Chapter 8

Lipase Applications in Biodiesel Production

Sevil Yücel, Pınar Terzioğlu and Didem Özçimen

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/52662

1. Introduction

Because of the global warming and depletion of fossil fuels, in recent years, intensive investigations are carried on for providing the greater use of sustainable biofuels instead of fossil fuels. Biomass, which various biofuels are produced from, has an important role among other alternative energy sources including wind energy, solar energy, geothermal energy, etc.

Biodiesel is one of the important biofuels and a clean energy source as an alternative to petroleum-based diesel fuels. Biodiesel has some advantages and disadvantages. Transportability, high combustion efficiency, low sulphur and aromatic content, high cetane number and biodegradability are advantages of the biodiesel [1]. Disadvantages of biodiesel are high viscosity, lower energy content, high cloud and pour point, high nitrogen oxide emission, lower engine speed and power, injector cooking, high price and engine erosion [2].

The flash point of biodiesel is higher than diesel fuel. This feature is important for fuel storage and transportation in the way of safety. Cetane number of biodiesel (~50) is higher than diesel fuel [3]. Biodiesel does not include aromatic and sulphur content and contains oxygen at the rate of 10-11% by mass [4]. Cetane number is an important factor to determine the quality of diesel fuel, especially ignition quality of diesel fuel. In other words, it determines the ignition tendency of fuel when being injected into engine. Ignition quality of biodiesel is determined by the structure of methyl ester [5].Viscosity is also an important factor for biodiesel. Viscosity affects mostly fuel injection equipment and the increase of fuel viscosity changes the viscosity at low temperatures. High viscosity has an negative effect on fuel spray atomization [6]. Amounts of elements and compounds in biodiesel and diesel fuel are present in Table 1 [7]. Biodiesel has more polar structure than diesel fuel because of the oxygen, which is an electronegative element present in its structure, and therefore biodiesel has higher viscosity comparing with diesel fuel. In addition, elemental oxygen content is responsible for lower heating value of biodiesel when compared with diesel fuel. [7-9]. Biodiesel

can be used in its pure form or when mixed with diesel fuel in certain proportions. Most common biodiesel blends are B2 (2 % biodiesel, 98 % diesel), B5 (5 % biodiesel, 95% diesel), B20 (20 % biodiesel, 80 % diesel) [10].

	Biodiesel Content (%)	Diesel Content (%)
Carbon	79.6	86.4
Hydrogen	10.5	13.6
Oxygen	8.6	-
Nitrogen	1.3	-
C/H	7.6	6.5
n-Aliphatics	15.2	67.4
Olephenics	84.7	3.4
Aromatics	-	20.1
Naphtens	-	9.1

Table 1. The comparison of elemental and chemical content of diesel and biodiesel [7]

The transesterification reaction can be influenced by several factors including molar ratio of alcohol, catalyst, presence of water, free fatty acid in oil samples, temperature, time and agitation speed. In this context, an understanding of the factors affecting the process is very important to make economically and environmentally biodiesel production [11].

To accelerate reaction rate, transesterification process is carried out in the presence of catalysts. So, biodiesel production is made by using chemical or enzymatic catalysts. Compared to chemical, enzymatic reaction is more attractive because of ability of make a high quality product, simplify the separation of products, mild reaction conditions, the reuse of the catalyst and especially environmental impact, although high conversion and reaction rate are obtained with chemical catalysts [11-14]. Lipase is important enzyme catalyst that catalyzes esterification and transesterification reaction to produce methyl esters (biodiesel). Figure 1 presents the enzymatic transesterification reaction [15].

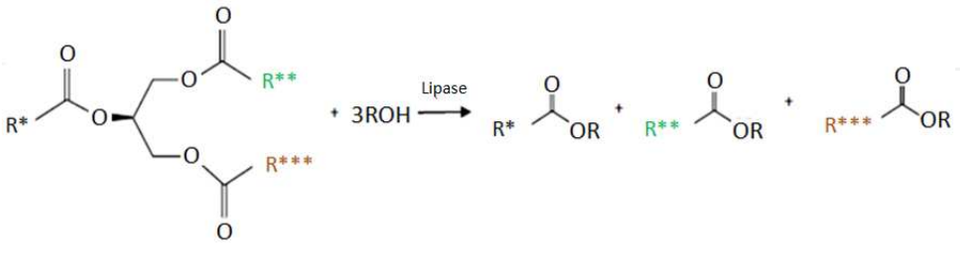

Figure 1. Enzymatic transesterification reaction [15].

In this study, enzymatic approach for biodiesel production was reviewed, and especially the usage of lipases in biodiesel production and factors affecting the effectiveness of lipase in reaction were explained in detail.

http://dx.doi.org/10.5772/52662

2. Lipases in biodiesel production

Biocatalyst based biotechnological applications are receiving increasing attention. Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are the important biocatalysts because of their excellent biochemical and physiological properties. Lipases are the hydrolytic enzymes that can be used in various industrial applications for alcoholysis, acidolysis, amynolysis and hydrolysis reactions. Biodiesel production is one of the stunning applications of lipase. Lipase catalyzed biodiesel production was reported first by Mittelbach [16]. Lipase-catalyzed transesterification takes place in two steps, which involves hydrolysis of the ester bond and esterification with the second substrate [15]. A ping-ping bi bi mechanism generally used for kinetic studies of enzyme catalyzed transesterification.

Lipases can be isolated from many species of plants (papaya latex, oat seed lipase, and castor seed lipase), animals (pig's and human pancreatic lipases), bacteria, filamentous fungi and yeast [17-19]. For industrial enzyme production generally microorganisms are preferred because of their shortest generation time [20]. The other advantages of microorganisms can be listed as high yield of conversion of substrate into product, great versatility to environmental conditions and, simplicity in genetic manipulation and in cultivation conditions [20]. Although lipases from different sources are able to catalyze the same reaction, bacterial and fungal lipases are mostly used in biodiesel production such as *Aspergillus niger,Candida antarctica, Candida rugosa, Chromobacterium Viscosum, Mucor miehei, Pseudomonas cepacia, Pseudomonas fluorescens, Photobacterium lipolyticum, Rhizopus oryzae, Streptomyces sp., and Thermomyces lanuginose* [21]. *Candida rugosa*, obtained from yeast, is the most used microorganism for lipase production [22]. Recently, *Streptomyces sp.* was investigated as a potent lipase producing microbe for biodiesel production and found applicable in the field of biodiesel [23].

Specificity of lipases has a great importance in the selection of the usage area of lipases. Lipases can be divided into three groups due to their specificity as 1,3-specific lipases, fatty acid-specific lipases and nonspecific lipases. Especially, 1,3-specific lipases which release fatty acids from positions 1 and 3 of a glyceride and hydrolyze ester bonds in these positions such as *Aspergillus niger, Rhizopus oryzae and Mucor miehei* catalyze transesterification reactions efficiently [20,24]. The study of Du et al. [25], showed that higher yield (90%) was achieved for biodiesel production by using a sn-1,3-specific lipase, *Thermomyces lanuginosa* immobilized on silica gel (Lipozyme TL IM). Thus, the use of sn-1,3- specific lipases can give rise to biodiesel yield of above 90% under appropriate conditions [24]. Substrate specificity of lipases is also a crucial factor towards the biodiesel production which acts on the choice of the proper enzyme based on the composition of raw materials by consisting in the capability of distinguishing structural features of acyl chains [20,24]. Lipases from *Pseudomonas fluorescens, Pseudomonas cepacia, Candida rugosa, Candida antarctica and Candida cylindracea* are suitable for transesterification reaction by displaying both wide substrate specificity and regiospecificity [24].

2.1. Immobilization of lipases

The immobilization of enzymes, which is attracting worldwide attention, was firstly reported in 1971 at Enzyme Engineering Conference [26]. During the past decade, chemical modification, physical modification, and gene expression techniques have been developed to obtain more economic, active, selective, or stable lipases. Immobilization is a modification method that can be defined as attaching the enzyme onto an insoluble solid support material [18]. By immobilization more operational and temperature stable lipases can be obtained and also lipases can be reused in the reactions. In addition, reusability of lipases will be a possible solution to the high cost of the enzymes and make them suitable for applications in industrial scale. The comparison of free enzymes and immobilized enzymes is given in Table 2. Methods for enzyme immobilization can be classified as adsorption, covalent bonding, entrapment, and cross-linking. The selection of method and support material is a prominent factor for obtaining an efficient lipase. The results of comparative studies revealed that the same lipase molecule can show very different catalytic activities after immobilization onto different supports [27].

Characteristics	Free Enzyme	Immobilized Enzyme
Price	High	Low
Efficiency	Low	High
Activity	Unstable	Stable
Reusability and recovery	Not possible	Possible
Tolerance to temperature, pH, etc.	Low	High
To separate from the substrate	Difficult	Easy
To separate from the product	Difficult	Easy

Table 2. The comparison of free enzyme and immobilized enzyme [19]

2.1.1. Adsorption technique

Adsorption is the adhesion of lipase on the surface of the adsorbent by weak forces, such as van der Walls, ionic and hydrophobic interactions, or dispersion forces [28]. Immobilization via adsorption method is the simply mixing of an aqueous solution of enzyme with the carrier material for a period and washing away the excess enzyme from the immobilized enzyme on the carrier after a time [29]. The level of adsorption is strictly related to the pH, temperature and ionic strength. Adsorption is the most widely employed method besides

other methods because of its special commercial advantages and simplicity. Adsorption is the only reversible enzyme immobilization method. The advantages of adsorption is mild and easy preparing conditions, low cost, no need for chemical additives, the carrier can be recovered for repeated use, and high activity [30].

Various types of carriers used in immobilization of lipases. Acrylic resin, celite, polypropylene and textile membrane are broadly used carriers. Some of the reported results of adsorption technique based immobilized enzymes used in biodiesel production are summarized in Table 3. As can be seen from table generally the biodiesel yields using the enzymes obtained by adsorption method are higher than 85%. Novozym 435 is a commercial lipase, which is obtained by immobilization of *Candida antartica* lipase on acrylic resin and is a good catalyst that provides biodiesel yield higher than 90% with vegetable oil or waste cooking oil as feedstock [31]. The other commercialized lipase is known as *Candida sp.* 99–125 lipase immobilized on textile membrane, which can catalyze lard, waste oil and vegetable oils with higher yields that is more than 87% [31]. Besides many advantages of immobilization by adsorption method, the main disadvantage is that desorption of the lipase from the carrier occurs because of the weak interactions between the enzyme and support.

2.1.2. Covalent binding technique

Another approach is covalent binding technique, which is the formation of covalent bonds between the aldehyde groups of support surface and active amino acid residues on the surface of the enzyme [29]. A variety of supports have been used such inorganic materials, natural polymers (agarose, chitin and chitosan), synthetic polymers (hydrophobic polypeptides,nylon fibers) and Eupergit® (made by copolymerization of N,N′-methylene-bis-(methacrylamide), glycidyl methacrylate, allyl glycidyl ether and methacrylamide) for immobilization of lipases by covalent binding [56].The main advantage of covalent binding method is obtaining thermal and operational stable enzymes because of strong interactions between the lipase and the carrier [31]. The comparison of biodiesel production performance using immobilized lipase via covalent binding method is summarized in Table 4. Chitosan is a promising carrier as a natural polymer due to its membrane forming and adhesion ability, high mechanical strength and facility of forming insoluble in water thermally and chemically inert films [57]. Xie and Wang [58], reported a technique for immobilization of *Candida rugosa* lipase on magnetic chitosan microspheres for transesterification of soybean oil.The immobilized enzyme was determined as an effective biocatalyst for the transesterification reaction due to giving a good conversion of soybean oil and retaining its activity during the four cycles [58].

Using two immobilized lipases with complementary position specificity instead of one lipase is a new approach to produce a cost effective biodiesel [19]. Lipase from *Rhizopus orizae* and *Candida rugosa* was covalently bound to the silica, which was used to produce biodiesel from crude canola oil. Under optimum conditions, the conversion rate of degummed crude canola oil to fatty acid methyl esters was 88.9%, which is higher than the conversion obtained by free enzyme mixture (84.25%) [59].

Lipase Source	Carrier	Acid/Oil Source	Alcohol	Maximum Performance (%)	Reference
Burkholderia sp. C20	Alkyl-functionalized Fe_3O_4–SiO_2	Olive	Methanol	92 (conversion)	[32]
Candida antartica	Acrylic resin	Soybean	Methanol	92(yield)	[33]
Candida antartica	Acrylic resin	Soybean and rapeseed	Methanol	98.4 (conversion)	[34]
Candida antartica	Acrylic resin	Soybean and rapeseed	Methanol	"/>95 (conversion)	[35]
Candida antarctica B	Granular activated carbon	Palm	Isobutanol	100 (conversion)	[36]
Candida sp. 99–125	Textile membrane	Lard	Methanol	87.4 (yield)	[37]
Candida sp. 99–125	Textile (cotton) membrane	Salad	Methanol	96 (conversion)	[38]
Candida sp. 99–125	Textile membrane	Crude rice bran	Methanol	87.4 (yield)	[39]
Candida rugosa and Pseudomonas fluorescens	Acurel	Palm	Ethanol	89 (yield)	[40]
Chromoacterlum viscosum	Celite-545	Jatropha	Ethanol	92 (yield)	[41]
Geobacillus thermocatenulatus	Poly-hydroxybutyrate beads	Babassu	Ethanol	100 (yield)	[42]
Pseudomonas aeroginosa	Celite	Soybean	Methanol	80(yield)	[43]
Pseudomonas cepacia	Celite	Jatropha	Ethanol	98 (yield)	[44]
Pseudomonas cepacia	Electrospun polyacrylonitrile fibers	Rapeseed	n-butanol	94 (conversion)	[45]
Pseudomonas cepacia	Polystyrene	Sapium sebiferum	Methanol	96.22 (yield)	[46]
Pseudomonas cepacia	Ceramic beads	Waste cooking	Methanol	40 (yield)	[47]
Pseudomonas fluorescens	Porous kaolinite particle	Triglyceride triolein	1-propanol	"/>90 (conversion)	[48]
Pseudomonas fluorescens and Pseudomonas cepacia	Polypropylene powder	Soybean	Methanol	58 37 (yield)	[49]
Penicillium expansum	Resin D4020	Waste	Methanol	92.8 (yield)	[50]
Rhizomucor miehei	Hydrophilic resins	Olive husk	Ethanol	-	[51]
Rhizomucor miehei	Silica	Waste cooking	Methanol	91.08 (yield)	[52]
Rhizopus oryzae	Macroporous resin HPD-400	Pistacia chinensis bge seed	Methanol	94 (yield)	[53]
Saccharomyces cerevisiae	Mg–Al hydrotalcite	Rape	Methanol	96 (conversion)	[5454]
Thermomyces lanuginosus (Lipozyme TL IM)	Hydrotalcite	Waste cooking	Methanol	95 (yield)	[55]

Table 3. Comparison of biodiesel production performance using immobilized lipase via adsorption method

http://dx.doi.org/10.5772/52662

Lipase Source	Carrier	Acid/Oil Source	Alcohol	Maximum Performance (%)	Reference
Burkholderia cepacia	Niobium Oxide (Nb_2O_5)	Babassu	Ethanol	74.13 (yield)	[60]
Burkholderia cepacia	Polysiloxane–Polyvinyl Alcohol (SiO_2–Pva)	Babassu Beef Tallow	Ethanol	100 89.7 (yield)	[60]
Candida rugosa	Chitosan Microspheres	Soybean	Methanol	87 (conversion)	[58]
Candida rugosa	Chitosan Powder	Rapeseed Soapstock	Methanol	95 (conversion)	[61]
Enterobacter aerogenes	Silica	Jatropha	Methanol	94 (yield)	[62]
Porcine pancreatic	Chitosan Beads	Salicornia	Methanol	55 (conversion)	[63]
Pseudomonas fluorescens	Toyopearl Af-Amino-650m Resin	Babassu	Ethanol	94.9 (yield)	[64]
Rhizopus oryzae	Resin Amberlite Ira-93	Pistacia Chinensis Bge Seed	Methanol	92 (yield)	[63]
Rhizopus oryzae	Polystyrene Polymer(Amberlite Ira-93)	Soybean	Methanol	90.05 (yield)	[65]
Rhizopus Orizae +*Candida rugosa*	Silica	-	Methanol	"/>98 (conversion)	[66]
Rhizopus orizae +Candida rugosa	Silica	Crude Canola	Methanol	88.9 (conversion)	[59]
Thermomyces lanuginosus	Olive Pomace	Pomace	Methanol	93 (yield)	[67]
Thermomyces lanuginosus	Polyglutaraldehyde Activated Styrene-Divinylbenzene Copolymer	Canola	Methanol	97 (yield)	[68]
Thermomyces lanuginosus	Toyopearl Af-Amino-650m Resin	Palm	Ethanol	100 (yield)	[64]
Thermomyces lanuginosus	Polyurethane Foam	Canola	Methanol	90 (yield)	[69]
Thermomyces lanuginosus	Aldehyde-Lewatit	Soybean	Ethanol	100 (conversion)	[70]
Thermomyces lanuginosus	Magnetic Fe_3O_4 Nano-Particles	Soybean	Methanol	90 (conversion)	[71]

Table 4. Comparison of biodiesel production performance using immobilized lipase via covalent binding method

2.1.3. Entrapment technique

Entrapment method is based on capturing of the lipase within a polymer network that retains the enzyme but allows the substrate and products to pass through [72]. This method can be simply defined as mixing an enzyme with a polymer solution and then crosslinking the polymer to form a lattice structure that captures the enzyme [29]. Entrapment is often used for industrial applications because the method is fast, cheap and can be carried out under mild conditions [73]. Entrapment can be divided into three categories such as gel or fiber entrapping and microencapsulation [74]. A number of supports have been investigated such as alginate, celite, carrageenan, resins, acrylic polymers etc. Some carriers used for entrapment and the biodiesel production yields obtained by these enzymes are displayed in Table 5. A disadvantage of entrapment method is the mass transfer problem due to the act of support as a barrier, so the lipase became effective only for low molecular weight substrates [19,75].

Lipase Source	**Carrier**	**Acid/Oil Source**	**Alcohol**	**Maximum Performance (%)**	**Reference**
Burkholderia cepacia	*K*-Carrageenan	Palm	Methanol	100 (conversion)	[76]
Burkholderia cepacia	Phyllosilicate Sol–Gel	Tallow and Grease	Ethanol	94 (yield)	[77]
Burkholderia cepacia	Mtms-Based Silica Monolith Coated With Butyl-Substituted Silicates	Jatropha	Methanol	95 (yield)	[78]
Candida antarctica	Celite®	Triolein	Methanol	60 (conversion)	[79]
Candida rugosa	Calcium Alginate Matrix	Palm	Ethanol	83 (yield)	[80]
Candida rugosa	Activated Carbon	Palm	Ethanol	85 (conversion)	[81]
Pseudomonas cepacia	Hydrophobic Sol–Gel	Soybean	Methanol	67 (conversion)	[82]
Pseudomonas fluorescens Mtcc 103	Alginate	Jatropha	Methanol	72 (yield)	[83]
Via Encapsulation Method					
Burkholderia cepacia	Silica Aerogels	Sunflower Seed	-	56 (conversion)	[84]
Burkholderia cepacia	*K*-Carrageenan	Palm	Methanol	100 (conversion)	[85]
Candida antartica	Silica Aerogels	Sunflower Seed	Methanol	90 (conversion)	[86]

Table 5. Comparison of biodiesel production performance using immobilized lipase via entrapment method

2.1.4. Cross linking technique

Cross-linking is another method for immobilization that can be defined as the interaction of a three dimensional network within enzyme, coupling reagent, and carrier [19]. The advantage of cross-linking is obtaining stable lipases due to the strong interaction between the lipase and the carrier. On the other hand, the cross-linking conditions are intense and the immobilized lipase shows lower activity [31].

The high free fatty acid content of waste cooking oil form water by esterification with alcohol which cause agglomeration of lipase and lowering biocatalysis efficiency [87]. Hence, free *Geotrichum sp.* lipase was not a suitable enzyme catalyst for transesterification of waste cooking oil. Yan et al. [87], report a modification procedure for preparation of cross-linked *Geotrichum sp.* The obtained lipase exhibited improved pH and thermostable stability compared to free lipase. The relative biodiesel yield was 85% for transesterification of waste cooking oil with methanol.

Kumari et al. [88] studied the preparation of *Pseudomonas cepacia* lipase cross-linked enzyme aggregates. It was shown that cross linked lipases has a greater stability than free enzymes to the denaturing conditions. The enzyme also used to catalyze madhuca indica oil, which's transesterification is difficult by chemical routes due to its high free fatty acid content. As a result, 92% conversion was obtained after 2.5 h.

Immobilization of *Candida rugosa* lipase on fine powder of Scirpus grossus L.f. by glutaraldehyde by cross linked technique for biodiesel production from palm oil, as already investigated by Kensingh et al. [89]. It was concluded that immobilized lipase yielded higher conversion of biodiesel than that of free lipase.

Lorena et al. [90] investigated the immobilization of the *Alcaligenes spp.* lipase on polyethylenimine agarose, glutaraldehyde agarose, octyl agarose, glyoxyl agarose, Sepabeads® by the aggregation and crosslinking method. The transesterification of canola oil was achieved with a yield 80% using a six-step addition of methanol and lipase immobilized on Sepabeads® by the aggregation method.

All these methods are shown schematically in Figure 2.

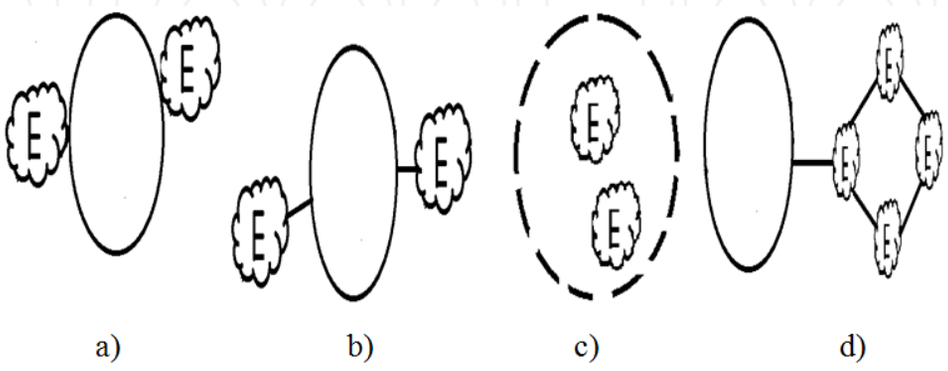

Figure 2. Schematic diagram of enzyme immobilization methods: a)Adsorption method b)Covalent binding method c)Entrapment method d) Crosslinking method

2.1.5. Whole cell immobilization

The applicability of lipases for the bulk production of fuels was limited significantly by the high cost of lipases [91]. Utilizing microbial cells such as fungi, bacteria, and yeasts cells containing intracellular lipase instead of extracellular lipases (free and immobilized lipase) is an easier and a cost effective way of enzymatic biodiesel production. Compared to conventional enzymatic processes, the use of whole cells provides excellent operational stability and avoids the complex procedures of isolation, purification and immobilization [91,92]. The general preparation steps for immobilized extracellular enzymes and whole cell enzymes showed in Figure 3. Biomass support particles have been used for immobilization of whole cells.

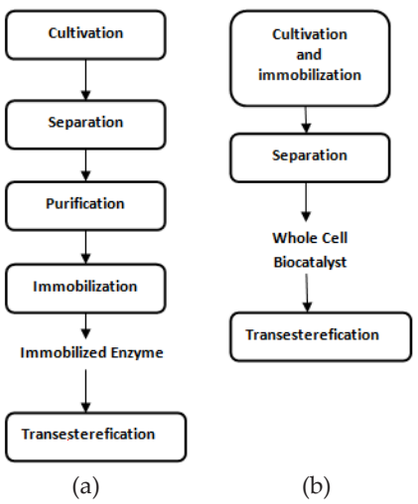

Figure 3. The preparation steps of a) immobilized extracellular lipase and b) whole cell biocatalyst

Aspergillus and Rhizopus have been most widely used as whole cell biocatalyst. Ban et al. [93], used first a whole cell biocatalyst, immobilized Rhizopus oryzae IFO4697 (a 1,3-positional specificity lipase) cells within biomass support particles, for the production of biodiesel and 91.1% methyl ester content was attained which was a similar result as that using the extracellular lipase. Many researchers have experimented on the use of whole cells to catalyze transesterification reaction summarized in Table 6.

A technique using glutaraldehyde cross-linking treatment on whole cell catalyst for methanolysis of soybean oil was developed by Sun et al. [94]. The glutaraldehyde cross linking treatment resulted in higher methanol tolerance and high catalytic activity (with the ratio of methanol to oil reaching 3). Also, a novel methanol addition strategy was proposed as stepwise addition of different amounts of methanol (1.0, 1.2, 1.5, and 2.0M equivalent of oil) every 24 h. It was found that the highest methyl ester yield could reach 94.1% after 24 h reaction by 1.2 mol, 1.5 M and 1.2 mol methanol additions at 0, 8, and 14 h. In general, the whole cell catalyzed process is slower than extracellular lipase catalyzed process. Sun et al. [94], also reported that the reaction time could be shortened by this way. It is clear that significant reduction in the cost of biodiesel production can be achieved by combining the whole cell biocatalyst process with stepwise addition of methanol.

Lipase Source	Carrier	Acid/Oil Source	Alcohol	Maximum Performance (%)	Reference
Aspergillus niger	BSPs[a]	Waste Cooking	Methanol	86.4 (yield)	[96]
Aspergillus niger	Polyurethane BSPs[a]	Palm	Methanol	>90 (yield)	[97]
Aspergillus niger	BSPs[a]	Palm	Methanol	87 (yield)	[98]
Aspergillus oryzae NS4	BSPs[a]	Soybean	Methanol	98 (conversion)	[99]
A. oryzae carrying r-CALB[b]	BSPs[a]	Palm Soybean	Methanol	85 90 (conversion)	[100]
Aspergillus oryzae expressing r-FHL[c]	BSPs[a]	Rapeseed	Methanol Ethanol 1-Propanol 1-Butanol	96 (yield) 94 (yield) 96 (yield) 97 (yield)	[101]
Escherichia coli BL21	-	Rapeseed	Methanol	97.7 (conversion)	[102]
Rhizopus chinensis CCTCC M201021	-	Soybean	Methanol	"/>86 (yield)	[103]
Rhizomucor miehei displaying Pichia pastoris	-	Soybean	Methanol	83.14 (yield)	[104]
Rhizopus oryzae IFO 4697	BSPs[a]	Refined Rapeseed Crude Rapeseed, Acidified Rapeseed	Methanol	~60(yield) ~60(yield) ~70(yield)	[105]
Rhizopus oryzae IFO 4697	BSPs[a]	Soybean	Methanol	~90(yield)	[106]
Rhizopus oryzae IFO 4697	BSPs[a]	Soybean	Methanol	~85(yield)	[107]
Rhizopus oryzae IFO4697	-	Soybean	Methanol	71 (conversion)	[108]
Rhizopus oryzae IFO 4697 and Aspergillus oryzae niaD300 (combined use)	BSPs[a]	Soybean	Methanol	~100 (conversion)	[109]

Lipase Source	Carrier	Acid/Oil Source	Alcohol	Maximum Performance (%)	Reference
Rhizopus oryzae ATCC 24563	-	Soybean (Free Fatty Acid Content 5.5%)	Methanol	97 (conversion)	[110]
Rhizopus oryzae IFO 4697	BSPs[a]	Soybean	Methanol	72 (yield)	[111]
Rhizopus oryzae	Polyurethane foam BSPs[a]	Soybean	Methanol	90 (conversion)	[112]
Rhizopus oryzae	BSPs[a]	Jatropha Curcas	Methanol	80 (conversion)	[113]
Rhizopus oryzae IFO 4697	-	Soybean	Methanol	86 (yield)	[114]
Rhizopus oryzae	BSPs[a]	Rapeseed	Methanol, Ethanol, 1-Propanol, 1-Butanol	83, 79, 93, 69 (yield)	[101]
Serratia marcescens YXJ-1002	-	Grease	Methanol	97 (yield)	[115]

[a]BSPs:Biomass support particles

[b]r-CALB: Candida antarctica lipase B

[c]r-FHL: Fusarium heterosporum lipase

Table 6. Comparison of biodiesel production performance using whole cell biocatalysts

Whole cell biocatalysts will be a way to industrialization of biodiesel production but the limited mass transfer efficiency of product and substrate is a hurdle to further investigations [95].

3. Feedstocks

The main aim of researches is to obtain a biodiesel, which will have a competitive price compared to other conventional sources of energy [116]. At this point, selecting the feedstock, represents more than 75-80% of the overall biodiesel production cost, is a vital step to ensure a cost effective biodiesel production. Different kinds of feedstock with varied range of edible and inedible vegetable oil, animal fats, waste oil, microbial oil and microalgae oil can be used for enzyme catalyzed transesterification [117].

3.1. Vegetable oils

Vegetable oils are candidates as alternative fuels for diesel engines with their high heat content [118]. But, direct use of vegetable oils is not possible because of the high kinematics viscosity of them which are varies in the range of 30–40 cSt at 38 °C and are about 10 times higher than of diesel fuel (Grade No. 2D) leads to many problems [118,119]. Therefore, modification of vegetable oil is necessary and the valuable product of this modification is named ''biodiesel''. The edible vegetable oils such as soybean [120,121], sunflower [122-124], palm [81,125], corn [126], cottonseed [127], canola [68,69,128] and olive [129,130] oils have been widely used in enzymatic transesterification. In developed countries, edible oils constitute more than 95% of biodiesel production feedstock because the produced biodiesel from these oils have properties very similar to petroleum-based diesel [131]. Also, the country and its climate, the oil percentage and the yield per hectare are effective parameters in selecting the potential renewable feedstock of fuel [118,132]. For example, while rapeseed oil prevailing the EU production, soybean oil prevailing the US and Latin American production, and palm oil mainly being used in Asia [133].

Inedible oils do not find a place in human consumption due to including toxic components. Therefore, inedible oils do not compete with food crops. Thus, inedible vegetable oils are an alternative feedstock for biodiesel production. Babassu (Orbinya martiana), Jatropha curcas (Linnaeus), neem (Azadiracta indica), polanga (Calophyllum inophyllum),karanja (Pongamia pinnata), rubber seed tree (Hevea brasiliensis), mahua (Madhuca indica and Madhuca longifolia), tobacco (Nicotina tabacum), silk cotton tree, etc. are promising inedible vegetable oil sources. Jatropha curcas is an attractive feedstock between various oil bearing seeds as it has been developed scientifically and found to give better biodiesel yield and productivity [134]. Crude Jatropha oil contains about 14% of free fatty acid that is too high for alkaline catalyzed biodiesel production [118]. However, high free acid content is not a problem in the production process of biodiesel via using enzyme catalysts. Besides Jatropha curcas, 26 species of fatty acid methyl ester of oils of including Azadirachta indica, Calophyllum inophyllum, and Pongamia pinnata were found most suitable for use as biodiesel, which adjust to the major specification of biodiesel standards of European Standard Organization, Germany, and USA [135]. Modi et al. reported conversion of crude oils of Pongamia pinnata (karanj), Jatropha curcas (jatropha) via immobilized Novozym 435 to biodiesel fuel with yield 90, and 92.7%, respectively [136].

3.2. Animal oils/fats

Animal fats are another group of feedstock for biodiesel production. Animal fats used to produce biodiesel via enzymatic route include lard [137], lamb meet [138] and beef tallow [139]. Animal fats are economically feasible feedstocks compared to vegetable oils. Animal fat methyl ester also has many favorably properties such as non-corrosive, high cetane number, and renewable [140,141]. However, animal fats saturated compounds lead to a tendency to oxidation and crystallization unacceptably at high temperatures [142].

3.3. Waste oils/fats

In general, around the world only half of the discharged edible oils recycled as animal feed or as raw material for lubricant and paint and the remainder is discharged into the environment [143]. Hence, the use of waste oils/fats for biodiesel production is very important to reduce and recycle the waste oil [143], to eliminate the environment and human health risk caused by waste oils [144] and to lower the biodiesel production cost. Waste cooking oil, animal fats, yellow grease, brown grease obtained from highly oxidized yellow grease or recovered waste grease from plumbing trap and waste sludge or soap-stock from the vegetable oil refining process were the major sources of waste oil have been used for biodiesel production [145]. The selection of a catalyst to be used for the production of biodiesel fuel is mostly influenced by the amount of free fatty acid content in various feedstocks [146]. The lipase-catalyzed reaction is a promising method for converting waste oils which contains high percentage of free fatty acids and high water content, into biodiesel with high yield [145]. It has been reported that Novozym 435 is capable of converting the used olive oils [129].

3.4. Algae oils

There is a considerable interest in the use of algae (micro and macro) oils for synthesis of biodiesel. Because these oils are cheap raw materials besides animal fats and have rapid growth rate and productivity when compared to conventional forestry, agricultural crops, high lipid content, tolerance for poor quality water, smaller land usage up to 49 or 132 times less when compared to rapeseed or soybean crops [142,147]. The smaller land usage brings the advantage of reducing the competition for arable soil with other crops, in particular for human consumption [147]. However, there are still some drawbacks for utilization of algae for biodiesel production. A considerable investment in technological development and technical expertise is needed to optimize the microalgae harvesting and oil extraction processes, to use cheap sources of CO_2 for culture enrichment [147]. Algae oils contain about 20-40% oil [148]. Several researchers have been experimented on microalgal oils as raw material for biodiesel production. Tran et al. [130], investigated the conversion of microalgal oil from Chlorella vulgaris ESP-31 to biodiesel by using immobilized Burkholderia lipase and a high fatty acid methyl esters conversion efficiency of 97.25 wt% oil (or 58.35 wt % biomass) was obtained for 48 h reaction. It is proposed that microalgal oil has good potential for application in the commercial production of biodiesel. The enzymatic conversion of microalgal oils to biodiesel in ionic liquids was firstly studied by Lai et al. [149]. Four microalgae two strains of Botryococcus braunii (BB763 and BB764), Chlorella vulgaris, and Chlorella pyrenoidosa have been catalyzed by two immobilized lipases, Penicillium expansum lipase and Candida antarctica lipase B (Novozym 435), in two solvent systems: an ionic liquid (1-butyl-3-methylimidazolium hexafluorophosphate, [BMIm][PF6]) and an organic solvent (tert-butanol). Penicillium expansum lipase was found more efficient for this application and the ionic liquid [BMIm] [PF6] showed a greater conversion yield (90.7% and 86.2%) obtained relative to the one obtained in the commonly used organic solvent tert-butanol (48.6% and 44.4%).

4. The effect of reaction parameterson enzymatic transesterification

4.1. The effect of temperature on enzymatic transesterification

Enzymatic transesterification takes place at low temperatures varying from 25 to 60°C. In general, initially the rate of reaction increases with rise in reaction temperature, because of an increase in rate constants with temperature and less mass transfer limitations [150,151]. Nevertheless, increased temperature after the optimum temperature promotes to denaturation and higher thermal deactivation of the enzyme, since it decreased the catalytic activity [152].

Various researches have been carried out to find out the effect of temperature on biodiesel production with immobilized enzymes. It is clear that immobilization provide more temperature resistance compared to free enzymes due to supplying a more rigid external backbone for lipase molecule [150,151]. However, optimum temperature is specific for each production. The studies about the effect of temperature for enzymatic transesterification are shown in Table 7.

Lipase	Oil Source	Alcohol	Performed Temperatures In The Range (°C)	Optimum Temperature (°C)	Reference
Immobilized Aspergillus niger	Palm	Methanol	25-50	40	[153]
Immobilized Aspergillus niger	Waste Cooking	Methanol	25-50	30	[154]
Immobilized Burkholderia cepacia	Babassu	Ethanol	39-56	39	[155]
Candida antarctica	Cotton Seed	T-Butanol	30-50	50	[156]
Candida antarctica	Acid	Methanol	30-50	30	[157]
Immobilized *Candida* Sp. 99–125	Salad	Methanol	27-50	40	[158]
Candida Sp. 99–125	Waste Cooking	Methanol	35-50	40-50	[159]
Immobilized Enterobacter aerogenes	Jatropha	T-Butanol	30-55	55	[160]
Immobilized Enterobacter aerogenes	Crude Rapeseed	Ethanol	25-50	35	[161]
Lipozyme RM IM	Soybean	Butanol	20-50	30	[162]
Lipozyme RM IM	Soybean	Methanol and Ethanol	40–60	50	[163]
Lipozyme RM IM	Soybean Oil Deodorizer Distillate	Ethanol	45-78	50	[164]
Lipozyme TL IM	Rapeseed	N-Butanol	30-60	40	[165]
Lipozyme TL IM	Soybean	Ethanol	20-50	35	[162]

Lipase	Oil Source	Alcohol	Performed Temperatures In The Range (°C)	Optimum Temperature (°C)	Reference
Lipozyme TL IM	Palm	Ethanol	30-78	50	[166]
Novozyme 435	Rapeseed	Methanol	25-55	40	[167]
Novozyme 435	Tung and Palm	Methanol and Ethanol	45-55	55	[168]
Novozym 435	Cottonseed	-(Dimethyl Carbonate As Organic Solvent)	30-55	50	[169]
Novozym 435	Canalo	Methanol	25-65	38	[170]
Novozym 435	Olive	Methanol	30-70	40	[129]
Novozym 435	Soybean	T-Amyl	30-60	40	[171]
Novozym 435	Sunflower	Methanol	25-65	45	[172]
Novozym 435	Stillingia	Methanol	30-60	40	[173]
Novozym 435	Cotton Seed	Methanol	30-70	50	[174]
Novozym 435, Lipozyme TL IM and Lipozyme RM IM	Soybean	Ethanol	25-60	25	[175]
Immobilized Penicillium expansum	Waste	T-Amyl	25-55	35	[176]
Immobilized Pseudomonas cepacia	Soybean	Methanol and Ethanol	25–60	35	[177]
Pseudomonas cepacia	Soybean	Methanol	20–60	30	[178]
Immobilized Pseudomonas fluorescens	Triolein	1-Propanol	40-70	60	[48]
Pseudomonas fluorescens	Soybean	Methanol	30-60	40	[49]
Rhizopus chinensis CCTCC M201021	Soybean	Methanol	30-40	30	[179]
Thermomyces lanuginosus	Canola	Methanol	30-70	40	[69]

Table 7. Data on optimum temperature for enzymatic biodiesel production

4.2. The effect of water content on enzymatic transesterification

Water content is one of the key factors for enzymatic transesterification reaction that have a strong effect on lipase's active three-dimensional conformational state [21,180]. Biocatalysts, needs a small amount of water to retain their activities [181]. Lipase has an unique feature on the water-oil interface, and the lipase activity depends on this interface. The presence of an oil–water interface required because it provides a suitable environment for enzyme activation which occurs due to the unmasking and restructuring the active site through confor-

mational changes of the lipase molecule [182,183]. When the addition of water increased, the amount of water available for oil to form oil–water droplets also increases, hence increasing the available interfacial area [182]. Thus, enzymatic activity can not be possible in a water free media. However, excess water cause reverse reaction of hydrolysis. The amount of required water, to provide an optimum enzyme activity, differs according to the type of enzyme and reaction medium composition. Enzymes, substrates, organic solvent and also immobilized support have a crucial role on optimal water activity for lipase [184]. Optimum water content not only provides keeping the hydrolysis of ester linkages at the minimum level, but also ensures the highest degree of transesterification [24]. Thus, a better control of water content is very important for enzymatic process.

Water activity (a_w) is defined as free (boundness) water in the system, which is a ratio of vapor pressure over the given system versus that over pure water [24]. Thermodynamic water activity is the best predictor of reaction rate that can be determined in any phase by different kinds of sensors such as holographic sensor, Weiss LiCl humidity sensor [180,185]. Also, several methods have been developed for control of water activity, for example, equilibration with saturated salt solutions [186], addition of salt hydrate pairs [187,188] and introduction of air or nitrogen into the reactor [189]. Recently, Peterson et. al. developed a practical way for control of water activity in large-scale enzymatic reactions by using a programmable logic controller. On the other hand, percentage water content is another expression which is used widely in transesterification, generally assayed by Karl-Fischer coulometer.

In general, lipases show higher activity with higher water activities in solvent free systems instead of Candida antarctica lipase (Novozym 435) [184]. For Candida sp. 99–125 lipase, the optimum water content is 10–20% based on the oil weight to maintain the highest transesterification activity [31].

Salis et al., investigated production of oleic acid alkyl esters by using Pseudomonas cepacia and determined that a_w in the range 0.4–0.6, 1-butanol:triolein 3:1 – were the best conditions to reach maximum enzymatic activity. It was also found that at the higher values of water activity, no hydrolysis reaction was occurred [190].

Noureddini and Philkana [82] tested immobilized Pseudomonas cepacia for the transesterification of soybean oil with methanol and ethanol and observed that increased addition of water provide a considerable increase in the ester yield. The optimal conditions were determined for processing 10 g of soybean oil by 475 mg lipase in 1 h as 1:7.5 oil/methanol molar ratio, 0.5 g water in the presence of methanol that resulted in 67 % yield and 1:15.2 oil/ethanol molar ratio, 0.3 g water in the presence of ethanol that resulted in 65% yield.

Al-Zuhair et al. studied the esterification of n-butyric acid with methanol in the presence of Mucor miehei lipase, and found similar results with literature [191] that higher water content, makes lipase more efficient [182].

Shah and Gupta used immobilized Pseudomonas cepacia lipase for ethanolysis of Jatropha oil and noted that the best yield 98% gained by in the presence of 4–5% (w/w) water in 8 h. The yield was only 70% in absence of water [44].

Kawakami et al. determined the effect of water content for transesterification of Jatropha oil and methanol to characterize Burkholderia cepacia lipase immobilized in an n-butyl-substituted hydrophobic silica monolith. The authors reported that biodiesel yield reached 90% with water content of 0.6% (w/w) after 12 h using a stoichiometric mixture of methanol and oil (3:1) [78].

Chen et al. investigated the effect of water content for production of biodiesel with oleic acid with methanol catalyzed by soluble lipase NS81020, produced by modification of Aspergillus oryzae microorganism, in the biphasic aqueous-oil systems and found that the esterification yield is low if the water was scant. The higher reaction rate and fatty acid methyl ester yield was obtained with 10 wt % water by oleic acid weight [192].

It is clear that during the past decade numerous investigations have been made to determine the optimal water content for transesterification. As a result, the necessary amount of water content is an important factor to create an interfacial surface between oil and water and to ensure optimal enzymatic activity. Also, water has a strong influence on structural integrity, active site polarity, and protein stability of lipase [21,193]. However, it differs from enzyme to reaction conditions.

4.3. The effect of acyl acceptors on enzymatic transesterification

Methanol, short chain alcohol, usually used as an acyl acceptor due to its low price and availability. Insoluble and a relatively high amount of methanol with respect to oil, have a negative influence on the stability of lipases and could be solved by a stepwise addition of the alcohols [15, 194]. To eliminate inhibitory effects of methanol some co-solvents are added to the reaction mixture. Tert-butanol is one of the important co-solvents which is added to enzymatic reaction. Usage of tert-butanol, a polar solvent, is also a possible solution for eliminating the inhibitory effects of methanol and glycerol (both of them soluble in tert-butanol) and suggested instead of using butanol [195]. Liu et al. [196], transesterified waste baked duck oil by three different commercial immobilized lipases (Novozym 435, Lipozyme TLIM and Lipozyme RMIM) with different monohydric alcohols (methanol, ethanol, propanol, isopropanol, isobutanol, isoamyl alcohol) and fusel oil-like alcohol mixture (containing 15% isobutanol, 80% isoamyl alcohol, 5% methanol) in solvent-free and tert-butanol systems. It was reported that each lipase presented a different kinetic pattern depending on the monohydric alcohols. The results showed that Lipozyme TL IM and Novozym 435 gave high conversion rate with isobutanol and isoamyl alcohol either in solvent-free or in tert-butanol system. Thus, the combined use of lipases, Novozym 435 and Lipozyme TLIM, as catalyst and fusel oil-like mixture as raw material for biodiesel synthesis was found effective in view of cost saving of biodiesel production [195].

Recently, novel acyl acceptors were investigated such as ethyl acetate, methyl acetate, butyl acetate, vinyl acetate [197], dimethyl carbonate [198]. Du and coworkers demonstrated the positive effect of methyl acetate, on enzymatic activity of Novozym 435 and found that lipase could be reused directly without any additional treatment [199]. The advantage of using methyl acetate is that the cost of the catalyst can be reduced dramatically due to the longer operational life and reusability of lipase. The byproduct of the system is triacetylgly-

cerol, which does not have any negative effect on the fuel property, and also no glycerol produced [200]. Hence, these advantages will provide industrial implementation of enzymatic biodiesel production. Dimethyl carbonate is another promising alternative acyl acceptor, which is eco-friendly, odorless, cheap, non-corrosive, and non-toxic [200]. The transesterification reaction is irreversible, because carbonic acid monoacyl ester, the intermediate compound, immediately decomposes to carbon dioxide and alcohol [200]. The fatty acid methyl ester yield is higher for lipase-catalyzed transesterification of vegetable oils with dimethyl carbonate besides conventional acyl acceptors (methanol and methyl acetate) [200]. Only, the higher price of acyl acceptor besides alcohols is a disadvantage [194].

4.4. Effects of the solvent on enzymatic transesterification reaction

In enzymatic transesterification reaction, excess of alcohol increases reaction efficiency, but if alcohol doesn't dissolve in reaction medium it can disrupt the enzyme activity. Methanol and vegetable oil in the values close to 1:1 molar ratio forms a solution in 40°C. Solvent is added into the reaction medium to increase the solubility of alcohol and thus it allows first step enzymatic transesterification by blocking degradation lipase catalytic activity [24]. To overcome deactivation of lipase activity and improve the lipase activity, various organic solvents have been used for enzymatic biodiesel synthesis. These solvents have been listed in Table 8. Cyclohexane, n-hexane, tert-butanol, petroleum ether, isooctane and 1,4-dioxane are mainly studied hydrophilic and hydrophobic organic solvents in enzymatic biodiesel production. In organic solvent medium, overall alcohol is added at the beginning of the reaction. In solvent free reaction medium, alcohol is added in several portions to prevent enzyme activity with high alcohol concentration [24].

Hexane is generally preferred because of its low cost and easily availability in the market. Some studies were performed in hexane solvent systems with soybean and tallow oil using monohydric alcohols [70,201, 202]. Nelson et al. performed transesterification of tallow with monohydric alcohols by Lipozyme IM 60 (M. miehei) and Novozyme SP435 (C. antarctica) in hexane and a solvent-free system. They compared the transesterification yields of two different systems. The yields with higher than 95% were obtained with methanol, ethanol and butanol with Lipozyme IM 60 lipase under hexane system (Table 8) while reaction yields under solvent-free system were 19% for methanol, 65.5% for ethanol, and 97.4% for isobutanol [201]. Similar results were found by Rodrigues et al. [70]. They compared the yields of transesterification of soybean with ethanol by Lipozyme TL IM. In the presence of n-hexane with 7.5:1 molar ratio of ethanol:soybean oil, the transesterification conversion was found to be as 100% while in solvent-free system the yield was 75%. At stoichiometric molar ratio, the yield was 70% conversion after 10 h of reaction in both systems. Transesterification conversion was obtained as 80% by three stepwise addition of ethanol, while a two step ethanolysis produced 100% conversion after 10 h of reaction in both solvent and solvent-free systems.

In enzyme catalyzed reaction, both alcohol amount and low glycerol solubility in biodiesel have negative effects on enzyme activity. Deposit of glycerol coating the immobilized catalyst is formed during the process, which reduces the enzymes activity [203]. The solubility of

methanol and glycerol in hydrophobic solvents is low. For this reason, this problem may occur in hydrophobic solvent system.

The enzymatic alcoholysis of triglyceride also was studied with petroleum ether, isooctane, cyclo hexane,1,4-dioxane (Table 4-2) [16,48,204]. Iso et al. [48], reported that when methanol and ethanol were used as alcohol in enzymatic transesterification, the reactions need an appropriate organic solvent [48]. On the other hand, the reaction could be performed without solvent when 1-propanol and 1-butanol was used. They also used, benzene, chloroform and tetrahydrofuran as solvent and immobilized P. fluorescens lipase as catalyst at 50°C to compare the results that of the 1,4 dioxane. The highest enzymatic activity was observed with 1,4-dioxane. The enzymatic activity increased with the high amount of 1,4-dioxane. But high conversion of oil (app.90%) to biodiesel was obtained with high proportion of 1,4 dioxane(90%). Although usage of high amount of solvents is not preferable in industry solvents can be recovered together with methanol after transesterifiation reaction.

Hydrophilic organic solvents can interact with water molecule in enzyme and this may affect the catalytic activity of enzyme. However, as shown in Table 8 high performance was ensured with hydrophilic solvents such as 1,4-dioxane and tert-butanol [48,156, 205-208]. Some studies were performed in the presence of t-butanol solvent because of positive effects on enzymatic catalyzed reaction. T-butanol has moderate polarity so methanol and glycerol are easily soluble in tertiary butanol. Solubility of methanol prevents enzyme inhibition and solubility of glycerol prevents accumulation on the enzyme carrier material. Another advantage of this solvent is sinteric hindrance. Due to this property, tert-butanol is not accepted by the lipase. High yield and conversions were obtained in the presence of t-butanol with various vegetable oils and immobilized lipases shown in Table 4-2. For example, Liu et al., [196] studied biodiesel synthesis by immobilized lipases in solvent-free and tert-butanol media. Each lipase showed a different conversion depending on the monohydric alcohols and immobilized lipase in solvent-free medium and tert-butanol system. For methanolysis, regardless of the lipase type, the conversion rate is higher in tert-butanol than that in solvent-free medium. Novozym 435 showed higher conversion rate with straight monoalcohols in tert-butanol medium. Lipozym RM IM and Lipozyme TL IM showed lower conversion with straight and branched monoalcohols (except methanol) in solvent free system. Similar results were obtained by Halim and Kamaruddin [208], in transesterification of waste cooking palm oil using various commercial lipases (Lipozyme RM IM, Lipozyme TL IM and Novozyme 435) in tert-butanol as reaction medium. Novozyme 435 was found to be more effective in catalyzing the transesterification with methanol in in-tert-butanol medium. It was also been demonstrated that even 3:1 methanol to oil molar ratio didn't inhibit the Novozyme 435 in tert-butanol system. Du et al. [209], showed that Lipozyme TL IM could be used without loss of lipase activity for 200 batches in tert-butanol system. Li et al. [210], used acetonitrileand tert-butanol mixture as co-solvent in transesterfication of stillingia oil with methanol. The highest biodiesel yield (90.57%) was obtained in co-solvent with 40% tert-butanol and 60% acetonitrile (v/v) with co-solvent. They also reported that co-solvent (as a mixture)enhance the tolerance of lipase to the methanol than the pure tert-butanol.

http://dx.doi.org/10.5772/52662

Solvent	Oil	Alcohol	Lipase	Temp/ Time	Reaction mixture	Performance (%)	Ref.
Tert-butanol	Cotton seed	Methanol	Novozyme 435 *(Candida antarctica)*	50 °C / 24h	13.5% meth., 54% oil 32.5% tert-butanol, Lipase:1.7% (wt of oil)	97 (yield)	[156]
Tert-butanol	Cotton seed	Methanol	Pancreatic lipase	37 °C / 4 h	Methanol :oil mol ratio:1:15 Lipase:0.5% enzyme (wt of oil) water conc.5% (wt of oil)	75–80 (conversion)	[205]
Tert-butanol	Rapeseed	Methanol	Novozyme435 & Lipozyme TL IM	35 °C / 12 h	Methanol: oil mol. ratio 4:1 tert-butanol/oil vol. 1:1 Lipase: 3% Lipozyme TL IM 1% Novozym 435 (wt of oil)	95 (conversion)	[206]
Tert-butanol	Soybean and deodorizer distillate	Methanol	Lipozyme TL IM Novozym 435	40°C/ 12 h	Methanol:oil molar ratio 3.6:1 Lipase :3% Lipozyme TL IM 2% Novozym 435 tert-butanol: 80% (wt of oil)	84 (yield)	[207]
Tert-butanol	Waste cooking palm	Methanol	Novozyme 435	40°C / 12 h	Methanol:oil mol. ratio 4:1, Lipase:4% (wt of oil)	88(yield)	[208]
Tert-butanol	Waste baked duck	Methanol	Novozym 435 Lipozyme TL IM	45 °C / 20 h	Methanol:oil mol. ratio 4:1, Lipase: 5 wt%(wt of oil)	85.4, 78.5, (conversion)	[196]
Hexane	Tallow	Methanol Ethanol Propanol	Lipozyme IM 60	45 C/ 5 h	0.34 M tallow in hexane (8 mL), Lipase: 10 (wt of oil) 200rpm	94.8, 98.0, 98.5 (conversion)	[201]

Solvent	Oil	Alcohol	Lipase	Temp/ Time	Reaction mixture	Performance (%)	Ref.
Hexane	Soybean	Methanol	Lipozyme IM 77	36.5°C/ 3h	Methanol :oil mol ratio:3.4:1 Lipase:0.9BAUN*of lipase; water 5.8% (wt% of oil)	92.2 (yield)	[202]
Hexane	Soybean	Ethanol	Lipozyme TL IM	30 °C/ 10 h	Ethanol:oil mol.ratio:7.5:1 Lipase: 15 %(wt of oil). 4% water	100 (conversion)	[70]
Cyclo hexane	Sunflower	Methanol	Lipase AK Lipozyme TL IM Lipozyme RM IM	40°C/ 24 h	Volume of organic solvent/ oil: 2 ml/0.2 mmol Lipase: 10% (wt of oil)	65, 75, 35 (conversion)	[204]
Acetonitrile 60%and 40% t-butanol (v/v)	Stillingia	Methanol	Novozym 435 and Lipozyme TL IM	40°C/ 24h	Methanol:oil mol ratio: 6.4:1 Lipase: 4% (w/w) of multiple-lipase (1.96% Novozym 435+2.04% Lipozyme TL IM)	90.57 (yield)	[210]
Petroleum ether	Sunflower	Ethanol	Lipozyme IM Lipase AK	45°C / 5h	Ethanol:oil mol. ratio:11:1 Lipase:20% (wt of oil)	82, 99, (yield)	[16]
I-octane	Sunflower	Methanol	Lipase AK Lipozyme TL, IM Lipozyme RM,IM	40 °C	Methanol: oil mol ratio::3:1 Vol. of organic solvent/oil: 2 ml/0.2 mmol	80, 65, 60, (yield)	[204]
1,4-dioxane	Triolein	Methanol	Lipase AK	50°C / 80h	Methanol:oil mol. ratio: 3:1 90% solvent	~70 (conversion)	[48]

*BAUN:Batch Acidolysis Units Novo

Table 8. Effect of the solvent on the performance of enzymatic transesterification reaction

Although positive effects of the usage of the solvents on the transesterification reaction, some drawbacks has also been known) such as; extra reactor volume, solvent toxicity and emissions, solvent recovery and loss cost [133].

4.5. The effect of molar ratio of alcohol to oil on enzymatic transesterification

Biodiesel yield always increased due to the molar excess of alcohol over fatty acids in triglycerides in traditional transesterification system [15]. The transesterification reaction is reversible and so, an increase in the amount of one of the reactants will result in higher ester yield and minimally 3 molar equivalents of methanol are required for the complete conversion of methyl ester [174]. Conversely, for enzyme catalyzed transesterification, insoluble excess methanol which exists as fine droplets demonstrates negative effects on enzyme activity and also decrease the production yield [211]. The reaction medium is an important factor during the determination of the optimum molar of alcohol to oil. The inactivation of lipases occurs by contact with insoluble alcohol because the highly hydrophilic alcohol eliminates the layer of essential water from the enzymes [212]. Thus, stepwise addition of alcohol is a potential approach for ratio optimizing the molar ratio in solvent free systems [15]. Whilst, higher reaction rates could be obtained with a slight excess of alcohol in organic solvent systems [15].

The two-step reaction system was reported to avoid the inactivation of the lipase by addition of excess amounts of methanol in the first-step reaction, and by addition of vegetable oil and glycerol in the second-step reaction [213]. Watanabe et al. [213], used a two-step reaction system for methyl esterification of free fatty acids and methanolysis of triacylglycerols using immobilized Candida antarctica lipase. The first step reaction was methyl esterification of free fatty acids that was performed by treating a mixture of 66 wt % acid oil and 34 wt % methanol with 1 wt % immobilized lipase. The second step reaction was conducted to convert triacylglycerols to fatty acid methyl esters. In this step, a mixture of 52.3 wt % dehydrated first-step product, 42.2 wt% rapeseed oil, and 5.5 wt% methanol using 6 wt% immobilized lipase in the presence of additional 10 wt % glycerol was treated. The contents of fatty acid methyl esters was 91.1wt.% after the second step reaction was repeated by the use of immobilized lipase for 50 cycles using recovered glycerol.

Moreno-Pirajan and Giraldo [81], added different amounts of alcohol varied from 2.7 to 13.7 molar equivalents for methanol and from 5.7 to 26.7 molar equivalents for ethanol, based on the moles of triglycerides toward the transesterification of palm oil catalyzed by Candida rugosa lipase and 10.4 molar ratio for all alcohols to palm oil was determined as optimal alcohol requirement resulted in 85 mol% of methyl esters yield with n-butanol.

Lipase catalyzed esterification of palmitic acid with ethanol in the presence of Lipozyme IM 20 in a solvent free medium was investigated by Vieira et al. [212]. Different acid/alcohol molar ratios were tried as 0.16, 0.5, 1.0, 1.5, and 1.84. The best result was obtained with 0.5 acid/alcohol molar ratio.

Zaidi et al. [214], explained the correlation existing between the kinetic parameters and the chain-length of the substrates in esterification of oleic acid using nylon-immobilized lipase in n-hexane. It is observed that the inhibition coefficient of the alcohol increased from 0.034

to 0.42 mol l^{-1}, when the number of carbon atoms increased from 1(methanol) to 18 (oleyl alcohol), respectively.

Dizge and Keskinler [69], used immobilized Thermomyces lanuginosus lipase to produce biodiesel with canola oil with methanol and investigated the role of substrate molar ratio. The biodiesel production was conducted at 1:1, 1:2,1:3,1:4,1:5;1:6 and 1:10 oil/alcohol molar ratios at 40°C. The highest methyl ester yield (85.8%) was obtained at the oil/methanol molar ratio of 1:6. Two important result from this study can be concluded as (i) an increase in the number of moles of methanol resulted in an increase in the ester production, (ii) when the formation of esters reached a maximum level the further increases in the methanol concentrations cause a decrease in the formation of esters due to enzyme inactivation.

Thus, the actual amount of alcohol needed varies significantly depending on the origin of the lipase and fat.

5. Reactors for enzymatic transesterification

Through the industrialization of enzymatic biodiesel production, it is necessary to show the applicability of enzymes in reactor systems. Various reactors, including batch reactors, packed bed reactors and supercritical reactors have been investigated by researchers. Most of the investigations on enzymatic synthesis of biodiesel have been performed in batch reactors and packed bed reactors.

Batch reactors are simple designs used in the laboratory. In batch reactors, methanol shows a good dispersion in the oil phase. But the physical agitation caused by shear stress from the stirring would disrupt the enzyme carrier which shortens the enzymes life [31]. On the other hand, batch operation is labor intensive, and not suitable for automation [215]. Packed bed reactors are alternative of batch reactors which are substantially faster and more economical continuous reactors [216]. A packed-bed reactor system is most widely used in biotechnology, as it is easy to operate and scale up these systems. In addition, these systems have high bed volume. The most important advantage of these systems is that it is lowering shear stress on immobilized enzymes which leads to long-term enzyme stability [217]. Furthermore, stepwise addition of alcohol can be performed to reduce the inactivation of the enzyme caused by excess alcohol. One of the encountered problems with an immobilized lipase is the inhibition of the enzyme due to the cloggage of the catalyst by accumulation of the glycerol by-product inside the reactor [218]. Also, the separation of glycerol which remains in the bottom of the reactor can be achieved in a simple way by using more than one column. Recently, a packed-bed reactor system, in which a reactant solution is pumped through a column containing biomass support particles immobilized recombinant Aspergillus oryzae and the effluent from the column is recycled into the same column with a stepwise addition of methanol was developed by Yoshida et al. [219]. In this system, lipase retains its activity for five batch cycles and 96.1% methyl ester content was obtained with a residence time of 140 min per pass and stepwise addition of 4.25 molar equivalents of methanol to oil for 6 passes. The methanolysis of soybean oil in packed bed reactor system using

Rhizopus oryzae whole cell was studied by Hama et al. [112]. The final methyl ester content was over 90% at a flow rate of 25 l/h in the first cycle and also, after 10 cycles approximately 80% conversion was achieved. Wang et al. [216], developed Pseudomonas cepacia lipase – Fe_3O_4 nanoparticle biocomposite based packed bed reactors. A single-packed-bed reactor and the four-packed-bed reactor were used to produce biodiesel by using refined soybean oil. A high conversion rate (over 88%, 192 h) and great stability was achieved with the four-packed-bed reactor compared to single-packed-bed reactor. It is considered that the four-packed-bed reactor supplied a longer residence time of the reaction mixture in the reactor and lowered the inhibition of the lipase by products [216]. By this way, the reaction efficiency was improved. Additionally, the cost of biodiesel production can be reduced by the effective recycling of the enzyme catalysts [184].

Supercritical reactors also have been investigated by researchers for enzymatic biodiesel production. D. Oliveira and J. V. Oliveira [220], produced biodiesel from palm kernel oil in the presence of Novozym 435 and Lipozyme IM in supercritical carbon dioxide in the temperature range of 40–70 °C and from 60 to 200 bar using a water concentration of 0–10 wt % and oil/ethanol molar ratios from 1:3 to 1:10. Lipozyme IM showed better results and the highest reaction conversion was obtained as 77.5 %. It was observed that lipase structure changed at pressures beyond 200 bar. Madras et al. [221], synthesized biodiesel from sunflower oil in supercritical carbon dioxide catalyzed by Novozym. However, the obtained conversions, when the reaction was conducted in supercritical methanol and ethanol at the optimum conditions, were 23 and 27%, respectively [221]. Enzymatic transesterification of lamb meat fat in supercritical carbon dioxide was investigated by Taher et al. [222].The maximum conversion (49.2%) was obtained at 50°C, with 50% Novozym 435 loading, 4:1 molar ratio, within 25 h reaction. Supercritical reactors could not commercialized according to the low conversion rate and cost of the system.

Consequently, packed bed reactor systems seem to be a practical transesterification reactor system with high transesterification efficiency. These systems will bring industrial scale up enzymatic biodiesel production in an economic way.

6. Conclusion

Today, the growing energy necessity and environmental pollution problem requires the use of renewable alternative energy sources to become less dependent on fossil resources. As known, biodiesel is an important alternative energy resource and seems to be the fuel of future because it is an environmentally friendly, nontoxic, renewable, and biodegradable fuel.

Conventionally, biodiesel production is achieved by mainly alkaline or acid catalysts. The interest in the use of biocatalyst for biodiesel production has been an increasing trend due to its many advantages.

Biodiesel have been shown to be effectively produced by enzymatic catalyst and also, numerous researches have been performed to obtain highly active lipases and to optimize

process conditions for biodiesel production. Besides many advantages, to produce biodiesel by enzyme catalysts on an industrial scale, it is necessary to reduce the high cost of enzymes and obtain lipases with better features. The immobilization of lipases and genetic engineering methods seems to be an attractive way to obtain more active, stable, and reusable lipases in organic solvents and alcohols. Also, selection of alternative acyl-acceptors is an option for eliminating the negative effects of methanol on lipase activity.

It can be concluded that in enzyme catalyzed biodiesel production significant progresses have been made but further improvements such as novel reactor design should be addressed and emphasized in the future research in order to ensure industrial enzymatic biodiesel production. By making novel improvements, much attention will be focused on enzyme usage in biodiesel production, and especially lipase reactions will be applied much more in this area.

Author details

Sevil Yücel[1*], Pınar Terzioğlu[1,2] and Didem Özçimen[1]

*Address all correspondence to: yuce.sevil@gmail.com syucel@yildiz.edu.tr

1 Yıldız Technical University, Faculty of Chemical and Metallurgical Engineering, Bioengineering Department, Istanbul, Turkey

2 Mugla Sıtkı Koçman University, Faculty of Sciences, Chemistry Department, Mugla, Turkey

References

[1] Demirbas A.Biodiesel: a realistic fuel alternative for diesel engine. London:Springer Publishing Co.;2008.

[2] Demirbas A. Progress and recent trends in biodiesel fuels. Energy Conversion and Management 2009; 50: 14–34.

[3] Demirbas M F, Balat M.Recent advances on the production and utilization trends of bio-fuels: a global perspective. Energy Convers Manage. 2006; 47: 2371–81.

[4] Chetri A B, Watts K C, Islam M R. Waste cooking oil as an alternate feedstock for biodiesel production. Energies 2008; 1: 3–18.

[5] Bamgboye A I, Hansen A C. Prediction of cetane number of biodiesel fuel from the fatty acid methyl ester (FAME) composition. Int Agrophys 2008; 22: 21–9.

[6] Balat M, Balat H. A critical review of biodiesel as a vehicular fuel. Energy Convers Manage, 2008; 49: 2727–41.

[7] Kulkarni BM, Bujar BG.Shanmukhappa S. Investigation of acid oil as a source of biodiesel. Indian J Chem Tech 2008; 15: 467–71.

[8] Gerpen JV, Shanks B, Pruszko R, Clements D, Knothe G.Biodiesel Production Technology. National Renewable Energy Laboratory. CO, USA; 2004.

[9] Capehart B L. Encyclopedia of energy engineering and technology. CRC Press, Taylor & Francis LLC; 2007.

[10] Pandey A. Handbook of plant-based biofuels.CRC Press, Taylor & FrancisLLC; 2008.

[11] Ghaly A E, Dave D, Brooks M S, Budge S.Production of Biodiesel by Enzymatic Transesterification: Review. American Journal of Biochemistry and Biotechnology 2010; 6 (2): 54-76.

[12] Mittelbach M, Remschmidt C. Biodiesel-the comprehensive handbook. Boersedruck Ges.m.b.H.69–80; 2004.

[13] Jegannathan KR, Abang S, Poncelet D, Chan E S, Ravindra P.Production of Biodiesel Using Immobilized Lipase—A Critical Review. Critical Reviews in Biotechnology 2008; 28: 253–264.

[14] Akoh C C, Chang S W, Lee G C, Shaw J F. Enzymatic approach to biodiesel production. J. Agric. Food. Chem. 2007; 55: 8995–9005.

[15] Fan X. Enzymatic biodiesel production – the way of the Future, Lipid Technology 2012; 24(2).

[16] Mittelbach M. Lipase catalyzed alcoholysis of sunflower oil. J. Am. Oil Chem. Soc. 1990;67:168-170.

[17] Hasan F, Shah A A, Javed S, Hameed A. Enzymes used in detergents: lipases. African Journal of Biotechnology 2010; 9(31) : 4836-4844.

[18] Villeneuve P, Muderhwa J M, Graille J, Haas MJ. Customizing lipases for biocatalysis: a survey of chemical, physical and molecular biological approaches. Journal of Molecular Catalysis B: Enzymatic 2000; 9 :113–148.

[19] Zhang B, Weng Y, Xu H, Mao Z. Enzyme immobilization for biodiesel production. Applied Microbiol Biotechnol 2012; 93:61–70. DOI 10.1007/s00253-011-3672-x

[20] Ribeiro B D, De Castro A M, Coelho M A Z, Freire D M G. Production and Use of Lipases in Bioenergy: A Review from the Feedstocks to Biodiesel Production. Enzyme Research 2011;1-16.

[21] Yahya A R M, Anderson W A, Moo-Young M. Ester synthesis in lipase catalyzed reactions. Enzyme and Microbial Technology 1998; 23:438–450.

[22] Freire D M G, Castilho L R. Lipases em Biocat´alise. Enzimas em Biotecnologia. Producao, Aplicacoes e Mercado 2008;1: 369–385.

[23] Cho S S, Park D J, Simkhada J R, Hong J H, Sohng J K, Lee O H, Yoo J C. A neutral lipase applicable in biodiesel production from a newly isolated Streptomyces sp. CS326. Bioprocess Biosyst Eng 2012; 35:227–234.

[24] Antczak M S, Kubiak A, Antczak T, Bielecki S. Enzymatic biodiesel synthesis – Key factors affecting efficiency of the process. Renewable Energy 2009; 34:1185–1194.

[25] Du W, Xu Y, Liu D, Li Z. Study on acyl migration in immobilized lipozyme TL catalyzed transesterification of soybean oil for biodiesel production. J Mol Catal B: Enzym 2005;37:68–71.

[26] Hartmeier W. Immobilized Biocatalysts: An Introduction. Springer-Verlag, Berlin; 1988.

[27] Gomes FM, De Paula A V, Silva SG, De Castro HF. Determinação das propriedades catalíticas em meio aquoso e orgânico da lipase de Candida rugosa imobilizada em celulignina quimicamente modificada por carbonildiimidazol. Quim. Nova 2006; 29, 710–718.

[28] Jegannathan KR, Abang S, Poncelet D, Chan ES, Ravindra P. Production of biodiesel using immobilized lipase-a critical review. Crit Rev Biotechnol 2008; 28(4):253–264.

[29] Costa S A, Azevedo H S, Reis R L. Biodegradable Systems in Tissue Engineering and Regenerative Medicine .Enzyme Immobilization in Biodegradable Polymers for Biomedical Applications, CRC Press LLC; 2005.

[30] Fukuda H, Kondo A, Noda H. Biodiesel fuel production by transesterification of oils. J Biosci Bioeng 2001; 92(5):405–416. doi:10.1016/S1389-1723(01)80288-7

[31] Tan T, Lu J, Nie K, Deng L, Wang F, Biodiesel production with immobilized lipase: A review. Biotechnology Advances 2010; 28: 628–634.

[32] Tran D T, Chen C L, Chang J S. Immobilization of Burkholderia sp. lipase on a ferric silica nanocomposite for biodiesel production. Journal of Biotechnology 2012; 158: 112– 119.

[33] Du W, Xu Y, Liu D, Zeng J. Comparative study on lipase-catalyzed transformation of soybean oil for biodiesel production with different acyl acceptors. Journal of Molecular Catalysis B: Enzymatic 2004; 30: 125–129.

[34] Shimada Y, Watanabe Y, Samukawa T, Sugihara A, Noda H, Fukuda H, Tominaga Y. Conversion of Vegetable Oil to Biodiesel Using Immobilized Candida antarctica Lipase. J9045, JAOCS 1999; 76: 789–793.

[35] Watanabe Y, Shimada Y, Sugihara A, Noda H, Fukuda H, Tominaga Y. Continuous Production of Biodiesel Fuel from Vegetable Oil Using Immobilized Candida antarctica Lipase. J9352, JAOCS 2000;77:355–360.

[36] Naranjo J, Córdoba A, Giraldo L, García V, Moreno-Parajàn J C. Lipase supported on granular activated carbon and activated carbon cloth as a catalyst in the synthesis of biodiesel fuel. J. Mol. Catal. B: Enzymatic2010; 66: 166–171.

[37] Lu J, Nie K, Xie F, Wang F, Tan T. Enzymatic synthesis of fatty acid methyl esters from lard with immobilized Candida sp. 99–125. Process Biochem 2007;42:1367–70.

[38] Nie K, Xie F, Wang F, Tan T. Lipase catalyzed methanolysis to produce biodiesel: optimization of the biodiesel production. J Mol Catal B Enzym 2006;43:142–7.

[39] Li Z, Deng L, Lu J, Guo X, Yang Z, Tan T. Enzymatic synthesis of fatty acid methyl esters from crude rice bran oil with immobilized Candida sp. 99-125. ChineseJournal of Chemical Engineering 2010; 18:870-875.

[40] Tongboriboon K, Cheirsilp P, H-Kittikun A. Mixed lipases for efficient enzymatic synthesis of biodiesel from used palm oil and ethanol in a solvent-free system. J. Mol. Catal. B: Enzymatic 2010; 67: 52–59.

[41] Shah S, Sharma S, Gupta M N.Biodiesel preparation by lipase-catalyzed transesterification ofjatropha oil. Energy & Fuels2004;18: 154-159.

[42] MendesA A, Oliveirac P C, Velezb A M, Giordanob R C, Giordanob R L C, De Castro H F.International Journal of Biological Macromolecules 2012; 50 :503– 511.

[43] Ji Q, Xiao S, He B, Liu X. Purification and characterization of an organic solvent-tolerant lipase from Pseudomonas aeruginosa LX1 and its application for biodiesel production. J. Mol. Catal. B: Enzymatic 2010; 66: 264–269.

[44] Shah S, Gupta MN. Lipase catalyzed preparation of biodiesel from Jatropha oil in a solvent free system. Process Biochem 2007;42:409–14.

[45] Sakai S, Liu Y, Yamaguchi T, Watanabe R, Kawabe M, Kawakami K. Production of butyl-biodiesel using lipase physically-adsorbed onto electrospun polyacrylonitrile fibers. Bioresource Technology 2010; 101 : 7344–7349.

[46] Li Q, Yan Y. Production of biodiesel catalyzed by immobilized Pseudomonas cepacia lipase from Sapium sebiferum oil in micro-aqueous phase. Applied Energy2010;87: 3148–3154.

[47] Al-Zuhair S. Enzymatic Production of Bio-Diesel from Waste Cooking Oil Using Lipase. The Open Chemical Engineering Journal 2008; 2: 84-88.

[48] Iso J M, Chen B, Eguchi M, Kudo T, Shrestha S. Production of biodiesel fuel from triglycerides and alcohol using immobilized lipase. Journal of Molecular Catalysis B: Enzymatic 2001; 16: 53–58.

[49] Salis A, Pinna M, Monduzzi M, Solinas V, Comparison among immobilized lipases on macroporous polypropylene toward biodiesel synthesis. Journal of Molecular Catalysis B: Enzymatic 2008; 54: 19–26.

[50] Li N, Zong M, Wu H. Highly efficient transformation of waste oil to biodiesel by immobilized lipase from Penicillium expansum. Process Biochemistry 2009; 44: 685–688.

[51] De Paola M G, Ricca E, Calabrò V, Curcio S, Iorio G. Factor analysis of transesterification reaction of waste oil for biodiesel production. BioresourceTechnology 2009; 100:5126–513.

[52] Chen Y, Xiao B,Chang J, Fu Y, Lv P, Wang X. Synthesis of biodiesel from waste cooking oil using immobilized lipase in fixed bed reactor. Energy Conversionand Management 2009; 50:668–673.

[53] Li X, He X Y, Li Z L, Wang Y D, Wang C Y, Shi H, Wang F. Enzymatic production of biodiesel from Pistacia chinensis bge seed oil using immobilized lipase. Fuel 2012; 92: 89–93.

[54] Zeng H, Liao K, Deng X, Jiang H, Zhang F. Characterization of the lipase immobilized on Mg–Al hydrotalcite for biodiesel. Process Biochemistry 2009; 44:791–798.

[55] Yagiz F, Kazan D, Akin N A. Biodiesel production from waste oils by using lipase immobilized on hydrotalcite and zeolites. Chem. Eng. J. 2007; 134:262–267

[56] Knezevic Z, Milosavic N, Bezbradica D, Jakovljevic Z, Prodanovic R. Immobilization of lipase from Candida rugosa on Eupergit® C supports by covalent attachment. Biochemical Engineering Journal 2006; 30: 269–278.

[57] Stoytcheva M, Montero G, Toscano L, Gochev V , Valdez B. The immobilized lipases inbiodiesel production. Biodiesel – Feedstocks and Processing Technologies,InTech.

[58] Xie W, Wang J. Immobilized lipase on magnetic chitosan microspheres for transesterification of soybean oil. Biomass and Bioenergy 2012; 3 6: 373-380.

[59] Jang M G, Kim D K, Park S C, Lee J S, Kim S W. Biodiesel production from crude canola oil by two-step enzymatic processes. Renewable Energy 2012; 42: 99-104.

[60] Da Ros P, Silva G, Mendes A, Santos J, Castro H. Evaluation of the catalytic properties of Burkholderia cepacia lipase immobilized on non-commercial matrices to be used in biodiesel synthesis from different feedstocks. Bioresource Technology 2010; 101:5508–5516.

[61] Shao P, Meng X, He J, Sun P. Analysis of immobilized Candida rugosa lipase catalyzed preparation of biodiesel from rapeseed soapstock. Food and BioproductsProcessing 2008; 86: 283–289.

[62] Kumari A,Mahapatra P, Garlapati V, Banerjee R. Enzymatic transesterification of Jatropha oil. Biotechnology for Biofuels2009; 2:1, doi:10.1186/1754-6834-2-1

[63] Desai P D, Dave A M, Devi S. Alcoholysis of salicornia oil using free and covalently bound lipase onto chitosan beads. Food Chemistry 2006; 95: 193–199.

[64] Mendes A, Giordano R C, Giordano R L C, Castro H. Immobilization and stabilization of microbial lipases by multipoint covalent attachment on aldehyderesin affinity: Application of the biocatalysts in biodiesel synthesis. J. Mol. Catal. B:Enzymatic 2011; 68: 109–115.

[65] Wang Y, Shen X, Li Z, Li X, Wang F, Nie X, Jiang J. Immobilized recombinant Rhizopus oryzae lipase for the production of biodiesel in solvent free system. Journal of Molecular Catalysis B: Enzymatic 2010; 67: 45–51.

[66] Ho L J, Lee D H, Lim J S, Um B H, Park C, Kang S W, Kim S W. Optimization of the process for biodiesel production using a mixture of immobilized Rhizopus oryzae and Candida rugosa lipases. J. Microbiol. Biotechnol. 2008; 18:1927–1931.

[67] Yücel Y. Biodiesel production from pomace oil by using lipase immobilized onto olive pomace. Bioresource Technology 2011; 102: 3977–3980.

[68] Dizge N, Keskinler B, Tanriseven A. Biodiesel production from canola oil by using lipase immobilized onto hydrophobic microporous styrene-divinylbenzene copolymer. Biochem Eng J 2009;44:220–5.

[69] Dizge N, Keskinler B. Enzymatic production of biodiesel from canola oil using immobilized lipase. Biomass and Bioenergy 2008; 32:1274–1278.

[70] Rodrigues R, Pessela B,Volpato G, Fernandez-Lafuente R, Guisan J, Ayub M. 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 2010; 45: 1268–1273.

[71] Xie W, Ma N. Enzymatic transesterification of soybean oil by using immobilized lipase on magnetic nano-particles. Biomass and Bioenergy2010; 34: 890-896.

[72] O'Driscoll, K. F. (1976) Techniques of enzyme entrapment in gels. In: Methods in Enzymology, volume XLIV, (Mosbach K., ed.), Academic Press, New York, NY, 169–183.

[73] Ikeda Y, Kurokawa Y, Nakane K, Ogata N.Entrap-immobilization of biocatalysts on cellulose acetate-inorganic composite gel fiber using a gel formation of cellulose acetate–metal (Ti, Zr) alkoxide.Cellulose2002; 9: 369–379.

[74] Brena B M, Batista-Viera F. Immobilization of Enzymes,Methods in Biotechnology: Immobilization of Enzymes and Cells, Second Edition, Totowa, NJ: Humana Press Inc.

[75] Murty V R, Bhat J, Muniswaran P K A. Hydrolysis of oils by using immobilized lipase enzyme: a review.Biotechnol. Bioprocess Eng. 2002; 7: 57-66.

[76] Jegannathan K, Jun-Yee L, Chan E, Ravindra P. Design an immobilized lipase enzyme for biodiesel production. J. Renewable and Sustainable Energy 2009; 1: 063101-1 -063101-8.

[77] Hsu A, Jones K, Marmer WN, Foglia TA. Production of alkyl esters from tallow and grease using lipase immobilized in a phyllosilicate sol–gel. J Am Oil Chem 2001;78:585–8.

[78] Kawakami K, Oda Y, Takahashi R. Application of a Burkholderia cepacia lipase immobilized silica monolith to batch and continuous biodiesel production with a stoi-

chiometric mixture of methanol and crude Jatropha oil. Biotechnology for Biofuels 2011; 4:42.

[79] Meunier S, Legge R. Evaluation of diatomaceous earth as a support for sol–gel immobilized lipase for transesterification. J. Mol. Catal. B: Enzymatic 2010; 62, 54–58.

[80] Sawangpanya N, Muangchim C, Phisalaphong M. Immobilization of lipase on $CaCO_3$ and entrapment in calcium alginate bead for biodiesel production,Sci. J. UBU 2010; 1(2): 46-51.

[81] Moreno-Pirajan J C, Giraldo L. Study of immobilized candida rugosa lipase for biodiesel fuel production from palm oil by flow microcalorimetry. Arabian Journal of Chemistry 2011; 4: 55–62.

[82] Noureddini H, Gao X, Philkana R S. Immobilized Pseudomonas cepacia lipase for biodiesel fuel production from soybean oil. Bioresource Technology 2005; 96: 769–777.

[83] Devanesan M G, Viruthagiri T, Sugumar N, Transesterification of jatropha oil using immobilized Pseudomonas fluorescens. African Journal of Biotechnology 2007; 6 (21): 2497-2501.

[84] Orçaire O, Buisson P, Pierre A. Application of silica aerogel encapsulated lipases in the synthesis of biodiesel by transesterification reactions. J. Mol. Catal. B:Enzymatic 2006; 42: 106–113.

[85] Jegannathan K R, Jun-Yee L, Chan E S, Ravindra P. Production of biodiesel from palm oil using liquid core lipase encapsulated in K-carrageenan.Fuel 2010; 89: 2272–2277.

[86] Nassreddine S, Karout A, Christ M, Pierre A. Transesterification of a vegetal oil with methanol catalyzed by a silica fibre reinforced aerogel encapsulated lipase. Applied Catalysis A: General 2008; 344: 70–77.

[87] Yan J, Yan Y, Liu S, Hu J, Wang G, Preparation of cross-linked lipase-coated microcrystals for biodiesel production from waste cooking oil. Bioresource Technology 2011; 102 : 4755–4758.

[88] Kumari V, Shah S, Gupta M N. Preparation of biodiesel by lipase-catalyzed transesterification of high free fatty acid containing oil from Madhuca indica. Energy & Fuels2007; 21:368-372.

[89] Kensingh P, Chulalaksananukul W, Charuchinda S. Lipase immobilization on Scirpus grossus L.f. fiber support byglutaraldehyde-crosslinked technique for biodiesel synthesis. Special Abstracts / Journal of Biotechnology 2010; 150S: 1–576.

[90] LorenS, Wilson L, Andrés I. Immobilization of the Alcaligenes spp. lipase to catalyze the transesterification of fatty acids to produce biodiesel. New Biotechnology 2009; 25S.

[91] Fukuda H, Hama S, Tamalampudi S, Noda H. Whole-cell biocatalysts for biodiesel fuel production. Trends in Biotechnology 2008;26:12.

[92] Li W, Du W, Liu D, Yao Y. Study on factors influencing stability of whole cell during biodiesel production in solvent-free and tert-butanol system. Biochemical Engineering Journal 2008; 41:111–115.

[93] Ban K, Kaieda M, Matsumoto T,Kondo A, Fukuda H. Whole-cell biocatalyst for biodiesel fuel production utilizing Rhizopus oryzae cells immobilized within biomass support particles. Biochem. Eng. J. 2001; 8:39–43.

[94] Sun T, Du W, Liu D, Dai L. Improved catalytic performance of GA cross-linking treated Rhizopus oryzae IFO 4697 whole cell for biodiesel production. Process Biochemistry 2010; 45: 1192–1195.

[95] Gog A, Roman M, Tos M, Paizs C, Irimie F D. Biodiesel production using enzymatic transesterification- Current state and perspectives. Renewable Energy 2012; 39: 10-16.

[96] Xıao M, Obbard J P. Whole cell-catalyzed transesterification of waste vegetable oil. GCB Bioenergy 2010; 2: 346–352.

[97] Xıao M, Qi C, Obbard J P. Biodiesel production using Aspergillus niger as a whole-cell biocatalyst in a packed-bed reactor.GCB Bioenergy 2011; 3: 293–298.

[98] Xıao M, Mathew S, Obbard J P. A newly isolated fungal strain used as whole-cell biocatalyst for biodiesel production from palm oil.GCB Bioenergy 2010; 2:45–51.

[99] Adachi D, Hama S, Numata T, Nakashima K, Ogino C, Fukuda H, Kondo A. Development of an Aspergillus oryzae whole-cell biocatalyst coexpressing triglyceride and partial glyceride lipases for biodiesel production. Bioresource Technology 2011; 102:6723–6729.

[100] Adachi D, Hama S, Nakashima K, Bogaki T, Ogino C, Kondo A. Production of biodiesel from plant oil hydrolysates using an Aspergillus oryzae whole-cell biocatalyst highly expressing Candida antarctica lipase B. Bioresource Technology 2012;in press: http://dx.doi.org/10.1016/j.biortech.2012.06.092

[101] Koda R, Numata T, Hama S, Tamalampudi S, Nakashima K, Tanaka T, Ogino C, Fukuda H, Kondo A. Ethanolysis of rapeseed oil to produce biodiesel fuel catalyzed by Fusarium heterosporum lipase-expressing fungus immobilized whole-cell biocatalysts. Journal of Molecular Catalysis B: Enzymatic 2010; 66: 101–104.

[102] Gao B, Su E, Lin J, Jiang Z, Ma Y, Wei D. Development of recombinant Escherichia coli whole-cell biocatalyst expressing a novel alkaline lipase-coding gene from Proteus sp. for biodiesel production. Journal of Biotechnology 2009; 139:169–175.

[103] He Q, Xu Y, Teng Y, Wang D. Biodiesel production catalyzed by whole-cell lipase from Rhizopus chinensis. Chin J Catal 2008; 29(1): 41–46.

[104] Huang D, Han S, Han Z, Lin Y. Biodiesel production catalyzed by Rhizomucor miehei lipase-displaying Pichia pastoris whole cells in an isooctane system. Biochemical Engineering Journal 2012; 63: 10– 14.

[105] Li W, Du W, Liu D. Rhizopus oryzae IFO 4697 whole cell catalyzed methanolysis of crude and acidified rapeseed oils for biodiesel production in tert-butanol system. Process Biochemistry 2007; 42: 1481–1485.

[106] Sun T, Du W, Zeng J, Dai L, Liu D. Exploring the effects of oil inducer on whole cell-mediated methanolysis for biodiesel production. Process Biochemistry 2010; 45: 514–518.

[107] Sun T, Du W, Zeng J, Liu D. Comparative study on stability of whole cells during biodiesel production in solvent-free system. Process Biochemistry 2011; 46: 661–664.

[108] Matsumoto T, Takahashi S, Kaieda M, Tanaka M U A, Fukuda H, Kondo A. Yeast whole-cell biocatalyst constructed by intracellular overproduction of Rhizopus oryzae lipase is applicable to biodiesel fuel production. Appl Microbiol Biotechnol 2001;57:515–520.

[109] Arai S, Nakashima K, Tanino T, Ogino C, Kondo A, Fukuda H. Production of biodiesel fuel from soybean oil catalyzed by fungus whole-cell biocatalysts in ionic liquids. Enzyme and Microbial Technology 2010; 46: 51–55.

[110] Lin Y H, Luo J J, Hwang S C J, Liau P R, Lu W J, Lee H T. The influence of free fatty acid intermediate on biodiesel production from soybean oil by whole cell biocatalyst. Biomass and Bioenergy 2011;35 : 2217-2223.

[111] Li W, Du W, Liu D. Optimization of whole cell-catalyzed methanolysis of soybean oil for biodiesel production using response surface methodology, Journal of Molecular Catalysis B: Enzymatic 2007;45 :122–127.

[112] Hama S, Yamaji H, Fukumizu T, Numata T, Tamalampudi S, Kondo A, Noda H, Fukuda H. Biodiesel-fuel production in a packed-bed reactor using lipase-producing Rhizopus oryzae cells immobilized within biomass support particles. Biochemical Engineering Journal 2007; 34: 273–278.

[113] Tamalampudi S, Talukder M R, Hama S, Numata T, Kondo A, Fukuda H. Enzymatic production of biodiesel from jatropha oil: a comparative study of immobilized-whole cell and commercial lipases as a biocatalyst. Biochemical Engineering Journal 2008; 39: 185–189.

[114] Zeng J, Du W, Liu X, Liu D, Dai L, Study on the effect of cultivation parameters and pretreatment on Rhizopus oryzae cell-catalyzed transesterification of vegetable oils for biodiesel production. Journal of Molecular Catalysis B: Enzymatic 2006; 43:15–18.

[115] Li A, Ngo T P N, Yan J, Tian K, Li Z. Whole-cell based solvent-free system for one-pot production of biodiesel from waste grease. Bioresource Technology 2012; 114: 725–729.

[116] Atabani A E, Silitonga A S, Badruddin I A, Mahlia TMI, Masjuki HH, Mekhilef S. A comprehensive review on biodiesel as an alternative energy resource and its characteristics. Renewable and Sustainable Energy Reviews 2012; 16: 2070– 2093.

[117] Demirbas A. Progress and recent trends in biodiesel fuels. Energy Conversion and Management 2009; 50:14–34.

[118] Koh M Y, Ghazi T I M. A review of biodiesel production from Jatropha curcas L. Oil. Renewable and Sustainable Energy Reviews 2011;15: 2240–2251.

[119] Singh S P, Singh D. Biodiesel production through the use of different sources and characterization of oils and their esters as the substitute of biodiesel: a review. Renewable and Sustainable Energy Reviews 2010;12:200–16.

[120] Mander P, Cho S S, Simkhada J R, Choi Y H, Park D J, Yoo J C. An organic solvent–tolerant lipase from Streptomyces sp. CS133 for enzymatic transesterification of vegetable oils in organic media. Process Biochemistry 2012; 47:635–642.

[121] Huang D, Han S, Han Z, Lin Y. Biodiesel production catalyzed by Rhizomucor miehei lipase-displaying Pichia pastoris whole cells in an isooctane system. Biochemical Engineering Journal 2012; 63: 10– 14.

[122] Karout A, Pierre A C. Partial transesterification of sunflower oil with ethanol by a silica fiber reinforced aerogel encapsulated lipase. J Sol-Gel Sci Technol 2009; 52:276–286.

[123] De los Ríos A P, Hernández Fernández FJ, Gómez D, Rubio M, Víllora G. Biocatalytic transesterification of sunflower and waste cooking oils in ionic liquid Media. Process Biochemistry 2011; 46: 1475–1480.

[124] Ognjanovic N, Bezbradica D, Knezevic-Jugovic Z. Enzymatic conversion of sunflower oil to biodiesel in a solvent-free system: Process optimization and the immobilized system stability. Bioresource Technology 2009; 100: 5146–5154.

[125] Talukder M M R, Das P, Fang T S, Wu J C. Enhanced enzymatic transesterification of palm oil to biodiesel. Biochemical Engineering Journal 2011; 55: 119–122.

[126] Mata T M, Sousa I R B G, Vieira S S, Caetano N S. Biodiesel Production from Corn Oil via Enzymatic Catalysis with Ethanol. Energy Fuels 2012;26: 3034–3041.

[127] Chattopadhyay S, Karemore A, Das S, Deysarkar A, Sen R. Biocatalytic production of biodiesel from cottonseed oil: Standardization of process parameters and comparison of fuel characteristics. Applied Energy 2011; 88: 1251–1256.

[128] Jang M G, Kim D K, Park S C, Lee J S, Kim S W. Biodiesel production from crude canola oil by two-step enzymatic processes. Renewable Energy 2012; 42: 99-104.

[129] Sanchez F, Vasudevan PT. Enzyme catalyzed production of biodiesel from olive oil. Appl Biochem Biotechnol2006; 135:1–14.

[130] Tran D T, Yeh K L, Chen C L, Chang J S. Enzymatic transesterification of microalgal oil from Chlorella vulgaris ESP-31 for biodiesel synthesis using immobilized Burkholderia lipase. Bioresource Technology 2012; 108:119–127.

[131] Leung D Y C, Wu X, Leung M K H. A review on biodiesel production using catalyzed transesterification. Applied Energy 2010;87:1083–95.

[132] Knothe G, Dunn R O. Biodiesel: an alternative diesel fuel from vegetable oils or animal fats, In: Industrial uses of vegetable oils. USA, Champaign. AOCS Press, ISBN 1-893997-84-7,2005.

[133] Nielsen P M, Brask J, Fjerbaek L. Enzymatic biodiesel production: Technical and economical considerations. Eur. J. Lipid Sci. Technol. 2008;110: 692–700.

[134] Jain S, Sharma MP. Prospects of biodiesel from Jatropha in India: a review. Renewable and Sustainable Energy Reviews 2010;14:763–71.

[135] Mohibbe A M, Amtul W, Nahar N M. Prospects and potential of fatty acid methyl esters of some non-traditional seed oils for use as biodiesel in India. Biomass Bioenergy 2005;29:293–302.

[136] Modi M K, Reddy J R C, Rao B V S K, Prasad R B N. Lipase-catalyzed mediated conversion of vegetable oils into biodiesel using ethyl acetate as acyl acceptor. Bioresour Technol 2007;98:1260–4.

[137] Lu J, Nie K, Xie F, Wang F, Tan T. Enzymatic synthesis of fatty acid methyl esters from lard with immobilized Candida sp. 99–125. Process Biochem 2007;42:1367–70.

[138] Al-Zuhair S, Hussein A, Al-Marzouqi A H, Hashim I. Continuous production of biodiesel from fat extracted from lamb meat in supercritical CO_2 media. Biochemical Engineering Journal 2012; 60: 106– 110.

[139] Da Ros P C M, De Castro H F, Carvalho A K F,. Soares C M F, De Moraes F F, Zanin G M. Microwave-assisted enzymatic synthesis of beef tallow biodiesel. Ind Microbiol Biotechnol 2012; 39:529–536.

[140] Guru M, Koca A, Can O, Cinar C, Sahin F. Biodiesel production from waste chicken fat based sources and evaluation with Mg based additive in a diesel engine. Renew Energy 2010;35:637–43.

[141] Balat M. Potential alternatives to edible oils for biodiesel production – A review of current work. Energy Conversion and Management 2011; 52: 1479–1492.

[142] Karatay S E, Donmez G. Microbial oil production from thermophile cyanobacteria for biodiesel production. Applied Energy 2011; 88: 3632–3635.

[143] Watanabe Y, Shimada Y, Sugihara A, Tominaga Y. Enzymatic conversion of waste edible oil to biodiesel fuel in a fixed-bed bioreactor. J9830, JAOCS 2001; 78: 703–707.

[144] Chen G, Ying M, Li W. Enzymatic conversion of waste cooking oils Intoalternative fuel-biodiesel. Applied Biochemistry and Biotechnology. 129–132: Humana press; 2006.

[145] Huynh L H, Kasim N S, Ju Y H. Biodiesel production from waste oils. Biofuels: Alternative Feedstocks and Conversion Processes. 375 -392, Elsevier Inc; 2011.

[146] Atadashi I M, Aroua M K, Aziz A R A, Sulaiman N M N, Production of biodiesel using high free fatty acid feedstocks. Renewable and Sustainable Energy Reviews 2012; 16: 3275– 3285.

[147] Mata T M, Martins A A, Caetano N S. Microalgae for biodiesel production and other applications: A review. Renewable and Sustainable Energy Reviews 2010;14: 217–232.

[148] Borugadda V B, Goud V V. Biodiesel production from renewable feedstocks: Status and opportunities. Renewable and Sustainable Energy Reviews 2012; 16: 4763–4784.

[149] Lai J Q, Hu Z L, Wang P W, Yang Z, Enzymatic production of microalgal biodiesel in ionic liquid [BMIm][PF6].Fuel 2012; 95: 329–333.

[150] Leca M, Tcacenco L, Micutz M, Staicu T.Optimization of biodiesel production by transesterification of vegetable oils using lipases.Romanian Biotechnological Letters 2010; 15(5): 5618-5630.

[151] Al-Zuhair S. Production of biodiesel: possibilities and challenges.Biofuels, Bioprod. Bioref.2007;1: 57–66.

[152] Luković N, Knežević-Jugović Z, Bezbradica D. Biodiesel fuel production by enzymatic transesterification of oils: recent trends, Challenges and Future Perspectives, Alternative Fuel,Intech

[153] Xıao M, Mathew S, Obbard JP. A newly isolated fungal strain used as whole-cell biocatalyst for biodiesel production from palm oil. GCB Bioenergy 2010; 2: 45–51.

[154] Xıao M, Obbard JP. Whole cell-catalyzed transesterification of waste vegetable oil. GCB Bioenergy 2010; 2 : 346–352.

[155] Freitas L, Da Ros PCM, Santos JC, Castro HF. An integrated approach to produce biodiesel and monoglycerides by enzymatic interestification of babassu oil (Orbinya sp). Process Biochemistry 2009; 44: 1068–1074.

[156] Royon D, Daz M, Ellenrieder G, Locatelli S. Enzymatic production of biodiesel from cotton seed oil using t-butanol as a solvent. Bioresour Technol2007; 98:648-653.

[157] Watanabe Y, Nagao T, Nishida Y, Takagi Y, Shimada Y. Enzymatic production of fatty acid methyl esters by hydrolysis of acid oil followed by esterification. J Am Oil Chem Soc 2007; 84:1015–1021. DOI 10.1007/s11746-007-1143-4

[158] Nie K, Xie F, Wang F, Tan T. Lipase catalyzed methanolysis to produce biodiesel: Optimization of the biodiesel production. Journal of Molecular Catalysis B: Enzymatic 2006; 43: 142–147.

[159] Chen Y, Xiao B, Chang J, Fu Y, Lv P, Wang X. Synthesis of biodiesel from waste cooking oil using immobilized lipase in fixed bed reactor. Energy Conversion and Management 2009; 50: 668–673.

[160] Kumari A, Mahapatra P, Garlapati V K, Banerjee R. Enzymatic transesterification of Jatropha oil.Biotechnology for Biofuels2009; 2:1.

[161] Zhang Z, Guan C. A promising alternate lipase for biodiesel fuel production. Front. Agric. China 2010; 4(2): 129–136, DOI 10.1007/s11703-010-0005-2.

[162] Rodrigues R C, Volpato G, Wada K, Ayub M A Z. Enzymatic synthesis of biodiesel from transesterification reactions of vegetable oils and short chain alcohols. J Am Oil Chem Soc 2008; 85:925–930 DOI 10.1007/s11746-008-1284-0

[163] Bernardes O L, Bevilaqua J V, Leal M C M R, Freire D M G, Langone M A P. Biodiesel fuel production by the transesterification reaction of soybean oil using immobilized lipase.Applied Biochemistry and Biotechnology 2007;136–140.

[164] Souza M S, Aguieiras E C G, Silva M A P, Langone M A P. Biodiesel synthesis via esterification of feedstock with high content of free fatty acids. Appl Biochem Biotechnol 2009;154:253–267.

[165] Kazanceva I, Makarevičienė V, Kazancev K. Application of biotechnological method to biodiesel fuel production using n-butanol.Environmental Research, Engineering and Management 2011;2(56): 35 – 42.

[166] Matassoli A L F, Corrêa I N S, Portilho M F, Veloso C O, Langone M A P. Enzymatic synthesis of biodiesel via alcoholysis of palm oil. Appl Biochem Biotechnol 2009; 155:347–355.

[167] Jeong G T, Park D H. Lipase-catalyzed transesterification of rapeseed oil for biodiesel production with tert-butanol. Appl Biochem Biotechnol 2008; 148:131–139, DOI 10.1007/s12010-007-8050-x

[168] Wang Y N, Chen M H, Ko C H, Lu P J, Chern J M, Wu C H, Chang F C. Lipase catalyzed transesterification of tung and palm oil for biodiesel. World Renewable Energy Congress 2011;Sweden,8-13 May.

[169] Su E Z, Zhang M J, Zhang J G, Gao J F, Wei D Z. Lipase-catalyzed irreversible transesterification of vegetable oils for fatty acid methyl esters production with dimethyl carbonate as the acyl acceptor. Biochemical Engineering Journal 2007; 36:167–173.

[170] Chang H M, Liao H F, Lee C C, Shieh C J. Optimised synthesis of lipase-catalyzed biodiesel by Novozym 435. J Chem Technol Biotechnol 2005; 80:307–312.

[171] Zheng Y, Quan J, Ning X, Zhu L M, Jiang B, He Z Y. Lipase-catalyzed transesterification of soybean oil for biodiesel production in tert-amyl alcohol. World J Microbiol Biotechnol 2009; 25:41–46.

[172] Ognjanovic N, Bezbradica D, Knezevic-Jugovic Z. Enzymatic conversion of sunflower oil to biodiesel in a solvent-free system: Process optimization and the immobilized system stability. Bioresource Technology 2009; 100: 5146–5154.

[173] Liu Y, Xin H l, Yan Y J. Physicochemical properties of stillingia oil: Feasibility for biodiesel production by enzyme transesterification. Industrial Crops and Products 2009; 30: 431–436.

[174] Köse O, Tüter M, Aksoy H A. Immobilized Candida antarctica lipase-catalyzed alcoholysis of cotton seed oil in a solvent-free medium. Bioresource Technology 2002; 83: 125–129.

[175] Hernandez-Martın E, Otero C. Different enzyme requirements for the synthesis of biodiesel: Novozym 435 and Lipozyme TL IM. Bioresource Technology 2008; 99: 277–286.

[176] Li N W, Zong M H, Wu H. Highly efficient transformation of waste oil to biodiesel by immobilized lipase from Penicillium expansum.Process Biochemistry 2009; 44: 685–688.

[177] Noureddini H, Gao X, Philkana RS.Immobilized Pseudomonas cepacia lipase for biodiesel fuel production from soybean oil. Bioresource Technology 2005; 96: 769–777.

[178] Gamba M, Lapis A A M, Dupont J. Supported ionic liquid enzymatic catalysis for the production of biodiesel. Adv. Synth. Catal. 2008; 350: 160 – 164.

[179] Qin H, Xu Y, Teng Y, Wang D. Biodiesel Production Catalyzed by whole-cell lipase from Rhizopus chinensis.Chinese Journal of Catalysis 2008; 29: 1.

[180] Petersson A E V, Adlercreutz P, Mattiasson B. A water activity control system for enzymatic reactions in organic media. Biotechnology and Bioengineering. 2007;97 (2): 1, ,DOI 10.1002/bit.21229.

[181] Bommarius A S, Riebel-Bommarius B R. Biocatalysts:Fundamentals and Applications. John Wiley & Sons, 266; 2000.

[182] Al-Zuhair S, Jayaraman K V, Krishnan S, Chan W H. The effect of fatty acid concentration and water content on the production of biodiesel by lipase. Biochemical Engineering Journal 2006; 30: 212–217.

[183] Al-Zuhair S, Hasan M, Ramachandran K B. Kinetic hydrolysis of palm oil using lipase. Proc Biochem2003; 38:1155–1163.

[184] Fjerbaek L, Christensen K V, Norddahl B. A review of the current state of biodiesel production using enzymatic transesterification. Biotechnology and Bioengineering 2009; 102 (5):1. DOI 10.1002/bit.22256

[185] Vermue M H, Tramper J. Bıocatalysıs ın non-conventıonal medıa: medıum engıneerıng aspects. Pure &Appl. Chem. 1995; 67(2): 345-373.

[186] Ujang Z, Vaidya A M. Stepped water activity control for efficient enzymatic interesterification. Appl Microbiol Biotechnol 1998; 50(3):318–322.

[187] Bell G, Halling P J, May L, Moore BD, Robb DA, Ulijn R, Valivety RH. 2001. Methods for measurement and control of water in non-aqueous biocatalysis. In: Vulfson EN,

Halling PJ, Holland HL, editors. Methods in Biotechnology. Totowa, NJ: Humana Press. p 105–126.

[188] Rosell C M, Vaidya A M. Twin-core packed-bed reactors for organic phase enzymatic esterification with water activity control. Appl Microbiol Biotechnol 1995; 44(3–4): 283–286.

[189] Won K, Lee S B. On-line conversion estimation for solvent-free enzymatic esterification systems with water activity control. Biotechnol Bioprocess Eng 2002; 7(2):76–84.

[190] Salis A, Pinna M, Monduzzi M, Solinas V. Biodiesel production from triolein and short chain alcohols through biocatalysis. Journal of Biotechnology 2005; 119: 291–299.

[191] Tweddell R J, Kermasha S, Combes D, Marty A. Esterification, interesterification activities of lipase from Rhizopus niveus and Mucor miehei in three different types of organic media: a comparitive study. Ezyme Microb. Technol. 1998; 22: 439–445.

[192] Chen X, Du W, Liu D. Effect of several factors on soluble lipase-mediated biodiesel preparation in the biphasic aqueous-oil systems. World J Microbiol Biotechnol 2008; 24:2097–2102,DOI 10.1007/s11274-008-9714-6

[193] Atadashi I M, Aroua M K, Abdul Aziz A R, Sulaiman N M N. The effects of water on biodiesel production and refining technologies: a review. Renewable and Sustainable Energy Reviews 2012; 16: 3456– 3470.

[194] Robles-Medina A, González-Moreno P A, Esteban-Cerdán L, Molina-Grima E. Biocatalysis: Towards ever greener biodiesel production. Biotechnology Advances 2009; 27: 398–408.

[195] Ranganathan S V, Narasimhan S L, Muthukumar K. An overview of enzymatic production of biodiesel.Bioresource Technology 2008; 99:3975–3981.

[196] Liu Y, Tan H, Zhang X, Yan Y, Hameed B H. Effect of monohydric alcohols on enzymatic transesterification for biodiesel production. Chemical Engineering Journal 2010;157:223–229.

[197] Jeong G T,Park D H. Synthesis of rapeseed biodiesel using short-chained alkyl acetates as acyl acceptor. Appl Biochem Biotechnol 2010; 161:195–208. DOI 10.1007/s12010-009-8777-7.

[198] Su E Z, Zhang M J, Zhang J G, Gaoa J F, Wei D Z. Lipase-catalyzed irreversible transesterification of vegetable oils for fatty acid methyl esters production with dimethyl carbonate as the acyl acceptor. Biochemical Engineering Journal 2007; 36: 167–173.

[199] Du W, Xu Y, Liu D, Zeng J. Comparative study on lipase-catalyzed transformation of soybean oil for biodiesel production with different acyl acceptors. Journal of Molecular Catalysis B: Enzymatic 2004; 30: 125–129.

[200] Xu Y, Du W, Liu D, Zeng J.A novel enzymatic route for biodiesel production from renewable oils in a solvent-free medium.Biotechnology Letters2003; 25:1239–1241.

[201] Nelson LA, Foglia A, Marmer WN. Lipase-catalyzed production of biodiesel. J Am Oil Chem Soc 1996;73:1191–5.

[202] Shieh C J, Liao H F, Lee C C. Optimization of lipase-catalyzed biodiesel by response surface methodology. Bioresource Technology 2003; 88: 103-106.

[203] Dossat V, Combes D, Marty A. Continuous enzymatic transesterication of high oleic sunfower oil in a packed bed reactor:Infuence of the glycerol production. Enzyme Microb. Technol. 1999 ; 25: 194–200.

[204] Soumanou MM, Bornscheuer UT. Improvement in lipase-catalyzed synthesis of fatty acid methyl esters from sunflower oil. Enz Microb Technol 2003;33(1):97–103.

[205] Chattopadhyay S, Karemore A, Das S, Deysarkar A, Sen R.Biocatalytic production of biodiesel from cottonseed oil: Standardization of process parameters and comparison of fuel characteristics.Applied Energy 2011; 88: 1251–1256.

[206] Li L, Du W, Liu D, Wang L, Li Z. Lipase-catalyzed transesterification of rapeseed oils for biodiesel production with a novel organic solvent as the reaction medium. J. Mol. Catal. B:Enzym. 2006 ;43 : 58–62.

[207] Wang L, Du W, Liu D, Li L, Dai N. Lipase-catalyzed biodiesel production from soybean oil deodorizer distillate with absorbent present in tert-butanol system. Journal of Molecular Catalysis B: Enzymatic 2006;43: 29–32.

[208] Halim S F Al, Kamaruddin A H. Catalytic studies of lipase on FAME production from waste cooking palm oil in a tert-butanol system. Process Biochemistry 2008; 43: 1436–1439.

[209] Du W, Liu D, Li L, Dai L. Mechanism exploration during lipase-mediated methanolysis of renewable oils for biodieselproduction in a tert-butanol system. Biotechnol Prog. 2007 ;23: 1087–1090.

[210] Li Q, Zheng J, Yan Y. Biodiesel preparation catalyzed by compound-lipase in co-solvent. Fuel Processing Technology 2010; 91:1229–1234.

[211] Ganesan D, Rajendran A, Thangavelu V. An overview on the recent advances in the transesterification of vegetable oils for biodiesel production using chemical and biocatalysts. Rev Environ Sci Biotechnol 2009;8:367–394.

[212] De Vieira A P A, Da Sılva MAP, Langone MAP, Biodiesel production via esterification reactions catalyzed by lipase. Latin American Applied Research 2006;36:283-288.

[213] Watanabe Y, Pinsirodom P, Nagao T, Asao Y, Kobayashi T , Nishida Y, Takagi Y, Shimada Y, Conversion of acid oil by-produced in vegetable oil refining to biodiesel fuel by immobilized Candida antarctica lipase, Journal of Molecular Catalysis B: Enzymatic 2007; 44 : 99–105.

[214] Zaidi A, Gainer J L, Carta G, Mrani A, Kadiri T, Belarbi Y, Mir A, Esterification of fatty acids using nylon-immobilized lipase in n-hexane: kinetic parameters and chain-length effects. Journal of Biotechnology 2002; 93: 209–216.

[215] Chen Y H, Huang Y H, Lin R H, Shang N C. A continous-flow biodiesel production process using a rotating packed bed. Bioresource Technology 2010; 101: 668–673.

[216] Wang X, Liu X, Zhao C, Ding Y, Xu P. Biodiesel production in packed-bed reactors using lipase–nanoparticle biocomposite.Bioresource Technology 2011; 102: 6352–6355.

[217] Hama S, Tamalampudi S, Yoshida A, Tamadani N, Kuratani N, Nodaa H, Fukuda H, Kondo A. Enzymatic packed-bed reactor integrated with glycerol-separating system for solvent-free production of biodiesel fuel.Biochemical Engineering Journal 2011;55: 66–71.

[218] Xu Y, Nordblad M,Woodley JM. A two-stage enzymatic ethanol-based biodiesel production in a packed bed reactor. Journal of Biotechnology2010; doi:10.1016/j.jbiotec.2012.05.017

[219] Yoshida A, Hama S, Tamadani N, Fukuda H, Kondo A. Improved performance of a packed-bed reactor for biodiesel production through whole-cell biocatalysis employing a high-lipase-expression system.Biochemical Engineering Journal 2012; 63: 76–80.

[220] Oliveira D, Oliveira J V. Enzymatic alcoholysis of palm kernel oil in n-hexane and SC CO_2. Journal of Supercritical Fluids 2001; 19 (2): 141–148.

[221] Madras G, Kolluru C, Kumar R. Synthesis of biodiesel in supercritical fluids. Fuel 2004;83 :2029–2033.

[222] Taher H, Al-Zuhair S, AlMarzouqui A, Hashim I. Extracted fat from lamb meat by supercritical CO2 as feedstock for biodiesel production. Biochemical Engineering Journal 2011; 55: 23–31.