes, moreover in particular lubrication processes where lubricant loss may occur, e.g. mist lubrication, chain lubrication, launch engine lubrication.

Lubricant	Thermal treatment (200 °C, 12 h)					Oxidative treatment (200 °C, 12 h, airflow 20 dm <sup>3</sup> /h)				
	VK <sub>100 °C</sub> (mm <sup>2</sup> /s)	VK <sub>40 °C</sub> (mm <sup>2</sup> /s)	VI <sub>E</sub>	Colour	Acid value (mg KOH/g)	VK <sub>100 °C</sub> (mm <sup>2</sup> /s)	VK <sub>40 °C</sub> (mm <sup>2</sup> /s)	VI <sub>E</sub>	Colour	Acid value (mg KOH/g)
DB-32	2.75	9.45	140	7	5.5	5.79	29.47	143	8	50.8
Biolubricant	2.29	6.41	209	0	0.1	5.57	25.91	162	8	10.6

Table 2. The effect of thermal and oxidative treatments

### 2.2.7 Study on toxicity of biolubricant by zebrafishes

To study the effect of the particular biolubricant on the water environment, its acute toxicity was assessed with Acute Fish Toxicity Test on Zebrafish (*Brachydanio rerio*), over an exposure period of 96 hours in a static system. A limit test was conducted according to the OECD Guidelines for the Testing of Chemicals, Procedure No. 203 (1992). In this kind of test the limit in LC<sub>50</sub> number is 100 mg/l. In the experiment two 20 l aquaria were used, in the control there was no biolubricant, while in the other aquarium its nominal concentration was 100 mg/l. Zebrafishes (7 fishes in both aquaria) were used in the test, which are extremely sensitive to the waste compounds in living waters. Analytical methods were applied to measure the parameters of the water (chemical properties, temperature, oxygen saturation, pH...etc.). The observation of fishes were carried out at regular intervals (3, 6, 24, 48, 72 and 96 hours) and mortality was determined.

As a result of the experiment no signs of reaction or mortality were detected. Thus the 24 h, 48 h, 72 h and 96 h LC<sub>50</sub> number of the particular biolubricant is > 100 mg/l. So it can be concluded that the biolubricant is not toxic for the living water.

## 3. Kinetics approach

Before developing a complete method for enzymatic manufacture of this biolubricant, a detailed kinetic analysis should be carried out on the reaction mechanism. Enzymatic esterification of fatty acids and ingredients of fusel oil was studied by (Gulati et al., 2003)

using lipase from *Aspergillus terreus*. They found that in n-hexane solvent the alcohols were able to react with the fatty acids (miristic acid, palmitic acid, stearic acid), except oleic acid. Using other lipase preparations (*Candida antarctica*, *Candida rugosa*, *Rhizomucor miehei*, *porcine pancreas*), however, made it possible the successful oleic acid esterification with similar low molecular weight alcohols. In Table 3 results published on the kinetics of enzymatic esterification of oleic acid with short chain alcohols in organic solvents are summarized.

Garcia et al. (1996) studied the kinetics of i-propyl-oleate formation by *Candida antarctica* lipase. The model used was an ordered bi-bi type containing 13 kinetic parameters. Thus the model seems too complicated having high uncertainty. Esterification of butyl alcohol by *Candida rugosa* lipase was studied by (Zaidi et al., 2002), where ping-pong bi-bi mechanism was assumed in the kinetical model with 5 parameters. However, the range of substrate concentration measured was quite narrow (0.1 – 1 mol/L), and the error of the modelling was found very high (28 %).

Alcohol	Enzyme	Model	Inhibition	Reference	Note
i-propyl alcohol	Novozym 435 <i>Candida antarctica</i>	ordered bi-bi 13 parameters	both S and P competitive	(Garcia et al., 1996)	too many parameters fitted to one measurement; high error of fitting
butyl alcohol	immobilised <i>Candida rugosa</i>	ping-pong bi-bi 5 parameters	both acid and alcohol	(Zaidi et al., 2002)	error is 28 %; narrow range of substrate concentration (0.1-1 mol/L)
ethyl alcohol	immobilised <i>Rhizomucor miehei</i>	random bi-bi 4 parameters	alcohol	(Oliveira et al., 2001)	Difference between the parameters is 15 order of magnitude; quasi one-substrate kinetics
ethyl alcohol	immobilised <i>Rhizomucor miehei</i>	Michaelis- Menten 2+1 parameters	alcohol	(Goddard et al., 2000)	Different kinetics for each alcohol concentration; pseudo one- substrate kinetics
ethyl alcohol	soluble, from porcine pancreas	ping-pong bi- bi 4 parameters	alcohol	(Hazirka et al., 2002)	Narrow range of substrate concentration (0.3- 0.8 mol/L)

Table 3. Kinetic studies on esterification of oleic acid

Immobilized *Rhizomucor miehei* lipase was applied for ethyl oleate synthesis by (Oliveira et al., 2001). In order to describe the kinetics, random bi-bio model was used, which contained 4 parameters. However, the difference between the parameter values determined was 15 order of magnitude, implying that the effect of certain parameter is nearly negligible comparing to the others. Esterification of ethyl alcohol and oleic acid by immobilized

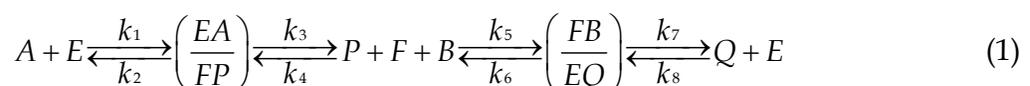
*Rhizomucor miehei* lipase was studied by (Goddard et al., 2000) as well. Michaelis-Menten model was used for the description of the reaction, however, different kinetics was used in each alcohol concentrations, which is considered as a pseudo-one substrate model.

Soluble porcine pancreatic lipase was applied for the ethyl oleate synthesis by (Hazarika et al., 2002). They assumed ping-pong mechanism, as well, containing 4 parameters, however the range of substrate concentration studied was even narrower (0.3 – 0.8 mol/L) than in case of (Zaidi et al., 2002). Description of the correct kinetics on the particular esterification reaction is even more difficult due to the various possible inhibition effects. As it is shown in Table 3, ethyl alcohol as a substrate was in all cases considered as inhibitor, while in the esterifications with other alcohols, both substrates were regarded as inhibitors.

As a summary, it seems from Table 3 that the kinetic models/parameters published so far can not be considered as a proper, detailed kinetic description of the enzymatic process for oleic acid esterification with short chain alcohols, moreover there has been no data found on esterification with i-amyl alcohol. Therefore our aim was to elaborate a proper, sophisticated model for this particular reaction.

### 3.1 Kinetics model

Kinetics of enzymatic reactions can be described by the well-known Michaelis-Menten model. For reactions having 2 substrates and 2 products (bi-bi reactions), its application is quite complicated since various mechanisms can be considered according to the order and rate both substrates' binding and products' releasing to/of the enzyme active sites (random, ordered, ping-pong...etc.). Since majority of the kinetical studies suggested ping-pong bi-bi mechanism for the enzymatic esterification of oleic acid and short chain alcohols, we also considered it as an initial point for the description. According to Cleland (Cleland, 1979) the ping-pong bi-bi mechanism can be outlined as follows:



In the first step the enzyme reacts with substrate A forming an AE enzyme-substrate complex, which is transformed into FP modified complex - by an internal rearrangement. Product P comes off the complex, then the modified enzyme molecule F is able to react with substrate B, forming a new enzyme-substrate complex FB. It is transformed into an enzyme-product complex EQ, then Q moves to the bulk solution. Finally, enzyme E becomes free and can react with another substrate A molecule. In the equation the reaction rate constants are shown ( $k_1 - k_8$ ), among them  $k_1, k_3, k_5$  and  $k_7$  belong to the towards direction of the reaction, while the others to the backwards direction (with negative sign). Summarising the steps, the formation rate of product can be written as follows:

$$v = \frac{(k_1 k_2 k_5 k_7 AB - k_2 k_4 k_6 k_8 PQ) E_0}{E + (EA + FP) + F + (FB + EQ)} \quad (2)$$

where  $E_0$  is the initial enzyme concentration and E is the actual enzyme concentration (the other capital letters mean concentration of the particular compound). This model, however, is too complicated to apply it in practice. To simplify the situation, the main parameters influencing the rate of product formation are selected, as follows (Janssen et al., 1996):

the concentration of substrate A (first),  
 the concentration of substrate B (second),  
 enzyme concentration,  
 the amount of products formed altogether.

Initial reaction rate can be described by including only the first three factors, taken into account the fact that product concentration is 0 at the beginning of the reaction:

$$v = \frac{V_m}{1 + \frac{K_A}{[A]} + \frac{K_B}{[B]}} \quad (3)$$

If substrate inhibition is considered, as well, the three-parameter equation should be completed with another constant (Dezbaradica et al., 2006) and the following equation can be used:

$$v = \frac{V_m}{1 + \frac{K_A}{[A]} \left( 1 + \frac{[B]}{K_{iB}} \right) + \frac{K_B}{[B]}} \quad (4)$$

This model contains four parameters, which can be further supplemented by the product inhibition factors. In this way two more parameters are added into the equation (Eq. 5). In the equation  $K'_{AB}$  parameter is the rate of the apparent product formation, the ping-pong parameter of the reaction.

$$v = \frac{V_m}{1 + \left( \frac{K_A}{[A]} + \frac{K'_{AB} * [P]}{[A] * [B]} \right) \left( 1 + \frac{[B]}{K_{iB}} \right) + \frac{K_B}{[B]} * \left( 1 + \frac{[P]}{K_{iP}} \right)} \quad (5)$$

where

$$V_m = \frac{k_3 k_7}{k_3 + k_7} \quad K_A = \frac{k_7 (k_2 + k_3)}{k_1 (k_3 + k_7)} \quad K_B = \frac{k_3 (k_6 + k_7)}{k_5 (k_3 + k_7)} \quad K_{iP} = \frac{k_3}{k_4}$$

$$K_{iB} = \frac{k_5}{k_6} \quad K'_{AB} = \frac{k_2 k_4 (k_6 + k_7)}{k_1 k_5 (k_3 + k_7)}$$

In our case, water is one of the products in esterification. But it is not only a product, a small amount of water should be present initially in the reaction mixture to keep in an active formation of the enzyme structure. In the beginning of the reaction, however, the initial concentration of water does not change significantly, therefore water content (P) can be considered as constant (its effect is negligible). Thus the equation can be simplified and a 5-parameter equation can be obtained:

$$v = \frac{V_m}{1 + \left( \frac{K_A}{[A]} + \frac{K_{AB}}{[A] * [B]} \right) \left( 1 + \frac{[B]}{K_{iB}} \right) + \frac{K_B}{[B]}} \quad (6)$$

It can be seen that the difference between the 4- and 5-parameter equations (Eqs. 4 and 6) is the KAB factor. Its influence is significant only in the case when concentrations of both substrates are very low. However, we do not plan to carry out measurements under these conditions, thus it is assumed that no significant difference will be experienced in the modelling results obtained by using the two systems.

### 3.2 Reaction rate determination

The operational parameters during the kinetical experiments were 200 rpm and 30 °C. 0.01 g immobilized lipase preparation was added to the incubated homogeneous reaction mixture to initiate the enzymatic reaction. Samples in duplicates were taken from the reaction mixture regularly.

Experiments using substrate concentrations in the range of 0.4 - 6.0 and 0.2 - 2.0 mol/L of i-amyl alcohol and oleic acid concentration, respectively, were carried out at 30 °C temperature, with an optimal initial 0.1 w/w % water content (determined earlier) (Koszorz et al., 2004) in n-heptane solvent. Progress curves: oleic acid consumptions as a function of time were measured.

From the data of progress curves the initial reaction rates were calculated by the Gregory-Newton method (Leskovac, 2003). The initial reaction rate data were modified taken into account the amount of enzyme used. Thus the reaction rate values were obtained as  $\mu\text{mol/s.genzyme}$  and summarised in Table 4.

$C_{\text{COA}} \backslash C_{\text{iAA}}$	0.4	1.0	2.0	4.0	6.0
0.2	4.90	5.13	5.45	5.57	3.50
0.5	7.91	7.91	8.26	7.13	7.31
1.0	9.68	12.56	12.80	9.69	10.80
1.5	11.25	15.15	18.45	15.30	13.72
2.0	9.78	14.46	17.65	14.50	14.31

Table 4. Intitial reaction rate data [ $\mu\text{mol/s.genzyme}$ ] using various oleic acid ( $C_{\text{COA}}$ ) and i-amyl alcohol concentrations ( $C_{\text{iAA}}$ ) (all the concentrations in mol/L)

### 3.3 Effect of immobilization on the mass transfer

Since immobilised enzyme preparation was used in the experiments, it was important to decide - before the detailed kinetical analysis - whether the reaction rates measured are the real values of enzymatic reaction or influenced significantly by the diffusion rates of the compounds (from the bulk phase to the solid particle and vice versa).

The reaction and the diffusion take place simultaneously and the rate limiting step is always the one which is slower. Using immobilised lipase preparations, rate of diffusion is usually not the limiting step (Letisse et al., 2003).

In our measurements Yadav-method (Yadav et al., 2003) was applied to determine the rate limiting step, using the Weisz-Prater criteria. This method is based on the calculation and

comparison of the two relevant relaxation times. The ratio of the relaxation time of biocatalysis rate,  $t_r$  and that of the diffusion rate,  $t_d$  shows which process should be considered as the limiting step.

The relaxation times can be defined as follows :

$$t_r = \frac{C_0}{r(C_0)} \text{ and } t_d = \frac{D}{(k_{sl})^2} \quad (7)$$

Oleic acid - having slower diffusivity - was chosen for the calculations and the highest reaction rate-substrate concentration value-pair was taken from Table 4. Thus  $t_r$  was calculate as:

$$t_r = \frac{C_0}{r(C_0)} = 2^5 \text{ s} \quad (8)$$

Diffusion constant (D) of oleic acid in n-heptane was determined according to Shibel (Perry, 1969):

$$D = k \frac{T}{\eta_b V_s^{1/3}} \quad (9)$$

$V_s$  molar volume density was estimated from its critical volume ( $V_c$ ):

$$V_s = 0,285V_c^{1,048} \quad (10)$$

$V_c$  of oleic acid is 1152 cm<sup>3</sup>/mol, thus  $V_s$  is obtained as 460 cm<sup>3</sup>/g. In this way D diffusion coefficient is calculated as 1.61x 10<sup>-5</sup> cm<sup>2</sup>/s.

The mass transfer coefficient can be calculated - based on the Sherwood number - from the diffusion coefficient and the particle size:

$$k_{sl} = 2D / d_k \quad (11)$$

The average diameter of Novozyme 435 immobilised lipase preparation is 0.06 cm, thus the value of the mass transfer coefficient is 5.3x 10<sup>-4</sup> cm/s.

The relaxation time for the diffusion is calculated as:

$$t_d = \frac{D}{(k_{sl})^2} = \frac{1,61 \cdot 10^{-5} [\text{cm}^2 / \text{s}]}{(5,3 * 10^{-4} [\text{cm} / \text{s}])^2} = 55,9 \text{ s} \quad (12)$$

Comparing the values of  $t_r$  and  $t_d$  it can be concluded that diffusion rate is three order of magnitude higher than the reaction rate, thus the rates measured in the enzymatic process can be considered as the real reaction rates.

### 3.4 Kinetical analysis

In Fig. 5a the initial reaction rates are presented as a function of oleic acid (substrate 1) concentration, while in Fig. 5b the same data are shown as a function of i-amyl alcohol (substrate 2) concentration. It can be clearly seen that the i-amyl alcohol has a considerably and oleic acid a slight inhibition effect on the enzymatic reaction.

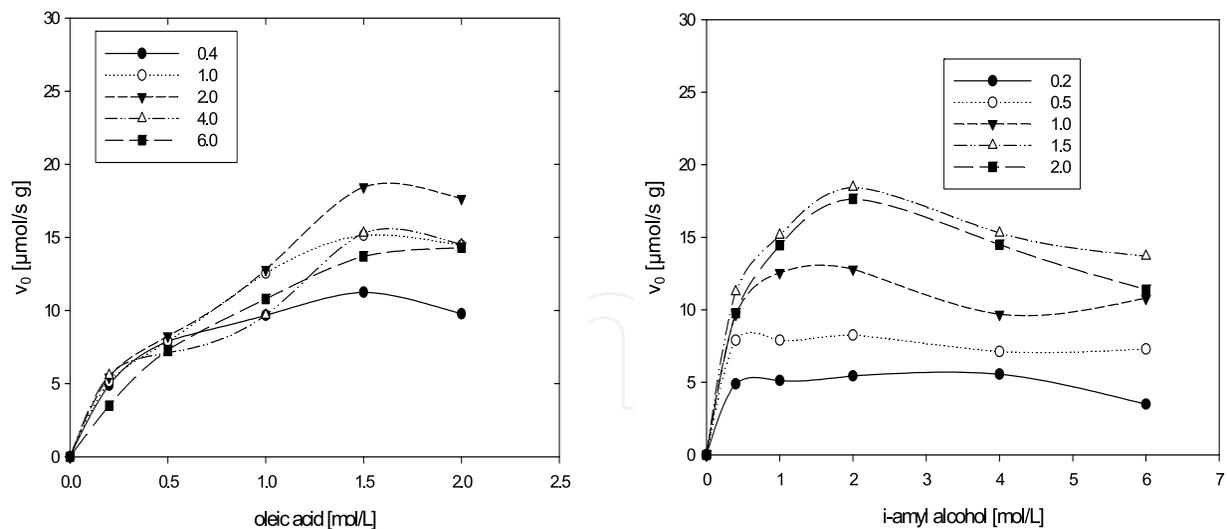


Fig. 5. Initial reaction rates as a (a) function of oleic-acid concentration at different i-amyl alcohol concentrations; (b) a function of i-amyl alcohol concentration at different oleic acid concentrations in mol/L

In the first step of the kinetic analysis the traditional linearization (graphical) methods were applied. Reciprocal data of the initial rates were plotted against the reciprocal data of the initial substrate concentrations (both) (Figs. 6a and 6b). It can be seen that in Fig. 6b in lower substrate concentrations the lines are parallel, implying ping-pong bi-bi mechanism. However, in higher substrate concentrations the lines steeply keep upwards to the ordinate, which means that inhibition (by the alcohol compound) occurred. Thus the kinetic parameters can not be determined graphically and the mechanism of inhibitions can not be doubtlessly decided.

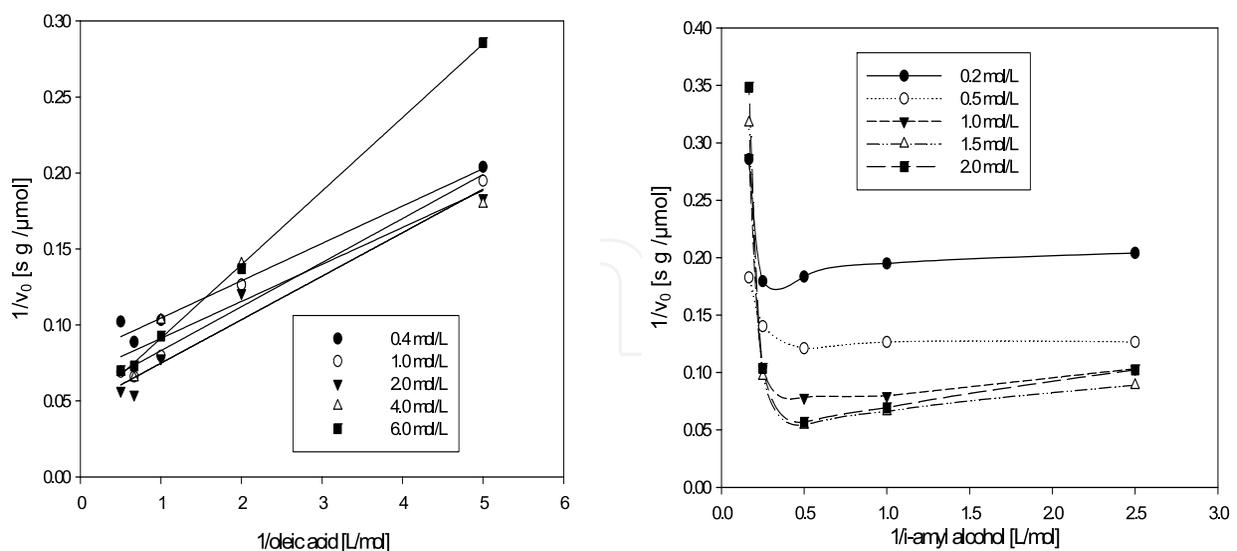


Fig. 6. (a) and (b) Lineweaver-Burk linearization of the reaction rate - substrate concentration data

Ping-pong bi-bi kinetic models having 3 (eq. 3), 4 (eq. 4.) and 5 (eq. 6.) parameters were used for the description of the enzymatic esterification and the parameters were calculated based

on the experimental data. In the calculation a variation of simplex method was applied, namely the Nelder-Niemand method (Bailey, 1986), which is more sensitive for the initial values of the parameters and slower than the original simplex method, but it provides more accurate final results.

Since the method is sensitive for the initial values of the parameters, a two-step method was elaborated. In the first step the values of kinetic parameters were estimated by a special programme, applying a simplified model (Luzenc4 programme) with no inhibition, using the experimental data. Thus the instability of the method was eliminated. The estimated kinetic parameters obtained were then used as initial parameters for the extended kinetic model. Having checked the kinetic results by comparing the experimental data, the program either refused the results and started another circle - modifying one or more parameters according to the Nelder-Niemand method - or accepted the results and presented.

During the procedure programme was run on data belonging to only one acid concentration, otherwise divergence occurred. In this way the parameters were obtained for every acid concentration, which were used in a final run of the programme, where errors were minimised.

In Table 3 values of the parameters determined are summarized. It can be seen from the value of  $r^2$ , that the four-parameter model (taking into account the inhibitions, as well) described better the kinetics of enzymatic i-amyl oleate synthesis than the three-parameter model.

Parameters	Three-parameter model	Four-parameter model	Five-parameter model
$V_m$ ( $\mu\text{mol/s g}$ )	23.5	30.8	29.9
$K_A$ (mol/L)	0.86	0.65	0.55
$K_B$ (mol/L)	0.19	0.58	0.53
$K_{iB}$ (mol/L)	-	3.2	2.7
$K_{AB}$ (mol/L)	-	-	0.055
$r^2$ (-)	0.952	0.975	0.975
ARE* (%)	12,2	3,1	2,8

Table 5. Parameters of the kinetical models

However, the five-parameter model is not more accurate than the four-parameter one (value of  $r^2$  is the same), therefore it is not reasonable to use the more complicated model. This theory evaluated using F-statistics (Bates, 1988) used significance level was  $P=0.05$ , as the result shows the 3 parameter model was significance different then the 4 and 5 parameters model while the 4 parameter and 5 parameters model are not significantly different. The results of the modelling were intended to compare with other literature data, however no similar results were found in published materials. Either the substrates of the esterification, or the enzyme applied were different, thus the parameters were not possible to compare.

Kinetic model for description of the enzymatic esterification of oleic acid and i-amyl alcohol was – according to our best knowledge – elaborated and presented in the first time in literature. The model is based on the ping-pong bi-bi mechanism and not only product, but substrate inhibition is taken into account, as well. The model fitted well to the experimental data (checked by  $r^2$  values), though the measurements covered an extremely wide concentration range.

#### 4. Reaction in ionic liquid

Ionic liquids can be suitable media for biocatalytic synthesis because of their enzyme stabilization effect, reusability and negligible vapour pressure (Yang & Pan, 2005). Many enzymes, especially lipases showed higher activity and greater selectivity in ionic liquids than in organic solvents (Jain et al., 2006). The stability of *Candida antarctica* lipase B (CALB) in ionic liquid was found to be higher than in organic solvent (Romero et al., 2005). The main advantages of ionic liquid + enzyme system are milder, lower reaction temperature comparing with conventional chemical synthesis, it needs less energy investment, can be better-controlled, attended with less side-reaction, furthermore the product more readily separable. In this work the purpose was to find a utilization of fusel oil where biolubricants can be manufactured in ionic liquid.

In organic solvents low conversions were achieved, therefore it became necessary to develop a better method, which is energy efficient, able to achieve higher conversion, furthermore environmental friendly, however waste materials may be as natural substrates. Our aim is to optimize the parameters of production, enhance the yield improving the advantages of enzyme+ionic liquid system.

##### 4.1 Ionic liquids

*Ionic liquids:* 1-butyl-3-methyl-imidazolium hexafluoro phosphate [bmim]PF<sub>6</sub>, 1-butyl-3-methyl-imidazolium tetrafluoro-borate [bmim]BF<sub>4</sub>, tributyl tetradecyl phosphonium dodecylbenzyl sulfonate (Cyphos-201), trihexyl tetradecyl phosphonium bis-(2,2,4-trimethylpentyl)-phosphate (Cyphos-104), tetradecyl phosphonium trihexyl decanoate (Cyphos-105) trihexyl tetradecyl phosphonium hexafluoride, phosphate (Cyphos-110) (IoLiTec GmbH, Germany)

*Reaction mixture composition:* Under the pre-investigations, the reaction mixture was the follow: 6.36 mmol of oleic acid, 36.95 mmol of isoamyl alcohol, 304.35 mmol n-hexane and 50 mg Novozym 435 lipase. In the course of the main experiment: 1.23 mmol of ionic liquid, 0.16 mmol oleic acid, 1.41 mmol isoamyl-alcohol were used. The reaction mixtures were shaken with an intensity of 150 rpm at 40, 50 and 60 °C temperatures for 4 hours.

##### 4.2 Effect of ionic liquid

As it well known ionic liquids are not only green solvents, they can have catalytic effect. Our aim was to investigate separately the enzyme stabilization effect, therefore preliminary experiments were necessary. Through these the most important investigated criterion was to verify if the ionic liquid itself had catalytic effect for the reaction without enzyme. Basic criterion was to be work in a two-phase reaction, where separation is easier, since the application of a new solvent can be avoid (Eckstein et al., 2004). Two of the investigated

ionic liquids (Cyphos-105 and Cyphos-110) were mixed with the substrates, so they were not investigated further. Henceforth the reactions were carried out at 50 °C, the ester yield was followed by GC and the decreasing oleic acid concentration using titrimetry. The percentage esterification was calculated from the values obtained for the blank and the test samples. The further phosphonium-type ionic liquids, Cyphos-201 and Cyphos-104 without the presence of enzyme greatly catalyze the process as it shown on Fig. 7.

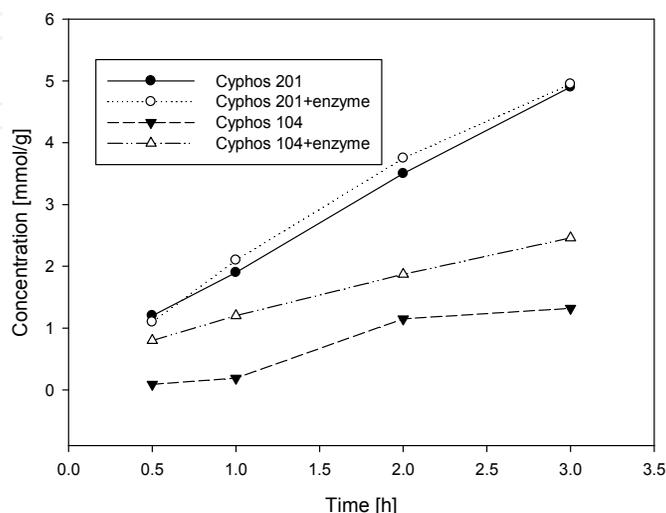


Fig. 7. Oleate ester concentration in case of using different phosphonium-type ionic liquids (reaction time: 180 min)

For imidazolium-cation containing ionic liquids literary data show that these types of ionic liquids are the most suitable for esterification, transesterification reactions (Moniruzzaman at al., 2010). Both investigated imidazolium-type ionic liquids were successfully applied in earlier experiments, where natural aroma esters production was the aim. In case of [bmim]BF<sub>4</sub> there were no significant differences in the detected oleate concentration if enzyme was added or not. For [bmim]PF<sub>6</sub> - without the presence of the enzyme only negligible product formation was observed, in the presence of enzyme, higher concentrations were achieved than in the experiments where n-hexane was used as solvent.

Therefore for further investigations this ionic liquid was chosen. In case of ionic liquids for water solubility cations are responsible. Comparing the same cation having [bmim]BF<sub>4</sub> and [bmim]PF<sub>6</sub> shows that while the first not miscible with water, the later has an unlimited solubility in water. Thus, the hydrophilic [bmim]BF<sub>4</sub> ionic liquid often distracts the absorbed water layer from the surface of the enzyme which should be necessary for the active conformation. Therefore the enzyme is deactivated (van Rantwijk & Sheldon, 2007). Further advance that in case of using [bmim]PF<sub>6</sub> side-reactions were not observed.

#### 4.3 Acid/alcohol molar ratio

The optimal parameters of the batch production were determined using experimental design software application. In doing so Statistica 8.0 program was applied. Based on earlier studies substrate molar ratio, amount of enzyme and ionic liquid were chosen as key factors. Each factor was prepared in two levels: -1 for low level and +1 for high level.

Concrete values were applied in a design matrix. It is evident that increasing reaction temperature enhances the reaction rate, that is way that its affect will be investigated later separately.

During the experiments different, software-defined combinations of the previously selected values of experimental parameters were investigated. Other parameters were fixed: temperature 50 °C, 150 rpm shaking intensity, 5 hours reaction time. Water content of the reaction mixture was also followed as an important parameter of esterification reactions which may shift the equilibrium, but there was not detectable concentration change using Carl-Fischer titration. The aim was to find the optimal parameter values of the isoamyl-oleate production. The results are shown on Fig. 8.

As it shown, increasing amount of ionic liquid results in higher oleate yield, which gives evidence for the advantageous enzyme stabilization effect. Complex investigation of the three chosen key factors shows that the highest ester conversion was obtained in the case of application the highest acid/alcohol molar ratio, amount of enzyme and ionic liquid. Relatively high yield was obtained also around medium values, around the center points.

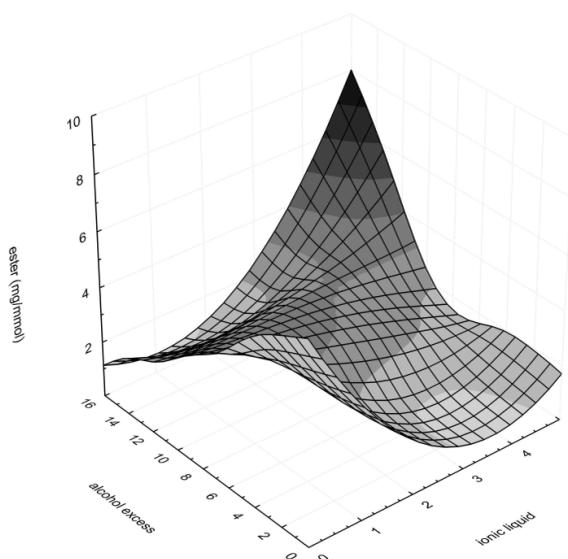


Fig. 8. Influence of the acid/alcohol molar ratio and amount of ionic liquid on the synthesis of isoamyl oleate

There was no inhibition effect observed neither in 1:16 acid/alcohol molar ratio. As none of the point of parameters could be an optimal value, because the highest yield belonging to the highest values, therefore further investigations will necessary to find the optimal values.

#### 4.4 Effect of temperature

In case of using conventional reaction media it is well known that increasing reaction temperature results the same yield in shorter time, but till a limit due to thermal deactivation of enzyme. Beside of the structure stabilization effect of ionic liquids the enzyme can be resist in the active conformation at higher temperatures (van Rantwijk & Sheldon, 2007). For these investigations that mixture was chosen, in which the highest oleate yield was obtained. The results are shown on Fig. 9.

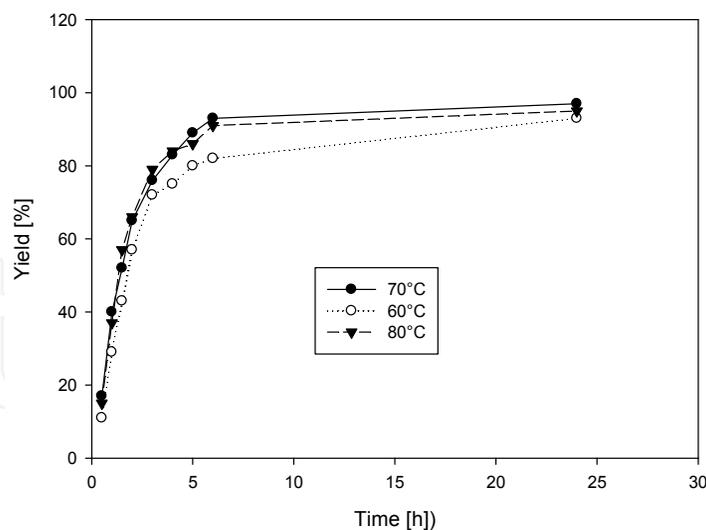


Fig. 9. Influence of reaction temperature on the synthesis of isoamyl-oleate

The yield-time functions in all cases show the saturation curve. Increasing the temperature equilibrium yield was not changed, but shorter time was necessary to achieve it. As it shown neither at 80 °C became the enzyme deactivation significant, but increasing up from 70 °C did not shorten the reaction time till achieving the equilibrium.

## 5. Conclusions

Our investigations have proven that an ester type biolubricant could be prepared from fusel oils and oleic acid by lipase enzyme in solvent-free system. Compared to the product obtained by acid catalysis, in the biolubricant there was no trace of oleic acid since complete conversion was achieved by continuous water removal by pervaporation. Our product was then tested in an acute toxicological procedure by zebrafishes, which has verified the assumption: the biolubricant is not toxic for the living water, so it is considered as an environmental safe product.

The tribological study has shown that the features of the biolubricant are similar to the DB 32 type synthetic reference lubricating oil, so it can be applied as a low viscosity lubricant, suitable even for special publication processes, where lubricant loss may occur. Although the biological degradation of the product has not been studied, it was manufactured from initial compounds having biological origin (oleic acid from plant oils and alcohols from a by-product in bioalcohol production), thus it is considered as a completely environmental-friendly product.

The kinetic model containing the parameters determined can be used in the particular enzymatic esterification reaction for calculations of the optimal conditions of various aspects, like highest yield, lowest acid residue, lowest amount of enzyme, shortest reaction time...etc. Moreover we are planning to apply the model for bioreactor design to realise continuous enzymatic i-amyl oleate synthesis by lipase.

Our work has shown that an ester type biolubricant could be prepared from isoamyl-alcohol and oleic acid by lipase enzyme in ionic liquid two phase system. Compared to the product obtained in solvent free system, higher conversion in shorter time was achieved. Despite the lack of water removal in the biolubricant there was no trace of oleic

acid since complete conversion was achieved. Determining the optimal reaction mixture composition high amount of ionic liquid and enzyme, large excess of alcohol was closest to the optimal. There was no inhibition effect neither at application of 1:16 acid/alcohol molar ratio. Increasing the temperature to 70 °C had a positive impact on the process, at 80 °C desactivation of the enzyme was not occurred, although we did not find higher yield.

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## 7. References

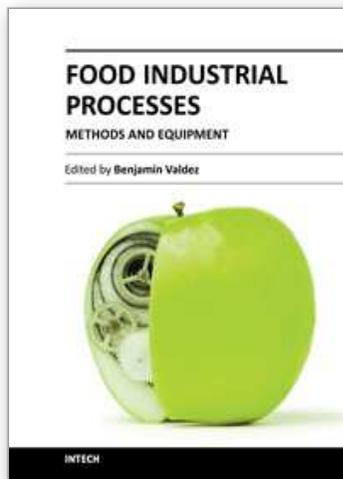
- Bailey, J.E., Ollis D.F., (1986) *Biochemical Engineering Fundamentals*, McGraw Hill Co. Singapore
- Bates D.M., Watts D.G., (1988) *Nonlinear regression analysis and its applications*, Wiley Interscience, New York, USA
- Bélafi-Bakó, K., Dombi, A., Szabó L., Nagy, E., (1994) Triacylglycerol hydrolysis by lipase in a flat membrane bioreactor *Biotechnol. Techn.* 8, pp. 671-674.
- Bezbradica, D., Mijin, D., Siler-Marinkovic, S., Knezevic, Z., (2006) The *Candida rugosa* lipase catalyzed synthesis of amyl isobutyrate in organic solvent and solvent-free system: A kinetic study, *J. Mol. Cat. B: Enzym.* 38 11-16.
- Cleland, W.W., (1979) *Optimizing Coupled Enzyme Assays*, *Anal. Biochem.* 99 142-145.
- Demirbas, A., (2000), *Energy from renewable sources* *Energ. Convers. Manage.* 42 pp. 1357-1378.
- Dossat, A., D. Combes and A. Marty, (2002) *J. Biotechnol.* 97, pp. 117-124.
- Eckstein M., Vilella M., Liese A., Kragl U. (2004) Use of an ionic liquid in a two-phase system to improve an alcohol dehydrogenase catalysed reduction. *Chem. Commun.* 9, 1084-1085.
- Filipini, M., T. Hogg, (1997), *Upgrading of Vegetable Wastes and Applications in the Food Industry. Med. Fac. Landbouww. Uni. Gent.* 62/a pp. 1329-1331.
- Garcia, T., Coteron, A., Martinez, M., (1996) Kinetic Modelling of Esterification Reactions Catalysed by Immobilized Lipases, *J. Aracil, Chem. Eng. Sci.* 51 2841-2846.
- Garcia, T., N. Sanchez, M. Martinez, J. Aracil, (1999) Enzymatic synthesis of fatty esters Part I. Kinetic approach *Enzyme Microbial Technol.* 25 584-590.
- Goddard, R., Bosley, J., Al-Duri, B., (2000) Esterification of oleic acid and ethanol in plug flow (packed bed) reactor under supercritical conditions: Investigation of kinetics, *J. Supercrit. Fluids.* 18 121-130.
- Gubicza L., Kabiri-Badr A., Keoves E., Belafi-Bako K. (2000) Large-scale enzymatic production of natural flavour esters in organic solvent with continuous water removal. *JOURNAL OF BIOTECHNOLOGY* 84:(2) pp. 193-196.
- Gubicza, L., Nemestóthy, N., Fráter T., Bélafi-Bakó K., (2003) Enzymatic esterification in ionic liquids integrated with pervaporation for water removal, *Green Chem.* 5 236-239.

- Gulati, R., P. Arya, B. Malhotra, A.K. Prasad, R.K. Saxena, J. Kumar, A.C. Watterson, V.S. Parmar, (2003) Novel biocatalytic esterification reactions on fatty acids: synthesis of sorbitol 1(6) - monostearate, *ARKIVOC* iii 159-170.
- Hazarika, S., Goswami, P., Dutta, N.N., Hazarika, A.K., (2002) Ethyl oleate synthesis by Porcine pancreatic lipase in organic solvents, *Chem. Eng. J.* 85 61-68.
- Jain N., Kumar A., Chaunhan S., Chaunhan S.M.S. (2005) Chemical and biochemical transformations in ionic liquids. *Tetrahedron* 61, 1015-1060.
- Janssen, A.E.M., Vaidya, A.E., Halling, P.J., (1996) Substrate specificity and kinetics of *Candida rugosa* lipase in organic media, *Enzyme. Microb. Tech.* 18 340-346.
- Koszorz, Z., Nemestóthy, N., Ziobrowski, Z., Bélafi-Bakó, K., Krupiczka, R., (2004), Influence of pervaporation process parameters on enzymatic catalyst deactivation, *Desalination*, 162 307-313.
- Leskovac V., (2003) *Comprehensive Enzyme Kinetics*, Kluwer Academic, New York, USA
- Letisse, F., Lamare, S., Legoy, M.D., Graber, D., (2003) Solid/gas biocatalysis: an appropriate tool to study the influence of organic components on kinetics of lipase-catalized alcoholysis, *BBA-Gen. Sub.* 1652 27-34.
- Linko, Y.-Y. M. Lämsä, X. Wu, E. Uosukainen, J. Seppälä and P. Linko, (1998) Biodegradable. Products by Lipase Biocatalysis *J. Biotechnol.* 66, pp. 41-50.
- Moniruzzaman M., Kazunori N., Noriho K., Masahiro G. (2010) Recent advances of enzymatic reactions in ionic liquids. *Biochem. Eng. Journal* 48., 295-314
- Oliveira, A.C., Rosa, M.F., Aires-Barros, M.R., Cabral, S.M.S., (2001) Enzymatic esterification of ethanol and oleic acid –a kinetic study, *J. Mol. Cat. B Enzym.* 11 999-1005.
- Özgülsün A., F. Karaosmanoglu and M. Tüter, (2000), Esterification reaction of oleic acid with fusel oil fraction for production of lubricating oil *JAOCs* 77 pp. 105-110.
- Özgülsün A., F. Karaosmanoglu, (1999), Environmentally friendly lubricating oil candidate *Appl. Biochem. Biotechnol.* 77 pp. 83-89.
- Perry J.H., (1969) *Chemical Engineers' Handbook*, McGraw-Hill, New York, USA
- Romero M.D., Calvo L., Alba C., Danesfar A., Ghaziaskar H.S. (2005) Enzymatic synthesis of isoamyl acetate with immobilized *Candida antarctica* lipase in n-hexane. *Enzyme Microb. Tech.* 37, 42-48.
- Van der Padt, A., J.J.W. Sewalt, K. van't Riet, (1993) On-line water removal during enzymatic triacylglycerol synthesis by means of pervaporation *J. Membr. Sci.* 80 199-208.
- Van Rantwijk F., Sheldon R.A. (2007) Biocatalysis in ionic liquids. *Chem. Rev.* 107, 2757-2785.
- Watanabe, Y., Shimada, Y., Sugihara A., Noda H., Fukuda H., and Tominaga Y., (2000) Continuous production of biodiesel fuel from vegetable oil using immobilized *Candida antarctica* lipase. *J. Am. Oil. Chem. Soc.* 77, pp. 355-360
- Willing, A., (1999) *Fett/Lipid* 101, pp. 192-198.
- Yadav, G.D., Lathi P.S., (2003) Kinetics and mechanism of synthesis of butyl isobutyrate over immobilised lipases, *Biochem. Eng. J.* 16 245-252.
- Yang Z., Pan W. (2005) Ionic liquids: Green solvents for nonaqueous biocatalysis. *Enzyme Microb. Tech.* 37, 19-28.

Zaidi, A., J.L. Gainer, G. Carta, A. Mrani, T. Kadiri, Y. Belarbi and A. Mir, (2002) Esterification of fatty acids using nylon-immobilized lipase in n-hexane: kinetic parameters and chain-length effects, *J. Biotechnol.* 93, pp. 209–216.

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