**Hydrolytic enzymes immobilization on silicate mesoporous materials or carbon nanomaterials: impact on N-acylation performances**

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**Highlights**

* Enzymes immobilized on carbon nanotubes and mesoporous silica SBA15 maintained their regioselectivity towards N-acylation of lysine.
* Enzymatic N-acylation performances depended on immobilization materials.
* Functionalization of SBA15 with APTES improved enzyme loading and N-acylation performances

**1. Introduction**

N-acylation reactions allow the synthesis of amino acids/peptide derivatives, called lipo-amino acids/peptides belonging to the class of surfactants used in food and non-food applications for their good foaming and emulsion stabilization properties. Furthermore, as amino acids and peptides are molecules that may possess biological activities, the acylated derivatives of these molecules may also exhibit these bioactivities, making them detergents of choice in the cosmetic or pharmaceutical formulations. Industrially, the chemical reaction conditions are well known with the Schotten-Baumann reaction, but require the use of fatty acid chlorides in the presence of an organic cosolvent and generate effluents rich in salts. An alternative to this chemical route may be the use of hydrolytic enzymes to catalyse this reaction in aqueous or non-aqueous solvents depending on the enzyme. The use of lipases in non-aqueous media such as organic solvents or ionic liquids have already been reported [1] and aminoacylases in aqueous medium have also been described for the synthesis of acylated amino acids [2]. However to develop enzymatic processes to replace the chemical ones, the immobilization of the biocatalyst is required to improve its stability and allow its recycling. In this context, the aim of this work is to develop immobilization materials and conditions to improve enzymatic performances of both lipase in non-aqueous system or aminoacylases in aqueous medium for the synthesis of acylated amino acids.

**2. Methods**

The lipase B of *Candida antarctica* and aminoacylases from *Streptomyces ambofaciens* were immobilized either on SBA15 or on carbon nanotubes by physisorption or chemisorption. Preliminary functionalizations of SBA15 with APTES (from 10 up to 50 %) were performed. The synthesis of lauroyl-lysine was carried out with L-lysine (0.12 M) and Lauric acid (0.24 M) in 2 mL of 2-methyl-2-butanol previously dehydrated on 4 Å molecular sieves for CALB and in Tris-HCl 25 mM NaCl 50 mM (pH 8) for aminoacylases. The acylation reaction was performed in an agitated parallel system (Carousel 12 Plus Reaction Station™, Radleys) and is initiated by the addition of lipase B of *C. antarctica* immobilized on carbon nanotubes by physi- or chemisorption or by the addition of aminoacylases immobilized on SBA15. The quantity of supported enzymes was determined depending on the immobilization yield in order to add 1 mg of protein in the reaction medium.

**3. Results and discussion**

The synthesis of lauroyl-lysine was investigated using the lipase B of *Candida antarctica* immobilized on carbon nanotubes and aminoacylases immobilized on mesoporous silicate SBA15 functionalized with APTES. Both enzymes immobilized on carbon nanotubes or SBA15 maintained their regioselectivity towards N-acylation in  or  position. By adsorption on SBA15, increased APTES concentration led to an improvement of aminoacylases loading up to 0.07 mg/mg material and increased N-acylation performances (Figure 1).

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Figure 1. Aminoacylases loading enzyme on SBA15 functionalized with different APTES concentrations and lauroyl-lysine concentration obtained after 48h reaction.

Alternatively, the N-acylation of lysine was carried out with CALB immobilized on carbon nanotubes. Enzyme loading reached 0.16 mg/mg carbon nanotubes by direct adsorption whereas 0.25 mg/mg carbon nanotubes can be achieved by covalent bonding. However, the activity of CALB covalently immobilized on carbon nanotubes was 20 fold less than with Novozyme 435.

**4. Conclusions**

The regioselectivity of N-acylation was maintained whatever the enzyme and immobilization support. However, the performances of both enzymes depended on immobilization supports by adsorption or covalent bond on SBA15 or carbon nanotubes.

**References**

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