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REVIEW ARTICLE

Lipase Immobilization on Siliceous Supports: Application to Synthetic Reactions

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Abstract: In the last quarter century, immobilization of lipases has rapidly grown into a field of converging knowledge on material science, chemical engineering, biochemistry, etc. The majority of the cumulative work on lipase immobilization has been done on silica (Si) based supports. Researchers have investigated the effect of different Si architectures such as monolith, particles and aerogels on the properties of the immobilized lipase. These heterogenous catalysts have proved efficient in synthetic reactions such as biodiesel formation, where, unlike other supports, siliceous materials are able to resist nonaqueous media, providing stability and reusability. The use of numerous sources of this type of enzymes assures the universality of silica for lipase immobilization. This work summarizes the immobilization strategies and functionalization methods on siliceous materials that have provided a fundamental technology base to exploit the power of lipases as biocatalyts.

Keywords: Lipase, enzyme immobilization, silica, biocatalysis, biodiesel production, siliceous materials.

1. INTRODUCTION

As a support for protein immobilization, silica provides a high degree of chemical, physical and biological resistance, along with high surface areas that can be easily modified with functional groups all of which are highly desirable properties in support for enzyme immobilization [1–3].

Enzyme immobilization adapts enzymes in order to exploit their excellent properties of selectivity and specificity in industrial applications. It enables enzyme recycling and can provide stabilization, two major advantages over the use of soluble enzymes [4]. Research on the application of lipases, the most frequently used enzymes in biotechnology, has not been oblivious to the evidence piled-up on the benefits of enzyme immobilization. In the last quarter century, immobilization of lipases has rapidly grown into a field of converging knowledge on material science, chemical engineering, biochemistry, etc. Majority of the cumulative work on lipase immobilization has been done on silica based supports. Confirmation of the impact of siliceous materials on the development of lipase immobilized preparations is evident by the analysis of the literature in the field (Fig. **1**).

Previous reports have concluded that many different chemical functionalization methods of silica used as a support for lipase immobilization have impacted the properties of the biocatalyst [5]. Furthermore, it is possible to finely tune the pore size, crystallinity and shape of silica particles, along with scalability of the synthesis of such supports [6]. Like many other supports utilized in enzyme immobilization, silica supports are generally porous materials. These materials have been synthesized as macroporous with pore diameters exceeding 50 nm, mesoporous with pore diameters between 50 and 2 nm and microporous with pore diameter smaller than 2 nm [7, 8]. The change in porosity may or may not provide an access to the enzymes in the inner portions of the preexisting silica materials or facilitate the exchange of the products and substrates to the enzyme molecules immobilized within the material.

Silica supports have not only been used to immobilize lipase as microscaled supports but also at the nanoscale range. Nanocarriers have an inherently large surface area that lead to high enzyme loading capacity and consequently high volumetric enzyme acti-vities [9]. Their physical properties such as high tensile strengths make them robust and resistant to breakage through mechanical shear in the running reactor thus making them suitable for multiple reuses. Configurations such as nanoparticles, nanowires and nanosheets are just a few examples of the nanosized silica based supports that have been implemented in lipase immobilization [10, 11].

Immobilized preparations of lipase on silica supports have been used in a plethora of industrial applications [12–15]. Particularly, their application in biodiesel production has attracted special attention due to their ability to resist harsh synthetic conditions such as absence of water and presence of organic solvents [10]. Enzymatic production of biodiesel emerged as a greener alternative to the conventional energy consuming and contaminating chemical processes. In spite of the multiple examples of immobilized lipase in biodiesel production, new innovative methods with higher activity and increased stability are required in order to improve the economic viability of the process. The increasing growth of the use of siliceous materials in the immobilization foresees a great future for the application of immobilized lipase as catalysts in industrial processes especially in biodiesel synthesis.

The importance of siliceous supports for enzyme immobilization in general, and for lipases in particular, justifies the need for an

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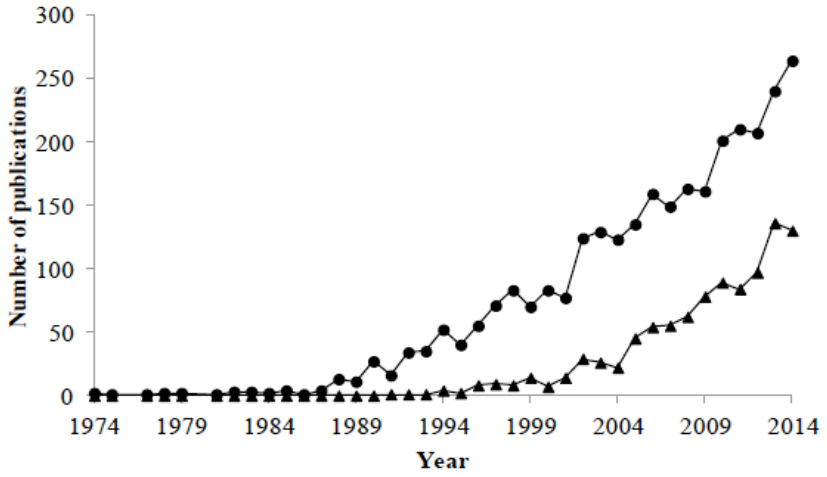
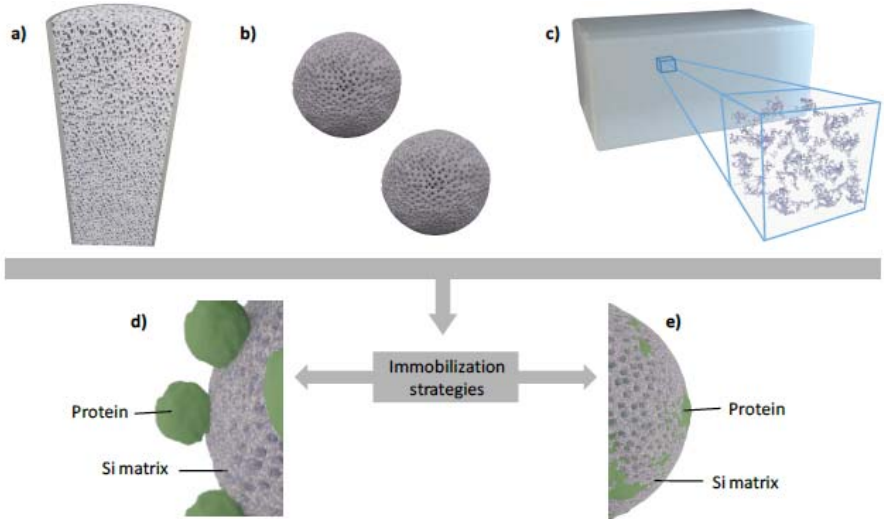


Fig. (1). Number of publications related to lipase immobilization (full circles) and lipase immobilization in siliceous supports (full triangles). Source: Scopus.



Scheme 1. Si architectures used for lipase immobilization (a) monolith, (b) particles, (c) aerogels, and strategies for lipase immobilization (d) Through interaction with the surface of the support, (e) through physical entrapment.

up-to-date revision of the work published on this subject. In this review, we will summarize the immobilization strategies and functionalization methods on siliceous materials that have provided a fundamental technology base to exploit the power of lipases as biocatalysts. A special focus will be paid on the application of siliceous-lipase heterogeneous catalysts in biodiesel production.

2. LIPASE IMMOBILIZATION ON PREFORMED SILICA SUPPORTS

Enzyme immobilization on siliceous supports relies mostly on two techniques depending on the presence or absence of the enzyme during the synthesis of the silica matrix. The most commonly used alternative is the preliminary synthesis of the support in the absence of the enzyme followed by enzyme immobilization onto the solid support. This strategy has been used with a variety of lipases from different sources, using diverse silica products and functionalizations of the silica matrix (Table **1**). The physicochemical properties of siliceous materials have facilitated multiple ways of functionalization which has provided the enzymatic preparations with different activity/stability properties [16–18].

Silica morphology. There are a number of silica structures able to support lipase immobilization (Scheme **1**). Silica particles stand out as the most for lipase immobilization. Their ubiquity responds to their commercial availability with well-defined physical characteristics, as well as their high degree of chemical, physical and biological resistance. Traditionally, particles with bigger diameter have stronger diffusion limitations of substrates, products and enzymes to be immobilized [12, 16, 19]. Frequently, silica particles can form agglomerates resulting from adsorption between them or the use of bifunctional reagents in functionalization. These agglomerations while increasing the apparent diameter of the particle, on the other hand, do not significantly increase the internal diffusion limitations. Furthermore, it has been hypothesized that substrates or products only have to diffuse through the smaller diameter of the individual particles of the agglomerate. Under this hypothesis, Fernández *et al.* [21] were able to prepare an immobilized preparation of *C. antarctica B* lipase loaded with 230 mg of enzyme/g of support. The agglomerates and the unbound particles showed similar catalytic activity and remain fully active and stable after 15 cycles in acetonitrile.

Another possibility in silica architecture for lipase immobilization is aerogels. These structures were first synthesized in 1931 by Samuel Kistler who defined them as the materials keeping their pore and network structure intact upon exchanging their pore liquid

Table 1. Immobilization strategies of lipases on preformed siliceous materials.

Silica support	Particle Diameter (μm)	Pore Diameter (nm)	Superficial Area (m^2/g)	Immobilization Strategy	mg of Immobilized Lipase/g Siliceous Support	Source of Immobilized Lipase	Ref.
Octyl-silica	60-200	15.9	73.2	Hydrophobic adsorption	-	*Pseudomonas fluorescens*	[20]
PSP (porous silica particles)	-	-	-	Covalent bonding via glutaraldehyde spacer arms, or directly coupled to epoxy-groups	86-15	Porcine pancreatic lipase	[60]
SBA-15	-	7.5	321	Covalent bonding	273	*Porcine pancreatic lipase*	[36]
SBA-15	-	-	-	Covalent bonding via isocyanate	280	*Mucor miehei*	[37]
SBA-15	-	8.5	700	Adsorption	0.5	*Mucor Miehei*	[38]
SBA-15	-	4.50	367	Adsorption	-	*Candida rugosa*	[39]
SBA-15	-	9	900	Adsorption	31		
Ms-3030 silica	-	33	280	Adsorption	34	*Candida antarctica*	[42]
Ms-3030 octyl silica	-	31	294	Adsorption	167		
MS3030 silica	70	-	254	Hydrophobic adsorption	230	*Candida antarctica*	[21]
MSU-H type mesoporous silicas	-	6/7.2/13.3	835/506/308	Adsorption followed by glutaraldehyde crosslinking	41.3/50.6/59.1	*Candida rugosa*	[47]
Octyl and thiol co-bonded silica (OTS)			280	Hydrophobic and strong cation exchange	62.5	*Candida rugosa*	[55]
Hierarchical porous silica	-	4-41	349	Hydrophobic adsorption followed by covalent bonding	-	*Pseudomonas stutzeri, Alcaligenes sp.*	[33]
MSU-H type mesoporous silica	-	-	-	Covalent bonding by glutaraldehyde crosslinking	64.5	*Candida rugosa*	[56]
Ordered mesoporous silica	-	4-14	589-860	Adsorption	-	*Candida antarctica*	[35]
Meso-macroporous silica	-	20	443	Covalent bonding	12 9.5	*Candida antarctica* *Alcaligenes.sp*	[32]
Silica gel	-	-	-	Adsorption	60	*Thermomyces lanuginosus*	[57]
Silica gel	-	-	-	Adsorption	25-125	*Bacillus cereus*	[23]
Silica gel	-	-	-	Metallic chelate adsorption and covalent bonding via epoxy groups	-	*Rhizomucor miehei*	[58]
SMB 300-5 silica gel	5	30	-	Covalent bonding	20.2	*Candida antarctica*	[59]
Epoxy-silica-polyvinyl alcohol composite	175	-	461	Covalent bonding	2.5	*Penicillium camembertii*	[30]
Ferric silica nanocomposite	-	4.5	202	Adsorption	29.5	*Burkholderia sp. C20*	[16]
Silica nanotubes	-	8.7	285	Adsorption		*Candida sp. 99-125*	[62]
Vesicular silica	-	15/20	314/362	Adsorption	44/48	*Candida rugosa*	[41]
Fumed silica	-	14.5	219	Adsorption	40		
Silica aerogel	-	5-100	110	Adsorption	10	*Rhizopus oryzae*	[22]
Monolithic silica	-	-	-	Covalent bonding by glutaraldehyde crosslinking	-	*Candida antarctica*	[25]

with gas [2]. Silica aerogels are extremely porous materials with highly specific surface areas. The unique properties of these highly porous materials are attributed to their irregular solid structure which can be tuned through the proper selection of their preparation conditions. By simple adsorption of lipase from *Rhizopus oryzae*, Kharrat *et al.* [22] recently prepared a stable immobilized enzyme able to produce butyl oleate. Using a similar immobilization strategy Lal Verma *et al.* [23] immobilized *B. cereus* lipase and catalyzed the production of 97 % (75 mM) isopropyl acetate in 9 h at 55°C in n-heptane. The preparation was able to resist 6 reuses under these conditions.

Silica monoliths with macropores (or throughpores) and mesopores have attracted much attention in the separation field [24] and lately, also in lipase immobilization [25, 26]. Compared to traditional particulate packings this structure enables high efficiency and low backpressure simultaneously for the flow through applications. The bimodal porous silica monolith is usually fabricated by a two-step method in which the hydrolysis and condensation of alkoxysilane occurs in the presence of polyethylene glycol forming a silica skeleton with macropores. Thereafter, when treated with a base, results in the formation of mesopores. The simplicity of this synthesis technique and the need for a scaffold structure for the monolith has potentiated its use in microreactors. Applications of immobilized lipase silica monolithic microreactors span from a range of hydrolytic and synthetic reactions with enzymes from different sources. All these applications shared the advantages of reduced reaction times, an enhanced control over reaction processes and the use of reduced reagent amounts [13, 27–29].

Immobilization strategies. Typically, silica surfaces can be functionalized using alkoxysilanes, that bind in a condensation reaction with surface silanol groups [6]. There is a wide variety of organosilanes available, allowing fine tuning of surface characteristics, like polarity or increased biocompatibility, and the ability to use those functional groups for different immobilization strategies.

Many authors have proven that the nature of the interaction between enzyme and support may have an impact on the properties of the final biocatalyst [30]. Zhang *et al.* demonstrated that porcine pancreatic lipase (PPL) could be covalently immobilized to various silica supports (nanoparticles with different functional groups attached through silanization), and applied with good results to biodegradable polymers synthesis [31]. The different supports used for PPL immobilization affected the biocatalyst performance according to the types of functional groups, but also their distribution on the carriers' surface consequently highlighting the importance of immobilization design to optimize the performance of the immobilized biocatalyst.

Bernal *et al.* [32] demonstrated that immobilized lipases from *Candida antartica B* and *Alcaligenes sp*. which were immobilized on hierarchical meso-macroporous silica activated with glyoxyl (aldehyde) groups, led to biocatalysts with high expressed activity (550 IU/g). This work also demonstrated that the stability of the biocatalysts at 60°C and pH 7 in non-reactive conditions improved dramatically after immobilization. This improvement was correlated to the nature of the support, given that lipases immobilized using the same chemistry to glyoxyl activated agarose were less stable. Further studies of Wilson´s research group explored the use of heterofunctional activated silica particles with glyoxyl and octyl groups [33]. Following a two-step immobilization strategy, the work capitalized on the affinity of lipases for hydrophobic surfaces. Once the lipases were hydrophobically adsorbed a change in the immobilization conditions (pH increase to pH 10) allowed the covalent immobilization of the adsorbed enzymes to the support. The use of both functionalities resulted in highly stable lipase preparations that were able to resist reuses at 40°C and the presence of pure acetone in the synthesis of lactulose palmitate using *Alcaligenes sp*. and *P. stutzeri* lipases.

Although, covalent interaction between lipases and siliceous supports may allow the preparation of stable immobilized biocatalysts, strategies that involved weaker interactions such as ionic or hydrophobic adsorption have also resulted in heterogeneous biocatalysts with increased stabilities and activities [20, 33]. Hydrophobicity and hydrophilicity of supports are crucial aspects of enzyme immobilization, specially working with lipases. Due to the hydrophobic nature of the active pocket of lipases and the lid that covers it, hydrophobic adsorption has been one of the preferred techniques for lipase immobilization. In this manner, the enzyme is adsorbed on the surface in a "open lid" position, therefore experiencing "interfacial activation". This has been extensively exploited to immobilize lipases, since it is reported that leads to hyperactivation of the enzyme, as well as it serves as a mean to selectively immobilize the lipases contained in a complex protein solution via hydrophobic adsorption. The synthesis and functionalization of the silica support will determine it hydrophobicity/hydrophilicity. Drozdz *et al.* [1] used multi-modal hierarchical pore structure silica to immobilize CALB. The functionalization of the support was done using various organosilanes to add hydrophobicity. Their results show that there is a strong relationship between the hydrophobicity of the functional groups and the amount of lipase that can be immobilized. More hydrophobicity results in higher loadings of lipase. Nevertheless, this does not necessarily mean better performance, since specific activity decreases due to multilayering occurring with heavy loadings. On the other hand, Boros *et al.* report that for immobilization of the same enzyme, silica gel functionalized with octyl groups underperformed the non treated counterparts [34].

A simple hydrophobic adsorption of *Pseudomonas fluorescens* lipase on octyl derivatized silica has allowed Lima *et al.* [20] to prepare a 12-fold more stable biocatalyst compared to its soluble counterpart, at 45°C and pH 8.0, in the presence of ethanol at 36 % (v/v). The preparation was tested in the transesterification of soybean oil using ethanol which performed better than the two commercially available immobilized preparations of lipases. Following the same immobilization strategy, Amurugam and Ponnusami [35] produced a biocatalyst with *Candida antarctica* lipase that was tested and produced good results in biodiesel production from *Calophyllum inophyllum* oil. Furthermore, the works of Lima *et al.* and Amurugam and Ponnusami demonstrated the operational stability of their biocatalyst by repeated use for more than 5 reuses with 100 % conversion in their respective transformations. These results excluded the possibility of enzyme leakage from the support which is a usual problem in adsorbed biocatalyst.

Influence of pore characteristics in siliceous materials for lipase immobilization. Pore size plays a fundamental role in the final results of immobilization [36–39]. Matsuura *et al.* [40] used their highly ordered mesoporous silica to determine the amount of lipase adsorbed on their support was related to the size and structure of the pore. Specifically, increasing the pore size from 4–7 nm and cubical as opposed to cylindrical porous structure in turn allowed more adsorption of the enzyme as well as higher activity. Wu *et al.* [41] in addition reported higher activity on larger pores with their *Candida rugosa* lipase immobilized on vesicular silica, thus owing these results to better transport of the substrate and product. Laszlo

Table 2. Strategies for the entrapment of lipases within siliceous materials.

Precursor	Source of Enzyme	Gelation Time	Additives During Synthesis	Pore Size	Specific Surface Area	Activity Immbilization Yield	Stability	Ref.
TMOS	*Rhizopus oryzae*	1 day	methyltrimethoxysilane (MTMS)-based	-	-	1000%	-	[69]
TMOS	*Burkholderia cepacia*	1 day	Methyltrimethoxysilane and Polyethylenglycol	9.24 nm	594.65 m2/g	4790%	100% residual activity after 12 hours at 60°C	[66]
TEOS	*Mucor miehei*	-	propyltrimethoxysilane (PTMS),	-	-	-	-	[17]
TEOS	*Candida rugosa*	seconds	Octyltrietoxysilane, calix[n]arene, calix[n]-NH2 and calix[n]-COOH	-	-	95.2% (for Calix[6]-NH2)	-	[70]
TEOS	*Burkholderia cepacia*	1 day	protic ionic liquids	3 nm	245 m2/g	1526%	-	[71]
TEOS	*Candida antarctica*	3 days	Poly-L-Lisine	2-40 nm	172 m2/g	70%	95% residual activity after 4 hours at 65°C	[44]

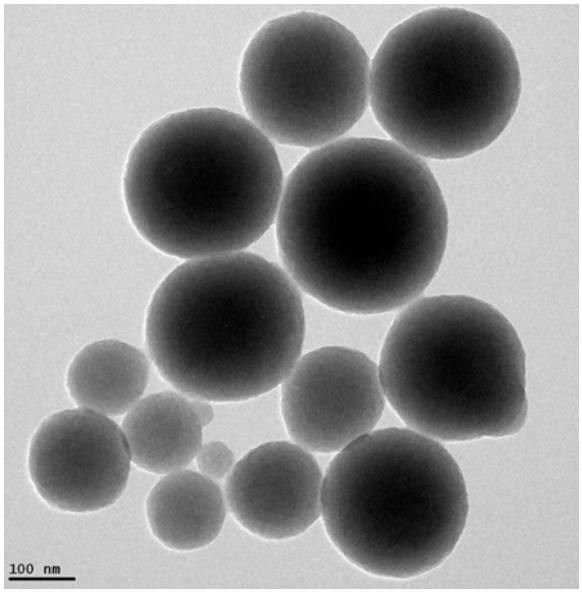


Fig. (2). Transmission electron microscopy of biomimetic silica nanoparticles.

et al. [42] found that pore structures influence enzymatic stability, being more stable in the ordered pore structures of SBA-15 silica as opposed to the amorphous features of MS-3030 silica. In addition, Bosley and Clayton [43], using hydrophobic controlled-pore glasses, found that for lipase activity to be independent of pore diameter it had to exceed 100 nm, while smaller pores significantly reduced the activity and below 35 nm the lipase from *Rhizomucor miehei* was not able to utilize the internal volume of the support. Kawachi *et al.* [44] immobilized a *Candida antarctica* lipase in a support with a pore diameter of 5 nm, and found it lost 30 % of activity, suggesting the pores were too tight for the enzyme, thus rigidifying its structure and decreasing activity. Independently, examination of pore size is a must in context to the development of an immobilization strategy. Smaller pores have the advantage of minimizing the leakage of enzymes which are not covalently bonded. Thus, proving enzymes immobilized by adsorption, which can be desorbed to the medium, can benefit from smaller pore sizes. Woong *et al.* [45] have reported that with repeated uses, *Pseudomonas cepacia* lipase immobilized on macroporous silica easily leached out in to the bulk solution, consequently, lowering the yield of the biocatalyst to levels below their mesoporous counterpart.

Salis *et al.* [46] examined various siliceous supports for lipase immobilization and found that higher loadings did not correlate with higher surface areas. Moreover, they report that the lipase first interacts with the free external adsorption sites mainly binding to the external surface of the particles, hindering the use of the internal surface. This phenomenon has also been reported by Yu *et al.* [47] explaining that the rapid adsorption on the external surface makes the diffusion to the internal pores of the silica particles slower due to blocking of the pore entrance by enzyme aggregation.

3. LIPASE IMMOBILIZATION BY ENTRAPMENT IN SILICEOUS MATERIALS

In situ immobilization happens when the enzyme is present in the reaction media during the synthesis of the support. In this type of immobilization, the enzyme is entrapped inside a porous network of the forming support during the process of silica condensation (Scheme **1**). The most common method for *in situ* immobilization is sol-gel entrapment [48]. For silica sol-gels the most commonly used precursors are tetramethoxy silane (TMOS) and tetraethoxy silane (TEOS). Some examples of lipases immobilized with the sol-gel method are shown in Table **2**.

A particular case of sol-gel silica synthesis is the biomimetic silicas. This type of synthesis is inspired upon natural biosilicification, [49–51]. It can be performed in near neutral pH and temperatures below 40°C in an aqueous medium, all of which are compatible for the entrapment of biomolecules like lipases (Fig. **2**) [52]. Entrapment in biomimetic silica has provided successful examples of lipase heterogenous preparations. *Candida antarctica* lipase has been immobilized in biomimetic silica with an efficiency close to 100%, displaying high levels of activity and improved thermal and

Table 3. Examples of biodiesel synthesis using Si immobilized lipases.

Source of Enzyme	Support	Type of Immobilization	Maximum Conversion (%)	Reuse	Source of Oil	Conditions	Ref
Pseudomonas cepacia	Magnetite coated with mesoporous silica	Covalent	54	55% of initial activiyy after 5 cylces	Soybean oil	methanol to oil molar ratio of 6:1, 40°C, 24 hours	[72]
Candida rugosa lipase and *Ryzopus oryzae*	Silica gel couples with glutaraldehyde	Covalent	99	90% incial activity after 35 cycles	Soybean oil	Methanol, 45°C, 3 hours	[67]
Rhizomucor miehei	Liposomes covered with microporous silica	Encapsulated	98	81% conversion after 5 cycles	Triolein	methanol to oil molar ratio of 6:1, 37°C, 3 hours	[73]
Pseudomonas fluorescens	Octyl functionalized silica	Hydrophobic adsoption	80	90% of initial activity after 5 cycles	Soybean oil	Ethanol to oil molar ratio of 7:1, 40°C, 48 hours	[20]
Pseudomonas fluorescens	epoxy silica-polyvinyl alcohol composite	Covalent	96		Beef tallow	ethanol to tallow molar ratio of 9:1, 45°C, 48 hours	[68]

pH stability [53]. *Pseudomonas cepacia* lipase was immobilized with a yield of 96%, and used for the synthesis of biodiesel obtaining a 79% conversion [54].

4. SYNTHETIC REACTIONS USING SILICA IMMOBILIZED LIPASES

Lipase immobilized preparations stand amongst the most important immobilized biocatalysts carrying out novel reactions in both aqueous and nonaqueous media. Particularly silica immobilized enzymes have been used in a myriad of synthetic reactions [55–59]: Bernal *et al.* [33] worked on the synthesis of a novel sugar ester with prebiotic characteristics, lactulose palmitate. Their use of heterofunctional silica as support resulted in almost 7-fold higher conversion than for the free lipase. Baeyer–Villiger oxidation (BVO) of cyclic ketones to lactones have been assayed by Drozdz *et al.* [1]. This work compares the same lipase from *Candida antarctica* on, the benchmark of immobilized lipases Novozyme 435, and a lipase immobilized in multi-modal hierarchical pore structure silica functionalized with alkyl chains. Their results showed that the silica immobilized catalyst produced a 99% yield in 29 hours, while the Novozyme 435 reached 90% conversion in 50 hours. Synthesis of polymers such as polycaprolactone (PCL) and poly(5,5- dimethyl-1,3-dioxan-2-one) have been synthesized by pancreatic porcine lipase covalently immobilized on porous silica particles [60]. Very interestingly, no polymers could be synthesized by the free enzyme. Khoobi *et al.* [61] report synthesis of ethyl valerate with esterification yields of 72.9% for the *Thermomyces lanuginose* lipase immobilized on silica gel as opposed to 37.7% for the free lipase. Kharrat *et al.* [22] immobilized *Rhizopus oryzae* lipase onto silica aerogels for n-butyl oleate synthesis by esterification of oleic acid with n-butanol. They obtained an 80% yield, compared to a 35% with the free lipase.

Additionally, an interesting feature of lipases is their ability for enantioselective hydrolysis or esterification. Bai *et al.* [62] report the use of a *Candida sp.* lipase immobilized on aminopropyl-grafted mesoporous silica nanotubes for the kinetic resolution of (R, S)-1-phenylethanol, by selective esterification. In this example, using capryilic acid as acyl donor, the lipase is highly selective for esterification of the R-enantiomer. Comparing the aminoporpyl-grafted support against the unmodified support, the author found a 22% increase in activity, which can be attributed to conformational changes observed by circular dichroism which suggested that portions of α helix were transformed into β sheets.

4.1. Examples in the Synthesis of Biodiesel

Biodiesel is a fuel consisting mainly of fatty acids monoalkylester. These are synthesized by alcoholysis of triacylglycerides, which produces fatty acid esters and glycerol as byproduct. It has recently become relevant given the diminishing oil reserves, increased awareness of environmental issues arising for the use of fossil fuels and increasing energy demands. In this context, biodiesel has found its way as a green alternative fuel, since it can be produced from oils and fats of biologic origin [63]. Industrially, biodiesel is most commonly produced by transesterification through homogeneous basic catalysis, using methanol and sodium or potassium hydroxide as catalyst. Despite being fast and relatively low cost, this method has several disadvantages: particularly, free fatty acids (more abundant in low quality cheap oils, which are the ideal feedstock for a greener approach) produce saponification with the catalyst and water. This complicates the downstream processing, as it complicate separation of biodiesel from glycerol due to gel formation, which requires large volumes of water for purification [64]. Lipases have the capacity to hydrolyze triacylglycerides to fatty acids and glycerol, providing and alternative as catalysts for biodiesel production. Thanks to high specificity and selectivity towards the transesterification products, secondary reactions that commonly hamper the homogeneous basic catalysis method can be avoided, lowering downstream processing costs [65]. Nevertheless, high cost of enzymes and slow reaction rates hinder their use at industrial scale. Immobilization of lipases on siliceous supports contributes to address this problem and has been utilized by different research groups in attempts to prove this type of supports as beneficial for biodiesel synthesis [26, 66] (Table **3**).

Lee *et al.* [67] proposes that enzymatic biodiesel production consists of three steps. The first is a slow rate determining step in which interfacial reaction occurs because of insolubility of alcohol and oils. As the reaction progresses the products (fatty acid alkyl esters and glycerol) act as emulsifiers and the interface disappears, becoming and homogeneous phase and increasing the reaction rate. Last, as glycerol concentration builds up, the alcohol moves to the glycerol layer and the rate of reaction is decreased. Lima *et al.* [20] have reported that using octyl functionalized silica, glycerol was not absorbed on silica surface. This proves to be an important advantage for batch and continuous use, since glycerol accumulation on the surface of the biocatalyst needs to be removed. Silica as a support can play an important role in the enzymatic synthesis of biodiesel. Macario *et al.* [18] report that the external mesoporours silica surface used for encapsulating the lipase, is able to adsorb the hydrophobic substrate, allowing for a higher fatty acid methyl esther yield as the free enzyme in the same time.

Viscosity is a very important property of biodiesel, since high viscosity leads to poor atomization of the fuel and operation of fuel injectors. An epoxy silica-polyvinyl alcohol composite was used by Silva *et al.* [68] to compared two different immobilized lipases *Burkholderia cepacia* and *Pseudomonas fluorescens* using beef tallow as substrate. Although performing similarly, *Pseudomonas fluorescens* lipase was superior in lowering the viscosity of the product, which also contributed to higher conversions. Therefore, importance lies in selecting enzymes that can process more efficiently viscous substrates such as beef tallow, resulting in a fuel with better properties.

5. CONCLUSIONS

The piled up knowledge on the preparation and use of silica immobilized lipase proves the impact of the use of this supports for biocatalytic applications. Specifically, for the synthetic reactions where, unlike other supports, siliceous materials are able to resist nonaqueous media, providing stability and reusability. The use of numerous sources of this type of enzymes assures the universality of silica for lipase immobilization. The potential for the application of enzymes in the synthesis of biodiesel remains a challenge and as such it requires to further explore additional lipase immobilization strategies where silica stand out as a promising support. We envision a future of work dedicated to material development with emphasis in nanosupports and hybrid structures and scale up applications of silica-immobilized enzymes for synthesis of biodiesel.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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