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Facile surface functionalization of multiwalled carbon nanotubes by soft dielectric barrier discharge plasma: Generate compatible interface for lipase immobilization

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ABSTRACT

To create compatible interface for enzyme immobilization, the surface of multi-walled carbon nanotubes (MWCNTs) was functionalized using soft technique dielectric barrier discharge plasma (DBDP) for carboxylation and amination; followed by further amidation of carboxyl group with alkylamine. Successful functionalization and enzyme immobilization were structurally confirmed using spectroscopic analysis Fourier-Transform Infrared Spectroscopy (FTIR) and X-ray Photoelectron Spectroscopy (XPS). The immobilization of *Candida rugosa* lipase (CRL) on functionalized MWCNTs was evidenced by clearly viewing with Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM) imaging. CRL showed more Freundlich equilibrium behavior upon immobilization on annealed and octadecylamidated MWCNTs, which suggested a multilayer adsorption; while upon physical adsorption on aminated and carboxylated MWCNTs, CRL, to more extent, demonstrated a Langmuir equilibrium property, producing an enzyme monolayer. It was proven that DBDP-mediated surface-functionalization could create compatible microenvironments for enzyme immobilization, resulted in improved specific activity and thermostability. The immobilized CRL on octadecylamidated MWCNTs displayed excellent reusability and operation stability, indicating its potential for industrial application.

1. Introduction

Immobilization of enzyme has been granted as an important cornerstone to pave the way for enzyme technology from lab curiosity to industrially viable process [1,2]. Besides its basic function to enable enzyme's easy separation and reusability, enzyme immobilization also adds advantages in enhancing enzyme stability against pH, temperature, organic solvents, and contaminants [3–5]. Use of nanostructured materials for enzyme immobilization has gained a wide popularity in recent years [6–10]. The key point to whirl this interest is their high ratios of surface area to volume, through which a high enzyme loading could be achieved and elevated specific catalytic activity might be obtained consequently [6–8,11,12]. Among the various nanostructured materials such as nanoparticles, mesostructures, nanofibers, and nanotubes, carbon nanotubes have been the focus of interest for many researchers [6–8,13–18]. Two main types of carbon nanotubes, single-walled carbon nanotubes and multiwalled carbon nanotubes (MWCNTs), have been used to immobilize enzymes [6–8]. Thanks to its easy preparation, low cost and better dispersibility, MWCNTs were more intensively investigated [19,20].

In principle, the carriers for enzyme immobilization should be chemically stable and inert to microbiological contamination [3–5]. On top of these basic properties, a good carrier should be able to create a compatible microenvironment to anchor enzyme where the enzyme molecule could be oriented and localized to maximize its catalytic activity [3–8,21]. Physical adsorption

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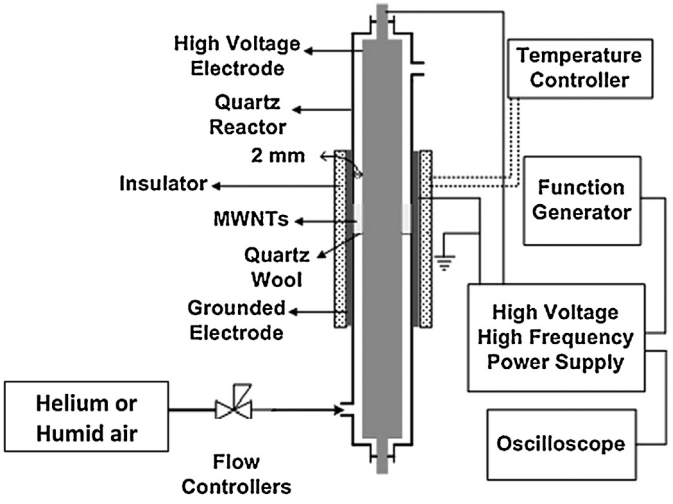
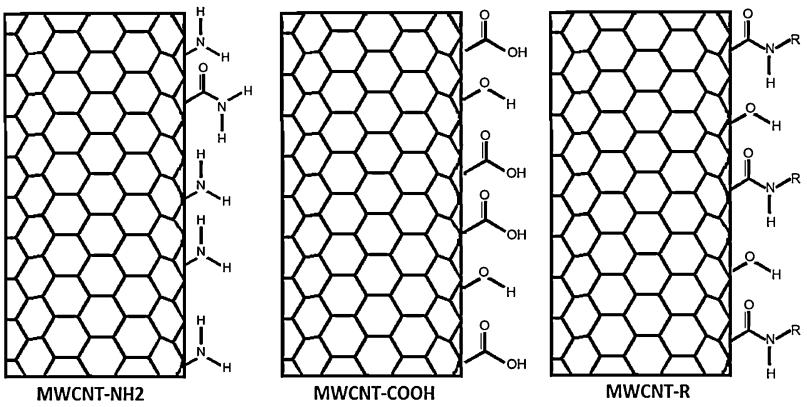
is a very widely used immobilized method which is assumed to preserves the conformational structure of enzyme in contrast to covalent conjugation of enzyme [3,5], however, this is not always the case for carbon nanotubes. Significant loss of alpha-helix content (corresponding structure change), when some enzyme upon adsorption on nanotubes, has observed by FTIR and circular dichroism spectroscopy analysis [22,23]. To cope with this problem, surface-functionalization of MWCNTs may present a possible solution to create an enzyme-preferable microenvironment [6,12,14,19,20,22–24]. For doing so, understanding of the surface chemistry of nanomaterials is crucial. It is well known that MWCNTs are composed entirely of sp^2 bonding, which leads to dominating hydrophobic and π–π interactions for the enzyme adsorbed on the surface of MWCNTs [6,25,26]. However, as a protein molecule, the molecular structure (consisting of hydrophilic/hydrophobic, acidic/basic, charged/uncharged side chains amino acids) and chemical interactions (electrostatic, H-bonding, van der Waals and salt bonding interaction etc.) of enzyme are rather complicated [27,28]. This molecular feature makes enzyme so delicate to enable a catalytic activity [3,5,27,28]. For MWCNTs, to alter/tune its strong surface hydrophobicity, introduction of hydrophilic groups is an obvious solution [6,12,15,19,20,22–24]. As such, two main technical approaches have been applied, namely, (1) CNTs were functionalized/coated with polymers/biomolecules; then the enzyme molecules were adsorbed/conjugated on the resulting polymer-functionalized CNTs [29,30]. This approach has also been used to tune surface property for other particle carriers [31,32]. (2) Functionalizing CNTs by chemical/physical methods, the enzyme was immobilized either by direct adsorption or covalent attachment through linking molecules [24,25,33,15]. The former approach is rather complicated and a decrease in enzyme activity was reported in some systems, suggesting a considerable fraction of enzyme molecules were probably embedded in the polymer and became less accessible. The latter process is rather straight forward, and in many cases enhanced activities [6,24] or good activity retention (up to 92% of native enzyme) [34] of immobilized enzyme was observed.

The current methods for functionalizing nanotubes covalently are acid (H_2SO_4/HNO_3) treatment (wet chemistry), caustic base (KOH/NaOH) treatment and high temperature vapors exposure; however, these approaches may significantly damage the structure of nanotubes depending on temperature and treatment time. Moreover, the corrosive chemical method generates large quantity of defects and corrosive residues to be removed, which might affect not only the stability of CNTs but also the activity of immobilized enzymes [35,36]. Therefore, development of a better and more effective functionalization method that can not only introduce high density and homogeneous surface functional groups but also has little or no structural damage to CNTs remains a major challenge [26,35–37]. An alternative method is the use of plasma technique which has considerable advantages. The nanotubes surface functionalization under plasma exposure can occur during a short period and low temperature, resulting in a less damage effect on nanotubes structure [26,36]. Being an attractive technique, plasma functionalization is flexible, quick and contaminant-free [37]. The exited molecules and radicals generated during plasma discharge attack C=C bond, creating open ends and defect sites as prime sites for functionalization. The discharges in different types of plasma (radio frequency, microwave, dielectric barrier (DBD), etc.) and in different gases (air, CO_2, NH_3, H_2O, etc.) have been utilized for surface modification of carbon-based materials. DBD plasma is a low-temperature process operated under an atmospheric-pressure condition; therefore the need for costly vacuum system can be avoided. Different functional groups could be generated by adjusting plasma parameters such as power, dielectric thickness, process duration and the gases used [38,39]. In the present work MWCNTs were firstly annealed through helium DBD plasma reactor operated at atmospheric pressure (Scheme 1). The resulting annealed MWCNTs were exposed to NH_3, thus nitrogen containing functional groups were attached to MWCNTs surfaces. The carboxyl groups onto the surface of MWCNTs were generated in DBD plasma reactor through passage of humid air. The carboxylated MWCNTs were further modified for amidation by reacting



Scheme 1. Schematic diagram of the experimental setup for the DBD plasma functionalization.



Scheme 2. Schematic diagram of functinalized MWCNTs.