Review Article

Production and Use of Lipases in Bioenergy: A Review from the Feedstocks to Biodiesel Production

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Received 30 January 2011; Accepted 28 April 2011

Academic Editor: Francisco Gírio

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Lipases represent one of the most reported groups of enzymes for the production of biofuels. They are used for the processing of glycerides and fatty acids for biodiesel (fatty acid alkyl esters) production. This paper presents the main topics of the enzyme-based production of biodiesel, from the feedstocks to the production of enzymes and their application in esterification and transesterification reactions. Growing technologies, such as the use of whole cells as catalysts, are addressed, and as concluding remarks, the advantages, concerns, and future prospects of enzymatic biodiesel are presented.

1. Lipid Feedstocks

The main feedstocks which present paramount importance for the application of lipases are fats and oils. Such materials are primarily composed of triglycerides, which are glycerol esters with saturated and unsaturated fatty acids, from vegetable, animal, or microbial origins. One of the distinguishable characteristics between fats and oils is the occurrence of unsaturated and saturated fatty acids in the triglycerides: higher saturated fatty acids content (as examples in Figure 1), higher melting point, and the presence of remaining solids at room temperature are characteristics of a fat; on the other hand, oils usually present higher occurrence of unsaturated fatty acids, remaining in liquid state at room temperature. In addition to triglycerides, vegetable oils can present di- and monoglycerides, free fatty acids (FFAs), phosphatides, and unsaponifiable matter, such as carotenoids, phytosterols, tocopherols, chlorophyll, triterpenic alcohols, and hydrocarbons [1–4].

The role of fats and oils in plants is related to energy reserve, regarding their occurrence in seeds, and protection against water loss (by wax formation) and against mechanical injuries (by hormone generation), when such components appear in the leaves and fruits [2, 5].

Worldwide production of fats and oils was estimated in 174.6 million tons for the season 2010/2011. From that, 86% represent vegetable oils (Table 1), with soybean, palm, rapeseed, and sunflower seed as the major resources [6, http://lipidlibrary.aocs.org/, 2011]. In Brazil, some oilcrops, such as castor bean (Ricinus communis), jatropha (Jatropha curcas), crambe (Crambe abyssinica), macaw palm (Acrocomia aculeata), and oiticica (Licania rigida), have been explored as alternatives for biodiesel production due to their high tolerance to drought and frost, higher productivity on low-fertility soils, and great potential for the sustainable development of Brazilian Northeast [7, http://www.ruralbioenergia.com.br/, 2009]. Moreover, the use of raw materials with appropriated physicochemical characteristics and widely available enables cost reduction for the production of the biofuel, since the feedstock cost represents 70–88% of the final price of biodiesel [8].

For the selection of a proper raw material for use as substrate for the production of biodiesel, some aspects should be observed, such as the following.
Table 1: World oilcrops distribution [6].

| Fats and oils       | World production (million tons) | Five major producers                                                                 |
|---------------------|---------------------------------|---------------------------------------------------------------------------------------|
| Animal fat          | 24.4                            | USA, China, Brazil, Germany, and France                                               |
| Coconut oil         | 3.7                             | Philippines, Indonesia, India, Vietnam, and Mexico                                      |
| Cottonseed oil      | 4.8                             | China, India, Pakistan, Uzbekistan, and USA                                           |
| Groundnut oil       | 5.3                             | China, India, Nigeria, Myanmar, and Sudan                                            |
| Linseed oil         | 0.6                             | China, Belgium, USA, Ethiopia, and India                                              |
| Maize oil           | 2.3                             | USA, China, Japan, Brazil, and South Africa                                          |
| Olive oil           | 2.9                             | Spain, Italy, Greece, Syrian Arab Republic, and Tunisia                               |
| Palm kernel oil     | 5.6                             | Indonesia, Malaysia, Nigeria, Thailand, and Colombia                                   |
| Palm oil            | 23.9                            | Malaysia, Nigeria, Thailand, Colombia, and Côte d’Ivoire                                |
| Rapeseed oil        | 21.2                            | China, Germany, India, Canada, and France                                            |
| Safflower oil       | 0.1                             | India, USA, and Argentina                                                            |
| Sesame oil          | 0.9                             | Myanmar, China, India, Sudan, and Japan                                               |
| Soybean oil         | 36.0                            | USA, China, Brazil, Argentine and India                                              |
| Sunflower oil       | 13.0                            | Russian Federation, Ukraine, Argentine, Turkey, and France                            |

Table 2: Nomenclature of fatty acids [1, 9].

| Common name                  | Systematic name                  | Chemical structure\(^{1}\) | Melting point (°C) |
|------------------------------|----------------------------------|-------------------------------|--------------------|
| Lauric acid                  | Dodecanoic acid                  | 12 : 0                        | 44.2               |
| Miristic acid                | Tetradecanoic acid               | 14 : 0                        | 54.4               |
| Palmitic acid                | Hexadecanoic acid               | 16 : 0                        | 62.9               |
| Palmitoleic acid             | 9-Hexadecenoic acid             | 16 : 1                        | −0.1               |
| Stearic acid                 | Octadecanoic acid               | 18 : 0                        | 70.1               |
| Oleic acid                   | 9-Octadecenoic acid             | 18 : 1                        | 16.3               |
| Elaidic acid                 | 9-Octadecenoic acid             | 18 : 1                        | 43.7               |
| Vaccenic acid                | 11-Octadecenoic acid            | 18 : 1                        | 44.0               |
| Linoleic acid                | 9, 12-Octadecadienoic acid      | 18 : 2                        | −6.5               |
| γ-Linolenic acid             | 6, 9, 12-Octadecatrienoic acid  | 18 : 3                        | −11.0              |
| α-Linolenic acid             | 9, 12, 15-Octadecatrienoic acid | 18 : 3                        | −12.8              |
| Arachidic acid               | Eicosanoic acid                 | 20 : 0                        | 76.1               |
| Gadoleic acid                | 9-Eicosenoic acid               | 20 : 1                        | 25.0               |
| Arachidonic acid             | 5, 8, 11, 14-Eicosatetraenoic acid | 20 : 4                   | −49.5              |
| Behenic acid                 | Docosanoic acid                 | 22 : 0                        | 80.0               |
| Erucic acid                  | 13-Docosenoic acid              | 22 : 1                        | 33.4               |

\(^{1}\) x : y nomenclature, where x represents the total number of carbon atoms and y represents the number of unsaturated bonds.

Figure 1: Schematic representation of a triglyceride with saturated fatty acids.

1.1. Fatty Acids Profile. The fatty acids profile varies greatly between fats and oils and can be referred by distinct nomenclatures (Table 2). It can comprise from high concentration of saturated fatty acids, like in palm seeds, such as coconut (Cocos nucifera), palm kernel (Elaeis guineensis), and babassu (Orbignya oleifera) (Table 3), as well as animal fats (Table 4), to high content of monounsaturated fatty acids, commonly in oleaginous fruits (Table 5). Certainly, there are some exceptions of typical profiles, such as castor bean oil, which has a high content of ricinoleic acid; crambe, with high quantity of erucic acid; palm, with similar quantities of saturated and unsaturated fatty acids (Table 3) [9].

Additionally to the vegetal and animal sources of lipids and fats presented in Tables 3–5, fatty acids can also come from microbial origin. As recently revised by Li et al. [21], yeasts from Cryptococcus, Lipomyces, Rhodospiridium, Rhodotorula, Trichosporon, and Yarrowia genera, as well as filamentous fungi and bacteria, can reach 53% of lipids content in its dry mass, with evidence for major appearance of palmitic and oleic acids. In another work, the profiles of fatty acids of the microalgae Spirulina sp., Scenedesmus
Table 3: Fatty acids profile of oilcrops [1, 9].

| Fatty acids1 | Palm kernel | Soybean | Jatropha curcas | Crambe | Rapeseed | Sunflower | Castor bean | Babassu |
|--------------|-------------|---------|----------------|--------|----------|-----------|-------------|---------|
| 12 : 0       | 41–55       | NR      | NR             | NR     | NR       | NR        | NR          | 40–55   |
| 14 : 0       | 14–18       | 7–14    | 10–17          | 1.8–2.0| 2.5–6.5  | 3.0–10.0  | 1.1         | 5.2–11.0|
| 16 : 0       | 6.5–10.0    | NR      | <0.5           | NR     | <0.6     | <0.1      | 0.2         | NR      |
| 18 : 0       | 1.3–3.0     | 1.4–5.5 | 5–10           | 0.7–1.0| 0.8–3    | 1–10      | 1           | 1.8–7.4 |
| 18 : 1       | 12–19       | 19–30   | 36–64          | 16.0–17.2| 53–70  | 14–35     | 3.33        | 9–20    |
| 18 : 2       | 1–3.5       | 44–62   | 18–45          | 8.0–8.7| 15–30    | 55–75     | 3.6         | 1.4–6.6 |
| 18 : 3       | NR          | 4–11    | NR             | 5.2–7  | 5–13     | <0.3      | 0.32        | NR      |
| 20 : 0       | NR          | <1.0    | NR             | 3.4    | 0.1–1.2  | <1.5      | 0.4         | NR      |
| 20 : 1       | NR          | <1.0    | NR             | 0.1–4.3| <0.5     | <0.9      | NR          | NR      |
| 22 : 0       | NR          | <0.5    | NR             | NR     | <0.6     | <1.0      | NR          | NR      |
| 22 : 1       | NR          | NR      | NR             | 56–66  | 0.7      | NR        | NR          | NR      |
| % oil        | 45–50       | 18–20   | 26–35          | 35–60  | 40–50    | 22–36     | 35–55       | 65–68   |

1 x : y nomenclature, where x represents the total number of carbon atoms and y represents the number of unsaturated bonds; NR: not reported.

Table 4: Fatty acid profile from animal origin [9].

| Fatty acids | Butter | Lard | Tallow |
|-------------|--------|------|--------|
| <14 : 0     | 11.0–23.8| 0.5 | 0.9    |
| 14 : 0      | 8.2–12.0| 1.3 | 3.0–3.7|
| 16 : 0      | 21.3–29.0| 23.8–25.0| 24.9–27.0|
| 18 : 0      | 9.8–13.0| 12.0–13.5| 7.0–18.9|
| 16 : 1      | 1.8–2.0| 2.7–3.0| 4.2–11.0|
| 18 : 1      | 20.4–28.0| 41.2–45.0| 36.0–48.0|
| 18 : 2      | 1.8 | 10.0–10.2| 3.1   |
| 18 : 3      | 1.2 | 1.0 | 0.6    |
| % fat       | 2–5   | 70–95| 70–95   |

These diversified profiles of fatty acids from different origins contribute for the generation of biofuels with different properties. For example, the higher the size of fatty acid hydrocarbon chain, the higher the cloud point and the cold filter plugging point. Therefore, due to the necessity of heating before ignition, it becomes difficult the use of a biodiesel with such characteristic in regions with low environment temperature.

Another factor concerning the use of unsaturated fatty acids for the production of biodiesel is that the fewer the double bonds in the molecules, the higher the cetane number of the biofuels (which, in turn, means a better quality of their combustion). Moreover, larger quantities of unsaturated bonds turn molecules more chemically unstable. This can cause some inconvenience due to the biofuel oxidation, degradation, and polymerization (resulting in low cetane number or formation of solid residues), if improperly stocked or transported. Then, in general, a biodiesel with high quantities of esters derived from monounsaturated fatty acids (e.g., oleic or ricinoleic acids) presents better results as a fuel [23].

Table 5: Fatty acids profile of oleaginous fruits.

| Fatty acids1 | Buriti | Olive | Avocado | Palm |
|--------------|--------|-------|---------|------|
| 12 : 0       | NR     | NR    | NR      | 0.1–1.0|
| 14 : 0       | 0.1    | 0.7   | <0.13   | 0.9–1.5|
| 16 : 0       | 17.3–19.3| 10–11.7| 19.8–22.7| 41.8–46.8|
| 18 : 0       | 1.9–2.0| 2.1 | 0.5–1.0 | 4.2–5.1|
| 20 : 0       | NR     | 0.48  | NR      | 0.2–0.7|
| 16 : 1       | NR     | 1.45  | 3.9–5.6 | 0.1–0.3|
| 18 : 1       | 73.3–78.7| 73.8–78 | 60–71 | 37.3–40.8|
| 18 : 2       | 2.4–3.9| 7.0–9.8| 7.1–15.3| 9.1–11.0|
| 18 : 3       | 2.2    | NR    | 0.4–1.0 | <0.6   |
| % oil        | 8–18   | 15–40 | 4–25    | 20–24  |

1 x : y nomenclature, where x represents the total number of carbon atoms and y represents the number of unsaturated bonds; NR: Not reported.

References [10, 11, 12].

1.2. Fats and Oils Processing. Animal fat processing is named rendering, where carcasses with fatty material are heated with hot water or steam to release fats, with subsequent separation by centrifugation or by surface removal. The vegetable oil processing is comprised of some steps, including mechanical pretreatment (cleaning, sorting, and comminution), heating, dehydration, mechanical pressing and/or solvent extraction, miscella distillation, meal desolventization, and refining [1, 9].

For biodiesel production, the oil refining processes play an important role in the yield of the conversion steps, since oil impurities, such as water, phosphatides, and pigments, can affect the conversion of triglycerides to esters due to excessive emulsification of the reaction mixture and difficulties in biodiesel separation, amongst others [1, 9].

obliquus, Chlorella vulgaris, and C. kessleri were determined [22]. The authors observed the prevalence of saturated fatty acids (lauric, miristic, palmitic, and stearic acids), with contributions of up to 46% for the total fatty acids content.
Table 6: Insight into recent literature on microbial lipase production.

| Microorganism       | Raw material                          | Type of fermentation | Maximum activity (time of fermentation) | Reference |
|---------------------|---------------------------------------|----------------------|-----------------------------------------|-----------|
| A. niger 11T53A14   | Wheat bran                            | SSF                  | 62.7 U·g^-1 (48 h)                      | [13]      |
| Penicillium sp.     | Olive oil                             | SmF                  | 21.0 U·mL^-1 (120 h)                    | [14]      |
| Rhizopus oryzae NRRL 3562 | Coconut oil                           | SSF                  | 96.2 U·g^-1 (115 h)                     | [15]      |
| Bacillus subtilis OCR-4 | Ground nut oil cake                   | SSF                  | 4.5 U·g^-1 (48 h)                       | [16]      |
| Burkholderia cepacia LTEB11 | Sugarcane bagasse and sunflower seed meal | SSF | 234 U·g^-1 (96 h)                      | [17]      |
| Rhizopus chinensis  | Wheat bran, wheat flour, and olive oil | SSF                  | 24.4 U·g^-1 (72 h)                      | [18]      |
| Pseudozyma hubeiensis HB85A | Soybean oil                       | SmF                  | 5.3 U·mL^-1 (18 h)                      | [19]      |
| P. chrysogenum     | Grease waste and wheat bran           | SSF                  | 46 U·mL^-1 (168 h)                      | [20]      |

SSF: Solid-state fermentation; SmF: Submerged fermentation.

Another important factor during feedstock processing is the valorization of coproducts. Such approach can contribute to the profits of an industrial plant, thus bettering the viability of biodiesel. As a classical example, soybean meal generated during soybean oil extraction is already used for protein and isoflavones extraction [24], and its main phospholipid, lecithin, separated in the degumming step, is used as natural emulsifier [25].

2. Enzyme Production and Characteristics

Lipases are enzymes classified as hydrolases (glycerol ester hydrolase, E.C. 3.1.1.3) and act on ester bonds of several compounds, with acylglycerols being the most proper substrates, catalyzing reactions of hydrolysis, synthesis, and trans- and interesterification (Figure 2). Lipases are more active in insoluble substrates, especially triglycerides made of long-chain fatty acids with over 10 carbon atoms, while esterases are active in soluble substrates, especially simple esters, such as ethyl acetate and triglycerides made of short-chain fatty acids with less than six carbon atoms. Esterases follow Michaelis-Menten kinetics, while lipases need a minimum substrate concentration to show high activity levels [26].

Due to the similarity of the catalytic triad found in lipases compared to those observed in serine proteases, the most widely accepted hypothesis is that the mechanism of lipase catalysis is similar to that of serine protease catalysis [27]. It is believed that the kinetic mechanism of lipases does not depend on the type of reaction being catalyzed (hydrolysis, acidolysis, transesterification, etc.).

The reaction begins with a nucleophilic attack on the carbon from the ester bond of the susceptible substrate by hydroxyl group in the serine residue of the active site, forming an acyl-enzyme complex and releasing alcohol from the lipid. Later, the acyl-enzyme complex is hydrolyzed, releasing the lipase regenerated. Figure 3 shows the stages of the reaction catalyzed by the lipase and its intermediates.

Furthermore, characteristics such as stability in the presence of organic solvents, no necessity of cofactors for their action and high enantioselectivity, turn lipases into a group of enzymes with one of the major technological interests [28–30].

Lipases occur widely in nature and can be produced by many microorganisms and higher eukaryotes. In animals, lipases obtained from pig and human pancreas are best known and more investigated than all other lipases. In these organisms, they are engaged in several lipid metabolism steps, including fat digestion, adsorption, reconstitution, and in lipoproteins metabolism. In plants, lipases are present in higher plants seeds, as castor bean and canola (Brassica napus). They are also found in several plants’ energy reserve tissues [28, 31–33]. However, for the production of industrial enzymes, microorganisms are the preferred source, once they have shortest generation time, high yield of conversion of substrate into product, great versatility to environmental conditions and, simplicity in genetic manipulation and in cultivation conditions. Due to habitats’ multiplicity, microorganisms usually produce various lipases types, with distinct specificity regarding to substrate utilization and also to optimum pH and temperature range. Lipases can be produced by bacteria, filamentous fungi, and yeasts, allowing these microorganisms to use lipids from animal or vegetable origin as carbon and energy sources for their growth. Though many microorganisms have been reported in literature as lipase producers, the genera Candida, Rhizopus, and Pseudomonas are considered the main industrial sources of lipases. The yeast Candida rugosa is the most employed microorganism for lipase production [30]. Table 6 gives an overview on recent literature regarding lipases production.

The use of lipases in industry is still limited by the cost of commercial enzymes, especially when large quantities of enzyme are required and when the final product is of low added value. There is therefore a considerable interest in reducing the cost of producing these biocatalysts. The use of solid-state fermentation (SSF) as a production system is one way of reducing enzyme production costs, especially because agroindustrial waste can be used as a culture medium.

A comparative economic analysis showed that the production of lipase from Penicillium restrictum by SSF is more economically feasible than its production by submerged
Other advantages of producing enzymes by SSF have been highlighted alongside the reduced production costs. In studies of lipase production by the fungus *Penicillium restrictum* using SSF and SmF, different significant physiologies were observed between the two systems when simple (oleic acid and glucose) and complex (olive oil and starch) sources of carbon were used, with a reduction in catabolite repression being observed for SSF [55].

Lipases from different microorganisms have been produced using SSF with different solid wastes, such as lipase from *Penicillium restrictum* in babassu cake [55, 56]; lipase from *P. simplicissimum* in babassu cake, soybean cake, and castor bean cake [57–61]; lipase from *Candida rugosa* in rice flour [62]; lipase from *Rhizopus homothallicus* in sugarcane bagasse [63, 64]; lipase from *Aspergillus niger* in wheat bran and sesame seed cake [65, 66]; lipase from *Rhizopus rhizopodiformis* and *Rhizomucor pusillus* in olive oil cake and sugarcane bagasse [67]; lipase from *Rhizopus oligosporus* in a variety of cakes [68]. These lipases were produced by SSF on a bench scale, mostly using tray bioreactors, and yielded high productivity rates.

**Table 7**: Sources of lipases and optimal conditions for their action.

| Sources                        | pH   | T (°C) | Reference   |
|--------------------------------|------|--------|-------------|
| *Candida rugosa*               | 5–8  | 35–50  | [34]        |
| *Pseudozyma antarctica A*      | 6–10 | 35–70  | [35]        |
| *Thermomyces lanuginosus*      | 6–9  | 30–50  | [36]        |
| *Aspergillus niger*            | 6–8  | 40–55  | [37]        |
| *Pseudomonas aeruginosa*       | 5.5–7.5 | 35–45 | [38]        |
| *Bacillus subtilis*            | 8–10 | 30–40  | [39]        |
| *Geotrichum candidum*          | 6.5–8.0 | 32–42 | [40, 41]    |
| *Streptomyces rimosus*         | 8.5–10.0 | 45–60 | [42]        |
| *Yarrowia lipolytica*          | 4–7  | 30–45  | [43–45]     |
| *Rhizopus niveus*              | 5–7  | 30–45  | [46]        |
| *Rhizomucor miehei*            | 6.5–7.5 | 30–40 | [47]        |
| Porcine pancreatin              | 6–9  | 40–55  | [48]        |
| Castor bean (*Ricinus communis*) | 4.0–4.5 | 30–35 | [49, 50]    |

Fermentation (SmF), with a production cost for the former being found to be 68% lower and a payback time of 1.5 years [54].

![Figure 2: Reactions catalyzed by lipases.](image-url)
There are no pre-established procedures in the literature for predicting the performance and design of SSF bioreactors. For this reason, large-scale systems have generally been developed from the results obtained from bench-scale or pilot systems. Ideally, a large-scale system should operate in the same way and with the same performance as a bench-scale system although this is often not the case for SSF processes [69]. The main limiting factor on scaling up such processes is heat transfer, which depends on the stage of fermentation, and the design and operation mode of the bioreactor [70–72]. Some mathematical models have been developed to describe the growth kinetics of the microorganisms under different operating conditions and to describe heat and mass transfer in tray bioreactors [73], fixed-bed bioreactors [70, 74], rotating drum bioreactors [75, 76], shaking reactors [77], and fluidized bed reactors. These models could be used as inputs for designing the scale-up of such systems.

In addition to the reduction of the costs related to fermentation step for industrial-scale production of lipases, the strategies to recover and purify lipases must also be as easy to scale up. Ideally, a large-scale system should ideally be easy to scale up.

Lipases are enzymes that are known to be strongly hydrophobic, because of the presence of alkyl groups on the surface of their structure [30]. Generally, a good first step for lipase purification is the use of hydrophobic–interaction chromatography. Normally, pre-purification involves precipitation with ammonium sulphate, and ion-exchange chromatography and gel filtration are also widely used [78–80].

Rua et al. [79] studied the production and purification of a thermostable alkaline lipase from Bacillus thermocatenuatus in Escherichia coli. The purification stages were done in butyl sepharose (hydrophobic bed) and TSK G3000 (gel filtration), giving a purification factor of 125 and a yield of 32%.

A lipase from Aspergillus niger F044 was purified by precipitation with ammonium sulphate, DEAE-Sepharose FF (ion exchange), and Sephadex G-75 (gel filtration). A yield of 33% was obtained, while the purification factor was 73 [37].

A lipase from Penicillium simplicissimum produced by submerged fermentation was purified in a five-step process [81]. First, the culture was concentrated using a 10 kDa membrane, then it was precipitated with ammonium sulphate. After concentration and pre-purification, the sample was injected in sequential chromatography steps on phenyl sepharose CL-4B (hydrophobic interaction), Ultrogel AcA-54 (gel filtration), and hydroxyapatite (ion exchange). The resulting purification factor was 788, and the yield was 20%.

In order to purify a lipase from Penicillium camemberti U-159, Isobe et al. [82] used ethanol precipitation and ammonium sulphate precipitation as the first and second steps. A sequence of chromatography steps followed, using DEAE-sepharose (ion exchange), amino octyl sepharose (hydrophobic interaction), hydroxyapatite (ion exchange), and concanavalin-A sepharose (affinity). The yield obtained was 27%, and the purification factor was 213.

Cunha et al. [83] studied the purification/immobilization of a “pool” of lipases from P. simplicissimum produced by SSF using babassu cake as a culture medium. The process undertaken by means of sequential immobilization in hydrophobic supports (butyl, phenyl, and octyl agarose) resulted in three fractions with distinct thermal stability, specificity, and enantioselectivity properties.

Depending on the source, lipases can present molar mass ranging from 20 to 75 kDa, enzymatic activity at pH between 4 and 9 and at temperatures since 27 until 70°C. Lipases

### Table 8: Reported conditions for enzymatic transesterification of some fats and oils [51–53].

| Alcohol      | Lipase source          | Feedstock      | Solvent      | Yield (%) |
|--------------|------------------------|----------------|--------------|-----------|
| Methanol     | *C. antarctica*        | Rapeseed oil   | Hexane       | 98        |
| Methanol     | *C. antarctica*        | Cottonseed oil | —            | 92        |
| Methanol     | *C. antarctica*        | Cottonseed oil | t-Butanol    | 97        |
| Methanol     | *C. antarctica*        | Degummed Soybean oil | —      | 94        |
| Methanol     | *T. lanuginosus*       | Soybean oil    | —            | 90        |
| Ethanol      | *P. cepacia*           | Tallow fat     | —            | 95        |
| Propanol     | *P. fluorescens*       | Sunflower oil  | 1,4-Dioxane  | >95       |
| 2-Ethyl-1-hexanol | *C. rugosa*         | Rapeseed oil   | —            | 97        |
| Methanol     | *P. cepacia*           | Palm kernel oil| —            | 15        |
| Ethanol      | *P. cepacia*           | Palm kernel oil| —            | 72        |
| Methanol     | *M. miehei*            | Soybean oil    | Hexane       | 75        |
| Ethanol      | *M. miehei*            | Soybean oil    | Hexane       | 97        |
| Methanol     | *M. miehei*            | Tallow fat     | Hexane       | 95        |
| Ethanol (96%)| *M. miehei*            | Tallow fat     | Hexane       | 98        |
| Anhydrous ethanol | *M. miehei*    | Tallow fat     | Hexane       | 68        |
| Propanol     | *M. miehei*            | Tallow fat     | Hexane       | 24        |
| Butanol      | *M. miehei*            | Tallow fat     | Hexane       | 20        |
| Propanol     | *C. antarctica*        | Tallow fat     | Hexane       | 61        |
| Butanol      | *C. antarctica*        | Tallow fat     | Hexane       | 84        |
are usually stable in neutral aqueous solutions at room temperature, presenting, in most cases, an optimal activity at 30–40°C. However, its thermostability varies considerably depending on the origin, and, according to Castro et al. [29], microbial lipases present the best thermostability.

Most commercial lipolytic preparations are composed by a mixture of various isozymes, in different proportions, such as those obtained from *Candida rugosa*, *Pseudozyma* (formerly *Candida*) *antarctica*, *Rhizopus niveus*, and *Chromobacterium viscosum*, among others. Each isoform has different properties (e.g., molar mass, specificity, stereoselectivity, glycosylation extension, and substrate preference) [28, 84, 85]. The main sources of lipases and their properties are described in Table 7.

For industrial applications, the specificity of lipase is a crucial factor. This enzyme can present specificity regarding the substrate (fatty acid or alcohol), including the differentiation of isomers. Lipases can be divided into three groups based on their specificity.

(i) Nonspecific lipases (such as those produced by *Candida rugosa*, *Staphylococcus aureus*, *Chromobacterium viscosum*, *Thermomyces lanuginosus*, and *Pseudomonas* sp.): They cleave acylglycerol molecules randomly generating FFAs and glycerol, as well as mono- and diglycerides as intermediates. In this case, the products are similar to those produced by chemical catalysis, but with less thermodegradation, due to the lower temperature used for the reaction, when compared to chemical processes [29, 46, 86].

(ii) 1,3-specific lipases (e.g., from *Aspergillus niger*, *Mucor javanicus*, *Rhizopus delemar*, *Rhizopus oryzae*, *Yarrowia lipolytica*, *Rhizopus niveus*, and *Penicillium roqueforti*): They release fatty acids from positions 1 and 3 of a glyceride and from, for this reason, products with different compositions of those obtained by nonregioselective lipases, or even by chemical catalysts. Generally, the hydrolysis of triglycerides to diglycerides is much faster than those into monoglycerides [29, 46, 87].

(iii) Fatty acid-specific lipases: they act specifically on the hydrolysis of esters, which have long-chain fatty acids with double bonds in cis position on carbon 9. Esters with unsaturated fatty acids, or without double bond in carbon 9, are slowly hydrolyzed. This type of specificity is not common among the lipases and probably the most studied example of this case is the lipase from *Geotrichum candidum* [29, 46, 87–89].

The study of substrate specificity is also of great importance for the application of lipases in biodiesel production, since it is a valuable input for the selection of the proper enzyme based on the composition of the raw material. Gutarra et al. [58] evaluated the substrate specificity of an acidic lipase produced by *Penicillium simplicissimum*, observing the highest activities on tricaprin (C8: 0) and tricaprycin (C10: 0), which were 83 and 92% higher, respectively, than those detected in the model substrate (olive oil).

Lipases can also be stereospecific, where one of the isomers of a racemate is hydrolyzed preferentially over another, or even the formation of one isomer can be catalyzed selectively from prochiral precursors such as meso-diester or meso-diol compounds. Some examples are lipases from *Burkholderia cepacia*, *Pseudozyma antarctica*, *Candida rugosa*, and *Rhizopus delemar* [88, 89].

### 3. Enzyme-Catalyzed Processes for the Production of Biodiesel

The main technology for biodiesel production in Brazil and in the world is homogeneous alkaline transesterification (or alcoholysis). In this reaction, an alcohol (usually methanol or ethanol), with a molar basis, is added to the oil or fat and, in the presence of a catalyst (Brønsted acids or bases), a mixture of glycerin and alkyl esters of fatty acids is generated, which is called biodiesel (Figure 4). However, alkaline catalysts, especially sodium hydroxide, became dominant for the production of biodiesel, due to their lower costs and faster kinetics [9, 23, 91, 92].

However, homogenous alkaline transesterification presents some disadvantages over enzyme-catalyzed processes, such as the need of raw materials (refined oils and alcohols) virtually free of fatty acids, phosphatides, and water; excess of alcohol and catalyst to avoid reversible reactions, which in turn makes difficult the separation of biodiesel and glycerin. Therefore, alternative catalysts have been studied, such as organic bases, metallic complexes, oxides, aluminosilicates, and enzymes. Their main characteristics are that they are easily recycled and the absence of soap formation, which facilitates the products separation at the end of alcoholysis [91–93].

The use of biocatalysis has, therefore, advantages over chemical processes, and these include esterification of both triglycerides and fatty acids; generation of a cleaner glycerol; reuse, mostly in the case of an immobilized lipase utilization. However, some problems still need to be resolved, as high cost of lipases and possible inhibition in the presence of short-chain alcohols, glycerol, and other impurities in the raw material [23, 91, 93, 94]. In the case of biocatalysis, the schematic flowsheet of Figure 4(a) can also be applied, but it can also be necessary to use immobilized enzymes, for the reasons shown above. Due to kinetic disadvantages, it can be necessary also to use more sequential reactors in order to achieve the residence time of the feedstock in the presence of the enzymes, for a desired conversion (Figure 4(b)). For biodiesel production by enzymatic catalysis, some factors should be considered and some topics should be covered, which can be divided into aspects for current and prospective approaches. These aspects are detailed as follows.

#### 3.1. Current Aspects

3.1.1. Refining Step. In enzymatic transesterification, higher yields are achieved for biodiesel production when refined oil is used, compared to crude oils. This is due to the presence of
Figure 3: Mechanism of the hydrolysis reaction of ester bonds catalyzed by esterases and lipases. The catalytic triad and water are shown in black; the oxyanion hole residues are in blue; the substrate is in red. (a) Nucleophilic attack of the serine hydroxyl on the carbonyl carbon of the susceptible ester bond; (b) tetrahedral intermediate; (c) acyl-enzyme intermediate and nucleophilic attack by water; (d) tetrahedral intermediate; (e) free enzyme [90].

phospholipids in the nonrefined oil, which affect the interaction between lipase and substrate, since they possibly occlude the pores of the support, in the case of using an immobilized enzyme. Therefore, at least the oil degumming step should be conducted before transesterification reactions, in order to obtain a better production of biodiesel [23, 91]. The oil degumming is traditionally done using chemical and physical processes, such as water degumming, ultrafiltration, and mainly acid (phosphorous or citric) treatment [95, 96]. However, since the 1990s, enzyme-catalyzed degumming has been reported as a potential alternative to the conventional processes, and this comprises the use of phospholipases, which are classified into four groups [97]. Phospholipases types A1 (E.C. 3.1.1.32) and A2 (E.C. 3.1.1.4) catalyze the cleavage of ester bonds in phospholipids, thus releasing FFAs and contributing for the increase of the overall yield of biodiesel. There are also phospholipases types C (E.C. 3.1.4.3) and D (E.C. 3.1.4.4), but these are involved in the breakdown of phosphate bonds in phospholipids and do not contribute to the increase in FFAs content of the oil [98]. Enzymatic degumming is done at mild temperature (40–45°C) and pH of about 4.5–5.0, for a period of 2–4 h [97, 99].

3.1.2. Free and Immobilized Enzymes. The use of free enzymes for biodiesel production results in technical limitations, and it is practically unreliable, due to impossibility of their recovery and reuse, which in turn increases the
production costs of the process, besides promoting the product contamination with residual enzyme. These difficulties can be overcome by the use of these enzymes in its immobilized form, allowing the reuse of biocatalyst several times, reducing costs, and further improving the quality of the product [100]. There are several techniques cited for lipases immobilization, such as adsorption, covalent bonding, entrapment, encapsulation, and cross-linking, but they will not be discussed in details in this paper, since there is a recently published review focusing on this issue [100]. In this context, Nielsen et al. [101] revised technical and economic aspects of biodiesel production, concluding that for enzyme-catalyzed biodiesel viability, using immobilized lipases, the enzymes must be stable for the production of 1200–7400 kg of biodiesel for each kg of enzyme preparation, depending on the substrate source and lipase used.

The confinement or physical location of an enzyme in a given region of a defined space, while maintaining their catalytic activity, which can be used repeatedly and continuously due to the ease of its separation from the reaction medium, comprises its immobilization [102].

The catalytic activities of enzymes and other features may change depending on the type of the immobilization technique (adsorption, covalent bound, entrapment, encapsulation, and cross-linking) and the interaction strength between enzyme and carrier used which may, in some cases, cause structural distortions in the protein. Still, the catalytic activity of the enzyme in a particular medium can be changed by increasing or decreasing stirring due to the support fragmentation by interaction between agitation system and support [100].

Thus, it is possible to occur some activity loss during transesterification reaction, even when immobilized lipases are used, and this is more probably due to enzyme leaching than to enzyme inactivation. On the other hand, if such leaching does not occur and the enzyme remains bound to the support, the increase of contact surface may help in raising mass transfer, thereby increasing the efficiency of the enzyme as a catalyst. Lipases from different sources have been immobilized and used in biodiesel production, but the most commonly reported were obtained from Pseudazyma antarctica and Thermomyces lanuginosus [8, 103].

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**Figure 4:** Simplified flowsheet for the production of biodiesel. (a) Classical industrial chemical process; (b) alteration in reactor design for biocatalysis.
The use of fixed bed reactors with immobilized lipases is a more suitable solution for continuous production of biodiesel, since the enzymes suffer lower shear stress compared with the batch process. Pandey [91] reported the methanolysis of a waste oil mixture (containing 1980 ppm of water and 2.5% FFA), using immobilized lipase from C. antarctica, and considering 3 steps of substrate addition. The highest biodiesel yield observed was 90.4%. In another example, the same author reported the ethanolysis of a fat in a recirculating packed bed reactor (flow of 1.8 L·h⁻¹) using a phyllosilicate sol-gel-immobilized lipase from Burkholderia cepacia, and in this case, a yield of 96% was observed. Over the subsequent four cycles evaluated, the yield was maintained in at least 90%.

3.1.3. Use of Solvents. The enzymatic production of biodiesel can be performed using organic solvents (usually hexane, heptanes, or petroleum ether) or simply using the mixture of substrates (lipids and alcohol) depending on the size of the chain of alcohol. If methanol or ethanol is used, a solvent can facilitate the oil solubility in alcohol and also decrease the viscosity of the reactional mixture, but there will be an additional cost for its removal (distillation or extraction) after the reaction [8, 94, 102].

Knothe et al. [23] reported the biodiesel production from sunflower oil using petroleum ether as solvent with immobilized lipase from Pseudomonas fluorescens, reaching 99% yield when the alcohol used was methanol, ethanol, or 1-butanol. In the absence of solvent, yields were reduced to 3%, 70, and 76%, respectively.

Soetaert and Vandamme [104] reported the use of the lipases from Mucor miehei and C. antarctica in the transesterification of various oils using hexane as solvent and found that the lipase from M. miehei is more efficient in converting primary alcohols (methanol, ethanol, propanol, and 1-butanol) with yields between 95 and 98%, whereas lipase from C. antarctica is more proper for the conversion of secondary alcohols (isopropanol and 2-butanol) with yields between 61 and 84%. In the absence of the solvent, the yields of methyl and ethyl esters decreased, particularly when methanol was used, with yield reduction up to 19%.

The use of solvents with intermediate polarity (such as t-butanol, 1,4-dioxane) has been suggested to achieve a better dissolution of the alcohol for transesterification (particularly methanol, due to its higher inhibitory effect over lipases) and oil, without affecting the lipase activity [102]. Ranganathan et al. [105] reported the use of t-butanol as a solvent for the transesterification of cottonseed oil using the commercial preparation Novozym 435 for 24 hours at 55°C and achieved a yield of 97%, maintaining the lipase activity by 95% of the initial activity during 500 h of continuous operation.

3.1.4. Type of Alcohol and Adsorption Agent. Many types of alcohol can be used in the reaction of enzymatic transesterification. Some examples are shown in Table 8.

The molar ratio between substrates is a variable with large influence on the biodiesel synthesis. Excess of alcohol, in relation to the stoichiometric ratio of 3/1, is used to ensure higher reaction rate and to minimize diffusional limitations. However, excessive levels of alcohol, mainly those with short chains, may inhibit the enzyme by increasing the polarity of the medium, which reduces the stabilization and removes the water layer associated with the immobilized enzymes [94]. This effect was observed by Brusamarello et al. [106], for the transesterification of soybean oil. The optimal ethanol to oil ratio was 6/1, where 93% conversion was achieved after 480 min of reaction. When increased ethanol to oil ratios (9/1 and 12/1) were used, the conversion, considering the same time of catalysis, dropped to 51 and 55%, respectively.

Hence, the gradual addition of alcohol can maintain lipase stable for a longer period. Pandey [91] reported that the gradual addition of methanol (in 3 steps, every 16 h of reaction), maintaining the same enzyme in the bioreactor, resulted in a yield of 98% of biodiesel, and that the conversion was kept over 95% during 50 cycles. Another aspect that may accelerate the methanolysis is the preincubation of lipase in ester or oil [104].

The addition of silica to the reaction medium provides a positive effect on yield, due to the absorption of glycerol and water, thus reducing lipase inhibition [23]. One example cited by Robles-Medina et al. [102] is the use of 6% (w/w) of silica-gel in the reaction mixture along with the commercial preparation Lipozyme TL. The use of silica increased the yield from 66 to 90%. Another possibility would be to remove the glycerol by dialysis or its dissolution in isopropanol or t-butanol [102, 105].

3.1.5. Alternative Donors of Acyl Group. As the use of methanol and ethanol can promote lipase inhibition, the use of alternative donors of acyl group, such as methyl acetate, ethyl acetate, and propan-2-ol, is being studied, since their use avoids the production of glycerol as a by-product, which blocks the porous support and lipase active sites. Novozym 435 was tested for biodiesel production using several oils and the donors of acyl groups cited above, and the results observed were yields always above 90% [53, 91].

3.1.6. Water Addition. Alcoholysis reactions do not involve water as a reagent. However, the control of water content in the reaction system is important for some reasons: lipase requires a minimum amount of water to maintain its active conformation; an excess of water may promote the hydrolysis of the substrate and generate diffusion limitations of substrate, thus reducing the biodiesel yield; the water can influence negatively the reaction when methanol or ethanol is used but does not affect the reaction when higher-chain alcohols are considered [93, 94, 102].

Drapcho et al. [93] reported the use of lipase from Pseudomonas cepacia immobilized on polymeric sol-gel matrix in the transesterification of tallow oil at 40°C for 1 h using a mixture of 10 g of fat, 3 g of lipolytic preparation, 3 g of methanol or 5 g ethanol, and different amounts of water. The authors observed that when water concentrations below 0.2% were used, the conversion was significantly decreased and, after the reuse of lipase during 11 cycles, the activity was decreased by 10%.
Pandey [91] reported the use of lipase from Chromobacterium viscosum in the transesterification of jatropha oil with a 10% enzyme dosage. When the biocatalyst in a free form was used, it was observed a yield of 62%, whereas when the enzyme was immobilized on Celite 545, 71% of yield was achieved. By adding 1% of water to the free enzymatic preparation and 0.5% of water to the immobilized enzyme, the biodiesel yields raised to 73% and 92%, respectively.

3.1.7. Temperature. Generally, the higher the temperature, the higher the reaction rate of alcoholysis or transesterification, until reaching the temperature of inactivation of lipases (usually above 60°C). This approach is valid mainly for systems in which the enzyme is used just once or few times. When enzyme reuse is considered, higher temperatures, may which be suitable for short-term use of the enzymes, may be not the most proper, since the half-life time of lipases decreases with increases in temperature.

Matassoli et al. [107] investigated the influence of temperature in ethanolysis of crude palm oil catalyzed by Lipozyme TL IM (3% w/w) with a molar ratio ethanol/oil of 3/1 and gradual addition of ethanol, observing the best result at 50°C. For the evaluation of the effect of temperature on lipase-catalyzed biodiesel production, a semiempirical model was proposed by Brusamarelo et al. [106]. The authors investigated soybean biodiesel production using the commercial product Novozym 435, within the range of 45–70°C, observing the highest yield (92%) when 65°C was used.

3.1.8. Enzyme Type and Dosage. The amount of enzyme added to reaction is also an important factor for biodiesel production, because it affects reaction rate (typically, the higher the enzyme dosage, the faster the reaction), but there is a limit in which the addition of enzyme does not alter anymore the rate of product formation or that the amount turns the process more economically prohibitive. In this context, enzyme-catalyzed biodiesel production was investigated using dosages of C. antarctica lipase B (Novozym 435) from 1 to 20% (w/w) [106]. The authors observed the highest conversion of triglycerides to ethyl esters (93%) when 10% (w/w) of the immobilized enzyme was used.

Regarding the effect of lipase specificity, Pandey [91] reported the use of some specific and non-specific lipases (from C. rugosa, P. cepacia, and P. fluorescens) in biodiesel production. The non-specific lipases promoted the highest yields of methyl esters when a molar ratio of 3/1 of methanol/oil was used. Specific lipases need gradual addition of methanol to achieve high yields (between 80 and 90%), and this is probably due to acyl migration of sn-2 to sn-1, which occurs spontaneously in glycerides [108].

3.2. Prospective Aspects

3.2.1. Whole Cells. For reduction of enzymatic processes costs, some researchers have studied microbial immobilization, such as fungal mycelia, bacteria, and yeasts cells, for their use as whole cell catalysts, taking advantage of functional proteins at the cell surface. From all whole cell support immobilization techniques, the most used is that named porous biomass support particles (BSPs) because it does not require chemical additives or cell preproduction; aseptic handling is unnecessary; higher enzyme production and rate of substrate mass transfer within BSP; the particles are reusable and resistant to mechanical shearing; the bioreactor scale-up is easy and presents lower costs compared to bioreactors used in other methods [8, 104].

The first example for biodiesel production using whole cell as biocatalysts was the Rhizopus oryzae mycelium immobilized in polyurethane foam [109]. The growth conditions were optimized regarding the production of intracellular lipase, as well as pretreatment methods and water content during methanolysis. The addition of substrates (olive oil or oleic acid) to the culture medium significantly improved lipase activity of the whole cell catalyst. The results for the obtaining of methyl esters of soybean oil using this catalyst at 32°C for 72 h (80–90% yield), when the addition of methanol to the system was implemented intermittently in the presence of 10–20% water, were very similar to those described with the use of extracellular lipases. Aiming to stabilize the R. oryzae cells, it was tested a cross-linking treatment with 0.1% gluteraldehyde, keeping the lipase dosage unaltered for 6 cycles. The yield of methyl ester varied between 70% and 83%, along 72 h of experiment. Without this treatment, the lipase activity decreased reaching a yield of 50% in the sixth batch [8, 91, 104].

In order to achieve higher yields of biodiesel using cells immobilized in BSP, Soetaert and Vandamme [104] used fixed bed systems. To increase the interfacial area between the reaction mixture and the whole cells, the former was emulsified by sonication before each batch cycle. When a gradual addition of methanol (ratio 4/1, methanol/oil) was conducted at a flow rate of 25 L·h⁻¹, a yield of 90% was obtained and maintained at about 80% for 10 batches. The authors attributed this decrease to the cell removal from the BSP, since it was detected a decrease of 56% in the cell concentration in the BSP between the first and the tenth batches.

As further examples, other freeze-dried whole cells, such as from R. chinensis mycelium, S. cerevisiae (containing intracellular R. oryzae lipase, ROL), and recombinant S. cerevisiae expressing the lipase gene of R. oryzae IFO 4697 (cell surface ROL), have been used as biocatalysts for the production of methyl biodiesel from soybean oil. In the absence of solvent, the yields observed for the cited examples were 86, 71, and 78%, respectively, after 165 h of reaction at 37°C [8, 102].

Salum et al. [110] showed that it is possible to decrease the costs associated to the synthesis of enzyme-catalyzed biodiesel, by using the fermented solids produced by cultivating Burkholderia cepacia LTEB11 on a mixture of sugarcane bagasse and sunflower seed meal. The authors used this fermented solids to catalyze the ethanolysis of soybean oil aiming to produce biodiesel in a fixed-bed reactor in a cosolvent-free system. They achieved 95% conversion after 46 h of reaction.

Although the use of whole cells does not require many of the steps related to the downstream process of biodiesel production, such as the isolation and purification of the enzyme.
3.2.2. Use of Acid Oils. Some waste oils, by-products from vegetable oils processing, may also be suitable raw materials for biodiesel production. These oils usually present high contents of FFAs, and some examples are the sunflower oil and corn oil, which have 55.6% and 75.3% of FFAs, respectively. Pandey [91] reported the esterification and transesterification of these oils in the presence of hexane using immobilized lipase from C. Antarctica and observed yields of 64% and 50% of methyl esters, while maintaining the lipase stable for over 100 cycles.

Hou and Shaw [52] reported that the esterification of acid oils is much faster than the transesterification of nonacid oils. In the former case, it was necessary only 3 h of reaction and 1% of lipase for the esterification of FFAs, where a yield of 95% was achieved, whereas for the latter case, the same yield was observed only after 30 h of methanolysis and using a higher enzyme dosage (4%). One disadvantage of the esterification reaction is the formation of water as a by-product, which often inhibits the reaction of triglycerides. One possible solution to this is to conduct the reaction in two separate stages: first, esterification of the FFAs in the mixture, with the evaporation of the generated water; then the methanolysis of the triglycerides. In the first step, the molar ratio of methanol/FFA should be low, such as 1/2 and low quantity of enzyme (0.5% w/w) is needed. In the second step, on the other hand, the molar ratio between methanol and triglycerides should be changed to 1/1, and the enzyme quantity should be increased to about 6% (w/w).

3.2.3. Hydroesterification and Hybrid Catalysis. Hydroesterification is a process that combines two basic processes, the hydrolysis of triglycerides and the esterification of fatty acids, in sequential reactions, in order to produce biodiesel.

Talukder et al. [112] studied the use of residual cooking oil for biodiesel production by enzymatic hydrolysis accompanied by chemical esterification. The C. rigosa lipase used completely hydrolyzed the oil after 10 h of reaction. The FFAs were converted into biodiesel by chemical esterification using Amberlyst 15 (acidic styrene divinylbenzene), and a 99% conversion into biodiesel was obtained after 2 h. In this work, there was a loss of enzyme activity, and the hydrolysis yield was decreased to 92% after five batch cycles.

Cavalcanti-Oliveira et al. [113] studied the use of a lipase from Thermomyces lanuginosus (TL 100 L) in the hydrolysis of soybean oil in a hydroesterification process. The lipase hydrolyzed 89% of the oil after 48 h of reaction. This stage was followed by the esterification of the FFAs with methanol, which was catalyzed by niobic acid in pellets. They obtained 92% conversion of the FFAs into fatty acid methyl esters after only 1 h of incubation. Sousa et al. [114] studied the lipase from jatropha seeds for the hydrolysis of different raw materials for biodiesel production using hydroesterification strategy. The best conversions were obtained using soybean oil and jatropha oil, obtaining up to 98% of FFA after 2 h. The esterification of the FFAs from the jatropha oil with methanol was catalyzed by niobic acid in pellets, obtaining up to 97% conversion into biodiesel after 2 h. The biodiesel obtained from this process fulfilled all the legal requirements for its commercial use.

4. Concluding Remarks

The use of enzyme catalysts (lipases) in biodiesel production is being increasingly studied because of the advantages that these catalysts present over chemically catalyzed and noncatalytic processes. Some of the advantages offered by the use of lipases are lower energy consumption; lower thermal degradation of substrates and products; versatility in the use of raw materials, including possibility to use acid oils without the decrease of process efficiency; easier purification of the alkyl esters (biodiesel) and separation of the coproduct (glycerol), especially if immobilized enzymes or whole cells are used; environmental benefits, due to biodegradability of the catalyst.

Nevertheless, some process conditions should be taken into account in order to have a feasible enzyme-based technology for the production of biodiesel, and these include the establishment of descriptive correlations between the enzyme dosage and the substrate source, in order to rationalize enzyme usage and optimize costs [106]; deep study of reaction conditions and their optimization; the selection of a proper biocatalyst which can be reused and maintain its stability over several cycles; product recovery strategies, especially when a cosolvent is used in the reaction.

The enzyme-based production of biodiesel is still under development, and it seems that there is a tendency for the use of conventional technologies as a new application for lipases, such as their immobilization in magnetic nanoparticles [115], microwave and ultrasound-assisted transesterification [116], esterification in pressurized fluids [117], and transesterification in supercritical fluids [116]. Although technical aspects of such strategies may lead to conversion improvement, economical considerations must be investigated in more details.

References

[1] F. D. Gunstone and F. B. Padley, Lipid Technologies and Applications, Marcel Dekker, New York, NY, USA, 1997.
[2] J. J. Salas, J. Sánchez, U. S. Ramli, A. M. Manaf, M. Williams, and J. L. Harwood, "Biochemistry of lipid metabolism in olive and other oil fruits," Progress in Lipid Research, vol. 39, no. 2, pp. 151–180, 2000.
Y. M. Choo, “Palmo i lc acarotenoids,” M. C. T. Damaso, M. A. Passianoto, S. C. Freitas, D. M. M. Singh, K. Saurav, N. Srivastava, and K. Kannabiran, E. Wolski, E. Menusi, M. Mazutti et al., “Response surface F. Danieli, “O ´oleo de abacate (Persea americana Mill) como L. M. P. Santos, “Nutritional and ecological aspects of buriti or aguaje (Mauritia flexuosa Linnaeus filius): a carotene-rich palm fruit from Latin America,” Ecology of Food and Nutrition, vol. 44, no. 5, pp. 345–358, 2005.

F. D. Gunstone, J. L. Harwood, and A. J. Dijkstra, “Lipid Function and Structure,” in Lipid Technology, Properties and Uses, Blackwell Publishing, Oxford, UK, 2002.

M. F. Ali, B. M. E. Ali, and J. G. Speight, Handbook of Industrial Chemistry—Organic Chemicals, McGraw-Hill, New York, NY, USA, 2005.

P. J. Eastmond and I. A. Graham, “Re-examining the role of the glyoxylate cycle in oilseeds,” Trends in Plant Science, vol. 6, no. 2, pp. 72–77, 2001.

Food and Agriculture Organization of The United Nations (FAO), Food Outlook, FAO, New York, NY, USA, 2010.

J. C. Melo, J. C. Teixeira, J. Z. Brito, J. G. A. Pacheco, and L. Stragevitch, “Produção de Biodiesel de Óleo de Óiticica,” in Proceedings of the 1 Congresso da Rede Brasileira de Tecnologia do Biodiesel, pp. 164–169, August 2006.

M. J. Dabdoub, J. L. Bronze, and M. A. Rampin, “Biodiesel: visão crítica do status atual e perspectivas na academia e na indústria,” Química Nova, vol. 32, no. 3, pp. 776–792, 2009.

F. D. Gunstone, J. L. Harwood, and A. J. Dijkstra, The Lipid Handbook, CRC Press, Boca Raton, FL, USA, 2007.

L. M. P. Santos, “Nutritional and ecological aspects of buriti or aguaje (Mauritia flexuosa Linnaeus filius): a carotene-rich palm fruit from Latin America,” Ecology of Food and Nutrition, vol. 44, no. 5, pp. 345–358, 2005.

F. D. Gunstone, J. L. Harwood, and A. J. Dijkstra, The Lipid Handbook, CRC Press, Boca Raton, FL, USA, 2007.

F. D. Gunstone, J. L. Harwood, and A. J. Dijkstra, The Lipid Handbook, CRC Press, Boca Raton, FL, USA, 2007.

F. D. Gunstone, J. L. Harwood, and A. J. Dijkstra, The Lipid Handbook, CRC Press, Boca Raton, FL, USA, 2007.

M. Singh, K. Saurav, N. Srivastava, and K Kannabiran, “Lipase production,” Bioresource Technology, vol. 102, no. 7, pp. 4909–4912, 2011.

Q. Li, W. Du, and D. Liu, “Perspectives of microbial oils for biodiesel production,” Applied Microbiology and Biotechnology, vol. 80, no. 5, pp. 749–756, 2008.

M. G. Morais and J. A. V. Costa, “Fatty acids profile of microalgae cultivated with carbon dioxide,” Ciência e Agrotecnologia, vol. 32, no. 4, pp. 1245–1251, 2008.
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Producção, purificação e caracterização da lipase de Geotrichum candidum obtida a partir de meios industriais, Dissertation. thesis, University of Campinas, Campinas, Brazil, 2006.

M. Abrimic, I. Lescic, T. Korica, L. Vitale, W. Saenger, and J. Pigac, “Purification and properties of extracellular lipase from Streptomyces rimosus,” Enzyme and Microbial Technology, vol. 25, no. 6, pp. 522–529, 1999.

J. Destain, D. Roblain, and P. Thonart, “Improvement of lipase production from Yarrowia lipolytica,” Biotechnology Letters, vol. 19, no. 2, pp. 105–107, 1997.

A. Aloulou, J. A. Rodriguez, D. Puccinelli et al., “Purification and biochemical characterization of the LIP2 lipase from Yarrowia lipolytica,” Biochimica et Biophysica Acta, vol. 1771, no. 2, pp. 228–237, 2007.

A. I. S. Brigida, P. F. F. Amaral, L. R. B. Gonçalves, and M. A. Z. Coelho, “Characterization of an Extracellular lipase from Yarrowia lipolytica,” in Proceedings of the European Congress of Chemical Engineering 6, (ECCE ’07), vol. 2, Norhaven Book, Copenhagen, Denmark, September 2007.

H. Uhlig, Industrial Enzymes and Their Applications, John Wiley & Sons, New York, NY, USA, 1998.

H. Abbas, A. Hiol, V. Deyris, and L. Comeau, “Isolation and characterization of an extracellular lipase from Mucor sp. strain isolated from palm fruit,” Enzyme and Microbial Technology, vol. 31, no. 7, pp. 968–975, 2002.

T. Godfrey and S. West, Industrial Enzymology, The Macmillan Press, London, UK, 1996.

P. J. Eastmond, “Cloning and characterization of the acid lipase from castor beans,” Journal of Biological Chemistry, vol. 279, no. 44, pp. 45540–45545, 2004.

E. D. C. Cavalcanti, Avaliação da atividade lipásica da semente dormente de Ricinus communis, Dissertation. thesis, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, 2006.

R. R. Costa Neto, Obtenção de estéreis alquímicos (Biodiesel) por via enzimática a partir de óleo de soja, Ph.D. thesis, Federal University of Santa Catarina, Florianópolis, Brazil, 2002.

C. T. Hou and J. F. Shaw, Biocatalysis and Bioenergy, John Wiley & Sons, Hoboken, NJ, USA, 2008.

A. Nag, Biofuels Refining and Performance, McGraw-Hill, New York, NY, USA, 2008.

L. R. Castilho, C. M. S. Polato, E. A. Baruque, G. L. Sant’Anna Jr., and D. M. G. Freire, “Economic analysis of lipase production by Penicillium restrictum in solid-state and submerged fermentations,” Biochemical Engineering Journal, vol. 4, no. 3, pp. 239–247, 2000.

L. A. I. Azeredo, P. M. Gomes, G. L. Sant’Anna Jr., L. R. Castilho, and D. M. G. Freire, “Production and regulation of lipase activity from Penicillium restrictum in submerged and solid-state fermentations,” Current Microbiology, vol. 54, no. 5, pp. 361–365, 2007.

M. B. Palma, A. L. Pinto, A. K. Gombert et al., “Lipase production by Penicillium restrictum using solid waste of industrial babassu oil production as substrate,” Applied Biochemistry and Biotechnology, vol. 84–86, pp. 1137–1145, 2000.

M. L. E. Gutarra, E. D. C. Cavalcanti, D. M. G. Freire, L. R. Castilho, and G. L. Sant’Anna Jr., “Lipase production by solid-state fermentation: cultivation conditions and operation of tray and packed-bed bioreactors,” Applied Biochemistry and Biotechnology, vol. 121, no. 1–3, pp. 105–116, 2005.

M. L. E. Gutarra, M. G. Godoy, F. Maugeri, M. I. Rodrigues, D. M. G. Freire, and L. R. Castilho, “Production of an acidic and thermotolerant lipase of the mesophilic fungus Penicillium simplicissimum by solid-state fermentation,” Bioresource Technology, vol. 100, no. 21, pp. 5249–5254, 2009.

E. D. C. Cavalcanti, M. L. E. Gutarra, D. M. G. Freire, L. R. Castilho, and G. L. Sant’Anna Jr., “Lipase production by solid-state fermentation in fixed-bed bioreactors,” Brazilian Archives of Biology and Technology, vol. 48, pp. 79–84, 2005.

M. Diluccio, E. Capra, N. P. Ribeiro, G. D. L. P. Vargas, D. M. G. Freire, and D. Oliveira, “Evaluation of lipase production by solid state fermentation by Penicillium simplicissimum using soy cake,” Applied Biochemistry and Biotechnology, vol. 113, pp. 173–180, 2004.

M. G. Godoy, M. L. E. Gutarra, A. M. Castro, O. L. M. Tavares, and D. M. G. Freire, “Adding value to a toxic residue from the biodiesel industry: production of two distinct pool of lipases from Penicillium simplicissimum in castor bean waste,” Journal of Industrial Microbiology & Biotechnology. In press.

P. V. Rao, K. Jayaraman, and C. M. Lakshmanan, “Production of lipase by Candida rugosa in solid state fermentation. I: determination of significant process variables,” Process Biochemistry, vol. 28, no. 6, pp. 385–389, 1993.

J. A. Rodriguez, J. C. Mateos, J. Nungaray et al., “Improving lipase production by nutrient source modification using Rhizopus homothallicus cultured in solid state fermentation,” Process Biochemistry, vol. 41, no. 11, pp. 2264–2269, 2006.

J. C. M. Mateos Diaz, J. A. Rodriguez, S. Roussos et al., “Lipase from the thermotolerant fungus Rhizopus homothallicus is more thermostable when produced using solid state fermentation than liquid fermentation procedures,” Enzyme and Microbial Technology, vol. 39, no. 5, pp. 1042–1050, 2006.

N. D. Mahadik, U. S. Puntambekar, K. B. Bastawde, J. M. Khire, and D. V. Gokhale, “Production of acidic lipase by Aspergillus niger in solid state fermentation,” Process Biochemistry, vol. 38, no. 5, pp. 715–721, 2002.

N. R. Kamini, J. G. S. Mala, and R. Puvanakrishnan, “Lipase production from Aspergillus niger by solid-state fermentation using gingelly oil cake,” Process Biochemistry, vol. 33, no. 5, pp. 505–511, 1998.

J. Cordova, M. Nemmaoui, M. Ismaili-Alaoui et al., “Lipase production by solid state fermentation of olive cake and sugar cane bagasse,” Journal of Molecular Catalysis. B, vol. 5, no. 1–4, pp. 75–78, 1998.

I. ul-Haq, S. Idrees, and M. I. Rajoka, “Production of lipases by Rhizopus oligosporous by solid-state fermentation,” Process Biochemistry, vol. 37, no. 6, pp. 637–641, 2002.

D. A. Mitchell, B. K. Losane, A. Durand et al., “General principles of reactors design and operation for SSC,” in
Solid Substrate Cultivation, H. Doelle, D. A. Mitchell, and C. E. Rols, Eds., pp. 115–139, Elsevier Applied Science, Amsterdam, The Netherlands, 1992.

[70] D. A. Mitchell, A. Pandey, P. Sangsurasak, and N. Krieger, “Scale-up strategies for packed-bed bioreactors for solid-state fermentation,” Process Biochemistry, vol. 35, no. 1–2, pp. 167–178, 1999.

[71] D. A. Mitchell, N. Krieger, D. M. Stuart, and A. Pandey, “New developments in solid-state fermentation II. Rational approaches to the design, operation and scale-up of bioreactors,” Process Biochemistry, vol. 35, no. 10, pp. 1211–1225, 2000.

[72] D. A. Mitchell, M. Berovic, and N. Krieger, “Overview of solid state bioprocessing,” Biotechnology Annual Review, vol. 8, pp. 183–225, 2002.

[73] F. D. H. Dalsenter, G. Viccini, M. C. Barga, D. A. Mitchell, and N. Krieger, “A mathematical model describing the effect of temperature variations on the kinetics of microbial growth in solid-state culture,” Process Biochemistry, vol. 40, no. 2, pp. 801–807, 2005.

[74] P. Sangsurasak and D. A. Mitchell, “Incorporation of death kinetics into a 2-dimenional dynamic heat transfer model for solid state fermentation,” Journal of Chemical Technology and Biotechnology, vol. 64, no. 3, pp. 253–260, 1995.

[75] M. T. Hardin, D. A. Mitchell, and T. Howes, “Approach to designing rotating drum bioreactors for solid-state fermentation on the basis of dimensionless design factors,” Biotechnology and Bioengineering, vol. 67, no. 3, pp. 274–282, 2000.

[76] M. A. I. Schutyser, P. Pagter, F. J. Weber, W. J. Briels, R. M. Boom, and A. Rinzema, “Substrate aggregation due to aerial hyphae during discontinuously mixed solid-state fermentation with Aspergillus oryzae: experiments and modeling,” Biotechnology and Bioengineering, vol. 83, no. 5, pp. 503–513, 2003.

[77] M. M. Santos, A. S. Rosa, S. Dal’Boit, D. A. Mitchell, and N. Krieger, “Thermal denaturation: is solid-state fermentation really a good technology for the production of enzymes?” Bioresource Technology, vol. 93, no. 3, pp. 261–268, 2004.

[78] A. Aloulou, J. A. Rodriguez, D. Puccinelli et al., “Purification and biochemical characterization of the LIP2 lipase from Yarrowia lipolytica,” Biochimica et Biophysica Acta, vol. 1771, no. 2, pp. 105–112, 1995.

[79] M. L. Rua, C. Schmidt-Dannert, S. Wahl, A. Sprauer, and R. D. Schmid, “Thermokalophilic lipase of Bacillus thermocatenulatus: large-scale production, purification and properties: aggregation behaviour and its effect on activity,” Journal of Biotechnology, vol. 56, no. 2, pp. 89–102, 1997.

[80] M. Teissere, M. Borel, B. Caillol, J. Nari, A. M. Gardies, and G. Noat, “Purification and characterization of a fatty acyl-ester hydrolase from post-germinated sunflower seeds,” Biochimica et Biophysica Acta, vol. 1255, no. 2, pp. 105–112, 1995.

[81] H. Sztajer, H. Lünßdorf, H. Erdmann, U. Menge, and R. Schmid, “Purification and properties of lipase from Penicillium simplicissimum,” Biochimica et Biophysica Acta, vol. 1124, no. 3, pp. 253–261, 1992.

[82] K. Ito, K. Nokihiara, S. Yamaguchi, T. Mase, and R. D. Schmid, “Crystallization and characterization of monoaoyl-glycerol and diacylglycerol lipase from Penicillium camemberti,” European Journal of Biochemistry, vol. 203, no. 1–2, pp. 239–237, 1992.

[83] A. G. Cunha, G. F. Lorente, M. L. E. Gutarra et al., “Separation and immobilization of lipase from Penicillium simplicissimum by selective adsorption on hydrophobic supports,” Applied Biochemistry and Biotechnology, vol. 156, no. 1–3, pp. 133–145, 2009.

[84] R. K. Saxena, A. Sheoran, B. Giri, and W. S. Davidson, “Purification strategies for microbial lipases,” Journal of Microbiological Methods, vol. 52, no. 1, pp. 1–18, 2003.

[85] P. D. Maria, J. M. Sánchez-Montero, J. V. Sinisterra, and A. R. Alcántara, “Understanding Candida rugosa lipases: an overview,” Biotechnology Advances, vol. 24, no. 2, pp. 180–196, 2006.

[86] P. F. F. Amaral, Produção de lipase de Yarrowia lipolytica em bioreator multifásico, Ph.D. thesis, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, 2007.

[87] F. V. P. Meirelles, Produção de lipase de Yarrowia lipolytica (IMUFJ/50682), Ph.D. thesis, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, 1997.

[88] M. F. C. P. J. S. Gama, Produção e caracterização de lipases de Penicillium restrictum, Dissertation, thesis, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, 2000.

[89] T. S. M. Martins, Produção e purificação de lipases de Yarrowia lipolytica (IMUFJ/50682), Dissertation, thesis, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, 2001.

[90] R. V. Almeida, Clonagem, expressão, caracterização e modelo estrutural de uma esterase termoestável de Pyrococcus furiosus, Ph.D. thesis, University of Rio de Janeiro, Rio de Janeiro, Brazil, 2005.

[91] A. Pandey, Handbook of Plant-Based Biofuels, CRC Press, Boca Raton, Fla, USA, 2009.

[92] P. A. Z. Suarez, A. L. F. Santos, J. P. Rodrigues, and M. B. Alves, “Biocombustíveis a partir de óleos e gorduras: desafios tecnológicos para viabilizá-los,” Química Nova, vol. 32, no. 3, pp. 768–775, 2009.

[93] C. M. Drapcho, N. P. Nhuan, and T. H. Walker, Biofuels Engineering Process Technology, McGraw-Hill, New York, NY, USA, 2008.

[94] R. C. Rodrigues, “Síntese de biodiesel através de transesterificação enzimática de óleos vegetais catalisada por lipase imobilizada por ligação covalente multipontual,” Ph.D. thesis, Federal University of Rio Grande do Sul, Porto Alegre, Brazil, 2009.

[95] M. Sadeghi, “Purification of soybean oil with phospholipase AI,” Theoretical and Experimental Chemistry, vol. 46, no. 2, pp. 132–137, 2010.

[96] B. Yang, R. Zhou, J. G. Yang, Y. H. Wang, and W. F. Wang, “Insight into the enzymatic degumming process of soybean oil,” Journal of the American Oil Chemist’s Society, vol. 85, no. 5, pp. 421–425, 2008.

[97] K. Clausen, “Enzymatic oil-degumming by a novel microbial phospholipase,” European Journal of Lipid Science and Technology, vol. 103, no. 6, pp. 333–340, 2001.

[98] L. De Maria, J. Vind, K. M. Oxenboll, A. Svendsen, and S. Patkar, “Phospholipases and their industrial applications,” Applied Microbiology and Biotechnology, vol. 74, no. 2, pp. 290–300, 2007.

[99] S. K. Roy, B. V. S. K. Rao, and R. B. N. Prasad, “Enzymatic degumming of rice bran oil,” Journal of the American Oil Chemist’s Society, vol. 79, no. 8, pp. 845–846, 2002.

[100] T. Tan, J. Lu, K. Nie, L. Deng, and F. Wang, “Biodiesel production with immobilized lipase: a review,” Biotechnology Advances, vol. 28, no. 5, pp. 628–634, 2010.

[101] P. M. Nielsen, J. Brask, and L. Fjerbaek, “Enzymatic biodiesel production: technical and economical considerations,” European Journal of Lipid Science and Technology, vol. 110, no. 8, pp. 692–700, 2008.
[102] A. Robles-Medina, P. A. Gonzalez-Moreno, L. Esteban-Cerdan, and E. Molina-Grima, “Biocatalysis: towards ever greener biodiesel production,” Biotechnology Advances, vol. 27, no. 4, pp. 398–408, 2009.

[103] W. Du, W. Li, T. Sun, X. Chen, and D. Liu, “Perspectives for biotechnological production of biodiesel and impacts,” Applied Microbiology and Biotechnology, vol. 79, no. 3, pp. 331–337, 2008.

[104] W. Soetaert and E. J. Vandamme, Biofuels, John Wiley & Sons, Hoboken, NJ, USA, 2009.

[105] S. V. Ranganathan, S. L. Narasimhan, and K. Muthukumar, “An overview of enzymatic production of biodiesel,” Bioresource Technology, vol. 99, no. 10, pp. 3975–3981, 2008.

[106] C. Z. Brusamarelo, E. Rosset, A. Césaro et al., “Kinetics of lipase-catalyzed synthesis of soybean fatty acid ethyl esters in pressurized propane,” Journal of Biotechnology, vol. 147, no. 2, pp. 108–115, 2010.

[107] A. L. F. Matassoli, I. N. S. Corrêa, M. F. Portilho, C. O. Veloso, and M. A. P. Langone, “Enzymatic synthesis of biodiesel via alcoholysis of palm oil,” Applied Biochemistry and Biotechnology, vol. 155, no. 1–3, pp. 347–355, 2009.

[108] Y.-D. Wang, X.-Y. Shen, Z.-L. Li et al., “Immobilized recombinant Rhizopus oryzae lipase for the production of biodiesel in solvent free system,” Journal of Molecular Catalysis B, vol. 67, no. 1-2, pp. 45–51, 2010.

[109] K. Ban, M. Kaieda, T. Matsumoto, A. Kondo, and H. Fukuda, “Whole cell biocatalyst for biodiesel fuel production utilizing Rhizopus oryzae cells immobilized within biomass support particles,” Biochemical Engineering Journal, vol. 8, no. 1, pp. 39–43, 2001.

[110] T. F. C. Salum, P. Villeneuve, B. Barea et al., “Synthesis of biodiesel in column fixed-bed bioreactor using the fermented solid produced by Burkholderia cepacia LTB11,” Process Biochemistry, vol. 45, no. 8, pp. 1348–1354, 2010.

[111] H. Fukuda, S. Hama, S. Tamalampudi, and H. Noda, “Whole-cell biocatalysts for biodiesel fuel production,” Trends in Biotechnology, vol. 26, no. 12, pp. 668–673, 2008.

[112] M. M. R. Talukder, J. C. Wu, and L. P. L. Chua, “Conversion of waste cooking oil to biodiesel via enzymatic hydrolysis followed by chemical esterification,” Energy and Fuels, vol. 24, no. 3, pp. 2016–2019, 2010.

[113] E. D. Cavalcanti-Oliveira, P. R. R. Silva, A. P. Ramos, D. A. G. Aranda, and D. M. G. Freire, “Study of soybean oil hydrolysis catalyzed by Thermomyces lanuginosus lipase and its application to biodiesel production via hydroesterification,” Enzyme Research, vol. 2011, Article ID 618692, 8 pages, 2011.

[114] J. S. Sousa, E. D. Cavalcanti-Oliveira, D. A. G. Aranda, and D. M. G. Freire, “Application of lipase from the physic nut (Jatropha curcas L.) to a new hybrid (enzyme/chemical) hydroesterification process for biodiesel production,” Journal of Molecular Catalysis B, vol. 65, no. 1–4, pp. 133–137, 2010.

[115] W. Xie and N. Ma, “Enzymatic transesterification of soybean oil by using immobilized lipase on magnetic nano-particles,” Biomass and Bioenergy, vol. 34, no. 6, pp. 890–896, 2010.

[116] A. P. Vyas, J. L. Verma, and N. Subrahmanyam, “A review on FAME production processes,” Fuel, vol. 89, no. 1, pp. 1–9, 2010.

[117] G. Kuhn, M. Marangoni, D. M. G. Freire et al., “Esterification activities of non-commercial lipases after pre-treatment in pressurized propane,” Journal of Chemical Technology and Biotechnology, vol. 85, no. 6, pp. 839–844, 2010.