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Application of magnetic nanostructures in biotechnological processes: Biodiesel production using lipase immobilized on magnetic carriers

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Abstract

Magnetic nanostructures have gained a remarkable interest in the last years both for basic research and applied studies. The use of magnetic nanostructures has been proven in biochemistry, biomedicine, and waste treatment among other fields. This broad range of applications is based on the fact that magnetic particles have very large magnetic moments, which allow them to be transported and driven by external magnetic fields. The magnetic nanostructures have also a great potential in biotechnological processes taking into account that they can be utilized as a carrier for enzymes during different biocatalytic transformations. In this way, the biocatalyst can be easily manipulated by a controlled magnetic field allowing it to be located permanently in the zone where the maximum concentration of reagents is present. In this work, some applications are presented. Particularly, the system composed of an immobilized lipase on a magnetic nanostructure for biodiesel production is analyzed. This system makes possible the intensification of the process due to the accomplishment of a reaction-extraction enzymatic process favoring the separation of the products formed during the transesterification reaction. In addition, the magnetic nature of the carrier permits the preferential location of the biocatalyst in the separation surface between the two liquid immiscible phases present in the system. Finally, techniques for the immobilization of different enzymes on magnetic carriers are described. This technology can offer innovative configurations allowing the intensification of enzymatic processes and the reduction of their costs.

Keywords: Biodiesel, reaction-extraction process, magnetic nanostructure, lipase.

1. Introduction

Magnetic particles (microspheres, nanospheres and ferrofluids) are widely studied for their applications in various fields in biology and medicine such as enzyme and protein immobilization, genes, radiopharmaceuticals, magnetic resonance imaging, diagnostics, immunoassays, RNA and DNA purification, magnetic cell separation and purification, magnetically controlled transport of anti-cancer drugs as well as hyperthermia generation (Ma, Zhang *et al.*, 2003).

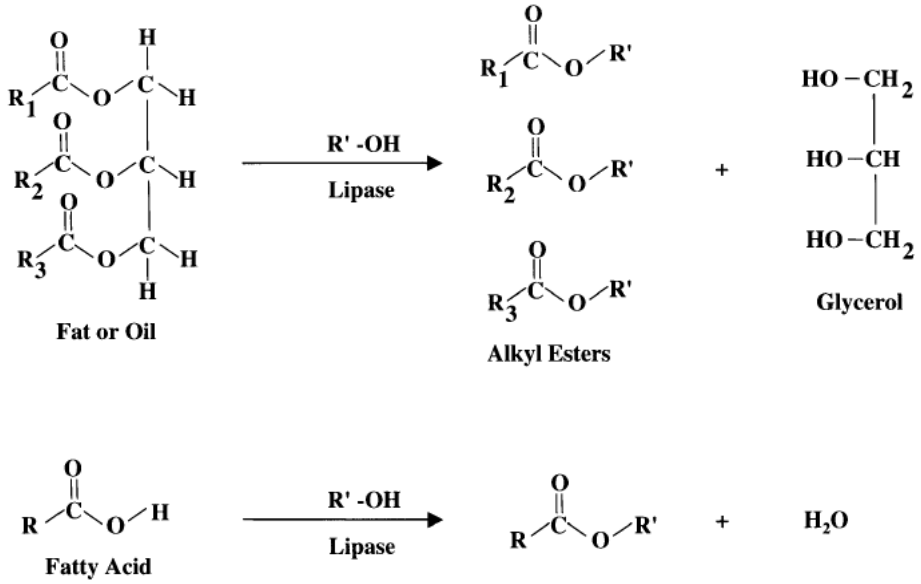
Applications in biotechnology impose strict requirements on the particles physical, chemical and pharmacological properties, including chemical composition, granulometric uniformity, crystal structure, magnetic behavior, surface structure, adsorption properties, solubility and low own toxicity. The following parameters of the nanomagnets are critical: (a) particle size (small as possible to improve tissular diffusion and to have long sedimentation times and high effective surface areas), (b) surface characteristics (easy encapsulation of the magnetic nanoparticles protects them from degradation and endows biocompatibility), and (c) good magnetic response (possibility of decreasing nanomagnets concentration in blood and therefore diminishing the associated side effects) (Tartaj, Morales *et al.*, 2005).

On the other hand, the use of biocatalysts in transforming fats, oils, partial glycerides and fatty acids into higer-value-added derivates is well documented (Hsu, Jones *et al.*, 2002). One area of interest is the utilization of immobilized lipases for catalyzing the synthesis of simple esters of vegetable oils (Clark, Wagner *et al.*, 1984; Selmi and Thomas, 1998; Shimada, Watanabe *et al.*, 1999) and other agricultural lipid feedstocks (Nelson, Foglia *et al.*, 1996; Foglia, Nelson *et al.*, 1997; Hsu, Jones *et al.*, 2001) (Scheme 1).

Some efforts have been made on the immobilization of lipase on the surface of ferromagnetic nanoparticles modified by polymer such as poly(ethylene glycol) and its copolymer with maleic acid (Tamaura, Takahashi *et al.*, 1986; Mihama, Yoshimoto *et al.*, 1988) and in magnetic sol-gel matrices (Kuncová and Sivel, 1997; Reetz, Zonta *et al.*, 1998; Chen and Lin, 2003; Zeng, Luo *et al.*, 2006). The former immobilization method seems to be more attractive because using magnetic nanoparticles as support not only yields a sufficiently large specific surface area for enzyme binding but also has no pore-diffusion resistance and fouling problem.

Therefore, the system composed of an immobilized lipase on a magnetic nanostructure can be applicable in reaction-extraction processes. Because the magnetic nature of the carrier permits the preferential location of the biocatalyst in the separation surface between the two liquid immiscible phases present in the system and an easy recover the biocatalysts of medium.

In the process reaction-extraction is used the immiscibility of liquid phase that can give naturally inside the reaction system or can be introduce with the addition of solvents (Samant, 1998), achieving the selective separation of intermediate compounds or products, prevent their later reaction. This in-situ separation carry to a reactive reconcentration which deceive (when are had reversible reactions) reach higher conversions. Besides the synergic effect reached by the reaction a separation combination, has the advantage of carry out the process in single equipment.



Scheme 1. Immobilized lipase-catalysed transesterification of triacylglycerols and etherification of fatty acids to simple alkyl esters (Hsu, Jones *et al.*, 2002).

2. Experimental

2.1 Synthesis of magnetite nanoparticles

Magnetite was made according to the method of Zeng (Zeng, Luo *et al.*, 2006). For the preparation of magnetic carriers, a mixture of $FeCl_2$ (0.2 mol/l) and $FeCl_3$ (0.3 mol/l) aqueous solution was added to a flask that containing stearic acid. Then the mixture was stirred vigorously for a while, and NaOH aqueous solution (4 mol/l) was dropped into the flask. A black precipitate was obtained. The precipitate was filtrated, washed and dried.

2.2 Silane-coated magnetite nanoparticles

Certain amount of Fe_3O_4 (magnetic particle) was suspended in distilled water. A mixture of [3-(2-aminoethylamino)propyl]trimethoxysilane (APTS), methanol and NaF (1%) aqueous solution was stirring for 10 minutes. Then tetraethyl orthosilicate was dropped slowly into the flask and stirred vigorously at room temperature for 24 hours. The precipitate was collected, washed and dried.

2.3 Lipase- immobilized

For the immobilization of the enzyme, glutaraldehyde was added to certain amount of particles, and stirred at room temperature. Then, certain amount of lipase *Candida Rugosa* was added to phosphate buffer solution and stirred until all the lipase was dissolved. To this solution was added the solution prepared previously, and stirred for several minutes at room temperature. The immobilized lipase was separated by

centrifugation and washed with phosphate buffer and dried. All portions of centrifugation were retained for the determination of protein concentration.

3. Results and discussion

3.1 Uncoated and coated nanoparticles magnetic

According to the XRD pattern (figure 1), the size of particle can be calculated in the following equation (Moore and Reynolds, 1997),

$$L = \frac{\lambda \cdot K}{\beta \cdot \cos\theta} \tag{1}$$

Where L is the mean diameter of particle, λ is the wavelength of copper anode (λ = 1.540562 Å), K is a constant (K = 1), β is full width at half maximum-FWHM (β = 1.606). The calculation result is 12.3 nm.

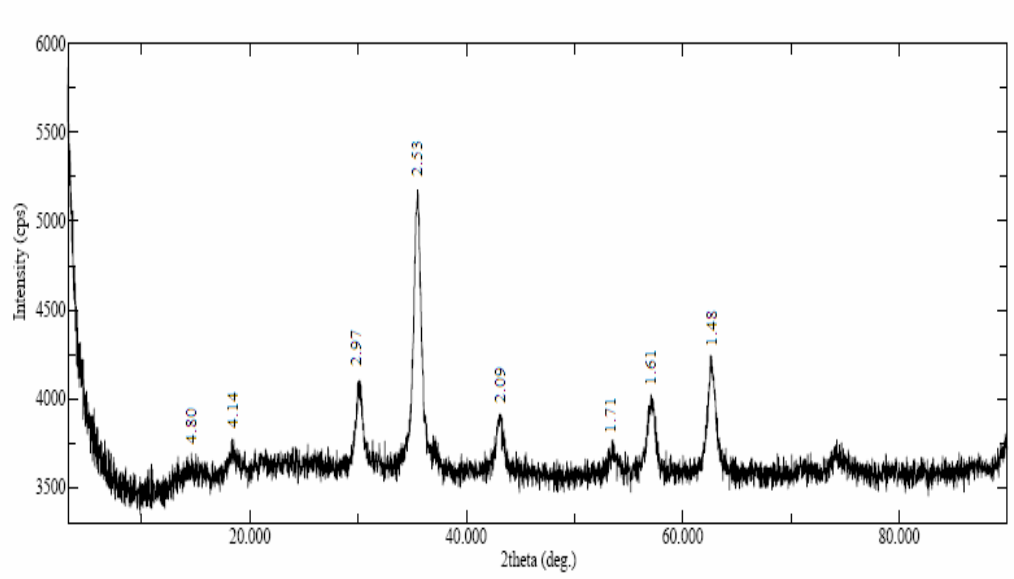


Figure 1. XRD pattern of the magnetite nanoparticles

In the figure 2 shown FT-IR spectra of uncoated and coated Fe_3O_4 nanoparticles. It can be seen that, compared with the uncoated sample, the coated Fe_3O_4 nanoparticles posses adsorption bands in 1068 cm^{-1} due to the stretching vibration of Si-O bond, band in 792 cm^{-1} due to the bending vibration of $–NH_2$ group. All these reveal the existence of APTS.

In addition, in figure 5 (a) and (b) the absorption bands near 3400 and 1630 cm^{-1} refer to the vibration of remainder H2O in the samples, bands near 2920 and 2850 cm^{-1} due to stretching vibration of C-H bond, bands near 570 cm^{-1} due to stretching vibration of Fe-O, bands near 1400 cm^{-1} due to stretching vibrations of N-H.

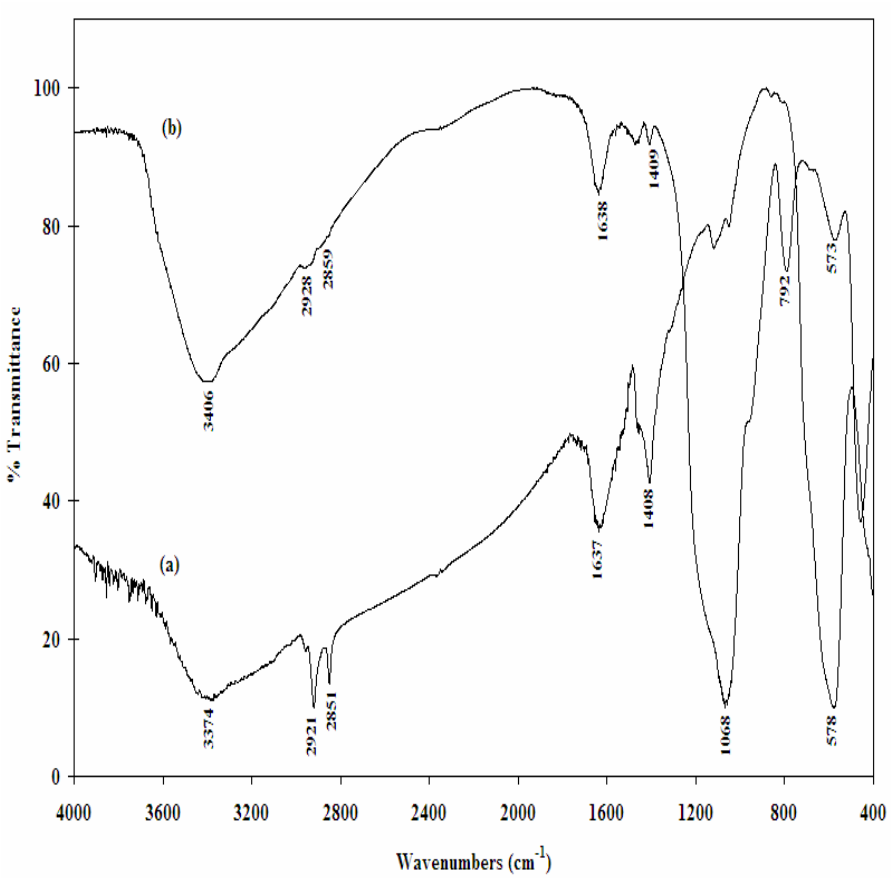


Figure 2. FT-IR spectra of the uncoated (a) and coated (b) magnetite nanoparticles

3.2 Immobilization of Lipase

The magnetite as support was selected because it has some advantages (Huang, Liao *et al.*, 2003): (i) higher specific surface area obtained for the binding of a larger amount of enzymes, (ii) lower mass transfer resistance and less fouling, and (iii) the selective separation of immobilized enzymes from a reaction mixture by the application of a magnetic field (Halling and Dunnill, 1980). Magnetite (Fe_3O_4) is one of the famous magnetic materials in common use. As a result of strong magnetic property and low toxicity, its applications in biotechnology and medicine have gained significant attention (Curtis and Wilkinson, 2001).

In addition and with the interest to increase this resistance, the addition of glutaraldehyde as covalent link allows improving the properties already mentioned and it increases the degree of acetylation of the support which leads to a bigger fixation of lipase and also to a greater degree of hydrofobicity avoiding the possible catalyst and poisoning (Shao-Hua and Wen-Teng, 2004).

Finally to carry out the fixation of the enzyme in the support has been used the impregnating technique, submerging the support with glutaraldehyde in a solution of known concentration and determining the percentage of immobilization of the

enzyme by means of espectofotometric tests; the obtained results indicate that the percentage of immobilization of the enzyme is of 40%. These results are corroborated with the Kjeldhal method which determined the total nitrogen (5.84%) that multiply for a constant (k = 6.5) gives the percentage of immobilization (38%). This result is similar to calculated for espectofotometric test.

4. Conclusions

Lipase was directly bound to Fe_3O_4 magnetic nanoparticles via glutaraldehyde activation. The analyses of XRD indicated the resultant magnetic nanoparticles were pure Fe_3O_4 with a mean diameter of 12.3 nm. FTIR spectra were utilized to prove the formation of Fe-O-Si chemical bonds. The percentage of lipase immobilization was 40% and this data were calculated for Kjeldhal method and espectofotometric test.
The enzyme-linked, it has been proved that these APTS-coated magnetite nanoparticles could significantly improve the protein immobilization.

The process of extractive reaction is begins developed for biodiesel production from palm or castor oil with ethanol catalyzed for immobilized lipase on magnetic particle. This process can be modeling and simulated with the principles of processes intensification and the methodology that has been developed for the Group Investigation: Chemical, Catalytic and Biotechnology Processes of the National University of Colombia at Manizales. The obtained results in the modeling, simulation and experimentation will make part of future publications.

Finally, this work will be helpful for the practical application of lipase and of other enzymes.

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