SURFACE MODIFICATION OF MULTIWALLED CARBON NANOTUBES BY CHEMICAL OXIDATION AND IMMOBILIZATION OF TYROSINASE

NUR ANIS BINTI MOHAMMAD SABRI

UNIVERSITI TEKNOLOGI MALAYSIA

SURFACE MODIFICATION OF MULTIWALLED CARBON NANOTUBES BY CHEMICAL OXIDATION AND IMMOBILIZATION OF TYROSINASE

NUR ANIS BINTI MOHAMMAD SABRI

A thesis submitted in fulfillment of the
requirements for the award of degree of
Master of Science (Chemistry)

Faculty of Science
Universiti Teknologi Malaysia

AUGUST 2012

Dedicated with love:
To my beloved daddy; Mohammad Sabri Ab Rahman,
To my adored mom; Mazni Ismail,
To my sweet little sisters and brother; Amira,Zamani,Salwana,Syukri
To my one and only, Fahmi Aizuddin Sha'ari

ACKNOWLEDGEMENT

An undying gratitude to Allah S.W.T for His blessing during my whole life and all that I have now. I feel so grateful to my beloved family, especially my parents with all their unconditional love and loyalty. Here, I would like to give my special thanks to all who's given their best in helping me during my project.

First and foremost, thanks to Associate Prof. Dr Zaiton Abdul Majid as my supervisor, for all the time, efforts and attention toward me and my project.

Then, to Dr Shafinaz Shahir and Associate Prof Dr Nor Aziah Buang as my co-supervisors for all their knowledge, advices and chances that had been given to me.

Last but not least, to all staff members and my friends who provided me with friendship and many kind of support along this very meaningful journey.

Thank you very much.

ABSTRACT

Studies on the development of interface between biological molecules and novel nanomaterials have attracted research worldwide. Carbon nanotubes (CNTs) have become an important matrix for the fabrication of biomaterials due to its unique properties. Surface properties of the CNTs and the medium of immobilization are critical in the immobilization of enzymes. In this study surface modification of multi-walled carbon nanotubes (MWCNTs) for carboxylic moieties attachment was accomplished by acid treatment and reaction with potassium permanganate ($KMnO_4$). The effect of these two oxidants on the surface modification of MWCNTs for tyrosinase immobilization was studied. Commercial MWCNTs were treated with either concentrated sulfuric acid - nitric acid mixture of ratio 3:1 or 0.1 M $KMnO_4$ via reflux, stirring and ultrasonication. The resulting surface modified MWCNTs were characterized with FT-IR spectrophotometer, XPS, and FESEM. The immobilized tyrosinase was tested for leaching assay and its catalytic activity towards phenol was analysed. The FTIR spectra of functionalized MWCNTs showed a significance peak in the range of 1700 cm^{-1} to 1729 cm^{-1} indicating the presence of carboxyl double bond, which confirmed the successful functionalization of MWCNTs (FCNTs) by chemical oxidation. The carboxylic peak of MWCNTs treated with $KMnO_4$ (FCNTK) showed higher intensity as compared to acid-treated MWCNTs (FCNTA). These results are supported with the shift of O 1s binding energy at 534.9 eV and shoulder of C 1s at 289.00 eV corresponding to carboxylic groups from XPS analysis. The immobilization of tyrosinase onto FCNTA is higher than FCNTK with high catalytic activity for phenol degradation. Further sorption study showed that FCNTA with immobilized tyrosinase (FCNTA-Ty) has higher sorption towards phenol as compared to FCNTA and pristine MWCNTs. The results illustrated that FCNTA-Ty, FCNTAs and MWCNTs had relatively well adsorption capacity for phenol as described by both Langmuir and Freundlich models. In addition, the adsorption kinetics for these CNTs were well fitted with the pseudo-second order model with reasonably good correlation coefficient. This study led to possible application of bioremediation of phenol in industrial sample by attaching the FCNTA-Ty onto chitosan.

ABSTRAK

Kajian berkenaan perkembangan hubungan antara molekul biologi dan bahan nano baru telah menarik minat sedunia. Tiubnano karbon (CNTs) menjadi matriks yang penting untuk pembuatan bahanbio kerana keunikan sifatnya. Sifat permukaan CNTs dan media nyahgerakan adalah kritikal bagi penyahgerakan enzim. Dalam kajian ini pengubahsuaian permukaan tiubnano karbon dinding berlapis (MWCNTs) dengan perlekatan kumpulan karboksilik dicapai melalui rawatan asid dan tindakbalas kalsium permanganat ($KMnO_4$). Kesan dua agen pengoksidaan ini terhadap pengubahsuaian permukaan MWCNTs untuk penyahgerakan tirosinase telah dikaji. MWCNTs komersial dirawat sama ada oleh campuran asid sulfurik dan asid nitrik pada nisbah 3:1 atau dengan 0.1M $KMnO_4$ melalui refluks, pengacauan, dan ultrsonikasi. MWCNTs dengan permukaan diubahsuai yang terhasil diuji dengan spektrofotometer FT-IR, XPS, dan FESEM. Tirosinase yang dinyahgerak diuji untuk asai penguraian dan aktiviti enzim terhadap fenol juga dianalisa. Spektra FTIR MWCNTs berfungsi menunjukkan puncak yang ketara pada julat 1700 cm^{-1} hingga 1729 cm^{-1} menunjukkan kewujudan ikatan berganda karboksil yang membuktikan kejayaan pengfungsian MWCNTs (FCNTs) melalui pengoksidaan kimia. Puncak karboksilik MWCNTs yang dirawat dengan $KMnO_4$ (FCNTK) menunjukkan kekuatan yang lebih tinggi berbanding MWCNTs yang dirawat asid (FCNTA). Keputusan ini disokong oleh anjakan tenaga ikatan O 1s pada 534.9 eV dan bahu C 1s pada 289.00 eV berkaitrapat dengan asid karboksilik melalui analisa XPS. Penyahgerakan tirosinase pada FCNTA adalah lebih tinggi berbanding FCNTK dengan aktiviti mangkin yang tinggi untuk penguraian fenol kepada kuinon. Kajian lanjut penyerapan menunjukkan FCNTA dengan tirosinase ternyahgerak (FCNTA-Ty) mempunyai serapan terhadap fenol yang lebih tinggi berbanding FCNTA dan MWCNTs asal. Keputusan menunjukkan FCNTA-Ty, FCNTAs dan MWCNTs mempunyai kapasiti serapan yang agak baik terhadap fenol oleh kedua-dua model Langmuir and Freundlich. Tambahan pula, kinetik serapan untuk semua CNTs ini sesuai dengan model aturan pseudo-kedua dengan pekali korelasi yang baik. Kajian ini membawa kepada kemungkinan aplikasi pembaikpulihbio fenol dalam sampel industri dengan mencantumkan FCNTA-Ty dengan kitosan.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

AH2	-	Chemical reductor
BSA	-	Bovine Serum Albumin
CTC	-	FCNT-Tyrosinase-Chitosan
CNT-Ty	-	Carbon Nanotubes-Tyrosinase
C-C	-	Carbon-Carbon
DOPA	-	Dihydroxyphenylalanine
EDTA	-	Etylendiaminetetraacetic acid
FESEM-EDX	-	Field Emission Scanning Electron Microscope – Energy Display Xray
FCNTs	-	Functionalized Carbon Nanotubes
FCNTAs	-	Functionalized Carbon Nanotubes by Acid
FCNTKs	-	Functionalized Carbon Nanotubes by Potassium Permanganate
FCNT-Ty	-	FCNTs Immobilized with Tyrosinase
FTIR	-	Fourier Transform Infrared
H_2SO_4	-	Sulfuric acid
HNO_3	-	Nitric acid
MWCNTs	-	Multiwalled Carbon Nanotubes
MnO_2	-	Manganese Dioxide
$KMnO_4$	-	Potassium Permanganate
STM	-	Scanning Tunneling Microscopy
SWCNTs	-	Single-walled Carbon Nanotubes
TPD	-	Temperature-programmed Desorption
XPS	-	X-Ray Photoelectron Spectroscopy
UV-Vis	-	Ultraviolet-Visible
°C	-	Degree Celcius

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Research Background

Nanotechnology is a strategic breakthrough technology that focused on generating, manipulating and fabricating nanomaterials at the scale of a billionth of a meter (Ratner *et al.*, 2010). This field of technology is employed into other areas to revolutionize them for a better utilization whether in fundamental studies or industrial application. Nanotechnology is a bridge to incorporate several diverse areas into one by fabricating new materials with new physical, chemical, or biological properties. One of the ultimate nanomaterials present today is carbon nanotubes (CNTs) which are carbon atoms in form of carbon tubules with hollowed centre with various special properties such as thermal conductivity, electrical conductivity, and also strength (Ebbesen, 1997).

CNTs have become the bridge in connecting material science, biotechnology and nanotechnology where their application varied from disease diagnosis, environmental analysis, to drug delivery (Pagona and Tagmatarchis, 2006). The same goes to both bioremediation and adsorption field, with research devoted to the development of surfaced-based bioremediation that enable selective remediation of biorecognition reaction. A genuine product that can be used as adsorbent and bioremediation will be very desirable. The selective remediation is achievable by using enzymes due to their reaction specificity towards certain substrates and produces few side reactions. Meanwhile, the CNTs are known for their adsorption ability for heavy metal, aromatic compounds and dye (Lu and Chiu, 2008; Shen,*et al.*, 2009; Zhu *et al.*, 2010).

Bio-adsorbent based on immobilized enzyme on sturdy material are pursued and idea on having CNT as support becomes a starting hypothesis with their steady characteristic and morphology. However, the stable chemical structure of CNTs make them difficult to form complexes with other elements. Hence, CNTs have to be functionalized before it can be used as a support for any enzyme. Functionalization of CNTs can be achieved by attaching groups with chemical functionalities such as carboxyl group onto the carbon walls or at the end of the tubules. This step is important to 'activate' the CNTs and overcome their difficulty to dissolve or disperse in solvent. This difficulty has limited their applications in many fields of interest.

The functionalization of CNTs by oxidation process is accomplished either by wet chemical methods, photo-oxidation, oxygen plasma, or gas treatment (Datsyuk and Kalyva, 2008). The wet chemical methods are usually chosen based on economical factor and ease of approach as compared to others. Several techniques usually involve in wet chemical methods such ultrasonication, reflux, stirring with their own advantages and side-effects. In this study, functionalizing of CNTs will be carried out using these techniques and their subsequent effectiveness toward enzyme immobilization will be compared. The advantages of using functionalized carbon nanotubes (FCNTs) as the support for enzyme immobilization include:

- The high surface area of FCNTs can provide a good immobilization area for enzyme loading
- The surface hydroxyl and carbonyl groups present on FCNTs can be readily used for enzyme attachment
- The chemical inertness of FCNTs can provide a secured environment for enzyme especially in severe reaction condition

Immobilization of enzyme or other biological compounds into inorganic support is not a new idea and had been applied in various fields for several reasons. The reasons are to improve the stability of enzyme in adverse reaction condition or in the presence of organic solvent, to separate the enzyme from its product stream, and also to allow repetitive usage of the enzyme. The main challenge in immobilization of biological compounds is to integrate them with the support matrix at the same time retaining most of their functions. This is because biological compounds especially enzymes, have their own special structure with specific function and to fully use these in fabricating new multifunctional nanomaterials is a great challenge.

Enzyme immobilization on support without damaging both enzyme and support will help in manufacturing adsorbent material (Xu *et al.*, 2005). Technique of immobilization would play a major role in protecting both enzyme and support during the process. Several techniques have been utilized to produce high immobilized enzyme and preserves its activity. The techniques include physical adsorption, covalent attachment, entrapment and encapsulation (de Faria *et al.*, 2007) . Physical adsorption and covalent attachment are two techniques with excellent enzyme immobilization with CNTs (Cui, 2008). Physical adsorption is the least complicated technique with adsorption occurs on the surface of support. The adsorption can be enhanced with hydrogen bonding between surface moieties of the support and the nitrogen or amine in enzyme. The covalent attachment of enzyme and CNTs only occur if the CNTs are functionalized with surface moieties that can promote covalent linkage. In FCNTs cases, usually physical adsorption and covalent attachment can occur simultaneously. However, the desirable physical adsorption can be promoted through immobilization condition. The other techniques are more suitable with polymer or inorganic support with special matrix or for short peptides only.

In this research, tyrosinase is chosen as enzyme of interest because of its wide applications especially in environmental and industrial field. Tyrosinase will be immobilized onto functionalized multi-walled carbon nanotubes to be employed as bio-adsorbent. The bio-adsorbent can be used for bioremediation of phenolic waste and its adsorption property will allow it to adsorb the waste simultaneously. Thus, it may reduce the amount of waste up to twice as much as other adsorbents. In addition, the immobilized tyrosinase with better stability in the form of bio-adsorbent can be used with highly acidic reaction condition.

1.2 Problem Statements

Functionalization of CNTs has been reported and it differs based on the future application purposes. For the purpose of enzyme immobilization, the frequently used functionalization is by oxidizing the CNTs through several techniques of wet chemical methods. The techniques usually involve are ultrasonication, reflux, stirring. However, each technique has its own advantages and disadvantages and the best technique of functionalization for tyrosinase immobilization is yet to be determined. Ultrasonication can produce high yield of FCNTs but at shorter length which is undesirable. Reflux and stirring can produce moderate yield of FCNTs and retain most of the CNTs physical properties. Thus, these two techniques and the mix technique of reflux and stirring will be studied in length to find the best oxidation technique of functionalization of MWCNTs for tyrosinase immobilization.

Phenol is a common pollutant found mainly in industrial effluent. The effluent has to be treated before it is discharged to avoid harmful consequences in overall water ecosystem. Phenol treatment could be achieved via bio-remediation and sorption. The remediation and sorption of phenol is accomplished by immobilizing tyrosinase onto carbon nanotubes. However, the characteristic of sorption by carbon nanotubes immobilized with tyrosinase towards phenol as its main analyte is ambivalent. As known by many CNTs also have the ability to adsorb elements and this has increased the need to identify whether decreasing amount of phenol was caused by CNTs or enzyme activity. In addition, the effect on tyrosinase activity after immobilization will be different than free enzyme. Hence, a close observation and investigation are required with stated problems as the parameters.

1.3 Objectives of the Research

The objectives of the study are:

1. To investigate techniques of functionalization of MWCNTs for tyrosinase immobilization and effect on its activity.
2. To assess sorption characteristic of immobilized tyrosinase towards phenol.

1.4 Scope of the Research

This research will encompass functionalization technique of MWCNTs, the sorption properties of CNTs, the immobilization of tyrosinase on the CNTs, and also possible application of tyrosinase immobilized onto FCNTs (CNT-Ty) for phenol removal. The functionalization of MWCNTs is via oxidative purification method or also acknowledged as carboxylation method. This method introduces carboxylic group onto CNTs by either liquid-phase or gas-phase oxidation process. The CNTs will be treated with strong oxidative agents such as nitric acid and sulfuric or mixture of both and also with potassium permanganate. The oxidation reaction will be done through reflux, stirring, and the mix techniques of reflux and stirring. The study will focus on investigating the best technique for tyrosinase immobilization. Characterization of CNTs on different stages will be executed by using Fourier Transform-Infra Red (FT-IR) spectrophotometer, X-ray Photoelectron Spectrophotometer (XPS) and Field Emission Scanning Electron Microscope – Energy Dispersive X-ray Analyzer (FESEM-EDX).

The sorption study by nanotubes will be limited only to phenol as tyrosinase's analyte or substrate. The sorption of phenol will be analyzed based on Langmuir and Freundlich isotherm. The adsorbate will be pristine MWCNTs, FCNTs, and CNT-Ty. The immobilization of tyrosinase will be done via physical adsorption onto the CNTs. Effect of parameters such as temperature; pH and incubation time on the adsorption process will be observed. In addition, the study on catalytic activity is also significant in this research. The enzymatic activity study before and after immobilization of tyrosinase will be analyzed using Ultra-violet/visible spectrophotometer based on amount of phenol degradation.

The possible application of CNT-Ty for phenol removal will be studied by intertwines the CNT-Ty onto chitosan beads. This is important to help in retrieving the CNT-Ty during the phenol removal process due to CNT-Ty small size. The beads will be immersed in known concentration of phenol solution. The phenol sorption will be analyzed to determine the efficiency of CNT-Ty in chitosan beads in removing the phenol.

REFERENCES

Agnihotri, S., Mota J.P.B.,Rostam-Abadi M.,and Rood M.J., (2006) Theoritical and Experimental Investigation of Morphology and Temeprature Effect on Adsorption of Organic Vapors in Singlewalled Carbon Nanotues. *Journal of Physical Chemistry*.110: 7640-7647

Balasubramanian, K. and Burghard, M. (2005), Chemically Functionalized Carbon Nanotubes. *Small,* 1: 180-192

Bethune, D.S., Johnson R.D., Zetti A. and Bando H., (1993) Cobalt-catalysed Growth of Carbon Nanotubes with Single Atomic Layer Walls; *Nature,* 363: 605-607.

Bisswanger, H., (2004) *Practical Enzymology*. Weinhem, Wiley-VCH Verlag GmbH & Co.

Chen, R.J., Zhang, Y., Wang, D., Dai, H., (2001), Noncovalent Sidewall Functionalization of Single-Walled Carbon Nanotubes for Protein Immobilization, *Journal of American Chemical Society* 123: 3838-3839

Chen Z., Thiel W., Hirsh A. (2003), Functionalization of Carbon Nanotube, *Physical Chemistry*. 4: 93-97

Chen, J., Hamon M.A., Hu H., Chen Y., Rao A.M., Eklund P.C. and Haddon R.C.(1998) Solution Properties of Single-walled Carbon Nanotubes. *Carbon* 282: 95-95

Cho, H.H., et al., 2010. Polymer Nanocomposite based on Functionalized Carbon Nanotubes. *Progress in Polymer Science* 35: 837-867.

Chu, A., Cook J., Heesom R.J.R., Hutchison J.L., Green M.L.H., and Sloan J., (1996) Filling of Carbon Nanotubes with Silver, Gold, and Gold Chloride. *Chemistry of Materials*.8: 2751-2754.

Collier C.P., (2006) *Carbon Nanotubes Tips for Scanning Probe Microscopy, Carbon Nanotubes : Properties and Applications*. Boca Raton, CRC Press.

Cui, D., Tian F. Ozkan C.S., Wang M., Gao H.(2008) Effect of Single Wall Carbon Nanotubes on Human HEK293 cells. *Toxicology Letter* 155: 73-85.

Daigle, F., Trudeau, F., Robinson, G., Smyth, M. R., and Leech, D.. (1998) Mediated Reagentless Enzyme Inhibition Electrodes. *Biosensors and Bioelectronics* 13(3): 417- 425.

Datsyuk, V.,Kalyva, M.,Papagelis K., Parthenios J., Tasis D.,Siokou A., Kallitsis I., and Galiotis C. (2008) Chemical Oxidation of Multi-walled Carbon Nanotubes. *Carbon.* 46: 833-840.

de Faria, R.O.,Moure V.R., Lopes M.A., Krieger N., and Mitchell D.A., (2007) The Biotechnological Potential of Mushroom Tyrosinase. *Food Technology and Biotechnology,* 45 : 287-294.

Dinadayalane T.C., Murray J.S., Concha C.M., Politzer P., and Leszczynski J., (2010) Reactivities of Sites on (5,5) Single-Walled Carbon Nanotubes with and without a Stone-Wales Defect *J. Chemical Theory Comput,*, 6(4): 1351–1357

Dresselhaus M.S. and Avoris P. (2001), *Carbon Nanotubes Synthesis, Structure, Properties and Applications.* Germany, Springer-Verlag Berlin Heidelberg.

Duran, N., and Esposito E. (2000). Potential Application of Oxidative Enzymes and Phenoloxidase-like Compounds in Wastewater and Soil Treatment : A review. *Applied Catalysis B : Environmental* 28(2): 83-99

Duran, N., A Rosa, M., D'Annibale, A., Ganfreda, L., (2002). Application of Laccase and Tyrosine (Phenol oxidases) Immobilized on Different Support: A review. *Enzyme and Microbial Technology,* 31(7): 907–931

Ebbesen, T.W., Ajayan P.M., Hiura H., Tanigaki K. (1994) Purification of Nanotubes. *Nature*. 367: 519-1519.

Gaibai E.(2004) Interactions of Metal Ions with Chitosan-based Sorbent: A review. *Separation and Purification Technology*. 38: 43-74.

Ge, B., Tan Y., Deng W., Xie Q., Huang J., and Yao s. (2009) Biofuel cell and Phenolic Biosensor Based on Acid-Resistant Laccase-glutaraldehyde Functionalized Chitosan-multiwalled Carbon Nanotubes Nanocomposite Film. *Biosensors and Bioelectronic*. 7: 2225-2231

Girelli, A. M., Mattei, E., and Messina, A. (2006) Phenols Removal by Immobilized Tyrosinase Reactor in On-line High Performance Liquid Chromatography. *Analytica Chimica Acta.*. 580(2): 271-277.

Gogotsi Y., (2006) *Nanomaterials Handbook*. Boca Raton, CRC Press.

Guo,T., (1995) Catalytic Growth of Single Walled Nanotubes by Laser Vaporization. *Chemical Physical Letter.* 243: 49.

Hamann, M.C.J., and Saville B., (1996) Enhancement of Tyrosinase Stability by Immobilization on Nylon-66. *Food Bioproduct Proc*ess 74: 47-52.

Hamon, M.A., Niyogi S., Hu H., Zhao B., Bhowmik P., Sen R., Itkis M.E., and Haddon R.C.(2002) Chemistry of Single-walled Carbon Nanotubes. *Acc.Chem. Res*. 35: 1105-1113.

Hou, P.X., Liu C., and Cheng H.M.,(2008) Purification of Carbon Nanotubes. *Carbon*. 46 : 2003-2025.

Hu, H., Ni Y., Montano V., Haddon R.C., and Parpura V. 2009 Chemically Functionalized Carbon Nanotubes as Substrates for Neuronal Growth. *Nano Letter*. 4: 507-511.

Illanes, A., (2008). *Enzyme Biocatalysis Principle and Applications.* Chile, Springer Science.

Jain A.K., Dubey V., Pharm M., Mehra N.K., Lodhi N., Nahar M., Mishra D.K., and Jain N.K., (2009) Carbohydrate-conjugated multiwalled Carbon Nanotubes: Development and Characterization. *Nanomed: Nanotechnology, Biology, and Medicine*. 5: 32-442.

Jishi R.A., Bragin J., and Lou L., (1995). Electronic Structure of Short and Long Carbon Nanotubes from First Principles. Physical Review B. 59: 9862-9863.

Joseyacaman, M., Miki-Yoshida M., Rendon L., and Santiesteban J.G., (1993) Catalytic Growth of Carbon Microtubules with Fullerene Structure. *Applied Physics Letter*. 62: 657-659.

Jung, Y. C., Muramatsu, H., Hayashi, T., Kim, J. H., Kim, Y. A., Endo, M. and Dresselhaus, M. S. (2010), Covalent Attachment of Aromatic Diisocyanate to the Sidewalls of Single- and Double-Walled Carbon Nanotubes. *European Journal of Inorganic Chemistry*, 2010: 4305–4308.

Karousis, N., Tagmatarchis N., and Tasis D., (2010) Current Progress on the Chemical Modofication of Carbon Nanotubes. *Chem Rev*. 110: 5366-5396.

Kim, Y.T., and Mitani, T., (2006) Surface Thiolation of Carbon Nanotubes as Support: A promising Route for the High Dispersion of Pt Nanoparticles for Electrolysts. *Journal of Catalysis.* 238: 394-401.

Kocabas, S.,Kopac, T., Dogu, G., and Dogu, T., (2008) Effect of Thermal Treatments and Palladium Loading on Hydrogen Sorption Characteristics of Single-Walled Carbon Nanotubes Int. J. Hydrogen Energy, 33: 1693 – 1699

Li, L., Feng, W. and Ji, P. (2011), Protein adsorption on functionalized multiwalled carbon nanotubes with amino-cyclodextrin. *AIChE Journal*, 57: 3507–3513

Liao, Q., Sun J., and Gao L., (2009) Degradation of Phenol by Heterogenous Fenton Reaction Using Multi-walled Carbon Nanotube Supported Fe2O3 Catalyst. *Journal of Colloid and Surfaces A: Physicochemical and Engineering Aspect.* 345: 95-100

Lin, D., and Xing, B., (2008) Adsorption of Phenolic Compounds by Carbon Nanotubes: Role of Aromaticity and Substituition of Hydroxyl Groups. *Environmental Science Technology.* 42: 7254-7259.

Lu, C., and Chiu, H.,(2008). Chemical Modification of Multiwalled Carbon Nanotubes for Sorption of Zn2+ from Aqueous Solutions. Chemical Engineering Journal. 139: 462-468.

Marin-Zamora, M.E., Rojas-Melgarejo F.,Garcia-Canovas F., and Garcia-Ruiz P.A, (2007) Effect of the Immobilization Supports on the Catalytic Properties of Immobilized Mushroom Tyrosinase : a Comparative Study Using Several Substrates. Journal of Biotechnology. 4: 388-396.

Nemes-Ineze, P., Tapaszto, L., Darabont, Al., Lambin, Ph., Biro, L.P., (2009), Scanning Tunneling Microscopy Observation of Circular Electronic Superstructures on Multiwalled Carbon Nanotubes Functionalized by Nitric Acid. *Carbon*. 4 : 764-768.

Norhafizah Binti Yasin (2009). Protein Released from Calcium Alginate Beads Coated with Chitosan. Bachelor of Science. Universiti Teknologi Malaysia, Skudai.

O'Connell M.J.,Bachillo S.M., Huffman C.B., Moore V.C., Strano M.S., Haroz E.H., Rialon K.L., Bol P.J. and Thestenson E.T., (2002) Band Gap Fluorescence from Individual Single-walled Carbon Nanotubes. *Science.* 297 : 593-596.

Pagona,G. and Tagmatarchis, N., 2006 Carbon Nanotubes : Materials for Medical Chemistry and Biotechnology Applications. *Current Medical Chem.Int.Ed.* 40: 4002-4005.

Pan B. and Xing B., (2008). Adsorption Mechanism of Organic Chemicals on Carbon Nanotubes. *Environmental Science Technology*. $2.pg 9005-9013.

Perez, J. P. H., Sanchez-Paniagua Lopez, M., Lopez-Cabaros, E., and Lopez-Ruiz, B. (2006). Amperometric Tyrosinase Biosensor Based on Polyacrylamide Microgels. *Biosensors and Bioelectronics*. 22(3): 429-439

Qiu, J., Gao Z., Bandosz T.J.,Zhao Z., and Han M. (2009) Investigation of Factors Affecting Asdorption of Transoition Metals on Oxidized Carbon Nanotubes. Journal of Hazardous Materials. 167 pg1-3.

Ratner, M.A., Vura-Weis J., Wasielewski M.R., (2010) Geometry and Electronic Coupling in Perylenediimide Stacks :Mapping Structure Change Transport Relationship. Journal of American Chemical Sociecty. 132: 1738.

Ren X., Chen, C., Nagatsu M.and Wang X., (2010) Carbon Nanotubes as Adsorbent in Environmental Pollution Management: A review. *Chemical Engineering Journal.*170: 395-410.

Shen, X.E., Shan X.Q., Dong M.D., Hua X.Y. and Owens G., (2009) Kineics and Thermodynamics of Sorption of Nitroaromatic Compounds to As-grown and

Oxidized Multiwalled Carbon Nanotubes. *Journal of Colloid and Interface Sciences*. 330: 1-8.

Sheng G.D., Shao D.D., Ren X.M., Wang X.Q., Li J.X., Chen Y.X., and Wang X.K.,(2010) Kinetics and Thermodynamic of Adsorption of Ionizable Aromatic Compounds from Aqueous Solution by As-prepared and Oxidized Multiwalled Carbon Nanotubes. *Journal of Hazardous Materials*. 8: 344-345

Vermisoglou, E.C., Georgekilas,V., Kouvelas E., Pilatos G., Viras K.,Romanos G., and Kanellopoulos N.K.,(2006) Sorption Properties of Modified Single-walled Carbon Nanotubes. Microporous and Mesoporous Materials 99: 98-105

Wade, L.G., (2006) *Organic Chemistry.*(6th ed.) USA. Prentice Hall.

Wiener N (1964). *Extrapolation, Interpolation, and Smoothing of Stationary Time Series.* Cambridge, Mass: MIT Press.

Xu, X.,Thwe MM., Shearwood C., Liao K.,(2002) Mechanical Properties and Interfacial Characteristics of Carbon Nanotube Reinforced Epoxy Thin Films. Applied Physical Letter. 81: 2833-2835.

Yakobson B.I, Avouris P.,(2001) Mechanical Properties of Carbon Nanotubes. *Applied Physics*. 80: 287-329.

Yaropolov, A.I., Yarapolov O.V.S, Vartanov S.S., and Varfolomeev S.D., (1995) Laccase, Properties, Catalytic Mechanism, and Applicabality. *Applied Biochem. Biotechnology.* 49: 257-280.

Zein, S.H.S., Chai S.P., Mohamed A.R.,(2007) The Effect of Reduction Temperature on Co-Mo/Al2O3 Catalysts for Carbon Nanotubes Formation.*Applied Catalysis A :General* 326: 173-179