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**Immobilization of lipases on hydrophobic supports: immobilization mechanism,
advantages, problems, and solutions**

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Abstract

Lipases are the most widely used enzymes in biocatalysis, and the most utilized method for enzyme immobilization is using hydrophobic supports at low ionic strength. This method allows the one step immobilization, purification, stabilization, and hyperactivation of lipases, and that is the main cause of their popularity. This review focuses on these lipase immobilization supports. First, the advantages of these supports for lipase immobilization will be presented and the likeliest immobilization mechanism (interfacial activation on the support surface) will be revised. Then, its main shortcoming will be discussed: enzyme desorption under certain conditions (such as high temperature, presence of cosolvents or detergent molecules). Methods to overcome this problem include physical or chemical crosslinking of the immobilized enzyme molecules or using heterofunctional supports. Thus, supports containing hydrophobic acyl chain plus epoxy, glutaraldehyde, ionic, vinylsulfone or glyoxyl groups have been designed. This prevents enzyme desorption and improved enzyme stability, but it may have some limitations, that will be discussed and some additional solutions will be proposed (e.g., chemical amination of the enzyme to have a full covalent enzyme-support reaction). These immobilized lipases may be subject to unfolding and refolding strategies to reactivate inactivated enzymes. Finally, these biocatalysts have been used in new strategies for enzyme coimmobilization, where the most stable enzyme could be reutilized after desorption of the least stable one after its inactivation.

Key words: lipase interfacial activation, lipase immobilization, intermolecular lipase crosslinking, heterofunctional supports, enzyme amination, acyl-vinylsulfone supports, enzyme coimmobilization

1. Introduction

1.1. Enzymes in industry

Modern industrial chemistry is pushed towards “greener” processes and the production of growingly complex substrates (e.g., chiral and multifunctional compounds regio and enantiomerically pure). This demand has produced a clear boost to biocatalysis as a way to maximize atomic economy using the selectivity and specificity of enzymes as catalysts (Castilla et al., 2018; Ferreira-Leitão et al., 2017; Jangale and Dalal, 2017; Krüger et al., 2018; Littlechild, 2017; Sheldon and Woodley, 2018; Sun et al., 2018). Enzyme biocatalysis is outstanding in this regard, as enzymes, when performing their physiological function, are highly selective and specific, usually under mild conditions such as at room temperature and atmospheric pressures, and in aqueous medium (Castilla et al., 2018; Ferreira-Leitão et al., 2017; Jangale and Dalal, 2017; Krüger et al., 2018; Littlechild, 2017; Sheldon, 2017, 2018; Sheldon and Woodley, 2018; Sun et al., 2018). This makes enzymes the recipients of many properties required by chemical industry.

However, it must be considered that enzymes are biological catalysts that have been designed to fulfill a physiological function. Thus, enzymes must respond to changes in the medium, and their stability should not be measured in months but in hours. That way, some enzyme properties are often far from industrial requirements, being unstable, inhibited by some components of the reaction, or water soluble (Schmid et al., 2001; Schoemaker et al., 2003). Furthermore, the selectivity and specificity of the enzyme on the applied reaction (often far from the physiological one) may be neither adequate for industrial purposes.

Fortunately, in the last years, a very rapid evolution (almost revolution) has been promoted in many areas related to the design of an industrial biocatalyst and has permitted to give answers to these enzyme-specific problems. Metagenomics permits to access all available biodiversity without the need for a cultivable organism to produce the enzyme(it may even occur that the given organism no longer exists) (Cowan et al., 2005, 2015; Daniel, 2005; Fernández-Arrojo et al., 2010; Ferrer et al., 2008; Peña-García et al., 2016; Simon and Daniel, 2011). Directed mutagenesis may permit, using proper protein modelling, to improve any enzyme property (de Graaf et al., 2007; van den Heuvel et al., 2001). Directed evolution permits to mimic natural evolution, but accelerating and focusing the process on both the target enzyme and the target enzyme property (Bornscheuer et al., 2019; Frey et al., 2019; Guangyue Li et al., 2018; Zeymer and Hilvert, 2018). Chemical modification has also evolved to produce more specific protein modifications (Boutureira and Bernardes, 2015; Cowan and Fernandez-Lafuente, 2011; Sakamoto and Hamachi, 2019; Spicer and Davis, 2014). The water enzyme solubility has been solved via immobilization techniques (Gonçalves et al., 2019; Sheldon and van Pelt, 2013; Zdarta et al., 2018). However, nowadays, the enzyme immobilization step has proved to be a powerful tool to improve many enzyme features, and an up-to-date immobilization must pursue improving some of the other enzyme limitations, such as its stability, activity, specificity, selectivity or resistance to inhibitors and chemical reagents (Bilal et al., 2018, 2019; Brady and Jordaan, 2009; Fernandez-Lafuente, 2009; Garcia-Galan et al., 2011; Iyer and Ananthanarayan, 2008; Mateo et al., 2007; Rodrigues et al., 2013). It has been reported that, if properly designed, it may be also used to purify the enzyme during the immobilization process (Barbosa et al., 2015). Recent advances in the coimmobilization of cofactors may facilitate the production of immobilized biocatalysts with possibilities near

to those inside natural cells (Benítez-Mateos et al., 2017, 2018; López-Gallego et al., 2017; Schmid-Dannert and López-Gallego, 2019; Velasco-Lozano et al., 2017). Very interestingly, immobilization is compatible with any other strategy designed to improve enzyme properties (Bernal et al., 2018b; Fernandez-Lafuente, 2009; Hernandez and Fernandez-Lafuente, 2011a; Rodrigues et al., 2011, 2014; Rueda et al., 2016c). That way, enzyme immobilization has become a very prominent step in the design of an industrial enzyme biocatalyst.

1.2. Lipases in biocatalysis

Among the enzymes with industrial interest, lipases enjoy a privileged position. Hydrolases (EC 3, according to the Enzyme Commission number) are undoubtedly the most used enzymes in biotransformations, due to their broad substrate specificity, commercial accessibility, absence of any cofactor dependence, and capability of working at high substrate concentrations, not only in their natural environment (aqueous solutions) but also in organic and neoteric solvents (Bertau and Jeromin, 2015; Bornscheuer and Kazlauskas, 2006; Busto et al., 2010; Kazlauskas, 2016; Lopez-Iglesias and Gotor-Fernandez, 2015; Mendez-Sanchez et al., 2016; Sirola et al., 2013). Among them, lipases (EC 3.1.1.3) are the most prominent group, according to the great number of reported processes in which they have been used, not only at lab scale (de Miranda et al., 2015; Reetz, 2002; Roy et al., 2013; Seddigi et al., 2017) but also at industrial level. Thus, it is not surprising to observe how the number of publications dealing with these enzymes is continuously growing, as depicted in Figure 1, extracted from Web of Science, using

“lipase” and “biocatal*” or “biotransform*” as search criteria. Clearly, the use of lipases in applied biocatalysis is still a hot topic, as it can be inferred from this figure, taking into account that the last data row only corresponds to a three-year period (from January 2016 to January 2019); therefore, we can foresee that by the end of 2020 the overall number of publications of this *lustrum* will far exceed 12.000.

The natural *in vivo* activity of lipases is the hydrolysis of triglycerides to free fatty acids and glycerol (Zechner et al., 2012); however, *in vitro* lipases have been used to efficiently catalyze a plethora of reactions, most of them somehow related to their natural role, such as esterifications (Aguieiras et al., 2019; Barsé et al., 2018; Chang and Ho, 2011; Chen et al., 2011; Cruz et al., 2018; de Lima et al., 2018; Duan et al., 2011; Fernandes et al., 2018; Foresti et al., 2009; Foukis et al., 2018; Gonçalves et al., 2008; Hamryszak et al., 2018; Huemmer et al., 2018; Kamaruddin et al., 2006; Kato et al., 2018; Kovalenko et al., 2018; Li et al., 2011; Liu et al., 2010; Parashar et al., 2018; Pereira et al., 2018b, 2018a; Spelbrink et al., 2018; Stergiou et al., 2013; Yang et al., 2018; Zare et al., 2019), transesterifications (Bäckvall et al., 2019; Basheer et al., 2018; Bauwelinck et al., 2018; Chado et al., 2018; Choi et al., 2018; Gumbyte et al., 2018; Jahangiri et al., 2018; Kolodziejska et al., 2018; Moreno-Perez et al., 2018; Poppe et al., 2018b; Rana et al., 2018; Tokuyama et al., 2018; Vasilescu et al., 2018; Yoshimura et al., 2018; Yu et al., 2018b), and acidolysis (or interesterification) (Abed et al., 2017, 2018; Bahari and Akoh, 2018; Chojnacka and Gładkowski, 2018; de Paula et al., 2018; Engelmann et al., 2018; He et al., 2017; Kavadia et al., 2018; Korma et al., 2018; Y. Li et al., 2018; Ribeiro et al., 2017; Rychlicka et al., 2018; Samoylova et al., 2017; Sivakanthan et al., 2019; Sneha and Jeyarani, 2018; Sousa et al., 2018; Speranza et al., 2018; Wang et al., 2017; Yamamoto et

al., 2018; Yu et al., 2018a; Yüksel and Şahin-Yeşilçubuk, 2018; Zaharudin et al., 2016; Zou et al., 2016b, 2016a).

As commented above, the attractiveness of lipases is caused by their specific mode of action, the so-called interfacial activation (see next section). In fact, the possibility of recognizing hydrophobic substrates and being active in bulk hydrophobic media, such as organic solvents, promoted the development of enzymology in what was called “non-conventional solvents” (Adlercreutz, 2008; Bornscheuer, 2010; Cao and Matsuda, 2016; Herbst et al., 2012; Kamal et al., 2013; Kumar et al., 2016; Roy et al., 2013; Salihu and Alam, 2015; Stepankova et al., 2013; Ueda, 2010; Wang et al., 2016; Zhen, 2005). Presently, the combination of lipases (and some biocatalysts) and bio-based derived green solvents is increasingly becoming trendy (Alcántara and Dominguez de Maria, 2018; Clarke et al., 2018; Hernáiz et al., 2010; Pace et al., 2012; Sethi et al., 2018; Sheldon and Brady, 2018; Sheldon and Woodley, 2018), due to the synergistic increase in the inherent process sustainability obtained by combining both aspects.

Interestingly, lipase capability for working in solvents different from water was described as a “promiscuous behavior” (Arora et al., 2014; Asler et al., 2010; Babbie et al., 2010; Bornscheuer and Kazlauskas, 2012; Brodzka et al., 2018; Busto et al., 2010; Carbonell and Faulon, 2010; Copley, 2017; Gatti-Lafranconi and Hollfelder, 2013; Gerlt, 2004; Green et al., 2010; Hult and Berglund, 2007; Humble and Berglund, 2011; Kapoor and Gupta, 2012; Kazlauskas, 2005; Khersonsky et al., 2006; Khersonsky and Tawfik, 2010; Lathe et al., 2015; Lopez-Iglesias and Gotor-Fernandez, 2015; O’Brien and Herschlag, 1999; Ondari and Walker, 2009; Sheldon and Brady, 2018; Xu and Lin, 2007). Furthermore, the catalytic flexibility of lipases clearly goes beyond the possibility of

employing them in non-aqueous media; in fact, lipases display an extremely wide substrate recognition capability, converting many different substrates. This may occur due to the physiological necessity of hydrolyzing tri, di and monoglycerides containing very different incoming fatty acids. Thus, they can be used for catalyzing (trans, inter) esterifications of a wide range of substrates, generally taking advantage of their inherent ability to recognize stereochemistry; indeed, triglycerides possess an stereogenic carbon atom at C2 of the glycerol moiety, provided that fatty acid residues at C1 and C3 are different (in other cases, if the acyl groups at C1 and C3 are the same, C2 is then a pseudo-asymmetric carbon). For this reason, the resolution of racemic mixtures of acids, alcohols, esters, amines or amides, or rather the desymetrization of this type of compounds bearing a prochiral centre, is a very usual process catalyzed by lipases (Cunha et al., 2015; de Miranda et al., 2015; Hoyos et al., 2016; Patel, 2012; Rodríguez-Mata and Gotor-Fernández, 2015; Seddigi et al., 2017). It is worth mentioning that going deeper into the knowledge of the molecular basis explaining the stereochemical recognition of (pro)chiral substrates is also a very attractive research area (Colombo et al., 2000; Cygler et al., 1994; Juhl et al., 2009; Li et al., 2008, 2015; Park et al., 2009; Rahman et al., 2012; Sin et al., 2009; Toledo et al., 2016; Trodler et al., 2009; Trodler and Pleiss, 2008).

Finally, the last type of enzymatic promiscuity of lipases is their ability to catalyze chemical reactions quite different from acyl-transfer processes, mainly C-C bond formation (Fu et al., 2018; He et al., 2010; Lopez-Iglesias and Gotor-Fernandez, 2015; Miao et al., 2015; Mitrev et al., 2016).

For all these reasons, as shown in Figure 1, the application of lipases in biotransformations is still an immensely attractive research field.

1.3. Interfacial activation of lipases

The natural function of lipases is the hydrolysis of oils and fats (Jaeger et al., 1999; Jaeger and Eggert, 2002). In this respect, triglycerides are very poorly water soluble molecules. However, lipases are able to fulfil this function, and that differentiates a lipase from a standard esterase: lipases are interfacial enzymes (Schmid and Verger, 1998; Verger, 1997). Most lipases have a structure that includes a polypeptide chain that has a hydrophobic face and a hydrophilic face (Brzozowski et al., 1991; Grochulski et al., 1993). In the so-called closed form of the lipases, the hydrophobic area of the lid interacts with the hydrophobic areas surrounding the active center, secluding it from the reaction medium. This lid is in some instances so small that does not fully seclude the lipase active center from the medium (e.g., lipase B from *Candida antarctica*) (Uppenberg et al., 1994), and in some other cases it is a sophisticated structure including two peptides (e.g., lipase from *Geobacillus thermocatenulatus*) (Carrasco-López et al., 2009).

This lid can move, exposing the active center and the large hydrophobic pocket to the medium. In homogenous saline aqueous medium, this form is unstable and the lipase tends to be in the closed form. However, in the presence of hydrophobic structures, the lipase becomes adsorbed via this large hydrophobic pocket, and that way the lipase may attack the insoluble drop of a triglyceride (Brzozowski et al., 1991; Grochulski et al., 1993). (Figure 2). From an applied point of view, that means that using a substrate with limited water solubility, the lipase follows standard saturation kinetics until reaching the formation of drops of substrate. In that moment, the lipase may become adsorbed on the drop of

substrate, the lipase open (and active) form is fixed, and the lipase activity experiences a boost in activity. For this reason, this step is called interfacial activation. This fact was known long before the real cause for this was identified (Chapus et al., 1976, 1978; Verger and De Haas, 1976).

This tendency of lipases to become adsorbed on any hydrophobic surface produces some problems. For example, lipases tend to form lipase-lipase dimers involving the open form of each of the lipase molecules (Fernández-Lorente et al., 2003; Palomo et al., 2003a) (Figure 3). This has been used to prepare immobilized lipases used as chromatographic matrices for lipase purification or immobilization (Palomo et al., 2004a, 2005a; Volpato et al., 2010) (Figure 4), but it may be a problem for lipase handling, because the properties of dimers and monomers may be very different (Fernández-Lorente et al., 2003; Palomo et al., 2003a; Wilson et al., 2006). Lipases can also become adsorbed on any other hydrophobic component of the extract, like a hydrophobic protein (Palomo et al., 2003c; Zhang et al., 2017). Again, this generates some problems as free and adsorbed lipase molecules may have fully different stability/activity behaviors. Moreover, lipases may become immobilized on any hydrophobic support following a similar behavior (Fernandez-Lafuente et al., 1998) (Figure 5). This subject will be the main topic of the current review.

1.4. Lipase immobilization

As any other enzyme, lipases need to be immobilized prior to most of their applications and it is interesting to take full advantage of this immobilization process in order to improve the enzyme properties, thus making the process more profitable than just

the simplification of enzyme recovery (Iyer and Ananthanarayan, 2008; Mateo et al., 2007; Rodrigues et al., 2013). Lipase immobilization, as in any other enzyme immobilization, should pursue not only the preparation of a heterogenous biocatalyst, but also should permit the enhancement of as many of the enzyme properties as possible, such as stability, activity or specificity (Bilal et al., 2018, 2019; Brady and Jordaan, 2009; Fernandez-Lafuente, 2009; Garcia-Galan et al., 2011; Iyer and Ananthanarayan, 2008; Mateo et al., 2007; Rodrigues et al., 2013).

In this regard, it is remarkable that, due to the great flexibility of the active center of lipases (this may be checked comparing the structured of the open and closed forms of lipases in the protein data bank), the features of these enzymes (including activity, selectivity or specificity) have been found to be easily tuned during immobilization (e.g., altering the degree of multipoint covalent attachment, the enzyme orientation or the enzyme environment) (Brem et al., 2012; Chaubey et al., 2006; dos Santos et al., 2015e; Fernandez-Lorente et al., 2008; Fernández-Lorente et al., 2001; Jia Liu et al., 2012; Mathesh et al., 2016; Palomo et al., 2002a, 2003b; Rodrigues et al., 2013; Tacias-Pascacio et al., 2017).

For this reason, the biocatalysts must be assayed exactly under the required conditions and in the target reaction. Thus, having a “universal” optimal biocatalyst is far from straightforward. However, differences between the biocatalysts properties for a specific process may become huge. In some cases this implies having different immobilized biocatalysts with the same enzyme but able to produce the S or the R isomer from a racemic mixture (Palomo et al., 2002b). To this end, all available immobilization techniques should be utilized to produce a large library of biocatalysts from a specific lipase (Rodrigues et al., 2013).

However, some points can complicate the preparation of these immobilized lipase biocatalysts. The tendency of some lipases to interact with hydrophobic components of the crude substrate (hydrophobic proteins, other lipase molecules) causes lipase immobilization to invoke some additional precautions (Fernández-Lorente et al., 2003; Palomo et al., 2003a, 2003c; Zhang et al., 2017) (Figure 6). As the lipase aggregation percentage may depend on the exact crude purity degree and concentrations, as well as on other immobilization conditions, reproducibility may not be simple. It has been suggested that using some detergents may permit to break these aggregates and ensure the immobilization of monomeric lipases (Palomo et al., 2003a) (Figure 7), but many lipases may become inactivated by these reagents (dos Santos et al., 2014; Fernandez-Lorente et al., 2007). Another problem is that the enzymes immobilized inside a porous catalyst cannot be subjected to interfacial activation by external interfaces; lipases will be only activated via interfacial activation if using nearly anhydrous media.

Both problems may be solved using hydrophobic supports for lipase immobilization: only the monomeric form of the lipase may become immobilized, and, as detailed below, the lipase is already in the open form (Manoel et al., 2015a).

1.5. Lipase immobilization via interfacial activation on hydrophobic supports

The immobilization of lipases on hydrophobic supports has been attributed to the interfacial activation of the lipases versus the support surface (Fernandez-Lafuente et al., 1998) (Figure 5). Therefore, this immobilization method only involves the percentage of lipase molecules that are in monomeric and open form, giving as a result the

immobilization of the open form of the monomeric lipase (Manoel et al., 2015a). This makes the opening of the lipase by other external interfaces unnecessary, and it becomes a guarantee of the immobilization of the lipases in monomeric and open form. The exact structure and properties of the support may condition the final properties of the lipases (Cabrera et al., 2009; Fernandez-Lorente et al., 2008; Hernandez et al., 2011a; Tacias-Pascacio et al., 2016), and there are also some reports suggesting that the immobilization conditions may also alter the final enzyme catalytic features (Silveira et al., 2017).

Not all lipases have the same affinity for a given support. There are some examples of lipases unable to become immobilized on a very hydrophobic support but immobilized on another less hydrophobic support (Palomo et al., 2005b; Segura et al., 2004, 2006). However, as a general rule the most hydrophobic support may immobilize more lipases than a less hydrophobic one. This can even permit the fractionation of mixtures of lipases as a function of their affinity for different supports (Brabcová et al., 2013; Fernández-Lorente et al., 2005; Sabuquillo et al., 1998; Volpato et al., 2011).

As a clear example of the simplicity and usefulness of this immobilization protocol, Novozym 435, that is the immobilized form of the lipase B from *Candida antarctica* and very likely the most utilized commercial immobilized biocatalysts, has been prepared using this strategy employing Lewatit VP OC 1600 as support (Bansode et al., 2017; Du et al., 2004; Endo et al., 2018; Galgali et al., 2018; Kaar et al., 2003; Ortiz et al., 2019; Polloni et al., 2017; Sbardelotto et al., 2018; Zhang et al., 2018).

Table 1 shows a compilation of the most utilized hydrophobic supports and the lipases that have been immobilized on them (from 2010 until present). These biocatalysts

have been applied in media that range from fully aqueous ones to anhydrous media, including in some cases ion liquids or supercritical fluids. It is possible to observe the great variety of materials used for lipase immobilization. Among the hydrophobic materials used, it is possible to find functionalized bentonites including both acid activated bentonite and organically modified bentonite with cetyltrimethyl ammonium bromide (Dong et al., 2013), octyl-Sepharose (Almeida et al., 2018; Barbosa et al., 2012b; Galvis et al., 2012; Ghattas et al., 2014; Habibi et al., 2013; Kurtovic et al., 2011; Manoel et al., 2015a; Ruiz et al., 2013; Volpato et al., 2011), Lewatit VP OC 1600 (a macroporous acrylic polymer resin) (Kurtovic et al., 2011), magnetic microspheres with carboxyl groups prepared by copolymerization of vinyl acetate, acrylamide, and acrylic acid (Zhang et al., 2012), hydrophobic silk fibers functionalized with methyl groups (Chen et al., 2012), sol-gel materials based on n-propyltrimethoxysilane/tetraethoxysilane (Hu et al., 2013), hydrophobic magnetic particles functionalized with the organosilane surfactant [3-(trimethoxysilyl) propyl] octadecyldimethylammonium chloride (Liu et al., 2012), D4020, a nonpolar hydrophobic resin (Liu et al., 2013), a NKA macroporous resin (polystyrene) (Liu et al., 2011; Su et al., 2014), nonwoven viscose fabric coated with methyl groups through treatment with polydimethylsiloxane (Weina Li et al., 2011), Sepabeads (macroporous acrylic support) activated with octadecyl groups (Hernandez et al., 2011b), silica aerogels modified with methyl groups (Gao et al., 2010), D152H (macroporous resin, acrylic-divinylbenzene) (Yan et al., 2013), small or large poly-hydroxybutyrate beads (Mendes et al., 2012), porous magnetic polymeric microspheres based on poly(methylmethacrylate-*co*-divinylbenzene)-Fe₃O₄ (Meng et al., 2013), octyl-agarose cross-linked with dextran sulfate (Pizarro et al., 2012), polyacrylonitrile nanofibers membrane (Gupta et al., 2013), MSU-H (Michigan State University) type mesoporous

silica (Yu et al., 2013), mesoporous silica with same pore size but with varying particle size (Gustafsson et al., 2012), styrene-divinylbenzene MCI GEL CHP20P porous support (de Abreu et al., 2014; Martins et al., 2013; Poppe et al., 2013), Diaion HP20LX (porous styrene-divinylbenzene beads) (Hernandez et al., 2011a), polysulfone globules (Gupta et al., 2010), MS-3030 silica derivatized with octyltriethoxysilane (Laszlo et al., 2011), Accurel MP 1000 (macroporous polymer of propylene) (Manoel et al., 2015b; Tecelão et al., 2012), Eupergit C (epoxy-activated acrylic beads) (Barbosa et al., 2010; Ruiz et al., 2013; Tecelão et al., 2012), Lewatit VP OC 1600 (DVB-crosslinked methacrylate polymer) (Tecelão et al., 2012), phosphorous-containing polyurethanes (Guncheva et al., 2011b), polyvinyl alcohol microspheres with different degrees of crystallinity (Bergamasco et al., 2013), octadecyl Sepabeads (Hernandez and Fernandez-Lafuente, 2011b), nanostructured SnO₂ material (Guncheva et al., 2011a), a macroporous resin (D3520, cross-linked polystyrene) (Zhao et al., 2013), Accurel MP macroporous polymer of propylene (Cavalcanti et al., 2018; Séverac et al., 2011), silica materials with multi-modal hierarchical pore structure and modified on the silica surface with organosilanes (methyl, octyl and hexadecyl groups) (Drożdż et al., 2013), hydroxyapatite (Dimitrijević et al., 2012), poly-(D,L)-lactic acid polymer (Kamel et al., 2011), mesoporous carbon beads (Quirós et al., 2011), macroporous resin DA-201 (Ning Liu et al., 2012), butyl-Toyopearl 650M (Volpato et al., 2011), mesoporous poly-methacrylate particles (Diaion® HP 2-MG) (Bassi et al., 2016), spherical beads made of octadecyl-functionalized methacrylic resins (Basso et al., 2016), treated silk woven fabric with amino-functional polydimethylsiloxane (Li et al., 2014), Purolite® A109, a polystyrenic macroporous resin (Mihailović et al., 2014), hydrophobic silk fibers functionalized with methyl groups (Tao et al., 2014), functional polyphosphazene cyclomatrix microspheres containing glucose and dodecyl moieties (Chen

et al., 2016), polymeric supports with core-shell morphology (polystyrene, methyl polymethacrylate, vinyl polyacetate) (Cunha et al., 2014), cellulose/lignin hydrogel beads (Park et al., 2015), matrix film of hydroxypropyl methyl cellulose (Badgujar and Bhanage, 2016), phenyl-Toyopearl, C18-Sepabeads (Ghattas et al., 2014), Immobead 150 (macroporous acrylic polymer) (de Abreu et al., 2014), mesoporous TiO_2 modified with comb polymer poly(sodium acrylate)-*g*-methoxy poly(ethylene oxide) (Wang et al., 2014), mesoporous hydrophobic poly-methacrylate particles (Diaion® HP 2-MG) (Lage et al., 2016), surface modified mesoporous silica with alkoxysilanes (C1-SiO_2 , C8-SiO_2 , C16-SiO_2 , SH-SiO_2 , Ph-SiO_2 , $\text{NH}_2\text{-SiO}_2$) (Zou et al., 2014), magnetic Fe_3O_4 nanoparticles (Xun et al., 2015), poly(urea-urethane) nanoparticles (Chiaradia et al., 2016), octadecyl groups-capped silica nanowires (Jin et al., 2018), mesoporous organosilica: virus-like organosilica nanoparticles (Jiang et al., 2019), chitosan functionalized with different alkyl chains (C4 , C8 , C12) (Urrutia et al., 2018), macroporous Purolite C-18 (Silveira et al., 2019), mesoporous Sepabeads C-18 (methacrylate resin containing octadecyl groups) (Moreno-Perez et al., 2018), octadecyl methacrylate (Verdasco-Martín et al., 2018), Diaion™ HP-20 (Vilas Bôas et al., 2018) resin (copolymer styrene-divinylbenzene), Lifetech™ ECR8804M (octadecyl methacrylate), Lifetech™ ECR8806M (octadecyl methacrylate), Lifetech™ ECR1090M (styrene) and Lifetech™ ECR1030M (DVB/methacrylic polymer) (Tacias-Pascacio et al., 2017), mesoporous TiO_2 functionalized with phenylaminopropyl trimethoxysilane (Gao et al., 2018), biomimetic silica nanoparticles functionalized with octyl trimethoxysilane and 3-glycidoxypopyl)methyldiethoxysilane (Cazaban et al., 2018), low density polyethylene (Accurel EP400) and high density polypropylene (Accurel EG100 and EP100) (Anand and Weatherley, 2018), silica hybrid functionalized via incorporation of β -cyclodextrin (Martin et al., 2018), magnetic

poly(styrene divinylbenzene) particles functionalized with oleic acid (Bento et al., 2017), pore-expanded mesoporous silica (SBA-15) (Rios et al., 2018), phenyl-modified ordered mesoporous silica (Zheng et al., 2017), magnetic mesoporous silica (Xue et al., 2018), ECR1030 resin (a divinyl benzene/acrylate polymer) (Li et al., 2017), polymer matrix consisting of hydroxypropyl methyl cellulose and polyvinyl alcohol (Mathpati et al., 2018), core-shell polymer supports based on poly(methyl methacrylate-*co*-divinylbenzene)/poly(methyl methacrylate-*co*-divinylbenzene) (Cipolatti et al., 2018), octadecyl-modified mesoporous-silica nanoparticles (Kalantari et al., 2017), stearic acid-modified silica nanoparticles (Singh and Mukhopadhyay, 2018), polymeric film made of hydroxypropyl methylcellulose and chitosan (Badgujar et al., 2017), Purolite C18 (octadecyl methacrylate) (Silveira et al., 2017), magnetic copolymer styrene-divinylbenzene (Silva et al., 2018), silica coated with octyl groups (Vescovi et al., 2017), polyhydroxybutyrate particles (Corradini et al., 2017), and Diatomite (Zhang et al., 2019). Table 1 shows also some of the uses of these biocatalysts.

2. Some clues on the mechanism of lipase immobilization on hydrophobic supports

As previously stated, lipase immobilization on hydrophobic supports has been attributed to the interfacial activation of the lipase versus the hydrophobic surface of the supports. This means that lipases become adsorbed on the hydrophobic supports as previously described in an oil drop. That is, lipase immobilization on hydrophobic support does not follow a conventional hydrophobic adsorption, but is a pseudo-affinity recognition of the hydrophobic surface of the support.. However, the proofs of this immobilization

mechanism are mainly indirect ones. We will summarize some of them here because they may be also relevant to maximize the profit of this immobilization strategy (Manoel et al., 2015a).

First, the immobilization of lipases on hydrophobic supports proceeds even at very low ionic strength. For this reason, the strategy may be used to purify lipases from other enzymes (Manoel et al., 2015a). The use of high ionic strength, far from improving lipase immobilization, makes it slower, and also reduces the purification achieved via this immobilization. At high ionic strength, the open form of the lipase will be disfavoured, and as this is the only form of the lipase able to become adsorbed via interfacial activation, lipase immobilization becomes slower. As a high ionic strength is a requirement to have a standard hydrophobic adsorption (Manoel et al., 2015a), we can discard that a simple hydrophobic adsorption may be responsible for this adsorption. In fact, many lipases may be immobilized on octyl-agarose beads even in 40% (v/v) glycerol or polyethyleneglycol (unpublished results from our research group). That is, the effect of ionic strength in the immobilization rate is the opposite of that expected in a standard hydrophobic adsorption, and it suggests that it is more rapid when the percentage of open forms of the enzyme is favored, that is, using low ionic strength or even some organic cosolvents (at moderate concentrations).

Immobilization on hydrophobic supports is very strong, but the lipases may be desorbed using a moderate concentration of detergents (much higher than the recommended one to regenerate the hydrophobic column) (Manoel et al., 2015a), permitting the reuse of the support. In many instances, a significant lipase hyperactivation is observed when they are immobilized on hydrophobic supports (Manoel et al., 2015a). However, that is not

a general rule and it depends on the support, enzyme and substrate (Rodrigues et al., 2015; Tacias-Pascacio et al., 2016). Considering the strong modulation of enzymes by immobilization or chemical modification, this is quite weak a proof of the interfacial activation produced by the immobilization, even taking into account that in general lipases increase their activity when immobilized on octyl agarose, except lipase B from *Candida antarctica* (Rueda et al., 2015b), that has not a blocking lid (Uppenberg et al., 1994).

One more interesting proof of the special mechanism of lipase immobilization on hydrophobic supports is the rate of inhibition with irreversible inhibitors (Carrasco-López et al., 2009; Manoel et al., 2015a). Lipases immobilized on octyl-agarose beads are rapidly inhibited and also maintain this inhibition rate even at high ionic strength, (while the inhibition rate of the covalently immobilized enzyme is slower. The same occurs with the activity, the covalent preparations rapidly decrease their activity with increasing ionic strength, while the enzyme immobilized on octyl agarose almost maintains its activity intact. This suggests that the enzyme immobilized on hydrophobic supports has the active center more exposed to the medium and that it cannot become closed when increasing the ionic strength (Manoel et al., 2015a). However, the covalent preparation of a lipase will have a tendency to become closed at high ion strength, lowering the activity and the irreversible inactivation rate (using irreversible inhibitors).

Thus, even though an interfacially activated lipase bonded to the hydrophobic support has not been “visualized”, there are many indirect evidences that fit with the mechanism of interfacial activation and suggest this immobilization mechanism. It is even more obvious that the mechanism of immobilization of lipases on hydrophobic supports is fully different to a conventional hydrophobic adsorption.

3. Advantages of the lipase immobilization on hydrophobic supports

This immobilization protocol has many advantages. The first one is that it is very simple, it may be performed at almost any pH (as long as the enzyme is soluble and stable), and other experimental conditions will not be as critical as in other immobilization protocols. It is very rapid and it readily coats the support surface with enzyme without any enzyme treatment (Fernandez-Lafuente et al., 1998).

Moreover, at low ionic strength, lipases are the only water soluble proteins that are massively immobilized on fairly hydrophobic supports. That way, we can use this immobilization strategy to get the one step immobilization/ purification of the lipase (Almeida et al., 2018; Bastida et al., 1998; Brabcová et al., 2013; de Abreu et al., 2014; Fernandez-Lafuente et al., 1998; Ghattas et al., 2014; Kerzel and Mersmann, 1992; Palomo et al., 2004b).

Additionally, since the open and monomeric form of the lipase is immobilized, we bypass all the problems raised in other immobilization protocols regarding immobilization of mixtures of monomers/dimers. Also, although the enzyme active center is confronted with the support surface (Manoel et al., 2015a), the acyl layer and the big pocket of the enzyme permits the entry of large substrates and very high activities may be achieved, in some cases higher than those obtained using the free lipase directly hyperactivated versus the drops of substrate (Bastida et al., 1998), even when the substrate is as large as a triglyceride (Al-Duri et al., 1995; Fernández-Lorente et al., 2011b, 2011a; Mahmood et al., 2008).

Moreover, it has been shown that the open and adsorbed form of lipases is very stable (Cygler and Schrag, 1999; Jaeger et al., 1993; Kim et al., 1997). In fact, they become more stable than multipoint covalently immobilized lipases where the conformational equilibrium is maintained (dos Santos et al., 2015e, 2015d, 2015c; Palomo et al., 2002c). Furthermore, as previously shown, these lipase forms are more resistant to changes in the reaction conditions, like changes in the ion strength. That way, we increase the range where lipases may be used (Manoel et al., 2015a) (Figure 8).

However, it seems that the stabilized open form of the lipase exhibited after lipase immobilization on hydrophobic supports causes this enzyme to be specially sensitive to the negative effects of phosphate anions and to the positive effect of some cations (Ca^{2+} and Mg^{2+}) on enzyme stability (Arana-Peña et al., 2018; Cipolatti et al., 2018; Fernandez-Lopez et al., 2015, 2016; Zaak et al., 2017b).

The use of a hydrophobic supports is convenient in certain cases. For example, in the production of biodiesel or in esterification reactions catalyzed by lipases, one of the main problems is the accumulation of hydrophilic compounds in the biocatalyst that can lead to enzyme inactivation. This may be reduced using a hydrophobic support. Marty and his group were pioneers in showing this advantage of very hydrophobic supports in biodiesel synthesis by reducing glycerin adsorption on the biocatalyst (Dossat et al., 1999; Marty et al., 1997; Séverac et al., 2011). Later, other researchers showed that these advantages constituted a general trend (e.g., prevention of water accumulation in esterification reactions) (Friedrich et al., 2013; Graebin et al., 2012; Martins et al., 2013).

In this sense, it was shown that the already very high stability of the lipase B from *Candida antarctica* in the presence of hydrogen peroxide could be further increased by immobilizing the enzyme on a very hydrophobic support (Hernandez et al., 2012). In this research, the moderately hydrophobic preparation Novozym 435 presented a stability in the presence of 1 M hydrogen peroxide similar to CALB immobilized on more hydrophobic supports (octadecyl Sepabeads or porous styrene-divinylbenzene beads) when the hydrogen peroxide concentration was increased to 10 M (Hernandez et al., 2011a; Hernandez and Fernandez-Lafuente, 2011b).

One final advantage of hydrophobic supports is that all these improvements of the enzyme properties are achieved using a reversible immobilization method (Virgen-Ortíz et al., 2017b). After enzyme inactivation to the desired level, it is not necessary to discard enzyme and support. The enzyme may be released from the support (using detergents, chaotropic reagents), leaving a clean support that can be reused until it is physically broken and the very small particles produce the blocking of the filters. That way, a critical aspect in choosing a hydrophobic support may not be the price, but the mechanical resistance of the support (dos Santos et al., 2015a; Garcia-Galan et al., 2011).

4. Problems of lipase immobilization on hydrophobic supports

Therefore, immobilization on hydrophobic supports at low ionic strength may permit the one step enzyme immobilization/purification/stabilization, and the support can be reused after desorbing the inactivated enzyme (Bastida et al., 1998; Fernandez-Lafuente et al., 1998; Palomo et al., 2002c). It looks like an unbeatable lipase immobilization

protocol. However, this immobilization protocol has a significant problem: the enzyme may be released to the medium under certain conditions, producing both biocatalyst operational inactivation and product contamination (Chen et al., 2007, 2008; Garcia-Galan et al., 2011; Hilterhaus et al., 2008; Kirk and Christensen, 2002; Nicolás et al., 2015; Petry et al., 2006; Saunders and Brask, 2010; Weinberger et al., 2018).

It has been reported that lipases may become released from the support at high temperature, although it is unclear if the enzyme is released from the support and then inactivated (the immobilized enzyme is much more stable) or if the lipase becomes inactivated and later it is then released to the medium (Rueda et al., 2015b). Even with this problem, as stated above, lipases immobilized on hydrophobic supports are more thermostable than multipoint covalently attached enzymes (dos Santos et al., 2015e, 2015d, 2015c; Palomo et al., 2002c).

Another cause for lipase desorption from hydrophobic supports is the presence of organic cosolvents (Rueda et al., 2015b; Saunders and Brask, 2010). This is an expected phenomenon, the hydrophobic solvents will reduce the strength of the adsorption of the enzyme on the hydrophobic support making enzyme desorption easier. This causes that in most instances, multipoint immobilized lipase biocatalysts are more stable in aqueous/organic solvents than the just interfacially activated enzyme on hydrophobic supports (dos Santos et al., 2015e, 2015d). Even ionically exchanged lipases may become more stable than the interfacially activated enzymes in these systems (Arana-Peña et al., 2018). It should be remarked that this problem is mainly related to systems consisting of aqueous medium containing high concentrations of organic cosolvent miscible with water,

in anhydrous medium lipase desorption is not so simple, as the enzyme is not soluble in an organic solvent.

Another additional problem of this immobilization technique is when the use of the biocatalysts involves detergent-like substrates or products (Virgen-Ortíz et al., 2017c) (Figure 9). Using Novozym 435, this desorption problem was reported long time ago using clearly amphipathic molecules (Hilterhaus et al., 2008; Nicolás et al., 2015; Ortiz et al., 2019). It must be considered that in many cases lipases will be used to modify triglycerides, and that free fatty acids, di or monoglycerides and phospholipids, may be considered molecules with detergent properties able to release the lipase from hydrophobic supports (Hilterhaus et al., 2008; Nicolás et al., 2015; Ortiz et al., 2019). Moreover, it has been recently shown that this effect may be found even using relatively small products, such as high concentrations of dibutylin or even diacetin (Hirata et al., 2016b, 2016a; Virgen-Ortíz et al., 2017c) (Figure 9).

In order to solve this problem without discarding all the other advantages from lipase immobilization on hydrophobic supports, researchers have looked for different solutions. For example, coating the whole immobilized biocatalyst with silicon has been attempted, although this is mainly addressed to solve the mechanical fragility of some supports (Wiemann et al., 2009). This was also found to reduce enzyme leakage (Wiemann et al., 2009). However, some strategies are directly related to the prevention of enzyme leakage, and these go from the modification of the already immobilized biocatalysts to get some enzyme-enzyme or enzyme-support crosslinking that may avoid (or at least reduce) the release of the adsorbed enzymes to the design of new heterofunctional supports with additional groups which promote enzyme adsorption through their interaction(s) with the

enzyme. This way, researchers have tried to maintain the good features of the immobilization protocol on hydrophobic supports (one step immobilization/purification/stabilization) while increasing the strength of the enzyme immobilization (establishing other enzyme-support interactions). In the next sections we will describe these strategies.

5. Physical or chemical intermolecular crosslinking

The crosslinking of several lipase molecules interfacially activated versus the support surface promotes that the lipase planar aggregate will become immobilized by multiple sites, and the release of the aggregate requires the simultaneous desorption of all involved proteins. The larger the aggregate is, the more difficult its release from the support becomes (Figure 10). If the intermolecular crosslinking of all immobilized enzyme molecules in a porous support is achieved, enzyme release will become impossible, although this task may be very difficult to achieve.

5.1. Glutaraldehyde

The first crosslinking strategy was using glutaraldehyde as crosslinking reagent (Kim et al., 2013; Pujari et al., 2006). This small reagent can only work in a massive enzyme molecules intermolecular crosslinking if the immobilized enzyme molecules are really close to each other (Fernandez-Lafuente et al., 1995). Immobilization using hydrophobic supports may allow this rapid immobilization. In fact, it has been described

that enzyme molecules become so near to one another that it can alter the stability properties of the biocatalyst (Fernandez-Lopez et al., 2017a; Zaak et al., 2017d). For example, Novozym 435 was treated with glutaraldehyde, but results were unsatisfactory and enzyme release was still a problem (Saunders and Brask, 2010). Later, using octyl-agarose, immobilized CALB was found to give some intermolecular crosslinking (Barbosa et al., 2012b). The percentage and size of the lipase aggregates can be modulated by the immobilization conditions (they were smaller when the immobilization rate decreased) or the loading of the support (Barbosa et al., 2012b). However, some monomeric lipase molecules can be found in all cases, showing that the intermolecular crosslinking was not fully effective, although the enzyme activity and stability increased. Many lipase dimers, trimers or tetramers could be found in most cases, and their concentration increased when the immobilization rate was increased (Barbosa et al., 2012b), but a massive crosslinking of all immobilized enzyme molecules could not be achieved.

This failure in obtaining a massive intermolecular crosslinking may be explained by the size of the crosslinking reagent: only groups located very near to one another can be involved in the crosslinking, and only if primary amino groups are confronted. This makes very difficult to achieve the total crosslinking of the immobilized enzyme molecules.

5.2. Aldehyde dextran

Aldehyde dextran has some advantages compared to glutaraldehyde. It is a multifunctional and large reagent and that way the problems derived from the requirements

of distance and alignments between the involved groups are significantly decreased (Fernández-Lafuente et al., 1999; Fuentes et al., 2004) (Figure 9).

There are some reports showing the use of this strategy. The first one permitted to hydrolyze an oil in the presence of organic cosolvents avoiding enzyme release (Fernández-Lorente et al., 2011b). Later, using aminated lipases, the modification with aldehyde dextran was used to even increase the enzyme thermostability (Orrego et al., 2018).

This modification proved to be effective, but it has a problem: it was so effective that the lipase immobilization becomes irreversible. After enzyme inactivation both enzyme and support must be discarded.

5.3. Poly-ionic polymers

As an alternative, the physical crosslinking of lipases immobilized on octyl agarose with large ionic polymers like polyethylenimine or dextran sulfate was studied. Polyethylenimine has been used in many instances to improve protein properties (Virgen-Ortíz et al., 2017a), here we will focus only on the paper related to preventing enzyme release (Figure 10). Results showed a decrease on the release of the enzyme to the medium in organic solvent or in thermal inactivations, accompanied by an increase in enzyme stability (Fernandez-Lopez et al., 2017b, 2018; Peirce et al., 2016a).

This was attributed to the physical intermolecular crosslinking of the immobilized enzyme molecules, but these polymers may have other beneficial effects for enzyme features (Virgen-Ortíz et al., 2017a). For this reason, researchers modified with

glutaraldehyde octyl-agarose lipase preparations previously coated with polyethylenimine, making the enzyme-polymer interactions irreversible. The results showed a massive intermolecular crosslinking, not observed using just glutaraldehyde, and strongly dependent on the enzyme loading (Zaak et al., 2017a). This result suggested that the main effect of the polymer coating was the physical crosslinking of the enzyme molecules and not just the beneficial effects of the PEI coating of the enzyme.

Thus, this simple ionic polymer coating, that in many instances improved enzyme activity, was found to significantly reduce enzyme leakage from the support and improve enzyme stability (mainly in organic medium, by coupling a lower enzyme desorption to a reduction of the organic solvents in the enzyme environments) (Fernandez-Lopez et al., 2017b, 2018; Peirce et al., 2016a).

Reversibility of the enzyme immobilization was maintained, although lipase desorption from the support became more difficult after its inactivation due to an increase of the enzyme-support interactions during protein unfolding (Virgen-Ortíz et al., 2017b).

6. Heterofunctional supports

Another approach to solve enzyme leakage from the hydrophobic supports is to modify the support to produce a heterofunctional support. The requirement is that the first immobilization must be via interfacial activation, and later, changing the conditions, other groups placed on the support may establish new interactions with the enzyme, making enzyme desorption impossible.

This strategy makes that the support activation and enzyme immobilization protocol may be more complex than the direct use of hydrophobic supports, but after immobilization the biocatalyst will not be submitted to any further modification. Obviously, both strategies may be combined if desired.

6.1. Glutaraldehyde and epoxy-acyl groups supports

The first report of a heterofunctional acyl-chemical reactivity used acyl- (amino/glutaraldehyde) moieties to have the lipases immobilized in a covalent way (Abaházi et al., 2014; Boros et al., 2013). These first papers did not analyze the main cause for enzyme immobilization (to check the fraction that remains just immobilized via interfacial activation) nor the effectiveness of the covalent immobilization. However, they showed that the enzyme immobilized on these supports has a better performance (e.g. it is resistant to detergent washing, suggesting a covalent immobilization) (Boros et al., 2013). Later, acyl-glutaraldehyde supports (octyl-silica-amino-glutaraldehyde) were used to immobilize lipase B from *Candida antarctica* (Vescovi et al., 2016). The immobilized enzyme was more stable than the enzyme immobilized in monofunctional octyl, amino-glutaraldehyde or in bifunctional octyl and glyoxyl, or octyl and epoxy groups. The biocatalyst was used in the synthesis of fructose oleate in tert-butyl alcohol with 70% of conversion and possibility of biocatalysts reuse (Vescovi et al., 2016). However, once again a deep study on the main cause for immobilization or the effectiveness of the covalent immobilization is lacking. Just epoxy-acyl supports were analyzed in this study. Another report used epoxy-acyl groups. In this research, lipase from *Rhizomucor miehei* was

immobilized on silica supports activated with both octyl-triethoxysilane and glycidoxypentyl-trimethoxysilane (Garmroodi et al., 2016). Enzyme leaching was reduced by this experiment. The researchers also analyzed the effect of epoxy to octyl ratio but they focused the studies mainly on enzyme activity recovery.

6.2. Glyoxyl-acyl groups supports.

The design of glyoxyl-acyl support was another answer to the problem of enzyme release. In the first approach, silica was modified with glyoxyl and octyl groups, and the enzymes immobilized on this support presented better performance than those prepared using only glyoxyl or only acyl groups (Bernal et al., 2014, 2015, 2018a, Guajardo et al., 2015a, 2015b). They did not show that the first cause for immobilization remained the interfacial activation nor did they analyze the effectiveness of the covalent immobilization, although direct immobilization via glyoxyl under the employed conditions could be considered negligible. Biocatalysts prepared following this strategy were successfully employed in diverse reactions, some related to detergents (acyl-sugars) showing that the enzyme was not released to the medium (Bernal et al., 2014, 2018a) or R- α -monobenzoate glycerol (Guajardo et al., 2015b).

Shortly after, octyl-glyoxyl agarose was utilized to immobilize several lipases (Rueda et al., 2015b; Suescun et al., 2015) (Figure 11). In this case, it was confirmed that lipase immobilization proceeded via a first interfacial activation versus the support, and also that a further incubation at alkaline pH (pH 10) was required to have some enzyme-support covalent immobilization (Rueda et al., 2015b; Suescun et al., 2015). However, even

after long incubation times, it was found that a significant percentage of lipase molecules remained just interfacially activated, without any covalent attachment (accounting for 30 to 5% of the enzyme molecules) (Rueda et al., 2015b; Suescun et al., 2015). This fraction could be discarded by washing the biocatalyst with detergents. The biocatalyst exhibited higher thermostability, and a much higher stability in the presence of organic cosolvents than the glyoxyl or octyl biocatalysts (Rueda et al., 2015b; Suescun et al., 2015). Very interestingly, these biocatalysts could be reused in certain reactions where just octyl-agarose immobilized enzymes were fully desorbed (Hirata et al., 2016b, 2016a).

6.2.1. Problems of the glyoxyl-acyl supports

These supports have several problems, added to the certain complexity of the immobilization problem. The first one, the immobilized enzymes may be unstable under the conditions required for glyoxyl/enzyme reaction (pH around 10) (Rueda et al., 2015b; Suescun et al., 2015). Secondly, a reversible immobilization method was transformed into an irreversible one, avoiding the support reuse after enzyme inactivation (Rueda et al., 2015b; Suescun et al., 2015). Thirdly, not all enzyme molecules become covalently immobilized on the support (Rueda et al., 2015b; Suescun et al., 2015). Finally, even though enzyme stability was enhanced, a multipoint covalent attachment could not be expected: first because the amount of Lys groups in the area near the support is scarce; second because not even one point immobilization was achieved for all enzyme molecules (Rueda et al., 2015b; Suescun et al., 2015). Researchers have offered solutions for each of these specific problems.

6.2.2. Solution to the immobilization of lipases on glyoxyl-acyl supports.

6.2.2.1. Increase enzyme stability at alkaline pH value

To solve the problem of enzyme stability at pH 10 or higher, the simplest strategy is to use some stabilizing reagent. The inactivation problem was very relevant only with some enzymes, as for example that from *Rhizomucor miehei* (RML), but in these cases around 50% of the initial activity was lost (Rueda et al., 2015b; Suescun et al., 2015). The utilized stabilizer must have some features: it must stabilize the enzyme at pH 10 while not interfering in the covalent reaction of the enzyme with the support.

This stability problem rose shortly after showing that some cations may stabilize lipases immobilized on hydrophobic supports (Fernandez-Lopez et al., 2015). These cations did not alter the reactivity of amino and glyoxyl groups. Thus the effect of these cations on enzyme stability at pH 10 was checked (Fernandez-Lopez et al., 2016). At this pH, a problem was the solubility of the cations, but a buffer that prevented cation precipitation was found (Fernandez-Lopez et al., 2016). Curiously, only Ca^{2+} remained able to stabilize the enzyme at pH 10 among the tested cations (Fernandez-Lopez et al., 2016). Finally, using Ca^{2+} , RML was immobilized on glyoxyl-octyl almost maintaining the lipase activity after alkaline incubation intact (Fernandez-Lopez et al., 2016).

6.2.2.2. Reuse of the support

6.2.2.2.1. Reactivation of the covalently immobilized and inactivated enzyme.

The use of acyl-supports allowed lipase desorption after inactivation, and that way the support can be reused after enzyme inactivation (Bastida et al., 1998; Fernandez-Lafuente et al., 1998; Sabuquillo et al., 1998) while the formation of secondary amino bonds between enzyme and support makes this unfeasible (the bonds are stable even at 210 °C in 1 M HCl) (Pedroche et al., 2007). This was an apparent disadvantage of the new protocols. However, the immobilization on hydrophobic supports was not compatible with the development of reactivation of inactivated lipases by unfolding of the inactivated lipases via incubation in chaotropic reagents and refolding by further incubation in aqueous medium (Rueda et al., 2015a; Suescun et al., 2015). Thus, enzymes immobilized on glyoxyl-octyl supports were submitted to several cycles of enzyme inactivation, unfolding and refolding, permitting to recover a large percentage of the lost activity (Rueda et al., 2015a; Suescun et al., 2015). Therefore, not only the support but the full biocatalyst can be reused when using glyoxyl-octyl supports in lipase immobilization. The problem found by the authors was that the recovered activity was not constant as it should occur if the enzyme molecules were fully unfolded and refolded in a similar support structure. This suggested that the enzyme could not be fully unfolded, or that the support structure (that was agarose) could be influenced by the incubation in guanidine (Zucca et al., 2016). The use of more rigid supports in these reactivation strategies using this kind of supports has not been reported.

6.2.2.2.2. Maintaining the reversibility of the immobilization

Another alternative is to maintain all enzyme-support interactions reversible, mixing interfacial activation (mainly hydrophobic) with other physical interactions of a fully different nature. Although this additional interaction could involve immobilized chelates (Zucca et al., 2016), thiol disulfide reactive groups (Brena et al., 1993; Grazú et al., 2003; Pavlovic et al., 2003), dyes (Yakup Arica et al., 1998), etc., the only one that we have been able to find is the use of long acyl moieties mixed with anion or cation groups, using agarose beads (Rueda et al., 2016d, 2016a).

That way, immobilization may become a mixed one, making enzyme release very difficult. This has reduced lipase desorption in thermal or organic solvent inactivation, and permitted the reuse of the support after incubation with guanidine (Rueda et al., 2015a, 2016d, 2016a). Curiously, in certain cases great enzyme stabilization was observed when compared to the hydrophobic support (Rueda et al., 2015a, 2016d, 2016a).

In another research effort, the lipase from *Candida rugosa* was immobilized on magnetic hollow mesoporous silica microspheres combining hydrophobic and anionic groups (Zheng et al., 2015). This biocatalyst was used in the transesterification of phytosterol with fatty acids and triglycerides in a solvent-free system, having yields over 90% and recycling the biocatalyst by 50 cycles.

6.2.2.3. Not all enzyme molecules are covalently attached to the support

This problem may not be very relevant at first glance (e.g., when only 5% of the enzyme is not covalently attached and may be released to the medium), but it may have relevance if we completely want to avoid any kind of enzyme contamination in the final

product. In order to prevent this, two solutions have been tried. The first one was the use of more reactive enzymes. The second one was the use of more reactive supports.

6.2.2.3.1. Increase of the lipase reactivity

To improve the chemical reactivity of the enzyme versus glyoxyl-acyl supports, a possibility is to genetically enrich the enzyme surface in Lys residues (Rueda et al., 2016c) on positions near the active center, that will be the relevant ones in enzyme covalent immobilization. However, although this seems to be likely and it has been used in other immobilization strategies (Abian et al., 2004; Cecchini et al., 2007; Serra et al., 2009), we have not found any report in the literature on the use of this strategy using glyoxyl-acyl supports. The solution found in the literature is the chemical amination of the enzyme, and this was exemplified using several lipases (Rueda et al., 2016b) (Figure 12). Although the reports suggested that it is possible to make this modification on the glyoxyl-octyl immobilized enzyme, taking advantage of the solid phase (Rueda et al., 2016b), chemical modification means that we need to globally modify the enzyme surface (not only the areas of interest for the immobilization) (Rueda et al., 2016b). Fortunately, chemical enzyme amination tends to have positive results on activity/stability of lipases immobilized on octyl agarose (Barbosa et al., 2012a; Galvis et al., 2012; Garcia-Galan et al., 2014; Pereira et al., 2015; Pizarro et al., 2012).

Thus, using ethylenediamine and the carbodiimide route, aminated lipases were immobilized on glyoxyl supports (Galvis et al., 2012; Rodrigues et al., 2009). This allowed to greatly increase the number of reactive groups of the enzyme, and also considering that these new amino groups presented a lower pK than that of the Lys (the pI was around 9.2)

(Fernandez-Lafuente et al., 1993), this could be advantageous to ensure the enzyme-support covalent reaction. The researchers found diverse advantages.

First, all enzyme molecules become covalently immobilized on the glyoxyl groups after the immobilization via interfacial activation. Second, this full covalent immobilization could be achieved even at pH 9. In some instances (e.g., lipase from *Rhizomucor miehei*), the aminated enzyme becomes much more stable at pH 10 and permitted the covalent immobilization after incubation at pH 10 without losing activity. Furthermore, a great stabilization was found, suggesting that some multipoint covalent attachment could be achieved (Rueda et al., 2016b).

This way, this chemical amination strategy seems to be very adequate to solve the problems of a partial covalent immobilization of the native enzymes on glyoxyl-octyl supports. Future efforts should be addressed to use genetic strategies to improve the chemical reactivity of lipases in areas near the active center versus the groups on the support.

6.2.2.3.2. Increase of the support reactivity

One simple way to enhance the chemical reactivity of the support may be to increase the number of glyoxyl groups, even decreasing the number of acyl chains. This study can be performed using home-made activated supports, but we have not found any report on this regard, just using epoxy groups (Garmroodi et al., 2016).

The other alternative is to change the glyoxyl moiety by another one that exhibits a higher reactivity. This is the case of the vinylsulfone group (dos Santos et al., 2015b).

6.2.2.3.2.1 Vinylsulfone-acyl groups supports

Supports activated with divinylsulfone have shown to be reactive under a wide range of pH values (immobilizing proteins even at pH 5) (dos Santos et al., 2015b; Santos-Moriano et al., 2016). Moreover, they can react with more groups than glyoxyl (moving from just primary amino groups to also include in the enzyme-support reaction thiol, imidazole, hydroxyl and even secondary amino groups) (Bale Oenick et al., 1990; Bryjak et al., 2008; dos Santos et al., 2015b; Labus et al., 2011; Lopez-Jaramillo et al., 2012; Medina-Castillo et al., 2012; Morales-Sanfrutos et al., 2010; Ortega-Muñoz et al., 2010; Prikryl et al., 2012). Moreover, it has a larger spacer arm than glyoxyl, facilitating the reaction with groups of the protein which may not be properly located, even with the steric hindrances generated by the acyl chains (dos Santos et al., 2015b).

Thus, octyl-agarose supports were treated with divinylsulfone, and used to immobilize several lipases and the results were quite spectacular (Albuquerque et al., 2016): all enzyme molecules were immobilized via interfacial activation and later they became covalently immobilized under a wide range of pH values and in a very rapid fashion, and the high stabilization achieved suggested even a certain multipoint covalent attachment (Albuquerque et al., 2016). Furthermore, the blocking step allowed to tailor the lipase environment, and these changes in the enzyme environment permitted a further tuning of the lipase catalytic properties (Albuquerque et al., 2016).

7. Combilipases

The simultaneous use of several lipases as a way to improve the full modification of oils has been proposed, for example, in biodiesel or fatty acid production. This is based on the fact that an oil is a heterofunctional substrate composed of many different glycerides, and very likely some of them may behave even as inhibitors of the best lipase for the main components of the oil. Thus, the use of mixtures of lipases in these reactions is becoming increasingly popular (Alves et al., 2014; Ibrahim et al., 2008; Poppe et al., 2015, 2018b, 2018a; Qiao et al., 2017; Rodrigues and Ayub, 2011).

In fact, this full modification of the oil may be considered a cascade reaction, going from triglyceride to glycerin and free fatty acids. In these reactions the coimmobilization may produce some kinetic advantages (Mayer et al., 2001; Rodrigues et al., 2013; van Dongen et al., 2009; Vriezema et al., 2007). We have found just one paper using coimmobilized enzymes in biodiesel production, and the coimmobilization was performed using silica/amino/glutaraldehyde (Lee et al., 2013). Unfortunately, the paper did not compare the results with individually immobilized enzymes.

From previous comments in this review, it may be assumed that lipases co-immobilization on hydrophobic supports may be a very general solution for lipases coimmobilization. However, we have not found any paper in this regard. Moreover, even if this is performed, it should be considered that one of the main problems related to enzyme coimmobilization remains: when the least stable enzyme is inactivated, all other enzymes must be discarded even if they remain fully active (Garcia-Galan et al., 2011).

To solve this problem, a new strategy has been recently proposed. It involves the immobilization of a very stable lipase via interfacial activation, its coating with polyethylenimine, and the coimmobilization of a galactosidase on the previous composite

via ion exchange (Peirce et al., 2016b) (Figure 13). This way, after galactosidase inactivation, it is possible to release the enzyme by incubation at high ionic strength, and load new galactosidase, enabling the reuse of the lipase. The release of the polymer was prevented via reaction with glutaraldehyde of the lipase/polymer composite (Zaak et al., 2017c). At first glance, this should be possible to perform using a very stable lipase (immobilized on the octyl support and coated with the ionic polymer) and a less stable lipase (immobilized via ion exchange on the polymer layer). However, we have not found any papers using this strategy.

Nevertheless, a proposal for using glyoxyl-acyl supports has been reported to fulfill these requirements. The most stable lipase could be immobilized on glyoxyl-octyl supports, and after reduction, the least stable lipase could be immobilized just via interfacial activation. That way, after inactivation of the least stable lipase, the biocatalyst was incubated in detergent and this inactive lipase was released, enabling the reuse of the most stable enzyme for several cycles (Arana-Peña et al., 2019). (Figure 14). This was showed using the very stable lipase B from *Candida antarctica* (covalently immobilized on octyl-glyoxyl) and the much less stable lipase from *Rhizomucor miehei* or the phospholipase Lacitase Ultra (just interfacially immobilized after reduction of the CALB-biocatalyst). (Arana-Peña et al., 2019).

This new research area is still in the starting grid and any future developments are hard to foretell.

8. Future trends

The advantages of hydrophobic supports to immobilize lipases make these supports prime candidates to maintain their supremacy in this area. It is unlikely that in a near future an immobilization strategy may in a general way permit the one step immobilization/purification/stabilization and even hyperactivation of lipases. Thus, most immobilization supports suppliers have a variety of hydrophobic supports, which are mainly recommended for lipases. It should not be overlooked that a hydrophobic surface is generally negative for enzyme stability; lipases are the exception, not the rule (dos Santos et al., 2015a; Mateo et al., 2002).

Evolutions of hydrophobic supports for immobilizing lipases may take different directions. For example, to prepare tailor-made lipase combilipases (as the same support may immobilize many different lipases) or lipase coimmobilized with other enzymes (after modifying the immobilized lipase with polyethylenimine) may have a great interest (Zaak et al., 2017c). Although this has been already reported in some instances (Peirce et al., 2016b; Zaak et al., 2017c), the possibilities are many and a deeper exploration must be carried out, as combilipases have proven to be a potent tool in reactions as interesting as oil hydrolysis or biodiesel production (Alves et al., 2014; Ibrahim et al., 2008; Poppe et al., 2015, 2018b, 2018a; Qiao et al., 2017; Rodrigues and Ayub, 2011).

A more systematic optimization of the heterofunctional supports preparation seems also lacking and necessary to see the real prospects of the technique. The role of the amount and the molar ratio between the long acyl chain and the secondary groups has not been properly analyzed regarding the interfacial activation and further enzyme-support interactions. Moreover, the role of the length of the spacer arms in these heterofunctional supports may be unexpected.

Additionally, to mix tailor-made supports with genetically engineered tailor-made enzymes, as it has been performed with other immobilization strategies (Godoy et al., 2011; Grazu et al., 2012; Grazi et al., 2010), may open new opportunities.

Thus, even heterofunctional hydrophobic supports have been subjected to a strong development after their relatively recent appearance. Some critical research must still be performed and perhaps this may further simplify the immobilization protocol or increase its potential to enhance lipase features.

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References

- Abaházi, E., Boros, Z., Poppe, L., 2014. Additives enhancing the catalytic properties of lipase from *Burkholderia cepacia* immobilized on mixed-function-grafted mesoporous silica gel. *Molecules* 19, 9818–9837.
- Abed, S.M., Wei, W., Ali, A.H., Korma, S.A., Mousa, A.H., Hassan, H.M., Jin, Q., Wang, X., 2018. Synthesis of structured lipids enriched with medium-chain fatty acids via solvent-free acidolysis of microbial oil catalyzed by *Rhizomucor miehei* lipase. *LWT - Food Sci. Technol.* 93, 306–315.
- Abed, S.M., Zou, X., Ali, A.H., Jin, Q., Wang, X., 2017. Synthesis of 1,3-dioleoyl-2-arachidonoylglycerol-rich structured lipids by lipase-catalyzed acidolysis of microbial oil from *Mortierella alpina*. *Bioresour. Technol.* 243, 448–456.
- Abian, O., Grazú, V., Hermoso, J., González, R., García, J.L., Fernández-Lafuente, R., Guisán, J.M., 2004. Stabilization of Penicillin G Acylase from *Escherichia coli*: Site-Directed Mutagenesis of the Protein Surface to Increase Multipoint Covalent Attachment. *Appl. Environ. Microbiol.* 70, 1249–1251.
- Adlercreutz, P., 2008. Fundamentals of Biocatalysis in Neat Organic Solvents, in: Carrea, G., Riva, S. (Eds.), *Organic Synthesis with Enzymes in Non-Aqueous Media*. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, pp. 3–24.
- Aguiéiras, E.C.G., de Barros, D.S.N., Fernandez-Lafuente, R., Freire, D.M.G., 2019. Production of lipases in cottonseed meal and application of the fermented solid as biocatalyst in esterification and transesterification reactions. *Renew. Energy* 130, 574–

581.

Al-Duri, B., Robinson, E., McNerlan, S., Bailie, P., 1995. Hydrolysis of edible oils by lipases immobilized on hydrophobic supports: Effects of internal support structure. *J. Am. Oil Chem. Soc.* 72, 1351–1359.

Albuquerque, T.L. d., Rueda, N., dos Santos, J.C.S., Barbosa, O., Ortiz, C., Binay, B., Özdemir, E., Gonçalves, L.R.B., Fernandez-Lafuente, R., 2016. Easy stabilization of interfacially activated lipases using heterofunctional divinyl sulfone activated-octyl agarose beads. Modulation of the immobilized enzymes by altering their nanoenvironment. *Process Biochem.* 51, 865–874.

Alcántara, A.R., Dominguez de Maria, P., 2018. Recent advances on the use of 2-methyltetrahydrofuran (2-MeTHF) in biotransformations. *Curr. Green Chem.* 5, 85–102.

Almeida, A.F. de, Terrasan, C.R.F., Terrone, C.C., Tauk-Tornisielo, S.M., Carmona, E.C., 2018. Biochemical properties of free and immobilized *Candida viswanathii* lipase on octyl-agarose support: Hydrolysis of triacylglycerol and soy lecithin. *Process Biochem.* 65, 71–80.

Alves, J.S., Vieira, N.S., Cunha, A.S., Silva, A.M., Ayub, M.A.Z., Fernandez-Lafuente, R., Rodrigues, R.C., 2014. Combi-lipase for heterogeneous substrates: a new approach for hydrolysis of soybean oil using mixtures of biocatalysts. *RSC Adv.* 4, 6863–6868.

Anand, A., Weatherley, L.R., 2018. The performance of microbial lipase immobilized onto polyolefin supports for hydrolysis of high oleate sunflower oil. *Process Biochem.* 68,

100–107.

Arana-Peña, S., Lokha, Y., Fernández-Lafuente, R., 2018. Immobilization of Eversa Lipase on Octyl Agarose Beads and Preliminary Characterization of Stability and Activity Features. *Catalysts* 8, 511.

Arana-Peña, S., Mendez-Sanchez, C., Rios, N.S., Ortiz, C., Gonçalves, L.R.B., Fernández-Lafuente, R., 2019. New applications of glyoxyl-octyl agarose in lipases co-immobilization: Strategies to reuse the most stable lipase. *Int. J. Biol. Macromol.* 131, 989-997

Arora, B., Mukherjee, J., Gupta, M., 2014. Enzyme promiscuity: using the dark side of enzyme specificity in white biotechnology. *Sustain. Chem. Process.* 2, 25.

Asler, I.L., Ivic, N., Kovacic, F., Schell, S., Knorr, J., Krauss, U., Wilhelm, S., Kojic-Prodic, B., Jaeger, K.-E., 2010. Probing Enzyme Promiscuity of SGNH Hydrolases. *Chembiochem* 11, 2158–2167.

Babtie, A., Tokuriki, N., Hollfelder, F., 2010. What makes an enzyme promiscuous? *Curr. Opin. Chem. Biol.* 14, 200–207.

Bäckvall, J.-E., Löfgren, J., Görbe, T., Oschmann, M., Swedendahl Humble, M., 2019. Transesterification of a Tertiary Alcohol by Engineered *Candida antarctica* Lipase A. *ChemBioChem* in press, 10.1002/cbic.201800792.

Badgujar, K.C., Bhanage, B.M., 2016. Lipase immobilization on hydroxypropyl methyl cellulose support and its applications for chemo-selective synthesis of β -amino ester compounds. *Process Biochem.* 51, 1420–1433.

- Badgujar, V.C., Badgujar, K.C., Yeole, P.M., Bhanage, B.M., 2017. Immobilization of *Rhizomucor miehei* lipase on a polymeric film for synthesis of important fatty acid esters: kinetics and application studies. *Bioprocess Biosyst. Eng.* 40, 1463–1478.
- Bahari, A., Akoh, C.C., 2018. Synthesis of a Cocoa Butter Equivalent by Enzymatic Interesterification of Illipe Butter and Palm Midfraction. *J. Am. Oil Chem. Soc.* 95, 547–555.
- Bale Oenick, M.D., Danielson, S.J., Daiss, J.L., Sunderberg, M.W., Sutton, R.C., 1990. Antigen-binding activity of antibodies immobilized on styrene copolymer beads. *Ann. Biol. Clin. (Paris)*. 48, 651–654.
- Bansode, S.R., Hardikar, M.A., Rathod, V.K., 2017. Evaluation of reaction parameters and kinetic modelling for Novozym 435 catalysed synthesis of isoamyl butyrate. *J. Chem. Technol. Biotechnol.* 92, 1306–1314.
- Barbosa, O., Ariza, C., Ortiz, C., Torres, R., 2010. Kinetic resolution of (R/S)-propranolol (1-isopropylamino-3-(1-naphthoxy)-2-propanol) catalyzed by immobilized preparations of *Candida antarctica* lipase B (CAL-B). *N. Biotechnol.* 27, 844–850.
- Barbosa, O., Ortiz, C., Berenguer-Murcia, Á., Torres, R., Rodrigues, R.C., Fernandez-Lafuente, R., 2015. Strategies for the one-step immobilization-purification of enzymes as industrial biocatalysts. *Biotechnol. Adv.* 33, 435–456.
- Barbosa, O., Ruiz, M., Ortiz, C., Fernández, M., Torres, R., Fernandez-Lafuente, R., 2012a. Modulation of the properties of immobilized CALB by chemical modification with 2,3,4-trinitrobenzenesulfonate or ethylenediamine. Advantages of using adsorbed

lipases on hydrophobic supports. *Process Biochem.* 47, 867–876.

Barbosa, O., Torres, R., Ortiz, C., Fernandez-Lafuente, R., 2012b. The slow-down of the CALB immobilization rate permits to control the inter and intra molecular modification produced by glutaraldehyde. *Process Biochem.* 47, 766–774.

Barsé, L.Q., Graebin, N.G., Cipolatti, E.P., Robert, J.M., Pinto, M.C., Pinto, J.C., Freire, D.M., Rodrigues, R.C., 2019. Production and optimization of isopropyl palmitate via biocatalytic route using home-made enzymatic catalysts. *J. Chem. Technol. Biotechnol.* 94, 389–397.

Basheer, S., Haj, M., Mohsen, U., Shehadeh, D., Hindawi, A., Masoud, E., Egbarieh, A., Masri, R., 2018. Enzymatic transesterification/esterification processes employing lipases immobilized on hydrophobic resins in the presence of water solutions. US20140186908A1.

Bassi, J.J., Todero, L.M., Lage, F.A.P., Khedy, G.I., Ducas, J.D., Custódio, A.P., Pinto, M.A., Mendes, A.A., 2016. Interfacial activation of lipases on hydrophobic support and application in the synthesis of a lubricant ester. *Int. J. Biol. Macromol.* 92, 900–909.

Basso, A., Hesseler, M., Serban, S., 2016. Hydrophobic microenvironment optimization for efficient immobilization of lipases on octadecyl functionalised resins. *Tetrahedron* 72, 7323–7328.

Bastida, A., Sabuquillo, P., Armisen, P., Fernández-Lafuente, R., Huguet, J., Guisán, J.M., 1998. A single step purification, immobilization, and hyperactivation of lipases via

interfacial adsorption on strongly hydrophobic supports. *Biotechnol. Bioeng.* 58, 486–493.

Bauwelink, J., Cornet, I., Wijnants, M., Dams, R., Tavernier, S., 2018. Investigation of the Enzyme-Catalysed Transesterification of Methyl Acrylate and Sterically Hindered Alcohol Substrates. *ChemistrySelect* 3, 5169–5175.

Benítez-Mateos, A.I., Contente, M.L., Velasco-Lozano, S., Paradisi, F., López-Gallego, F., 2018. Self-Sufficient Flow-Biocatalysis by Coimmobilization of Pyridoxal 5'-Phosphate and ω -Transaminases onto Porous Carriers. *ACS Sustain. Chem. Eng.* 6, 13151–13159.

Benítez-Mateos, A.I., San Sebastian, E., Ríos-Lombardía, N., Morís, F., González-Sabín, J., López-Gallego, F., 2017. Asymmetric Reduction of Prochiral Ketones by Using Self-Sufficient Heterogeneous Biocatalysts Based on NADPH-Dependent Ketoreductases. *Chem. - A Eur. J.* 23, 16843–16852.

Bento, H.B.S., de Castro, H.F., de Oliveira, P.C., Freitas, L., 2017. Magnetized poly(STY-co-DVB) as a matrix for immobilizing microbial lipase to be used in biotransformation. *J. Magn. Magn. Mater.* 426, 95–101.

Bergamasco, J., de Araujo, M. V., de Vasconcellos, A., Luizon Filho, R.A., Hatanaka, R.R., Giotto, M. V., Aranda, D.A.G., Nery, J.G., 2013. Enzymatic transesterification of soybean oil with ethanol using lipases immobilized on highly crystalline PVA microspheres. *Biomass and Bioenergy* 59, 218–233.

Bernal, C., Illanes, A., Wilson, L., 2015. Improvement of Efficiency in the Enzymatic

- Synthesis of Lactulose Palmitate. *J. Agric. Food Chem.* 63, 3716–3724.
- Bernal, C., Illanes, A., Wilson, L., 2014. Heterofunctional hydrophilic-hydrophobic porous silica as support for multipoint covalent immobilization of lipases: Application to lactulose palmitate synthesis. *Langmuir* 30, 3557–3566.
- Bernal, C., Poveda-Jaramillo, J.C., Mesa, M., 2018a. Raising the enzymatic performance of lipase and protease in the synthesis of sugar fatty acid esters, by combined ionic exchange -hydrophobic immobilization process on aminopropyl silica support. *Chem. Eng. J.* 334, 760–767.
- Bernal, C., Rodríguez, K., Martínez, R., 2018b. Integrating enzyme immobilization and protein engineering: An alternative path for the development of novel and improved industrial biocatalysts. *Biotechnol. Adv.* 36, 1470–1480.
- Bertau, M., Jeromin, G.E., 2015. Hydrolysis and Transacylation: Esterases, Lipases, Phosphatases, and Phosphoryl Transferases, in: Faber, Fessner, Turner (Eds.), *Biocatalysis in Organic Synthesis*. Georg Thieme Verlag, Stuttgart, pp. 129–187.
- Bilal, M., Asgher, M., Cheng, H., Yan, Y., Iqbal, H.M.N., 2019. Multi-point enzyme immobilization, surface chemistry, and novel platforms: a paradigm shift in biocatalyst design. *Crit. Rev. Biotechnol.* 39, 202–219.
- Bilal, M., Rasheed, T., Zhao, Y., Iqbal, H.M.N., Cui, J., 2018. “Smart” chemistry and its application in peroxidase immobilization using different support materials. *Int. J. Biol. Macromol.* 119, 278–290.
- Bornscheuer, U., Kazlauskas, R.J., 2006. Hydrolases in organic synthesis. regio- and

stereoselective biotransformations, 2nd ed. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.

Bornscheuer, U.T., 2010. Lipases, Synthesis of Chiral Compounds, Aqueous and Organic Solvents, in: Encyclopedia of Industrial Biotechnology. John Wiley & Sons, Inc., Hoboken, NJ, USA.

Bornscheuer, U.T., Hauer, B., Jaeger, K.E., Schwaneberg, U., 2019. Directed Evolution Empowered Redesign of Natural Proteins for the Sustainable Production of Chemicals and Pharmaceuticals. *Angew. Chemie Int. Ed.* 58, 36–40.

Bornscheuer, U.T., Kazlauskas, R.J., 2012. Enzymatic Catalytic Promiscuity and the Design of New Enzyme Catalyzed Reactions, in: Enzyme Catalysis in Organic Synthesis. Wiley-VCH Verlag GmbH & Co. KGaA, pp. 1693–1733.

Boros, Z., Weiser, D., Márkus, M., Abaháziová, E., Magyar, Á., Tomin, A., Koczka, B., Kovács, P., Poppe, L., 2013. Hydrophobic adsorption and covalent immobilization of *Candida antarctica* lipase B on mixed-function-grafted silica gel supports for continuous-flow biotransformations. *Process Biochem.* 48, 1039–1047.

Boutureira, O., Bernardes, G.J.L., 2015. Advances in Chemical Protein Modification. *Chem. Rev.* 115, 2174–2195.

Brabcová, J., Demianová, Z., Vondrášek, J., Jágr, M., Zarevúcka, M., Palomo, J.M., 2013. Highly selective purification of three lipases from *Geotrichum candidum* 4013 and their characterization and biotechnological applications. *J. Mol. Catal. B Enzym.* 98, 62–72.

- Brady, D., Jordaan, J., 2009. Advances in enzyme immobilisation. *Biotechnol. Lett.* 31, 1639–1650.
- Brem, J., Turcu, M.C., Paizs, C., Lundell, K., Toşa, M.-I., Irimie, F.-D., Kanerva, L.T., 2012. Immobilization to improve the properties of *Pseudomonas fluorescens* lipase for the kinetic resolution of 3-aryl-3-hydroxy esters. *Process Biochem.* 47, 119–126.
- Brena, B.M., Ovsejevi, K., Luna, B., Batista-Viera, F., 1993. Thiolation and reversible immobilization of sweet potato δ -amylase on thiosulfonate-agarose. *J. Mol. Catal.* 84, 381–390.
- Brodzka, A., Koszelewski, D., Zysk, M., Ostaszewski, R., 2018. The mechanistic promiscuity of the enzymatic esterification of chiral carboxylic acids. *Catal. Commun.* 106, 82–86.
- Bryjak, J., Liesiene, J., Kolarz, B.N., 2008. Application and properties of butyl acrylate/pentaerythrite triacrylate copolymers and cellulose-based Granocel as carriers for trypsin immobilization. *Colloids Surf. B Biointerfaces* 61, 66–74.
- Brzozowski, A.M., Derewenda, U., Derewenda, Z.S., Dodson, G.G., Lawson, D.M., Turkenburg, J.P., Bjorkling, F., Huge-Jensen, B., Patkar, S.A., Thim, L., 1991. A model for interfacial activation in lipases from the structure of a fungal lipase-inhibitor complex. *Nature* 351, 491–494.
- Busto, E., Gotor-Fernandez, V., Gotor, V., 2010. Hydrolases: catalytically promiscuous enzymes for non-conventional reactions in organic synthesis. *Chem. Soc. Rev.* 39, 4504–4523.

- Cabrera, Z., Fernandez-Lorente, G., Fernandez-Lafuente, R., Palomo, J.M., Guisan, J.M., 2009. Novozym 435 displays very different selectivity compared to lipase from *Candida antarctica* B adsorbed on other hydrophobic supports. *J. Mol. Catal. B Enzym.* 57, 171–176.
- Cao, C., Matsuda, T., 2016. Chapter 3 - Biocatalysis in Organic Solvents, Supercritical Fluids and Ionic Liquids A2 - Goswami, Animesh, in: Stewart, J.D. (Ed.), *Organic Synthesis Using Biocatalysis*. Academic Press, pp. 67–97.
- Carbonell, P., Faulon, J.L., 2010. Molecular signatures-based prediction of enzyme promiscuity. *Bioinformatics* 26, 2012–2019.
- Carrasco-López, C., Godoy, C., de las Rivas, B., Fernández-Lorente, G., Palomo, J.M., Guisán, J.M., Fernández-Lafuente, R., Martínez-Ripoll, M., Hermoso, J.A., 2009. Activation of bacterial thermo alkalophilic lipases is spurred by dramatic structural rearrangements. *J. Biol. Chem.* 284, 4365–4372.
- Castilla, I., Woods, D., Reen, F.J., O’Gara, F., 2018. Harnessing Marine Biocatalytic Reservoirs for Green Chemistry Applications through Metagenomic Technologies. *Mar. Drugs* 16, 227.
- Cavalcanti, E.D.C., Aguiéiras, É.C.G., da Silva, P.R., Duarte, J.G., Cipolatti, E.P., Fernandez-Lafuente, R., da Silva, J.A.C., Freire, D.M.G., 2018. Improved production of biolubricants from soybean oil and different polyols via esterification reaction catalyzed by immobilized lipase from *Candida rugosa*. *Fuel* 215, 705–713.
- Cazaban, D., Illanes, A., Wilson, L., Betancor, L., 2018. Bio-inspired silica lipase

- nanobiocatalysts for the synthesis of fatty acid methyl esters. *Process Biochem.* 74, 86–93.
- Cecchini, D.A., Serra, I., Ubiali, D., Terreni, M., Albertini, A.M., 2007. New active site oriented glyoxyl-agarose derivatives of *Escherichia coli* penicillin G acylase. *BMC Biotechnol.* 7, 54.
- Chado, G.R., Holland, E.N., Tice, A.K., Stoykovich, M.P., Kaar, J.L., 2018. Modification of Lipase with Poly(4-acryloylmorpholine) Enhances Solubility and Transesterification Activity in Anhydrous Ionic Liquids. *Biomacromolecules* 19, 1324–1332.
- Chang, C.-S., Ho, S.-C., 2011. Enantioselective esterification of (R,S)-2-methylalkanoic acid with *Carica papaya* lipase in organic solvents. *Biotechnol. Lett.* 33, 2247–2253.
- Chapus, C., Semeriva, M., Bovier-Lapierre, C., Desnuelle, P., 1976. Mechanism of pancreatic lipase action. 1. Interfacial activation of pancreatic lipase. *Biochemistry* 15, 4980–4987.
- Chapus, C., Semeriva, M., Charles, M., Desnuelle, P., 1978. Adsorption and Activation of Pancreatic Lipase at Interfaces, in: Gatt, S., Freysz, L., P., M. (Eds.), *Enzymes of Lipid Metabolism. Advances in Experimental Medicine and Biology*. Springer, Boston, MA, pp. 57–68.
- Chaubey, A., Parshad, R., Koul, S., Taneja, S.C., Qazi, G.N., 2006. Enantioselectivity modulation through immobilization of *Arthrobacter* sp. lipase: Kinetic resolution of fluoxetine intermediate. *J. Mol. Catal. B Enzym.* 42, 39–44.

- Chen, B., Hu, J., Miller, E.M., Xie, W., Cai, M., Gross, R.A., 2008. *Candida antarctica* Lipase B Chemically Immobilized on Epoxy-Activated Micro- and Nanobeads: Catalysts for Polyester Synthesis. *Biomacromolecules* 9, 463–471.
- Chen, B., Liu, H., Guo, Z., Huang, J., Wang, M., Xu, X., Zheng, L., 2011. Lipase-catalyzed esterification of ferulic Acid with oleyl alcohol in ionic liquid/isooctane binary systems. *J. Agric. Food Chem.* 59, 1256–1263.
- Chen, B., Miller, E.M., Miller, L., Maikner, J.J., Gross, R.A., 2007. Effects of macroporous resin size on *Candida antarctica* lipase B adsorption, fraction of active molecules, and catalytic activity for polyester synthesis. *Langmuir* 23, 1381–1387.
- Chen, B., Yin, C., Cheng, Y., Li, W., Cao, Z., Tan, T., 2012. Using silk woven fabric as support for lipase immobilization: The effect of surface hydrophilicity/hydrophobicity on enzymatic activity and stability. *Biomass and Bioenergy* 39, 59–66.
- Chen, C., Zhu, X., Gao, Q., Fang, F., Wang, L., Huang, X., 2016. Immobilization of lipase onto functional cyclomatrix polyphosphazene microspheres. *J. Mol. Catal. B Enzym.* 132, 67–74.
- Chiaradia, V., Soares, N.S., Valério, A., de Oliveira, D., Araújo, P.H.H., Sayer, C., 2016. Immobilization of *Candida antarctica* Lipase B on Magnetic Poly(Urea-Urethane) Nanoparticles. *Appl. Biochem. Biotechnol.* 180, 558–575.
- Choi, N., No, D.S., Kim, H., Kim, B.H., Kwak, J., Lee, J.-S., Kim, I.-H., 2018. In situ lipase-catalyzed transesterification in rice bran for synthesis of fatty acid methyl ester. *Ind. Crops Prod.* 120, 140–146.

- Chojnacka, A., Gładkowski, W., 2018. Production of Structured Phosphatidylcholine with High Content of Myristic Acid by Lipase-Catalyzed Acidolysis and Interesterification. *Catalysts* 8, 281.
- Cipolatti, E.P., Pinto, M.C.C., Robert, J. de M., Silva, T.P. da, Beralto, T. d. C., Santos Jr, J.G.F., Castro, R. d. P.V. de, Fernandez-Lafuente, R., Manoel, E.A., Pinto, J.C.C. d. S., Freire, D.M.G., 2018. Pilot-Scale Development of Core-Shell Polymer Supports for the Immobilization of Recombinant Lipase B from *Candida antarctica* and their Application in the Production of Ethyl Esters from Residual Fatty Acids. *J. Appl. Polym. Sci.* 135, 46727.
- Clarke, C.J., Tu, W.-C., Levers, O., Bröhl, A., Hallett, J.P., 2018. Green and Sustainable Solvents in Chemical Processes. *Chem. Rev.* 118, 747–800.
- Colombo, G., Ottolina, G., Carrea, G., 2000. Modelling of enzyme properties in organic solvents. *Monatshefte Für Chemie* 131, 527–547.
- Copley, S.D., 2017. Shining a light on enzyme promiscuity. *Curr. Opin. Struct. Biol.* 47, 167–175.
- Corradini, M.C.C., Costa, B.M., Bressani, A.P.P., Garcia, K.C.A., Pereira, E.B., Mendes, A.A., 2017. Improvement of the enzymatic synthesis of ethyl valerate by esterification reaction in a solvent system. *Prep. Biochem. Biotechnol.* 47, 100–109.
- Cowan, D., Meyer, Q., Stafford, W., Muyanga, S., Cameron, R., Wittwer, P., 2005. Metagenomic gene discovery: Past, present and future. *Trends Biotechnol.* 23, 321–329.

- Cowan, D.A., Fernandez-Lafuente, R., 2011. Enhancing the functional properties of thermophilic enzymes by chemical modification and immobilization. *Enzyme Microb. Technol.* 49, 326–346.
- Cowan, D.A., Ramond, J.-B., Makhalanyane, T.P., De Maayer, P., 2015. Metagenomics of extreme environments. *Curr. Opin. Microbiol.* 25, 97–102.
- Cruz, M., Pinho, S.C., Mota, R., Almeida, M.F., Dias, J.M., 2018. Enzymatic esterification of acid oil from soapstocks obtained in vegetable oil refining: Effect of enzyme concentration. *Renew. Energy* 124, 165–171.
- Cunha, A.G., Besteti, M.D., Manoel, E.A., Da Silva, A.A.T., Almeida, R. V., Simas, A.B.C., Fernandez-Lafuente, R., Pinto, J.C., Freire, D.M.G., 2014. Preparation of core-shell polymer supports to immobilize lipase B from *Candida antarctica*: Effect of the support nature on catalytic properties. *J. Mol. Catal. B Enzym.* 100, 59–67.
- Cunha, R., Ferreira, E.A., Oliveira, C.S., Omori, A.T., 2015. Biocatalysis for desymmetrization and resolution of stereocenters beyond the reactive center: How far is far enough? *Biotechnol. Adv.* 33, 614–623.
- Cygler, M., Grochulski, P., Kazlauskas, R.J., Schrag, J.D., Bouthillier, F., Rubin, B., Serreqi, A.N., Gupta, A.K., 1994. A structural basis for the chiral preferences of lipases. *J. Am. Chem. Soc.* 116, 3180–3186.
- Cygler, M., Schrag, J.D., 1999. Structure and conformational flexibility of *Candida rugosa* lipase. *Biochim. Biophys. Acta* 1441, 205–214.
- Daniel, R., 2005. The metagenomics of soil. *Nat. Rev. Microbiol.* 3, 470–478.

- de Abreu, L., Fernandez-Lafuente, R., Rodrigues, R.C., Volpato, G., Ayub, M.A.Z., 2014. Efficient purification-immobilization of an organic solvent-tolerant lipase from *Staphylococcus warneri* EX17 on porous styrene-divinylbenzene beads. *J. Mol. Catal. B Enzym.* 99, 51–55.
- de Graaf, C., Oostenbrink, C., Keizers, P.J., van Vugt-Lussenburg, B.A., van Waterschoot, R.B., Tschirret-Guth, R., Commandeur, J.M., Vermeulen, N.E., 2007. Molecular Modeling-Guided Site-Directed Mutagenesis of Cytochrome P450 2D6. *Curr. Drug Metab.* 8, 59–77.
- de Lima, L., Mendes, A., Fernandez-Lafuente, R., Tardioli, P., Giordano, R., 2018. Performance of Different Immobilized Lipases in the Syntheses of Short- and Long-Chain Carboxylic Acid Esters by Esterification Reactions in Organic Media. *Molecules* 23, 766.
- de Miranda, A.S., Miranda, L.S.M., de Souza, R., 2015. Lipases: Valuable catalysts for dynamic kinetic resolutions. *Biotechnol. Adv.* 33, 372–393.
- de Paula, A. V, Nunes, G.F.M., de Castro, H.E., dos Santos, J.C., 2018. Performance of packed bed reactor on the enzymatic interesterification of milk fat with soybean oil to yield structure lipids. *Int. Dairy J.* 86, 1–8.
- Dimitrijević, A., Veličković, D., Bihelević, F., Bezbradica, D., Jankov, R., Milosavić, N., 2012. One-step, inexpensive high yield strategy for *Candida antarctica* lipase A isolation using hydroxyapatite. *Bioresour. Technol.* 107, 358–362.
- Dong, H., Li, Y., Sheng, G., Hu, L., 2013. The study on effective immobilization of lipase

on functionalized bentonites and their properties. *J. Mol. Catal. B Enzym.* 95, 9–15.

- dos Santos, J.C.S., Barbosa, O., Ortiz, C., Berenguer-Murcia, A., Rodrigues, R.C., Fernandez-Lafuente, R., 2015a. Importance of the Support Properties for Immobilization or Purification of Enzymes. *ChemCatChem* 7, 2413–2432.
- dos Santos, J.C.S., Garcia-Galan, C., Rodrigues, R.C., de Sant'Ana, H.B., Gonçalves, L.R.B., Fernandez-Lafuente, R., 2014. Stabilizing hyperactivated lecithase structures through physical treatment with ionic polymers. *Process Biochem.* 49, 1511–1515.
- dos Santos, J.C.S., Rueda, N., Barbosa, O., Fernández-Sánchez, J.F., Medina-Castillo, A.L., Ramón-Márquez, T., Arias-Martos, M.C., Millán-Linares, M.C., Pedroche, J., Yust, M. del M., Gonçalves, L.R.B., Fernandez-Lafuente, R., 2015b. Characterization of supports activated with divinyl sulfone as a tool to immobilize and stabilize enzymes via multipoint covalent attachment. Application to chymotrypsin. *RSC Adv.* 5, 20639–20649.
- dos Santos, J.C.S., Rueda, N., Gonçalves, L.R.B., Fernandez-Lafuente, R., 2015c. Tuning the catalytic properties of lipases immobilized on divinylsulfone activated agarose by altering its nanoenvironment. *Enzyme Microb. Technol.* 77, 1–7.
- dos Santos, J.C.S., Rueda, N., Sanchez, A., Villalonga, R., Gonçalves, L.R.B., Fernandez-Lafuente, R., 2015d. Versatility of divinylsulfone supports permits the tuning of CALB properties during its immobilization. *RSC Adv.* 5, 35801–35810.
- dos Santos, J.C.S., Rueda, N., Torres, R., Barbosa, O., Gonçalves, L.R.B., Fernandez-Lafuente, R., 2015e. Evaluation of divinylsulfone activated agarose to immobilize

- lipases and to tune their catalytic properties. *Process Biochem.* 50, 918–927.
- Dossat, V., Combes, D., Marty, A., 1999. Continuous enzymatic transesterification of high oleic sunflower oil in a packed bed reactor: Influence of the glycerol production. *Enzyme Microb. Technol.* 25, 194–200.
- Drożdż, A., Chrobok, A., Baj, S., Szymańska, K., Mrowiec-Białoń, J., Jarzębski, A.B., 2013. The chemo-enzymatic Baeyer–Villiger oxidation of cyclic ketones with an efficient silica-supported lipase as a biocatalyst. *Appl. Catal. A Gen.* 467, 163–170.
- Du, W., Xu, Y., Liu, D., Zeng, J., 2004. Comparative study on lipase-catalyzed transformation of soybean oil for biodiesel production with different acyl acceptors. *J. Mol. Catal. B Enzym.* 30, 125–129.
- Duan, Z.-Q., Du, W., Liu, D.-H., 2011. The mechanism of solvent effect on the positional selectivity of *Candida antarctica* lipase B during 1,3-diolein synthesis by esterification. *Bioresour. Technol.* 102, 11048–11050.
- Endo, Y., Hatanaka, T., Maeda, K., Arafune, K., Yamamoto, T., Itoh, K., Kuramochi, H., Kashino, Y., Ifuku, K., 2018. Use of ethanol with triolein for fatty acid ethyl ester as biodiesel fuel in a Novozym®435 fixed-bed reactor. *Biomass and Bioenergy* 108, 433–438.
- Engelmann, J.I., Silva, P.P., Igansi, A. V., Pohndorf, R.S., Cadaval, T.R.S., Crexi, V.T., Pinto, L.A.A., 2018. Structured lipids by swine lard interesterification with oil and esters from common carp viscera. *J. Food Process Eng.* 41, e12679.
- Fernandes, K.V., Papadaki, A., Cavalcanti da Silva, J.A., Fernandez-Lafuente, R.,

- Koutinas, A.A., Guimaraes Freire, D.M., 2018. Enzymatic esterification of palm fatty-acid distillate for the production of polyol esters with biolubricant properties. *Ind. Crops Prod.* 116, 90–96.
- Fernández-Arrojo, L., Guazzaroni, M.-E.E., López-Cortés, N., Beloqui, A., Ferrer, M., 2010. Metagenomic era for biocatalyst identification. *Curr. Opin. Biotechnol.* 21, 725–733.
- Fernandez-Lafuente, R., 2009. Stabilization of multimeric enzymes: Strategies to prevent subunit dissociation. *Enzyme Microb. Technol.* 45, 405–418.
- Fernandez-Lafuente, R., Armisen, P., Sabuquillo, P., Fernández-Lorente, G., Guisán, J.M., 1998. Immobilization of lipases by selective adsorption on hydrophobic supports. *Chem. Phys. Lipids* 93, 185–197.
- Fernández-Lafuente, R., Rodríguez, V., Mateo, C., Penzol, G., Hernández-Justiz, O., Irazoqui, G., Villarino, A., Ovsejevi, K., Batista, F., Guisán, J.M., 1999. Stabilization of multimeric enzymes via immobilization and post-immobilization techniques. *J. Mol. Catal. B Enzym.* 7, 181–189.
- Fernandez-Lafuente, R., Rosell, C.M., Rodriguez, V., Guisan, J.M., 1995. Strategies for enzyme stabilization by intramolecular crosslinking with bifunctional reagents. *Enzyme Microb. Technol.* 17, 517–523.
- Fernandez-Lafuente, R., Rosell, C.M., Rodriguez, V., Santana, C., Soler, G., Bastida, A., Guisan, J.M., 1993. Preparation of activated supports containing low pK amino groups. A new tool for protein immobilization via the carboxyl coupling method.

Enzyme Microb. Technol. 15, 546–550.

Fernandez-Lopez, L., Bartolome-Cabrero, R., Rodriguez, M.D., dos Santos, J.C.S., Rueda, N., Fernandez-Lafuente, R., 2015. Stabilizing effects of cations on lipases depend on the immobilization protocol. RSC Adv. 5, 83868–83875.

Fernandez-Lopez, L., Pedrero, S.G., Lopez-Carrobles, N., Gorines, B.C., Virgen-Ortíz, J.J., Fernandez-Lafuente, R., 2017a. Effect of protein load on stability of immobilized enzymes. Enzyme Microb. Technol. 98, 18–25.

Fernandez-Lopez, L., Pedrero, S.G., Lopez-Carrobles, N., Virgen-Ortíz, J.J., Gorines, B.C., Otero, C., Fernandez-Lafuente, R., 2017b. Physical crosslinking of lipase from *Rhizomucor miehei* immobilized on octyl agarose via coating with ionic polymers: Avoiding enzyme release from the support. Process Biochem. 54, 81–88.

Fernandez-Lopez, L., Rueda, N., Bartolome-Cabrero, R., Rodriguez, M.D., Albuquerque, T.L., dos Santos, J.C.S., Barbosa, O., Fernandez-Lafuente, R., 2016. Improved immobilization and stabilization of lipase from *Rhizomucor miehei* on octyl-glyoxyl agarose beads by using CaCl_2 . Process Biochem. 51, 48–52.

Fernandez-Lopez, L., Virgen-Ortíz, J.J., Pedrero, S.G., Lopez-Carrobles, N., Gorines, B.C., Otero, C., Fernandez-Lafuente, R., 2018. Optimization of the coating of octyl-CALB with ionic polymers to improve stability and decrease enzyme leakage. Biocatal. Biotransformation 36, 47–56.

Fernández-Lorente, G., Betancor, L., Carrascosa, A. V., Guisán, J.M., 2011a. Release of Omega-3 Fatty Acids by the Hydrolysis of Fish Oil Catalyzed by Lipases Immobilized

on Hydrophobic Supports. J. Am. Oil Chem. Soc. 88, 1173–1178.

- Fernandez-Lorente, G., Cabrera, Z., Godoy, C., Fernandez-Lafuente, R., Palomo, J.M., Guisan, J.M., 2008. Interfacially activated lipases against hydrophobic supports: Effect of the support nature on the biocatalytic properties. Process Biochem. 43, 1061–1067.
- Fernández-Lorente, G., Filice, M., Lopez-Vela, D., Pizarro, C., Wilson, L., Betancor, L., Avila, Y., Guisan, J.M., 2011b. Cross-linking of lipases adsorbed on hydrophobic supports: Highly selective hydrolysis of fish oil catalyzed by RML. J. Am. Oil Chem. Soc. 88, 801–807.
- Fernández-Lorente, G., Ortiz, C., Segura, R.L., Fernández-Lafuente, R., Guisán, J.M., Palomo, J.M., 2005. Purification of different lipases from *Aspergillus niger* by using a highly selective adsorption on hydrophobic supports. Biotechnol. Bioeng. 92, 773–779.
- Fernandez-Lorente, G., Palomo, J.M., Cabrera, Z., Fernandez-Lafuente, R., Guisán, J.M., 2007. Improved catalytic properties of immobilized lipases by the presence of very low concentrations of detergents in the reaction medium. Biotechnol. Bioeng. 97, 242–250.
- Fernández-Lorente, G., Palomo, J.M., Fuentes, M., Mateo, C., Guisan, J.M., Fernandez-Lafuente, R., Fernández-Lorente, G., Palomo, J.M., Fuentes, M., Mateo, C., Guisán, J.M., Fernández-Lafuente, R., 2003. Self-assembly of *Pseudomonas fluorescens* lipase into bimolecular aggregates dramatically affects functional properties. Biotechnol. Bioeng. 82, 232–237.

- Fernández-Lorente, G., Terreni, M., Mateo, C., Bastida, A., Fernández-Lafuente, R., Dalmases, P., Huguet, J., Guisán, J.M., 2001. Modulation of lipase properties in macro-aqueous systems by controlled enzyme immobilization: enantioselective hydrolysis of a chiral ester by immobilized *Pseudomonas lipase*. *Enzyme Microb. Technol.* 28, 389–396.
- Ferreira-Leitão, V.S., Cammarota, M.C., Aguiéiras, E.C.G., de Sá, L.R.V., Fernandez-Lafuente, R., Freire, D.M.G., 2017. The protagonism of biocatalysis in green chemistry and its environmental benefits. *Catalysts* 7, 9.
- Ferrer, M., Beloqui, A., Timmis, K.N., Golyshin, P.N., 2008. Metagenomics for mining new genetic resources of microbial communities. *J. Mol. Microbiol. Biotechnol.* 16, 109–123.
- Foresti, M.L., Galle, M., Ferreira, M.L., Briand, L.E., 2009. Enantioselective esterification of ibuprofen with ethanol as reactant and solvent catalyzed by immobilized lipase: experimental and molecular modeling aspects. *J. Chem. Technol. Biotechnol.* 84, 1461–1473.
- Foukis, A., Gkini, O.A., Stergiou, P.-Y., Papamichael, E.M., 2018. New insights and tools for the elucidation of lipase catalyzed esterification reaction mechanism in n-hexane: The synthesis of ethyl butyrate. *Mol. Catal.* 455, 159–163.
- Frey, R., Hayashi, T., Buller, R.M., 2019. Directed evolution of carbon–hydrogen bond activating enzymes. *Curr. Opin. Biotechnol.* 60, 29–38.
- Friedrich, J.L.R., Peña, F.P., Garcia-Galan, C., Fernandez-Lafuente, R., Ayub, M.A.Z.,

- Rodrigues, R.C., 2013. Effect of immobilization protocol on optimal conditions of ethyl butyrate synthesis catalyzed by lipase B from *Candida antarctica*. J. Chem. Technol. Biotechnol. 88, 1089–1095.
- Fu, Y., Fan, B., Chen, H., Huang, H., Hu, Y., 2018. Promiscuous enzyme-catalyzed cascade reaction: Synthesis of xanthone derivatives. Bioorg. Chem. 80, 555–559.
- Fuentes, M., Segura, R.L., Abian, O., Betancor, L., Hidalgo, A., Mateo, C., Fernandez-Lafuente, R., Guisan, J.M., 2004. Determination of protein-protein interactions through aldehyde-dextran intermolecular cross-linking. Proteomics 4, 2602–2607.
- Galgali, A., Gawas, S.D., Rathod, V.K., 2018. Ultrasound assisted synthesis of citronellol laurate by using Novozym 435. Catal. Today 309, 133–139.
- Galvis, M., Barbosa, O., Torres, R., Ortiz, C., Fernandez-Lafuente, R., 2012. Effect of solid-phase chemical modification on the features of the lipase from *Thermomyces lanuginosus*. Process Biochem. 47, 460–466.
- Gao, S., Wang, Y., Wang, W., Luo, G., Dai, Y., 2010. Enhancing performance of lipase immobilized on methyl-modified silica aerogels at the adsorption and catalysis processes: Effect of cosolvents. J. Mol. Catal. B Enzym. 62, 218–224.
- Gao, Z., Chu, J., Jiang, T., Xu, T., Wu, B., He, B., 2018. Lipase immobilization on functionalized mesoporous TiO₂: Specific adsorption, hyperactivation and application in cinnamyl acetate synthesis. Process Biochem. 64, 152–159.
- Garcia-Galan, C., Berenguer-Murcia, Á., Fernandez-Lafuente, R., Rodrigues, R.C., 2011. Potential of Different Enzyme Immobilization Strategies to Improve Enzyme

Performance. Adv. Synth. Catal. 353, 2885–2904.

Garcia-Galan, C., dos Santos, J.C.S., Barbosa, O., Torres, R., Pereira, E.B., Corberan, V.C.,

Gonçalves, L.R.B., Fernandez-Lafuente, R., 2014. Tuning of Lecitase features via solid-phase chemical modification: Effect of the immobilization protocol. Process Biochem. 49, 604–616.

Garmroodi, M., Mohammadi, M., Ramazani, A., Ashjari, M., Mohammadi, J., Sabour, B.,

Yousefi, M., 2016. Covalent binding of hyper-activated *Rhizomucor miehei* lipase (RML) on hetero-functionalized siliceous supports. Int. J. Biol. Macromol. 86, 208–215.

Gatti-Lafranconi, P., Hollfelder, F., 2013. Flexibility and Reactivity in Promiscuous

Enzymes. Chembiochem 14, 285–292.

Gerlt, J.A., 2004. How enzymes evolve: Insights from natural and unnatural promiscuity.

Abstr. Pap. Am. Chem. Soc. 228, U181–U181.

Ghattas, N., Filice, M., Abidi, F., Guisan, J.M., Ben Salah, A., 2014. Purification and

improvement of the functional properties of *Rhizopus oryzae* lipase using immobilization techniques. J. Mol. Catal. B Enzym. 110, 111–116.

Godoy, C.A., Rivas, B.D.L., Grazú, V., Montes, T., Guisán, J.M., López-Gallego, F., 2011.

Glyoxyl-disulfide agarose: A tailor-made support for site-directed rigidification of proteins. Biomacromolecules 12, 1800–1809.

Gonçalves, L.R.B., Rodrigues, D.S., Cavalcante, G.P., Silva, G.F., Ferreira, A.L.O., 2008.

Effect of additives on the esterification activity of immobilized *Candida antarctica*

lipase. World J. Microbiol. Biotechnol. 24, 833–839.

- Gonçalves, M.C.P., Kieckbusch, T.G., Perna, R.F., Fujimoto, J.T., Morales, S.A.V., Romanelli, J.P., 2019. Trends on enzyme immobilization researches based on bibliometric analysis. Process Biochem. 76, 95–110.
- Graebín, N.G., Martins, A.B., Lorenzoni, A.S.G., García-Galan, C., Fernández-Lafuente, R., Ayub, M.A.Z., Rodrigues, R.C., 2012. Immobilization of lipase B from *Candida antarctica* on porous styrene-divinylbenzene beads improves butyl acetate synthesis. Biotechnol. Prog. 28, 406–412.
- Grazú, V., Abian, O., Mateo, C., Batista-Viera, F., Fernández-Lafuente, R., Guisán, J.M., 2003. Novel bifunctional epoxy/thiol-reactive support to immobilize thiol containing proteins by the epoxy chemistry. Biomacromolecules 4, 1495–1501.
- Grazu, V., López-Gallego, F., Guisán, J.M., 2012. Tailor-made design of penicillin G acylase surface enables its site-directed immobilization and stabilization onto commercial mono-functional epoxy supports. Process Biochem. 47, 2538–2541.
- Grazú, V., López-Gallego, F., Montes, T., Abian, O., González, R., Hermoso, J.A., García, J.L., Mateo, C., Guisán, J.M., 2010. Promotion of multipoint covalent immobilization through different regions of genetically modified penicillin G acylase from *E. coli*. Process Biochem. 45, 390–398.
- Green, K.D., Chen, W., Houghton, J.L., Fridman, M., Garneau-Tsodikova, S., 2010. Exploring the Substrate Promiscuity of Drug-Modifying Enzymes for the Chemoenzymatic Generation of N-Acylated Aminoglycosides. Chembiochem 11,

119–126.

Grochulski, P., Li, Y., Schrag, J.D., Bouthillier, F., Smith, P., Harrison, D., Rubin, B.,

Cygler, M., 1993. Insights into interfacial activation from an open structure of

Candida rugosa lipase. *J. Biol. Chem.* 268, 12843–12847.

Guajardo, N., Bernal, C., Wilson, L., Cabrera, Z., 2015a. Asymmetric hydrolysis of

dimethyl-3-phenylglutarate in sequential batch reactor operation catalyzed by

immobilized *Geobacillus thermocatenulatus* lipase. *Catal. Today* 255, 21–26.

Guajardo, N., Bernal, C., Wilson, L., Cabrera, Z., 2015b. Selectivity of R- α -monobenzoate

glycerol synthesis catalyzed by *Candida antarctica* lipase B immobilized on

heterofunctional supports. *Process Biochem.* 50, 1870–1877.

Gumbyte, M., Makareviciene, V., Skorupskaite, V., Sendzikiene, E., Kondratavicius, M.,

2018. Enzymatic microalgae oil transesterification with ethanol in mineral diesel fuel

media. *J. Renew. Sustain. Energy* 10, 013105.

Guncheva, M., Dimitrov, M., Zhiryakova, D., 2011a. Novel nanostructured tin dioxide as

promising carrier for *Candida rugosa* lipase. *Process Biochem.* 46, 2170–2177.

Guncheva, M., Tashev, E., Zhiryakova, D., Tosheva, T., Tzokova, N., 2011b.

Immobilization of lipase from *Candida rugosa* on novel phosphorous-containing

polyurethanes: Application in wax ester synthesis. *Process Biochem.* 46, 923–930.

Gupta, A., Dhakate, S.R., Pahwa, M., Sinha, S., Chand, S., Mathur, R.B., 2013. Geranyl

acetate synthesis catalyzed by *Thermomyces lanuginosus* lipase immobilized on

electrospun polyacrylonitrile nanofiber membrane. *Process Biochem.* 48, 124–132.

- Gupta, S., Singh, K., Bhattacharya, A., 2010. Lipase immobilization on Polysulfone globules and their performances in olive oil hydrolysis. *Int. J. Biol. Macromol.* 46, 445–450.
- Gustafsson, H., Johansson, E.M., Barrabino, A., Odén, M., Holmberg, K., 2012. Immobilization of lipase from *Mucor miehei* and *Rhizopus oryzae* into mesoporous silica—The effect of varied particle size and morphology. *Colloids Surf. B Biointerfaces* 100, 22–30.
- Habibi, Z., Mohammadi, M., Yousefi, M., 2013. Enzymatic hydrolysis of racemic ibuprofen esters using *Rhizomucor miehei* lipase immobilized on different supports. *Process Biochem.* 48, 669–676.
- Hamryszak, L., Grzesik, M., Witczak, T., 2018. Kinetics of lauric acid esterification with n-hexanol. *Przem. Chem.* 97, 1562–1564.
- He, T., Li, K., Wu, M.Y., Feng, X.W., Wang, N., Wang, H.Y., Li, C., Yu, X.Q., 2010. Utilization of biocatalytic promiscuity for direct Mannich reaction. *J. Mol. Catal. B Enzym.* 67, 189–194.
- He, Y., Qiu, C., Guo, Z., Huang, J., Wang, M., Chen, B., 2017. Production of new human milk fat substitutes by enzymatic acidolysis of microalgae oils from *Nannochloropsis oculata* and *Isochrysis galbana*. *Bioresour. Technol.* 238, 129–138.
- Herbst, D., Peper, S., Niemeyer, B., 2012. Enzyme catalysis in organic solvents: influence of water content, solvent composition and temperature on *Candida rugosa* lipase catalyzed transesterification. *J. Biotechnol.* 162, 398–403.

- Hernáiz, M., Alcántara, A.R., García, J.I., Sinisterra, J. V, 2010. Applied Biotransformations in Green Solvents. Chem. - A Eur. J. 16, 9422–9437.
- Hernandez, K., Berenguer-Murcia, A., Rodrigues, R.C., Fernandez-Lafuente, R., 2012. Hydrogen Peroxide in Biocatalysis. A Dangerous Liaison. Curr. Org. Chem. 16, 2652–2672.
- Hernandez, K., Fernandez-Lafuente, R., 2011a. Control of protein immobilization: Coupling immobilization and site-directed mutagenesis to improve biocatalyst or biosensor performance. Enzyme Microb. Technol. 48, 107–122.
- Hernandez, K., Fernandez-Lafuente, R., 2011b. Lipase B from *Candida antarctica* immobilized on octadecyl Sepabeads: A very stable biocatalyst in the presence of hydrogen peroxide. Process Biochem. 46, 873–878.
- Hernandez, K., Garcia-Galan, C., Fernandez-Lafuente, R., 2011a. Simple and efficient immobilization of lipase B from *Candida antarctica* on porous styrene-divinylbenzene beads. Enzyme Microb. Technol. 49, 72–78.
- Hernandez, K., Garcia-Verdugo, E., Porcar, R., Fernandez-Lafuente, R., 2011b. Hydrolysis of triacetin catalyzed by immobilized lipases: Effect of the immobilization protocol and experimental conditions on diacetin yield. Enzyme Microb. Technol. 48, 510–517.
- Hilterhaus, L., Thum, O., Liese, A., 2008. Reactor concept for lipase-catalyzed solvent-free conversion of highly viscous reactants forming two-phase systems. Org. Process Res. Dev. 12, 618–625.
- Hirata, D.B., Albuquerque, T.L., Rueda, N., Sánchez-Montero, J.M., Garcia-Verdugo, E.,

- Porcar, R., Fernandez-Lafuente, R., 2016a. Advantages of Heterofunctional Octyl Supports: Production of 1,2-Dibutylin by Specific and Selective Hydrolysis of Tributyrin Catalyzed by Immobilized Lipases. *ChemistrySelect* 1, 3259–3270.
- Hirata, D.B., Albuquerque, T.L., Rueda, N., Virgen-Ortíz, J.J., Tacias-Pascacio, V.G., Fernandez-Lafuente, R., 2016b. Evaluation of different immobilized lipases in transesterification reactions using tributyrin: Advantages of the heterofunctional octyl agarose beads. *J. Mol. Catal. B Enzym.* 133, 117–123.
- Hoyos, P., Pace, V., Hernáiz, M.J., Alcántara, A.R., 2016. Dynamic Kinetic Resolution via Hydrolase–Metal Combo Catalysis, in: *Green Biocatalysis*. John Wiley & Sons, Inc, pp. 373–396.
- Hu, Y., Jiang, X., Wu, S., Jiang, L., Huang, H., 2013. Synthesis of vitamin E succinate by interfacial activated *Candida rugosa* lipase encapsulated in sol-gel materials. *Chinese J. Catal.* 34, 1608–1616.
- Huemmer, M., Kara, S., Liese, A., Huth, I., Schrader, J., Holtmann, D., Hümmer, M., Kara, S., Liese, A., Huth, I., Schrader, J., Holtmann, D., 2018. Synthesis of (-)-menthol fatty acid esters in and from (-)-menthol and fatty acids - novel concept for lipase catalyzed esterification based on eutectic solvents. *Mol. Catal.* 458, 67–72.
- Hult, K., Berglund, P., 2007. Enzyme promiscuity: mechanism and applications. *Trends Biotechnol.* 25, 231–238.
- Humble, M.S., Berglund, P., 2011. Biocatalytic promiscuity. *European J. Org. Chem.* 3391–3401.

- Ibrahim, N.A., Guo, Z., Xu, X., 2008. Enzymatic Interesterification of Palm Stearin and Coconut Oil by a Dual Lipase System. *J. Am. Oil Chem. Soc.* 85, 37–45.
- Iyer, P. V, Ananthanarayan, L., 2008. Enzyme stability and stabilization-Aqueous and non-aqueous environment. *Process Biochem.* 43, 1019–1032.
- Jaeger, K.-E., Dijkstra, B.W., Reetz, M.T., 1999. Bacterial Biocatalysts: Molecular Biology, Three-Dimensional Structures, and Biotechnological Applications of Lipases. *Annu. Rev. Microbiol.* 53, 315–351.
- Jaeger, K.-E., Eggert, T., 2002. Lipases for biotechnology. *Curr. Opin. Biotechnol.* 13, 390–397.
- Jaeger, K.-E., Ransac, S., Koch, H.B., Ferrato, F., Dijkstra, B.W., 1993. Topological characterization and modeling of the 3D structure of lipase from *Pseudomonas aeruginosa*. *FEBS Lett.* 332, 143–149.
- Jahangiri, A., Møller, A.H., Danielsen, M., Madsen, B., Joernsgaard, B., Vaerbak, S., Adlercreutz, P., Dalsgaard, T.K., 2018. Hydrophilization of bixin by lipase-catalyzed transesterification with sorbitol. *Food Chem.* 268, 203–209.
- Jangale, A.D., Dalal, D.S., 2017. Green synthetic approaches for biologically relevant organic compounds. *Synth. Commun.* 47, 2139–2173.
- Jiang, Y., Liu, H., Wang, L., Zhou, L., Huang, Z., Ma, L., He, Y., Shi, L., Gao, J., 2019. Virus-like organosilica nanoparticles for lipase immobilization: Characterization and biocatalytic applications. *Biochem. Eng. J.* 144, 125–134.
- Jin, Q., Li, X., Deng, C., Zhang, Q., Yi, D., Wang, X., Tang, Y., Wang, Y., 2018. Silica

- nanowires with tunable hydrophobicity for lipase immobilization and biocatalytic membrane assembly. *J. Colloid Interface Sci.* 531, 555–563.
- Juhl, P.B., Trodler, P., Tyagi, S., Pleiss, J., 2009. Modelling substrate specificity and enantioselectivity for lipases and esterases by substrate-imprinted docking. *BMC Struct. Biol.* 9, 39.
- Kaar, J.L., Jesionowski, A.M., Berberich, J.A., Moulton, R., Russell, A.J., 2003. Impact of Ionic Liquid Physical Properties on Lipase Activity and Stability. *J. Am. Chem. Soc.* 125, 4125–4131.
- Kalantari, M., Yu, M., Yang, Y., Strounina, E., Gu, Z., Huang, X., Zhang, J., Song, H., Yu, C., 2017. Tailoring mesoporous-silica nanoparticles for robust immobilization of lipase and biocatalysis. *Nano Res.* 10, 605–617.
- Kamal, M.Z., Yedavalli, P., Deshmukh, M. V, Rao, N.M., 2013. Lipase in aqueous-polar organic solvents: Activity, structure, and stability. *Protein Sci.* 22, 904–915.
- Kamaruddin, A.H., Ong, A.L., Bhatia, S., Long, W.S., Lim, S.T., Kumari, R., 2006. Performance of free *Candida antarctica* lipase B in the enantioselective esterification of (R)-ketoprofen. *Enzyme Microb. Technol.* 39, 924–929.
- Kamel, G., Bordi, F., Chronopoulou, L., Lupi, S., Palocci, C., Sennato, S., Verdes, P. V., 2011. Adsorption of *Candida rugosa* lipase at water-polymer interface: The case of poly(dl)lactide. *Surf. Sci.* 605, 2017–2024.
- Kapoor, M., Gupta, M.N., 2012. Lipase promiscuity and its biochemical applications. *Process Biochem.* 47, 555–569.

- Kato, G., Sato, A., Tokuyama, H., 2018. Continuous Esterification using Lipase-Entrapped Amphiphilic Copolymeric Gel Beads. *Kagaku Kogaku Ronbunshu* 44, 185–188.
- Kavadia, M.R., Yadav, M.G., Odaneth, A.A., Lali, A.M., 2018. Synthesis of designer triglycerides by enzymatic acidolysis. *Biotechnol. Reports* 18, e00246–e00246.
- Kazlauskas, R., 2016. Hydrolysis and Formation of Carboxylic Acid and Alcohol Derivatives, in: Goswami, A., Stewart, J.D. (Eds.), *Organic Synthesis Using Biocatalysis*. Elsevier, pp. 127–148.
- Kazlauskas, R.J., 2005. Enhancing catalytic promiscuity for biocatalysis. *Curr. Opin. Chem. Biol.* 9, 195–201.
- Kerzel, P., Mersmann, A., 1992. Adsorption behaviour of lipase from *Staphylococcus carnosus* on a hydrophobic adsorbent. *J. Chromatogr. B Biomed. Sci. Appl.* 584, 109–113.
- Khersonsky, O., Roodveldt, C., Tawfik, D.S., 2006. Enzyme promiscuity: evolutionary and mechanistic aspects. *Curr. Opin. Chem. Biol.* 10, 498–508.
- Khersonsky, O., Tawfik, D.S., 2010. Enzyme Promiscuity: A Mechanistic and Evolutionary Perspective. *Annu. Rev. Biochem.* 79, 471–505.
- Kim, K.K., Song, H.K., Shin, D.H., Hwang, K.Y., Suh, S.W., 1997. The crystal structure of a triacylglycerol lipase from *Pseudomonas cepacia* reveals a highly open conformation in the absence of a bound inhibitor. *Structure* 5, 173–185.
- Kim, S.H., Kim, S., Park, S., Kim, H.K., 2013. Biodiesel production using cross-linked *Staphylococcus haemolyticus* lipase immobilized on solid polymeric carriers. *J. Mol.*

- Catal. B Enzym. 85–86, 10–16.
- Kirk, O., Christensen, M.W., 2002. Lipases from *Candida antarctica* : Unique Biocatalysts from a Unique Origin. Org. Process Res. Dev. 6, 446–451.
- Kolodziejska, R., Studzinska, R., Pawluk, H., 2018. Lipase-catalyzed enantioselective transesterification of prochiral 1-((1,3-dihydroxypropan-2-yl)oxy)methyl)-5,6,7,8-tetrahydroquinazoline-2, 4(1H,3H)-dione in ionic liquids. Chirality 30, 206–214.
- Korma, S.A., Zou, X., Ali, A.H., Abed, S.M., Jin, Q., Wang, X., 2018. Preparation of structured lipids enriched with medium- and long-chain triacylglycerols by enzymatic interesterification for infant formula. Food Bioprod. Process. 107, 121–130.
- Kovalenko, G.A., Perminova, L. V, Beklemishev, A.B., Mamaev, A.L., Patrushev, Y. V, 2018. Biocatalytic Heterogeneous Processes of the Esterification of Saturated Fatty Acids with Aliphatic Alcohols. Catal. Ind. 10, 68–74.
- Krüger, A., Schäfers, C., Schröder, C., Antranikian, G., 2018. Towards a sustainable biobased industry – Highlighting the impact of extremophiles. N. Biotechnol. 40, 144–153.
- Kumar, A., Dhar, K., Kanwar, S.S., Arora, P.K., 2016. Lipase catalysis in organic solvents: Advantages and applications. Biol. Proced. Online 18, 1–11.
- Kurtovic, I., Marshall, S.N., Zhao, X., 2011. Hydrophobic immobilization of a bile salt activated lipase from Chinook salmon (*Oncorhynchus tshawytscha*). J. Mol. Catal. B Enzym. 72, 168–174.
- Labus, K., Turek, A., Liesiene, J., Bryjak, J., 2011. Efficient *Agaricus bisporus* tyrosinase

- immobilization on cellulose-based carriers. *Biochem. Eng. J.* 56, 232–240.
- Lage, F.A.P., Bassi, J.J., Corradini, M.C.C., Toderó, L.M., Luiz, J.H.H., Mendes, A.A., 2016. Preparation of a biocatalyst via physical adsorption of lipase from *Thermomyces lanuginosus* on hydrophobic support to catalyze biolubricant synthesis by esterification reaction in a solvent-free system. *Enzyme Microb. Technol.* 84, 56–67.
- Laszlo, J.A., Jackson, M., Blanco, R.M., 2011. Active-site titration analysis of surface influences on immobilized *Candida antarctica* lipase B activity. *J. Mol. Catal. B Enzym.* 69, 60–65.
- Lathe, R., Kotelevtsev, Y., Mason, J.I., 2015. Steroid promiscuity: Diversity of enzyme action. *J. Steroid Biochem. Mol. Biol.* 151, 1–2.
- Lee, J.H., Kim, S.B., Yoo, H.Y., Lee, J.H., Han, S.O., Park, C., Kim, S.W., 2013. Co-immobilization of *Candida rugosa* and *Rhizopus oryzae* lipases and biodiesel production. *Korean J. Chem. Eng.* 30, 1335–1338.
- Li, C., Feng, X.-W., Wang, N., Zhou, Y.-J., Yu, X.-Q., 2008. Biocatalytic promiscuity: the first lipase-catalysed asymmetric aldol reaction. *Green Chem.* 10, 616–618.
- Li, D., Wang, W., Liu, P., Xu, L., Faiza, M., Yang, B., Wang, L., Lan, D., Wang, Y., 2017. Immobilization of *Candida antarctica* Lipase B Onto ECR1030 Resin and its Application in the Synthesis of n-3 PUFA-Rich Triacylglycerols. *Eur. J. Lipid Sci. Technol.* 119, 1700266.
- Li, G., Wang, J., Reetz, M.T., 2018. Biocatalysts for the pharmaceutical industry created by structure-guided directed evolution of stereoselective enzymes. *Bioorg. Med. Chem.*

26, 1241–1251.

Li, W., Chen, B., Tan, T., 2011. Comparative study of the properties of lipase immobilized on nonwoven fabric membranes by six methods. *Process Biochem.* 46, 1358–1365.

Li, W., Li, R., Yu, X., Xu, X., Guo, Z., Tan, T., Fedosov, S.N., 2015. Lipase-catalyzed Knoevenagel condensation in water-ethanol solvent system. Does the enzyme possess the substrate promiscuity? *Biochem. Eng. J.* 101, 99–107.

Li, W., Shen, H., Tao, Y., Chen, B., Tan, T., 2014. Amino silicones finished fabrics for lipase immobilization: Fabrics finishing and catalytic performance of immobilized lipase. *Process Biochem.* 49, 1488–1496.

Li, W.N., Chen, B.Q., Tan, T.W., 2011. Esterification synthesis of ethyl oleate in solvent-free system catalyzed by lipase membrane from fermentation broth. *Appl. Biochem. Biotechnol.* 163, 102–111.

Li, Y., Zhao, J., Xie, X., Zhang, Z., Zhang, N., Wang, Y., 2018. A low trans margarine fat analog to beef tallow for healthier formulations: Optimization of enzymatic interesterification using soybean oil and fully hydrogenated palm oil. *Food Chem.* 255, 405–413.

Littlechild, J.A., 2017. Improving the ‘tool box’ for robust industrial enzymes. *J. Ind. Microbiol. Biotechnol.* 44, 711–720.

Liu, C.-H., Huang, C.-C., Wang, Y.-W., Lee, D.-J., Chang, J.-S., 2012. Biodiesel production by enzymatic transesterification catalyzed by *Burkholderia* lipase immobilized on hydrophobic magnetic particles. *Appl. Energy* 100, 41–46.

- Liu, D., Trodler, P., Eiben, S., Koschorreck, K., Müller, M., Pleiss, J., Maurer, S.C., Branneby, C., Schmid, R.D., Hauer, B., 2010. Rational Design of *Pseudozyma antarctica* Lipase B Yielding a General Esterification Catalyst. *ChemBioChem* 11, 789–795.
- Liu, J., Bai, S., Jin, Q., Zhong, H., Li, C., Yang, Q., 2012. Improved Catalytic Performance of Lipase Accommodated in the Mesoporous Silicas with Polymer-Modified Microenvironment. *Langmuir* 28, 9788–9796.
- Liu, N., Wang, Y., Zhao, Q., Cui, C., Fu, M., Zhao, M., 2012. Immobilisation of Lecitase® Ultra for production of diacylglycerols by glycerolysis of soybean oil. *Food Chem.* 134, 301–307.
- Liu, T., Liu, Y., Wang, X., Li, Q., Wang, J., Yan, Y., 2011. Improving catalytic performance of Burkholderia cepacia lipase immobilized on macroporous resin NKA. *J. Mol. Catal. B Enzym.* 71, 45–50.
- Liu, Y., Guo, C., Sun, X.-T., Liu, C.-Z., 2013. Improved performance of *Yarrowia lipolytica* lipase-catalyzed kinetic resolution of (R,S)-2-octanol by an integrated strategy of interfacial activation, bioimprinting and immobilization. *Bioresour. Technol.* 142, 415–419.
- López-Gallego, F., Jackson, E., Betancor, L., 2017. Heterogeneous Systems Biocatalysis: The Path to the Fabrication of Self-Sufficient Artificial Metabolic Cells. *Chem. - A Eur. J.* 23, 17841–17849.
- Lopez-Iglesias, M., Gotor-Fernandez, V., 2015. Recent advances in biocatalytic

promiscuity: hydrolase-catalyzed reactions for nonconventional transformations.

Chem. Rec. 15, 743–759.

Lopez-Jaramillo, F.J., Ortega-Munõz, M., Megia-Fernandez, A., Hernandez-Mateo, F., Santoyo-Gonzalez, F., 2012. Vinyl sulfone functionalization: A feasible approach for the study of the lectin-carbohydrate interactions. *Bioconjug. Chem.* 23, 846–855.

Mahmood, I., Guo, C., Xia, H., Ma, J., Jiang, Y., Liu, H., 2008. Lipase Immobilization on Oleic Acid–Pluronic (L-64) Block Copolymer Coated Magnetic Nanoparticles, for Hydrolysis at the Oil/Water Interface. *Ind. Eng. Chem. Res.* 47, 6379–6385.

Manoel, E.A., dos Santos, J.C.S., Freire, D.M.G., Rueda, N., Fernandez-Lafuente, R., 2015a. Immobilization of lipases on hydrophobic supports involves the open form of the enzyme. *Enzyme Microb. Technol.* 71, 53–57.

Manoel, E.A., Ribeiro, M.F.P., dos Santos, J.C.S., Coelho, M.A.Z., Simas, A.B.C., Fernandez-Lafuente, R., Freire, D.M.G., 2015b. Accurel MP 1000 as a support for the immobilization of lipase from *Burkholderia cepacia*: Application to the kinetic resolution of myo -inositol derivatives. *Process Biochem.* 50, 1557–1564.

Martin, L.S., Ceron, A., Oliveira, P.C., Zanin, G.M., de Castro, H.F., 2018. Different organic components on silica hybrid matrices modulate the lipase inhibition by the glycerol formed in continuous transesterification reactions. *J. Ind. Eng. Chem.* 62, 462–470.

Martins, A.B., Friedrich, J.L.R., Cavaleiro, J.C., Garcia-Galan, C., Barbosa, O., Ayub, M.A.Z., Fernandez-Lafuente, R., Rodrigues, R.C., 2013. Improved production of butyl

- butyrate with lipase from *Thermomyces lanuginosus* immobilized on styrene-divinylbenzene beads. *Bioresour. Technol.* 134, 417–422.
- Marty, A., Dossat, V., Condoret, J.-S., 1997. Continuous operation of lipase-catalyzed reactions in nonaqueous solvents: Influence of the production of hydrophilic compounds. *Biotechnol. Bioeng.* 56, 232–237.
- Mateo, C., Abian, O., Fernández-Lorente, G., Pedroche, J., Fernández-Lafuente, R., Guisan, J.M., Tam, A., Daminati, M., 2002. Epoxy Sepabeads: A novel epoxy support for stabilization of industrial enzymes via very intense multipoint covalent attachment. *Biotechnol. Prog.* 18, 629–634.
- Mateo, C., Palomo, J.M., Fernandez-Lorente, G., Guisan, J.M., Fernandez-Lafuente, R., 2007. Improvement of enzyme activity, stability and selectivity via immobilization techniques. *Enzyme Microb. Technol.* 40, 1451–1463.
- Mathesh, M., Luan, B., Akanbi, T.O., Weber, J.K., Liu, J., Barrow, C.J., Zhou, R., Yang, W., 2016. Opening Lids: Modulation of Lipase Immobilization by Graphene Oxides. *ACS Catal.* 6, 4760–4768.
- Mathpati, A.C., Kalghatgi, S.G., Mathpati, C.S., Bhanage, B.M., 2018. Immobilized lipase catalyzed synthesis of n -amyl acetate: parameter optimization, heterogeneous kinetics, continuous flow operation and reactor modeling. *J. Chem. Technol. Biotechnol.* 93, 2906–2916.
- Mayer, S.F., Kroutil, W., Faber, K., 2001. Enzyme-initiated domino (cascade) reactions. *Chem. Soc. Rev.* 30, 332–339.

- Medina-Castillo, A.L., Morales-Sanfrutos, J., Megia-Fernandez, A., Fernandez-Sanchez, J.F., Santoyo-Gonzalez, F., Fernandez-Gutierrez, A., 2012. Novel synthetic route for covalent coupling of biomolecules on super-paramagnetic hybrid nanoparticles. *J. Polym. Sci. Part A Polym. Chem.* 50, 3944–3953.
- Mendes, A.A., Oliveira, P.C., Vélez, A.M., Giordano, R.C., Giordano, R.D.L.C., de Castro, H.F., 2012. Evaluation of immobilized lipases on poly-hydroxybutyrate beads to catalyze biodiesel synthesis. *Int. J. Biol. Macromol.* 50, 503–511.
- Mendez-Sanchez, D., Lopez-Iglesias, M., Gotor-Fernandez, V., 2016. Hydrolases in organic chemistry. recent achievements in the synthesis of pharmaceuticals. *Curr. Org. Chem.* 20, 1186–1203.
- Meng, X., Xu, G., Zhou, Q.L., Wu, J.P., Yang, L.R., 2013. Improvements of lipase performance in high-viscosity system by immobilization onto a novel kind of poly(methylmethacrylate-co-divinylbenzene) encapsulated porous magnetic microsphere carrier. *J. Mol. Catal. B Enzym.* 89, 86–92.
- Miao, Y., Rahimi, M., Geertsema, E.M., Poelarends, G.J., 2015. Recent developments in enzyme promiscuity for carbon-carbon bond-forming reactions. *Curr. Opin. Chem. Biol.* 25, 115–123.
- Mihailović, M., Stojanović, M., Banjanac, K., Carević, M., Prlainović, N., Milosavić, N., Bezbradica, D., 2014. Immobilization of lipase on epoxy-activated Purolite® A109 and its post-immobilization stabilization. *Process Biochem.* 49, 637–646.
- Mitrev, Y.N., Mehandziyski, A.Y., Batovska, D.I., Liese, A., Galunsky, B., 2016. Original

enzyme-catalyzed synthesis of chalcones: Utilization of hydrolase promiscuity. *J.*

Serbian Chem. Soc. 81, 1231–1237.

Morales-Sanfrutos, J., Lopez-Jaramillo, J., Ortega-Muñoz, M., Megia-Fernandez, A.,

Perez-Balderas, F., Hernandez-Mateo, F., Santoyo-Gonzalez, F., 2010. Vinyl sulfone:

A versatile function for simple bioconjugation and immobilization. *Org. Biomol.*

Chem. 8, 667–675.

Moreno-Perez, S., Luna, P., Señorans, J., Rocha-Martin, J., Guisan, J.M., Fernandez-

Lorente, G., 2018. Enzymatic transesterification in a solvent-free system: synthesis of

sn-2 docosahexaenoyl monoacylglycerol. *Biocatal. Biotransformation* 36, 265–270.

Nicolás, P., Lassalle, V.L., Ferreira, M.L., 2015. About the role of typical

spacer/crosslinker on the design of efficient magnetic biocatalysts based on nanosized

magnetite. *J. Mol. Catal. B Enzym.* 122, 296–304.

O'Brien, P.J., Herschlag, D., 1999. Catalytic promiscuity and the evolution of new

enzymatic activities. *Chem. Biol.* 6, R91–R105.

Ondari, M.E., Walker, K.W., 2009. Using enzyme promiscuity in the biocatalysis of second

generation taxanes. *Abstr. Pap. Am. Chem. Soc.* 238.

Orrego, A., Ghobadi, R., Moreno-Perez, S., Mendoza, A., Fernandez-Lorente, G., Guisan,

J., Rocha-Martin, J., 2018. Stabilization of Immobilized Lipases by Intense

Intramolecular Cross-Linking of Their Surfaces by Using Aldehyde-Dextran

Polymers. *Int. J. Mol. Sci.* 19, 553.

Ortega-Muñoz, M., Morales-Sanfrutos, J., Megia-Fernandez, A., Lopez-Jaramillo, F.J.,

- Hernandez-Mateo, F., Santoyo-Gonzalez, F., 2010. Vinyl sulfone functionalized silica: A “ready to use” pre-activated material for immobilization of biomolecules. *J. Mater. Chem.* 20, 7189–7196.
- Ortiz, C., Ferreira, M.L., Barbosa, O., dos Santos, J.C.S., Rodrigues, R.C., Berenguer-Murcia, Á., Briand, L.E., Fernandez-Lafuente, R., 2019. Novozym 435: the “perfect” lipase immobilized biocatalyst? *Adv. Synth. Catal.* Accepted for publication
- Pace, V., Hoyos, P., Castoldi, L., Domínguez de María, P., Alcántara, A.R., 2012. 2-Methyltetrahydrofuran (2-MeTHF): A Biomass-Derived Solvent with Broad Application in Organic Chemistry. *ChemSusChem* 5, 1369–1379.
- Palomo, J.M., Fernández-Lorente, G., Mateo, C., Fuentes, M., Fernández-Lafuente, R., Guisan, J.M., 2002a. Modulation of the enantioselectivity of *Candida antarctica* B lipase via conformational engineering. Kinetic resolution of ($\pm$)- α -hydroxy-phenylacetic acid derivatives. *Tetrahedron: Asymmetry* 13, 1337–1345.
- Palomo, J.M., Fernandez-Lorente, G., Mateo, C., Ortiz, C., Fernandez-Lafuente, R., Guisan, J.M., 2002b. Modulation of the enantioselectivity of lipases via controlled immobilization and medium engineering: hydrolytic resolution of mandelic acid esters. *Enzyme Microb. Technol.* 31, 775–783.
- Palomo, J.M., Fuentes, M., Fernández-Lorente, G., Mateo, C., Guisan, J.M., Fernández-Lafuente, R., 2003a. General trend of lipase to self-assemble giving bimolecular aggregates greatly modifies the enzyme functionality. *Biomacromolecules* 4, 1–6.
- Palomo, J.M., Muñoz, G., Fernández-Lorente, G., Mateo, C., Fuentes, M., Guisan, J.M.,

- Fernández-Lafuente, R., 2003b. Modulation of *Mucor miehei* lipase properties via directed immobilization on different hetero-functional epoxy resins: Hydrolytic resolution of (R,S)-2-butyroyl-2-phenylacetic acid. *J. Mol. Catal. B Enzym.* 21, 201–210.
- Palomo, J.M., Muñoz, G., Fernández-Lorente, G., Mateo, C., Fernández-Lafuente, R., Guisán, J.M., 2002c. Interfacial adsorption of lipases on very hydrophobic support (octadecyl-Sepabeads): Immobilization, hyperactivation and stabilization of the open form of lipases. *J. Mol. Catal. B Enzym.* 19–20, 279–286.
- Palomo, J.M., Ortiz, C., Fernández-Lorente, G., Fuentes, M., Guisán, J.M., Fernández-Lafuente, R., 2005a. Lipase-lipase interactions as a new tool to immobilize and modulate the lipase properties. *Enzyme Microb. Technol.* 36, 447–454.
- Palomo, J.M., Ortiz, C., Fuentes, M., Fernandez-Lorente, G., Guisan, J.M., Fernandez-Lafuente, R., 2004a. Use of immobilized lipases for lipase purification via specific lipase-lipase interactions. *J. Chromatogr. A* 1038, 267–273.
- Palomo, J.M., Peñas, M.M., Fernández-Lorente, G., Mateo, C., Pisabarro, A.G., Fernández-Lafuente, R., Ramírez, L., Guisán, J.M., 2003c. Solid-phase handling of hydrophobins: Immobilized hydrophobins as a new tool to study lipases. *Biomacromolecules* 4, 204–210.
- Palomo, J.M., Segura, R.L., Fernández-Lorente, G., Pernas, M., Rua, M.L., Guisán, J.M., Fernández-Lafuente, R., 2004b. Purification, immobilization, and stabilization of a lipase from *Bacillus thermocatenuatus* by interfacial adsorption on hydrophobic supports. *Biotechnol. Prog.* 20, 630–635.

- Palomo, J.M., Segura, R.L., Mateo, C., Terreni, M., Guisan, J.M., Fernández-Lafuente, R., 2005b. Synthesis of enantiomerically pure glycidol via a fully enantioselective lipase-catalyzed resolution. *Tetrahedron: Asymmetry* 16, 869–874.
- Parashar, S.K., Srivastava, S.K., Dutta, N.N., Garlapati, V.K., 2018. Engineering aspects of immobilized lipases on esterification: A special emphasis of crowding, confinement and diffusion effects. *Eng. Life Sci.* 18, 308–316.
- Park, J.H., Ha, H.J., Lee, W.K., Genereux-Vincent, T., Kazlauskas, R.J., 2009. Molecular Basis for the Stereoselective Ammoniolysis of N-Alkyl Aziridine-2-Carboxylates Catalyzed by *Candida antarctica* Lipase B. *ChemBioChem* 10, 2213–2222.
- Park, S., Kim, S.H., Kim, J.H., Yu, H., Kim, H.J., Yang, Y.-H., Kim, H., Kim, Y.H., Ha, S.H., Lee, S.H., 2015. Application of cellulose/lignin hydrogel beads as novel supports for immobilizing lipase. *J. Mol. Catal. B Enzym.* 119, 33–39.
- Patel, R.N., 2012. 9.12 Industrial Applications of Biocatalytic Hydrolysis (Esters, Amides, Epoxides, Nitriles) and Biocatalytic Dynamic Kinetic Resolution, in: Editors-in-Chief: Erick, M.C., Hisashi, Y. (Eds.), *Comprehensive Chirality*. Elsevier, Amsterdam, pp. 288–317.
- Pavlovic, E., Oscarsson, S., Quist, A.P., 2003. Nanoscale Site-Specific Immobilization of Proteins through Electroactivated Disulfide Exchange. *Nano Lett.* 3, 779–781.
- Pedroche, J., del Mar Yust, M., Mateo, C., Fernández-Lafuente, R., Girón-Calle, J., Alaiz, M., Vioque, J., Guisán, J.M., Millán, F., 2007. Effect of the support and experimental conditions in the intensity of the multipoint covalent attachment of proteins on

glyoxyl-agarose supports: Correlation between enzyme-support linkages and thermal stability. *Enzyme Microb. Technol.* 40, 1160–1166.

- Peirce, S., Tacias-Pascacio, V., Russo, M., Marzocchella, A., Virgen-Ortíz, J., Fernandez-Lafuente, R., 2016a. Stabilization of *Candida antarctica* Lipase B (CALB) Immobilized on Octyl Agarose by Treatment with Polyethyleneimine (PEI). *Molecules* 21, 751.
- Peirce, S., Virgen-Ortíz, J.J., Tacias-Pascacio, V.G., Rueda, N., Bartolome-Cabrero, R., Fernandez-Lopez, L., Russo, M.E., Marzocchella, A., Fernandez-Lafuente, R., 2016b. Development of simple protocols to solve the problems of enzyme coimmobilization. Application to coimmobilize a lipase and a β -galactosidase. *RSC Adv.* 6, 61707–61715.
- Peña-García, C., Martínez-Martínez, M., Reyes-Duarte, D., Ferrer, M., 2016. High throughput screening of esterases, lipases and phospholipases in mutant and metagenomic libraries: A review. *Comb. Chem. High Throughput Screen.* 19, 605–615.
- Pereira, G.N., Holz, J.P., Giovannini, P.P., Oliveira, J.V., de Oliveira, D., Lerin, L.A., 2018a. Enzymatic esterification for the synthesis of butyl stearate and ethyl stearate. *Biocatal. Agric. Biotechnol.* 16, 373–377.
- Pereira, G.N., Holz, J.P., Lerin, L., Picoli Zenevich, M.C., de Oliveira, D., Vladimir de Oliveira, J., 2018b. Solvent-Free Production of Ethylene Glycol Monostearate through Enzymatic Esterification. *Ind. Eng. Chem. Res.* 57, 6627–6632.

- Pereira, M.G., Facchini, F.D.A., Polizeli, A.M., Vici, A.C., Jorge, J.A., Pessela, B.C.,
 Fernández-Lorente, G., Guisán, J.M., De Moraes Polizeli, M.D.L.T., 2015.
 Stabilization of the lipase of *Hypocrea pseudokoningii* by multipoint covalent
 immobilization after chemical modification and application of the biocatalyst in oil
 hydrolysis. *J. Mol. Catal. B Enzym.* 121, 82–89.
- Petry, I., Ganesan, A., Pitt, A., Moore, B.D., Halling, P.J., 2006. Proteomic methods
 applied to the analysis of immobilized biocatalysts. *Biotechnol. Bioeng.* 95, 984–991.
- Pizarro, C., Brañes, M.C., Markovits, A., Fernández-Lorente, G., Guisán, J.M., Chamy, R.,
 Wilson, L., 2012. Influence of different immobilization techniques for *Candida*
cylindracea lipase on its stability and fish oil hydrolysis. *J. Mol. Catal. B Enzym.* 78,
 111–118.
- Polloni, A.E., Chiaradia, V., Figura, E.M., de Paoli, J.P., de Oliveira, D., de Oliveira, J.V.,
 de Araujo, P.H.H., Sayer, C., 2017. Polyesters from Macrolactones Using Commercial
 Lipase NS 88011 and Novozym 435 as Biocatalysts. *Appl. Biochem. Biotechnol.* 1–
 14.
- Poppe, J.K., Garcia-Galan, C., Matte, C.R., Fernandez-Lafuente, R., Rodrigues, R.C.,
 Ayub, M.A.Z., 2013. Optimization of synthesis of fatty acid methyl esters catalyzed
 by lipase B from *Candida antarctica* immobilized in hydrophobic supports. *J. Mol.*
Catal. B Enzym. 94, 51–56.
- Poppe, J.K., Matte, C.R., de Freitas, V.O., Fernandez-Lafuente, R., Rodrigues, R.C., Ayub,
 M.A.Z., 2018a. Enzymatic synthesis of ethyl esters from waste oil using mixtures of
 lipases in a plug-flow packed-bed continuous reactor. *Biotechnol. Prog.* 34, 952–959.

Poppe, J.K., Matte, C.R., Fernandez-Lafuente, R., Rodrigues, R.C., Ayub, M.A.Z., 2018b.

Transesterification of Waste Frying Oil and Soybean Oil by Combi-lipases Under Ultrasound-Assisted Reactions. *Appl. Biochem. Biotechnol.* 186, 576–589.

Poppe, J.K., Matte, C.R., Peralba, M.D.C.R., Fernandez-Lafuente, R., Rodrigues, R.C.,

Ayub, M.A.Z., 2015. Optimization of ethyl ester production from olive and palm oils using mixtures of immobilized lipases. *Appl. Catal. A Gen.* 490, 50–56.

Prikryl, P., Lenfeld, J., Horak, D., Ticha, M., Kucerova, Z., 2012. Magnetic bead cellulose as a suitable support for immobilization of α -chymotrypsin. *Appl. Biochem. Biotechnol.* 168, 295–305.

Pujari, N.S., Vaidya, B.K., Bagalkote, S., Ponrathnam, S., Nene, S., 2006. Poly(urethane methacrylate-co-glycidyl methacrylate)-supported-polypropylene biphasic membrane for lipase immobilization. *J. Memb. Sci.* 285, 395–403.

Qiao, H., Zhang, F., Guan, W., Zuo, J., Feng, D., 2017. Optimisation of combi-lipases from *Aspergillus niger* for the synergistic and efficient hydrolysis of soybean oil. *Anim. Sci. J.* 88, 772–780.

Quirós, M., García, A.B., Montes-Morán, M.A., 2011. Influence of the support surface properties on the protein loading and activity of lipase/mesoporous carbon biocatalysts. *Carbon N. Y.* 49, 406–415.

Rahman, M.Z.A., Salleh, A., Rahman, R., Rahman, M.B.A., Basri, M., Leow, T.C., 2012.

Unlocking the mystery behind the activation phenomenon of T1 lipase: A molecular dynamics simulations approach. *Protein Sci.* 21, 1210–1221.

- Rana, Q.U.A., Laiq Ur Rehman, M., Irfan, M., Ahmed, S., Hasan, F., Shah, A.A., Khan, S., Badshah, M., 2018. Lipolytic bacterial strains mediated transesterification of non-edible plant oils for generation of high quality biodiesel. *J. Biosci. Bioeng.* in press, 10.1016/j.jbiosc.2018.11.001.
- Reetz, M.T., 2002. Lipases as practical biocatalysts. *Curr. Opin. Chem. Biol.* 6, 145–150.
- Ribeiro, M.D.M.M., Ming, C.C., Lopes, T.I.B., Grimaldi, R., Marsaioli, A.J., Gonçalves, L.A.G., 2017. Synthesis of structured lipids containing behenic acid from fully hydrogenated *Crambe abyssinica* oil by enzymatic interesterification. *J. Food Sci. Technol.* 54, 1146–1157.
- Rios, N.S., Pinheiro, M.P., Lima, M.L.B., Freire, D.M.G., da Silva, I.J., Rodríguez-Castellón, E., de Sant’Ana, H.B., Macedo, A.C., Gonçalves, L.R.B., 2018. Pore-expanded SBA-15 for the immobilization of a recombinant *Candida antarctica* lipase B: Application in esterification and hydrolysis as model reactions. *Chem. Eng. Res. Des.* 129, 12–24.
- Rodrigues, R.C., Ayub, M.A.Z., 2011. Effects of the combined use of *Thermomyces lanuginosus* and *Rhizomucor miehei* lipases for the transesterification and hydrolysis of soybean oil. *Process Biochem.* 46, 682–688.
- Rodrigues, R.C., Barbosa, O., Ortiz, C., Berenguer-Murcia, Á., Torres, R., Fernandez-Lafuente, R., 2014. Amination of enzymes to improve biocatalyst performance: coupling genetic modification and physicochemical tools. *RSC Adv.* 4, 38350–38374.
- Rodrigues, R.C., Berenguer-Murcia, Á., Fernandez-Lafuente, R., 2011. Coupling Chemical

Modification and Immobilization to Improve the Catalytic Performance of Enzymes.

Adv. Synth. Catal. 353, 2216–2238.

- Rodrigues, R.C., Godoy, C.A., Volpato, G., Ayub, M.A.Z., Fernandez-Lafuente, R., Guisan, J.M., 2009. Immobilization–stabilization of the lipase from *Thermomyces lanuginosus*: Critical role of chemical amination. Process Biochem. 44, 963–968.
- Rodrigues, R.C., Hernandez, K., Barbosa, O., Rueda, N., Garcia-Galan, C., dos Santos, J.C.S., Berenguer-Murcia, A., Fernandez-Lafuente, R., 2015. Immobilization of proteins in poly-styrene-divinylbenzene matrices: Functional properties and applications. Curr. Org. Chem. 19, 1707–1718.
- Rodrigues, R.C., Ortiz, C., Berenguer-Murcia, Á., Torres, R., Fernández-Lafuente, R., 2013. Modifying enzyme activity and selectivity by immobilization. Chem. Soc. Rev. 42, 6290–6307.
- Rodríguez-Mata, M., Gotor-Fernández, V., 2015. Resolution of Alcohols, Amines, Acids, and Esters by Nonhydrolytic Processes. Biocatal. Org. Synth.
- Roy, I., Mukherjee, J., Gupta, M.N., 2013. High Activity Preparations of Lipases and Proteases for Catalysis in Low Water Containing Organic Solvents and Ionic Liquids, in: Guisan, J.M. (Ed.), Immobilization of Enzymes and Cells. pp. 275–284.
- Rueda, N., Albuquerque, T., Bartolome-Cabrero, R., Fernandez-Lopez, L., Torres, R., Ortiz, C., dos Santos, J., Barbosa, O., Fernandez-Lafuente, R., 2016a. Reversible Immobilization of Lipases on Heterofunctional Octyl-Amino Agarose Beads Prevents Enzyme Desorption. Molecules 21, 646.

- Rueda, N., dos Santos, J.C.S., Ortiz, C., Barbosa, O., Fernandez-Lafuente, R., Torres, R., 2016b. Chemical amination of lipases improves their immobilization on octyl-glyoxyl agarose beads. *Catal. Today* 259, 107–118.
- Rueda, N., dos Santos, J.C.S., Ortiz, C., Torres, R., Barbosa, O., Rodrigues, R.C., Berenguer-Murcia, Á., Fernandez-Lafuente, R., 2016c. Chemical Modification in the Design of Immobilized Enzyme Biocatalysts: Drawbacks and Opportunities. *Chem. Rec.* 16, 1436–1455.
- Rueda, N., dos Santos, J.C.S., Rodriguez, M.D., Albuquerque, T.L., Barbosa, O., Torres, R., Ortiz, C., Fernandez-Lafuente, R., 2016d. Reversible immobilization of lipases on octyl-glutamic agarose beads: A mixed adsorption that reinforces enzyme immobilization. *J. Mol. Catal. B Enzym.* 128, 10–18.
- Rueda, N., dos Santos, J.C.S., Torres, R., Barbosa, O., Ortiz, C., Fernandez-Lafuente, R., 2015a. Reactivation of lipases by the unfolding and refolding of covalently immobilized biocatalysts. *RSC Adv.* 5, 55588–55594.
- Rueda, N., dos Santos, J.C.S., Torres, R., Ortiz, C., Barbosa, O., Fernandez-Lafuente, R., 2015b. Improved performance of lipases immobilized on heterofunctional octyl-glyoxyl agarose beads. *RSC Adv.* 5, 11212–11222.
- Ruiz, M., Galvis, M., Barbosa, O., Ortiz, C., Torres, R., Fernandez-Lafuente, R., 2013. Solid-phase modification with succinic polyethyleneglycol of aminated lipase B from *Candida antarctica*: Effect of the immobilization protocol on enzyme catalytic properties. *J. Mol. Catal. B Enzym.* 87, 75–82.

- Rychlicka, M., Niezgoda, N., Gliszczyńska, A., 2018. Lipase-Catalyzed Acidolysis of Egg-Yolk Phosphatidylcholine with Citronellic Acid. New Insight into Synthesis of Isoprenoid-Phospholipids. *Molecules* 23, 314.
- Sabuquillo, P., Reina, J., Fernandez-Lorente, G., Guisan, J.M., Fernandez-Lafuente, R., 1998. "Interfacial affinity chromatography" of lipases: Separation of different fractions by selective adsorption on supports activated with hydrophobic groups. *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.* 1388, 337–348.
- Sakamoto, S., Hamachi, I., 2019. Recent Progress in Chemical Modification of Proteins. *Anal. Sci.* 35, 5–27.
- Salihu, A., Alam, M.Z., 2015. Solvent tolerant lipases: A review. *Process Biochem.* 50, 86–96.
- Samoylova, Y. V., Piligaev, A. V., Sorokina, K.N., Parmon, V.N., 2017. Enzymatic Interesterification of Sunflower Oil and Hydrogenated Soybean Oil with the Immobilized Bacterial Recombinant Lipase from *Geobacillus stearothermophilus* G3. *Catal. Ind.* 9, 62–70.
- Santos-Moriano, P., Monsalve-Ledesma, L., Ortega-Muñoz, M., Fernandez-Arrojo, L., Ballesteros, A.O., Santoyo-Gonzalez, F., Plou, F.J., 2016. Vinyl sulfone-activated silica for efficient covalent immobilization of alkaline unstable enzymes: application to levansucrase for fructooligosaccharide synthesis. *RSC Adv.* 6, 64175–64181.
- Saunders, P., Brask, J., 2010. Improved Immobilization Supports for *Candida antarctica* Lipase B, in: Loos, K. (Ed.), *Biocatalysis in Polymer Chemistry*. Wiley-VCH Verlag

GmbH & Co. KGaA, Weinheim, Germany, pp. 65–82.

- Sbardelotto, C., Piazza, S., Nyari, N., Dallago, R., Oliveira, D., Oliveira, V., Zeni, J., Cansian, R., Paroul, N., 2018. Optimization of solvent-free geranyl butanoate production using novozyme 435 and homemade polyurethane immobilized Novozyme NZL-102-lyo-Hq as catalysts. *Quim. Nova* 41, 905–911.
- Schmid-Dannert, C., López-Gallego, F., 2019. Advances and opportunities for the design of self-sufficient and spatially organized cell-free biocatalytic systems. *Curr. Opin. Chem. Biol.* 49, 97–104.
- Schmid, A., Dordick, J.S., Hauer, B., Kiener, A., Wubbolts, M., Witholt, B., 2001. Industrial biocatalysis today and tomorrow. *Nature* 409, 258–268.
- Schmid, R.D., Verger, R., 1998. Lipases: Interfacial enzymes with attractive applications. *Angew. Chemie, Int. Ed.* 37, 1609–1633.
- Schoemaker, H.E., Mink, D., Wubbolts, M.G., 2003. Dispelling the Myths--Biocatalysis in Industrial Synthesis. *Science* 299, 1694–1697.
- Seddigi, Z.S., Malik, M.S., Ahmed, S.A., Babalghith, A.O., Kamal, A., 2017. Lipases in asymmetric transformations: Recent advances in classical kinetic resolution and lipase-metal combinations for dynamic processes. *Coord. Chem. Rev.* 348, 54–70.
- Segura, R.L., Betancor, L., Palomo, J.M., Hidalgo, A., Fernández-Lorente, G., Terreni, M., Mateo, C., Cortés, A., Fernández-Lafuente, R., Guisán, J.M., 2006. Purification and identification of different lipases contained in PPL commercial extracts: A minor contaminant is the main responsible of most esterase activity. *Enzyme Microb.*

Technol. 39, 817–823.

- Segura, R.L., Palomo, J.M., Mateo, C., Cortes, A., Terreni, M., Fernández-Lafuente, R., Guisan, J.M., 2004. Different properties of the lipases contained in porcine pancreatic lipase extracts as enantioselective biocatalysts. *Biotechnol. Prog.* 20, 825–829.
- Serra, I., Ceechini, D.A., Ubiñe, D., Manazza, E.M., Albertini, A.M., Terreni, M., 2009. Coupling of Site-Directed mutagenesis and immobilization for the rational design of more efficient biocatalysts: The case of immobilized 3G3K PGA from *E. coli*. *European J. Org. Chem.* 1384–1389.
- Sethi, M.K., Chakraborty, P., Shukla, R., 2018. CHAPTER 2 Biocatalysis - A Greener Alternative in Synthetic Chemistry, in: *Biocatalysis: An Industrial Perspective*. The Royal Society of Chemistry, pp. 44–76.
- Séverac, E., Galy, O., Turon, F., Pantel, C.A., Condoret, J.S., Monsan, P., Marty, A., 2011. Selection of CalB immobilization method to be used in continuous oil transesterification: Analysis of the economical impact. *Enzyme Microb. Technol.* 48, 61–70.
- Sheldon, R.A., 2018. Metrics of Green Chemistry and Sustainability: Past, Present, and Future. *ACS Sustain. Chem. Eng.* 6, 32–48.
- Sheldon, R.A., 2017. The E factor 25 years on: the rise of green chemistry and sustainability. *Green Chem.* 19, 18–43.
- Sheldon, R.A., Brady, D., 2018. The limits to biocatalysis: pushing the envelope. *Chem. Commun.* 54, 6088–6104.

- Sheldon, R.A., van Pelt, S., 2013. Enzyme immobilisation in biocatalysis: why, what and how. *Chem. Soc. Rev.* 42, 6223–6235.
- Sheldon, R.A., Woodley, J.M., 2018. Role of Biocatalysis in Sustainable Chemistry. *Chem. Rev.* 118, 801–838.
- Siirola, E., Frank, A., Grogan, G., Kroutil, W., 2013. C–C Hydrolases for Biocatalysis. *Adv. Synth. Catal.* 355, 1677–1691.
- Silva, M.V.C., Aguiar, L.G., de Castro, H.F., Freitas, L., 2018. Optimization of the parameters that affect the synthesis of magnetic copolymer styrene-divinylbenzene to be used as efficient matrix for immobilizing lipases. *World J. Microbiol. Biotechnol.* 34, 169.
- Silveira, E.A., Moreno-Perez, S., Basso, A., Serban, S., Pestana-Mamede, R., Tardioli, P.W., Sanchez-Farinas, C., Castejon, N., Fernandez-Lorente, G., Rocha-Martin, J., Guisan, J.M., 2019. Biocatalyst engineering of *Thermomyces lanuginosus* lipase adsorbed on hydrophobic supports: Modulation of enzyme properties for ethanolysis of oil in solvent-free systems. *J. Biotechnol.* 289, 126–134.
- Silveira, E.A., Moreno-Perez, S., Basso, A., Serban, S., Pestana Mamede, R., Tardioli, P.W., Sanchez Farinas, C., Rocha-Martin, J., Fernandez-Lorente, G., Guisan, J.M., 2017. Modulation of the regioselectivity of *Thermomyces lanuginosus* lipase via biocatalyst engineering for the Ethanolysis of oil in fully anhydrous medium. *BMC Biotechnol.* 17, 88.
- Simon, C., Daniel, R., 2011. Metagenomic Analyses: Past and Future Trends. *Appl.*

- Environ. Microbiol. 77, 1153–1161.
- Sin, G., Woodley, J.A., Gernaey, K. V, 2009. Application of Modeling and Simulation Tools for the Evaluation of Biocatalytic Processes: A Future Perspective. Biotechnol. Prog. 25, 1529–1538.
- Singh, A.K., Mukhopadhyay, M., 2018. Immobilization of lipase on carboxylic acid-modified silica nanoparticles for olive oil glycerolysis. Bioprocess Biosyst. Eng. 41, 115–127.
- Sivakanthan, S., Jayasooriya, A.P., Madhujith, T., 2019. Optimization of the production of structured lipid by enzymatic interesterification from coconut (*Cocos nucifera*) oil and sesame (*Sesamum indicum*) oil using Response Surface Methodology. LWT - Food Sci. Technol. 101, 723–730.
- Sneha, R., Jeyarani, T., 2018. Lipase-catalysed acidolysis of mango kernel fat with capric acid to obtain medium- and long-chain triacylglycerols. Int. J. Food Sci. Technol. 53, 1527–1534.
- Sousa, V., Campos, V., Nunes, P., Pires-Cabral, P., 2018. Incorporation of capric acid in pumpkin seed oil by sn -1,3 regioselective lipase-catalyzed acidolysis. OCL 25, A302.
- Spelbrink, R.E.J., Giuseppin, M.L.F., Egmond, M.R., 2018. Lipase in short-chain esterification of fatty acids. US 10034485.
- Speranza, P., Leão, K.M.M., Narciso Gomes, T.S., Reis, L.V.C., Rodrigues, A.P., Alves Macedo, J., Ribeiro, A.P.B., Alves Macedo, G., 2018. Improving the chemical properties of Buriti oil (*Mauritia flexuosa* L.) by enzymatic interesterification. Grasas

y Aceites 69, 282.

Spicer, C.D., Davis, B.G., 2014. Selective chemical protein modification. *Nat. Commun.* 5, 4740.

Stepankova, V., Bidmanova, S., Koudelakova, T., Prokop, Z., Chaloupkova, R., Damborsky, J., 2013. Strategies for Stabilization of Enzymes in Organic Solvents. *ACS Catal.* 3, 2823–2836.

Stergiou, P.Y., Foukis, A., Filippou, M., Koukouritaki, M., Parapouli, M., Theodorou, L.G., Hatziloukas, E., Afendra, A., Pandey, A., Papamichael, E.M., 2013. Advances in lipase-catalyzed esterification reactions. *Biotechnol. Adv.* 31, 1846–1859.

Su, F., Li, G., Zhang, H., Yan, Y., 2014. Enhanced Performance of *Rhizopus oryzae* Lipase Immobilized on Hydrophobic Carriers and Its Application in Biorefinery of Rapeseed Oil Deodorizer Distillate. *BioEnergy Res.* 7, 935–945.

Suescun, A., Rueda, N., dos Santos, J.C.S., Castillo, J.J., Ortiz, C., Torres, R., Barbosa, O., Fernandez-Lafuente, R., 2015. Immobilization of lipases on glyoxyl-octyl supports: Improved stability and reactivation strategies. *Process Biochem.* 50, 1211–1217.

Sun, H., Zhang, H., Ang, E.L., Zhao, H., 2018. Biocatalysis for the synthesis of pharmaceuticals and pharmaceutical intermediates. *Bioorg. Med. Chem.* 26, 1275–1284.

Tacias-Pascacio, V.G., Peirce, S., Torrestiana-Sanchez, B., Yates, M., Rosales-Quintero, A., Virgen-Ortíz, J.J., Fernandez-Lafuente, R., 2016. Evaluation of different commercial hydrophobic supports for the immobilization of lipases: tuning their

stability, activity and specificity. RSC Adv. 6, 100281–100294.

- Tacias-Pascacio, V.G., Virgen-Ortíz, J.J., Jiménez-Pérez, M., Yates, M., Torrestiana-Sanchez, B., Rosales-Quintero, A., Fernandez-Lafuente, R., 2017. Evaluation of different lipase biocatalysts in the production of biodiesel from used cooking oil: Critical role of the immobilization support. Fuel 200, 1–10.
- Tao, Y., Cui, C., Shen, H., Liu, L., Chen, B., Tan, T., 2014. Enhancing trimethylolpropane esters synthesis through lipase immobilized on surface hydrophobic modified support and appropriate substrate feeding methods. Enzyme Microb. Technol. 58–59, 60–67.
- Tecelão, C., Guillén, M., Valero, F., Ferreira-Dias, S., 2012. Immobilized heterologous *Rhizopus oryzae* lipase: A feasible biocatalyst for the production of human milk fat substitutes. Biochem. Eng. J. 67, 104–110.
- Tokuyama, H., Naito, A., Kato, G., 2018. Transesterification of triolein with ethanol using lipase-entrapped NIPA-co-PEGMEA gel beads. React. Funct. Polym. 126, 83–86.
- Toledo, M., Collins, S., Llerena Suster, C., Briand, L., 2016. Molecular recognition of an acyl-enzyme intermediate on the lipase B of *Candida antarctica*. Abstr. Pap. Am. Chem. Soc. 252.
- Trodler, P., Pleiss, J., 2008. Modeling structure and flexibility of *Candida antarctica* lipase B in organic solvents. BMC Struct. Biol. 8, 9.
- Trodler, P., Schmid, R.D., Pleiss, J., 2009. Modeling of solvent-dependent conformational transitions in *Burkholderia cepacia* lipase. BMC Struct. Biol. 9, 38.
- Ueda, M., 2010. Development of Revolutionary Enzymatic Reactions in Organic Solvents

with Molecular Display. *Yakugaku Zasshi-Journal Pharm. Soc. Japan* 130, 1479–1485.

Uppenberg, J., Hansen, M.T., Patkar, S., Jones, T. a, 1994. The sequence, crystal structure determination and refinement of two crystal forms of lipase B from *Candida antarctica*. *Structure* 2, 293–308.

Urrutia, P., Arrieta, R., Alvarez, L., Cardenas, C., Mesa, M., Wilson, L., 2018.

Immobilization of lipases in hydrophobic chitosan for selective hydrolysis of fish oil: The impact of support functionalization on lipase activity, selectivity and stability. *Int. J. Biol. Macromol.* 108, 674–686.

van den Heuvel, R.H.H., Laane, C., van Berkel, W.J.H., 2001. Exploring the Biocatalytic Potential of Vanillyl-Alcohol Oxidase by Site-Directed Mutagenesis. *Adv. Synth. Catal.* 343, 515–520.

van Dongen, S.F.M., Nallani, M., Cornelissen, J.J.L.M., Nolte, R.J.M., van Hest, J.C.M., 2009. A Three-Enzyme Cascade Reaction through Positional Assembly of Enzymes in a Polymersome Nanoreactor. *Chem. - A Eur. J.* 15, 1107–1114.

Vasilescu, C., Todea, A., Paul, C., Benea, I.C., Nan, A., Turcu, R., Peter, F., 2018. New multilayer magnetic biocatalyst for esterification and transesterification reactions. *N. Biotechnol.* 44, S73–S73.

Velasco-Lozano, S., Benítez-Mateos, A.I.A.I., López-Gallego, F., 2017. Co-immobilized Phosphorylated Cofactors and Enzymes as Self-Sufficient Heterogeneous Biocatalysts for Chemical Processes. *Angew. Chemie Int. Ed.* 56, 771–775.

- Verdasco-Martín, C.M., Garcia-Verdugo, E., Porcar, R., Fernandez-Lafuente, R., Otero, C., 2018. Selective synthesis of partial glycerides of conjugated linoleic acids via modulation of the catalytic properties of lipases by immobilization on different supports. *Food Chem.* 245, 39–46.
- Verger, R., 1997. “Interfacial activation” of lipases: Facts and artifacts. *Trends Biotechnol.* 15, 32–38.
- Verger, R., De Haas, G.H., 1976. Interfacial Enzyme Kinetics of Lipolysis. *Annu. Rev. Biophys. Bioeng.* 5, 77–117.
- Vescovi, V., dos Santos, J.B.C., Tardioli, P.W., 2017. Porcine pancreatic lipase hydrophobically adsorbed on octyl-silica: A robust biocatalyst for syntheses of xylose fatty acid esters. *Biocatal. Biotransf.* 35, 298–305.
- Vescovi, V., Kopp, W., Guisán, J.M., Giordano, R.L.C., Mendes, A.A., Tardioli, P.W., 2016. Improved catalytic properties of *Candida antarctica* lipase B multi-attached on tailor-made hydrophobic silica containing octyl and multifunctional amino-glutaraldehyde spacer arms. *Process Biochem.* 51, 2055–2066.
- Vilas Bôas, R.N., Ceron, A.A., Bento, H.B.S., de Castro, H.F., 2018. Application of an immobilized *Rhizopus oryzae* lipase to batch and continuous ester synthesis with a mixture of a lauric acid and fusel oil. *Biomass and Bioenergy* 119, 61–68.
- Virgen-Ortíz, J.J., dos Santos, J.C.S., Berenguer-Murcia, Á., Barbosa, O., Rodrigues, R.C., Fernandez-Lafuente, R., 2017a. Polyethylenimine: a very useful ionic polymer in the design of immobilized enzyme biocatalysts. *J. Mater. Chem. B* 5, 7461–7490.

- Virgen-Ortíz, J.J., Pedrero, S., Fernandez-Lopez, L., Lopez-Carrobes, N., Gorines, B., Otero, C., Fernandez-Lafuente, R., 2017b. Desorption of Lipases Immobilized on Octyl-Agarose Beads and Coated with Ionic Polymers after Thermal Inactivation. Stronger Adsorption of Polymers/Unfolded Protein Composites. *Molecules* 22, 91.
- Virgen-Ortíz, J.J., Tacias-Pascacio, V.G., Hirata, D.B., Torrestiana-Sanchez, B., Rosales-Quintero, A., Fernandez-Lafuente, R., 2017c. Relevance of substrates and products on the desorption of lipases physically adsorbed on hydrophobic supports. *Enzyme Microb. Technol.* 96, 30–35.
- Volpato, G., Filice, M., Ayub, M.A.Z., Guisan, J.M., Palomo, J.M., 2010. Single-step purification of different lipases from *Staphylococcus warneri*. *J. Chromatogr. A* 1217, 473–478.
- Volpato, G., Filice, M., de las Rivas, B., Rodrigues, R.C., Heck, J.X., Fernandez-Lafuente, R., Guisan, J.M., Mateo, C., Ayub, M.A.Z., 2011. Purification, immobilization, and characterization of a specific lipase from *Staphylococcus warneri* EX17 by enzyme fractionating via adsorption on different hydrophobic supports. *Biotechnol. Prog.* 27, 717–723.
- Vriezema, D.M., Garcia, P.M.L., Sancho Oltra, N., Hatzakis, N.S., Kuiper, S.M., Nolte, R.J.M., Rowan, A.E., van Hest, J.C.M., 2007. Positional Assembly of Enzymes in Polymersome Nanoreactors for Cascade Reactions. *Angew. Chemie Int. Ed.* 46, 7378–7382.
- Wang, B., Li, W., Wu, B., He, B., 2014. Efficient resolution of 3-phenoxy-1,2-propanediol by immobilized lipase on amphiphilic comb polymer modified TiO₂. *J. Mol. Catal. B*

Enzym. 106, 105–110.

Wang, S., Meng, X., Zhou, H., Liu, Y., Secundo, F., Liu, Y., 2016. Enzyme Stability and Activity in Non-Aqueous Reaction Systems: A Mini Review. *Catalysts* 6, 32.

Wang, X., Chen, Y., Zheng, L., Jin, Q., Wang, X., 2017. Synthesis of 1,3-distearoyl-2-oleoylglycerol by enzymatic acidolysis in a solvent-free system. *Food Chem.* 228, 420–426.

Weinberger, S., Pellis, A., Comerford, J.W., Farmer, T.J., Guebitz, G.M., 2018. Efficient physisorption of candida antarctica lipase B on polypropylene beads and application for polyester synthesis. *Catalysts* 8.

Wiemann, L.O., Nieguth, R., Eckstein, M., Naumann, M., Thum, O., Ansorge-Schumacher, M.B., 2009. Composite Particles of Novozyme 435 and Silicone: Advancing Technical Applicability of Macroporous Enzyme Carriers. *ChemCatChem* 1, 455–462.

Wilson, L., Palomo, J.M., Fernández-Lorente, G., Illanes, A., Guisán, J.M., Fernández-Lafuente, R., 2006. Effect of lipase-lipase interactions in the activity, stability and specificity of a lipase from *Alcaligenes* sp. *Enzyme Microb. Technol.* 39, 259–264.

Xu, J.M., Lin, X.F., 2007. Catalytic promiscuity of enzyme and its new progress in organic synthesis. *Chinese J. Org. Chem.* 27, 1473–1478.

Xue, P., Hu, C.-M., Yan, X.-H., Fang, G.-L., Shen, H.-F., 2018. Enhancement of activity and reusability of lipase immobilized on magnetic mesoporous silica for the resolution of racemic secondary alcohols. *J. Chinese Chem. Soc.* in press, doi: 10.1002/jccs.201800193.

- Xun, E., Wang, Z., Zhao, J., Guo, J., 2015. Kinetic resolution of (R,S)-2-(2-chloro-1-hydroxyethyl) thiophene via immobilizing lipase from *Alcaligenes sp.* onto magnetic nanoparticles. *J. Chem. Technol. Biotechnol.* 90, 492–499.
- Yakup Arica, M., Denizli, A., Baran, T., Hasirci, V., 1998. Dye derived and metal incorporated affinity poly(2-hydroxyethyl methacrylate) membranes for use in enzyme immobilization. *Polym. Int.* 46, 345–352.
- Yamamoto, Y., Yoshida, H., Nagai, T., Hara, S., 2018. Preparation of Chiral Triacylglycerols, sn-POO and sn-OOP, via Lipase-mediated Acidolysis Reaction. *J. Oleo Sci.* 67, 207–214.
- Yan, Y., Zhang, X., Chen, D., 2013. Enhanced catalysis of *Yarrowia lipolytica* lipase LIP2 immobilized on macroporous resin and its application in enrichment of polyunsaturated fatty acids. *Bioresour. Technol.* 131, 179–187.
- Yang, Y., Wang, D., Zhang, Z., Xu, Y., 2018. Activation of Esterification Activity of *Rhizopus Chinensis* Lipase in Organic Media by Surfactant. *Chem. J. Chinese Univ.* 39, 1948–1953.
- Yoshimura, Y., Saito, A., Mori, M., Osaki, S., Miyazaki, T., Mori, H., 2018. Immobilization of lipase on the surface of a micro tube and continuous transesterification. *J. Flow Chem.* 8, 45–50.
- Yu, D., Qi, X., Jiang, Y., Zou, D., Wang, L., Jiang, L., Qin, L., 2018a. Preparation of Margarine Stock Rich in Naturally Bioactive Components by Enzymatic Interesterification. *J. Oleo Sci.* 67, 29–37.

- Yu, D., Zhang, X., Zou, D., Wang, T., Liu, T., Wang, L., Elfalleh, W., Jiang, L., 2018b. Immobilized CALB Catalyzed Transesterification of Soybean Oil and Phytosterol. *Food Biophys.* 13, 208–215.
- Yu, W.H., Fang, M., Tong, D.S., Shao, P., Xu, T.N., Zhou, C.H., 2013. Immobilization of *Candida rugosa* lipase on hexagonal mesoporous silicas and selective esterification in nonaqueous medium. *Biochem. Eng. J.* 70, 97–105.
- Yüksel, A., Şahin-Yeşilçubuk, N., 2018. Use of Echium oil fatty acids and tricaprylin as substrates of enzymatic interesterification for the production of structured lipids. *Grasas y Aceites* 69, 236.
- Zaak, H., Fernandez-Lopez, L., Otero, C., Sassi, M., Fernandez-Lafuente, R., 2017a. Improved stability of immobilized lipases via modification with polyethylenimine and glutaraldehyde. *Enzyme Microb. Technol.* 106, 67–74.
- Zaak, H., Fernandez-Lopez, L., Velasco-Lozano, S., Alcaraz-Fructuoso, M.T., Sassi, M., Lopez-Gallego, F., Fernandez-Lafuente, R., 2017b. Effect of high salt concentrations on the stability of immobilized lipases: Dramatic deleterious effects of phosphate anions. *Process Biochem.* 62, 128–134.
- Zaak, H., Kornecki, J.F., Siar, E.-H., Fernandez-Lopez, L., Corberán, V.C., Sassi, M., Fernandez-Lafuente, R., 2017c. Coimmobilization of enzymes in bilayers using pei as a glue to reuse the most stable enzyme: Preventing pei release during inactivated enzyme desorption. *Process Biochem.* 61, 95–101.
- Zaak, H., Siar, E.-H., Kornecki, J.F., Fernandez-Lopez, L., Pedrero, S.G., Virgen-Ortíz,

- J.J., Fernandez-Lafuente, R., 2017d. Effect of immobilization rate and enzyme crowding on enzyme stability under different conditions. The case of lipase from *Thermomyces lanuginosus* immobilized on octyl agarose beads. *Process Biochem.* 56, 117–123.
- Zaharudin, N.A., Rashid, R., Esivan, S.M.M., Othman, N., Idris, A., 2016. Review on the potential use of waste cooking palm oil in the production of high oleic palm oil via enzymatic acidolysis. *J. Teknol.* 78, 85–99.
- Zare, M., Golmakani, M.-T., Niakousari, M., 2019. Lipase synthesis of isoamyl acetate using different acyl donors: Comparison of novel esterification techniques. *LWT - Food Sci. Technol.* 101, 214–219.
- Zdarta, J., Meyer, A., Jesionowski, T., Pinelo, M., 2018. A General Overview of Support Materials for Enzyme Immobilization: Characteristics, Properties, Practical Utility. *Catalysts* 8, 92.
- Zechner, R., Zimmermann, R., Eichmann, T.O., Kohlwein, S.D., Haemmerle, G., Lass, A., Madeo, F., 2012. FAT SIGNALS - Lipases and Lipolysis in Lipid Metabolism and Signaling. *Cell Metab.* 15, 279–291.
- Zeymer, C., Hilvert, D., 2018. Directed Evolution of Protein Catalysts. *Annu. Rev. Biochem.* 87, 131–157.
- Zhang, D.-H., Yuwen, L.-X., Xie, Y.-L., Li, W., Li, X.-B., 2012. Improving immobilization of lipase onto magnetic microspheres with moderate hydrophobicity/hydrophilicity. *Colloids Surf. B Biointerfaces* 89, 73–78.

- Zhang, K., Jin, Z., Wang, P., Zheng, S.-P., Han, S.-Y., Lin, Y., 2017. Improving the catalytic characteristics of lipase-displaying yeast cells by hydrophobic modification. *Bioprocess Biosyst. Eng.* 40, 1689–1699.
- Zhang, Y., Cheng, F., Yan, H., Zheng, J., Wang, Z., 2018. The enzymatic resolution of 1-(4-chlorophenyl)ethylamine by Novozym 435 to prepare a novel triazolopyrimidine herbicide. *Chirality* 30, 1225–1232.
- Zhang, Y., Zhao, Y., Gao, X., Jiang, W., Li, Z., Yao, Q., Yang, F., Wang, F., Liu, J., 2019. Kinetic model of the enzymatic Michael addition for synthesis of mitomycin analogs catalyzed by immobilized lipase from *T. laibacchii*. *Mol. Catal.* 466, 146–156.
- Zhao, X., Fan, M., Zeng, J., Du, W., Liu, C., Liu, D., 2013. Kinetics of lipase recovery from the aqueous phase of biodiesel production by macroporous resin adsorption and reuse of the adsorbed lipase for biodiesel preparation. *Enzyme Microb. Technol.* 52, 226–233.
- Zhen, Y., 2005. Fundamentals of biocatalysis in organic solvents. *Prog. Chem.* 17, 924–930.
- Zheng, M., Mao, L., Huang, F., Xiang, X., Deng, Q., Feng, Y., 2015. A mixed-function-grafted magnetic mesoporous hollow silica microsphere immobilized lipase strategy for ultrafast transesterification in a solvent-free system. *RSC Adv.* 5, 43074–43080.
- Zheng, M., Xiang, X., Wang, S., Shi, J., Deng, Q., Huang, F., Cong, R., 2017. Lipase immobilized in ordered mesoporous silica: A powerful biocatalyst for ultrafast kinetic resolution of racemic secondary alcohols. *Process Biochem.* 53, 102–108.

- Zou, B., Hu, Y., Cui, F., Jiang, L., Yu, D., Huang, H., 2014. Effect of surface modification of low cost mesoporous SiO₂ carriers on the properties of immobilized lipase. *J. Colloid Interface Sci.* 417, 210–216.
- Zou, X., Jin, Q., Guo, Z., Huang, J., Xu, X., Wang, X., 2016a. Preparation of 1, 3-dioleoyl-2-palmitoylglycerol-rich structured lipids from basa catfish oil: Combination of fractionation and enzymatic acidolysis. *Eur. J. Lipid Sci. Technol.* 118, 708–715.
- Zou, X., Jin, Q., Guo, Z., Xu, X., Wang, X., 2016b. Preparation of human milk fat substitutes from basa catfish oil: Combination of enzymatic acidolysis and modeled blending. *Eur. J. Lipid Sci. Technol.* 118, 1702–1711.
- Zucca, P., Fernandez-Lafuente, R., Sanjust, E., 2016. Agarose and Its Derivatives as Supports for Enzyme Immobilization. *Molecules* 21, 1577.

Figure legends

Figure 1: Publications (from 1952 to January 2019) containing “lipase” and “biocatal*” or “biotransform*”. Source, Web of Science

Figure 2. Interfacial activation of lipases versus drops of insoluble substrate.

Figure 3. Trend of lipases to form lipase-lipase dimers.

Figure 4. Use of immobilized lipase as chromatographic matrix in lipase purification.

Figure 5. Lipase immobilization via interfacial activation on hydrophobic supports.

Figure 6. Problems caused by the tendency of lipase to dimerize during immobilization.

Figure 7. Use of detergents during lipase immobilization prevents the immobilization of lipase dimers.

Figure 8. Effect of ionic strength on the conformational equilibrium of lipases immobilized via interfacial activation or using conventional supports.

Figure 9. Desorption of lipases from hydrophobic supports during hydrolysis of hydrophobic substances that produce emulgent molecules.

Figure 10. Physical or chemical crosslinking of immobilized lipases to prevent enzyme desorption.

Figure 11. Use of heterofunctional octyl-glyoxyl agarose beads to get interfacially activated plus covalently attached lipase molecules.

Figure 12. Chemical amination of lipases to increase their reactivity versus glyoxyl groups

on heterofunctional octyl-glyoxyl supports.

Figure 13. Use of polyethylenimine (PEI) to get the coimmobilization of beta-galactosidase and lipase, enabling the reuse of lipase after galactosidase inactivation.

Figure 14. Use of heterofunctional octyl-glyoxyl supports to coimmobilize different lipases, enabling the reuse of the most stable lipase.

Table 1. Hydrophobic support materials for enzyme immobilization and their applications

Support	Lipase source	Catalyzed reaction	Reference
Octyl Sepharose	Lipase from <i>Candida viswanathii</i>	Hydrolysis of triacylglycerol and soy lecithin	(Almeida et al., 2018)
Low density polyethylene (Accurel EP400) and high density polypropylene (Accurel EG100 and EP100)	Lipase from <i>Candida rugosa</i> OF360	Hydrolysis of sunflower oil	(Anand and Weatherley, 2018)
Hydroxypropyl methyl cellulose support	Lipase from <i>Pseudomonas fluorescens</i>	Synthesis of β -amino ester	(Badgujar and Bhanage, 2016)
Polymeric film made of hydroxypropyl methylcellulose and chitosan	Lipase from <i>Rhizomucor miehei</i>	Synthesis of butyl dodecanoate	(Badgujar et al., 2017)
Eupergit C and partially modified Eupergit C support	Lipase from <i>Candida antarctica</i> B	Kinetic resolution of racemic (R/S)-propranolol	(Barbosa et al., 2010)
Octyl-agarose	Lipase B from <i>Candida antarctica</i>	Hydrolysis of R/S methyl mandelate	(Barbosa et al., 2012a)
Mesoporous poly-methacrylate particles (Diaion® HP 2-MG)	Lipase from <i>Thermomyces lanuginosus</i>	Synthesis of a lubricant ester: <i>n</i> -octyl oleate	(Bassi et al., 2016)
Spherical beads made of octadecyl functionalized methacrylic resins	Lipase B from <i>Candida antarctica</i> , lipases from <i>Rhizomucor miehei</i> and <i>Thermomyces lanuginosus</i>	Synthesis of propyl laurate	(Basso et al., 2016)
Magnetic poly(styrene	Lipase from <i>Candida</i>	Synthesis of <i>n</i> -	(Bento et al., 2017)

divinylbenzene) particles functionalized with oleic acid	<i>rugosa</i> (Type VII)	butyl butyrate	
Polyvinyl alcohol microspheres	<i>Rhizomucor miehei</i> lipase	Transesterification of soybean oil to fatty acid ethyl esters	(Bergamasco et al., 2013)
Accurel® MP1000 (polypropylene porous beads)	Lipase from <i>Candida rugosa</i>	Production of biolubricant base oils	(Cavalcanti et al., 2018)
Biomimetic silica nanoparticles functionalized with octyl trimethoxysilane and 3-glycidoxypyl)methyldiethoxysilane	Lipase from <i>Thermomyces lanuginosus</i>	Synthesis of biodiesel	(Cazaban et al., 2018)
Silk fiber treated with polydimethylsiloxane	Lipase from <i>Candida</i> sp.99-125	Hydrolysis of olive oil	(Chen et al., 2012)
Functional polyphosphazene cyclomatrix microspheres containing glucose and dodecyl moieties	<i>Candida rugosa</i> lipase	Hydrolysis of glycerol triacetate	(Chen et al., 2016)
Poly(urea-urethane) nanoparticles	Lipase B from <i>Candida antarctica</i>	Synthesis of geranyl oleate and geranyl propionate	(Chiaradia et al., 2016)
Core-shell polymer supports based on poly(methyl methacrylate- <i>co</i> -divinylbenzene)/poly(methyl methacrylate- <i>co</i> -divinylbenzene)	Recombinant lipase B from <i>Candida antarctica</i>	Esterification of palm fatty acid distillate and soybean fatty acid distillate	(Cipolatti et al., 2018)
Polyhydroxybutyrate particles	Lipase from <i>Thermomyces lanuginosus</i>	Synthesis of ethyl valerate	(Corradini et al., 2017)
Polymeric supports with core-shell morphology (polystyrene, methyl polymethacrylate, vinyl polyacetate)	Lipase B from <i>Candida antarctica</i>	Esterification of oleic acid with ethanol. Kinetic	(Cunha et al., 2014)

		resolution of ($\pm$)- 1,2-O- isopropylidene- 3,6-di-O-benzyl- <i>myo</i> -inositol	
Immobead 150 (macroporous acrylic polymer), MCI GEL CHP20P (styrene-divinylbenzene)	Lipase from <i>Staphylococcus warneri</i> EX17	Synthesis of ethyl butyrate	(de Abreu et al., 2014)
Hydroxyapatite	Lipase A from <i>Candida antarctica</i>	Purification of lipase	(Dimitrijević et al., 2012)
Composite bentonite	Bovine pancreatic lipase	Hydrolysis of olive oil	(Dong et al., 2013)
Silica materials with multi-modal hierarchical pore structure and modified with organosilanes	<i>Candida antarctica</i> lipase B	Baeyer-Villiger oxidation of cyclic ketones to lactones	(Drożdż et al., 2013)
Octyl-Sepharose	Lipase from <i>Thermomyces lanuginosus</i>	Hydrolysis of <i>p</i> -nitrophenyl butyrate	(Galvis et al., 2012)
Silica aerogels modified with methyl group	<i>Candida rugosa</i> lipase	Hydrolysis of olive oil	(Gao et al., 2010)
Mesoporous TiO ₂ functionalized with phenylaminopropyl trimethoxysilane	Lipase from <i>Burkholderia ambifaria</i> YCJ01	Synthesis of cinnamyl acetate	(Gao et al., 2018)
Phenyl-Toyopearl, octyl-Sepharose, C18-Sepabeads	<i>Rhizopus oryzae</i> lipase	Purification and hydrolysis of racemic 2-O-butyryl-2-phenylacetic acid	(Ghattas et al., 2014)
Nanostructured tin dioxide, polypropylene	<i>Candida rugosa</i> lipase	Synthesis of isoamyl acetate	(Guncheva et al., 2011a)
Phosphorous-containing polyurethanes	<i>Candida rugosa</i> lipase	Esterification of palmitic acid with cetyl	(Guncheva et al., 2011b)

Polysulfone globules	Lipase from <i>Rhizomucor miehei</i>	alcohol Hydrolysis of olive oil	(Gupta et al., 2010)
Polyacrylonitrile nanofibers membrane	<i>Thermomyces lanuginosus</i> lipase	Synthesis of geranyl acetate	(Gupta et al., 2013)
Mesoporous silica	Lipases from <i>Mucor miehei</i> and <i>Rhizopus oryzae</i>	Hydrolysis of 4-nitrophenyl acetate	(Gustafsson et al., 2012)
Octyl-sepharose	<i>Rhizomucor miehei</i> lipase	Hydrolysis of racemic ibuprofen esters	(Habibi et al., 2013)
Octadecyl Sepabeads	Lipase B from <i>Candida antarctica</i>	Hydrolysis of <i>rac</i> -methyl mandelate	(Hernandez and Fernandez-Lafuente, 2011b)
Diaion HP20LX (porous styrene-divinylbenzene beads)	Lipase B from <i>Candida antarctica</i>	Hydrolysis of triacetin, <i>rac</i> -methyl mandelate, and butyl acetate	(Hernandez et al., 2011a)
Sepabeads (macroporous acrylic support) activated with octadecyl groups	Lipase B from <i>Candida antarctica</i> and lipase from <i>Rhizomucor miehei</i>	Regioselective hydrolysis of triacetin	(Hernandez et al., 2011b)
Sol-gel materials based on <i>n</i> -propyltrimethoxysilane/tetraethoxysilane	<i>Candida rugosa</i> lipase	Synthesis of vitamin E succinate	(Hu et al., 2013)
Mesoporous organosilica: virus-like organosilica nanoparticles	Lipase B from <i>Candida antarctica</i>	Esterification of levulinic acid and <i>n</i> -lauryl alcohol	(Jiang et al., 2019)
Octadecyl groups-capped silica nanowires	Lipase from <i>Burkholderia cepacia</i>	Transesterification of racemic 1-	(Jin et al., 2018)

		phenylethanol with vinyl acetate	
Octadecylalkyl-modified mesoporous-silica nanoparticles	Lipase from <i>Candida rugosa</i>	Hydrolysis of 4-nitrophenyl palmitate	(Kalantari et al., 2017)
Poly-(D,L)-lactic acid polymer	<i>Candida rugosa</i> lipase	Studies of interactions between lipase and polymeric surfaces	(Kamel et al., 2011)
Octyl-Sepharose and Lewatit VP OC 1600	Lipase from Chinook salmon	Hydrolysis of milkfat/ghee and fish oil emulsions	(Kurtovic et al., 2011)
Mesoporous hydrophobic poly- methacrylate particles (Diaion® HP 2-MG)	Lipase from <i>Thermomyces lanuginosus</i>	Synthesis of isoamyl oleate (biolubricant)	(Lage et al., 2016)
MS-3030 silica derivatized with octyltriethoxysilane	<i>Candida antarctica</i> lipase B	Hydrolysis of tributyryl	(Laszlo et al., 2011)
Nonwoven viscose fabric coated with methyl groups	Crude lipase <i>Candida</i> sp. 99-125	Hydrolysis of olive oil and the Esterification of lauric acid	(Weina Li et al., 2011)
Treated silk woven fabric with amino-functional polydimethylsiloxane	Lipase from <i>Candida</i> sp. 99-125	Esterification of dodecanoic acid and lauryl alcohol	(Li et al., 2014)
ECR1030 resin, a divinyl benzene/acrylate polymer	Lipase B from <i>Candida antarctica</i>	Synthesis of <i>n</i> -3 polyunsaturated fatty acids-rich triacylglycerols	(Li et al., 2017)
Macroporous resin NKA (polystyrene)	Lipase from <i>Burkholderia cepacia</i>	Synthesis of methyl esters	(Liu et al., 2011)
Hydrophobic magnetic particles	Lipase from	Synthesis of	(Liu et al.,

	<i>Burkholderia</i> sp.	biodiesel	2012)
D4020 resin	<i>Yarrowia lipolytica</i> lipase	Kinetic resolution of (R,S)-2-octanol	(Liu et al., 2013)
Octyl agarose beads	Lipases from <i>Thermomices</i> <i>lanuginosus</i> and <i>Pseudomonas</i> <i>cepacia</i>	Hydrolysis of methyl mandelate	(Manoel et al., 2015a)
AccurelMP 1000 (macroporous polymer of propylene)	Lipase from <i>Burkholderia</i> <i>cepacia</i>	Kinetic resolution of 1,3,4-tri-O- benzyl- <i>myo</i> - inositol	(Manoel et al., 2015b)
Silica hybrid functionalized via incorporation of β -cyclodextrin	Lipase from <i>Burkholderia</i> <i>cepacia</i>	Transesterificatio n of palm kernel oil with ethanol	(Martin et al., 2018)
Styrene-divinylbenzene porous support	Lipase from <i>Thermomyces</i> <i>lanuginosus</i>	Synthesis of butyl butyrate	(Martins et al., 2013)
Polymer matrix consisting of hydroxypropyl methyl cellulose and polyvinyl alcohol	Lipase from <i>Burkholderia</i> <i>cepacia</i>	Synthesis of <i>n</i> - amyl acetate using <i>n</i> -amyl alcohol and vinyl acetate	(Mathpati et al., 2018)
Poly-hydroxybutyrate beads	Lipases from <i>Thermomyces</i> <i>lanuginosus</i> , <i>Pseudomonas</i> <i>fluorescens</i> , <i>Geobacillus</i> <i>thermocatenulatus</i> , and type B from <i>Pseudozyma</i>	Hydrolysis of olive oil and biodiesel synthesis	(Mendes et al., 2012)

	<i>antarctica</i>		
Poly(methylmethacrylate-co divinylbenzene) encapsulated porous magnetic microsphere	<i>Mucor javanicus</i> lipase	Esterification of glycerol and oleic acid	(Meng et al., 2013)
Purolite® A109, polystyrenic macroporous resin	Lipase from <i>Candida</i> sp. L3170	Hydrolysis of <i>p</i> -nitrophenyl butyrate	(Mihailović et al., 2014)
Sepabeads C-18 (methacrylate resin containing octadecyl groups)	Lipases from <i>Rhizomucor miehei</i> , <i>Alcaligenes</i> sp. and <i>Candida antarctica</i> -fraction B	Transesterification of docosahexaenoic acid ethyl ester with glycerol	(Moreno-Perez et al., 2018)
Macroporous resin DA-201	Lecitase ultra (phospholipase)	Glycerolysis of soybean oil	(Ning Liu et al., 2012)
Cellulose/lignin hydrogel beads	Lipase from <i>Candida rugosa</i>	Hydrolysis of <i>p</i> -nitrophenyl butyrate	(Park et al., 2015)
Octyl-agarose cross-linked with dextran sulfate	Lipase from <i>Candida cylindracea</i>	Hydrolysis of fish oil	(Pizarro et al., 2012)
Styrene-divinylbenzene MCI GEL CHP20P porous support	Lipase B from <i>Candida antarctica</i>	Synthesis of fatty acid methyl esters using soybean oil	(Poppe et al., 2013)
Mesoporous carbon beads	Lipases A and B from <i>Candida antarctica</i>	Kinetic resolution of (±)-1-phenylethanol	(Quirós et al., 2011)
Pore-expanded mesoporous silica (SBA-15)	Recombinant <i>Candida antarctica</i> lipase B	Production of methyl and ethyl butyrate, hydrolysis of ethyl hexanoate	(Rios et al., 2018)
Octyl Sepharose beads 4BCL and Eupergit C	Lipase B from <i>Candida antarctica</i>	Hydrolysis of R and S methyl	(Ruiz et al., 2013)

		mandelate.	
		Enantioselective transesterification of (R/S)-propanolol and atenolol with vinyl acetate	
Accurel MP (macroporous polymer of propylene)	Lipase B from <i>Candida antarctica</i>	Continuous oil transesterification	(Séverac et al., 2011)
Magnetic copolymer styrene-divinylbenzene	Lipases from <i>Burkholderia cepacia</i> and <i>Pseudomonas fluorescens</i>	Transesterification of coconut oil with ethanol	(Silva et al., 2018)
Purolite C18 (octadecyl methacrylate)	Lipase from <i>Thermomyces lanuginosus</i>	Ethanolysis of sunflower oil	(Silveira et al., 2017)
Macroporous Purolite C-18, mesoporous Sepabeads C-18	Lipase from <i>Thermomyces lanuginosus</i>	Ethanolysis of sunflower oil	(Silveira et al., 2019)
Stearic acid - modified silica nanoparticles	Lipases from <i>Candida antarctica</i> and <i>Candida rugosa</i>	Glycerolysis reaction using olive oil	(Singh and Mukhopadhyay, 2018)
Lifetech™ ECR8804M (octadecyl methacrylate), Lifetech™ ECR8806M (octadecyl methacrylate), Lifetech™ ECR1090M (styrene) and Lifetech™ ECR1030M (DVB/methacrylic polymer)	Lipase from <i>Thermomyces lanuginosus</i>	Production of biodiesel	(Tacias-Pascacio et al., 2017)
Hydrophobic silk fibers functionalized with methyl groups	<i>Candida</i> sp. 99-125 lipase	Synthesis of tri-substituted trimethylolpropane esters	(Tao et al., 2014)

AccurelMP 1000 (polypropylene)	Heterologous	Synthesis of	(Tecelão et al., 2012)
Eupergit C (epoxy-activated acrylic beads)	<i>Rhizopus oryzae</i> lipase	human milk fat substitutes	
Lewatit VP OC 1600 (DVB-crosslinked methacrylate polymer)			
Chitosan functionalized with different alkyl chains (C4, C8, C12)	Lipase B from <i>Candida antarctica</i> and lipase from <i>Rhizomucor miehei</i>	Hydrolysis of fish oil	(Urrutia et al., 2018)
Octadecyl methacrylate	Lipase from <i>Rhizomucor miehei</i>	Synthesis of conjugated linoleic acid partial glycerides via glycerol esterification	(Verdasco-Martín et al., 2018)
Silica coated with octyl groups	Porcine pancreatic lipase	Synthesis of fatty acid esters of D-xylose	(Vescovi et al., 2017)
Diaion™ HP-20 resin: copolymer styrene-divinylbenzene	Lipase from <i>Rhizopus oryzae</i>	Esterification of isoamyl alcohol with lauric acid	(Vilas Bôas et al., 2018)
Octyl-Sepharose, butyl-Toyopearl 650M	Lipase 45-kDa fraction from <i>Staphylococcus warneri</i> EX17	Hydrolysis of <i>p</i> -nitrophenyl butyrate	(Volpato et al., 2011)
Mesoporous TiO ₂ modified with comb polymer poly(sodium acrylate)- <i>g</i> -methoxy poly(ethylene oxide)	Lipase from <i>Burkholderia ambifaria</i> YCJ01	Resolution of 3-phenoxy-1,2-propanediol	(Wang et al., 2014)
Magnetic mesoporous silica	Lipase from <i>Burkholderia cepacia</i>	Transesterification resolution of racemic aromatic secondary alcohols to synthesize chiral	(Xue et al., 2018)

Magnetic Fe ₃ O ₄ nanoparticles	Lipase from <i>Alcaligenes</i> sp. (lipase QL)	intermediates Kinetic resolution of (R, S)-2-(2-chloro-1-hydroxyethyl) thiophene	(Xun et al., 2015)
D152H (macroporous resin, acrylic-divinylbenzene)	<i>Yarrowia lipolytica</i> lipase LIP2	Enrichment of docosahexaenoic acid from <i>Chlorella protothecoides</i> oil	(Yan et al., 2013)
MSU-H type mesoporous silica	<i>Candida rugosa</i> lipase	Esterification of conjugated linoleic acid and ethanol	(Yu et al., 2013)
Poly(vinyl acetate-acrylamide-acrylic acid) magnetic microspheres	Lipase from <i>Candida rugosa</i>	Hydrolysis of olive oil	(Zhang et al., 2012)
Diatomite	Lipase from <i>Trichosporon laibacchii</i>	Synthesis of 2-methyl-3-n-butylaminoyl-1-hydro-4-quinone	(Zhang et al., 2019)
Macroporous resin (D3520, cross-linking polystyrene)	Lipase from <i>Aspergillus niger</i>	Preparation of biodiesel	(Zhao et al., 2013)
Phenyl-modified ordered mesoporous silica	Lipase from <i>Burkholderia cepacia</i>	Resolution of 1-phenylethanol	(Zheng et al., 2017)
Surface modified mesoporous silica with alkoxysilanes (C1-SiO ₂ , C8-SiO ₂ , C16-SiO ₂ , SH-SiO ₂ , Ph-SiO ₂ , NH ₂ -SiO ₂)	Porcine pancreas lipase	Hydrolysis of triacetin	(Zou et al., 2014)

Lipases immobilization on hydrophobic supports via interfacial activation is described in this review

This protocol permits the one step immobilization, purification, stabilization and hyperactivation of lipases

Lipases may be released from the support under certain conditions

Intermolecular crosslinking may prevent enzyme release

Heterofunctional supports prevent enzyme release but they have some limitations

There are many ways of taking full advantage of the new heterofunctional supports

SOURCE: Web of Science

Figure 1

Figure 2

Dimer of lipase molecules
with altered properties

Figure 3

Figure 4

Figure 5

LIPASE IN CONFORMATIONAL EQUILIBRIUM

Figure 6

LIPASE IN CONFORMATIONAL EQUILIBRIUM

Figure 7

Figure 8

Figure 9

Figure 10

**Lipase immobilization via interfacial
activation at pH 7**

**IRREVERSIBLE IMMOBILIZATION
VIA SECONDARY AMINO BONDS
CHEMICALLY INERT SUPPORT**

Figure 11

Figure 12

LIPASE IMMOBILIZED VIA INTERFACIAL ACTIVATION

IMMOBILIZED
LIPASE
REUSE

GALACTOSIDASE
AND PEI
DESORPTION
AT HIGH IONIC
STRENGTH

LIPASE IMMOBILIZED VIA INTERFACIAL ACTIVATION
COATED WITH PEI

GALACTOSIDASE IMMOBILIZED
VIA ION EXCHANGE

GALACTOSIDASE
INACTIVATION

Figure 13

**IMMOBILIZATION OF THE MOST
STABLE LIPASE ON
GLYOXYL OCTYL AGAROSE
VIA INTERFACIAL ACTIVATION**

**IMMOBILIZATION OF THE LEAST
STABLE LIPASE ON
REDUCED OCTYL GLYOXYL AGAROSE
VIA INTERFACIAL ACTIVATION**

Figure 14