Review Article

Enzymatic biodiesel production: Technical and economical considerations

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It is well documented in the literature that enzymatic processing of oils and fats for biodiesel is technically feasible. However, with very few exceptions, enzyme technology is not currently used in commercial-scale biodiesel production. This is mainly due to non-optimized process design and a lack of available cost-effective enzymes. The technology to re-use enzymes has typically proven insufficient for the processes to be competitive. However, literature data documenting the productivity of enzymatic biodiesel together with the development of new immobilization technology indicates that enzyme catalysts can become cost effective compared to chemical processing. This work reviews the enzymatic processing of oils and fats into biodiesel with focus on process design and economy.

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1 Introduction

Governmental as well as financial incentives have been the background for the recent expansion of biodiesel production. The interest in biodiesel is due to the advantages of biodiesel with respect to:

- reduction of greenhouse gas emissions
- energy security
- use of renewables
- reduced CO, hydrocarbons, NO_x and particles in exhaust emission
- applicability in the existing transport sector (in contrast to other "eco-fuels" such as hydrogen)

The production of biodiesel in Europe was 4.9 million tons in 2006 whereas US production reached 0.9 million tons [1]. Today, smaller amounts are produced in South America and Asia, but the grouping of production and consumption is changing. To reduce CO_2 emissions, it has been decided by EU directive to include 5.75% biofuel (bioethanol and biodiesel) in transport sector fuels by 2010. Additionally, in January 2008 the EU proposed an additional requirement for 10% biofuels by 2020. With this demand, it is of great importance that the production process is as efficient and sustainable as possible.

Today, essentially all biodiesel is produced using chemical catalysts. The alkaline-catalyzed process requires raw materials of high and uniform quality. The use of low-quality oil requires extra process steps to eliminate the free fatty acids (FFA) from the oil before it enters the alkaline-catalyzed process. The FFA are then converted to biodiesel using a troublesome acid-catalyzed process. In contrast, the essential feature of an enzyme-catalyzed process is that, by selection of the right enzyme composition, it is possible to make a continuous single-step process for biodiesel, even with very high FFA content in the oil. This allows utilization of low-quality and non-food oils without a negative impact on the environment.

The amount of literature on enzymatic biodiesel production has increased a lot within the last years and excellent reviews can be found [2–6]. These papers cover the available literature extensively, typically with a focus on properties of different enzymes and alcohols in laboratory-scale batch experiments. A few review articles discuss the issues of scale-up, economy, and the perspectives of commercialization [7–9]. Also general books and reviews on biodiesel often include a short paragraph on enzymatic catalysis [10]. The consensus conclusion in the literature seems to be that the enzyme cost must be lower to make the process cost effective. The question

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is then how cheap the enzymes need to be to reach a breakeven in biodiesel production cost compared to chemical catalysis. This is a difficult estimate as the chemical and enzymatic process will be very different. If we look solely at the catalyst costs, the calculation is today 0.35 USD/lb catalyst times 0.0314 lb catalyst/lb feedstock divided by 0.982 lb biodiesel/lb feedstock yield, corresponding to 25 USD/ton biodiesel [1]. Of course, it is a very rough assumption only to compare the catalyst cost when the enzyme-catalyzed process offers several additional benefits:

- compatibility with variations in the quality of the raw material
- fewer process steps
- higher quality of glycerol
- improved phase separation (no emulsification from soaps)
- reduced energy consumption and wastewater volumes

This review discusses the technical possibilities of enzymatic processing in biodiesel production and relates it to the prospects for a favorable process economy compared to current biodiesel processes.

2 Biodiesel fundamentals

Unmodified vegetable oils can be used as fuel in combustion engines. However, their high viscosity leads to poor atomization in the combustion chamber, and hence to operational problems such as engine deposits. Various approaches have been made to convert vegetable oils into fuels, including hydrogenation. The approach of transforming the oil to simple alkyl esters in a transesterification reaction with alcohols such as methanol or ethanol was early recognized [11]. The term biodiesel most often refers to such fatty acid alkyl esters. In fact, the biodiesel standards (DIN 51606, EN 14214, and ASTM D6751) require or indirectly specify that biodiesel should be fatty acid methyl esters (FAME). This is most likely dictated more by the technological feasibility of chemical production of esters rather than the properties of the biodiesel itself. Recently, also a "biofuel" prepared by partial enzymatic transesterification of glycerides has been suggested [12, 13]. The resulting mixture of fatty acid alkyl esters and partial glycerides is claimed to have good fuel properties, although it does not live up to the current biodiesel standards. In this review, we will use the term biodiesel broadly also for fatty acid esters of other small alcohols:

Glycerides + FFA + alcohol → fatty acid alkyl esters (biodiesel) + glycerol + water

Research into biodiesel started in the 1980's [14] and expanded rapidly in the 1990's. Commercial-scale production has been growing steadily since the late 1990's. Biodiesel is miscible with petrodiesel in all ratios and is today most often used in blends, such as B20 with 20% biodiesel.

2.1 Feedstocks

The choice of feedstock for today's commercial biodiesel plants depends largely on geography, with rapeseed oil dominating the EU production, soybean oil dominating the US and Latin American production, and palm oil mainly being used in Asia. The use of virgin, edible oils for fuel production is controversial, and with increasing prices, there is a growing interest in alternative feedstocks. This includes high-yielding, non-edible tropical crops such as *Jatropha curcas*. On a longer term, oil from marine microalgae has been proposed. Algae have oil productivities (L/ha/year) that far exceed that of any land-based crops, which is indisputably needed if we imagine biofuels to fully meet our global demand for transport fuels [15].

The composition of the feedstocks varies substantially, depending on source and refining. Typical compositions of oils and fats that can be used for production of biodiesel are presented in Table 1.

Virgin oils will easily serve as feedstock for an enzymatic biodiesel production. In fact, the literature describes enzymatic biodiesel synthesis from practically all the known plant oils (soybean, rapeseed, palm, sunflower, olive, peanut, *Jatropha*, *etc.*). However, for a first introduction of enzyme technology to the industry, it appears that the largest benefits can be obtained with low-cost, low-quality oils with high FFA content. With feedstocks such as animal fat (lard, tallow, poultry), used oils, acid oils (from soapstock splitting in chemical oil refining), and fatty acid distillates (from deodor-

Table 1. Composition (in wt-%) of biodiesel feedstocks [16].

	Rapeseed	Soybean	Palm	Tallow	Palm fatty acid distillate	Used cooking oil
Triglycerides	96.0	98.6	87.0	74.0	8.0	62.0
Diglycerides	2.0	0.8	6.0	12.0	5.0	16.0
Monoglycerides	0.5	0.1	2.0	4.0	2.0	7.0
FFA	1.5	0.5	5.0	10.0	85.0	15.0

izer/fatty acid stripping), enzymatic biodiesel production is sound from a technical, economical as well as sustainability point of view.

2.2 Alcohols

A range of alcohols as well as a few esters [17] have been investigated for biodiesel synthesis in the literature. Examples can be found for the use of all the smaller alcohols, *i.e.* methanol, ethanol, 1- and 2-propanol, and butanol isomers [18, 19]. Today, commercial biodiesel is FAME, *i.e.* made with methanol. Methanol has good reactivity in the alkaline synthesis reaction and, most importantly, it is in most regions the cheapest alcohol (Brazil and India being noteworthy exceptions). With increasing oil/gas prices and increasing production of bioethanol, the price structure could, however, change in the future. Part of the added cost of a heavier alcohol is also compensated for by the increased mass (and volumetric) gain of biodiesel. With ethanol *vs.* methanol, the increased weight is that of one methylene group, corresponding to approx. 5% of the biodiesel weight. Larger alcohols have certain advantages over methanol, especially in an enzymatic process. They have higher solubility in the oil and are more compatible with enzymes (methanol has a denaturing effect, *vide infra*). Furthermore, the larger alcohols are reported to yield biodiesel with superior low-temperature properties, *e.g.* lower crystallization temperature [19–21].

3 Enzymatic biodiesel production

A range of lipases and other esterases have been tested for enzymatic biodiesel synthesis. In nature, these enzymes hydrolyze triglycerides to FFA and partial glycerides. Mechanistically, a serine residue in the enzyme active site forms, as the first step, a covalent intermediate ester with the acyl donor. The second step is when water enters to hydrolyze the intermediate ester, thereby liberating the free enzyme and FFA. With other nucleophiles present, other reactions are possible. Hence, with an alcohol instead of water, a transesterification reaction is the result. This is utilized in enzymatic biodiesel production.

Furthermore, enzymes catalyze by nature both a "forward" ester hydrolysis reaction and a "reverse" condensation reaction (between a carboxylic acid and an alcohol). The equilibrium can be controlled by water and alcohol concentrations. Thereby, with a high alcohol/water ratio, lipases will condense FFA with alcohols to their ester product, *i.e.* biodiesel. This is fundamentally the key advantage of an enzymatic biodiesel process that triglycerides (and partial glycerides) as well as FFA can be efficiently transformed into biodiesel under the same mild conditions.

The *Candida antarctica* B-lipase (CALB) has been investigated extensively in biodiesel applications, though reports also describe benefits of other lipases, *e.g.* from *Candida rugosa* (CRL) [22], *Rhizomucor miehei* (RML) [2], *Burkholderia* (*Pseudomonas*) *cepacia* (PCL) [23, 24], and *Thermomyces lanuginosa* (TLL) [25]. Many of these enzymes are commercially available, also in immobilized form. Immobilized CALB, RML, and TLL are commercialized by Novozymes as "Novozym 435" (previously "SP435"), "Lipozyme RM IM", and "Lipozyme TL IM", respectively. Interestingly, it has been suggested that a mixture of two lipases with different substrate specificities (*e.g.* 1,3-specific and a non-specific lipase) will act synergistically [22]. Alternatively, one lipase could have high activity on FFA while the other has high activity on triglycerides. The CALB-TLL system seems to be an example of the latter (Fig. 1) [25–27]. Here, CALB very quickly esterifies FFA, but has relatively low activity on glycerides. TLL has the opposite preference.

From the numerous literature reports [2–6] describing biodiesel synthesis with a range of enzymes, using different feedstocks and alcohols under different conditions, it can be difficult to generalize on reaction conditions and the product quality obtained. Besides the lack of information on enzyme re-usability, academic studies frequently report long reaction times and a biodiesel product characterized only by its alkyl ester content. Several papers report FAME yields of >97%,

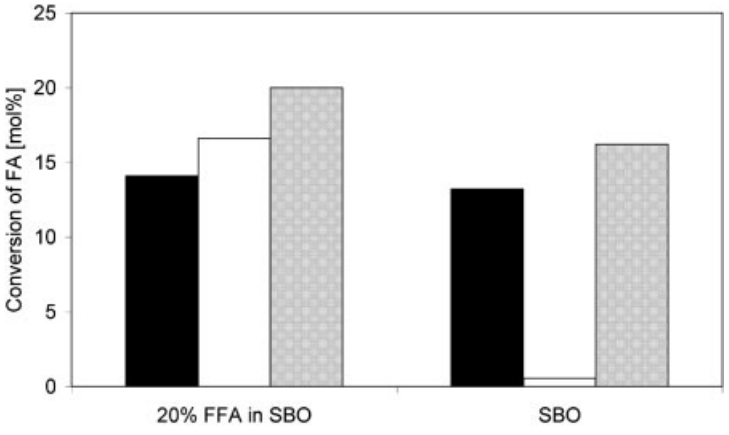

Figure 1. Synergistic effect of biodiesel formation from soybean oil (SBO) with and without added FFA when using two different lipases, alone or in combination [26]. Conditions: MeOH/FA = 0.44 (molar ratio), 30% water, 50 °C, 3 h, using liquid formulated enzymes. Black: TLL-type, 50 ppm; white: CALB, 50 ppm; gray: TLL-type + CALB, 25 + 25 ppm.

but otherwise do not discuss if the obtained product is within the given specifications (EN 14214). With low-quality oils (used cooking oil, *etc.*), a final distillation of the product will most likely be required. This is also often the case when such feedstocks are converted to biodiesel with chemical catalysis.

Beside the FFA content in the enzymatic biodiesel feedstock, it is important also to consider other components or impurities. This is especially the case with low-quality oils. However, also a common component such as phospholipids has been reported to inhibit lipase activity. This has been illustrated in experiments with three different qualities of soybean oil (crude, degummed, and refined) using immobilized CALB [28]. Hence, it is suggested that the raw material for enzyme-catalyzed biodiesel production should be degummed. Alternatively, enzymatic degumming and biodiesel synthesis can be performed in one process step [29].

3.1 Free enzymes

Examples can be found in the literature on the use of freeze-dried enzyme powder for biodiesel synthesis. It is, however, strongly recommended only to use such unformulated enzyme preparations with care and not in any larger scale due to safety concerns (enzyme dust is allergenic if inhaled). As an alternative, stabilized liquid enzyme formulations are commercially available. These contain an aqueous solution of the enzyme with added stabilizers to prevent enzyme denaturation (*e.g.* glycerol or sorbitol) as well as preservatives to prevent microbial growth (*e.g.* benzoate). An advantage of non-immobilized enzyme formulations is that the addition of an extra (solid) phase to the reaction system is avoided. This is important as the biodiesel reactants often already exist as two immiscible phases, and multiple phases in general result in slower reactions. The carrier itself, as well as the immobilization process, adds significantly to the cost of immobilized enzymes. Hence, free enzymes are significantly cheaper than their immobilized counterparts. They are, however, still considerably more expensive than the chemical catalysts used for biodiesel production. Therefore, to be competitive, enzyme re-use is a necessity.

When free enzymes are used in a biodiesel process, the enzymatic activity can be partially recovered in the glycerol phase. However, the build-up of glycerol limits the possible number of re-uses. A counter-current design of oil and glycerol phase has been suggested as a solution to this (Fig. 2) [26]. In summary, good yields are reported from literature examples describing the use of free enzymes, but all seem to be based on single-use batch experiments with no attempts to recover the enzyme activity [24, 30, 31]. This proves that commercial-scale biodiesel production with free enzymes could become reality, but today a "turn-key solution" does not exist.

3.2 Immobilized enzymes

The use of immobilized enzymes in oil and fat processing is experiencing significant growth due to new technology developments that have enabled cost-effective interesterification of triglycerides (to modify melting properties) for margarine and shortenings [32]. A fundamental advantage of immobilized enzymes is that they can be recovered and re-used from a batch process by simple filtration. Further, packing of immobilized enzymes in columns allows for easy implementation of a continuous process. Immobilizing enzymes generally also has a positive effect on the operational stability of the catalyst (compared to free enzymes), it makes handling easier (compared to free enzyme powder), and it allows operation under low-water conditions (compared to liquid formulated enzymes). The critical issues of water content, temperature stability, stability towards alcohols, use of solvents, and glycerol build-up are discussed below in relation to enzymatic biodiesel production with immobilized enzymes.

3.2.1 Water content

Low water content in the oil phase is required to drive the biodiesel reaction to high conversion. Water removal is, however, a balance, as very low water activity will inactivate the enzyme catalyst (CALB has been shown to work under much lower water concentrations than other lipases) [33]. The

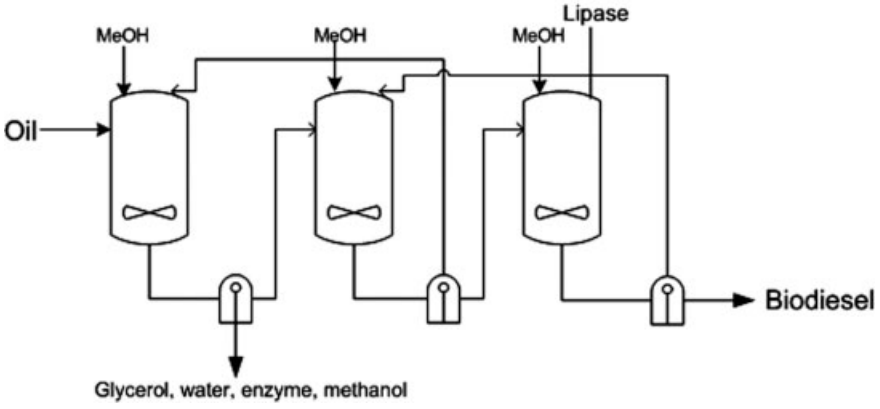

Figure 2. Counter-current process for the production of biodiesel using liquid lipase formulations and stepwise methanol addition [26].

importance of removing water during esterification of high-FFA feedstock was demonstrated in a novel approach by Watanabe and coworkers [34]. In this experiment, glycerol was added to absorb water from the esterification of an acid oil hydrolysate with methanol. In different experiments, the positive effect of adding molecular sieves for water removal has been demonstrated by Du *et al.* who esterified soybean oil deodorizer distillate with methanol and achieved significantly higher conversion rates compared to control experiments without water elimination [35]. Also Li and coworkers found reduced performance with higher content of water in oils (refined rapeseed oil and used cooking oil) in experiments using a combination of CALB and TLL lipases with *t*-butanol as solvent [25]. Elimination of the water in this process significantly increased conversion.

3.2.2 Destabilizing effect of alcohols

Immobilized lipases are, in general, rather thermostable in oils. The commercial process for enzymatic interesterification is generally performed at 70 °C [32]. Short-chain alcohols, however, have a negative impact on the stability of the lipases, and this destabilizing effect increases with increasing temperature. Watanabe and coworkers demonstrated the negative effect of methanol, ethanol and *n*-propanol at different temperatures for immobilized CALB [28, 36]. It was shown that when using methanol above 30 °C, the enzyme was inactivated, while ethanol and especially *n*-propanol gave better results. The destabilizing effect of alcohols on lipases hence seems to decrease with increasing alcohol molecular weight. The apparent connection between the solubility of the alcohol in oil and the destabilizing effect of the oil has been noted by several groups, and was recently illustrated by Jachmanián *et al.* with phase diagrams for the biodiesel reaction system components (oil, alcohol, alkyl ester, glycerol) [37]. The solubility of methanol in oil corresponds to approx. 1/3 equivalent (1/3 the amount needed to esterify all fatty acids) and detailed schemes have been developed for stepwise addition of methanol to the reaction mixture [4].

3.2.3 Solvents

There are several examples on the use of organic solvents in biodiesel synthesis reactions. Oliveira *et al.* used hexane in the synthesis of ethyl esters with CALB and RML [38]. Recently, *t*-butanol has been explored as solvent by Du *et al.* [25, 39] as well as by Royon *et al.* [40]. Due to steric hindrance, this alcohol is not accepted by the lipases in the esterification reaction, and as a solvent it has the ability to dissolve oil, alcohol and glycerol at the relevant concentrations and temperatures. Solubilization of methanol has a positive effect on the enzyme stability, and solubilization of glycerol prevents deposition on the enzyme carrier material, which in several examples has been reported to limit the possible number of re-uses of immobilized enzymes [41]. Du *et al.* demonstrated the significant variation in lipase stability when using different solvents, with *t*-butanol being superior [39]. For industrial-scale production, it should, however, be taken into consideration that, even if solvents have benefits, it will be a solution that introduces other problems like reduction of capacity (as the solvent takes up volume), environmental issues (toxicity, emissions) and costs (recovery and losses). These negative issues have to be balanced with the positive effects.

4 Enzymatic biodiesel process design considerations

The process setup is very important as it has to take into account the discussed technical issues, *e.g.* homogeneity of reaction/product mixture, solubility of alcohol, stability of enzyme, recovery of enzyme, *etc.* There are several different process designs to be considered: batch, continuous stirred-tank reactors and packed-bed reactors. These will briefly be outlined in the following paragraphs.

4.1 Batch process

The batch process is a typical process used in the laboratory due to the simple setup. The process can be operated with the addition of all components from the start, whereas stepwise addition of especially methanol is recommended. The batch process is useful to collect data about the process, as for instance productivity of the enzyme. Negative elements of this process setup in large scale are the large tank volume required, the long reaction time, and the fact that the process is not continuous. Another very important fact to consider is the gradual decline in enzyme activity as the number of re-uses increases. When the enzyme activity decreases, the reaction time must be increased accordingly, to keep a constant high degree of conversion. With time, the capacity of the plant will decrease and eventually become unacceptably low. That is when the enzyme is replaced. The difficult decision is the compromise between capacity and cost of catalyst. It is hard to imagine a plant allowed to operate down to 20% enzyme activity, which might be required to reach the necessary productivity.

4.2 Continuous stirred-tank process

A continuous stirred-tank reactor is a container with a continuous supply of feed and withdrawal of product. The design requires multiple tanks in series to assure the same degree of conversion for the same reaction time, meaning the total tank volume will also be large. The advantage of such a system is that the capacity of the plant can be more constant as the tanks can hold enzymes of different age/activity. This also implies that the enzyme can be used more effectively until the activity has become very low. Another advantage of this design is the possibility of introducing separation steps between the tanks to eliminate the glycerol formed. This was suggested by Chris-

tensen *et al.* in the counter-current continuous stirred-tank reactor setup using liquid formulated enzyme (*vide supra*) [26].

4.3 Packed-bed columns

A system of packed-bed columns with immobilized enzymes results in a well-defined contact time between the liquid reactants and the solid catalyst. Furthermore, with this setup the enzyme-to-substrate ratio will be high at any specific time, and the whole system can be designed to be relatively compact. Commercial-scale precedence for this technology already exists for enzymatic interesterification of oils [32]. For enzymatic biodiesel (FAME) production, Shimada and coworkers have outlined a packed-bed design and discussed how to solve some of the technical issues with the technology [4]. Hence, the issue with inactivation of the enzyme by addition of alcohol in concentrations higher than the solubility was solved by stepwise addition before each column. In a similar way, the glycerol produced in the reaction is removed between the columns (Fig. 3).

5 Processing economy

As reviewed, most of the literature investigates immobilized enzymes due to the easier handling and re-use. On the other hand, as mentioned, the immobilized products have a significantly higher price per "activity unit" compared to liquid products. It is difficult to make general comparisons between cost prices of liquid formulated *vs.* immobilized enzymes, as this will depend very much on the cost price of the immobilization. The immobilized lipase that has been extensively used (Novozym 435) has a high price per kilogram, meaning that a very high productivity is required for the process to be cost effective. One the other hand, new immobilization technology resulted in a much lower selling price for the immobilized lipase that was recently successfully introduced for interesterification. If the immobilization technology can be leveraged into enzymatic biodiesel production, it gives promise of more cost-effective immobilized enzymes also for this industry.

As discussed, the most critical issue for the application of enzymes in biodiesel production is the cost of the catalyst. A few studies in the literature present data that can be used for the calculation of the productivity (kg biodiesel/kg enzyme) (Table 2). The studies are based on repeated usage of the enzyme where the activity is still preserved after a high number of re-uses and the calculated productivities should therefore be seen as minimum numbers. From these data, a maximum price of the enzyme can be calculated, provided that the catalyst cost should be the same as when using chemical catalysis, *i.e.* 25 USD/ton biodiesel. It appears that enzyme prices from 12 to 185 USD/kg can be accepted, depending on the

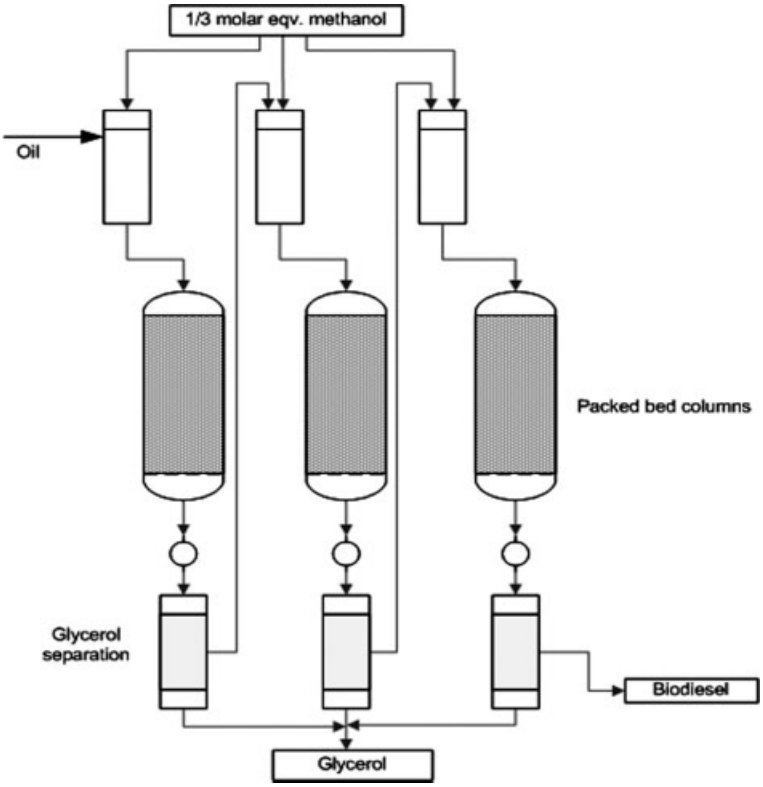

Figure 3. Packed-bed process for the production of biodiesel using stepwise addition of methanol and separation of glycerol between columns [4].

Table 2. Collection of studies from which productivity (kg biodiesel/kg enzyme) can be calculated. The data are used to calculate the maximum enzyme cost for breakeven with the cost of chemical catalysts in biodiesel production.

Reference	Oil	Enzyme	Yield [%]	Productivity [kg biodiesel/kg enzyme]	Calculated[§] max. enzyme cost [USD/kg enzyme]
[42]	soy	CALB	>96	1200	30
[43]	soy	CALB	>97	470	12
[40]	cottonseed	CALB	95	2000	50
[44]	soy	CALB	>70	5400	135
[25]	rapeseed	CALB + TLL	95	4250	106
[45]	acid oil	CALB	>71	7400	185
[45]	acid oil	CALB	>90	1700	43

[§] Productivity (kg biodiesel/kg enzyme) multiplied with a catalyst cost of 0.025 USD/kg biodiesel.

productivity in the application. When comparing these calculated enzyme prices to the price of the immobilized lipase for interesterification, it falls within a similar range.

Of course, a full economic analysis of enzymatic *vs.* chemical catalysis for biodiesel production would require a series of assumptions and is outside the scope of this review. Both capital and operating costs will depend highly on the chosen process design and its implications on purification steps, *etc*. However, in general terms, processing costs will be a function of factors such as [46]:

- cost of oil; usage of lower-cost high-FFA oil can dramatically impact the overall economy
- cost of alcohol
- cost of pre-processing steps
- process yield
- cost of waste product handling
- value of glycerol stream
- cost of post-treatment stages

As outlined in the introduction, enzyme technology will positively impact many of these factors.

Overall, the increased raw material opportunities offered by enzyme technology, combined with the recent advancements in enzyme application technologies and the significant reduction of immobilized enzyme cost, due to the use of inexpensive support materials, are factors likely to make enzymatic biodiesel production commercially viable.

6 Conclusions

The desire to develop renewable fuels and to become less dependent on fossil resources has led to a need of research in biodiesel production, including the possibility to use enzymes as catalyst rather than chemicals. Many papers over the last 10 years have discussed the technical issues in using lipases and concluded that one major problem for successful introduction to the market has been the relatively high price of the enzymes. This price has been dictated by the production costs of the lipases and especially the immobilization costs. However, it now seems possible that new immobilization technology can change the situation and bring enzyme costs down to a level comparable to chemical catalysis costs, *i.e*. 25 USD/ton biodiesel produced. It must be emphasized that there still is work to be done with respect to up-scaling the process layout from laboratory or pilot scale to production. In this respect, the use of immobilized enzymes with packed-bed technology seems to hold considerable potential.

Conflict of interest statement

The authors have declared no conflict of interest.

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