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Investigation of the transesterification efficiency of different immobilized lipase enzymes

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The alkaline catalyzed transesterification has several disadvantages. The aim of our experiments was to eliminate these problems and to investigate the possibilities of the enzyme catalyzed transesterification of Hungarian, appropriately pre-treated rapeseed oil. Transesterification efficiencies of three commercially available immobilized lipases (*Candida antartica* (Novozym 435), *Rhizomucor miehei* (Lipozyme RM IM) and *Thermomyces lanuginosus* (Lipozyme TL IM)) were investigated (temperature: 30-70°C, methanol:triglyceride molar ratio: 4:1, amount of catalyst: 6-12% based on the amount of the total reaction mixture, methanol feed: more than 1 portions). Based on the properties of the products we established that yields of the methyl ester phases and their methyl ester contents were significantly different in case of the applied lipase enzymes. The highest product yields (97.4-98.5% of the theoretical value) and methyl ester content (>98%) were achieved by the application of *Candida antartica* (Novozym 435). The quality properties – except the CFPP values – of these biodiesels satisfied the requirements of the EN 14214:2003 standard.

1. Introduction

Recently, research and production of biofuels is in the focus of mankind, due to the non-uniform distribution and the depletion of crude oil reserves, the periodically and rapidly changing crude oil prices, the utilization of renewable energy sources, to replace import energy carriers, to reduce the import dependence, the political potential to support rural population, utilization of fallow lands, the lower lifecycle carbon-dioxide emission, and the contribution to the protection of soil and water sources.

Application of transportation fuels containing biomass-derived components has emerged into focus in the last couple of years. The main reason of this tendency is the energy policy of the European Union, declared in Directive 2003/30/EC of the European Council and Parliament.

Member states should ensure that a minimum proportion of biofuels and other renewable fuels are placed on their markets, and shall set national indicative targets. A reference value for these targets shall be 2.0%, calculated on the basis of energy content, of all petrol and diesel for transport purposes placed on their markets by 31 December 2005 (Directive 2003/30/EC, 2003).

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By 2005 1.0% of the fuels consummated by the EU was biomass derived, thanks to the 2003/30/EC directive of the European Union which helped to merge the use of biofuels (COM(2006) 845 final, 2007).
The most recent proposal of the European Union is to raise the share of biofuels to 10% by 2020 when the following conditions are accomplished:

- sustainable production,
- the second generation biofuels are commercially available,
- the motor fuel standards are modified according to this (COM(2006) 848 final, 2006).

This proposed value can be reached by fuel purpose utilization of chemically converted vegetable oils and other triglyceride containing feedstock (fish oil, used frying oil, animal fat, algae oil, etc.):

- transesterification to biodiesel fuels,
- production of fuel blending components by different cracking and hydrotreating processes (engine gasoline, JET, diesel fuel).

Recently, among these methods the use of biodiesels gained by the transesterification of triglycerides by methanol is the most preferred.
Nowadays, the industrial production of biodiesel fuel is performed by transesterification of triglycerides using alkaline catalysts, but the enzyme-catalyzed transesterification has emerged into focus in the last years. Primarily the applicability of lipase enzymes are investigated for the enzyme-catalyzed transeterification of triglycerides from different sources. But the experimental results are insufficient, especially in solvent-free medium (Fukuda et al., 2001, Iso et al., 2001, Köse et al., 2002, Shimada et al., 2002, Soumanou and Bornscheuer, 2003).

2. Experimental

The main goal of our experiments was to investigate the possibilities of the enzyme catalyzed transesterification of appropriately pre-treated, Hungarian rapeseed oil in solvent free medium. Transesterification efficiency of three commercially available immobilized lipases (E.C. 3.1.1.3. subgroup) *Candida antartica* (Novozym 435), *Rhizomucor miehei* (Lipozyme RM IM) and *Thermomyces lanuginosus* (Lipozyme TL IM) were investigated (temperature: 30-70°C, methanol:triglyceride molar ratio: 4:1, amount of catalyst: 6-12% based on the amount of the total reaction mixture, methanol feed: more equal portions). The operational parameters during our experimental work were based on our previous results (Bélafi-Bakó et al., 2002, Kovács et al., 2007).

2.1 Experimental apparatus

The enzyme catalyzed transesterification was carried out in heated shaker equipment with a capacity of 9 Erlenmeyer flasks (New Brunswick G24). Simultaneously all feedstocks can be put into the shaker, so the same parameters for each sample can be assured.

2.2 Materials and their preparation

During the transesterification reactions Hungarian rapeseed oil (Table 1) and analytical grade methanol (SPEKTRUM 3D) were used. Before the transesterification the first

step was the pretreatment of the different vegetable oils with Tonsil® adsorption clay and adequate volume of pertfil filter aid.

Table 1 The fatty acid composition of the rapeseed oil

Fatty acid composition, %	Rapeseed oil
Myristic acid (C14:0*)	0.0
Palmitic acid (C16:0)	4.6
Palmitoleic acid (C16:1)	0.2
Stearic acid (C18:0)	1.7
Oleic acid (C18:1)	61.1
Linoleic acid (C18:2)	20.5
Linolenic acid(C18:3)	9.5
Arachidic acid (C20:0)	0.6
Eicosenoic acid (C20:1)	1.5
Behenic acid (C22:0)	0.3

*The first number represents the number of carbon atoms and the second means the number of double bonds in the molecule

The investigated enzyme catalysts were the macroporous resin immobilized lipase *Candida antarctica* (Novozym 435) (activity: 7000 PLU/g, density: 430 kg/m^3, diameter: 0.3-0.9 mm), acrylic resin immobilized *Thermomyces lanuginosus* (Lipozyme TL IM) (activity: 250 IUN/g, density: 400 kg/m^3, diameter: 0.4-0.9 mm) and anion-exchange resin immobilized *Rhizomucor miehei* (Lipozyme RM IM) (activity: 150 IUN/g, density: 415 kg/m^3, diameter: 0.3-1.0 mm).

2.3 Test method

The main properties of the feeds and products were determined by standard test methods specified by EN 14214: 2004 (Automotive fuels. Fatty acid methyl esters (FAME) for diesel engines. Requirements and test methods), complying with the specified precision data.

2.4 Results and discussion

In case of enzymatic catalysis first of all the temperatures had to be determined whereon the selected immobilized lipase enzymes (*Candida antarctica, Rhizomucor miehei, Thermomyces lanuginosus*) provided the highest activity.

During the transesterification experiments 44 g of vegetable oil and 3 g of immobilized lipase (6 % of the total amount of reactants) were used. The applied methanol-to-triglyceride molar ratio was 4:1 (6.4 g methanol) instead of the stochiometric ratio of 3:1, considering that excess methanol favors the progress of the reaction. This amount was added to the reaction mixture in 3 equal parts because the stepwise addition is necessary to prevent the inhibiting effect of the methanol. For the complete (enzyme-catalyzed) transesterification of different triglycerides at least stochiometric quantity of methanol is needed. According to the experiments of us and other researchers (Shimada et al., 2002) this stochiometric quantity of methanol can not dissolve in the vegetable oils, used cooking oils and in animal fats. In case of the alcohols containing three or more carbon atoms this problem do not occur, these alcohols can totally dissolve in the

different triglycerides. In our previous experiments we noticed that above 0,5 methanol/fatty acid molar ratio the rate of the enzyme-catalyzed reaction decreased significantly, the immobilized *Candida antarctica* lipase enzyme partially inactivated. Probably the insoluble methanol present in the mixture builds drops and disperses in the oil. These methanol drops can contact the lipase enzyme and neutralize it, so this leads to the enzyme deactivation.

After the reactions the ester containing phase was separated and the excess of methanol was removed by vacuum distillation. Thereafter, the yield of the products (methyl ester phase and glycerol phase) and the methyl ester content of the ester phase were determined.

Based on our experimental results it was found that the highest product yields (> 95%) and methyl ester content were achieved by the application of *Candida antartica* and *Thermomyces lanuginosus* at 50°C, meanwhile with *Rhizomucor miehei* at 40 °C (Figure 1).

In the next step of our experiments we investigated the effect of the number of methanol feeds and effect of the catalyst amount on the methyl ester content of the products in case of the three different enzymes at the optimal temperature. The parameters of the experiments can be seen in Table 2.

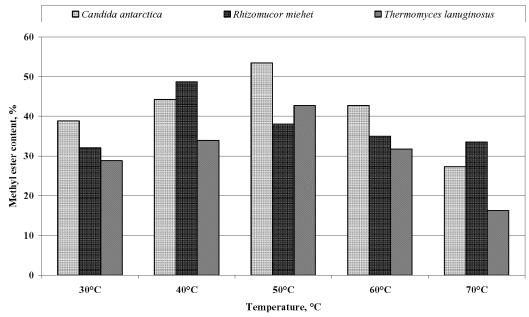

Figure 1: Methyl ester of the products after 16 hours reaction time in different temperatures

Table 2 Parameters of the experiments

Experiment	Amount of enzyme, %	Number of methanol feeds
M-1	6	1
M-2	6	2
M-3	6	3
M-4	6	4
M-8	6	8
E-6	6	8
E-9	9	8
E-12	12	8

The effect of the number of methanol feeds on the methyl ester content of the products can be seen in Fig. 2.-4. From the results it can be concluded that the more number of

methanol feeds were applied the higher the conversion was. The reason is that the enzyme deactivation level decreased with the decreasing methanol concentration of the reaction mixture caused by the more methanol feeds. Figure 5.-7. illustrate the effect of the amount of enzyme on the methyl ester content of the products in case of the three applied enzymes. Based on the results it can be concluded that by increasing the amount of the applied catalyst (6-12 %) the methyl ester contents of the products increased in case of all three lipases.

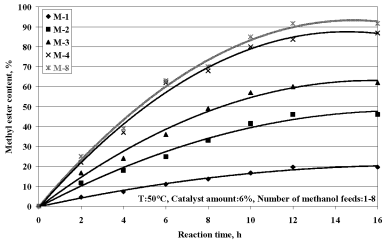

Figure 2: The effect of the methanol feeding on the methyl ester content of the products (Candida antarctica)

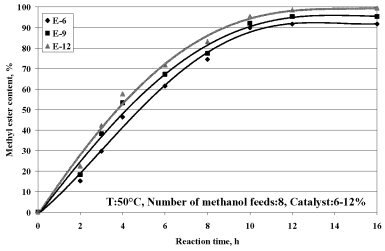

Figure 5: The effect of the catalyst amount on the methyl ester content of the products (Candida antarctica)

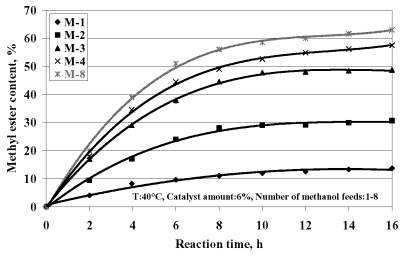

Figure 3: The effect of the methanol feeding on the methyl ester content of the products (Rhizomucor miehei)

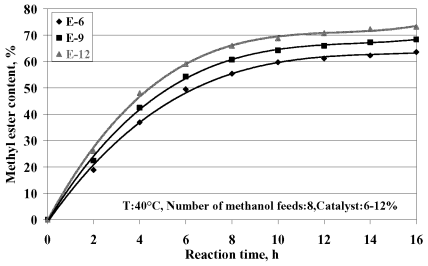

Figure 6: The effect of the catalyst amount on the methyl ester content of the products (Rhizomucor miehei)

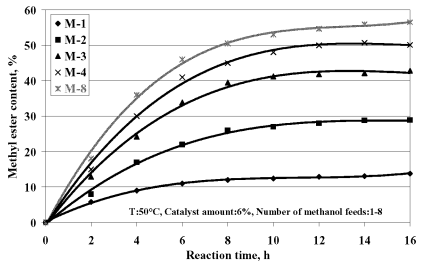

Figure 4: The effect of the methanol feeding on the methyl ester content of the products (Thermomyces lanuginosus)

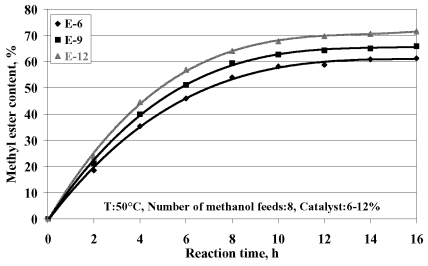

Figure 7: The effect of the catalyst amount on the methyl ester content of the products (Thermomyces lanuginosus)

3. Conclusion

Based on our experimental results it was found that the yield of the methyl ester phase approached the theoretical value to 97-98.5% when we applied *Candida antarctica* and the methyl ester changed between 91.7 and 99.4 %. The change of the number of methanol feeds and the amount of enzyme had favorable effect on the methyl ester content of the products in case of the three immobilized lipase.

The favorable temperature was 40°C when we applied *Rhizomucor miehei* – Lipozyme RM IM immobilized lipase. In case of the other two enzymes (*Thermomyces lanuginosus* – Lipozyme TL IM and *Candida antarctica* - Novozym 435) the advantageous temperature was 50°C.

Based on our experimental results it was found that the highest methyl ester content was achieved when we applied advantageous temperature, stepwise methanol addition and 12% enzyme of the total amount of reactants and Novozym 435 immobilized lipase catalyst. In case of the other two enzymes (*Thermomyces lanuginosus* – Lipozyme TL IM and *Rhizomucor miehei* – Lipozyme RM IM) so high methyl ester content could not be reached even after 24 hours reaction time.

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