Biotechnology Advances 28 (2010) 628–634

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Biodiesel production with immobilized lipase: A review

Tianwei Tan [a,*], Jike Lu [a,b], Kaili Nie [a], Li Deng [a], Fang Wang [a]

[a] Beijing Bioprocess Key Laboratory, College of Life Science and Technology, Beijing University of Chemical Technology, Beijing, 100029, PR China
[b] Bioengineering Department, Zhengzhou University, Zhengzhou, 450001, PR China

ARTICLE INFO

Available online 24 May 2010

Keywords:
Biodiesel
Immobilized lipase
Production
Review

ABSTRACT

Fatty acid alkyl esters, also called biodiesel, are environmentally friendly and show great potential as an alternative liquid fuel. Biodiesel is produced by transesterification of oils or fats with chemical catalysts or lipase. Immobilized lipase as the biocatalyst draws high attention because that process is “greener”. This article reviews the current status of biodiesel production with immobilized lipase, including various lipases, immobilization methods, various feedstocks, lipase inactivation caused by short chain alcohols and large scale industrialization. Adsorption is still the most widely employed method for lipase immobilization. There are two kinds of lipase used most frequently especially for large scale industrialization. One is *Candida antartica* lipase immobilized on acrylic resin, and the other is *Candida* sp. 99–125 lipase immobilized on inexpensive textile membranes. However, to further reduce the cost of biodiesel production, new immobilization techniques with higher activity and stability still need to be explored.

Contents

1. Introduction

Due to limited energy reserves and the increasing environmental pressure on green-house gases coming from the fossil fuels, biodiesel (Fatty acid alkyl esters, FAAEs), is becoming the hot topic of every country's policy agenda (Jegannathan et al., 2008). Biodiesel has drawn attention as a non-toxic, biodegradable and renewable source of fuel and energy with significantly lower exhaust emissions of particulate matter and green-house gases such as CO, CO_2 and SO_x. Therefore biodiesel is environmentally friendly and shows great potential as an alternative liquid fuel and energy product (Lu et al., 2007).

Biodiesel is produced by esterification of fatty acids or transesterification of oils and fats with short chain alcohols. Methanol is mostly used because of its lower cost compared with other alcohols, so FAAEs most commonly refers to fatty acid methyl esters (FAMEs). The synthesis is classified as chemical or enzymatic production according to the catalysts employed in the process. Short time and high yields are obtained when chemical transesterification is applied. However, drawbacks such as high energy requirements, difficulties in the recovery of the catalyst and glycerol and potential pollution to the environment are major disadvantages in alkali or acid catalyzed

---

* Corresponding author. Tel./fax: +86 10 6441 6691.
E-mail addresses: twtan@mail.buct.edu.cn, ljk002004@163.com (T. Tan).

0734-9750/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.biotechadv.2010.05.012

processes (Lara Pizarro and Park, 2003). Lipases can be used to catalyze the reaction in mild conditions and an increasing number of researchers have reported on its use (Lu et al., 2008; Shimada et al., 2002). One of the bottlenecks for industrial application of lipase is the high cost of the biocatalyst. Immobilization methods have been introduced to improve lipase stability and allow for repeated utilization (Cao, 2005; Salis et al., 2008).

This article provides a review of the enzymatic production of biodiesel, including various immobilization methods, different feedstocks and the corresponding catalytic process, current industrial production, as well as the prospects of enzymatic biodiesel industrialization in the future.

2. Lipase and its immobilization

Lipases (EC 3.1.1.3) are widely employed to catalyze hydrolysis, alcoholysis, esterification and transesterification of carboxylic esters. Lipases have excellent catalytic activity and stability in non-aqueous media, which facilitate the esterification and transesterification process during biodiesel production (Villeneuve et al., 2000). Immobilized enzymes are defined as “enzymes physically confined or localized in a certain defined region of space with retention of their catalytic activities, and which can be used repeatedly and continuously” (Jegannathan et al., 2008). There are several methods for lipase immobilization, including adsorption, covalent bonding, entrapment, encapsulation, and cross-linking. These immobilization methods have been employed to improve lipase stability for biodiesel production in recent years, and this is discussed in the following sections.

2.1. Adsorption

Adsorption is the attachment of lipase on the surface of the carrier by weak forces, such as van der Walls, hydrophobic interactions or dispersion forces (Jegannathan et al., 2008). Adsorption can be prepared under mild conditions without major activity loss and the associated process is relatively easy and low cost. Moreover, the carrier can easily be recovered for repeated immobilization. With these advantages, adsorption is still the most widely employed method for lipase immobilization. Much recent research has been focused on this method and reported results are summarized in Table 1.

The immobilized lipases employed for biodiesel production are derived from different sources, for example: *Candida antartica* (Du et al., 2004; Shimada et al., 1999; Wang et al., 2007; Watanabe et al., 2000), *Candida* sp. 99–125 (Lu et al., 2007, 2008, 2009, 2010; Lv et al., 2008; Nie et al., 2006; Tan et al., 2006), *Pseudomonas fluorescens* (Iso et al., 2001; Salis et al., 2008; Soumanou and Bornscheuer, 2003), *Pseudomonas cepacia* (Salis et al., 2005; Shah and Gupta, 2007), *Porcine pancreatic* (Yesiloglu, 2004), *Rhizomucor Miehei* and *Chromobacterium viscosum* (Shah et al., 2004; Shieh et al., 2003; Yesiloglu, 2004) etc. The carriers used include acrylic resin, textile membrane, polypropylene, celite and diatomaceous earth. In general, all the biodiesel yields using the adsorption technique are higher than 80% with vegetable oil or waste cooking oil as feedstock. There are two kinds of lipase used most frequently, especially for large scale industrialization. One is the *Candida antartica* lipase immobilized on acrylic resin, which is known by its commercial name Novozym 435. Novozym 435 can catalyze vegetable oil and cooking oil with yield higher than 90%, and the lipase shows quite excellent catalytic properties in *t*-butanol solvent (Royon et al., 2007). The other is the *Candida* sp. 99–125 lipase immobilized on cheap textile membrane. This immobilized lipase textile can catalyze lard, waste oil and various vegetable oil with yield higher than 87%. A comparison between these two lipases is summarized in Table 2.

Although adsorption has its special commercial advantages for its high activity toward biodiesel production at low cost, the lipase may

Table 1
Biodiesel production with various immobilized lipase (Jegannathan et al., 2008).

Immobilized method	Carrier used	Lipase origin	Oil	Acyl acceptors	Yield (%)	References
Adsorption	Acrylic resin	*Candida antartica*	Vegetable oil, waste cooking oil	Methanol, 1-propanol, methyl acetate	>90	(Du et al., 2004; Shimada et al., 1999; Wang et al., 2007; Watanabe et al., 2000)
Adsorption	Textile membrane	*Candida* sp. 99–125	Lard, waste oil, salad oil	Methanol	>87	(Lu et al., 2007, 2008, 2010; Lv et al., 2008; Nie et al., 2006; Tan et al., 2006)
Adsorption	Toyonite 200-M, polypropylene	*Pseudomonas fluorescens*	Vegetable oil	Methanol	>87	(Iso et al., 2001; Salis et al., 2008; Soumanou and Bornscheuer, 2003)
Adsorption	Celite, Diatomaceous earth	*Pseudomonas cepacia,*	Jatropha oil, vegetable oil	Ethanol, 2-butanol	>98	(Salis et al., 2005; Shah and Gupta, 2007)
Adsorption	Anion resin, celite-545	*Porcine pancreatic, Rhizomucor Miehei, Chromobacterium viscosum*	Sunflower oil, soybean oil, Jatropha oil	Ethanol, methanol	>80	(Shah et al., 2004; Shieh et al., 2003; Yesiloglu, 2004)
Covalent bond	Silica-PVA styrene-divinylbenzene	*Burkholderia cepacia, Thermomyces lanuginosus*	Babassu oil, canola oil	Ethanol, methanol	>97%	(Dizge et al., 2009a,b; Freitas et al., 2009)
Entrapment	Hydrophobic sol–gel support	*Pseudomonas cepacia*, NS44035	Soybean oil, triolein,	Methanol, ethanol	60	(Meunier and Legge, 2010; Noureddini et al., 2005)
Cross-linking	Glutaraldehyde	*Pseudomonas cepacia*	Mahua oil	Ethanol	92	Kumari et al. (2007)

Table 2
Comparisons of immobilized *Candida antartica and Candida* sp. 99–125.

Lipases	Carrier used	Substrate	Organic solvent	Effect of water on yield	Yield (%)	Stability	Cost	References
Candida antartica	Acrylic resin	Vegetable oil, waste cooking oil	Hydrophobic solvents, solvent free, *t*-butanol	No water added	>90	500 h	High	(Du et al., 2004; Royon et al., 2007; Shimada et al., 1999; Wang et al., 2007; Watanabe et al., 2000; Wu and Zong, 2007)
Candida sp. 99–125	Textile membrane	Lard, waste oil, salad oil	Hydrophobic solvents, solvent free	10% wt.% to the oil	>87	210 h	Low	(Lu et al., 2007, 2008, 2010; Lv et al., 2008; Nie et al., 2006; Tan et al., 2006)

be stripped off from the carrier because of the weak adhesion forces between the enzyme and support. The immobilized lipase may not be stable enough to prevent lipase from desorption during the catalytic process, though enzyme inactivation is not the main reason for lower activity (Jegannathan et al., 2008).

2.2. Other immobilization techniques

Besides adsorption, there are several other techniques for lipase immobilization such as covalent bonding, entrapment, encapsulation and cross-linking. A comparison of these different immobilized methods is summarized in Table 3.

Lipase immobilization by covalent bonding to the solid carrier has the expected advantage of irreversible binding of the lipase to the support matrix (Trevan, 1988a,b). With the covalent bond method, the strong interactions between the lipase and the support make enzyme leaching uncommon during the catalytic process. Lipase from *Thermomyces lanuginosus* was immobilized by covalent attachment onto polyglutaraldehyde activated styrene-divinylbenzene (STY-DVB) copolymer, which was used to catalyze methanolysis of canola oil for biodiesel synthesis. Under the optimized conditions, the maximum biodiesel yield was 97% at 50 °C in 24 h reaction. The immobilized enzyme retained its activity during the 10 repeated batch reactions (Dizge et al., 2009b).

Entrapment of a lipase entails capture of the lipase within a matrix of polymer (Jegannathan et al., 2008). In theory the entrapped enzyme is not attached to the polymer; its free diffusion is merely restrained. Virtues of the entrapment method for immobilizing lipase are that it is fast, cheap, very easy and usually involves mild conditions (Trevan, 1988a,b). *P. cepacia* was entrapped in hydrophobic sol–gel, and the immobilized lipase could catalyze biodiesel production with soybean oil as feedstock. The final conversion was around 67% (Noureddini et al., 2005). Lipase (NS44035) from Novozymes North America Inc. was entrapped in a celite supported sol–gel to prepare the immobilized lipase. Then the lipase was then employed for methanolysis of triolein with an approximately 60% conversion (Meunier and Legge, 2010). The conversion catalyzed by entraped lipase has so far proven to be relatively low. The reason might be due to the poor diffusion and erosion of lipase from the surface of the support during the processing procedures (Jegannathan et al., 2008).

3. Catalytic process

3.1. Feedstock

Biodiesel is produced by methanolysis (for FAME) of the oils and fats from various origins. Therefore theoretically, any form of oils and fats coming from the animals, plants or even microorganisms can be used as feedstock for biodiesel production. Currently the main biodiesel feedstock are classified into three categories: (1) plant oil such as soybean oil (Lv et al., 2008; Silva et al., 2007), jatropha oil (Shah et al., 2004; Tamalampudi et al., 2008), palm oil (Halim et al., 2009; Sim et al., 2009; Talukder et al., 2006), cottonseed oil and sunflower oil etc (Dizge et al., 2009a,b; Soumanou and Bornscheuer, 2003; Wu et al., 2007). (2) animal fat such as tallow (Da Cunha et al., 2009), lard and grease etc. (Lee et al., 2002; Ngo et al., 2008) and (3) waste cooking oil and industrial waste oil (Dizge et al., 2009a,b; Lara Pizarro and Park, 2003). Fatty acid distributions in some common oil sources are shown in Table 4.

Every country develops biodiesel feedstock according to its national conditions (Min and Zhang, 2006). The United States mainly uses genetically modified soybean oil as raw material, while the European Union and Canada use rapeseed oil to produce biodiesel. Some South-eastern Asian countries such as Malaysia and Indonesia are abundant in palm oil, so these countries develop biodiesel from palm oil. Although China is a large agriculture country, the food supply system is still a big problem facing the whole country. In order to follow principle of never competing with grain land, China's recent biodiesel development is based on waste cooking oil. Jatropha oil will become another choice for China due to some unique advantages. Jatropha tree can grow on waste land with a minimum water and fertilizer demand, and the oil is non-edible because of the presence of some antinutritional factors such as toxic phorbol esters (Jegannathan et al., 2008; Shah et al., 2004). Other non-food oil sources such as

Table 3
Comparison of different lipase immobilized methods.

Methods	Advantages	Disadvantages
Adsorption	Preparing conditions are mild and easy with low cost. The carrier can be regenerated for repeated use.	The interaction between the lipase and the carrier is weak, so the immobilized lipase was sensitive to pH, ionic strength and temperature etc. The adsorption capacity is small and the protein might be stripped off from the carrier.
Covalent bond	The immobilized lipase is rather stable because of the strong forces between the protein and the carrier.	The preparation conditions are rigorous, so the lipase might lose its activity during the immobilized process. Some coupling reagents are toxic.
Cross-linking	The interaction between the lipase and the carrier is strong and the immobilized lipase is stable.	The cross-linking conditions are intense and the mechanical strength of the immobilized lipase is low.
Entrapment	The entrapment conditions are moderate, and the immobilized method is applicable to a wide range of carrier and lipases.	This immobilized lipase always has the mass transfer restriction during the catalytic process, so the lipase is only effective for low molecular weight substrates.

Table 4
Structure of fatty acids and their distributions in raw materials of biodiesel (Ma and Hanna, 1999).

Fatty acid	Formula	Soybean	Cottonseed	Palm	Lard	Tallow	Coconut
Lauric	12:0	0.1	0.1	0.1	0.1	0.1	46.5
Myristic	14:0	0.1	0.7	1.0	1.4	2.8	19.2
Palmitic	16:0	10.2	20.1	42.8	23.6	23.3	9.8
Stearic	18:0	3.7	2.6	4.5	14.2	19.4	3.0
Oleic	18:1	22.8	19.2	40.5	44.2	42.4	6.9
Linoleic	18:2	53.7	55.2	10.1	10.7	2.9	2.2
Linolenic	18:3	8.6	0.6	0.2	0.4	0.9	0

micro-algae oil and microbial oil also have significant potential for they have short production cycles and they can be produced by fermentation using inexpensive sources, such as CO_2 or waste water (Um and Kim, 2009; Xue et al., 2006, 2008).

3.2. Lipase inactivation caused by alcohols

For biodiesel synthesis, at least a stoichiometric amount of methanol is required for the complete conversion of triacylglycerols (TAGs) to their corresponding FAMEs. However, methanolysis is decreased significantly by adding >1/2 molar equivalent of methanol at the beginning of the enzymatic process. This inactivation caused by the polar short chain alcohols was the major obstacle for the enzymatic biodiesel production (Shimada et al., 2002). To solve this problem, researchers select from the following three options, methanol stepwise addition, acyl acceptor alterations and solvent engineering (Tables 5).

The stepwise addition of methanol was the most common strategy in earlier studies. With immobilized *Candida antarctica* lipase as catalyst, methaolysis of vegetable oil for boidiesel production was conducted in Shimada' research (Shimada et al., 1999). The first step of the reaction was conducted at 30 °C for 10 h in a mixture of oil/methanol (1:1, mol/mol) and 4% immobilized lipase with shaking at 130 oscillations/min. After more than 95% methanol was consumed in ester formation, a second molar equivalent of methanol was added and the reaction continued for 14 h. The third molar equivalent of methanol was finally added and the reaction continued for 24 h (total reaction time, 48 h). This three-step process converted 98.4% of the oil to its corresponding methyl esters (Shimada et al., 1999). The methanol stepwise addition strategy was also effective for other lipases such as *Candida* 99–125 (Lu et al., 2007), *Pseudomonas fluorescens* (Soumanou and Bornscheuer, 2003), *Rhizopus orzyae* (Chen et al., 2006) etc. Currently, a methanol stepwise addition strategy is still the first choice as it can achieve a high yield using simple operating conditions.

When methanol is replaced by a different acyl acceptor as methyl acetate or ethyl acetate, the lipase inactivation caused by short chain alcohol is avoided. With commercial Novozym 435 as catalyst, using methyl acetate as the acyl acceptor, a yield of 92% of methyl ester could be obtained with a molar ratio of methyl acetate to oil of 12:1, and methyl acetate showed no negative effect on enzymatic activity. There is almost no detected loss in lipase activity, even after being continuously used for 100 batches (Du et al., 2004). Although methyl acetate has its special advantage as the acyl acceptor for biodiesel production, the low reaction rate and the high cost are major constraints for industrial applications.

Since the immobilized lipase might be inactivated by the insoluble methanol in the system, it is reasonable to solve the problem through solvent selection to improve methanol solubility. *t*-butanol is a good solvent of the substrate methanol, and many previous studies are focused on this solvent. Using immobilized *Candida antarctica* lipase as catalyst in *t*-butanol solvent, biodiesel production from cottonseed oil was studied (Royon et al., 2007). In a batch system, lipase inhibition caused by un-dissolved methanol was eliminated by adding *t*-butanol to the reaction medium. This also produced a noticeable increase of reaction rate and ester yield. A methanolysis yield of 97% was observed after 24 h at 50 °C with a reaction mixture containing 32.5% *t*-butanol, 13.5% methanol, 54% oil and 0.017 g enzyme $(\text{g oil})^{-1}$. With the same mixture, a 95% ester yield was obtained using a one step fixed bed continuous reactor with a flow rate of $9.6\ \text{ml h}^{-1}\ (\text{g enzyme})^{-1}$. Experiments with the continuous reactor over 500 h did not show any appreciable decrease in ester yields (Royon et al., 2007). Other solvents such as 1, 4-dioxane and ionic liquids were also examined as solvents for lipase catalyzed biodiesel production (Ha et al., 2007; Iso et al., 2001). Although these solvents might solve the problem of lipase inactivation caused by methanol, difficulties in recovering the solvent would make these methods less competitive at industrial scale.

3.3. Effect of water content

For the lipase-catalyzed biodiesel production in predominantly non-aqueous media, water plays multiple roles and it has strong influence on the catalytic activity and stability of the lipase (Lu et al., 2009). Some essential water is needed to keep the enzyme active in organic solvents. On the other hand, water might take part in the transesterification, thus influencing the equilibrium (Kaieda et al., 1999). Lipase possesses the unique feature of acting at the interface between an aqueous and an organic phase, so the lipase activity generally depends on the interfacial area. Water facilitates an increase in the available interfacial area, thus it helps to maintain lipase activity. However, excess water might make the lipase more flexible and lead to some unintended side-reactions such as hydrolysis, especially in the transesterification process. Consequently the optimum water content required to maximize enzymatic activity can be determined for most lipases, and the amount for a certain reaction depends on the feedstock, the lipase, the immobilized support and the organic solvent employed (Jegannathan et al., 2008; Lu et al., 2009). For commercial Novozym 435 lipase, no extra water was added into the system for high biodiesel yield (Du et al., 2004; Royon et al., 2007; Shimada et al., 1999; Watanabe et al., 2000), while the optimum water amount required for *Candida* sp. 99–125 lipase to maintain the highest transesterification activity is 10–20% based on the oil weight (Lu et al., 2007, 2008, 2009; Lv et al., 2008; Nie et al., 2006; Tan et al., 2006).

Table 5
Summary of the options to avoid lipase inactivation caused by methanol.

Options	Operating conditions	Yield (%)	Advantages	Disadvantage	References
Methanol stepwise addition	Three-step or two-step methanol addition	>87	Higher yield is obtained without inactivation to the lipase	The operation is relative complicated in large scale production.	(Chen et al., 2006; Lu et al., 2007; Shimada et al., 1999; Soumanou and Bornscheuer, 2003)
Acyl acceptor alterations	Methyl acetate, acetate ethyl	>90	No inactivation effect occurs and no glycerol is produced.	The reaction rate is low and the acyl acceptor cost is high.	Xu et al. (2003)
Solvent engineering	With *t*-butanol, 1,4-dioxane, ionic liquid as solvents	>80	Good solvents of methanol and glycerol, so methanol inactivation and glycerol deposit are avoided.	Increment of the solvent recovery cost.	(Ha et al., 2007; Iso et al., 2001; Royon et al., 2007)

Table 6
Different reactors used for enzymatic production of biodiesel.

Reactors	Advantages	Disadvantages	Yield (%)	References
Batch reactor	High degree of substrate dispersion, with simple structure and easy to control,	Low reaction efficiency, the carrier can be disrupted by the shear force	>87	(Iso et al., 2001; Lara Pizarro and Park, 2003; Lu et al., 2007; Shah et al., 2004; Soumanou and Bornscheuer, 2003; Yesiloglu, 2004)
PBR	Continuous production with high efficiency, reduced product inhibition	High pressure drop, axial concentration distribution, difficulty in temperature control and lipase replacement	>90	(Hama et al., 2007; Shimada et al., 2002)

4. Reactors and industrialization

Most of the established biodiesel production lines are based on chemical methods. The catalysts used are acid catalyst, such as H_2SO_4, or alkaline catalysts, such as NaOH and sodium methoxide. The alkaline method is better than acid catalysis due to the high FAME yield and short reaction times. Generally, large molar ratio of methanol to oil is needed for alkaline catalysis process to achieve high yield, and a distillation process will be needed for methanol recovery and biodiesel refining. The production process for alkaline method is shown in Fig. 1.

Chemical methods give high conversion of triacylglycerols (TAG) to methyl esters (biodiesel) in relatively short times (4 hours to 10 hours). However, they have drawbacks such as high energy consumption, difficulty in recovering the glycerol and significant amount of alkaline waste water. The fatty acid alkaline salts (soaps) are by-products which have to be removed by washing with water. The chemical catalysis process is still the most popular method for industrial scale use due to the high cost of lipase.

Although many papers have been published about enzymatic production of biodiesel, most of the experiments were done in the laboratory with batch reactors. Methanol shows a high degree of dispersion in the oil phase in a batch reactor. However, shear stress from the stirring would disrupt the enzyme carrier by the physical agitation. So the immobilized might not be reused for a long period of time. A packed bed reactor (PBR) is a continuous operation without separation of the catalyst from the reaction product. The by-product glycerol remains in the bottom of the PBR because of its high viscosity. The aggregated glycerol might deposit on the surface of the immobilized lipase, thus decreasing the catalytic efficiency. So glycerol must be eliminated in a timely manner during the process. A three step methanolysis of vegetable oil in a PBR packed with *Candida antarctica* lipase, as shown in Fig. 2 (Shimada et al., 2002). A flow rate of 6 ml/h was used for the three reaction steps, and 1/3 molar equivalent of methanol to oil was added at each step. The glycerol was separated by gravity sedimentation after each step. The conversion to FAMEs reached 90%, and the threes step reaction was continuously used for 100 days (Shimada et al., 2002). Hama et al. developed a PBR system using *Rhizopus oryzae* whole-cell as biocatalyst for biodiesel production by soybean oil methanolysis. A high methyl ester content of over 90% was achieved at a flow rate of 25 l/h in the first cycle of repeated batch methanolysis and a high value of approximately 80% was maintained after the tenth cycle. Comparison with conducting the methanolysis reaction in a shaken bottle suggests that the PBR enhances repeated batch methanolysis by protecting the immobilized cells from physical damage and excess amounts of methanol (Hama et al., 2007). Nie et al. conducted continuous transesterification on PBR with lipase *Candida* sp. 99–125 and a hydrocyclone was set after PBR to separate glycerol (Nie et al., 2006). The final conversion to FAME from plant oil and waste oil

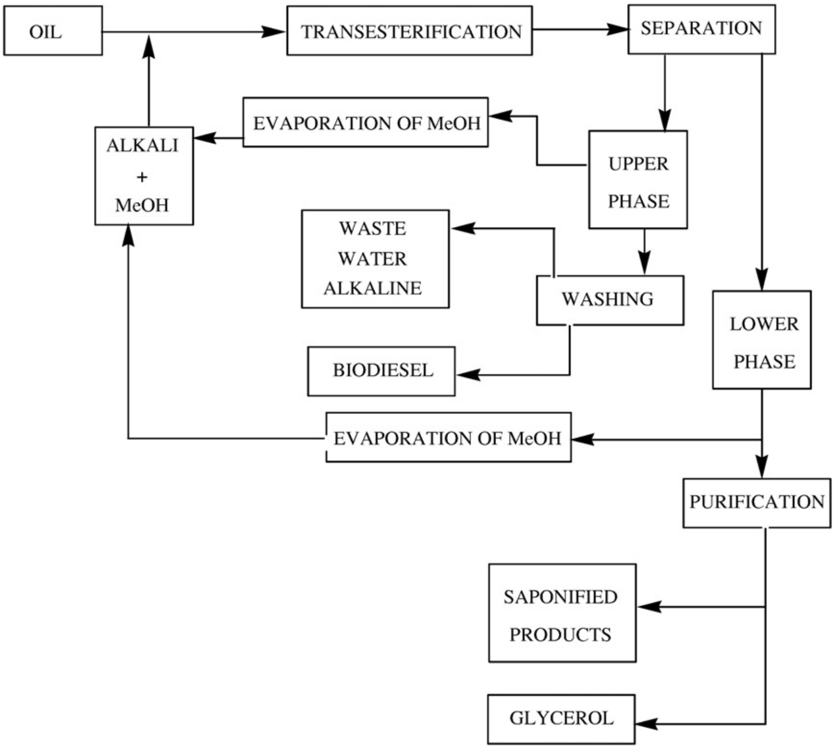

Fig. 1. Flow sheet of biodiesel production with chemical methods.

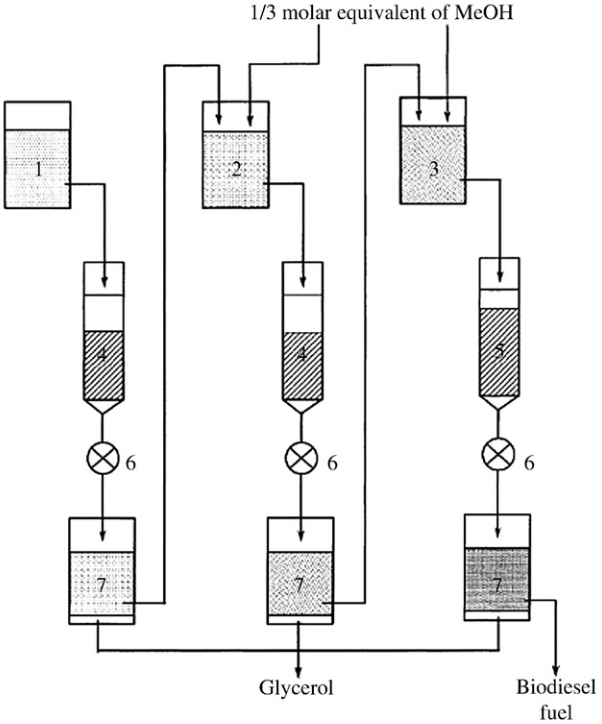

Fig. 2. Three step methanolysis of oil for biodiesel production in PBR (Shimada et al., 2002). 1-3, Storage vessel of first-step substrate (oil and 1/3 molar equivalent of MeOH); 4, PBR packed with 3 g immobilized *Candida antarctica* lipase; 5, PBR packed with 4.5 g immobilized *Candida antarctica* lipase; 6, peristaltic pump; 7, receiver of eluted reaction mixture.

under the optimal condition was 90% and 92%, respectively. The life of the immobilized lipase was more than 10 days (Nie et al., 2006). A comparison of the two reactors is summarized in Table 6.

In 2007, Lvming Co. Ltd. established an enzymatic production line with capacity of 10,000 tons in Shanghai, China. The factory employed the technique from Beijing University of Chemical Technology, with immobilized lipase *Candida* sp. 99–125 as catalyst. A high acid value (AV>160mmgKOH/g) waste cooking oil from Shanghai was used as raw material. The enzyme dosage was 0.4% to the weight of oil. The process was conducted in a stirred tank reactor, and a centrifuge was used to separate glycerol and water. Yields of FAMEs achieved 90% under optimal conditions. The cost of lipase was 200 CNY/t biodiesel. Another factory that conducted enzymatic catalysis in China was Hainabaichuan Co. Ltd., Hunan Province. The factory used the technology of Tsinghua University, with commercial Novozyme 435 used as the catalyst.

5. Conclusions and prospects

Immobilized lipase catalyzed biodiesel production is a very interesting topic in recent years as it allows the use of mild reaction conditions that are consistent with a green process. Novozym 435 and *Candida* sp. 99–125 immobilized on textile membrane are the most common employed lipase to catalyze biodiesel production from various sources of oils such as lard, soybean oil, cottonseed oil and waste oil. The low cost of immobilized *Candida* sp. 99–125 lipase is rather competitive for industrial use. The feedstock accounts for more than 70% of the total production cost. In the future, jatropha oil, microbial oil, micro-algae oil and waste oil will become the main feedstock for biodiesel production. New immobilization techniques with higher activity and stability at low cost still need to be explored and developed. If the cost of the catalyst lipase is reduced, the industrial production of biodiesel using enzymatic methods will soon have a bright future.

Acknowledgements

This research was financially supported by the National High Technology Research and Development Program of China (2006AA020203, 2009AA03Z232), the National Nature Science Foundation of China (20576013), the National Science Found for Distinguished Young Scholars of China (20325622), the State Key Development Program for Basic Research of China (2007CB714304), the Natural Science Foundation of Beijing, China (2071002).

References

Cao L. Immobilised enzymes: science or art? Curr Opin Chem Biol 2005;9:217–26.
Chen G, Ying M, Li W. Enzymatic conversion of waste cooking oils into alternative fuel—Biodiesel. Appl Biochem Biotechnol 2006;132:911–21.
Da Cunha ME, Krause LC, Moraes MSA, Faccini CS, Jacques RA, Almeida SR, et al. Beef tallow biodiesel produced in a pilot scale. Fuel Process Technol 2009;90:570–5.
Dizge N, Aydiner C, Imer DY, Bayramoglu M, Tanriseven A, Keskinler B. Biodiesel production from sunflower, soybean, and waste cooking oils by transesterification using lipase immobilized onto a novel microporous polymer. Bioresour Technol 2009a;100:1983–91.
Dizge N, Keskinler B, Tanriseven A. Biodiesel production from canola oil by using lipase immobilized onto hydrophobic microporous styrene-divinylbenzene copolymer. Biochem Eng J 2009b;44:220–5.
Du W, Xu Y, Liu D, Zeng J. Comparative study on lipase-catalyzed transformation of soybean oil for biodiesel production with different acyl acceptors. J Mol Catal B Enzym 2004;30:125–9.
Freitas L, Da Rós PCM, Santos JC, de Castro HF. An integrated approach to produce biodiesel and monoglycerides by enzymatic interestification of babassu oil (Orbinya sp). Process Biochem 2009;44:1068–74.
Ha SH, Lan MN, Lee SH, Hwang SM, Koo Y-M. Lipase-catalyzed biodiesel production from soybean oil in ionic liquids. Enzyme Microb Technol 2007;41:480–3.
Halim SFA, Kamaruddin AH, Fernando WJN. Continuous biosynthesis of biodiesel from waste cooking palm oil in a packed bed reactor: optimization using response surface methodology (RSM) and mass transfer studies. Bioresour Technol 2009;100:710–6.
Hama S, Yamaji H, Fukumizu T, Numata T, Tamalampudi S, Kondo A, et al. Biodiesel-fuel production in a packed-bed reactor using lipase-producing *Rhizopus oryzae* cells immobilized within biomass support particles. Biochem Eng J 2007;34:273–8.
Iso M, Chen B, Eguchi M, Kudo T, Shrestha S. Production of biodiesel fuel from triglycerides and alcohol using immobilized lipase. J Mol Catal B Enzym 2001;16:53–8.
Jegannathan KR, Abang S, Poncelet D, Chan ES, Ravindra P. Production of biodiesel using immobilized lipase — a critical review. Crit Rev Biotechnol 2008;28:253–64.
Kaieda M, Samukawa T, Matsumoto T, Ban K, Kondo A, Shimada Y, et al. Biodiesel fuel production from plant oil catalyzed by Rhizopus oryzae lipase in a water-containing system without an organic solvent. J Biosci Bioeng 1999;88:627–31.
Kumari V, Shah S, Gupta MN. Preparation of biodiesel by lipase-catalyzed transesterification of high free fatty acid containing oil from *Madhuca indica*. Energ Fuel 2007;21:368–72.
Lara Pizarro AV, Park EY. Lipase-catalyzed production of biodiesel fuel from vegetable oils contained in waste activated bleaching earth. Process Biochem 2003;38:1077–82.
Lee K-T, Foglia T, Chang K-S. Production of alkyl ester as biodiesel from fractionated lard and restaurant grease. J Am Oil Chem Soc 2002;79:191–5.
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.
Lu J, Nie K, Wang F, Tan T. Immobilized lipase Candida sp. 99–125 catalyzed methanolysis of glycerol trioleate: Solvent effect. Bioresour Technol 2008;99:6070–4.
Lu J, Chen Y, Wang F, Tan T. Effect of water on methanolysis of glycerol trioleate catalyzed by immobilized lipase Candida sp. 99–125 in organic solvent system. J Mol Catal B Enzym 2009;56:122–5.
Lu J, Deng L, Zhao R, Zhang R, Wang F, Tan T. Pretreatment of immobilized Candida sp. 99–125 lipase to improve its methanol tolerance for biodiesel production. J Mol Catal B Enzym 2010;62:15–8.
Lv P, Wang X, Yuan Z, Tan T. Conversion of soybean oil to biodiesel fuel with immobilized *Candida* lipase on textile cloth. Energ Source Part A 2008;30:872–9.
Ma F, Hanna MA. Biodiesel production: a review. Bioresour Technol 1999;70:1-15.
Meunier SM, Legge RL. Evaluation of diatomaceous earth as a support for sol–gel immobilized lipase for transesterification. J Mol Catal B Enzym 2010;62:54–8.
Min E, Zhang L. Development of biodiesel industrial chain. Beijing: China petrochemical press; 2006.
Ngo HLN, Zafiropoulos NA, Foglia TA, Samulski ET, Lin W. Efficient two-step synthesis of biodiesel from greases. Energ Fuel 2008;22:626–34.
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.
Noureddini H, Gao X, Philkana RS. Immobilized *Pseudomonas cepacia* lipase for biodiesel fuel production from soybean oil. Bioresour Technol 2005;96:769–77.
Royon D, Daz M, Ellenrieder G, Locatelli S. Enzymatic production of biodiesel from cotton seed oil using *t*-butanol as a solvent. Bioresour Technol 2007;98:648–53.
Salis A, Pinna M, Monduzzi M, Solinas V. Biodiesel production from triolein and short chain alcohols through biocatalysis. J Biotechnol 2005;119:291–9.
Salis A, Pinna M, Monduzzi M, Solinas V. Comparison among immobilised lipases on macroporous polypropylene toward biodiesel synthesis. J Mol Catal B Enzym 2008;54:19–26.

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

Shah S, Sharma S, Gupta MN. Biodiesel preparation by lipase-catalyzed transesterification of jatropha oil. Energ Fuel 2004;18:154–9.

Shieh CJ, Liao HF, Lee CC. Optimization of lipase-catalyzed biodiesel by response surface methodology. Bioresour Technol 2003;88:103–6.

Shimada Y, Watanabe Y, Samukawa T, Sugihara A, Noda H, Fukuda H, et al. Conversion of vegetable oil to biodiesel using immobilized *Candida antarctica* lipase. J Am Oil Chem Soc 1999;76:789–93.

Shimada Y, Watanabe Y, Sugihara A, Tominaga Y. Enzymatic alcoholysis for biodiesel fuel production and application of the reaction to oil processing. J Mol Catal B Enzym 2002;17:133–42.

Silva C, Weschenfelder TA, Rovani S, Corazza FC, Corazza ML, Dariva C, et al. Continuous production of fatty acid ethyl esters from soybean oil in compressed ethanol. Ind Eng Chem Res 2007;46:5304–9.

Sim JH, Harun KA, Bhatia S. Effect of mass transfer and enzyme loading on the biodiesel yield and reaction rate in the enzymatic transesterification of crude palm oil. Energ Fuel 2009;23:4651–8.

Soumanou MM, Bornscheuer UT. Improvement in lipase-catalyzed synthesis of fatty acid methyl esters from sunflower oil. Enzyme Microb Technol 2003;33:97-103.

Talukder MMR, Puah SM, Wu JC, Won CJ, Chow Y. Lipase-catalyzed methanolysis of palm oil in presence and absence of organic solvent for production of biodiesel. Biocatal Biotransform 2006;24:257–62.

Tamalampudi S, Talukder MR, 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. Biochem Eng J 2008;39:185–9.

Tan T, Nie K, Wang F. Production of biodiesel by immobilized Candida sp. lipase at high water content. Appl Biochem Biotechnol 2006;128:109–16.

Trevan MD. Enzyme immobilization by covalent bonding. New Protein Techniques. Humana Press; 1988a. p. 495–510.

Trevan MD. Enzyme immobilization by entrapment new protein techniques. New Protein Techniques. Humana Press; 1988b. p. 491–4.

Um B-H, Kim Y-S. Review: a chance for Korea to advance algal-biodiesel technology. J Ind Eng Chem 2009;15:1–7.

Villeneuve P, Muderhwa JM, Graille J, Haas MJ. Customizing lipases for biocatalysis: a survey of chemical, physical and molecular biological approaches. J Mol Catal B Enzym 2000;9:113–48.

Wang J-X, Huang Q-D, Huang F-H, Wang J-W, Huang Q-J. Lipase-catalyzed production of biodiesel from high acid value waste oil using ultrasonic assistant. Chin J Biotechnol 2007;23:1121–8.

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. J Am Oil Chem Soc 2000;77:355–60.

Wu H, Zong M-H. Ultraclean Transportation Fuels. Washington, DC: American Chemical Society; 2007.

Wu Q, Chen H, Han M, Wang D, Wang J. Transesterification of cottonseed oil catalyzed by Bronsted acidic ionic liquids. Ind Eng Chem Res 2007;46:7955–60.

Xu Y, Du W, Liu D, Zeng J. A novel enzymatic route for biodiesel production from renewable oils in a solvent-free medium. Biotechnol Lett 2003;25:1239–41.

Xue F, Zhang X, Luo H, Tan T. A new method for preparing raw material for biodiesel production. Process Biochem 2006;41:1699–702.

Xue F, Miao J, Zhang X, Luo H, Tan T. Studies on lipid production by *Rhodotorula glutinis* fermentation using monosodium glutamate wastewater as culture medium. Bioresour Technol 2008;99:5923–7.

Yesiloglu Y. Immobilized lipase-catalyzed ethanolysis of sunflower oil. J Am Oil Chem Soc 2004;81:157–60.