LIPASE IMMOBILIZATION ON PRISTINE AND MODIFIED CARBON NANOTUBES IN ORDER TO OBTAIN AN EFFICIENT AND ENVIRONMENTALLY FRIENDLY BIOCATALYSTS

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INTRODUCTION AND OBJECTIVE

Nanobiocatalysis, which refers to the application of enzymes immobilized on nanomaterials, is a rapidly growing research field. Among the various nanostructured materials that might be used as novel supports for enzyme immobilization and stabilization, multi-walled carbon nanotubes (MWCNTs) are of great interest to many research centers worldwide due to their stability, high adsorption capacity, improved retention of catalytic activity, biocompatibility, and commerciall availability at a relative lower price, which makes them more feasible for industrial applications. The addition of poly(dimethylsiloxane) (PDMS) to MWCNTs improves the mechanical, electrical, and thermal properties of the resulting nanocomposites. Moreover, lipases are well-known interfacially active catalysts and exhibit their catalytic abilities at the interface between the organic phase containing hydrophobic substrates and aqueous phase, so they can be activated at the hydrophobic–hydrophilic interface.

In the present work, for the first time, the methodology to design and characterize an alternative, highly stable, and active nanobiocatalyst based on *Candida antarctica* lipase B (CALB) immobilized onto pristine and poly(dimethylsiloxane) modified MWCNTs is presented and discussed. The idea was to combine the textural properties as well as functionality of PDMS modified MWCNTs with lipase activity to obtain a novel type of biocatalyst dedicated to biotechnological applications. As a result, high enzyme loading, its improved stability and reusability, as well as activity of the biocatalyst produced, were expected. The innovative nature of the presented study is based not only on application of a novel, previously undescribed support material for lipase immobilization, but also on the possibility to use MWCNTs surface modifying agents at various molecular weights to examine their effects on enzyme loading and catalytic activity.

EXPERIMENTAL

I. Preparation of MWCNTs/PDMS Nanocomposites

PDMS-100 (Mw ≈ 3410 g/mol) and PDMS-12500 (Mw ≈ 39500 g/mol) fluids were physically adsorbed onto pristine multiwalled carbon nanotubes (P-MWCNTs) in the amount of 40 wt.%. Before adsorption, the samples were dried at 110 °C for 2 h. A hexane solution of PDMS (1 wt.% PDMS) was prepared, and its estimated amount was added to a fixed amount of dry carbon powder material. The suspension was mechanically stirred and finally dried at room temperature for 48 h and then at 80 °C for 3 h. All samples modified with PDMS in the amount of 40 wt.% were in the form of powder similar to P-MWCNTs, while neat PDMS-100 and PDMS-12500 were liquids. The prepared polymer nanocomposites were marked as MWCNTs/PDMS-100(40) and MWCNTs/PDMS-12500(40), respectively.

II. Lipase Immobilization

The pristine MWCNTs and MWCNTs/PDMS nanocomposites were used as supports for the immobilization of *Candida antarctica* lipase B (CALB). In all experiments, 100 mg of P-MWCNTs or modified MWCNTs (MWCNTs/PDMS-100(40) and MWCNTs/PDMS-12500(40)) were added to 5 mL of lipase solution at concentration of 5 mg/mL in 50 mM phosphate buffer solution at pH 7. The samples were then shaken for 24 h using a KS 4000i Control incubator (IKA Werke GmbH, Staufen im Breisgau, Germany) at ambient temperature. Next, samples were centrifuged (4000 rpm at 4 °C) using an Eppendorf 5810 R centrifuge (Hamburg, Germany) and furthermore washed several times with 50 mM phosphate buffer in order to remove unbounded lipase. The samples were labelled as CALB@P-MWCNTs, CALB@MWCNTs/PDMS-100(40), and CALB@MWCNTs/PDMS-12500(40), respectively.

RESULTS

Analysis of Nanocomposites before and after Lipase Immobilization

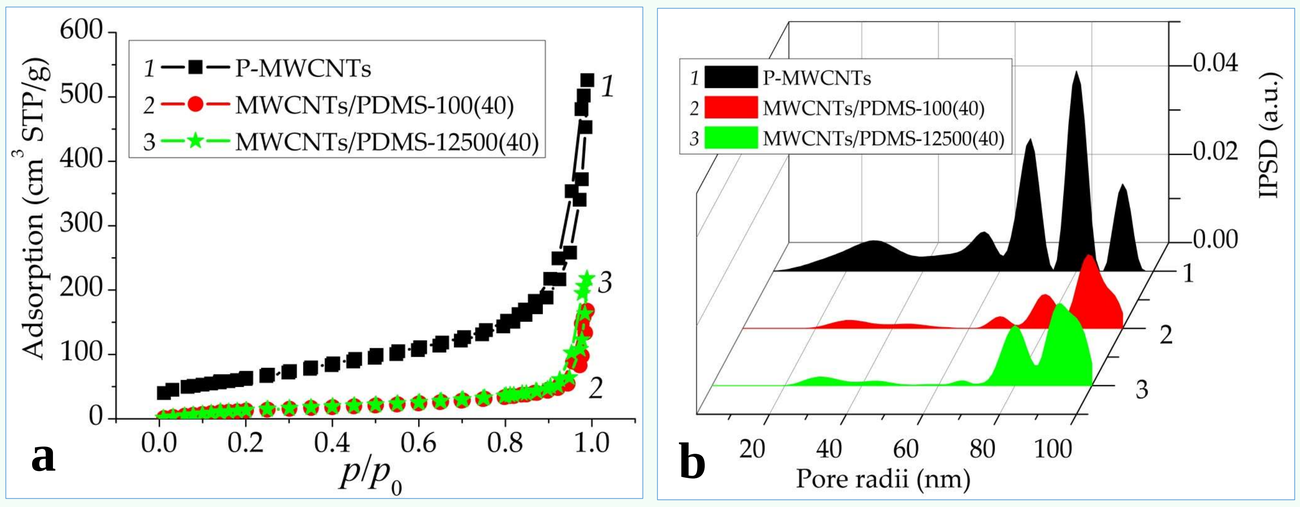

Figure 1. **(a)** Nitrogen adsorption–desorption isotherms and **(b)** incremental pore size distributions for P-MWCNTs (curve 1) and MWCNTs/PDMS nanocomposites (curves 2, 3)

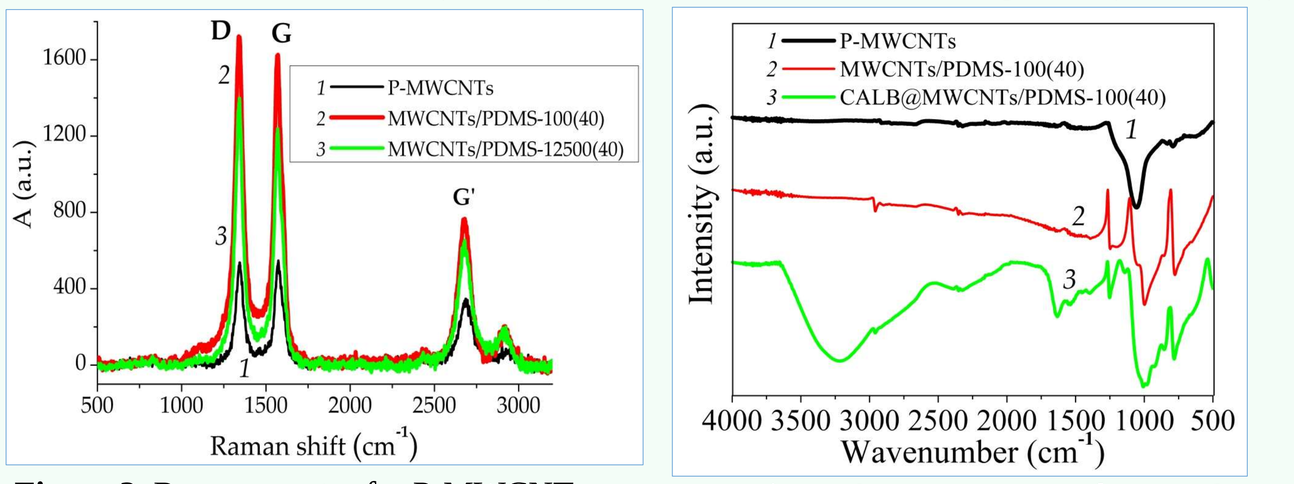

Figure 2. Raman spectra for P-MWCNTs (curve 1) and MWCNTs/PDMS nanocomposites (curves 2, 3) *(A_G/A_D for P-MWCNTs is 1.1 A_G/A_D for MWCNTs/PDMS is 0.95)*

Figure 3. FTIR spectra of P-MWCNTs (curve 1) and MWCNTs/PDMS-100 nanocomposites, before and after lipase immobilization (curves 2, 3)

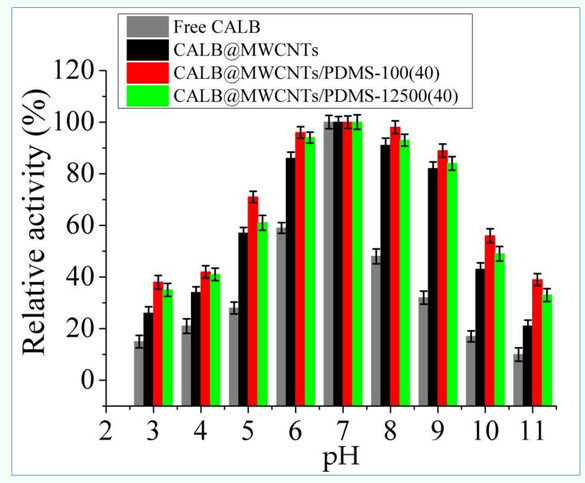

Figure 5. pH profiles of free lipase and enzyme immobilized onto P-MWCNTs and MWCNTs/PDMS nanocomposites

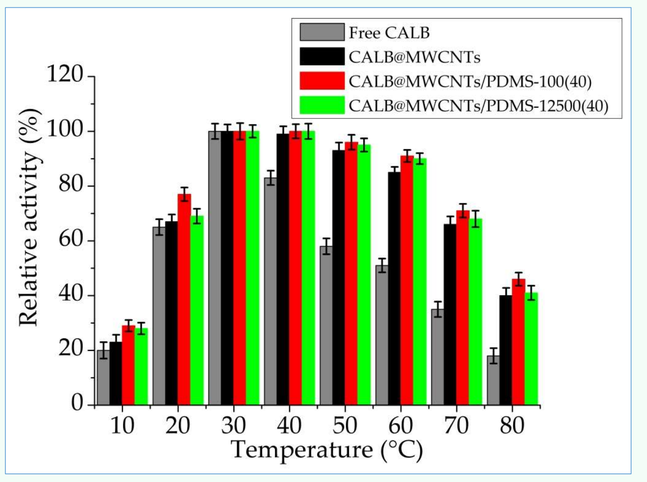

Figure 6. Temperature profiles of free lipase and enzyme immobilized onto P-MWCNTs and MWCNTs/PDMS nanocomposites

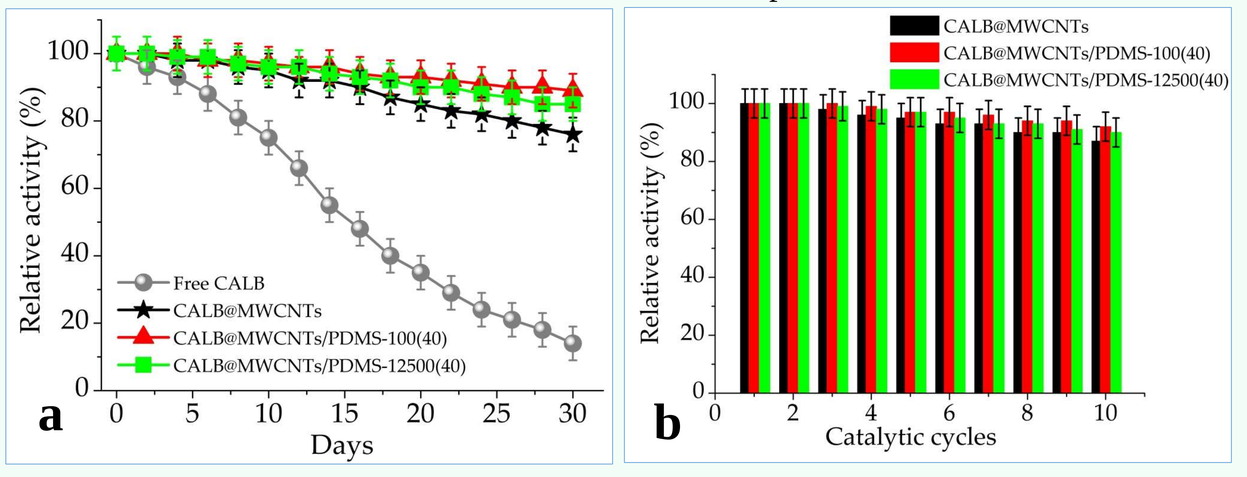

Figure 7. (**a**) Storage stability and (**b**) reusability of free lipase and enzyme immobilized onto P-MWCNTs and MWCNTs/PDMS nanocomposites

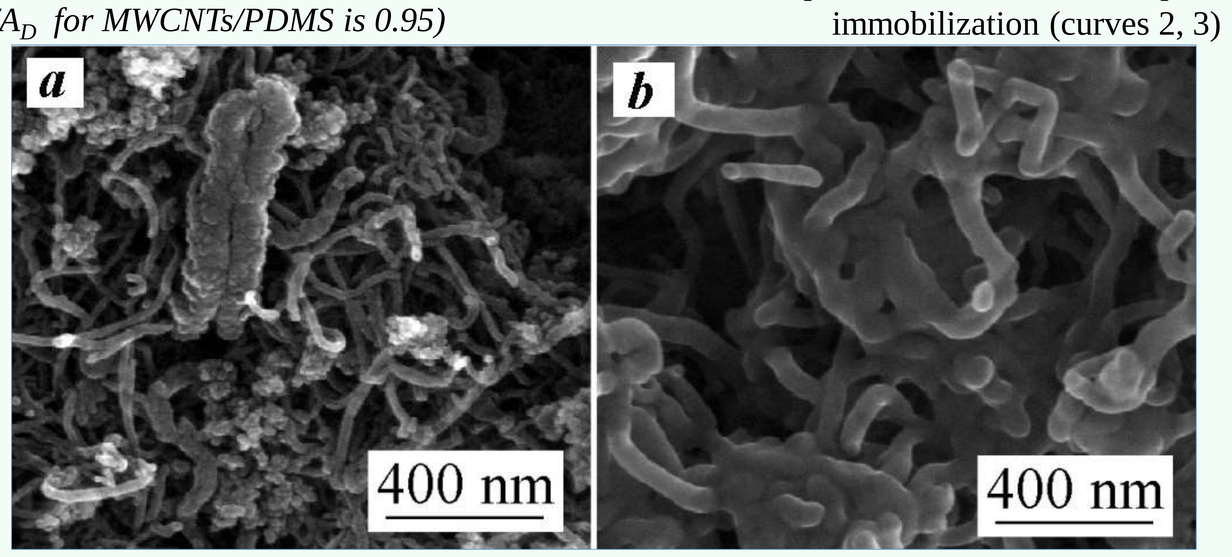

Figure 4. SEM images of **(a)** P-MWCNTs and **(*b*)** MWCNTs/PDMS-100(40) nanocomposite

CONCLUSIONS

It was proved that the textural characteristics of MWCNTs change after the modification with polymer, and that the prepared nanocomposites are characterized by a different graphitization degree, which results from, e.g., surface modification of carbon nanotubes with polymer - a lower graphitization degree; graphitic layers are semi-crystalline and possess many defects related to the introduction of new functional groups to carbon nanotube surfaces. Effective MWCNTs modification with PDMS as well as enzyme loading were confirmed by bands present on FTIR spectra, characteristic for both modifier and biomolecule structures, which all together confirmed relative high potential of synthesized MWCNTs-based materials as a support for lipase immobilization. Enzyme loaded onto P-MWCNTs and MWCNTs/PDMS nanocomposites showed significantly higher relative activity over wider pH and temperature ranges as compared to free counterpart. Moreover, significant improvement of thermal stability and enzyme half-life of the lipase after immobilization was observed. It was confirmed that after immobilization, the external backbone for the enzyme structure was provided due the formation of stable enzyme–support interactions, which stabilize the enzyme structure and protect biocatalyst against denaturation under harsh reaction conditions. This fact suggests wide application potential of designed novel types of biocatalytic systems in various /biotechnological applications.

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