MACROJOURNALS

The Journal of **Macro**Trends in Technology and Innovation

Functionalization of Multi -Walled Carbon Nanotubes for Lipase Immobilization

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Abstract

Multi-walled carbon nanotubes (MWCNT) were functionalized through dielectric barrier discharge plasma (DBD) reactor in ambient temperature and pressure in short time. Different functional groups as well as carboxyl and hydroxyl were introduced to the carbon nanotubes' surface by using different plasma medium. Candida rugosa lipase was immobilized physically in different weight ratio of enzyme to support. Conversion of oleic acid and butanol to butyl oleate ester was measured for immobilized lipase.

Keywords: *lipase; DBD plasma; multi-walled carbon nanotubes functionalization*

1. INTRODUCTION

Nanobiocatalysis is a rapidly growing research field which refers to the application of enzymes immobilized materials. Nanostructured materials presents some advantages over the bulk solid materials, namely the high surface area which can lead to higher enzyme loading, the nanoscale dispersion and the ease of surface functionalization (Kim et al., 2008). Carbon anotubes(CNTs) have attracted considerable interest among nanostructured materials for their unique mechanical, thermal and electrical properties as well as their biocompability (Kuchibhatl, 2007). Lipases can interact with the wall of CNT through hydrophobic and π-π stacking interaction which hydrophobicity of surface can improve their activity .The surface chemistry of the functionalized CNTs can affect their dispersability and interactions with enzymes, thus alter the biological activity of the immobilized enzymes [Feng and , 2011; Pavlidiset al., 2010). There are

not enough studies on the surface chemistry of nanomaterials as well as methods of functionalizing their surface on structure and function of conjugated enzymes. The current methods for functionalize nanotubes covalently, are acid treatment (wet chemistry) and high temperature vapors exposure which these approaches may damage the structure of nanotubes depending on temperature and treatment time. An alternative to these methods is the use of plasma method which can have considerable advantages. The nanotubes surface functionalization under plasma exposure can occur during a short period and low temperature. Therefore it has less damage effect on nanotubes structure (Pourfayaz, 2010).

In the present work MWCNTs were functionalized through Helium DBD plasma (method 1) reactor operated at atmospheric pressure which was followed by exposure to NH3, where attachment of nitrogen containing functional groups on the MWCNTs surfaces became possible. Further work was to functionalize MWCNTs in H2O DBD plasma (methode2)reactor through passage of humid air, where carboxyl groups' attachment on the surfaces was demonstrated. The work was extended on the latter matrix by introducing octadecylamine as a chemical type of amidation (methode3).

Immobilization of commercial candida rugosa lipase onto these functionalized MWCNTs was carried out and the enzyme activities were determined considering formation of butyl oleate ester in hexane solvent as a model system. The enzymatic activity was then measured according to the standard procedure.

2.MATERIALS AND METHODS

2.1. Materials

MWCNT with Purity: > 95 wt% and OD 30-50 nm was purcahased fro US nano. Lipase from Candida rugosa was purchased from Sigma. All chemicals used were of analytical grade.

2.2. MWCNTs Functionalization

A quartz DBD reactor with inlet and outlet diameter of 15 and 19 mm was used. Total flow rate of inlet gas was 50 sccm. Plasma voltage was 9 kV and frequency was held at kHz for 4 min reaction time.

2.2.1. DBD plasma under air and H2O

80 mg MWNTs Were put in the reactor and exposed to humid air with volume rate of 50 cm3/min. Then plasma of air and H2O created for 4 min processing time (Ono and Oda, 2000).

2.2.2. DBD plasma under helium and NH3

80 mg MWNTs Were put in the reactor and exposed to helium with volume rate of 50 cm3/min. The plasma of helium created during 4 min processing time which then followed by exposing to NH3 flow at 300˚C for 1 h (Pourfayaz, 2010).

2.2.3. Chemical amidation

0.22 g of air and H2O plasma treated MWNTs were heated with 0.5 g octadecylamine at 90˚C for 96 h. After cooling to room temperature, the black solid washed with ethanol and centrifuged. This washing operation was repeated five times and followed by washing with THF three times. The product then washed with deionized water and acetone, and the functionalized MWNT material produced was dried in a vacuum oven at 70˚C (Mark et al., 1999).

2.3. Enzyme Immobilization

Lipase was immobilized on carbon nanotubes by physical adsorption. In a typical procedure, 50 mg of nanotubes in 40 ml of phosphate buffer (100 mM, pH 7.5) were sonicated for 30 min. then 10 ml of enzyme solution containing 25-150 mg was added and the mixture incubated under stirring for 2 h at 20˚C and 200 rpm and then overnight at 4˚C. The bioconjugates were separated by centrifugation from the supernatant and then they were washed twice with buffer solution to remove loosely bound enzyme. The immobilized enzyme was dried over silica gel at 4˚C and stored at 4˚C (Plvidis, 2012). The amount of immobilized lipase was determined based on Lowry method (Lowry et al., 1951).

2.4. Esterification Activity

In order to determine the enzyme activity, Esterification of oleic acid with butyl alcohol was followed. For esterification acitivity Reaction required 0.32 ml oleic acid, 5 ml hexane and 5 mg of free or 10 mg immobilized lipase. The mixture was prepared in 37˚C. After preparing the enzyme solution, 0.095 ml butyl alcohol was added. The reaction was stopped by centrifugation at 3000 rpm. The quantity of oleic acid was measured by spectrophotometric assy. 0.1 ml of sample was dissolved in 5 ml isoocatane in test tube. Then I.0 ml of cupric-acetate (5% w/v) reagent was added and was mixed vigorously for 90 sec. The absorbance was measured at 715 nm A standard curve of 2.0-50 µmol of oleic acid was determined (Lowry and Tinsley, 1976; Kwon and Rhee, 1986).

2.5. Characterization

FTIR spectra were measured using KBr pellets for characterization functional groups on MWCNTs.

3. RESULTS

3.1. Characterization

To distinguish the chemical moieties on the MWCNTs'surfaces, the FTIR results, in the present study, are shown in Fig.1 For instance stretching vibration of carbonyl of carboxylic acid group shows band at wave numbers of 1763 and 1163 cm-1 , where the band at 1620 cm -1 are assigned to carbonyl group of amides, respectively. While stretch of C-O band of the carboxylic acid is shown at 1163 cm -1 and these band at 1620 is due to N-H bending of the amides. Stretch of N-H groups of the amines occurs in the range of 3500-3300 cm -1 and this band in MWCNT-NH2 spectrum is seen in 3444 cm -1.similar band characteristic are also seen in the

other FTIR spectra. It is important to realize some functional groups such as NH and OH groups may overlap. The FTIR spectrum MWCNT-R shows bands at wave numbers of 3748 , 3444 cm-1 which are assigned to NH stretch mono substituted in amides and bands at 1641, 1544 cm-1 are due to carbonyl stretch and NH bending in amides respectively. C-C stretch in alkanes shows many picks but these are not interpretatively useful. H2 and CH3 bands typically assigned in alkanes (1450 and 1375 cm-1) are not present in the relevant spectra. There are some bands for annealed MWCNT that shows adsorption of water vapor and carbon dioxide in the air (Pavia et al, 2001).

3.2. Immobilization yield

Fig.2 shows the constant yield of immobilization while the weight ratio of enzyme to support is increased and no trend of decrease in support matrices for receiving enzyme is seen. Presence of alkyl group on MWCNT has made them more eligible in forming hydrophobic interaction with lipase. Despite the fluctuation of the immobilization yield for the other support, no apparent decrease was observed in the loading.

3.3. Esterification activity

Fig. 3 shows results of the oleic acid conversion to butyl oleate using butanol. Hydrophilic group like amine and carboxyl attached on carbon nanotube surface resulted in more enzyme attachment and increasing lipase activity. Carboxyl groups can cause covalent attachment of lipase which may
affect the immobilization and enzyme performance (Shi et al., 2007). MWCNT-R on which the capacity of the tubes towards the lipase molecules increased, because of, introducing octadecylamine on the MWCNT showed higher amount of the enzymatic reaction. It shows that role of octadecylamine for introducing more hydrophobicity as well as creating more distance for lipase on support improved the esterification activity.

4.CONCLUSIONS

Less damage occurred during functionalizing MWCNT by plasma method resulted in high lipase loading. Introduction of alkyl group to MWCNT increased surface hydrophobicity which resulted in creating better environment for lipase attachment. Hydrophobic property and role
of alkyl group as spacer arm as well as more amount of immobilized lipase let to better activity.

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Figures

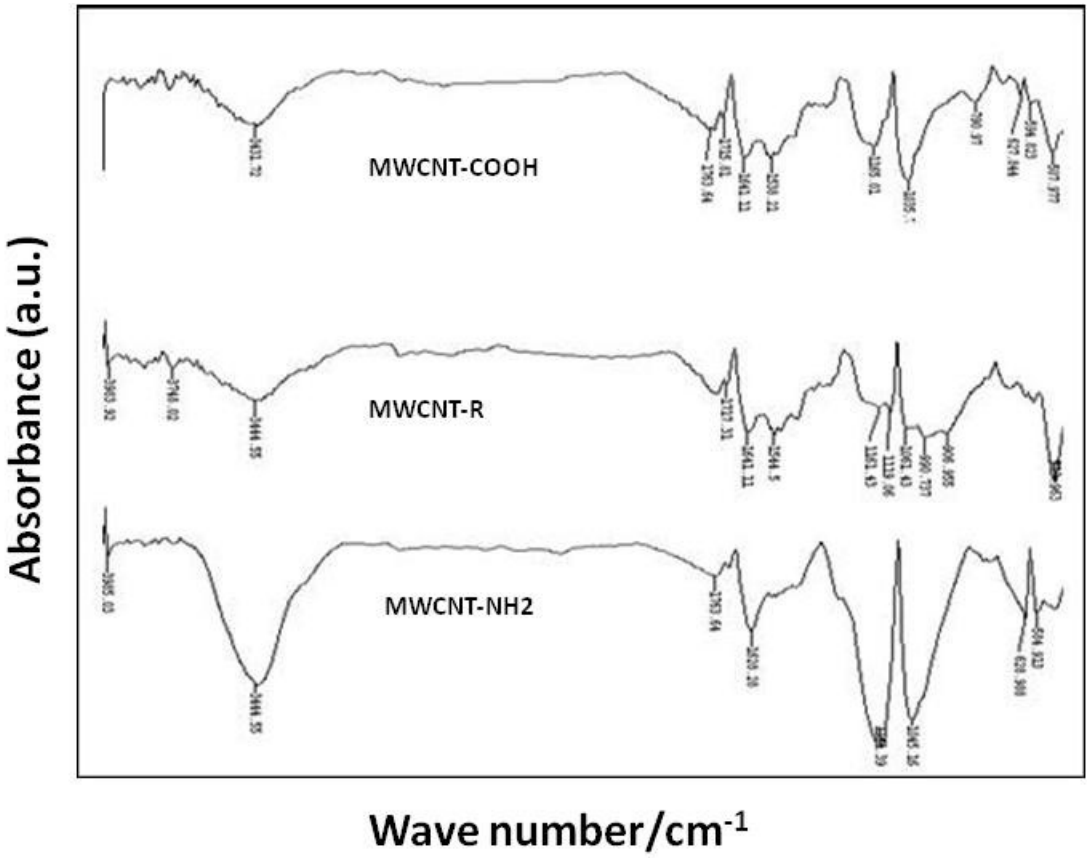

Fig. 1. FTIR spectra of functionalized MWCNTs.

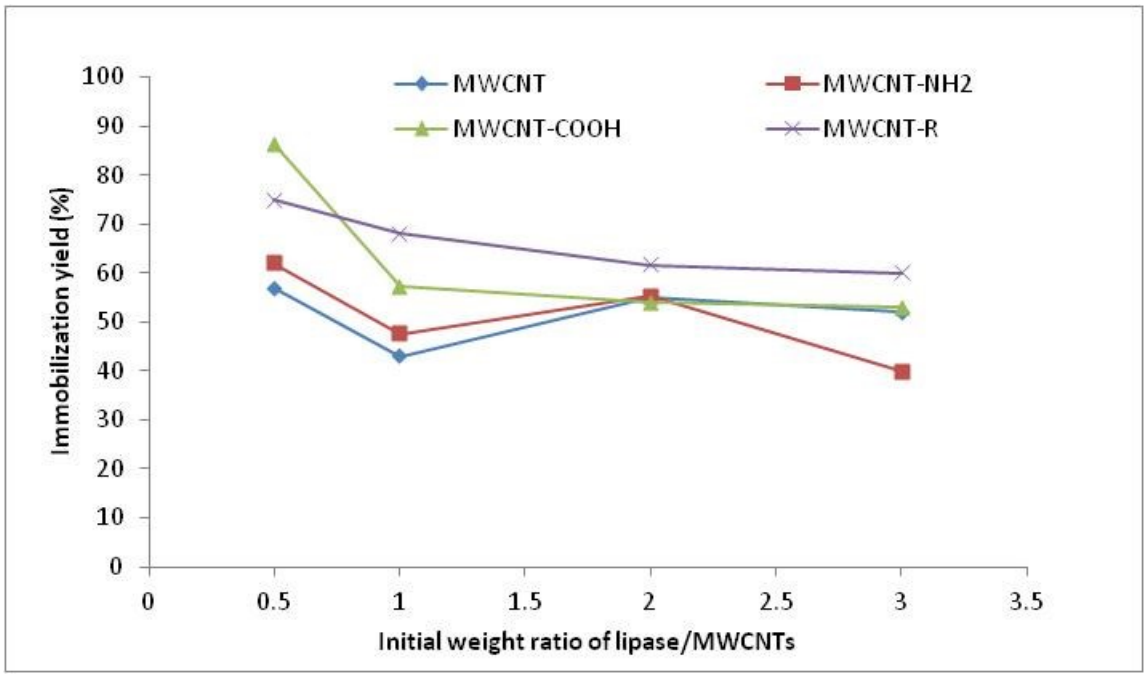

Fig.2. Immobilization yield of Lipase

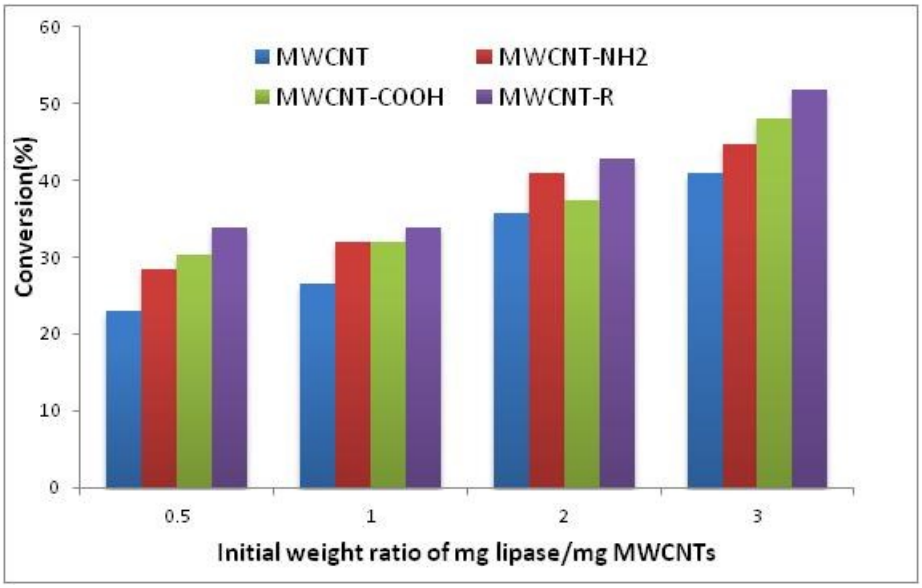

Figure 3. Conversion of oleic acid to the butyl oleate.