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REVIEW ARTICLE

Support engineering: relation between development of new supports for immobilization of lipases and their applications

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Abstract The growing interest in processes with the use of immobilized lipases guides to the development of new supports. In that way, the design and characterization of new supports for lipase immobilization have been increasingly popular in literature. Efforts to obtain ''the perfect support'' (a not accomplished yet) are described in this paper. Obviously, the choice and development of a support is directly related to the process in which it will be used, considering different factors as the media where the immobilzed enzyme will be used (whether aqueous, free or with solvents), potency of agitation, reactor configuration or substrates/products that will be involved. The present work discusses the use of some techniques of support synthesis in the case of core-shell particles, such as: miniemulsion, microemulsion, suspension, dispersion, the use of heterofunctional supports, whole-cell and processes of coimobilization. Some analytical tools for the investigation of enzyme immobilization are also presented, such as fourier transform infrared spectroscopy, as well as support characteristics that may be relevant for its final performance (e.g., specific surface area, particle diameter and particle size distribution and confocal laser scanning microscope).

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Introduction

The increasing and notable interest in enzyme immobilization is based on already widely discussed and proven factors, such as the possibility of improving the stability of the biocatalyst under different environmental conditions, improving mass transfer (case of the use of nanoparticles) and reuse in reactions of interest (Cipolatti, Silva, et al., 2014; Fernandez-Lafuente, Armisén, Sabuquillo, Fernández-Lorente, & Guisán, 1998; Guisan & Blanco, 1987; Rodrigues et al., 2010). A search made in a scientific research platform about enzyme immobilization publications shows the growing interest in the area, that is reflected through the increasing number of studies conducted and published in international journals on this matter (Fig. 1). The graph shows the number of publications since 1974, with a notable increase from 2004. The plotted data indicate that this area is still rising.

In some studies about enzyme immobilization, the focus is in the development of the support. The supports may be rigid or flexible, porous or non porous, macroporous particles, nanoparticles or membranes, among others (Cipolatti et al., 2016; Gumí, Paolucci-Jeanjean, Belleville, & Rios, 2007; Huckel, Wirth, & Hearn, 1996; Sato, Kawakami, & Tokuyama, 2014; Zang et al., 2014).

Some commercial supports can be too expensive for large-scale application, or did not offer the improvement in enzyme properties demanded for a immobilized biocatalyst (Barbosa et al., 2015; Mateo, Palomo, Fernandez-Lorente, Guisan, & Fernandez-Lafuente, 2007; Rodrigues, Ortiz, Berenguer-Murcia, Torres, & Fernández-Lafuente, 2013) and this is one of the main reasons why researchers are looking for cheaper and more efficient alternatives. There is also an interest in the new, for discovering new materials, nanocomposites, which provide an efficient immobilization, but also help in the better understanding of the enzyme-support interactions and the ability of the materials to act as supports.

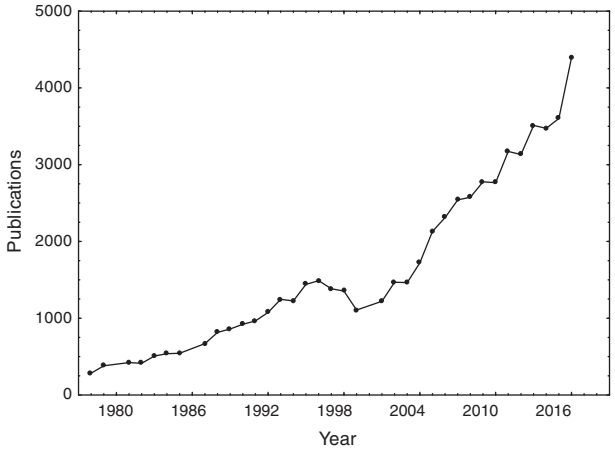

Figure 1 Publications on enzyme immobilization over the years (ScienceDirect, accessed in November, 2016). Keywords: enzyme, immobilization.

Methodologies for the development of supports

Miniemulsion

The miniemulsion technique for the synthesis of supports for enzyme immobilization is still scarcely used; few papers are cited in the literature using this technique (Cipolatti et al., 2014, 2015; Fritzen-Garcia et al., 2013; Valério et al., 2015). Fig. 2 shows an example, CALB enzyme immobilization in poly(methyl methacrylate) (PMMA) nanoparticles obtained by miniemulsion polymerization.

Considering an easy manipulation of the conditions and monomers used in the miniemulsion process, besides the low cost of the polymers, this may be a promising method for the synthesis of supports for immobilization of enzymes. Miniemulsion is classically defined as a relatively stable aqueous dispersion of oil droplets within the 50–500 nm size range prepared by a system containing oil, water, surfactant and a ''cosurfactant'' agent (Landfester, Bechthold, Tiarks, & Antonietti, 1999). The miniemulsion polymerization aims to initiate polymerization when the droplets are already stable to avoid secondary nucleation and minimizing the mass transport (Antonietti & Landfester, 2002). This technique allows drugs, oils or other substances can be incorporated into drops, maintaining its characteristics from the dispersion to obtain the nanoparticles (Landfester, 2009; Valério, Araújo, & Sayer, 2013a; Valério, da Rocha, Araújo, & Sayer, 2014). Typically, the preparation of nanoparticles in miniemulsion systems includes three stages: pre-emulsion of two heterogeneous phases to prepare (macro)emulsions, homogenization of gross emulsions for the miniemulsions and reaction to yield nanoparticles (Qi, Cao, & Ziener, 2014). The nanoparticles can be formed with the use of high pressure homogenizer or ultrasound. This method can be used for encapsulating materials in polymeric nanoparticles (Landfester, 2009).

Furthermore, the miniemulsion has the advantage that the final product can be obtained in one reaction step. The synthesis of PU (polyurethane) nanoparticles in one step consists in to add the monomers (diisocyanate and polyols), chain extender and other reaction components to the reactor simultaneously to form the final product (Cipolatti et al., 2014; Valério, Araújo, & Sayer, 2013b). The miniemulsions can still be classified as direct or reverse, depending on the polarity of the dispersed and continuous phases. In direct miniemulsion, the polarity of the continuous phase is greater than in the dispersed phase, whereas in the inverse miniemulsion the polarity of the continuous phase is lower than in the dispersed phase. In direct miniemulsion, an aqueous solution of surfactant is commonly used as a continuous phase. In reverse miniemulsions, a hydrophobic surfactant solution is used as a continuous phase. Most commonly hydrophobic solvents used are cyclohexane, toluene, hexadecane and isopar M (a hydrocarbons mixture of C12–C14). Systems with direct miniemulsions are used to prepare hydrophobic nanoparticles, whereas the inverse produces hydrophilic particles (Qi et al., 2014).

The polymerization in miniemulsion also has advantages such as the non-excessive use of surfactant, sufficient colloidal stability and incorporation of hydrophobic

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