

## BIOLUBRICANT PRODUCTION IN IONIC LIQUIDS BY ENZYMATIC ESTERIFICATION

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Esterification of oleic acid with short carbon chain alcohols gives biofuels, with long chain alcohols biolubricants can be produced. Previously biolubricants were successfully produced from fusel oil and isoamyl-alcohol substrates by enzyme catalysed esterification, but solution of polar substrates was problematic, that was solved by using ionic liquids. The optimal reaction parameters such as substrate molar ratios, initial water content, amount of enzyme and type of ionic liquid were determined by experimental design using Statistica software. Some of the ionic liquids have catalytic effect by itself, therefore control reactions were necessary to determine which ionic liquid has negligible catalytic effect. In five of the six investigated ionic liquid the yield was the same without enzyme, while in case of B[mim]PF<sub>6</sub>, the yield was negligible. In shorter time higher yield was achieved in presence of ionic liquid against other methods. Increasing the temperature results higher yield till a limit, higher temperature does not make more product, but thermal desactivation of the enzyme was not occur.

**Keywords:** enzyme biocatalysis, biolubricant, oleate production, ionic liquid two phase system, lipase, esterification

### Introduction

Crude oil is a limited source for fuels, lubricants. Furthermore, it is not renewable and environmentally friendly. Petroleum based lubricants usually deposited in the environment, endangering our planet. To solve this problem, lubricants should be manufactured from plant oil derivatives. At the last ten years more and more product appeared on the market which based on plant oils, for example biodiesel components and biolubricants [1]. There are several industrial application possibilities for fatty acid esters, as natural compounds [2]. Esterification of plant oils, mainly occurs oleic acid and other long-chain fatty acids (12–20 carbon atoms) reaction. Chain length of the alcohol substrate determines the usage of the product. Short-chain (3-5 carbon atoms) and low molecular weight alcohols: biodiesel, biofuel components can be produced [3], beyond fuel production, significant users the food, detergent, cosmetic and pharmaceutical industries [4]. Long-chain alcohol (5 to 12 carbon atoms) reactions give lubricants [5]. The demands against biolubricants are that they should provide maximal protection during the usage, do not pollute the environment and do not accumulate. Usage of biolubricants can reduce the chemical risk, because of the possibility of biodegradability [6]. Important physical effect of ester group is to reduce the volatility and increase the flash point [7], as well as providing a good lubricating ability, because the polar ester group facilitates the bonding to the metal surface. Good

viscosity-temperature characteristic is their typical property and due to the high molecular weight, low evaporation loss, as well. General expectations over against lubricants that tolerate the mechanical effects and stress, biolubricants can resist these criteria. The disadvantages of biolubricants are low oxidative stability and poor cold flow properties [8]. They have wide area of application, machinery moving parts friction between the surfaces, at not too high temperature locations can be used with considerable lubricant loss, as well as in areas with increased exposition to nature, into the bypass. Examples for application: the transmission system of military vehicles, in several other outdoor activities: forestry machines, chain saws, mining, railway vehicles and installations, water transport, fisheries and agricultural machines [9]. Fusel oil is a by-product of distilleries, its average composition is 10% ethanol, 13% n-propanol, 15% i-butanol, 51% isoamyl-alcohol, 11% miscellaneous alcohols and water. Nowadays fusel oil is usually burned at the distilleries to complete the energy necessities. The ionic liquids are organic salts which are built up by cations and anions (not including neutral molecules), they have low-melting point, and up to 300–400 °C they remain liquid without dissolution. They are non-volatile, non-flammable, possessing excellent thermal and chemical stability. Due to low melting point they are usually liquids at room temperature. Furthermore, these cations and anions are almost unlimited variable, there is a possibility to prepare the best medium important for the reaction, taking into consideration for example the solubility of substrates [10]. Ionic liquids are organic

salts, which are empirically built up by cations and anions, do not contain neutral components. They have good thermal stability and low vapour pressure, different combinations of cations and anions can generate wide scale of properties, therefore they are considered as modern, environmentally friendly reaction media. They are non-volatile and flammable, therefore they begun to replace the conventional organic solvents as reaction media. Ionic liquids can be suitable media for biocatalytic synthesis because of their enzyme stabilization effect, reusability and negligible vapour pressure [11]. Many enzymes, especially lipases showed higher activity and greater selectivity in ionic liquids than in organic solvents [12]. The stability of *Candida antarctica* lipase B (CALB) in ionic liquid was found to be higher than in organic solvent [13]. 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 [12,13]. In this work the purpose was to find a utilization of fusel oil where biolubricants can be manufactured in ionic liquid. In our earlier work several enzymatic esterification reactions were investigated in solvent free system and in ionic liquids as well. In the earlier course of our experiments mixture of oleates were prepared successfully using fusel oil and oleic acid as substrates and enzyme catalytic esterification as method in different solvents and in solvent free system, as well [14]. At these experiments 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.

## Materials and methods

**Enzyme:** triacylglycerol hydrolase, E.C. 3.1.1.3., Novozym 435, Novo Nordisk (Bagsværd, Denmark). PLU 1U = 7000 mol/g, 15 min, 1 atm (PLU: propyl laurate unit), water content 1-2%

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

**Chemicals:** 96% oleic acid (Merck, Germany), isoamyl-alcohol 96% modeling fusel oil (Scharlau GmbH), n-hexane (Reanal), ethyl alcohol, diethyl ether, potassium hydroxide (Spectrum 3D).

**GC analysis:** GOW-MAC Series 600 gas chromatograph, HP-FFAP column (Macherey-Nagel), head pressure: 140 kPa, He flow rate: 12 cm<sup>3</sup>/min, H<sub>2</sub>

flow rate: 30 cm<sup>3</sup>/min, air flow rate: 300 cm<sup>3</sup>/min, 1:6 split-ratio, starting time and temperature: 130 °C, 3 min, end temperature and time: 240 °C, 5 min, heating rate 10 °C/min, injection temperature: 250 °C, FID detector. At every measurement point three parallel measurements were made, in each 4 µl samples were taken directly from the reaction mixture.

**Measuring of acid content:** The fatty acid concentration was determined by conventional acid-base titration with 0.1 M ethanolic potassium hydroxide solution.

**Shaking, incubation:** All reactions were carried out in flasks in an IKA KS 4000 shaking incubator.

**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 (this mixture was applied only for calibrating the analytical methods). 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.

**Experimental design:** Statistica 8.0 software

## Results and discussion

The esterification reaction of oleic acid with the isoamyl-alcohol occurs as follows:



In this reversible reaction the molar ratio of substrates, temperature, amount of enzyme and ionic liquid are the variables affecting the conversion.

### Effect of ionic liquid

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 criterium 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 [15]. 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. 1.

## Acid / alcohol molar ratio

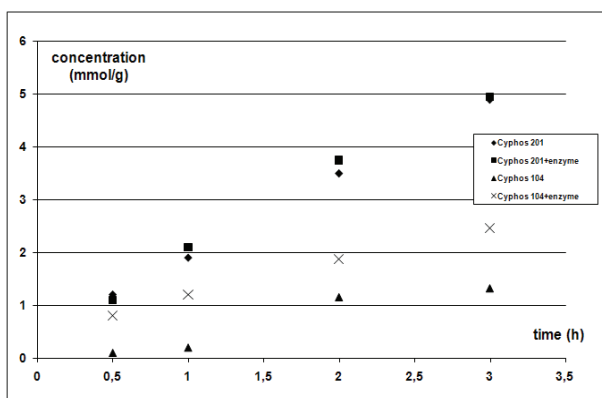


Figure 1: Oleate ester concentration in case of using different phosphonium-type ionic liquids (reaction time: 180 min)

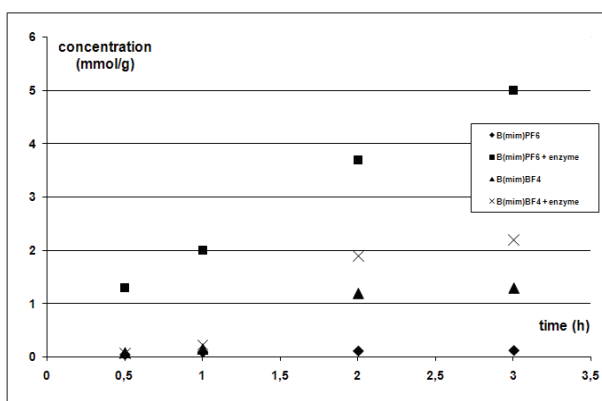


Figure 2: Oleate ester concentration in case of using different imidazolium-type ionic liquids (reaction time: 180 min)

For imidazolium-cation containing ionic liquids literary data show that these types of ionic liquids are the most suitable for esterification, transesterification reactions [16]. Both investigated imidazolium-type ionic liquids were successfully applied in earlier experiments, where natural aroma esters production was the aim. As the results show on Fig. 2 in case of B[mim]BF<sub>4</sub> there were no significant differences in the detected oleate concentration if enzyme was added or not. For B[mim]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 B[mim]BF<sub>4</sub> and B[mim]PF<sub>6</sub> shows that while the first not miscible with water, the later has an unlimited solubility in water. Thus, the hydrophilic B[mim]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 [17]. Further advance that in case of using B[mim]PF<sub>6</sub> side-reactions were not observed.

Acid/alcohol molar ratio is one of the most important parameters in enzymatic esterifications. As the reaction is reversible, an increase in the amount of one of the substrates 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 in a given reaction.

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 [14]. 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. 3, Fig. 4 and Fig. 5.

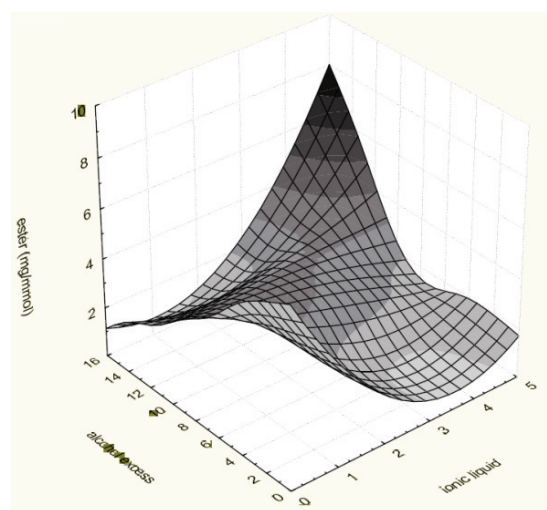


Figure 3: Influence of the acid/alcohol molar ratio and amount of ionic liquid on the synthesis of isoamyl-oleate

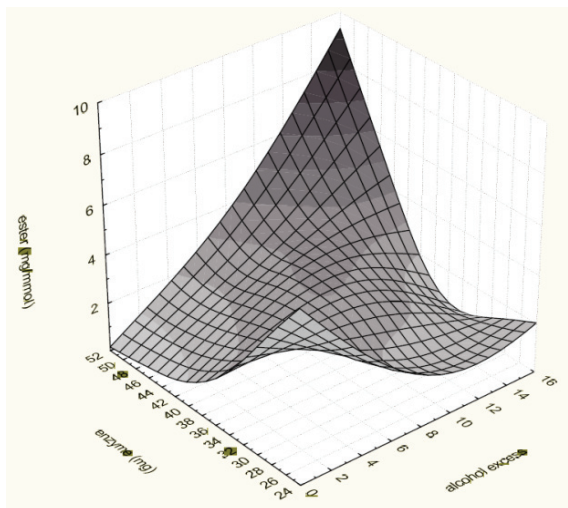


Figure 4: Influence of the acid/alcohol molar ratio and amount of enzyme on the synthesis of isoamyl-oleate

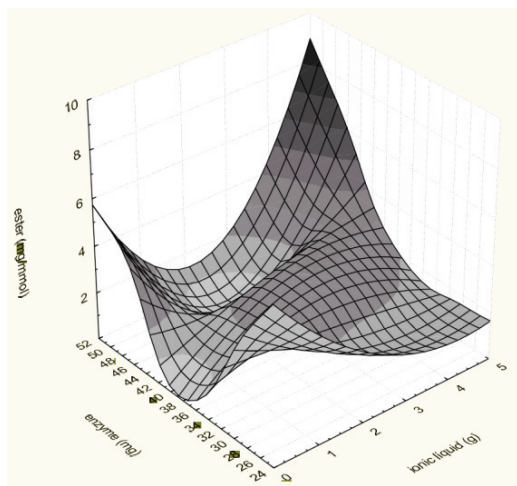


Figure 5: Influence of the amount of enzyme and ionic liquid on the synthesis of isoamyl-oleate

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. 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.

#### *Effect of temperature*

In case of using conventional reaction media the increasing reaction temperature results the same yield in shorter time, but till a limit due to thermal desactivation of enzyme. Beside of the structure stabilization effect of ionic liquids the enzyme can be resist in the active conformation at

higher temperatures [17]. For these investigations that mixture was chosen, in which the highest oleate yield was obtained. The results are shown on Fig. 6.

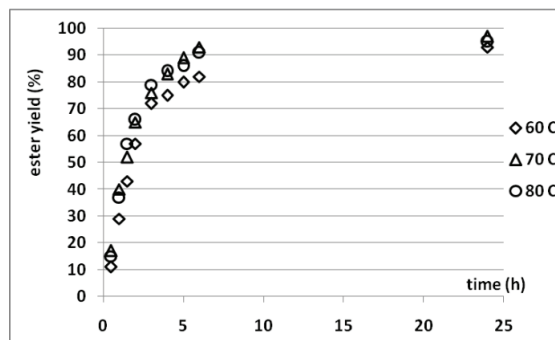


Figure 6: Influence of reaction temperature on the synthesis of isoamyl-oleate

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 desactivation significant, but increasing up from 70 °C did not shorten the reaction time till achieving the equilibrium.

## Conclusions

Our investigations have proven 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 the yield was unchanged.

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