Chapter from the book *Biodiesel - Feedstocks and Processing Technologies*
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1. Introduction

The leading standard setting organization ASTM International, formerly ASTM (American Society of Testing and Materials), defines biodiesel as a fuel comprised of mono-alkyl esters of long chain fatty acids (ASTM D6751). It is usually manufactured by triglycerides transesterification with methanol or ethanol in the presence of a catalyst, according to the following reaction:

\[
\begin{align*}
\text{triglycerides (vegetable oil, animal fat)} & \quad \text{alcohol} \quad \text{mono-alkyl esters (biodiesel)} \quad \text{glycerol} \\
\text{CH}_2\text{-O-C-R}_1 & \quad \text{O} \quad \text{R}_1\text{-C-O-R} & \quad \text{CH}_2\text{OH} \\
\text{CH}_2\text{-O-C-R}_2 & \quad \text{O} \quad \text{R}_2\text{-C-O-R} & \quad \text{CH}_2\text{OH} \\
\text{CH}_2\text{-O-C-R}_3 & \quad \text{O} \quad \text{R}_3\text{-C-O-R} & \quad \text{CH}_2\text{OH} \\
\end{align*}
\]

Recently, biodiesel production by lipase catalyzed transesterification has been suggested as a promising alternative to the conventional chemical catalysis, in spite of the high conversion and reaction rates of the latter (Akoh et al., 2007; Bajaj et al., 2010; Bisen et al., 2010; Demirbas, 2009; Fjørbaek et al., 2009; Fukuda et al., 2001, 2009; Ghaly et al., 2010; Helwani et al., 2009; Jegannathan & Abang, 2008; Man Xi Ao et al., 2009; Marchetti et al., 2007; Ranganathan et al., 2008; Robles-Medina et al., 2009; Semwal et al., 2011). The enzymatic process enables eliminating the drawbacks of the alkali- or acid-catalyzed transesterification, namely: product contamination, wastewater release, free fatty acids and water interferences, and difficult glycerol recovery. Nevertheless, the commercialization of
the lipase-catalyzed biodiesel synthesis remains problematic, because of the cost of the enzyme: approximately 1000 USD per kg of Novozym 435 lipase. Therefore, the implementation of strategies, such as enzyme immobilization, for the development of economic and effective enzyme based technologies for biodiesel production is of crucial importance. Enzyme immobilization ensures several issues: repetitive and continuous use of the enzyme and its stabilization, localization of the interaction, prevention of product contamination, reduction of effluent problems and material handling, and effective control of the reaction parameters (D’Souza, 1982). All these aspects are reflected on the production cost.

The present review is intended to provide an overview on the use of immobilized lipases in biodiesel production, the techniques applied for enzyme immobilization, and the factors affecting the process.

2. Lipases mode of action and classification

Lipases (EC 3.1.1.3 triacylglycerol acylhydrolase) represent a group of water soluble enzymes that originally catalyze the hydrolysis of ester bonds in water insoluble lipid substrates, acting at the interface between the aqueous and the organic phases. This unique heterogeneous reaction is feasible because of: (i) the specific lipases molecule 3D structure consisting of three domains: “contact domain”, responsible for distinguishing of substrate surface, “hydrophobic” domain, responsible for extracting of one substrate molecule and its association with the “functional” domain, and “functional” domain, containing the catalytic triad Ser, Hys and Asp/Glu; (ii) the transition from closed to open conformation in the presence of the lipidic phase (Guncheva & Zhiryakova 2011; Panalotov & Verger, 2000). Enzymatic action of lipases on the substrate is a result of a nucleophilic attack on the carbonyl carbon atom from ester groups. Some lipases are also able to catalyze the processes of esterification, interesterification, transesterification, acidolysis, amyolysis and may show enantioselective properties (Hasan et al., 2009). The mechanism of the lipase catalyzed transesterification of triglycerides with an alcohol to produce biodiesel (Fjerbaek et al., 2009) could be presented by the following sequence of reactions:

\[
\begin{align*}
E + TG & \quad \rightleftharpoons \quad E-TG \quad \rightleftharpoons \quad DG + AcE \quad \rightleftharpoons \quad \text{alcohol} \quad \rightleftharpoons \quad \text{AcE-alcohol} \\
E + DG & \quad \rightleftharpoons \quad E-DG \quad \rightleftharpoons \quad MG + AcE \quad \rightleftharpoons \quad \text{H}_2\text{O} \quad \rightleftharpoons \quad E + FFA \\
E + MG & \quad \rightleftharpoons \quad E-MG \quad \rightleftharpoons \quad G + AcE \quad \rightleftharpoons \quad \text{biodiesel + E}
\end{align*}
\]

with: E-enzyme; TG-triglyceride; DT-diglyceride; MG-monoglyceride; G-glycerol; AcE-acylated enzyme; FFA-free fatty acid.

According to the origin lipases are plant, animal and microbial. The mostly used lipases in biodiesel production are of bacterial and fungal origin, such as: Candida antarctica (Novozym 435), Candida Rugosa (Lipase AY), Pseudomonas cepacia (Lipase PS), Pseudomonas fluorescens (lipase AK), Pseudomonas aeruginosa, and Thermomyces lanuginose (Lipozime TL), among other. The catalytic properties and potential applications of Bacillus lipases are extensively reviewed by Guncheva & Zhiryakova (2011). Among the available lipase producing microorganisms, filamentous fungi belonging to various species of genera Aspergillus (Adinarayana et al., 2004; Karanam, & Medicrlera, 2008), Rhizopus (Hiol et al., 2000; Shukla
& Gupta, 2007), *Penicillium* (Chahinian et al, 2000; Lima et al., 2003; Vardanega et al., 2010), and *Trichoderma* (Kashmiri et al., 2006; Rajesh et al., 2010) are described as the most prospective lipase producers. Only microbial lipases are a matter of practical interest for biodiesel production, because only microbial lipases are produced in industrial scale. The application of the microbial lipases, all together with their immobilization which allows the regeneration and the reuse of the enzyme preparation in several working cycles reduces the production costs, and respectively the final cost of biodiesel. A review on microbial lipase production with emphasis on lipase engineering and use of mathematical models for process improvement and control is provided by Treichel et al. (2010).

Some fungi cultured by the authors as powerful producers of lipase of use in biodiesel synthesis are shown in Fig. 1.

![Fig. 1. Filamentous fungi belonging to various species of genera Aspergillus and Penicillium, considered as prospective lipase producers.](image)

**3. The immobilized lipases in biodiesel production**

The term “enzyme immobilization” was defined at the first Enzyme Engineering Conference held at Hennicker, NH, USA, in 1971. It describes “enzymes physically confined at or
localized in a certain region of space with retention of their catalytic activity and which can be used repeatedly and continuously” (Powel, 1996). It is considered that lipase immobilization induces the enzyme conformational change required to enable the free access of substrate to the active centre. Especially, hydrophobic supports allow the adsorption of the open form of the lipases via interfacial activation, mimicking the lipophilic substrate (Ahn et al., 2010; Rodrigues & Fernandez-Lafuente, 2010; Salis et al., 2008; Séverac et al., 2011).

The revision of the literature covering the period 2005-2011 demonstrates that a large variety of matrices have been used for lipases immobilization, and that the main methods applied include adsorption, entrapment and/or encapsulation, and covalent attachment.

3.1 Lipases immobilization by adsorption

Physical adsorption is considered as the simplest method for enzyme immobilization. Enzyme fixation is performed through hydrogen bonds, salt linkages, and Van der Waal’s forces. The process is carried out in mild conditions, without or with minimum support activation and clean up procedures application, and in the absence of additional reagents. Thus, it is economic and allows preserving enzyme activity and specificity. The chemical composition of the carrier, the molar ratio of hydrophilic to hydrophobic groups, as well as the particle size and the surface area determine the amount of enzyme bound and the enzyme behaviour after immobilization. Some of the most commonly used carriers for lipases immobilization by adsorption are listed in Table 1.

Data shown in Table 1 indicate that among the variety of lipase immobilization supports, Accurel has found a large application. Accurel is the trade name of a group of macroporous polymers. As carriers for lipase immobilization are used the polypropylene based hydrophobic Accurel MP (MP1000 with particle size below 1500 µm, Accurel MP1001 with particle size below 1000 µm and Accurel MP1004 with particle size below 400 µm), and Accurel EP-100. On the most hydrophobic support tested, Accurel MP1001, no glycerol adsorption was observed (Séverac et al., 2011). It has been demonstrated (Salis et al., 2009) comparing the catalytic efficiencies (activity/loading) of eight lipases, that they show a different level of adaptation to the support. Immobilized \textit{Pseudomonas fluorescens} lipase is the most active biocatalyst, followed by immobilized \textit{Pseudomonas cepacia} lipase. The other lipases tested (from \textit{Rhizopus oryzae}, \textit{Candida rugosa}, \textit{Mucor javanicus}, \textit{Penicillium roquefortii}, \textit{Aspergillus niger}, \textit{Penicillium camembertii}), are inactive toward biodiesel synthesis in the described conditions.

Enzyme immobilization on Accurel could be performed by direct contact between the lipase solution and the support (Cheirsilp et al., 2008). However, it has been confirmed that ethanol pre-treatment improves the immobilization process by inducing a better penetration of the enzyme solution inside the hydrophobic Accurel and by reducing the enzyme thermodynamic activity, thus forcing the adsorption process (Foresti, & Ferreira, 2004). Enzyme adsorption with previous ethanol treatment of the support is carried out via: (i) wetting the support with, sequentially: ethanol, aqueous ethanol solution and finally with water, with intermediary filtration, or (ii) a single wetting with ethanol and then a direct contact with the enzyme solution without removing ethanol.

Accurel, due to its hydrophobic properties, should stabilise the enzyme in its open (active) conformation. Thus, it is considered as efficient for lipase immobilization.

Other synthetic polymers used for lipases immobilization comprise: hydrophobic polystyrene macroporous resin (Li & Yan, 2010), electrospun polyacrylonitrile nanofibers
with higher porosity and interconnectivity compared with other nanostructured carriers (Sakai et al., 2010), polymethacrylate (Salis et al., 2009), etc. The naturally occurring materials used as carriers for lipase immobilization include: activated carbon (Moreno-Paraján & Giraldo, 2011; Naranjo et al., 2010) and carbon cloth (Naranjo et al., 2010), celite (Ji et al., 2010; Shah & Gupta, 2007); hydrotalcite (Yagiz et al., 2007; Zeng et al., 2009), zeolites (Yagiz et al., 2007), etc. The role of the nature of the support surface on the loading and the activity, as well as on the operational stability of the immobilized enzyme has been investigated in details.

<table>
<thead>
<tr>
<th>Carrier</th>
<th>Immobilized lipase origin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accurel</td>
<td>Candida Antartica</td>
<td>Séverac et al., 2011; Tongboriboon et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Candida rugosa</td>
<td>Salis et al., 2008; Tongboriboon et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas cepacia</td>
<td>Salis et al., 2008; Tongboriboon et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas sp.</td>
<td>Cheirsilp et al., 2008</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas fluorescens</td>
<td>Salis et al., 2008, 2009</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas fluorescens</td>
<td>Tongboriboon et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Mucor javanicus</td>
<td>Salis et al., 2008</td>
</tr>
<tr>
<td></td>
<td>Penicillium roqueforti</td>
<td>Salis et al., 2008</td>
</tr>
<tr>
<td></td>
<td>Penicillium camembertii</td>
<td>Salis et al., 2008</td>
</tr>
<tr>
<td></td>
<td>Rhizopus oryzae</td>
<td>Salis et al., 2008</td>
</tr>
<tr>
<td></td>
<td>Thermomyces lanuginosus</td>
<td>Tongboriboon et al., 2010</td>
</tr>
<tr>
<td>Activated carbon</td>
<td>Candida Antartica</td>
<td>Naranjo et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Candida rugosa</td>
<td>Moreno-Paraján &amp; Giraldo, 2011</td>
</tr>
<tr>
<td>Celite</td>
<td>Candida rugosa</td>
<td>Shah &amp; Gupta, 2007</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas cepacia</td>
<td>Shah &amp; Gupta, 2007</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas aeruginosa</td>
<td>Ji et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas fluorescens</td>
<td>Shah &amp; Gupta, 2007</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>Pseudomonas cepacia</td>
<td>Li &amp; Yan, 2010</td>
</tr>
<tr>
<td>Carbon cloth</td>
<td>Pseudomonas cepacia</td>
<td>23 Naranjo et al., 2010</td>
</tr>
<tr>
<td>Poly(acrylonitrile)</td>
<td>Pseudomonas cepacia</td>
<td>16 Sakai et al., 2010</td>
</tr>
<tr>
<td>Ceramics</td>
<td>Pseudomonas cepacia</td>
<td>Shah &amp; Gupta, 2007</td>
</tr>
<tr>
<td>Pre-treated textile</td>
<td>Candida sp.</td>
<td>Chen et al., 2009; Li et al., 2010; Lu et al., 2007 and 2010</td>
</tr>
<tr>
<td>Hydrophilic resins</td>
<td>Rhizomucor miehei</td>
<td>De Paola et al., 2009</td>
</tr>
<tr>
<td>Silica</td>
<td>Rhizomucor miehei</td>
<td>Chen et al., 2009</td>
</tr>
<tr>
<td>Mg-Al hydrotalcites</td>
<td>Pseudomonas fluorescens</td>
<td>Salis et al., 2009</td>
</tr>
<tr>
<td>Resin D4020</td>
<td>Saccharomyces cerevisiae</td>
<td>Zeng et al., 2009</td>
</tr>
<tr>
<td>Polymethacrylate</td>
<td>Penicillium expansum</td>
<td>Li et al., 2009</td>
</tr>
<tr>
<td>Organosilicate</td>
<td>Pseudomonas fluorescens</td>
<td>Salis et al., 2009</td>
</tr>
<tr>
<td>Hydrotalcite</td>
<td>Thermomyces lanuginosus</td>
<td>Yagiz et al., 2007</td>
</tr>
<tr>
<td>Zeolites</td>
<td>Thermomyces lanuginosus</td>
<td>Yagiz et al., 2007</td>
</tr>
</tbody>
</table>

Table 1. Carrier used for lipases immobilization by adsorption.

### 3.2 Lipases immobilization by entrapment and/or encapsulation

Entrapment involves capture of the enzyme within a matrix of a polymer, although enzyme encapsulation refers to the formation of a membrane-like physical barrier around the enzyme preparation (Cao, 2005). The matrix is usually formed during the process of the immobilization. The enzyme entrapped in a gel matrix can be further encapsulated. Both processes require simple equipment and relatively inexpensive reagents. It is supposed that
enzymes immobilized by entrapment and/or encapsulation are more stable than the physically adsorbed ones. At the same time the immobilized enzymes maintain their activity and stability.

Numerous materials and techniques have been used for lipases entrapment and/or encapsulation. Some of the immobilization matrices developed during the last years (2005-2011) are enumerated in Table 2.

<table>
<thead>
<tr>
<th>Carrier</th>
<th>Immobilized lipase origin</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>κ-carrageenan</td>
<td>Candida Antarctica</td>
<td>Jegannathan et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Candida rugosa</td>
<td>Jegannathan et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Burkholderia cepacia</td>
<td>Jegannathan et al., 2009, 2010</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas fluorescens</td>
<td>Jegannathan et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Aspergillus niger</td>
<td>Jegannathan et al., 2010</td>
</tr>
<tr>
<td>Silica gel</td>
<td>Thermomyces lanuginosus</td>
<td>Khor et al., 2010</td>
</tr>
<tr>
<td></td>
<td>R. miehei</td>
<td>Macario et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas cepacia</td>
<td>Noureddini et al., 2005</td>
</tr>
<tr>
<td>Celite supported sol-gel</td>
<td>Candida Antarctica</td>
<td>Meunier &amp; Legge, 2010</td>
</tr>
<tr>
<td></td>
<td>Lipase NS44035</td>
<td>Meunier &amp; Legge, 2010</td>
</tr>
<tr>
<td>Silica aerogel</td>
<td>Candida Antarctica</td>
<td>Nassreddine et al., 2008</td>
</tr>
<tr>
<td></td>
<td>Candida Antarctica</td>
<td>Orçaire et al., 2006</td>
</tr>
<tr>
<td></td>
<td>Burkholderia cepacia</td>
<td>Orçaire et al., 2006</td>
</tr>
</tbody>
</table>

Table 2. Carrier used for lipases immobilization by entrapment and/or encapsulation.

For instance, a simple technique for lipase encapsulation in κ-carrageenan by co-extrusion was suggested by Jegannathan et al. (2009, 2010). Carrageenan has been selected because of its availability, biodegradability, low cost, and lack of toxicity. It was found that at optimized reaction conditions a methyl ester conversion up to 100% could be achieved in transesterification of palm oil using the liquid core encapsulated lipase PS from *Burkholderia cepacia*. The immobilized lipase was stable and retained 82% relative transesterification activity after five cycles.

Another technique for lipase immobilization by entrapment and/or encapsulation, which has received a considerable attention in recent years, is the sol-gel process. The method involves an aqueous solution of the enzyme, a catalyst (NaOH, NaF, HCl), and an inorganic-organic matrix precursor (alkoxysilane). The hydrolysis and condensation of the precursor result in an amorphous silica matrix that covers the enzyme. The method has been applied for *R. miehei* lipase encapsulation within the micellar phase of a surfactant that is self-assembled with silica (Macario et al., 2009). It has been demonstrated that the enzyme preserves its mobility and activity. More over, because of the activation of the enzyme catalytic centre by the hydrophobic groups of the surfactant, the immobilized lipase was more active than its free form. In addition, the obtained ordered mesoporous structure improved the stability of the enzyme and decreased the rate of leaching.

Comprehensive characterization of sol–gel immobilized lipase has been performed by Noureddini et al. (2005). Lipase PS was entrapped within a sol–gel polymer matrix, prepared by polycondensation of hydrolyzed tetramethoxysilane and isobutyltrimethoxysilane. The immobilized lipase was stable and more active than the free lipase toward the transesterification of soybean oil.

Various supports could be used to improve the stability of the entrapped/encapsulated enzymes. Celite supported lipase sol–gels were investigated aiming such problems as
activity, stability and reusability of the enzyme (Meunier & Legge, 2010). The three types of Celite considered (R633, R632, and R647) were compared to unsupported lipase sol–gels. It has been established that sol–gel immobilized lipase supported on Celite R632 allowed achieving an average conversion of 60% per gram of material for 6 h, and exhibited an average initial lipase activity comparable to that of the unsupported sol–gel formulation. Orçaire et al. (2006) report a technique for encapsulation of Candida Antarctica and Burkholderia cepacia lipases in silica aerogels reinforced with silica quartz fibre felt and dried by the CO$_2$ supercritical technique. The aerogel encapsulation permits maintaining the enzymes in a dispersion state similar to the dispersion prevailing in an aqueous solution, even in organic media, while agglomeration of the lipase occurs if it is used directly in the organic solvent. At present, sol-gel enzyme entrapment/encapsulation is considered to be the most successful immobilization technique for lipase immobilization.

### 3.3 Lipases immobilization by covalent attachment

Covalent attachment is a result of a chemical reaction between the active amino acid residues outside the active catalytic and binding site of the enzyme, and the active functionalities of the carrier (Cao, 2005). Although drastic and complicated, and strongly affected by the carriers’ properties, covalent attachment is the most efficient technique for enzyme immobilization. Some carriers used for covalent lipase immobilization are displayed in Table 3.

<table>
<thead>
<tr>
<th>Carrier</th>
<th>Immobilized lipase origin</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olive pomace</td>
<td><em>Thermomyces lanuginosus</em></td>
<td>Yücel, 2011</td>
</tr>
<tr>
<td>Resins</td>
<td><em>Thermomyces lanuginosus</em></td>
<td>Mendes et al., 2011</td>
</tr>
<tr>
<td></td>
<td><em>Pseudomonas fluorescens</em></td>
<td>Mendes et al., 2011</td>
</tr>
<tr>
<td>Polymers</td>
<td><em>Thermomyces lanuginosus</em></td>
<td>Dizge et al., 2008, 2009a, 2009b</td>
</tr>
<tr>
<td>Polyurethane foam</td>
<td><em>Thermomyces lanuginosus</em></td>
<td>Dizge &amp; Keskinler, 2008</td>
</tr>
<tr>
<td>Nb$_2$O$_5$ and SiO$_2$-PVA</td>
<td><em>Burkholderia cepacia</em></td>
<td>Da Rys et al., 2010</td>
</tr>
<tr>
<td>Chitosan</td>
<td><em>Candida rugosa</em></td>
<td>Shao et al., 2008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ting et al., 2008</td>
</tr>
<tr>
<td>Lewatit</td>
<td><em>Thermomyces lanuginosus</em></td>
<td>Rodrigues et al., 2010</td>
</tr>
<tr>
<td>Silica</td>
<td><em>Rhizopus orizae+Candida rugosa</em></td>
<td>Lee and al., 2008</td>
</tr>
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<td></td>
<td><em>Enterobacter aerogenes</em></td>
<td>Kumari et al., 2009</td>
</tr>
<tr>
<td>Magnetic nanostructures</td>
<td><em>Candida rugosa</em></td>
<td>Dussan et al., 2007, 2010</td>
</tr>
<tr>
<td></td>
<td><em>Thermomyces lanuginosus</em></td>
<td>Xie &amp; Ma, 2010</td>
</tr>
</tbody>
</table>

Table 3. Carrier used for lipases immobilization by covalent attachment

Yücel (2011) reports a method for *Thermomyces lanuginosus* lipase covalent binding on polyglutaraldehyde-activated olive pomace powder. The technique is cost effective, because of the low price of the support and because of the strong covalent bond formed, leading to enzyme stabilization without loss of activity, allowing the multiple reuse of the enzyme. Immobilized lipase was stable for 10 batches of pomace oil transesterification retaining more than 80% residual activity.

Among the other naturally occurring materials, chitosan is considered as appropriate for enzyme binding. Its membrane forming and adhesion ability, high mechanical strength and facility of forming insoluble in water thermally and chemically inert films make it suitable for lipase immobilization. For instance, *Candida rugosa* type VII lipase was fixed onto chitosan beads using a binary method consisting in the follows: (i) lipase
immobilization onto the hydroxyl groups of chitosan by activation with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; (ii) immobilization of additional lipase molecules through their amino groups to chitosan by cross-linking with glutaraldehyde. The immobilized enzyme has been used to catalyse the hydrolysis of soybean oil. Then, the feedstock containing free fatty acids, mono-, di- and triglycerides was esterified with methanol in the presence of an acid catalyst to produce biodiesel. It has been demonstrated that the enzymatic/acid-catalyzed hybrid process uses milder reaction conditions and allows avoiding the inactivation of the immobilized enzyme by polar compounds and increase biodiesel yields.

A new method for the synthesis of hydrophobic microporous matrices for enzyme immobilization, namely styrene-divinylbenzene-polyglutaraldehyde and poly(styrene-divinylbenzene)-polyglutaraldehyde copolymers, applying High Internal Phase Emulsions (HIPE) technique has been developed by Dizge et al. (2008, 2009a, 2009b). *Thermomyces lanuginosus* lipase was successfully attached to the support by covalent binding. According to the authors, the copolymers could be prepared in a short time and in large amounts and shapes. The immobilization efficiency, defined as the ratio of the activity of the immobilized enzyme to the activity of the free enzyme was found to vary from 80% to 89%. The immobilized enzyme retained its activity during 10-15 repeated batch reactions. Promising results in terms of enzyme thermal and operational stability improvement have been obtained using as supports for lipases immobilization silanized *Nb₂O₅* and *SiO₂*-PVA (Da Rys et al., 2010), and glutaraldehyde or ethanolamine activated silica gels (Lee and al., 2008; Kumari et al., 2009). However, the stability of the immobilized enzyme depends not only on the chemical/physical nature of the carrier, but also on the binding mode, the binding number, and the position of the binding on the enzyme surface (Cao, 2005), among other. Mendes et al. (2011) and Rodrigues et al. (2010) demonstrate that the multipoint covalent attachment of *Thermomyces lanuginosus* lipase on Toyopearl AF-amino-650M resin and on aldehyde-Lewatit is an efficient strategy for enzyme stabilization. It has been demonstrated that *Thermomyces lanuginosus* lipase immobilized on glyoxyl-resin is between 27 and 31 times more stable than the soluble lipase (Mendes et al., 2011).

Another important issue provided by enzyme immobilization concerns the localization of the interaction in the zone where the maximum concentration of reagents is present and/or at the interface between the immiscible heterogeneous phases, regarding lipases. For this purpose, lipases (from *Candida rugosa* and *Thermomyces lanuginosus*) have been immobilized on magnetic nanostructures (Fe₃O₄) and localized by application of a magnetic field. In addition, the method favours the simple and fast separation of the enzyme from the reaction mixture. Thus, it allows the intensification of the process and the reduction of the production costs.

### 3.4 Cells immobilization

The technological and economic advantages of immobilized cells over immobilized enzymes are well known (D’Souza, 1982): higher operational stability, higher yields of enzyme activity after immobilization, greater resistance to environmental perturbations, greater potential for multistep processes, and lower effective enzyme cost (enzyme purification and extraction are avoided). Despite of these benefits, only few investigations on the use of immobilized cells in biodiesel synthesis are reported until now (Fukuda et al., 2009; Hama et al., 2006, 2007; Li et al., 2007; Oda et al., 2005; Tamalampudi et al., 2008). The research efforts were focused on the immobilization no more than of *Rhysopus oryzae* within porous biomass.
support particles. The fixation was achieved spontaneously during batch cultivation. The applied technique (Atkinson et al., 1979) offers numerous advantages over other methods: the particles are reusable and mechanically resistant; additional reagents, aseptic handling of particles, and preproduction of cells are not necessary. It has been demonstrated that *Rhysopus oryzae* cells immobilized within biomass support particles can be used as low cost catalyst for biodiesel production.

4. Conclusion

Enzymatic approach to biodiesel production offers several advantages over the chemical catalysis currently applied. It is more efficient because of the enzyme specificity and selectivity, involves less energy consumption because of the mild reaction conditions, and is environmentally friendly because of the limited release of side products or wastes. Catalyst immobilization presents a number of additional benefits, such as repeated use of the enzymes, enhancement of their thermal and operational stability, localization of the interaction, effective control of the reaction parameters, etc., thus reducing the production cost and making the enzyme biodiesel synthesis an attractive alternative to other technologies. The present review provides an overview on the techniques applied for lipases immobilization.

5. References


ASTM D6751: American Society for Testing and Materials standard specification for biodiesel fuel (B 100) blend stock for distillate fuels


The Immobilized Lipases in Biodiesel Production


The book "Biodiesel: Feedstocks and Processing Technologies" is intended to provide a professional look on the recent achievements and emerging trends in biodiesel production. It includes 22 chapters, organized in two sections. The first book section: "Feedstocks for Biodiesel Production" covers issues associated with the utilization of cost effective non-edible raw materials and wastes, and the development of biomass feedstock with physical and chemical properties that facilitate it processing to biodiesel. These include Brassicaceae spp., cooking oils, animal fat wastes, oleaginous fungi, and algae. The second book section: "Biodiesel Production Methods" is devoted to the advanced techniques for biodiesel synthesis: supercritical transesterification, microwaves, radio frequency and ultrasound techniques, reactive distillation, and optimized transesterification processes making use of solid catalysts and immobilized enzymes. The adequate and up-to-date information provided in this book should be of interest for research scientist, students, and technologists, involved in biodiesel production.

How to reference
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