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Covalently immobilized lipase on aminoalkyl-, carboxy- and hydroxy-multi-wall carbon nanotubes in the enantioselective synthesis of Solketal esters

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ABSTRACT

Aiming at the preparation of efficient, stable on storage and recyclable nanobiocatalysts for enantioselective transesterification, alkaline lipase from *Pseudomonas fluorescens* was covalently immobilized (up to 8.5 wt.%) on functionalized multi-wall carbon nanotubes (f-MWCNTs). f-MWCNTs were synthesized *via*: (a) (2 + 1)-cycloaddition of a nitrene to the *C*-sp^2 nanotube walls (3.2 mmol g^{-1}, a novel synthetic approach) and, (b) oxidative treatments, *i.e.* Fenton reagent (3.5 mmol g^{-1}) and nitrating mixture (2.5 mmol g^{-1}), yielding aminoalkyl-, hydroxyl- and carboxyl-MWCNTs, respectively. Amino- and epoxy- functionalized mesoporous silica (f-SBA-15) were used as the reference supports. Transesterification of vinyl *n*-butyrate by racemic Solketal with a chromatographically (GC) traced kinetics was selected as the model reaction. The studies revealed that different chemical functionalization of morphologically identical nanotube supports led to various enzyme loadings, catalytic activities and enantioselectivities. MWCNT-NH_2-based nanobiocatalyst was found to be the most active composite among all of the tested systems (yield 20%, t = 0.5 h, 1321 U g^{-1}), *i.e.* 12 times more active than the native enzyme. In turn, lipase immobilized on MWCNT-COOH emerged as the most enantioselective system (*ex aequo* with SBA-NH_2) (ee_R = 74%, t = 0.5 h at yield of 3–5%). The activity of the MWCNT-NH_2-based nanobiocatalyst after 8 cycles of transesterification dropped to 60% of its initial value, whereas for SBA-NH_2-based composite remained unchanged. Importantly, stability on storage was fully maintained for all MWCNT-based nanobiocatalysts or even 'extra-enhanced' for MWCNT-OH.

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1. Introduction

Biocatalysis has recently emerged as an attractive method for the synthesis of valuable commodity chemicals for diverse industrial applications, *e.g.* in the production of pharmaceuticals, cosmetics, bio-fuels and foods [1]. There is a *significant interest* in such applications of *enzymes* due to their high chemo-, regio- and stereoselectivity. However, the enzymes in their native form are rather unstable and susceptible to deactivation or denaturation under technological conditions. Fortunately, those disadvantages can be omitted using immobilization of enzymes on solid supports and, particularly, on nanosupports [2]. The enzyme immobilization has been a popular strategy for most of the large-scale applications due to facile recycling of the catalyst which enabled continuous operation and purification of the products [3]. Moreover, the immobilized enzyme, as opposed to its native form, often reveals better thermal and pH stability as well as exhibits higher resistance to organic solvents [4–6]. Of the possible routes for immobilization, *i.e.* non-covalent and covalent, the latter might be the first choice route since the energy of adhesion and hence recyclability as well as higher activity due to conformational changes are critical [7].

Functionalized polymeric matrices have been the most commonly used carriers in the immobilization of enzymes due to easiness in reacting with protein groups to form stable linkages [8,9]. Also, siliceous materials display a considerable potential as supports. Silica gels or mesoporous silicates are environmentally friendly, structurally stable and resistant to microbial attacks [10,11]. Their surface can be densely covered with covalently

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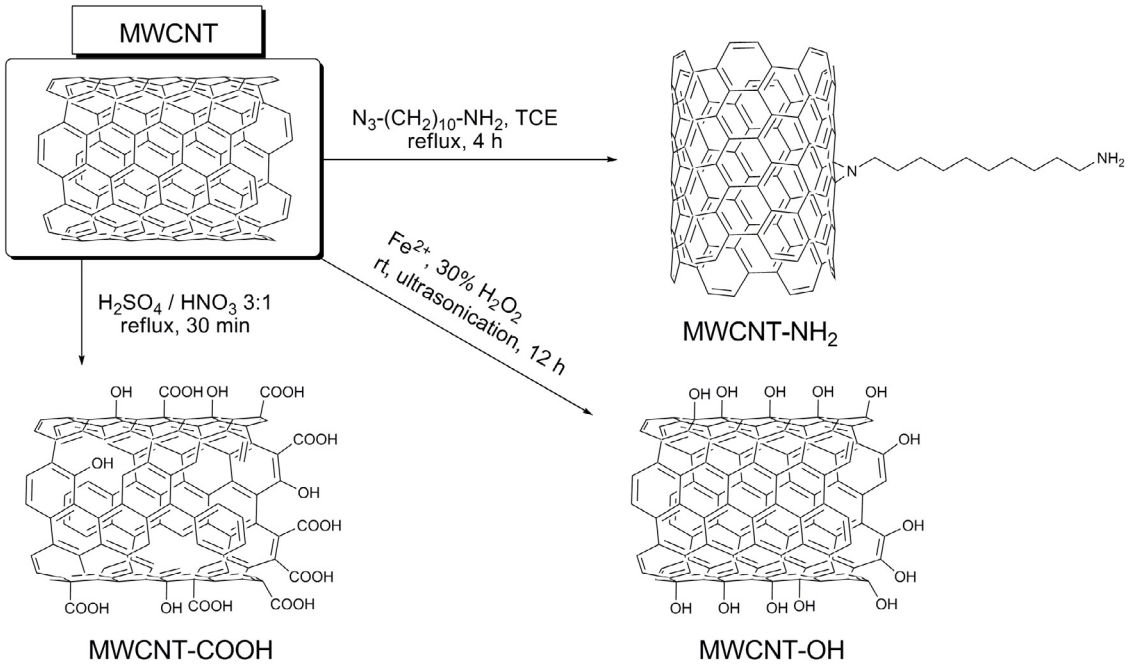
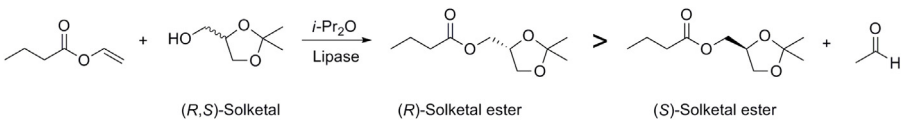


Fig. 1. Chemical modifications of MWCNTs towards f-MWCNTs as lipase supports—based on the nitrene chemistry, oxidation under harsh conditions and via Fenton reagent; for clarity only the outer nanotube wall is shown; oxidation of MWCNTs using nitrating mixture yielded thinned and the most defectuous graphene walls full of grooves rich in functional groups.



Scheme 1. Enantioselective transesterification of (*R,S*)-Solketal towards its *n*-butyrate catalyzed by lipase from *Pseudomonas fluorescens* as the model reaction.

anchored functional groups. Additionally, by virtue of large surface area and the presence of pores with diameters larger than those of enzyme molecules, a significant contact area can be achieved [11–13]. With the rapid development of nanotechnology, nanomaterials have also emerged as very attractive carriers for immobilization [14]. Among them, multi-wall carbon nanotubes (MWCNTs) represent an important group of carbon-based nanosupports [15] since they exhibit high surface area, excellent physicochemical stability, tunable functionalization, unusual mechanical resistance, inexpensive accessibility and, last but not least, lower cytotoxicity than their single-wall (SWCNTs) counterparts [16,17]. MWCNTs also balance key factors determining efficiency of the biocatalysts, including mass transfer resistance and effective enzyme loadings [18]. Furthermore, functionalization can affect their dispersibility and interactions with enzymes. It was possible to significantly enhance activity of the enzymes after their immobilization on MWCNTs [19–21]. f-MWCNTs offer an unusually high potential as carriers for enzymes. Numerous representative examples can be recalled here to illustrate this behavior with lipase from *Candida antarctica* covalently attached to MWCNTs as one of them [22]. In those studies, it was shown that 78% of activity of the catalytic system was retained even after 50 cycles. *Thermomyces lanuginosus* lipase was covalently immobilized on a f-MWCNT support using glutaraldehyde as a cross-linking agent [23]. As compared to the native enzyme, catalytic activity of this composite (expressed as V_{max}) was 14% higher. Also its thermal stability was improved – at 80 °C activity of the immobilized enzyme was 2 times higher. Nanobiocatalysts constructed *via* covalent immobilization of lipases from *Candida rugose* and *C. antarctica* onto amine f-MWCNTs revealed 29% higher initial activity than the corresponding non-covalent hybrids [24]. Moreover, at nearly identical enzyme loadings, MWCNTs emerged as significantly more activating supports (by 1200%) than similarly functionalized graphene oxide. The enhancement was attributed to geometry of the nanostructure and hence a higher α-helix content in the MWCNT-composites. In other studies, lipase from *Rhizopus arrhizus* was covalently attached to MWCNTs *via* amide bonds and the composite was characterized by 36% higher activity (at optimal temperature 55 °C) than the native enzyme and retained its stereoselectivity at the content of unchanged α-helix of 62% [25].

Up to date, however, no comparative studies between lipase covalently immobilized on various f-MWCNTs has been performed. Particularly, primary amine groups – a strong nucleophilic anchoring center for covalent immobilization – born on a hydrophobic extension linker have not been studied yet. Moreover, stereoselective processes still lack clear technological premises in the application of MWCNTs as lipase supports. This gap is even more evident if we consider low-cost and rapidly increasing production capacity of MWCNTs enabling their effective and long-awaited transfer to the industrial biotechnology. In our last work [26], we have presented non-covalent immobilization of lipase from *P. fluorescens* on pristine versus oxidized MWCNTs and silica materials using the adsorption technique. Those studies were very recently extended to numerous acetate esters by Badgujar et al. [27]. Both works confirmed that adsorption emerged as the appropriate technique of lipase immobilization due to its simplicity, low cost, and importantly, significantly enhanced activity, stereoselectivity and stability (no decline in the activity up to 10 cycles). Here, we have investigated transesterification of Solketal catalyzed by commercial lipase from *P. fluorescens* covalently immobilized on

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