We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

3

Biodiesel Fuel Production by Enzymatic Transesterification of Oils: Recent Trends, Challenges and Future Perspectives

Nevena Luković, Zorica Knežević-Jugović and Dejan Bezbradica
Faculty of Technology and Metallurgy, University of Belgrade
Serbia

1. Introduction

Liquid fuels have been used for many years as the most dominant and basic fuel for motor engines. However, declining fossil fuel resources as well as the tendency for developing new renewable biofuels have shifted the interest of the society towards finding novel alternative fuel sources. Biodiesel (monoalkyl esters of long-chain fatty acids) has a great potential as an alternative diesel fuel. From an environmental point of view it shows clear advantages over conventional fuel: it comes from renewable sources, and hence does not contribute to new carbon dioxide emission, it is biodegradable, its combustion products have reduced levels of particulates, sulphur oxides, carbon oxides, nitrogen oxides, and therefore, significantly reduces pollution (Al-Zuhair, 2007; Salis et al., 2005). One of the advantages of biodiesel in comparison to other biofuels is that biodiesel can be pumped, stored and handled using the same infrastructure employed for conventional diesel fuel (Robles-Medina et al., 2009). Also, major advantage of biodiesel as an alternative fuel is that its energy content is similar to conventional fuels, so it can be used either on its own or mixed with conventional diesel fuel, with no need of altering existing engines (Bozbas, 2005). European countries have recognized need for alternative fuels and issued the Directive on the Promotion of the use of biofuels and other renewable fuels for transport (2003/30/EC). The Directive stipulates that EU countries should replace 5.75% of fossil fuels with alternative, biofuels until 31. December 2010. This Directive has been amended by Directive 2009/28/EC which also promotes the usage of energy from renewable sources (aims at achieving a 20% share of energy from renewable sources in the EU's final consumption of energy by 2020). In 2005, the estimated world production of biodiesel was 2.92 million tones of which 87% was obtained in EU. More importantly, between 2000 and 2005 world production increased threefold, indicating that share of biodiesel in global fuel production will significantly increase in future (Mousdale, 2008).

There are several possible processes for biodiesel synthesis: pyrolysis, the use of microemulsions and transesterification. Though pyrolysis, due to reduced viscosity, gives good quality fuel it still produces more biogasoline than biodiesel fuel. Fuel produced by microemulsion creates engine performance problems (Fukuda et al., 2001; Ma and Hanna, 1999). Conventionally, biodiesel is produced by transesterification of triacylglycerol (TAG) and short chain alcohols, commonly methanol, in the presence of an acid or an alkaline

catalyst (Marchetti et al., 2007). By this process the flashpoint of biodiesel is lowered and the cetane number is improved (Parawira, 2009). Transesterification is a three-step consecutive reaction, in which diglycerides and monoglycerides are formed as an intermediate compounds (Figure 1. and Figure 2.). Three moles of biodiesel and one mole of glycerol are produced for every mole of TAG that undergoes completely conversion. It is a reversible reaction and accordingly, excess alcohol is used to shift the equilibrium to the products side.

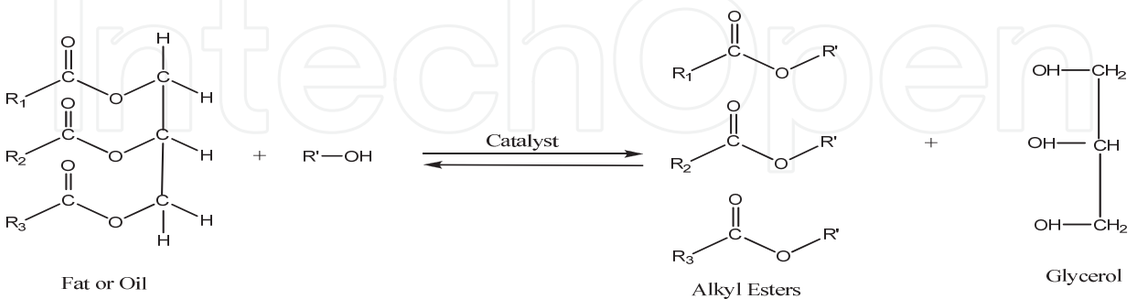


Fig. 1. General equation of transesterification of triacilglycerols with alcohol

This approach of the biodiesel synthesis has been used on industrial scale for decades, but there are several problems associated with this kind of production. Complex removal of catalyst, excessive energy requirements, recovery of glycerol, undesirable side reactions and the cost of the refined feedstock are the major drawbacks for such chemical process. Not only that the preliminary processing of feedstock is required (to reduce soap formation), but waste water must be treated as well (Fjerbaek et al., 2008). There have been several attempts to overcome these problems and to develop the alternative methodologies for biodiesel production. Nowadays, the biological production of biodiesel with lipases has received great consideration and it is undergoing a rapid development.

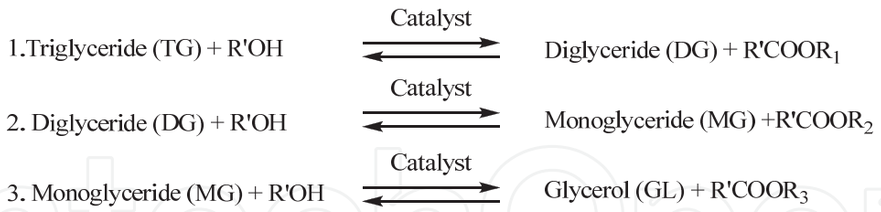


Fig. 2. Transesterification of triacilglycerols with alcohol- Three consecutive and reversible reactions

The main advantages of employment of lipases as biocatalyst are mild reaction conditions and easy recovery of glycerol without purification or chemical waste production and production of very high purity product. In addition, free fatty acids content in the oil can be completely converted to methyl esters, with no soap formation, and thus increasing the biodiesel yield and reducing the costs for fuel purification. This characteristic of the enzymes allows the usage of materials with high free fatty acids (FFA) or high water content such as non-edible oils, waste cooking oils and industrial waste oil. Transesterification of triglycerides using lipases is considered to be one of the most efficient processes of biodiesel production from waste oil. It is clear that production process for alkaline method is more complex than enzymatic procedure as it is shown in Figure 3. and Figure 4.

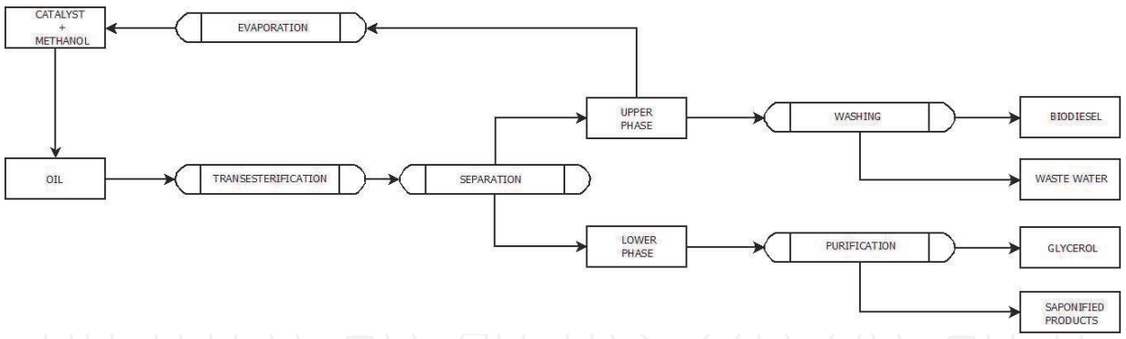


Fig. 3. Biodiesel production by alkali process

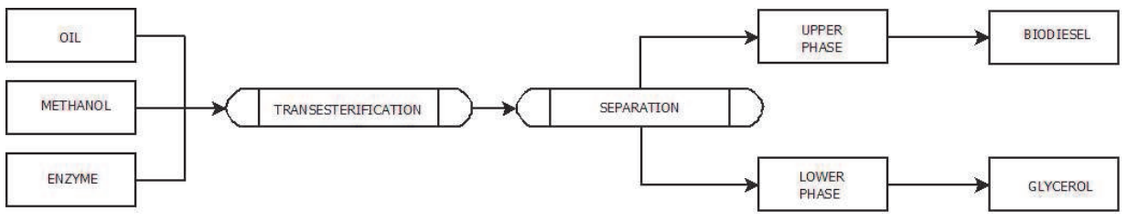


Fig. 4. Enzymatic biodiesel production

Despite numerous advantages, enzymatic processes have drawbacks such as: low reaction rate, enzyme cost for industrial scale use in comparison to alkali catalyst, low enzyme stability in the presence of excess methanol (Bajaj et al., 2010; Fjerbaek et al., 2008). In order to make enzymatic transesterification competitive on industrial scale there are several issues that have to be addressed: solvent engineering, lipases immobilization, selection of acyl acceptor, and selection of the reactor system. In this chapter we will try to review offered solutions to the current problems and to look for new perspectives in the industrial application of enzymatic biodiesel synthesis.

2. Lipases as biocatalysts in biodiesel synthesis

Lipases (triacylglycerol hydrolase, EC 3.1.1.3.) are enzymes that catalyze the hydrolysis of carboxylic ester link in the triacylglycerol molecule to form free fatty acids, di- and monoglycerides and glycerol. Although their natural function is to catalyze hydrolysis of ester links, they can also catalyze the esterification, the creation of this link between alcohol hydroxyl groups and carboxyl groups of carboxylic acids. Therefore, since they can catalyze hydrolysis, alcoholysis, esterification and transesterification they have a wide spectrum of biotechnological applications.

Lipases are also highly specific as chemo-, regio- and enantioselective catalysts. Thanks to direct evolution and protein engineering it is possible to enhance catalytic potential of lipases and to 'tailor' them to specific application and process conditions, enabling further expansion of their industrial use (B van Beilen and Li, 2002; Kirk et al., 2002). Among lipases of plant, animal and microbial origins, most commonly used are microbial lipases. They have numerous advantages over lipases from animals and plants. Using microorganisms it is possible to achieve a higher yield of enzymes, and to genetically manipulate the producing strain in obtaining a low-cost lipase with desired properties for the conversion of natural fats and oils into biodiesel. In addition, the enzymatic yield is independent of

potential seasonal variations and it is possible to achieve rapid growth of microorganism in low-cost culture media (Gupta et al., 2004).

Lipases	Oil	Acyl acceptor	Solvent	Time	Yield	Reference
Candida antarctica B	Waste cooking palm oil	Methanol	*tert*-butanol	4h	79.1%	Halim et al. (2009)
Thermomyces lanuginosa	Soybean oil	Ethanol	*n*-hexane/solvent free	10h	70%-100%	Rodrigues et al. (2010)
Candida rugosa *Pseudomonas fluorescens* *Mucor javanicus* *Burkholderia cepacia*	*Jatropha* seed oil	Ethanol	solvent-free	8h	98%	Shah and Gupta (2007)
Candida antarctica	Cotton seed oil	Methanol	*tert*-butanol	24h	97%	Royon et al. (2007)
Rhizomucor miehei *Penicilium cyclopium*	Soybean oil	Methanol	solvent-free	12h	68%-95%	Guan et al. (2010)
Candida antarctica B	Soybean oil	Methyl acetate	solvent-free	14h	92%	Du et al. (2004)
Thermomyces lanuginosa	Sunflower oil Soybean oil Waste cooking oil	Methanol	solvent-free	24h	90%-97%	Dizge et al. (2009)
Candida antarctica	Sunflower oil	Methyl acetate	solvent-free	12h	>95%	Ognjanovic et al. (2009)
Candida antarctica	Cotton seed oil	Methanol, Propanol, Butanol, Amyl alcohol	solvent-free	7h	91.5%	Kose et al. (2002)
Candida antarctica	Rapeseed oil	Methanol	solvent-free	24h	91.1%	Watanabe et al. (2007)
Thermomyces lanuginosa *Candida antarctica*	Rapeseed oil	Methanol	*tert*-butanol	12h	95%	Li et al. (2006)
Candida antarctica	Jatropha seed oil Karanj oil Sunflower oil	Ethyl acetate	solvent-free	12h	>90%	Modi et al. (2007)

Lipases	Oil	Acyl acceptor	Solvent	Time	Yield	Reference
Rhizomucor miehei	Sunflower oil	Methanol	n-hexane	24h	>80%	Soumanou and Bornscheuer (2003)
Thermomyces lanuginosa	Sunflower oil	Methanol	solvent -free	24h	>90%	
Pseudomonas fluorescens						
Candida sp. 99-125	Waste cooking oil	Methanol	*n*-hexane	10h	91.08%	Chen et al. (2009)
Penicillium expansum	Waste oil	Methanol	*t*-amyl alcohol	24h	92,80%	Li et al. (2009)
Bacillus subtilis	Waste cooking oil	Methanol	solvent-free	72h	>90%	Ying and Chen (2007)
Enterobacter aerogenes	Jatropha seed oil	Methanol	tert-butanol	48h	94%	Kumari et al. (2009)
Criptococcus SP-2	Rice bran oil	Methanol	solvent-free	120h	80,20%	Kamini and Iefuji (2001)
Burkholderia cepacia	Soybean oil	Methanol	solvent-free	90h	>80%	Kaieda et al. (2001)

Table 1. Biodiesel production with various lipases

Reaction system for lipase is a two-phase system consisting of aqueous phase with dissolved enzyme and an organic phase with dissolved substrate. Lipases have a specific reaction mechanism due to the fact that active site of the enzyme is covered by amphiphilic peptide loop that acts like a lid. This disables the substrate molecule to bind to the enzyme active site, resulting with negligible activity of lipases in aqueous solutions (in the absence of an interphase or organic solvent). When contact occurs with a lipid/water interface, lid undergoes a conformational rearrangement which renders the active site accessible to the substrate (Schmid and Verger, 1998).

A large number of lipases from different sources have been utilized for biodiesel synthesis as shown in Table 1. *Candida antarctica* B lipase has been the most studied enzyme for biodiesel production in various reaction systems. It catalyzes acyl transfer reactions of various oils and acyl acceptors (alcohols or esters) showing high stability in organic solvents and broad substrate specificity. However, the lipase from *Pseudomonas* has also many advantages considering enzyme stability in a water-containing system in the presence of methanol. Kaieda et al. (2001) reported that the lipase from *P. cepacia* has much higher methanol resistance than those from *Rhizopus oryzae, P. roqueferoti*, *C. lipolytica* and even *C. antarctica*, making it more attractive for use as a biocatalyst in methanolysis reaction processes. Soumanou and Bornscheuer (2003) confirmed that lipase from *P. fluorescens* showed the greatest methanol resistance among the tested lipases, and a rather high yield of biodiesel (>90%) was obtained even at 4.5 M equivalents of methanol in the solvent free system. Other lipases also have been reported to be an efficient biocatlyst for the methanolysis reaction in the solvent-free system. For example, Kamini and Iefuji (2001) found that a crude lipase from the yeast *Cryptococcus* spp. S-2 efficiently catalyzed the methanolysis of vegetable oil even in a water-containing system without an organic solvent and in a single step, avoiding the stepwise addition of methanol. In general, lipases produced from yeast and fungi are most commonly used due to their low price and accessibility, although several bacterial lipases such as those from Pseudomonas sp., *Enterobacter aerogenes*, *Bacillus subtilis*, and others have been thoroughly investigated because

of their superior methanol resistance (Al-Zuhair, 2007; Jaeger and Eggert, 2002; Noureddini et al., 2005). The main characteristic of lipases for biodiesel production from triacylglycerols is that they should be nonstereospecific so that all tri-, di- and monoglycerides can be converted to fatty acid alkyl esters. It is also an imperative that they should catalyze the esterification of free fatty acids (Fjerbaek et al., 2009). In addition, commercially available lipases (Lipozyme, Novozyme) appear to be more suitable catalysts for large scale application, since the use of free *Pseudomonas* or other lipases requires the additional immobilization step (Kose et al., 2002; Samukawa et al., 2000; Watanabe et al., 2001; Soumanou and Bornscheuer, 2003; Ognjanović et al., 2008).

2.1 Lipases immobilization

One of the main obstacles for industrial application of lipases is the high cost of biocatalysts. Therefore, immobilization of lipases, which allows their reusability, is a necessity to make them more attractive for industrial biodiesel processes. The aim of immobilization is to enhance lipases properties such as thermostability and activity in non-aqueous media, and to improve handling, recovery and recycling of biocatalyst. Recycling of immobilized enzymes greatly reduces the cost of the production, so the most promising immobilization supports and methods could make the enzymatic biodiesel production competitive to chemical processes.

By definition, the immobilization of enzymes is localization or confinement of an enzyme on to a solid support or on a carrier matrix. There are a number of supports that can by used for immobilization and the selection depends on several factors: thermal stability, chemical durability, mechanical strength, lipase type, type of the reaction system, ease of regeneration, loading capacity and cost (Jegannathan and Abang, 2008). Generally, immobilization is accompanied by changes in enzyme activity, optimum pH, temperature and stability. The productivity of an immobilized system is evaluated through both, activity and stability of a biocatalys. Often, the loss of enzyme activity is compensated by enhanced operational stability, which in overall makes the immobilization method a viable procedure. Methods for enzyme immobilization can be classified as physical adsorption, entrapment, covalent bonding and encapsulation, each with its advantages and disadvantages. Different immobilization techniques of lipases used as catalysts in biodiesel production are summarized in Table 2.

Adsorption is the most widely used method for lipase immobilization primarily because procedure is easy, conditions are mild and the method itself is cheap (Hilal et al., 2006; Nie et al., 2006; Yagiz et al., 2007). This technique involves no toxic chemicals, supports can be regenerated for repeated use, and there is no major activity loss. Various supports have been used such as: textile membrane, alumina, ceramics, sepharose, sepadex, cellulose, hydrotalcite, different type of zeolites, celite, silica gel, polyethylene, polypropylene and others. The nature of support strongly dictates catalytic properties of the lipase such as activity, selectivity, stability (Knežević et al. 2004). Yang et al. explored the influence of pore diameter of nonpolar and weakly polar resin on degree of immobilization and reported that the degree of immobilization increases with increasing pore diameter. Also, the higher lipase activity was obtained employing nonpolar resins (Yang et al. 2006). It has been documented that lipase activity is generally higher with hydrophobic support. At low ionic strength, hydrophobic areas surrounding the active site are adsorbed on hydrophobic support, leaving stabilized the open form of the lipase (Mateo et al. 2007; Petkar et al. 2006).

Shah and Gupta immobilized *Burkholderia cepacia* on celite and concluded that the immobilized lipase performed better than free enzyme, under the same conditions. The immobilized enzyme gave a higher biodiesel yield and, in general, immobilized enzymes are known to give better catalytic performances in non-aqueous media (Shah and Gupta, 2007). However, reusability of immobilized lipases has been reported to decrease after four cycles. Since the enzymes are attached on the surface of support by weak forces (van der Walls, hydrophobic interactions, hydrogen bonds) they are easily stripped off. This leads to inevitable loss of catalytic activity which is not caused by enzyme inactivation. This is the main reason why the immobilization of lipases by adsorption is not the best solution for industrial application.

Immobilization method	Carier used	Source of lipases	Oil	Acyl acceptor	Yield	Reference
Adsorption	Celit	*Burkholderia cepacie*	Jatorpha oil	Ethanol	98%	Shah and Gupta (2007)
Adsorpiton	Cotton membrane	*Candida* sp. 99-125	Waste oil	Methanol	92%	Nie et al. (2006)
Adsorption	Hydrotalcite and zeolites	*Thermomyces lanuginosus*	Waste oil	Methanol	92,80%	Yagiz et al. (2007)
Adsorption	Nonpolar resin	*Candida* sp. 99-125	Soybean oil	Methanol	97,30%	Yang et al. (2006)
Adsorption	Toyonite 200-M	*Burkholderia fluorescens*	Safflower oil	Methanol		Iso et al. (2011)
Adsorption	Polypropylene support, Accurel MP 100	*Burkholderia cepacia*	Mahua oil	Ethanol	96%	Kumari et al. (2007)
Cross-linked enzyme aggregates (CLEAs)	None	*Burkholderia cepacia*	Mahua oil	Ethanol	92%	Kumari et al. (2007)
Protein-coated microcrystals (PCMCs)	None	*Burkholderia cepacia*	Mahua oil	Ethanol	99%	Kumari et al. (2007)
Entrapment	Hydrophobic sol-gel support	*Burkholderia cepacie*	Soybean oil	Methanol, ethanol	65%	Noureddini et al. (2005)
Entrapment	Phyllosilicate sol-gel matrix	*Burkholderia cepacie*	Restaurant grease	Methanol, ethanol	87-95%	Hsu et al. (2002)
Adsorption	Macropouros polypropylene	*Burkholderia fluorescens Burkholderia cepacie*	Soybean oil	Methanol	98%	Salis et al. (2008)
Entrapment	Phyllosilicate sol-gel matrix	*Burkholderia cepacia*	Restaurant grease	Ethanol	96%	Hsu et al. (2004)
Encapsulation	Silica aerogels	*Burkholderia cepacia Candida antarctica*	Sunflower oil	Methyl acetate		Orcaire et al. (2006)

Immobilization method	Carier used	Source of lipases	Oil	Acyl acceptor	Yield	Reference
Covalent bond	Aldehyde-Lewatit	*Thermomyces lanuginosus*	Soybean oil	Ethanol	75%-100%	Rodrigues et al. (2010)
Covalent bond	Silica -PVA composite	*Burkholderia cepacia*	Babassu oil	Ethanol	98%	Freitas et al. (2009)
Covalent bond	Novel microporous polymeric matrix (basically copolimer of styrene cross-linking with divinilbenzene)	*Thermomyces lanuginosus*	Sunflower oil	Methanol	63,80%	
			Soybean oil		55,50%	Dizge et al. (2009)
			Waste cooking oil		50,90%	
Covalent bond	Chitin activated with hexamethylenediamine (HEMDA)	*Candida rugosa*	Butyric acid	Butanol	187	Gomes et al. (2004)
Covelent bond	Amino-functionalized magnetic nanoparticles	*Thermomyces lanuginosa*	Soybean oil	Methanol	94%	Xie and Ma (2009)
Covelent bond	Silica-PVA composite	*Burkholderia fluorescens*	Palm oil	Ethanol	98%	Moreira et al. (2007)

Table 2. Different techniques of the immobilization of lipases used in biodiesel synthesis

Lipases immobilized by entrapment are more stable than physically adsorbed lipase. Entrapment of lipase includes capture of the lipase within a matrix of a polymer. The porose nature of the matrix allows the retention of the enzyme within the support, also enabling substrate and product diffusion. This method uses a relatively simple procedure and a number of natural and organic supports: alginate, agarose, gelatin, phyllosilicate sol-gel matrix. A variety of methods have been documented. For example, Meunier and Legger developed a procedure for entrapment of lipase from *Candida antarctica* on sol-gel supported on diatomaceous earth (Meunier and Legger, 2010). Although the loading of the enzymes and the activity of immobilized enzymes were high, the low conversion of triacylglycerol was noticeable. The ester yield in the reaction of triolein and methanol using lipase from *C. antarctica* immobilized in particles obtained via sol-gel process was around 60%. The low conversions are result of a poor diffusion and enzyme leakage which are a common problem in gel entrapped systems. However, recent advances in materials synthesis have allowed the preparation of an efficient immobilized lipase system suitable for designing a bioreactor for the continuous production of biodiesel. Namely, Hsu et al. (2002) have developed an original procedure to entrap lipase from *P. cepacia* (PS-30) within a phyllosilicate sol-gel matrix (IM PS-30) (Hsu et al. 2002; 2004). Lipase prepared in this way exhibit remarkable stability under normally denaturing conditions. Namely, the immobilized IM PS-30 lipase has shown to be more active biocatalyst and gave higher conversion to ester than both commercial *C. antarctica* lipase supported on a macroporous acrylic resin and *P. cepacia* lipase supported physically on granulated silica. The immobilized *Burkholderia cepacia* was used in transesterification of soybean oil and a conversion of around 95% after 48 h of reaction was achieved (Hsu et al., 2002).

Encapsulation is the confinement of enzyme within a porous membrane forming a bilayer (Jegannathan et al. 2008). In a way, it provides a cage, which prevents enzyme leaching making the resulting immobilized enzyme a highly reusable biocatalyst. Encapsulation can be carried out by using natural polymers, such as alginate and carrageenan, synthetic polymers (photo-cross linkable resins and polyurethane polymers), acrylic polymers, hydrogels, microemulsion based gels and those obtained by sol–gel methods (Yadav and Jadhav, 2005). For the use in biodiesel synthesis lipases from *Burkholderia cepacia* and *C. antarctica* were encapsulated in silica aerogels reinforced with silica quartz fiber felt and dried by the CO_2 supercritical technique (Orcaire et al. 2006). Under optimum reaction conditions final conversion of 56% was achieved and the operational stability of the immobilized lipase was high. However, with the increase of the substrate concentration limitation by diffusion occurs. The solution is to produce an encapsulated enzyme with smaller size to overcome the mass transfer problems, and to use the enzymes of highest purity (Jegannathan et al. 2008).

The main advantage of covalent immobilization is the irreversible bonding of the lipase to the support matrix, thereby preventing leaching of the enzyme in the reaction system. The immobilization by covalent bonding is the most complex one. Since the supports don't have the reactive groups but hydroxyl, amino, amide and carboxy groups, they have to be activated for immobilization of enzymes. Therefore, covalent immobilization usually consists of two stages: activation or modification of the support and attachment of the enzyme on the activated support. Activation of the support implies chemical reaction between support and an activating agent, creating new (usually electrophilic) groups on its surface that show a great affinity towards functional groups (usually nucleophilic) of the enzyme. According to the presence of certain groups, appropriate method of chemical activation is chosen. It is very important that the immobilization of lipase by covalent attachment to a support should involve only functional groups of the lipase that are not essential for its catalytic activity (Knežević et al. 2006; Prlainović et al. 2011). When it comes to supports with carboxy groups, enzymes are immobilized on the polymers through their amino groups, forming a peptide bond, with the presence of carbodiimide as a carboxy activator group. Covalent immobilization via the carboxy group of supports is often used in immobilization of lipase on the polymer derivates of acrylic acid. It must be noted that these active derivatives enable mild covalent coupling of lipase. Polymers containing amino groups can be activated by the introduction of the diazo groups. Enzymes are then easily linked through their α- or ε-amino groups and less by sulfhydryl, hydroxyl, imidazole or carboxy groups. One of the most commonly used methods of activating supports with amino groups is with glutaraldehyde. Recently different authors have focused their attention on developing a large number of supports containing epoxy groups. These groups can react with various nucleophilic groups of amino acids residues (amino, hydroxyl or sulfhydryl group) at very mild conditions: neutral pH and temperatures between 4 and 25°C (Mateo et al. 2000; Bezbradica et al. 2009). In this way, a vast number of very strong bonds are formed, and a considerable increase of stability is achieved due to multiple bonding. In addition, polymers containing epoxy groups can be easily modified by other activating agents such as glutaraldehyde, if the lipase activity decreases due to the formation of undesirable interactions between the enzyme and supports due to short distance (Bayramoglu et al. 2005). Immobilization methods are highly specific and should be optimized for a particular lipase support system. For the use in biodiesel synthesis, lipase from *Thermomyces lanuginosus* has been stabilized by coupling the chemical amination of the enzyme surface to the multipoint covalent attachment of the modified enzyme to glyoxyl-

agarose beads (Rodrigues et al. 2010). The immobilized lipase proved to be very stable and active in transesterification reaction. It presented high reaction activity in the presence of excess alcohol under the optimal conditions. This method is an excellent step in reducing the cost of biodiesel by reducing the cost of enzyme, making it competitive with the chemical process. Lipases from *Thermomyces lanuginosus* and *Pseudomonas fluorescens* were immobilized by multipoint covalent attachment on Toyopearl AF-amino-650 resin. Glutaraldehyde, glycidol and epicchlololohydrin were used as activating agents and better catalytic properties were found when the support was activated with glycidol and epichlorohydrin. *T. lanuginosus* immobilized on glyoxyl-resin obtained derivates with the highest hydrolytic activity and thermal stability, almost 30 times more stable than soluble lipase. This lipase was successfully used for transesterification of palm oil with methanol, with the yield ranging from 93.5 to 100% (Mendes et al., 2011).

2.2 Pretreatment of lipase

Pretreatments of lipase by various methods have a goal of improving enzyme activity, enzyme stability and, especially important for biodiesel synthesis, to improve methanol tolerance. Researches have shown that when immobilized enzyme is successfully pretreated enzyme activity is increased and the ability of the enzyme to resist deactivation by methanol is higher, thereby enabling multiple reuses of enzymes for industrial production. Generally, pre-treatment reagents can be classified as: 1) substrates or their analogues; 2) organic solvents; 3) salts; 4) enzyme lyoprotectants such as crown ethers (Lu et al. 2010). Treatments with different reagents have, more or less, the same goal, keeping the conformation of lipases in its active form, by making the conformational change of active site from closed to open form. The most commonly used method is the usage of substrates or their analogues as pre-treatment reagents. They have the role of enhancing enzyme activity in organic solvents by molecular imprinting. The formed complex enzyme-imprinter keeps the enzyme in its active conformation and therefore retains higher activity in the nonaqueous environment as compared to the nonimprinted enzyme (Rich et al. 2002). Lu et al. pretreated immobilized lipase form *Candida* sp. 99-125 with methanol solutions within volume concentration range 10-20%. This treatment enhanced the enzyme activity and methanol tolerance in three step methanol addition and even in one step methanol addition. However, there were no enhancements for both, the initial rates and the equilibrium biodiesel yields with lipase pretreated with short chain alcohols: *n*-propyl alcohol, *n*-butanol, isopropyl alcohol, *t*-butanol, isobutyl alcohol. The most promising results were achieved when the lipase was treated with 1mM solution of $CaCl_2$ and $MgCl_2$,, since lipase activity, methanol tolerance and operational stability were drastically improved. It is assumed that salts could incorporate with the protein to form a more stable molecule, which could resist conformational change induced by high methanol concentration (Lu et al. 2010). Lipases from different origins show to have distinct properties and one activation methods might not be versatile for other lipases. The immobilized lipase from *C. antarctica* was pretreated, immersed, in alcohols: isopropanol, 2-butanol, *t*-butanol. The activity of the commercial immobilized enzyme, Novozyme 435, increased about tenfold in comparison to the enzyme not subjected to any pre-treatment and the methyl ester yield was about 7 to 10 times higher (Chen and Wu, 2003). Smukawa et al. achieved 97% biodiesel yield in methanolysis of plant oil after 3.5 hours with preincubated Novozym 435. Methanolysis progressed much faster when Novozym 435 was preincubated in methyl oleate for 0.5h and subsequently in soybean oil for 12h (Samukawa et al. 2000). It is clear that these procedures can significantly increase

productivity of industrial enzyme preparations and make enzymatic processes for industrial biodiesel production economically feasible.

3. Key operational variables

A number of different factors influence the enzymatic biodiesel synthesis: oil source, reaction temperature, choice of acyl acceptors, acyl acceptors to oil molar ratio, amount of water in the system or water activity, and presence of organic solvent in the mixture. Optimal parameters for enzymatic transesterification vary depending on the origin and type of lipase, type of oil source, and reactor type.

Reaction temperature may vary from 23 to 50°C. Optimal temperature for methanolysis of sunflower oil is 50°C when *T. lanuginose* is used as biocatalyst, but when *R. miehei* is used in the same reaction, temperature optimum lower than 40 °C has been reported (Soumanou and Bornscheuer, 2003). In general, increasing the temperature leads to an increase of the reaction rate of biodiesel production. When the optimum is reached, further increase of temperature, leads to decreased catalytic activity of the enzyme due to denaturation and inactivation. The researches have shown that immobilization of enzymes shift temperature optimum to higher values in comparison to free enzymes. It seems that immobilization provides a more rigid external backbone for lipase molecule, leading to the increase of the temperature optima and higher reaction rates (Al-Zuhair, 2007).

One of the key factors of enzymatic ester synthesis is the water content in the system. Lipases need an optimal small amount of water to maintain the activity in the organic media. Nevertheless, increased water concentration has an unfavourable effect on the equilibrium conversion, since it promotes reverse reaction of hydrolysis. The amount of water in the system should be a compromise between minimizing hydrolysis and maximizing lipase activity for the transesterification reaction and it should be determined for a particular reaction system (Chowdary, 2002; Noureddini et al., 2005). Many studies have shown that immobilized enzymes show highest activity in low water system. Tamalampudi et al. showed that, in biodiesel synthesis using lipase from *C. antarctica* (CAL-B), the rate of methanolysis decreased with the increase of the water content, reaching the FAME content of 75% when no water was added in the system. Similar results were achieved using the same lipase in the transesterification of sunflower oil, where yield of over 90% was achieved in an anhydrous reaction medium (Ognjanović et al., 2009). It has been shown that many immobilized lipases contain sufficient amount of water to preserve the catalytic conformation. Another problem occurs when waste oils are used, since the content of free fatty acids (FFA) is increased compared to refined oils. The esterification of FFA releases water, which can shift the reaction equilibrium towards ester hydrolysis. In these cases, molecular sieves are used for the control of water activity and increase ester yields by removing water produced by esterification. However, when lipases are immobilized on hydrophilic support, the molecular sieves are not needed, since, in that case, they have a negligible impact on methyl esters yield (Hsu et al., 2002).

There are many possible raw materials with a potential to obtain biodiesel. Generally, the main feedstock for biodiesel production can be divided in: 1) Vegetable oils such as sunflower oil (Dizge et al., 2009; Modi et al., 2007), soybean oil (Guan et al., 2010; Rodrigues et al., 2010), rapeseed oil (Li at al., 2006; Watanabe et al., 2007), jatropha oil (Shah and Gupta, 2007; Tamalampudi et al. 2008), cotton seed oil (Royon et al.,2007); 2) Animal fats such as tallow, lard (Da Cunha et al.,, 2009; Ngo et al. 2008); 3) Waste cooking oils and industrial

waste oils (Chen et al., 2009; Halim et al., 2009).The fatty acid composition of animal fats is not favourable for biodiesel production, since they contain predominantly saturated fatty acids. The limited supplies of animal fats and high melting points of obtained esters, which require addition of very powerful freeze protectants, make animal fats substrate of minor importance. On the other hand, vegetable oils are renewable and virtually inexhaustible energy source. Depending on availability of raw materials in different countries, various oils have been used: soybean oil is predominantly used in the United States, rapeseed oil and sunflower oil are the main feedstock for biodiesel synthesis in many European countries. Coconut oil and palm oil are used in Asian countries (Demirbas, 2009). Figure 5. and Figure 6. shows the usage of oils for biodiesel production as well as world production by feedstock in 2007 (International Grains Council, 2007).

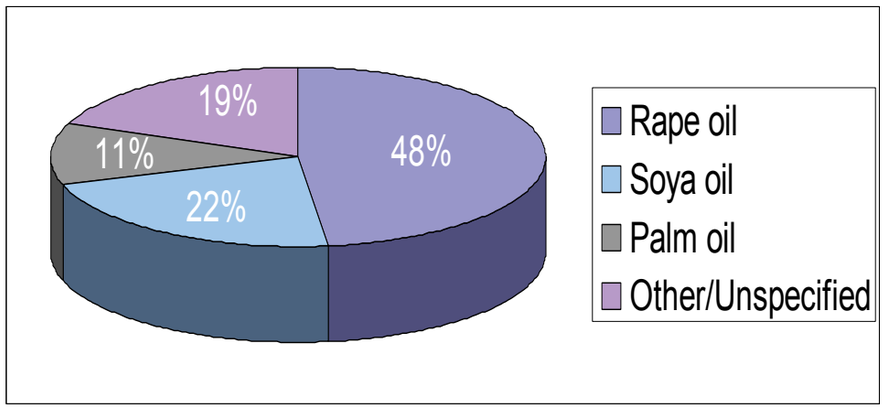


Fig. 5. Vegetable oil in use for biodiesel production

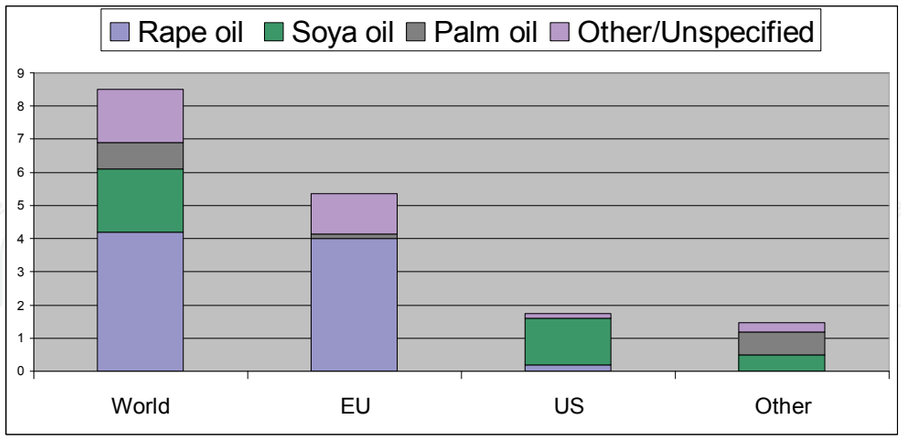


Fig. 6. Biodiesel world production by feedstock

However, edible oils are not in surplus supply and the cost of oil sources accounts for a large part in biodiesel production. In order to make the biodiesel production viable the solution is to develop a production based on waste cooking oils where no competition with food production takes place. But the amount of waste oils alone is not sufficient to meet demands. The optimal solution is to use non-edible oils which can not be used for human

consumption because of the presence of some antinutritional factors, or toxic components. The most suitable oils are those from crops with the highest productivity pre hectare, low production cost and that can grow on waste land, such as *Jatropha* oil or rapeseed oil (Shah and Gupta, 2007; Tan et al., 2010). It is important to know that the quality and the properties of biodiesel are greatly influenced by the fatty acid composition of fats and oils used for its synthesis. Fatty acid composition for different vegetable oils is shown in Table 3.

Oil	C 16:0	C 16:1	C 18:0	C 18:1	C 18:2	C 18:3	C 20:0	C 20:1	Others	Ratio SFA/UFA
Almond	6,5	0,5	1,4	70,7	20			3,5	0,9	7,9/91,2
Borage	12,9	0,2	4,3	19,1	39	18,7	0,3		2	17,5/82,5
Corn	11,7		1,9	25,2	60,5	0,5	0,2			13,8/86,2
Cotton seed	28,3		0,9	13,3	57,5					29,2/70,8
Jatropha	16,4	1	6,2	37	39,2		0,2			22,8/77,2
Olive	11,8	1,5	2,7	74,1	8,5	0,7	0,4	0,3		14,9/85,1
Palm	42,6	0,3	4,4	40, 5	10,1	0,2			1,9	47/51,1
Canola	3,5		0,9	64,4	22,3	8,2			0,7	4,4/94,9
Soybean	11,4		4,4	20,8	53,8	9,3	0,3			16,1/83,9
Sunflower	7,1		4,7	25,5	62,4		0,3			12,1/87,9

Table 3. Fatty acid composition for different vegetable oils (Robles–Medina et al., 2007)

Biodiesel produced from oils that have high content of unsaturated fatty acids show higher pour and cloud point properties, making it appropriate for both cold and warm weather. However, the quality of such biodiesel is lower since it has lower cetane number and combustion temperature (Demirbas, 2008; Ghaly, 2010). Then again, biodiesel produced from oils with long chain fatty acids (longer than 18 carbons) has high cetane number and combustion temperature, but low cloud and pour point and greater viscosity. So the choice of feedstock should be a compromise between the unsaturation and the length of fatty acid chain (Robles-Medina et al., 2009).

3.1 Solvent engineering and acyl acceptor selection

The majority of enzymatic syntheses of biodiesel are performed in organic solvents. The yield of biodiesel is greatly influenced by the type of organic solvent present in the reaction system. Immobilized lipases showed high degree of efficiency in the presence of non-polar solvents (Soumanou and Bornscheuer, 2003). The polar, less hydrophobic solvents are not suitable for biocatalytic processes since they can distort water microlayer around the enzyme influencing its native structure, thereby, leading to denaturation. It has been shown that the highest biodiesel yield with lipase from *C. antarctica* was achieved with *n*-hexane as solvent. The lowest yields were obtained with polar solvents such as acetone (Nie et al. 2006). High yields were achieved in the transesterification reaction with immobilized lipase from *Pseudomonas fluorescens* with 1, 4-dioxane as solvent (Iso et al. 2001). *Tert*-butanol is also commonly used solvent for biodiesel enzymatic synthesis. It has been shown that the presence of *t*-butanol significantly reduces the negative effects caused by both methanol and glycerol, due to *t*-butanol ability to dissolve both methanol and glycerol. In the

transesterification of rapeseed oil with Lipozyme TI LM and Novozyme 435 as catalyst, and under optimal condition, 90% yield was achieved (Li et la. 2006). *Tert*-butanol was used as a solvent in the enzymatic production of biodiesel from cotton oil with lipase from *C. antarctica*, in the batch reactor and a packed bed reactor. In both cases, the yield was over 90% (Royon et al. 2007).

However, despite the promising results, use of solvents should be avoided because of their toxicity and flammability, damaging effects on the environment and consequential requirement for solvent removal. From the economic point of view, the use of organic solvents is unfavorable due to the necessity of their removal from the final product which further increases production cost. Thus, to enable enzymatic processes to be competitive, enzymatic solvent-free systems have been developed. It has been shown that there are many benefits in using the solvent-free system in comparison with the organic solvent system, including the costs reduction and the improvement in the process control (Kose et al. 2002; Selmi and Thomas, 1998). The main drawback in industrial implementation of lipases into the solvent-free biodiesel synthesis is rather low enzyme stability in the presence of excess methanol, since several studies reported that a high methanol concentration could lead to serious inactivation of lipase (Kose et al. 2002; Royon et al. 2007). Methanol is the most popular alcohol used in transesterification process because of its relatively low price in comparison to other alcohols. In a typical methanolysis reaction, the reaction mixture consists of two phases due to low solubility of methanol in oil, leading to the inactivation of enzyme and the decreased fatty acid methyl ester yield. To minimize the enzyme inactivation, the solvent-free reaction systems with a stepwise addition of methanol have been developed. The most common way is a three step addition of methanol in accordance with the reaction dynamics, enabling conversions as high as 98.4% after 48 h (Shimada et al., 1999). Several other studies confirmed that the three-step addition of methanol is superior to the one-step addition (Shimada et al., 2002; Watanabe et al., 2001, 2002). A group of researches has performed the methanolysis of plant oils by lipase immobilized from *C. antarctica* in batch conditions with two-step (Watanabe et al., 2000) and three-step (Watanabe et al., 1999) addition of methanol (50% or 33% of stoichiometric quantity required) with a yield of 95% and 98.4%. However, operational stability of lipases in repeated cycles of methanolysis is not very high, indicating that an important parameter affecting the economic feasibility of the process is the biocatalysts stability and reusability over an extended period of time. Biocatalyst susceptibility towards methanol shifted interest of scientific community into selecting new acyl acceptors for transesterification reaction.

Several studies have focused their attention on branched and long chain alcohols. Experiment showed that increase of the number of carbon atoms increased the cetane number as well as heat content of the fuel. Also, fatty acid esters of secondary or branched-chain alcohols can be used as fuel additives since they decrease the solidification point, and consequently, the high cloud point and pour point (Salis et al. 2005; Watanabe et al. 2007). Kose et al. dealt with the alcoholysis of cotton seed oil with primary and secondary alcohols by using Novozyme 435 lipase. They analyzed the effect of alcohol types on alcoholysis on cotton seed oil indicating that the highest yield was obtained with isoamyl alcohol (Kose et al., 2002). Ognjanović et al. investigated the influence of methanol, 2-propanol and *n*-butanol on biodiesel synthesis. Operational stability of lipase from *C. antarctica* was investigated in a three-step addition of alcohol in a solvent-free system. With all three acyl acceptors a high initial yield was achieved but lipase exhibited poor activity during the repeated experiments. The replacement of methanol with less polar alcohols resulted in only slight

increase in retained activity, but the significant inactivation of lipase still occurs. This might be due to the inactivation effect caused by alcohol and the negative effect caused by byproduct glycerol adsorbed on the surface of the immobilized lipase (Ognjanović et al., 2009). Byproduct glycerol is hydrophobic and insoluble on oil, so it is easily adsorbed onto the surface of the immobilized lipase, also exhibiting a negative effect on lipase activity and operational stability (Du et al., 2004). Most recent studies are focusing their interest on implementing methyl acetate and ethyl acetate as an acyl acceptors in biodiesel synthesis (Modi et al., 2007; Xu et al., 2005). It has been shown that methyl acetate has no negative effect on lipase activity. The usage of methyl acetate also eliminates the risk of deactivation of enzyme by glycerol, since no glycerol is produced in the reaction. In this reaction, instead of glycerol, the byproduct is triacetin, which has a greater value than glycerol. It can be used as a fuel additive, as an antiknock agent which can reduce engine knocking in gasoline, and to improve cold and viscosity properties of biodiesel (Du et al., 2004; Wang et al., 2006). Since methyl acetate has no negative effect on enzyme stability a stepwise addition of methyl acetate is not needed. This greatly reduces the reaction time and simplifies the procedure. Ognjanović et al. reported the transesterification of sunflower oil with methyl acetate by using the immobilized *C. antarctica* (Novozyme 435) in the solvent-free system (Ognjanović et al., 2009). They have explored the substrate ratio on biodiesel production and concluded that equilibrium conversion increases as methyl acetate concentration increases. A large excess of methyl acetate is required in order to shift the interesterfication in the forward direction. The highest methyl ester yield of 98% was obtained at 12:1 molar ratio of methyl acetate to oil, at 45°C and 3% of enzyme based on oil weight. The operational stability of lipase was found to be constant over 7 repeated cycles (200h), whereas the activity of immobilized enzyme decreased rapidly when methanol was used, and after the third cycle it was below 5%. Du et al. also used methyl acetate in the transesterification of soybean oil utilizing Novozym 435 as biocatalyst. This research also showed that in contrary to what occurred when using methanol, large excess of methyl acetate showed no inhibitory effect on lipase (Du et al., 2004). They achieved conversion of 92%, and there was no loss of the enzyme activity even after being continuously used for 100 bathes. Ethyl acetate has been used as acyl acceptor for interesterification of jatropha and sunflower oil with Novozym 435 as biocatalyst. The operational stability of lipase was unchanged for 12 cycles, whereas under the same conditions, when ethanol was used as acyl acceptor, lipase lost all its activity after 6 cycles (Modi et la., 2007).

4. Reactor configuration and industrial application

Important aspect of lipase implementation in biodiesel industrial production is development of an efficient immobilized reactor system. On a laboratory scale most commonly used systems are stirred batch reactors. The advantage of this reactor system is easy handling and control, as well as the simple configuration of the reactor. There are numerous studies on enzymatic transesterification in various reactor configurations with the emphasis on determining the best operational parameters, but there isn't sufficient information on scale-up systems (Du et al., 2007; Talukder et al., 2009). Packed bed reactor (PBR) systems are frequently applied for the large-scale enzymatic reactions. They enable continuous production with high efficiency, and reduced shear denaturation of the biocatalyst. PBR are more suitable for long lasting and industrial production since it allows the reuse of enzyme without the need of a prior separation and the immobilized enzyme is subjected to fewer

shear stress, in comparison to stirred batch reactors,. The main advantage of packed bed reactors over fluidized bed reactors or continuous stirred tank reactor is the higher conversion per weight of catalyst. Basic construction of packed bed reactors (on a laboratory scale) is a glass column, packed with immobilized lipases with the reaction mixture placed in thermostat water bath, and pumped upward through the column by a peristaltic pump. Attention has turned to determining the operational and hydrodynamic conditions that maximize the yield in an industrially feasible immobilized system. Chen et al. reported an efficient system for continual production of biodiesel fuel by methanolysis of waste cooking oil (WCO) using immobilized *Candida* lipase in a three-step fixed bed reactor (Chen et al., 2009). They have explored the effect of lipase, solvent, water, temperature, operational stability and flow of the reaction mixture on the synthesis of biodiesel. The reaction was carried with hexane as a solvent, since waste cooking oil was not completely dissolved in methanol. Solvent improved the transfer of the substrate and the products, and reduced the toxic effect of methanol on immobilized lipases by decreasing methanol concentration. A crucial parameter of packed bed reactors are the amount of enzyme and the flow rate. The effect of flow rate on the enzyme efficiency is not straightforward. When the flow rate is low, methanol is in prolonged contact with the enzyme, which can lead to reduced enzyme activity by denaturation. On the other hand, too high flow rates reduce time of contact between the lipase and substrate, significant portion of substrates fail to bind at the enzyme active site, leading to inevitable decrease in biodiesel yield. The optimum reaction conditions were 25:15:10:100 weight ratio of lipase/hexane/water/WCO, temperature of 45°C and reactant flow of 1, 2mlmin^{-1}. The achieved yield was 91.08%, however, it decreased to 76.74% over 100h. There are two possible reasons for such poor stability: the influence of glycerol adsorbed on the surface of the immobilized lipase and the negative effect of methanol on the enzyme. Glycerol must be eliminated in a timely manner during the process in order to achieve better stability. Another solution is to use a different solvent since glycerol is insoluble in n-hexane, so it remains in the reactor adsorbed onto the enzymatic support, inevitably leading to decrease in enzymatic activity.

Packed bed reactor has been tested in laboratory scale by Severac et al. for biodiesel production from high-oleic sunflower oil using *t*-butanol as co-solvent for butanol. Initial substrate concentrations and their molar ratio were optimized by using Novozym 435 as biocatalyst. The crucial point of control is the partition of polar compounds between the reaction medium and the supported enzyme (Severac et al., 2011). Since *tert*-butanol has the ability to dissolve both, polar and nonpolar compound, it is a perfect solvent for this production. The best results were obtained for an initial oil concentration of 500mM and a molar ratio of oil/butanol, 1:5, with estimated productivity of 13,8 tons year^{-1} kg Novozym 435 with 96.5% yield. It is important to notice that system operated without the loss of activity for more than 50 days (over 1200h), making this procedure an effective approach for biodiesel synthesis. One crucial difference between these systems is the choice of catalyst: *Candida* sp. 99-125 immobilized on textile cloth versus commercial immobilized *C. antarctica* on macroporous acrylic resign. The support for *C. antarctica* is hydrophobic and limits the adsorption of polar components presented in the medium such as glycerol in the continuous transesterification conditions (Severac et al., 2011). The selection of catalyst needs to be a compromise between activity, stability, and price. It seems that, although the catalytic life of immobilized *Candida* sp. 99-125 is shorter, the cost of catalyst is significantly lower making it rather competitive for industrial use.

Similar PBR was optimized by Hailm et al. for continuous synthesis of biodiesel from waste cooking oil with methanol using *t*-butanol as solvent. The packed bed reactor consisted of two series of Pyrex column where the substrate mixture was fed upwards through the column using a peristaltic pump (Halim et al. 2009). With Novozyme 435 as biocatalyst, the FAME yield was 80% and the operational stability was longer than 120h. Halim et al. investigated the effect of mass transfer. It seems that at low velocity of substrate the mass transfer could dominate the yield, whereas at high velocities the reaction could dominate the yield. By analyzing two proposed models it was concluded that transesterification reaction of waste cooking oil in a continuous packed bed reactor occurs under mass transfer control conditions. It was suggested that mass transfer rate can be increased using conventional methods, such as enhancement of active area per unit volume of immobilized enzyme.

It was previously reported that, due to internal mass transport limitation, packed bed reactor don't seem to be an option for solvent free enzymatic FAME production (Fjerbaek et al., 2008). There are several problems to address: due to the high viscosity of solvent free systems pressure drop becomes significant. In order to minimize the pressure drop packed bed reactors need to operate at low flow velocities, the size of carrier must be increased or solvent must be added. With increasing carrier particle diameter the pressure drop decreases, but the internal mass transfer rate in biocatalyst decreases, which can affect the overall reaction rate. Despite these apparent limitations several PBR system have been developed for solvent free reactions (Hajar et al., 2009; Hama et al., 2007; Shimada et al., 2002; Ognjanović et al., 2009). The usage of solvents only increases production costs as they have to be removed and purified for recycling. Shimada et al. developed methanolysis of vegetable oil in a PBR packed with *C. antarctica* lipase. Since the influence of methanol in a solvent free system is more detrimental on lipase activity, stepwise addition of methanol is required. In this case glycerol was separated by sedimentation after each step. The conversion reached 90% and a three step reaction was used for 100 days (Shimada et al. 2002). Enzymatic methanolysis of canola oil in the solvent-free system was studied in a PBR using Novozyme 435 with small pieces of loofa (Hajar et al., 2009). Loofa was used for several reasons: it prevents enzyme compression especially for viscous flows, due to structure porosity it facilitates oil passing through the reactor, reduces the accumulation of glycerol and provides better enzyme distribution. As it was previously reported, *C. antarctica* lipase was inactivated by adding more than 1.5 molar equivalents of methanol to the oil in a solvent-free system. Stepwise addition of methanol was required and the appropriate volume of methanol was added at three stages: at the beginning of the reaction and after 24 and 48h. The achieved yield of methyl ester in this PBR configuration was above 95% and biocatalyst retained constant activity for more than 400h. Hajar et al., using response surface methodology, also investigated the influence of flow rate. Fluid dynamics and operating regime of the reactor system need to be investigated with special concern when solvent-free systems are applied. At lower flow rates mass transfer of the oil to the surface of lipase is decreased. Due to insufficient agitation rate hydrophilic layer of methanol and glycerol is formed around lipase. By increasing flow rates exposure of lipase to substrate is improved, thus improving the biodiesel yield. The feasibility of another PBR construction for solvent free system was investigated with methyl acetate as acyl acceptor (Ognjanović et al., 2009). Based on the best results achieved in a batch reactor (3% of the immobilized lipase based on the oil weight, and 12:1 methyl acetate to oil molar ratio) the packed bed reactor was constructed to perform the transesterification of sunflower oil with

commercial *C. antarctica* B lipase immobilized on acrylic resin. The kinetics in the packed bed reactor system proved to have a slightly better profile than in a batch stirred tank reactor. Almost complete conversion of the oil to methyl esters (96.25%) was achieved in 8 hours. The yield of biodiesel remained the same for eight cycles (93.6 ± 3.75%) showing that the enzyme did not loose its activity even in a large excess of methyl acetate. With a proper selection of the reactor configuration and the operating conditions, the immobilized enzyme might offer a great prospect to ensure the high conversion values, improved enzyme stability, and thus prolonged operation times and cost-effective industrial enzymatic processes.

A very interesting and important study was conducted by Sotoft et al. They carried out the process simulation and economical evaluation of an enzymatic biodiesel production plant (Sotoft et al., 2010). Since there is, to date, only one plant in the world that uses enzymatic transesterification of biodiesel these kinds of studies are very important to make a correct evaluation of the industrial potential of a particular process. The study evaluated several important and relevant scenarios for enzyme catalyzed biodiesel production processes. Enzymatic biodiesel production from rapeseed oil and methanol has been investigated for solvent-free and cosolvent production processes. Study included two different production scales (8 and 200 mil.kg biodiesel/year) and different enzyme prices: current price 762.7€/kg enzyme and assumed price of enzyme in the future 7.627€/kg enzyme. The process simulations were carried out in Aspen Plus and Aspen Icarus Process Evaluator. Based on the simulations, the solvent free process is viable for a larger scale production of 200 mil.kg biodiesel/year with the current enzyme price. Also the continuous operation is the only realistic option with stepwise addition of methanol. Byproduct sale is also an important part of process economy. For a solvent free operation the cost of raw materials is distributed as: 50% enzyme, 47% oil and 3% methanol. The influence of enzyme cost is lower for cosolvent operation due to the improved enzyme performance. The estimated product price was 0.73-1.49€/kg biodiesel with the current enzyme price and 0.05-0.75€/kg with the enzyme price for a future for solvent-free processes.

5. Conclusion

The production and consumption of biodiesel will inevitably rise in future due to low environmental impact, ease of handling, and possibility of use without need for major adjustments of existing engines of motor vehicles. Although the majority of biodiesel manufacturers still employ a base-catalyzed process, biocatalytic methods based on activity of various microbial lipases offer several advantages, such as mild reaction conditions and high selectivity of biocatalysts in comparison with chemical catalysts. These advantages have good prospects to cause significant increase of economical feasibility of biodiesel production, since mild conditions decrease energy costs, while high selectivity leads to avoiding of unwanted by-products formation, simpler downstream processing, and easier separation of valuable by-product – glycerol. The main obstacle of biocatalytic process application on industrial level is high costs for enzyme formulations, but simultaneous efforts in optimization of different parameters of biocatalytic process led to significant increase of productivity. Various microbial lipase producers with strong affinity towards catalysis of methanolysis reaction and low susceptibility towards presence of methanol have been identified during previous two decades. The stability of lipases has been further increased by pre-treatment of lipases, selection of adequate immobilization procedures or

use of whole-cell biocatalysts, leading to prolonged activity of biocatalyst. Detrimental effects of methanol on lipase activity opened new area of investigation – selection of alternative acyl-acceptors, such as higher or branched alcohols, and esters. Investigations in this area led not only to prolonged stability of biocatalyst, but to additional valuable by-products and even biodiesel of improved fuel properties. Additionally, the reduction of oil costs has been performed by development of processes with waste oil and further increase of productivity was achieved by application of novel reactor designs. It can be concluded that promising novel findings obtained in optimization of lipase-based processes of biodiesel production indicate that biocatalysis has great potential in biodiesel synthesis.

6. Acknowledgment

The authors are grateful to the Ministry of Science, Republic of Serbia for financial support (Project III 46010).

7. References

Al-Zuhair, S. (2007). Production of biodiesel: possibilities and challenges. *Biofuels, Bioproducts and Biorefining*, Vol. 1, (July 2007), pp. 57-66, ISSN 1932-1031

B van Beilen, J. & Li, Z., (2002). Enzyme technology: an overview. *Current Opinion in Biotechnology,* Vol. 13, No. 4, (August 2002), pp. 338-344, ISSN 0958-1669

Bajaj, A., Lohan P., Jha P.N. & Mehrotra R. (2010). Biodiesel production through lipase catalyzed transesterification: An overview. *Journal of Molecular Catalysis B: Enzymatic,* Vol. 62, No. 1, (October 2009), pp. 9-14, ISSN 1381-1177

Bayramoglu, G., Kaya, B. & Anca, M.Y. (2005) Immobilization of *Canida rugosa* lipase onto spacer-arm attached poly(GMA-HEMA-EGDMA) microspheres. *Food Chemistry,* Vol. 92, No. 2, (September 2005), pp. 261-268, ISSN 0308-8146

Bezbradica D., Mijin D., Mihailović M. & Knežević-Jugović Z. (2009) Microvawe-assisted immobilization of lipase from *Candida rugosa* on Eupergit supports, *Journal of Chemical Technology and Biotechnology* Vol. 84, No. 11, (November 2009), pp. 1642-1648, ISSN 0268-2575

Bozbas, K. (2008). Biodiesel as an alternative motor fuel: Production and policies in the European Union. *Renewable and Sustainable Energy Reviews,* Vol. 12, No. 2, (February 2008), pp. 542-552, ISSN 1364-0321

Chen, J-W. & Wu, W-T. (2003) Regeneration of immobilized *Candida antarctica* lipase for transesterification, *Journal of Bioscience and Bioengineering,* Vol. 95, No. 5, (January 2003), pp. 466-469, ISSN1389-1723

Chen, Y., Xiao, B., Chang, J., Fu, Y., Lv, P. & Wang, X. (2009). Synthesis of biodiesel from waste cooking oil using immobilized lipase in fixed bed reactor, *Energy Conversion and Management,* Vol. 50, No. 3, (March 2009), pp. 668-673, ISSN 0196-8904

Chowdary, G.V. & Prapulla, S.G. (2002). The influence of water activity on the lipase catalyzed synthesis of butyl butyrate by transesterification, *Process Biochemistry,* Vol. 38, No. 3, (November 2002), pp. 393-397, ISSN 1359-5113

Da Cunha, M.E., Krause, L.C., Moraes, M.S.A., Faccini, C.S., Jacques, R.A., Almeida, S.R., Rodrigues, M.R.A. & Caramao, E.B. (2009) Beef tallow biodiesel produced in a pilot scale, *Fule Processing Technology,* Vol. 90, No. 4, (April 2009), pp. 570-575, ISSN 0378-3820

Demirbas, A. (2008) Relationships derived from physical properties of vegetable oil and biodiesel fuels, *Fuel,* Vol. 87, No. 8-9, (July 2008), pp. 1743-1748, ISSN 0016-2361

Demirbas, A. (2009). Progress and recent trends in biodiesel fuels, *Energy Conversion and Management,* Vol. 50, No. 1, (January 2009), pp. 14-34, ISSN 0196-8904

Dizge, N., Aydiner, C., Imer, D.Y., Bayramoglu, M., Tanriseven, A. & Keskinler, B. (2009) Biodiesel production from sunflower, soybean and waste cooking oils by transesterification using lipase immolbilized onto a novel microporous polymer, *Bioresource Technology,* Vol. 100, No. 6, (March 2009), pp. 1983-1991, ISSN 0960-8524

Du, W., Xu, Y., Liu D. & Zeng, J. (2004). Comparative study on lipase-catalyzed transformation of soybean oil for biodiesel production with different acyl acceptors, *Journal of Molecular Catalysis B: Enzymatic,* Vol. 30, No. 3-4, (August 2004), pp. 125-129, ISSN 1381-1177

Du, W., Liu, D., Li, L. & Dai, L. (2007) Mechanism exploration during lipase-mediated methanolysis of renewable oils for biodiesel production in a *tert*-butanol system, *Biotechnology progress*, Vol. 23, No. 5, (Septemeber 2007), pp. 1087- 1090, ISSN 8756-7938

Fjerbaek, L., Christensen, K.V. & Norddahl, B. (2009). A review of the current state of biodiesel production using enzymatic transesterification. *Biotechnology and Bioengineering*, Vol. 102, No. 5, pp. 1298-1315, ISSN 0006-3592

Freitas, L., Da Ros, P.C.M., Santos, J.C. & de Castro, H.F. (2009) An integrated approach to produce biodiesel and monoglycerides by enzymatic interestification of babassu oil (*Orbinya* sp). *Process Biochemistry*, Vol. 44, No. 10, (October 2009), pp. 1068-1074, ISSN 1359-5113

Fukuda, H., Kondo A. & Noda H. (2001). Biodiesel fuel production by transesterification of oils. *Journal of Bioscience and Bioengineering,* Vol. 92, No. 5, (September 2001), pp. 405-416, ISSN 1389-1723

Ghaly, A.E., Dave, D., Brooks, M.S. & Budge, S. (2010) Production of biodiesel by enzymatic transesterification: Review, *American Journal of Biochemistry and Biotechnology,* Vol. 6, No. 2, pp. 54-76, ISSN 1553-3468

Gomes, F.M., Pereira, E.B. & Castro, H.F., (2004). Immobilization of lipase on chitin and ıts use in nonconventional biocatalysis. *Biomacromolecules*, Vol. 5, No. 1, (January 2004), pp. 17-23, ISSN 1525-7797

Guan, F., Peng, P., Wang, G., Yin, T., Peng, Q., Huang, J., Guan, G. & Li, Y. (2010). Combination of two lipases more efficiently catalyzes methanolysis of soybean oil for biodiesel production in aqueous medium, *Process Biochemistry,* Vol. 45, No. 10, (October 2010), pp. 1667-1682, ISSN 1359-5113

Gupta, R., Gupta, N. & Rathi P. (2004). Bacterial lipases: an overview of production, purification and biochemical properties. *Applied Microbiology and Biotechnology,* Vol. 64, No. 6, (February 2004), pp. 763-781, ISSN 0175-7598

Hajar, M., Shokorollahzadeh, S., Vahabzadeh, F. & Monazzami, A. (2009) Solvent-free methanolysis of canola oil in a packed-bed reactor with use of Novozyme 435 plus loofa, *Enzyme and Microbial Technology,* Vol. 45, No. 3, (September 2009), pp. 188-194, ISSN 0141-0229

Halim, S.F.A., Kamaruddin, A.H. & Fernando, W.J.N. (2009). Continuous biosynthesis of biodiesel from waste cooking palm oil in a packed bed reactor: Optimization usig

response surface methodology (RSM) and mass transfer studies. *Bioresource Technology,* Vol. 100, No. 2, (January 2009), pp. 710-716, ISSN 0960-8524

Hama, S., Yamaji, H., Fukumizu, T., Numata, T., Tamalampudi, S., Kondo, A., Noda, H. & Fukuda, H. (2007) Biodiesel-fuel production in a packed-bed reactor using lipase-producting *Rhizopus oryzae* cells immobilized within biomass support particles, *Biochemical Engineering Journal,* Vol. 34, No. 3, (June 2007), pp. 273-278, ISSN 1369-703X

Hilal, N., Kochkodan, V., Nigmatullin, R., Goncharuk, V. & Al-Khatib, L. (2006). Lipase-immobilized biocatalytic membranes for enzymatic esterification: Comparison of various approaches to membrane preparation, *Journal of Membrane Science,* Vol. 268, No. 2, (January 2006), pp. 198-207, ISSN 0376-7388

Hsu, A-F., Jones, K., Foglia T.A. & Marmer, W.N. (2002). Immobilized lipase-catalysed production of alkyl esters of restaurant grease as biodiesel. *Biotechnology and Applied Biochemistry,* Vol. 36, No. 3, (December 2002), pp. 181- 186, ISSN 0885-4513

Hsu, A-F., Jones, K., Foglia, T.A. & Marmer, W.N. (2004). Continuous production of ethyl esters of grease using an immobilized lipase. *Journal of American Oil Chemical Society,* Vol. 81, No. 8, (August 2004), pp. 749-752, ISSN 0003-021X

Iso, M., Chen, B., Eguchi, M., Kudo, T., Shrestha S. (2001). Production of biodiesel fuel from triglycerides and alcohol using immobilized lipase. *Journal of Molecular Catalysis B: Enzymatic,* Vol. 16, No. 1, (November 2001), pp. 53-58, ISSN 1381-1177

Jaeger, K.E. & Eggert, T. (2002). Lipases in biotechnology. *Current Opinion in Biotechnology,* Vol. 13, No. 4, (August 2002), pp. 390-397, ISSN 0958-1669

Jegannathan, K.R. & Abang, S., (2008). Production of biodiesel using immobilized lipase-A critical review. *Critical Reviews in Biotechnology,* Vol. 28, No. 4, pp. 253-264, ISSN 0738-8551

Kaieda, M., Samukawa, T., Kondo, A. & Fukuda, H. (2001). Effect of methanol and water contents on production of biodiesel fuel from plant oil catalyzed by various lipases in a solvent-free system. *Journal of Bioscience and Bioengineering*, Vol. 91, No. 1, pp. 12-15, ISSN 1389-1723

Kamini, N.R. & Iefuji, H. (2001). Lipase catalyzed methanolysis of vegetable oils in aqueous medium by *Cryptococcus* spp. S-2. *Process Biochemistry*, Vol. 37, No. 4, (December 2001), pp. 405-410, ISSN 1359-5113

Kirk, O., Borchert, T.V. & Fuglsang, C.C. (2002). Industrial enzyme application. *Current Opinion in Biotechnology,* Vol. 13, No. 4, (August 2002), pp. 345-351, ISSN 0958-1669

Knežević, Z., Šiler-Marinković, S. & Mojović, Lj. (2004). Immobilized lipases as practical catalysts. *Acta Periodica Technologica,* Vol. 35, pp. 151-164, ISSN 1450-7188

Knežević, Z., Milosavić, N., Bezbradica, D., Jakovljević, Ž. & Prodanović, R. (2006) Immobilization of lipase from *Candida rugosa* on Eupergit C supports by covalent attachment, *Biochemical Engineering Journal*, Vol. 30, No. 3, (June 2006), pp. 269-278, ISSN 1369-703X

Kose, O., Tuter, M. & Aksoy, H.A. (2002). Immobilized *Candida antarctica* lipase-catalyzed alcoholysis of cotton seed oil in a solvent-free medium. *Bioresource Technology*, Vol. 83, No. 2, (June 2002), pp. 125-129, ISSN 0960-8524

Kumari, A., Shah, S. & Gupta, M.N. (2007). Preparation of Biodiesel by Lipase-Catalyzed Transesterification of High Free Fatty Acid Containing Oil from Madhuca indica. *Energy and Fuels* Vol. 21, No. 1, (January 2007), pp. 368-371, ISSN 0087-0624

Kumari, A., Mahapatra, P., Garlapati, V.K. & Banerjee, R. (2009) Enzymatic transesterification of Jatropha oil, *Biotechnology for Biofuels,* Vol. 2, No. 1, (January 2009), pp. 1-7, ISSN 1754-6834

Li, L., Du W., Liu, D., Wang, L. & Li, Z. (2006) Lipase-catalyzed transesterification of rapeseed oils for biodiesel production with a novel organic solvent as the reaction medium. *Journal of Molecular Catalysis B: Enzymatic,* Vol. 43, No. 1-4, (December 2006), pp. 58-62, ISSN 1381-1177

Li, N., Zong, M. & Wu, H. (2009) Highly efficient transformation of waste oil to biodiesel by immobilized lipase from *Penicillium expansum*. *Process Biochemistry*, Vol. 44, No. 6, (June 2009), pp. 685-688, ISSN 1359-5113

Lu, J., Deng, l., Zhao, R., Zang, R., Wang, F. & Tan, T. (2010) Pretreatment of immobilized *Candida* sp. 99-125 lipase to improve its methanol tolerance for biodiesel production. *Journal of Molecular Catalysis B: Enzymatic,* Vol. 62, No. 1, (January 2010), pp. 15-18, ISSN 1381-1177

Ma, F. & Hanna, M.A. (1999). Biodiesel production: a review. *Bioresource Technology*, Vol. 70, No. 1, (Octobar 1999), pp. 1-15, ISSN 0960-8524

Marchetti, J.M., Miguel, V.U. & Erazzu, A.F. (2005). Possible methods for biodiesel production. *Renewable and Sustainable Energy Reviews,* Vol.11, No. 6, (August 2005), pp. 1300-1311, ISSN 1364-0321

Mateo, C., Abian, O., Fernandez-Lafuente, R. & Guisan, J.M. (2000) Increase in conformational stability of enzymes immobilized on epoxy-activated supports by favoring additional multipoint covalent attachment. *Enzyme and Microbial Technology,* Vol. 26, No. 7, (April 2000), pp. 509-515, ISSN 0141-0229

Mateo, C., Palomo, J.M., Fernandez-Lorente, G., Guisan, J. & Fernandez-Lorente, R. (2007) Improvement of enzyme activity, stability and selectivity via immobilization techniques. *Enzyme and Microbial Technology,* Vol. 40, No. 6, (May 2007), pp. 1451-1463, ISSN 0141-0229

Mendes, A., Giordano, R., Giordano, R. & de Castro, H. (2011). Immobilization and stabilization of micribial lipases by multipoint covalet attachment on aldehyde-resin affinity: Application of the biocatalysts in biodiesel synthesis. *Journal of Molecular Catalysis B: Enzymatic,* Vol. 68, No. 1, (January 2011), pp. 109-115, ISSN 1381-1177

Meunier, S. & Legge, R. (2010) Evaluation of diatomaceous earth as a support for sol-gel immobilized lipase for transesterification. *Journal of Molecular Catalysis B: Enzymatic,* Vol. 62, No. 1, (January 2010), pp. 54-58, ISSN 1381-1177

Modi, M. K., Reddy J.R.C., Rao, B.V.S.K. & Prasad, R.B.N. (2007) Lipase-mediated conversion of vegetable oils into biodiesel usig ethyl acetate as acyl acceptor. *Bioresource Technology*, Vol. 98, No. 6, (April 2007), pp. 1260-1264, ISSN 0960-8524

Moreira, A.B.R., Perez, V.H., Zanin, G.M. & Castro, H.F. (2007) Biodiesel Synthesis by Enzymatic Transesterification of Palm Oil with Ethanol Using Lipases from Several Sources Immobilized on Silica–PVA Composite. *Energy & Fuels,* Vol. 21, No. 36, (November 2007), pp. 3689–3694, ISSN 0887-0624

Mousdale, D. M. (2008) *Biofuels – Biotechnology, Chemistry, and Sustainable Development*, CRC Press, ISBN-13: 978-1-4200-5124-7, United States of America

Ngo, H.L., Zafiropoulos, N.A., Foglia, T.A., Samulski, E.T. & Lin, W. (2008) Efficient two-step synthesis of biodiesel from greases, *Energy and Fuels,* Vol. 22, No. 1, (January 2008), pp. 626-634, ISSN 0887-0624

Nie, K., Xie, F., Wang, F. & Tan, T. (2006) Lipase catalyzed methanolysis to produce biodiesel: Optimiyation of the biodiesel production, *Journal of Molecular Catalysis B: Enzymatic,* Vol. 43, No. 1-4, (December 2006), pp. 142-147, ISSN 1381-1177

Noureddini, H., Gao, X. & Philkana, R.S. (2005). Immobilized *Pseudomonas cepacia* lipase for biodiesel fule production from soybean oil, *Bioresource Technology,* Vol. 96, No. 7, (May 2005), pp. 769-777, ISSN 0960-8524

Ognjanović, N., Šaponjić, S., Bezbradica, D. & Knežević-Jugović, Z. (2008). Lipase-catalyzed biodiesel synthesis with different acyl acceptors. *Acta Periodica Technologica*, Vol. 39, pp. 161-169, ISSN 1450-7188

Ognjanović, N., Bezbradica, D. & Knežević, Z. (2008). Optimization of the production of biodiesel by a commercial immobilized lipase in a solvent-free system using a response surface methodology. *Journal of the Serbian Chemical Society,* Vol. 73, No. 2, pp. 147-156, ISSN 0352-5139

Ognjanović, N., Bezbradica, D. & Knežević-Jugović, Z. (2009). Enzymatic conversion of sunflower oil to biodiesel in a solvent-free system: Process optimization and immobilized system stability. *Bioresource Technology*, Vol. 100, No. 21, (November 2009), pp. 5146-5154, ISSN 0960-8524

Orcaire, O., Buisson, P. & Pierre, A. (2006) Application of silica aerogel encapsulated lipases in the synthesis of biodiesel by transesterification reaction. *Journal of Molecular Catalysis B: Enzymatic,* Vol. 42, No. 3-4, (November 2006), pp. 106-113, ISSN 1381-1177

Parawira, W. (2009). Biotechnological production of biodiesel fuel using biocatalysed transesterification: a review. *Critical Reviews in Biotechnology,* Vol. 29, No. 2, (September 2009), pp. 82-93, ISSN 0738-8551

Petkar, M., Lali, A., Caimi, P. & Daminati, M. (2006) Immobilization of lipases for non-aqueous synthesis, *Journal of Molecular Catalysis B: Enzymatic,* Vol.39, No. 1-4, (May 2006), pp. 83-90, ISSN 1381-1177

Prlainović, N., Knežević-Jugović, Z., Mijin, D. & Bezbradica, D. (2011) Immobilization of lipase from *C. rugosa* on Sepabeads: the effect of lipase oxidation by periodates. *Bioprocess Biosystems Engineering* (in press, DOI 10.1007/s00449-011-0530-2)

Rich, J., Mozahev, V., Dordick, J., Clark, D. & Khmelnitsky, Y. (2002) Molecular imprinting of enzymes with water insoluble ligands for nonaqueous biocatalysis. *Journal of the American Chemical Society,* Vol. 124, No. 19, (May 2002), pp. 5254-5255, ISSN 0002-7863

Robles-Medina, A., Gonzales-Moreno, P.A., Esteban-Cerdan, L. & Molina-Grima, E. (2009) Biocatalysis: Towards ever greener biodiesel production. *Biotechnology Advances*, Vol. 27, No. 4, (October 2008), pp. 398-408, ISSN0734-9750

Rodrigues, R.C., Pessela, B.C.C., Volpato, G., Fernandez-Lafuente, R., Guisan J.M. & Ayub, M.A.Z. (2010). Two step ethanolysis: A simple and efficient way to improve the enzymatic biodiesel synthesis catalyzed by an immobilized-stabilized lipase from *Thermomyces lanuginosus*. *Process Biochemistry,* Vol. 45, No. 8, (August 2010), pp. 1268-1273, ISSN 1359-5113

Royon, D., Daz, M., Ellenrieder, G. & Locatelli, S. (2007). Enzymatic production of biodiesel from cotton seed oil using t-butanol as a solvent. *Bioresource Technology,* Vol. 98, No. 3, (February 2007), pp. 648-653, ISSN 0960-8524

Salis, A., Pinna, M., Monduzzi, M. & Solina, V. (2005) Biodiesel production from triolein and short chain alcohols through biocatalysis. *Journal of Biotechnology*, Vol. 119, No. 3, (September 2005), pp. 291-299, ISSN 0168-1656

Salis, A., Pinna, M., Monduzzi, M. & Solina, V. (2008) Comparison among immobilised lipases on macroporous polypropylene toward biodiesel synthesis. *Journal of Molecular Catalysis B: Enzymatic,*Vol. 54, No. 1-2, (July 2008), pp. 19-26, ISSN 1381-1177

Samukawa, T., Kaieda, M., Matsumoto, T., Ban, K., Kondo, A., Shimada, Y., Noda, H. & Fukuda, H. (2000) Pretreatment of immobilized *Candida antarctica* for biodiesel fuel production from plant oil. *Journal of Bioscience and Bioengineering,* Vol. 90, No. 2, (May 2000), pp. 180-183, ISSN 1389-1723

Schmid, R.D. & Verger, R. (1998) Lipases: interfacial enzymes with attractive applications. *Angewandte Chemie International Edition,* Vol. 37, No. 12, (December 1998), pp. 1608-1633, ISSN 1433-7851

Selmi, B. & Thomas, D., (1998) Immobilized lipase-catalyzed ethanolysis of sunflower oil in a solvent-free medium. *Journal of the American Oil Chemists' Society,* Vol. 75, No. 8, (August 1998), pp. 691-695, ISSN 0003-021X

Severac, E., Galy, O., Turon, F., Monsan, P. & Marty, A. (2011) Continuous lipase-catalyzed production of esters from crude high-oleic sunflower oil. *Bioresource Technology,* Vol. 102, No. 8, (April 2011), pp. 4954-4961, ISSN 0960-8524

Shah S. & Gupta M.N., (2007) Lipase catalyzed preparation of biodiesel from *Jatropha* oil in a solvent free system. *Process Biochemistry,* Vol. 42, No. 2, (March 2007), pp. 409-414, ISSN 1359-5113

Shimada, Y., Watanabe, Y., Samukawa, T., Sugihara A. & Noda, H. (1999) Conversion of vegetable oil to biodiesel usin immobilized *Canida antarctica* lipase. *Journal of the American Oil Chemists' Society,* Vol. 76, No. 7,(July 1999), pp. 789-793, ISSN 0003-021X

Shimada, Y., Watanabe, Y., Sugihara, A. & Tominanga, Y., (2002) Enzymatic alcoholysis for biodiesel fuel production and application of the reaction to oil processing. *Journal of Molecular Catalysis B: Enzymatic,* Vol.17, No. 3-5, (June 2002), pp. 133-142, ISSN 1381-1177

Sotoft, L.F., Rong, B.G., Christensen, K.V. & Norddahl, B. (2010) Process simulation and economical evaluation of enzymatic biodiesel production plant. *Bioresource Technology*, Vol. 101, No. 14, (July 2010), pp. 5266-5274, ISSNv0960-8524

Soumanou, M. & Bornscheuer, U.T. (2003). Improvement in lipase-catalyzed synthesis of fatty acid methyl esters from sunflower oil. *Enzyme and Microbial Technology*, Vol. 33, No. 1, (July 2003), pp. 97-103, ISSN 0141-0229

Talukder, M.R., Wu, J.C., Nguyen, T.B.V., Fen, N.M. & Melissa, Y.L.S. (2009) Novozyme 435 for production of biodiesel from unrefined oil: Comparison of the methanolysis methods. *Journal of Molecular Catalysis B: Enzymatic,* Vol.60, No. 3-4, (October 2009), pp.106-112, ISSN 1381-1177

Tamalampudi, S., Talukder, M.R., Hama, S., Numata, T., Kondo, A. & Fukuda, H. (2008). Enzymatic production of biodiesel from Jatropha oil: A comparative study of

immobilized-whole cell and commercial lipases as a biocatalyst. *Biochemical Engineering Journal,* Vol. 39, No. 1, (April 2008), pp. 185-189, ISSN 1369-703X

Tan, T., Lu, J., Nie, K., Deng, L. & Wang, F. (2010) Biodiesel production with immobilized lipase: A review. *Biotechnology Advances,* Vol. 28, No. 5, (September-October 2010), pp. 628-634, ISSN 0734-9750

Wang, L., Du, W., Liu, D. & Dai, N. (2006). Lipase-catalyzed biodiesel production from soybean oil deodorizer distillate with absorbent present in *tert*-butanol system. *Journal of Molecular Catalysis B: Enzymatic,* Vol.43, No. 1-4, (December 2006), pp. 29-32, ISSN 1381-1177

Watanabe, Y., Samukawa, T., Sugihara, A., Noda, H., Fukuda, H. & Tominaga, Y. (1999) Conversion of vegetable oil to biodiesel using immobilized *Candida antarctica* lipase. *Journal of the American Oil Chemists' Society,* Vol. 76, No. 7, (July 1999), pp. 789-793, ISSN 0003-021X

Watanabe, Y., Shimada, Y., Sugihara, A., Noda, H., Fukuda, H. & Tominaga, Y. (2000) Continuous production of biodiesel fuel from vegetable oil using immobilized *Candida antarctica* lipase. *Journal of the American Oil Chemists'Society,* Vol. 77, No. 4, (April 2000), pp. 355-360, ISSN 0003-021X

Watanabe, Y., Shimada, Y., Saguhara, A. & Tominaga, Y. (2001) Enzymatic conversion of waste edible oil to biodiesel fuel in a fixed bed reactor, *Journal of the American Oil Chemists' Society,* Vol. 78, No. 7, (July 2001), pp. 701-707, ISSN 0003-021X

Watanabe, Y., Shimada, Y., Sugihara, A. & Tominaga, Y. (2002) Conversion of degummed soybean oil to biodiesel fuel with immobilized *Candida antarctica* lipase, *Journal of Molecular Catalysis B: Enzymatic,* Vol.17, No. 3-5, (June 2002), pp. 151-155, ISSN 1381-1177

Watanabe, Y., Pinsirodom, P., Nagao, T., Yamauchi, A., Kobayashi, T., Nishida, Y., Takagi, Y. & Shimada, Y. (2007) Conversion of acid oil by-produced in vegetable oil refining to biodiesel fuel by immobilized *Candida antarctica* lipase. *Journal of Molecular Catalysis B: Enzymatic,* Vol.44, No. 3-4, (March 2007), pp. 99-105, ISSN 1381-1177

Xie, W. & Ma, N. (2009). Immobilized Lipase on Fe_3O_4 Nanoparticles as Biocatalyst for Biodiesel Production. *Energy & Fuels*, Vol. 23, No. 3, (January 2009), pp. 1347-1353, ISSN 0887-0624

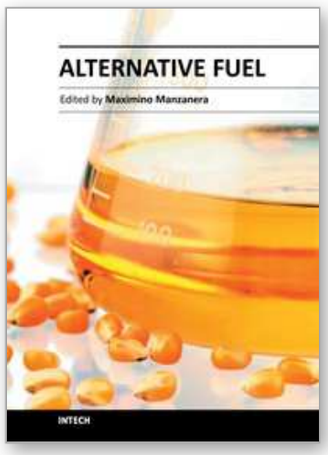
Xu, Y., Du, W. & Liu, D. (2005) Study on the kinetics of enzymatic interesterification of triglycerides for biodiesel production with methyl acetate as the acyl acceptor. *Journal of Molecular Catalysis B: Enzymatic,* Vol. 32, No. 5-6, (March 2005), pp. 241-245, ISSN 1381-1177

Yadav, G. & Jadhav, S. (2005) Synthesis of reusable lipases by immobilization on hexagonal mesoporous silica and encapsulation in calcium alginate: Transesterification in non-aqueous medium. *Microporous and Mesoporous Materials,* Vol. 86, No. 1-3, (November 2005), pp. 215-222, ISSN 1387-1811

Yagiz, F., Kazan, D. & Akin, A.N. (2007) Biodiesel production from waste oils by using lipase immobilized oh hydrotalcite and zeolites. *Chemical Engineering Journal,* Vol. 134, No. 1-3, (November 2007), pp. 262-267, ISSN 1385-8947

Yang, G., Tian-Wei, T., Kai-li, N. & Fang, W. (2006). Immobilization of lipase on macroporous resin and its application in synthesis of biodiesel in low aqueous media. *Chinese Journal of Biotechnology,* Vol. 22, No. 1, (January 2006), pp. 114-118

Ying, M. & Chen, G. (2007). Study on the Production of Biodiesel by Magnetic Cell Biocetalyst Based on Lipase-Producing Bacillus subtilis, *Applied Biochemistry and Biotechnology*, Vol. 137-140, No. 1-12, (April 2007), pp. 793- 804, ISSN 0273-2289



Alternative Fuel

Edited by Dr. Maximino Manzanera

ISBN 978-953-307-372-9
Hard cover, 346 pages
Publisher InTech
Published online 09, August, 2011
Published in print edition August, 2011

Renewable energy sources such as biodiesel, bioethanol, biomethane, biomass from wastes or hydrogen are subject of great interest in the current energy scene. These fuels contribute to the reduction of prices and dependence on fossil fuels. In addition, energy sources such as these could partially replace the use of what is considered as the major factor responsible for global warming and the main source of local environmental pollution. For these reasons they are known as alternative fuels. There is an urgent need to find and optimise the use of alternative fuels to provide a net energy gain, to be economically competitive and to be producible in large quantities without compromising food resources.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Nevena Luković, Zorica Knežević-Jugović and Dejan Bezbradica (2011). Biodiesel Fuel Production by Enzymatic Transesterification of Oils: Recent Trends, Challenges and Future Perspectives, Alternative Fuel, Dr. Maximino Manzanera (Ed.), ISBN: 978-953-307-372-9, InTech, Available from: http://www.intechopen.com/books/alternative-fuel/biodiesel-fuel-production-by-enzymatic-transesterification-of-oils-recent-trends-challenges-and-futu

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen